WESTERN SECTION
AMERICAN SOCIETY OF ANIMAL SCIENCE
Committee Assignments 2004 - 2005
*** Denotes Committee Chair**

Executive
1. E. E. Grings, President (06, USDA-ARS, Miles City)*
2. J. Thompson, President-Elect (07, Oregon State Univ.)
3. J. C. Whittier, Past-President (05, Colorado State Univ.)
4. T. Ross, Secretary-Treasurer (08, New Mexico State Univ.)
5. D. Hallford, ASAS Board Director (07, New Mexico State Univ.)
6. B. L. Christensen, Industry Director (05, Alltech)
7. K. C. Olson, A&C Chair (05, Utah State Univ.)

Awards
1. J. Thompson (05, Oregon State Univ.)*
2. R. P. Ansotegui (05, Montana State Univ.)
3. D. E. Hawkins (05, New Mexico State Univ.)
4. T. DelCurto (06, Oregon State Univ.)
5. M. K. Peterson (06, New Mexico State Univ.)

Symposium
1. T. W. Geary (05, USDA-ARS, Miles City)*
2. P. D. Burn (05, Univ. Northern Colorado)
3. J. E. Sawyer (05, Texas A & M)
4. T. Engle (06, Colorado State University)
5. M. Wise (06, New Mexico State University)
6. M. MacNeil (06, USDA-ARS, Miles City)

Advising and Coordinating
1. K. C. Olson (05, Utah State Univ.)*
2. C. P. Mathis (05, New Mexico State Univ.)
3. F. M. Mitioehner (05, Univ. California, Davis)
4. G. E. Sides (05, Pfizer)
5. J. B. Taylor (05, USDA-ARS, Dubois)
6. J. M. Rumph (06, Montana State Univ.)
7. S. J. Filley (06, Oregon State Univ.)
8. J. B. Lamb (07, BYU-Idaho)
9. J. Sprinkle (07, Univ. Arizona)
10. S. I. Paisley (07, Univ. Wyoming)
11. J. B. Glaze (07, Univ. Idaho)
12. L. B. Bruce (07, Univ. Nevada)
13. S. Daugherty (07, Cal Poly)
14. J. Busboom (07, Washington Sate University)
15. D. Garrick (07, Colorado State Univ.)

Paper Competition
1. D. H. Crews (06, Ag Canada, Lethbridge)*
2. J. Berardinelli (05, Montana State Univ.)
3. D. McLean (05, Washington State Univ.)
4. C. A. Loest (05, New Mexico State Univ.)
5. R. M. Enns (06, Colorado State Univ.)
6. P. A. Ludden (06, Univ. Wyoming)
7. D. W. Bohnert (07, Oregon State Univ.)
8. T. Bodine (07, Western Feed Supplements. Washington)

Academic Quadrathalon
1. N.A. Irlbeck (Colorado State Univ.)*
2. J.B. Lamb (BYU – Idaho)
3. W. E. Plummer (Cal Poly State Univ.)
4. C. W. Hunt (Univ. Idaho)
5. L. M. Surber (Montana Sate University)
6. P.D. French (Oregon State University)
7. S. Soto-Navarro (New Mexico State University)
8. S. Wickler (Cal Poly, Pomona)
9. R. D. Weidmeier (Utah State Univ.)
10. D. C. Rule (Univ. Wyoming)

Extension
1. H. H. Patterson (05, South Dakota State Univ.)*
2. J. A. Scanga (05, Colorado State Univ.)
3. W. F. Gipp (05, Montana State Univ.)
4. D. Zobell (06, Utah Sate Univ.)
5. R. Zinn (06, Univ. California, Davis)
6. M. Encinias (07, New Mexico State Univ.)
7. R. Hathaway (07, Oregon State Univ.)

Necrology
1. P. G. Hatfield (05, Montana State Univ.)

Nominating
1. J. C. Whittier (05, Colorado State Univ.)
2. B. L. Christensen (05, Alltech)
3. P. G. Hatfield (05, Montana State Univ.)

Minutes of the Western Section of the
American Society of Animal Science
Business Meeting
June 18, 2004
Oregon State University
Corvallis, Oregon

President Jack Whittier called the meeting to order at 8:37 a.m.

Acceptance of the minutes of the 2003 business and awards meeting.
The minutes of the 2003 business meeting were approved as printed in the 2004 Proceedings of the Western Section of the American Society of Animal Science.

Advisory and Coordinating Committee Report
Clay Mathis, Chair

Issues Addressed:

WSASAS Strategic Plan

The committee evaluated the need to organize a revision of the Strategic Plan.

Consensus

The Strategic Plan is sufficient with well-stated goals. The A&C Committee recommends that the Western Section evaluate the section's efforts to achieve the goals stated in the Strategic Plan.

Web Site

The committee evaluated the need to make the Western Section meeting schedule available on the web site further in advance of the meeting, and discussed the need for an editorial proof reading of the Western Section Constitution posted on the web site.

Consensus

The A&C Committee recommends that the Executive committee request a proof reading of the Western Section Constitution and work with the host institution to post a general (skeleton) schedule when meeting registration information becomes available.

Graduate Student Competition

The committee discussed several issues surrounding the graduate student competition.

Consensus

The A&C Committee recognizes that scheduling challenges exist when the number of competitors is high, but is opposed to setting any limits on the number of oral presentations in the Graduate Student Competition because all facets of the competition greatly help the Western Section achieve goals listed in the Strategic Plan under increasing membership and participation.

The A&C Committee recommends that in the event that the Executive Committee or Graduate Student Competition Committee entertains the need for limiting the number of oral presentations in the future, the historical number of graduate students competing each year is considered to evaluate how frequently a significant scheduling issue occurs.

Academic Quadrathlon Report
Lisa Surber, Chair

The 2004 Regional Academic Quadrathlon contest was hosted by New Mexico State University on March 26 & 27, 2004. Six teams participated in this year's contest and they were as follows; Brigham Young University – Idaho, Colorado State University, Montana State University, New Mexico State University, University of Idaho, and Utah State University. Dr. Carl Hunt and was responsible for the written examination. Dr. Randy Weidmeier was in charge of the oral presentation. Dan Rule was responsible for the quiz bowl portion of the contest. Dr. Patrick French secured over $2,000 in book awards. Monsanto Company provided $5,000 in scholarships for this year's contest; first place team members received $600, second place team members received $400 and third place team members received $250. The contest results were as follows:

Quiz Bowl
1st Place Utah State University
2nd Place Colorado State University
3rd Place Montana State University

Written Examination
1st Place University of Idaho
2nd Place Utah State University
3rd Place Brigham Young University – Idaho

Discussion
1st Place Montana State University
2nd Place New Mexico State University
3rd Place Utah State University

Practicum
1st Place Utah State University
2nd Place New Mexico State University
3rd Place University of Idaho

Overall Placing
1st Place Utah State University
2nd Place New Mexico State University
3rd Place University of Idaho

The AQ Advisor Recognition Award was given to Dr. Carl Hunt of UI for his support and commitment to the AQ program. Dr. Hunt has served as UI advisor for many years and organized the written exam portion of the Regional AQ contest.

Last year's first place team was from Colorado State University and they competed in the National Collegiate Quiz Bowl during the National Cattlemen's Beef Association convention in Phoenix, Arizona and placed 1st. The 2004 overall winner will represent the Western Section at next years NCBA meeting, which will be held in February 2005 in San Antonio, Texas.

The 2005 Regional Academic Quadrathlon contest will be hosted by Utah State University and will be held on March 25 & 26, 2005.

Graduate Student Competition
Paul Ludden, Chair

Twelve manuscripts and oral presentations by graduate students from 7 institutions were evaluated by the 8-member committee. Committee members included:

Dr. Jim Berardinelli, Montana State University
Dr. Denny Crews, AAFC-LRC
Dr. Mark Enns, Colorado State University.
Dr. Clint Loest, New Mexico State University
Dr. Paul Ludden, University of Wyoming (2004 Committee Chair)
Dr. Derek McLean, Washington State University
Dr. Roy Silcox, Brigham Young University

Dr. Mark Wise, New Mexico State University

Due to unforeseen circumstances, Dr. Roy Silcox was unable to attend the meetings in Corvallis, but did submit scores for the written manuscripts. Dr. Dale ZoBell (Utah State University) served as a substitute for Dr. Silcox in evaluating the oral presentations. Dr. Clint Loest was unable to attend the meetings, and did not participate in the written or oral evaluation of the 2004 competition.

Winners of the 2004 Graduate Competition were announced at the Awards Barbeque. Individual award winners for the 2004 competition were:

1st place ($500): Scott L. Lake, University of Wyoming
2nd place ($300): John S. Nelson, New Mexico State University
3rd place ($200): Craig A. Gifford, New Mexico State University

The 2004 Graduate Student Institutional Award was also presented at the Awards Barbeque. A check for $2000 was presented by Connie Larson of Zinpro Corporation to the graduate students from New Mexico State University as winners of the 2004 Award. The students from NMSU had the highest average score among institutions with at least two competitors, which included Montana State University (3), New Mexico State University (3), and the University of Wyoming (2).

A proposed amendment to the WSASAS Bylaws/Constitution modifying the eligibility requirements for participation in the Graduate Competition was prepared and introduced to the Executive Committee for consideration at the Annual Business meeting. A system for notification of authors that a paper has been submitted for presentation in the Graduate Competition was also discussed. This notification system may also serve as a means of verifying eligibility, and may be developed on the advice of and/or action by the Executive Committee.

By unanimous vote, the committee makes the following recommendations. Dr. Denny Crews, AAFC-LRC, will serve as chair of the Graduate Competition Committee for the 2005 meetings in Las Cruces, NM. The terms of Dr. Mark Wise and Dr. Roy Silcox on the committee have been fulfilled and are in need of replacement to maintain an 8-member committee. The committee would further welcome the appointment of more than two new members pending willing participation. All other members of the committee listed above will remain on the committee for the 2005 meetings.

Proposed by-law amendment regarding membership in the Western Section and eligibility for elective office, awards and graduate student paper competition.

Motion: Change ARTICLE II: Membership Section 2 to read as follows:

Eligibility for Elective Office, Awards, and Graduate Student Paper Competition: Any qualified member who is located in the geographical region designated by the Bylaws of ASAS as part of the Western Section will be automatically eligible for election to office in the Western Section, nomination for Western Section awards, and participation in the Graduate Student Paper Competition, unless that individual has chosen to be considered a member of another geographical section of ASAS in accordance with ASAS Bylaws regarding sectional membership. Any qualified member who is not in the geographical region outlined as part of the Western Section by the ASAS Bylaws regarding

membership, but who has chosen to be a member of the Western Section according to ASAS bylaws regarding sectional membership, will be eligible for election to office and nomination for awards in the Western Section after a period of four years of membership in the Western Section. Graduate students outside the geographical region outlined as part of the Western Section by the ASAS Bylaws regarding membership will be eligible to participate in the Graduate Student Paper Competition provided that individual has chosen to be considered a member of the Western Section in accordance with ASAS Bylaws regarding sectional membership at the time of abstract submission.

This motion was voted upon and passed.

Awards
Elaine Grings, Chair
This was the first year that WSASAS award nominations were submitted and reviewed on the ASAS web site.
Committee Members:
Dr. Raymond Ansotegui, Montana State University
Dr. Dean Hawkins, New Mexico Sate University
Dr. Mark Peterson, New Mexico Sate University
Dr. Tim DelCurto, Oregon State University
Dr. Elaine Grings, USDA-ARS, Committee Chair

Distinguished Service Award:
Recipient: Dr. Martin Vavra
US Forest Service
La Grande, OR
Sponsor: DSM Nutritional Products

Distinguished Teacher Award:
Recipient: Dr. Janice Bowman
Montana State University
Bozeman, MT
Sponsor: Elanco Animal Health

Extension Award:
Recipient: Dr. Wayne Gipp
Montana State University
Bozeman, MT
Sponsor: Fort Dodge Animal Health

Young Scientist Award:
Recipient: Dr. D.H. 'Denny' Crews
Agriculture and Agri-Food Canada
Lethbridge Alberta
Sponsor: Western Section ASAS

Applied Animal Science Award
Bret Christensen, Chair

Winners of the Applied Animal Science Award sponsored by Alltech.

First Place:($500) Ward, E.H. and H.H. Patterson, SDSU - Effects of Thiamin Supplementation on performance and health of growing steers consuming high sulfate water.

Second Place: ($300) Merrill, M.L., R.P.Ansotegui, J.A. Paterson, and T.W. Geary - MSU Effect of Flunixin Meglumine on early embryonic mortality in stressed beef females.

Third Place: ($200) Rainey, B.M., J.A. Paterson, M.C. King, and W.T. Choat - MSU Effect of light test weight barley vs. wheat middlings on ADG and carcass characteristics of early-weaned calves.

Nominating
Pat Hatfield, Chair

Nominations for the 2004 WSASAS elections were:

President elect: James Thompson, Oregon State University
Secretary-Treasurer: Tim Ross, New Mexico State University
ASAS Western Section Director: Dennis Hallford, New Mexico State University
Ken Olson, Utah State University

Results of the 2004 WSASAS election were:
President elect: James Thompson
Secretary-Treasurer: Tim Ross
ASAS Western Section Director: Dennis Hallford

Necrology
Jim Oltjen, Chair

None

2004 WSASAS Financial Report
Jim Thompson Sec-treasurer

Cash on Hand at December 31,2002		**36,016.26**
Revenue and Support		
Donations - General	3,561.82	
Donations - Awards	3,600.00	
Meeting Registrations	31,999.93	
Ticketed Events	12,514.99	
Proceedings	15,930.00	
ASAS-Symposium Support	1,500.00	
ASAS-Dues	1,140.00	
Interest Income	1,979.31	
Miscellaneous Income	5,146.70	
Total Revenue and Support		77,372.75
Expense		
Call for Papers/Abstracts	222.00	
Awards/Plaques	4,977.07	
Quadrathalon	6,904.70	
Convention Fees	48,740.36	
Proceedings	4,548.81	
Postage/Supplies	64.58	
Telephone	28.05	
Staff Support	1,498.31	
Total Expenses		66,983.88
Net Revenue over Expense		10,388.87
Cash on Hand as of December 31, 2003		**46,405.13**

Old Business:
None

New Business
Jack Whittier turned the gavel over to President –Elect Elaine Grings. Elaine Grings then presented President Whittier a plaque in appreciation of his service. The meeting was adjourned at 9:10 a.m.

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RANDOM MUSING ON LAND GRANT UNIVERSITIES: THEIR RELEVANCE AND FUTURE

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Abstract: Real and potential decreases in funding for Land Grant Universities from both national and state sources provide impetus to examine the relevance and future of the Land Grant system; and to question certain aspects of its current structure and function. The Morrill Act of 1862 provided the beginnings of a revolution in higher education that would support the agricultural and mechanical revolutions in this country. The Hatch Act of 1887 provided the research component for the Land Grants with the creation of agricultural experiment stations. Also significant to modern day universities was the G.I. Bill of Rights of 1944 which changed the tradition of who could go to college. The post war era, particularly the coming of the "space age" saw a dramatic change in university priorities from teaching to research. Declining support for public universities by state legislatures and the increasing availability of and need for grant funds exacerbated the situation. Today Land Grant Universities are at a cross roads as the power of traditional clientele in state legislatures declines, as the ability of the universities to attract or even accept new clientele for support is lacking, and as the courage of administrators and faculty to break with traditionalism is absent. The future of the Land Grant Universities lies in the combined ability of both faculty and administration to adapt to a changing clientele and initiate constructive change.

ACKNOWLEDGEMENTS
The author wishes to extend his appreciation to the Western Section American Society of Animal Science for the Distinguished Service Award and to DSM Nutritional Products, Inc. for sponsoring the award. I would also like to thank Dr. Kelvin Koong for providing critical discussion regarding this manuscript.
Key Words: Land Grant Universities, teaching, research, extension

Introduction

Real and potential decreases in funding for Land Grant Universities from both national and state sources provides impetus to examine the relevance and future of the land grant system; and to question certain aspects of its current structure and function. The role of Land Grant Universities, that which comprises teaching, research, and extension, is unique as a mission for a university. The Morrill Act of 1862 (and later 1890) with funding through land sales for the establishment of Land Grant Colleges provided the beginnings of a revolution in higher education that would support the agricultural and mechanical revolutions in this country (Boyer 1990). The Hatch Act of 1887 added the research component through federal funding of the agricultural experiment stations. American higher education, once devoted to the intellectual and moral development of students now added service as a mission (Boyer 1990). Service was more formally defined by the Smith Lever Act of 1914 which established the Cooperative Extension Service. Not only was a college education possible for non-elite young people but learning was brought directly to the farmer (Boyer 1990). With the passage of the G. I. Bill of Rights in 1944, higher education moved completely from an educational system for the elite to a mass system, which changed forever the entire tradition of who should go to college (Boyer 1990).

Post-war America went on a technology binge with the coming of the “space age” and research priorities increased. Unfortunately, as a more diverse student body was entering the land grant universities, this new emphasis in research began narrowing the standards used to measure academic prestige (Boyer 1990). Professors were expected to obtain grants, conduct research, and publish results. At the same time in many states, support for higher education was declining, forcing more reliance on grants and the overhead derived from them. Publishing became the driving force in a successful application for tenure. In just a few decades the professoriate has become more hierarchical and restrictive; and the emphasis has changed from undergraduate education to graduate education and research (Boyer 1990).

Today, in most states land grant institutions struggle for identity with a clientele base that is changing from rural to urban; and with declining political clout at state and national levels. Also, large corporate agricultural operations may not need research and extension to assist in their viability, they have their own staffs. Land Grant Universities seem locked into their traditional clientele and uninterested or unable to expand that base to include non-traditional clientele. As any aging entity tends to do, land grant universities often cling to their traditional structure of delivering teaching, research and extension; new models of organization and delivery are notably absent. Changes are usually superficial and really done to maintain the status quo. The future of the land grant universities may be in the ability of both faculty and administration to adapt to changing clientele and initiate constructive change in program delivery. Change should not be considered a journey from point A to point B but an ongoing trajectory with only modifications in direction based on periodic assessments of success.

In this paper I would like to provide my opinions on some of the short comings of current land grant university structure and function, and offer some thoughts on where the future might lie.

Discussion

Teaching. Undergraduate teaching is perhaps the most important function of university faculty. It is also the one where exhibited excellence does not get the credit it deserves. Teaching duties, especially introductory courses, are often "dumped" on new-hire assistant professor level faculty or contract adjunct instructors. The most important students to engage in our field are the ones just beginning. Maximizing interest in the profession in the freshman and sophomore years is critical to student retention and developing a better attitude to learning. Teaching begins with what the teacher knows (Boyer 1990). Boyer goes on to say that those who teach must, above all, be well informed, and steeped in the knowledge of their fields. Therefore full professors with years of experience should be at the forefront of undergraduate teaching; but not to the neglect of graduate level teaching. Young faculty should have more freedom to broaden their experience base through research or extension activities before they begin to teach introductory courses at the undergraduate level.

Students in today's Land Grant University are more than likely to have not come from a production agriculture background. Course structure has to accommodate this lack of essential basic knowledge (what is a steer? a gilt?). In many cases, curricula in agricultural disciplines has not changed dramatically in decades. The original land grant mission was built on a liberal education, but agricultural schools have oriented it toward training and technology (CAHA 1993). Leininger (2005) reported that merely changing the names of courses increased enrollment in those courses. Some universities are now changing names of departments and undergaraduate majors to reflect a broader focus (McComb 2005). Curricula in land grant universities should be re-evaluated and "modernized" to meet current job market needs (McComb 2005) and to stimulate interest by the students. Every agriculture major should have good problem solving skills, be able to cope with change, communicate with different kinds of people, understand the political process, and know how to relate his/her specific training to broad public policy issues (CAHA 1993). McComb (2005) echoed this thought when stating that campuses must find a balance of disciplinary strengths while having the capacity to address interdisciplinary issues and opportunities. For example, 20 years ago an animal science graduate did not have to defend animal agriculture to animal rights advocates or explain how BSE got into our food supply.

Extension. The Smith Lever Act of 1914 Launched the Cooperative Extension Service. The model of a general agricultural agent, a home economist and a 4-H agent for each county was excellent for 1914 rural America. At that time farmers had little formal education, many could be classified as subsistence or barely above that level, wives stayed at home and processed many farm products for both home use and sale (canning, butter making, etc.), and rural children had almost no formal activities other than 4-H available. Today's farm family is much different in level of farm production and education of farmer and spouse. Additionally, one or both family members may be forced to "work in town" to support continuance of the farming operation (Hanson 1996). Farms are much more business than subsistence. Farm children have a great variety of activities tied with schools and non-school organizations; as well as the internet. And yet the extension model has not changed. The county agent in the western U.S. of today must be an expert in a variety of crop and animal production systems, an ecologist, expert in water quality, facilitator of confrontational public meetings, and may still get stuck judging market hogs as some county fair. It is time we recognized that the job has become impossible. The challenge is to make extension programs effective given the complexity of the issues and the number osf stakeholders (McComb 2005). We need to pursue new models of technology transfer. Impeding that change, in many cases, are county commissioners who contribute to extension budgets and demand agents be housed in their counties in return for continued support. A critical need of extension is management of exiting information (McComb 2005). Research is seldom archived and accessible. Data banks could be developed to facilitate the use of met-analysis and other data-mining techniques to address future problems (McComb 2005).

Research. Boyer (1990) flatly states that all faculty should establish their credentials as researchers. Whether or not they choose specialized, investigative work on an ongoing basis, every scholar must, demonstrate the capacity to do original research, study a serious intellectual problem, and present to colleagues the results. Certainly for faculty with teaching and/or extension appointments this would be demonstrated in the dissertation and afterward expectations for scholarship would be remaining professionally alive in their field of teaching and/or extension. For faculty with research FTE, however, this would be demonstrated on a continuing basis through the production of peer reviewed publications.

The decline in base funding for agricultural research and the resultant increasing need to capture grant funds has greatly weakened the quality of research at Land Grant Universities. Most grants run on a 2, 3, or 5-year timeframe, coinciding with M.S. and PhD. degrees (or a combination of both, 5 years). In many cases of research this is simply not enough time to sufficiently sample temporal variability effects (variation in annual precipitation on crop production). Long-term research requires stable base funding and freedom from the pressures of counting publications for tenure, promotion, or salary increases; neither exists in great quantity in today's academic world.

Contemporary research questions commonly are too complex for one scientist or a single discipline. Interdisciplinary teams are required. Within the university system, there is no consistent process for forming these teams and no consistent form of reward for the scientists who somehow manage to accomplish team

efforts (McComb 2005). In fact, the structure of discipline departments provides an effective barrier to cooperation. Departments are more often in competition with one another for scant funds and function like independent bandit kingdoms.

The prime need for interdisciplinary and transdisciplinary research teams is the issue of sustainability. Defined broadly, sustainability should be at the top of research agendas (McMichael et al. 2003). The authors go on to state that the inability of key scientific disciplines to engage interactively is an obstacle to the actual attainment of sustainability. And further, that such approaches (interdisciplinary) must be unconstrained by traditional disciplinary domains and concepts. Such approaches may prove difficult to achieve within conventional university departments, and purpose-built interdisciplinary centers will therefore be needed (McMichael et al. 2003). What better entity than the land grant universities to collectively address sustainability! It just might be the silver bullet to save the land grants.

Clientele. "Land grant research and teaching have pursued efficiency and mechanized production of food at the expense of small farmers, rural communities, and high quality food. The accountability project blames declining rural populations, disappearing small towns, and rural poverty in part on the policies of land grant universities." So states Marston (1992) in referencing James Hightowers's book *Hard tomatoes, Hard Times* (1973). That may or may not be the case. But the issue of who are the clientele of the Land Grant Institutions is an important question and critical to survival.

Too often faculty become overly involved with the industry they think they serve. They move from providing information to improve the industry to advocates of the industry. Sometimes this latter relationship is nurtured by the industry itself through political pressure on Deans or legislators. Faculty or departments involved in advocacy may provide for industry preference for one department over another, and create a cascading effect of credibility loss throughout a college of agriculture. Prime examples of this occur in grazing issues in the West. It is important to note that probably an equal number of university faculty exist that are advocates for special interest groups totally opposed to agricultural production. Again, grazing issues in the West offer the prime example.

Advocacy may increase status with a narrow clientele group but alienate a large number of other citizens. This begs the question: who are the clientele of the land grant universities? Marston (1992), expanding Hightower's (1973) list, included large farmers, chemical and equipment manufacturers, agribusiness, ranchers, loggers, water developers, and so forth as the current clientele list. Now, quite frankly, I see no reason why these groups should not be on a clientele list, as long as faculty do not provide advocacy to these groups and as long as this list is not exclusive of others. The problem is that land grants have not actively pursued new clientele, and, in fact, have often clearly resisted. Oregon is currently in the midst of a state budget crisis. There is now no extension office in the county that is home to 25 percent of the population.

As the number of people involved in agriculture declines and as non-ag industries increase in the western states, the power of rural legislators to provide funding for agriculture and forestry research and extension will decline. This has already begun at the national level as the proposed dismantling of the Hatch Act indicates. The general citizenry of any state should be engaged and convinced that the land grant institution is there to provide a bettering of their lives. Remember, the West, and indeed the entire US, is made up of city dwellers that know very little about agriculture and agricultural research. The concept of sustainability is again an excellent example of a research direction that applies to all the citizens of a state.

Traditionalism. One of the greatest impediments to progress and even survival is the inability to change and adapt; to stay with the traditional way of thinking and doing. Land Grant faculties and administrators are often guily of operating this way. We have been traditional in our thinking (what we teach, research and deliver) and in how we are organized. I have already stated my belief that departmental structure is one of the greatest impediments to interdisciplinary research.

Internal review (CAHA 1993) recognized that academic departments and individual faculty tend to work within, and get their rewards from, their respective disciplines. There are few rewards for interdisciplinary or integrative research. In the CAHA (1993) report, The Chair of that group, reflecting back on the report, stated that institutions were more likely to protect turf than to change, even when, and if, presented with good evidence that a new vision is needed (Box 2004). That report (CAHA 1993) listed several shortcomings of Land Grant Universities. Among them were: reluctance to acknowledge change, manage and predict it, and anticipate future change with preparations to address the next generation of problems; maintaining ties only with traditional funding sources, commodity groups and production agriculture; funding usually for narrow discipline oriented research is often directed by commodity groups; and little funding is developed for broad, integrative research and extension efforts. The western land grant university deans, however, thought the report too negative and it was dismissed in favor of a marketing program to sell the positive aspects of agriculture (Box 2004). Even when and if, a dean has the courage to suggest new structures other than the standard discipline departments, the faculty will rise up and decry that as heresy; even to the extent of engaging clientele and legislators to suppress the idea. Box (2004) went on to say that land grant schools have lost funding and students in the area of natural resource management because non-land grant schools developed curricula to educate students in how to deal with change [non-traditional curricula], and their graduates now take jobs once available to land grant graduates.

Conclusions

The President's proposed FY 2006 budget calls for a reduction and realignment of $143 million Hatch formula funding. This has been discussed previously, but FY 2006 is the first appearance in a budget. Recent interest in this move was stimulated by a Congressionally mandated task force (Stokstad 2004) of USDA appointed scientists that pondered a national institute of food and agricultural science. USDA preferred another approach, boosting the National Research Initiative, and that is where half of the cut Hatch funds will go. Will this reduction really take place? Will there continue to be an erosion of Hatch formula funds? Where does that leave land grant universities? And the real question: Are land grant universities still relevant?

Marston (1992) observed that Land Grant Universities, through their ties with the land and the rural people, have the potential for providing a model of reform and renewal. Dr. John H. Gibbons, President Clinton's science advisor was quoted by Broad and Glanz (2003) as saying "Science is still the wellspring of new options. How else are we going to face the issues of the 21st century on things like environment, health, security, food, and energy?" The land grant faculty that made up CAHA (1993) stated that the land grant university model should have a comparative advantage over other organizations since its mission relates to societal issues through developing new knowledge, teaching future leaders, helping apply knowledge, and providing feedback from citizen to academician. Additionally, they felt agricultural colleges can reestablish their role only if they recognize the changing societal demands on the land, enhance their areas of comparative advantage and work for mutual complimentary benefits between old and new constituencies.

Among the CAHA (1993) recommendations to universities were 1) encourage leadership at all levels of the university to create a shared vision of how the agricultural college can relate to changing social values and public concerns; 2) create a pool of funds, obtainable on a competitive basis, for integrative and interdisciplinary efforts; 3) restructure the rewards system to give credit to team efforts and other non-traditional efforts; 4) work with a broad range of publics; 5) diversify the faculty; 6) examine teaching programs to assure they are adequately preparing students. Additionally, CAHA (1993) recommended to all western land grants to: 1) work to get funds for coping with change into the highest priority among national agricultural priorities; 2) create west-wide ad hoc committees as needed to develop strategies and cooperative plans for actions on specific issues; 3) take collective action to make regional projects more effective; and 4) create a pool of funds for competitive grants directed toward interdisciplinary research.

Throughout this discussion I have mentioned the lack of integrative multidisciplinary research. CAHA (1993) made this a major issue as I have indicated. Provenza (2004) suggested that by interacting with others of disparate background, we can create crucial blends in science and management, but we typically do not integrate among diverse scientific disciplines or between science and management.

Finally I would like to paraphrase the CAHA (1993) report and say the ability of agricultural colleges to cope with changing publics, changing societal values, and changing economies is central to its being able to handle issues. If land grant universities are successful in identifying what society expects then they can be effective today and tomorrow. If they ignore public concern for new issues and listen only to their traditional clientele groups they will find themselves at odds with the people land grant universities are supposed to serve. If they refuse to acknowledge or are insensitive to societal changes they will become irrelevant.

Implications

Agricultural faculty within Land Grant Universities are faced with decreasing funding but are still expected to deliver teaching, extension and research products. There is a need to develop new models for all three services and develop new clientele. Ignoring the need for change will continue the downward spiral of funding at both state and national levels.

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CHARACTERISTICS AND POTENTIAL NUTRITIVE VALUE OF SAGEBRUSH-GRASSLAND VEGETATION COLLECTED FROM SITES CONTINUOUSLY GRAZED, RESTED FOR ONE YEAR AND ONE YEAR POST-DISTURBANCE

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ABSTRACT: Objectives were to assess initial impacts of rangeland management practices on characteristics and nutritive value of native sagebrush-grassland vegetation. Plots were randomly assigned to one of three treatments in a split-block experiment: continually grazed by beef cattle from June through October (Grazed), rested from grazing for one year (Rested), and interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*) and rested from grazing for one year (Disturbed-rested). Dominant plant species at peak production included Wyoming big sagebrush (*Artemisia tridentata*; ARTR), western wheatgrass (*Pascopyrum smithii*; PASM), prairie junegrass (*Koeleria pyramidata*; KOPY), Sandberg′s bluegrass (*Poa secunda*; POSE), rubber rabbitbrush (*Chrysothamnus nauseousus*; CHNA), and hood′s flox (*Phlox hoodii*; PHHO). Bare ground tended to be greater ($P = 0.07$) for the Disturbed-rested treatment. Aerial cover was less ($P = 0.03$) for Disturbed-rested because mean and relative cover of ARTR decreased ($P = 0.02$) and mean cover of POSE tended to decrease ($P = 0.09$) with this treatment. However, relative cover ($P < 0.001$) and DM yield ($P = 0.01$) of KOPY were greatest for Disturbed-rested. Although DM yield of PASM also increased ($P = 0.01$) with the Disturbed-rested treatment, total DM yield did not differ ($P = 0.42$) among treatments. Nutritive value was not influenced ($P = 0.22$ to 0.99) by treatment; however, nutritional value of new growth varied ($P < 0.001$) among the plant species. Relative feed value was greater for CHNA than ARTR, which was greater than the grasses but relative feed value did not differ among grass species. Crude protein was generally greater for shrubs than grasses. Within grasses, CP content was greater for KOPY and POSE than PASM. Disturbing rangelands by interseeding with yellow-flowering alfalfa resulted in greater quantities of grazable forage without major alterations in rangeland productivity.

Key Words: Rangelands, Nutritive value, Community characteristics

Introduction

Utilization of rangeland forage by grazing livestock is an integral component of agricultural systems in the western US. Rangelands are typically deficient in soil N, exhibit low productivity, and have poor forage quality throughout much of the year. The USDA-NRCS (1998) suggests that 67% of native rangelands could benefit from improved management or renovation. Resting rangelands from grazing has been proposed as a management strategy to increase native forage production and improve range condition (Gardner, 1950; Frischknecht and Plummer, 1955; Robertson, 1971). Due to the success experienced by a rancher in South Dakota (Smith, 1997), recent attention has focused on interseeding yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*) as a method to alleviate soil N deficiencies and increase forage production and quality on rangelands. Although most fixed N is tied up in the alfalfa plant biomass, N is leaked into the soil through root exudation and root turnover, making it available for the native rangeland vegetation (Schuman et al., 2002). Mortenson et al. (2004) demonstrated that interseeding yellow-flowering alfalfa increased total soil N and overall forage production of mixed-grass prairie. Crude protein content and in vitro digestibility of native forages were enhanced in range sites where yellow-flowering alfalfa had been established for longer than 15 years (Hess et al., 2004). Although information is emerging on the practice of interseeding yellow-flowering alfalfa into mixed grass communities of the Northern Great Plains, we are not aware of information on interseeding yellow-flowering alfalfa into sagebrush-grasslands. Therefore, our objectives were to assess initial impacts of interseeding yellow-flowering alfalfa into sagebrush-grasslands and one year rest from livestock grazing on rangeland characteristics and nutritive value of native vegetation.

Materials and Methods

General

The study site was established in 2003 within an 1,800 ha pasture on the University of Wyoming's McGuire Ranch, approximately 56 km northeast of Laramie. Elevation is 2,203 m with Bonjea sandy loam as the major soil type. Precipitation, temperature, and wind speed were recorded on site from May, 2003 to May, 2004 using a Vantage Pro Model WWN-WM2 weather station (Davis Instruments, San Francisco, CA).

Nine, 100 m^2 plots were randomly assigned to one of three treatments in a replicated, split-block designed experiment. Treatments included continuous season-long (June through October) grazing by beef cattle (Grazed), rested from grazing for one year before collection (Rested), and interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*) and rested from grazing for one year (Disturbed-rested). On April 23, 2003, a Truax no-till drill modified with a chisel opener was used to under cut native sod and prepare an opening 5 cm deep and 15 cm wide for

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interseeding yellow-flowering alfalfa at a rate of 0.5 kg/ha. Permanent enclosures were constructed around plots that included a resting period to prevent disturbance associated with grazing by livestock and indigenous ungulates. Schuman et al. (2002) suggested that three years may be required for establishment of yellow-flowering alfalfa. Therefore, data reported herein may serve only as initial or baseline information.

Rangeland Assessment and Vegetation Collection

Sampling occurred during peak production from one of the three blocks (each treatment was represented in each block) spanning a three week period (June 28 to July 14, 2004). Using a modified Daubenmire (1959) approach, three transects (80 m) were randomly located within each plot and permanently established using steal rebar. Each transect consisted of 10, 0.25 m^2 quadrats systematically located for a total of 30 sampling quadrats per plot. Percent species canopy cover, frequency, above-ground biomass, litter, bare ground, and rock were determined within each 0.25 m^2 quadrat. Above-ground biomass for each species was determined by clipping each quadrat at ground level and combining quadrat samples within each transect. Mean cover of all species and relative cover by species were calculated on a quadrat basis from the individual species data. Dominant species included Wyoming big sagebrush (*Artemisia tridentata*; ARTR, shrub), western wheatgrass (*Pascopyrum smithii*; PASM, cool-season perennial grass), prairie junegrass (*Koeleria pyramidata*; KOPY, cool-season perennial grass), Sandberg's bluegrass (*Poa secunda*; POSE, cool-season perennial grass), rubber rabbitbrush (*Chrysothamnus nauseousus*; CHNA, shrub), and hood's flox (*Phlox hoodii*; PHHO, perennial forb). Only the recent meristematic growth was collected from shrubs.

Laboratory Analyses

Pooled vegetation samples (by transect) were dried in a 50^{o}C forced air oven, and ground through a 1 mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). Samples were analyzed for DM and ash (AOAC, 1990), NDF and ADF (ANKOM 200 fiber analyzer, ANKOM Technology Fairport, NY), and N (LECO model FP-528 Nitrogen Determinator, LECO, St. Joseph, MI). Nitrogen was further fractionated into NDF-associated N (**NDIN**), ADIN, and B_3 (Sniffen et al., 1992). Relative feed value and DMD were estimated from fiber content using equations of Linn and Martin (1989).

Statistics

Mean canopy cover, relative cover, DM yield per individual species, and nutritive values were analyzed as a 3 (treatment) × 6 (dominant plant species) factorial arrangement in a split-block design. Data were analyzed using PROC MIXED of SAS (SAS Institute, Cary NC) with block and the block × treatment interaction included in the RANDOM statement. Total DM yield and data for other rangeland characteristics were analyzed as a randomized complete block design using PROC MIXED with block as a random effect. Following a significant preliminary F-test, the LSMEANS option was used to separate means.

Results and Discussion

Vegetation and Non-vegetation Community Characteristics

There were no differences (P = 0.12 to 0.96) in mean cover, relative cover, and yield for all plant species collected from the Grazed and Rested treatments (Table 1). Although it is probable that yield will increase after one year's rest (Frischknect and Plummer, 1955), Rice and Westoby (1978) demonstrated that production of dominant shrubs and perennial grasses of semiarid shrublands was not affected by resting from grazing for up to 15 years. The Disturbed-rested treatment tended to increase (P = 0.07) the proportional area of bare ground by decreasing (P = 0.03) vegetation cover (Table 2), as indicated by a decrease in mean (P = 0.02) and relative cover (P = 0.02) of ARTR and a trend toward decreased (P = 0.09) mean cover of POSE. Reduced cover of ARTR and POSE also seemed to be associated with increased (P < 0.001) relative cover of KOPY and greater (P = 0.01) DM yield of KOPY and PASM in the Disturbed-rested treatment. This may be explained by the competitive nature of range plants. Köchy and Wilson (2000) suggested that shrubs suppress grass production by reducing available soil N and their secondary growth creates greater biomass and height, eventually displacing grasses. Therefore, when ARTR is removed an increase in production of perennial grasses is anticipated (Frischknecht and Plummer, 1955). However, the lack of difference in DM yield of ARTR (P = 0.42) and POSE (P = 0.25) suggests that a reduction in cover of these species was offset by increased production. This is consistent with Köchy and Wilson (2000) who suggest that competition exists between plant species as well as among individual plants of the same species. The initial disturbance associated with interseeding of yellow-flowering alfalfa may therefore increase production of perennial grasses without altering sagebrush productivity.

Nutritive Value

Nutritive value of sagebrush-grassland species were not influenced (P = 0.22 to 0.99) by treatment (data not shown); however, nutritional value varied (P < 0.001) among individual plant species (Table 3). Crude protein content was generally greater for shrubs than grasses. Within the grasses, CP was greater (P< 0.001) for KOPY and POSE compared with PASM. Lyons et al. (1996) reported that native perennial grasses ranged from 2 to 25% CP during the growing season. Cook and Harris (1950) observed that CP of grasses on Utah summer range was about 6% and leaf CP material (~ 12%) was greater than stem CP (~ 4%). Initiation of growth for cool-season perennial grasses typically starts in May and June when temperatures are approximately 12 ^{o}C (Toole, 1976). High temperatures recorded on site reached 12 ^{o}C during April; however, average temperature did not reach 12^{o}C until late June which corresponded with the sampling period. Accumulation of leaf material was most likely limited until late June, which may explain CP content of perennial grasses within the lower range reported by Lyons et al. (1996). Although shrubs generally had greater CP than grasses, ADIN was often greater for shrubs compared with grasses.

Fiber-associated CP (Van Soest et al., 1991) has been used to describe CP fractions that are slowly degraded in the rumen and unavailable to ruminants (NRC, 1996). The NDIN fraction represents CP that is not degraded in the rumen. The bound fraction (fraction C) is estimated by ADIN, which is not available for digestion. The B_3 fraction

is determined by the difference between NDIN and ADIN and assumed to have an intestinal digestibility of 80%. Within the grass species, NDIN was less ($P < 0.001$) for PASM compared with POSE and KOPY; however, the B_3 fraction was greatest ($P \leq 0.001$) for KOPY. Although fractionation of CP using the fiber-associated determinations does not completely account for the dynamics of ruminal fermentation and postruminal digestion (Sniffen et al., 1992), strategic protein supplementation for grazing cattle may be justified due to apparent differences in CP availability.

Due to less ($P < 0.001$) fiber, relative feed value was greater ($P = 0.001$) for CHNA than ARTR. Relative feed value of shrubs was greater ($P < 0.001$) than grasses. However, relative feed value did not differ ($P = 0.41$ to 0.88) among the perennial grass species. Bhat et al. (1990) also reported that shrubs have relatively high nutrient content. Greater relative feed value estimates for shrubs is likely attributed to higher cell soluble contents in actively growing plant material of shrubs compared with perennial grasses (Lyons et al., 1996). Greater nutritional values for shrubs, however, must be interpreted with caution. For example, the actual nutritive value of big sagebrush is greatly reduced (Ngugi et al., 1995) because substances in sagebrush cause deleterious effects on digestibility (Johnson et al., 1976).

In summary, the process of interseeding yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*) results in reduced aerial cover of dominant shrub species without impacting productivity. Reduced competition from shrub species promotes increased production of less dominant cool-season perennial grasses. Resting a site from grazing for a period of one year did not alter plant community characteristics or productivity of native rangeland.

Implications

Disturbing sagebrush-grassland rangelands by interseeding with yellow-flowering alfalfa altered the physical structure of sagebrush-grasslands, but forage production was largely unaffected because of compensatory responses by some perennial grasses. Livestock managers should be aware of differences in potential digestibility of crude protein among the various prominent grasses when designing feed supplements.

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Table 1. Characteristics of dominant plant species collected from the McGuire Ranch in southeastern Wyoming, 2004

	Mean aerial cover, %					Relative aerial cover, %					Yield, kg/ha				
Item[a]	G[b]	R[c]	DR[d]	SE[e]	*P*	G	R	DR	SE[e]	*P*	G	R	DR	SE[e]	*P*
ARTR	10.7[z]	9.3[z]	6.2[y]	1.3	0.02	29.6[z]	29.3[z]	20.7[y]	4.6	0.02	156.5	170.1	202.0	42.0	0.52
CHNA	3.5	3.6	3.1	0.9	0.75	9.5	10.6	11.0	2.1	0.72	65.7	96.9	86.2	25.4	0.28
KOPY	2.5	2.6	3.0	0.5	0.24	6.8[y]	7.9[y]	10.6[z]	1.2	< 0.01	35.4[y]	40.3[y]	65.1[z]	10.7	0.01
PASM	2.9	2.5	2.7	0.3	0.43	7.8	8.0	9.6	0.7	0.17	103.5[y]	93.7[y]	147.9[z]	23.5	0.01
PHHO	3.0	2.8	2.8	0.3	0.75	8.3	8.6	10.1	1.1	0.22	48.0	46.2	54.0	7.0	0.60
POSE	9.5[z]	8.3[z]	6.4[y]	1.7	0.09	25.2	25.4	21.3	3.0	0.33	135.3	156.0	159.8	13.8	0.25

[a]ARTR = Wyoming big sagebrush (*Artemisia tridentata*), CHNA = rubber rabbitbrush (*Chrysothamnus nauseousus*), KOPY= prairie junegrass (*Koeleria pyramidata*), PASM = western wheatgrass (*Pascopyrum smithii*), PHHO = hood's flox (*Phlox hoodii*),and POSE = Sandberg's bluegrass (*Poa secunda*).
[b]G = continuous season-long (June through October) grazing by beef cattle.
[c]R = rested from grazing for one year before collection.
[d]DR = interseeded with yellow-flowering alfalfa then rested from grazing for one year before collection.
[e]n=27.
[zy]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 2. Effect of treatment on rangeland characteristics

	Treatment[a]				
Item	Grazed	Rested	Disturbed-rested	SE	*P*
Yield, kg/ha	544.36	603.2	714.91	87.0[b]	0.42
Vegetation cover, %	36.4[z]	32.2[zy]	29.1[y]	2.6[c]	0.03
Rock, %	6.8	8.1	7.7	1.1[c]	0.62
Litter, %	15.1	16.4	15.7	0.8[c]	0.44
Bare ground, %	41.6[y]	43.2[y]	47.5[z]	2.0[c]	0.07

[a]Continuous season-long (June through October) grazing by beef cattle (Grazed), rested from grazing for one year before collection, and interseeded with yellow-flowering alfalfa (Rested), then rested for one year before collection (Disturbed-rested)
[b]n = 9
[c]n = 27
[z,y]Means within a row lacking a common superscript differ ($P < 0.05$)

Table 3. Nutritional indices of dominant plant species collected from the McGuire Ranch in southeastern Wyoming, 2004

	Species[a]							
Item	ARTR	CHNA	KOPY	PASM	PHHO	POSE	SE[b]	*P*
Ash, %DM	8.31[w]	13.28[y]	9.82[xw]	8.02[w]	26.53[z]	11.15[yx]	1.1	<0.0001
NDF, %DM	41.58[x]	37.92[w]	66.81[z]	65.72[z]	59.32[y]	64.78[z]	1.1	<0.0001
ADF, %DM	32.19[w]	29.72[v]	42.82[y]	39.05[x]	55.13[z]	40.92[yx]	2.7	<0.0001
CP, %DM	11.29[y]	12.82[z]	10.92[yx]	9.28[w]	7.84[v]	10.62[x]	0.2	<0.0001
NDIN[c], %CP	42.20[y]	34.27[x]	49.79[z]	26.88[w]	38.11[yx]	35.95[x]	1.9	<0.0001
ADIN, %CP	17.75[y]	14.67[yx]	12.26[xw]	7.35[v]	21.94[z]	10.42[wv]	3.9	<0.0001
B_3[d], %CP	24.45[y]	19.60[yx]	37.53[z]	19.53[yx]	16.16[x]	25.53[y]	3.7	<0.0001
DMD[e], %	63.83[y]	65.75[z]	55.55[w]	58.48[x]	45.95[v]	57.02[xw]	2.1	<0.0001
RFV[f]	143.06[y]	165.59[z]	77.62[x]	82.91[x]	72.30[x]	81.95[x]	7.2	<0.0001

[a]ARTR = Wyoming big sagebrush (*Artemisia tridentata*), PASM = western wheatgrass (*Pascopyrum smithii*), KOPY = prairie junegrass (*Koeleria pyramidata*), POSE = Sandberg's bluegrass (*Poa secunda*), CHNA = rubber rabbitbrush (*Chrysothamnus nauseousus*), and PHHO = hood's flox (*Phlox hoodii*).
[b]n=54
[c]NDIN = neutral detergent insoluble N.
[d]B_3 = protein fraction assumed to be available for digestion in the lower digestive tract.
[e]DMD = dry matter disappearance [88.9 – (0.779 x ADF %)].
[f]RFV = relative feed value [(DMD x DMI)/1.29].
[z,y,x,w,v]Means within a row lacking a common superscript differ ($P < 0.05$).

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ASSESSING STORAGE OF WET BREWERS' GRAINS WITH STRAWS

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ABSTRACT: The objective of this study was to evaluate the ensiling properties of wet brewers' grain (WBG) alone and with various levels of straw so it can be used as part of the sole ration for ruminants. The WBG was ensiled alone and was mixed with one of two straws, wheat and tall fescue, at 20%, 30% or 40% WBG (as is basis), respectively. Final mixtures were brought to 65% moisture content with water. Treatments were replicated three times. Treatments were thoroughly mixed, vacuum sealed, and ensiled for 30 days. After ensiling, bags were opened and measured for dry matter and pH. Samples were freeze dried and analyzed for: ethanol, ammonia–N, lactic acid, acetic acid, propionic acid, butyric acid, and proximate analysis. Compared to the straw treatments, pH was lower ($P<0.01$) for WBG. Wet brewers' grain was higher ($P<0.01$) in CP than straw treatments. Across straw treatments, CP was lower than expected. Ethanol production was high ($P<0.05$) for WBG versus straw treatments. Ammonia-N was higher ($P<0.05$) in WBG when compared to straw treatments. Acetic acid in WBG was higher ($P<0.05$) versus straw treatments. Wheat straw was lower ($P<0.05$) in acetic acid than grass straw. Lactic acid in WBG versus straw treatments showed no difference ($P=0.30$). Comparing straw treatments for lactic acid content, wheat straw was lower ($P<0.01$) than grass straw. Using proper storage techniques, WBG alone can be used as an effective and nutritious feed. It can be mixed at high percentage (30-40%) of WBG to straw to be cost effective and offer a complete ration.

Keywords: Wet brewers' grain, Straw silage, By-products

Introduction

Wet brewers' grain (WBG) has been used as a livestock feed since the production of the first beer (Chandler, 1991). The WBG is sterile when it leaves the brewery. The material is produced from food-grade quality products that have been subjected to extensive heating for extended periods of time. This heating during production results in increased palatability and the establishment of high levels of bypass proteins (Stengel, 1991). Wet brewers' grain is a concentrated source of digestible fiber (NDF), ranging from 75-80% moisture, and is high in CP at approximately 26% on a DM basis (Chandler, 1991). Since, WBG is high in NDF content and low in starch content there is less energy loss from ruminal methane in comparison to high starch feeds (McDonald et al., 1995). Little is known about WBG in mixed silages along with its efficacy in current production systems. Historically, research was conducted on the ensiling of straight WBG (Harrison, 1996). European research evaluated the effects of absorbents to reduce effluent loss, with straw being the most absorbent but resulting in a reduction of straw digestibility (Harrison, 1996). A high amount of grass seed straw is produced in the Pacific Northwest. In 1999, approximately 900,000 tons of grass seed straw was available in Oregon (86%), Washington (8%) and Idaho (6%). It is a commonly harvested forage fed throughout the Willamette Valley, OR and its use would increase if nutrient value is improved. Grass seed straw is typically around 7-10% CP (Bohnert et al., 2003). Producers also use lower quality forages such as wheat straw. The objectives of this study are to achieve a cost-effective, locally available feed to benefit producers and formulate nutritionally adequate silage to be used as a total mixed ration (TMR) for ruminant species.

Materials and Methods

Experimental treatments

Wet brewers' grain was collected on April 15th, 2004 from Rogue Brewery in Newport, OR. The WBG was ensiled alone and was also mixed with one of two straw treatments, wheat and tall fescue, at 20% (20:80), 30% (30:70), and 40% (40:60) WBG, respectively. The ingredients were thoroughly mixed in each respective treatment by use of the Uebler® mixer. Each silage treatment (3 replicates) was randomly assigned to one of twenty one bags; which had been altered to measure internal temperature and gas production throughout the ensiling process. Each bag contained 2.3-6.8 kg of the assigned silage treatment. Bags were then vacuum sealed to ensure complete oxygen removal and to provide an anaerobic environment to begin the ensiling process. Over the first 5 days, temperature and gas production were monitored at 8 hour intervals. After that period, temperature was monitored every 24 hours for the remainder of the 30 day period.

Analytical procedures

At the end of 30 days of ensiling, bags were opened and the pH was determined. Fifty grams of wet sample were homogenized with 100 mL of deionized water for 5 min in a laboratory blender (Waring Blender 700, New Hartford, CN) and pH was measured. The remaining silage from each bag was freeze dried and stored for later analysis. Thawed silage was prepared for fermentation analysis by adding 250 g of distilled water to 30 g of silage and homogenized for 5 min in laboratory blender

(Waring Blender 700, New Hartford, CN). The silage was analyzed for ethanol, lactic acid, acetic acid, propionic acid, and butyric acid by gas chromography (Hewlett Packard 5890, with a capillary column, and helium gas as carrier gas; Horney et al, 1996). Total acids were computed as the sum of lactic acid and volatile fatty acids. Ammonia-N was measured by a phenol hypochlorite assay as described by Broderick and Kang (1980) with the use of UV spectrometer. All silages were analyzed for DM (AOAC, 1990). CP content was determined by the Kjedahl method (Buchi 322, Brinkman, Switzerland). Neutral Detergent Fiber (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) was analyzed using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport NY).

Statistical analysis

Data were analyzed as a completely randomized design using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model included all replications, treatment, straw type, percentages, and straw type X percentage interaction. Preplanned orthogonal contrasts were used to compare treatment means: 1) linear effect of WBG vs. Grass & Wheat; 2) linear effect of straw type and percentage; 3) quadratic effect of straw type and percentage.

Results and Discussion

Wet Brewers' grain vs. Straw Treatments

Compared to straw treatments, WBG was lower ($P<0.05$; Table 1) for DM, NDF, and ADF. As expected, WBG was higher ($P<0.05$) in CP compared to straw treatments (Table 1). Fermentation results show that WBG was higher ($P<0.05$) in ammonia-N, ethanol, and acetic acid production (Table 4)[F1]. This could be due to additional fermentation of the WBG. Wet brewers' grain was lower ($P<0.05$) in pH, lactic:acetic ratio, and total acids (Table 2). The natural buffering capacity of straw treatments may have contributed to higher pH compared to WBG (3.88 vs 3.49). The lactic:acetic ratio and total acids were lower for WBG due to the low amount of lactic acid being produced compared to the straw treatments. There was no difference ($P=0.30$) in lactic acid production comparing WBG (7.1) to straw treatments (7.96). Butyric Acid was only detectable in WBG at 0.13%. Propionic acid was greater ($P<0.05$) for WBG versus straw treatments (0.09 vs 0.01%). There was no difference ($P>.05$) in temperature.

Straw treatments

The 20:80 WBG/wheat is believed to have natural buffering capacity as a result of the 80% wheat straw in the silage, therefore slowing down the ensiling process. This resulted in a straw type x percentage interaction ($P<0.05$) and a quadratic effect ($P<0.05$) in DM, CP, NDF, ADF, and ammonia-N (Tables 3, 4). The quadratic effect in DM, NDF, and ADF is due to the higher wheat straw percentage, causing higher DM, NDF and ADF values (Table 3). The lower values for CP and ammonia-N can be attributed to a higher percentage straw in the 20:80 WBG/wheat, since wheat straw is low in nitrogen content. One assumption for the difference in expected CP vs. actual CP among straw type percentages(Table 5), is that when mixing, water escaped through holes in the Uebler along with pulling any WBG effluent that wasn't absorbed by the straw. The effluent is believed to contain a high amount of soluble protein. There was a linear effect ($P<0.01$) in pH, ethanol, lactic acid, lactic:acetic ratio, and total acids (Table 4). The decreasing straw percentages lead to linear decrease in pH for silages (Table 4). This is credited to the natural buffering capacity of the straw. The ethanol, lactic acid, lactic:acetic ratio, and total acids increased linearly as the WBG percentage increased. This is a result of higher fermentation with high ratios of WBG to straw and the lowering of silage pH. Acetic acid only differed ($P<0.01$) among straw types with wheat straw being lower. There was no difference ($P>.05$) among treatments in temperature.

Implications

Wet brewers' grain has potential for use as silage when ensiled alone or with straws. Mixing with locally available straws can be cost effective while offering a complete ration[F2]. When mixed with wheat straw, a higher ratio of WBG to straw is desirable. Ensiling of WBG by itself proved effective only if storage is adequate to not allow effluent runoff and prevent spoilage.

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Table 1. Chemical Composition of ensiled wet brewers' grain (WBG) vs. WBG/Straw silages

Diet	DM	CP (%)	NDF (%)	ADF (%)
WBG	18.75 b	28.30 [b]	52.30 [b]	24.08 [b]
Straw Treatments	39.59 [c]	8.50 [c]	66.77 [c]	38.49 [c]
SEM [a]	2.86	0.54	0.99	0.68

[a] SEM, Standard Error of difference of the least square means
[b,c] Means without common superscript differ $P < 0.05$

Table 2. Fermentation Analysis of ensiled wet brewers' grain (WBG) vs. WBG/straw silages [a]

Diet	pH	Ammonia-N, ppm	Ethanol, %	Acetic Acid, %	Lactic Acid, %	Lactic:acetic Ratio	Total Acids, %
WBG	3.49 [c]	601.8 [c]	6.02 [c]	0.93 [c]	7.10 [c]	7.66 [c]	8.52 [c]
Straw Treatments	3.88 [d]	346.4 [d]	0.91 [d]	0.69 [d]	7.96 [c]	11.29 [d]	8.65 [d]
SEM [b]	0.07	31.39	0.25	0.07	0.77	1.11	0.81

[a] Based on Dry Matter Basis
[b] SEM, Standard Error of difference of the least square means
[c,d] Means without common superscript differ $P < 0.05$

Table 3. Chemical Composition of WBG/Straw silages

Diet	DM	CP (%)	NDF (%)	ADF (%)
20:80 Wheat	49.46 [b]	4.39 [b]	73.65 [b]	45.42 [b]
30:70 Wheat	35.72 [c]	8.10 [c]	68.80 [c]	41.15 [c]
40:60 Wheat	35.70 [c]	7.89 [c]	69.18 [c]	41.80 [c]
20:80 Grass	37.02 [c]	10.12 [d]	62.77 [d]	33.78 [d]
30:70 Grass	40.00 [c]	10.36 [d]	63.20 [d]	34.38 [d]
40:60 Grass	39.64 [c]	10.11 [d]	63.02 [d]	34.40 [d]
SEM [a]	1.62	0.38	0.92	0.65

[a] SEM, Standard Error of difference of the least square means
[b,c,d] Means without common superscript differ $P < 0.05$

Table 4. Fermentation Analysis of WBG/straw silages [a]

Diet	pH	Ammonia-N, ppm	Ethanol, %	Acetic Acid, %	Lactic Acid, %	Lactic: acetic Ratio	Total Acids, %
20:80 Wheat	4.10 [c]	227.4 [c]	0.64 [c]	0.56 [cd]	4.09 [c]	7.28 [c]	4.65 [c]
30:70 Wheat	3.81 [d]	417.0 [d]	0.82 [cd]	0.44 [c]	4.36 [c]	9.94 [cd]	4.80 [c]
40:60 Wheat	3.76 [d]	372.9 [d]	0.88 [cd]	0.50 [c]	6.55 [c]	13.17 [d]	7.05 [c]
20:80 Grass	3.88 [d]	388.3 [d]	0.97 [cd]	0.82 [de]	10.14 [d]	12.39 [d]	10.96 [d]
30:70 Grass	3.87 [d]	332.0 [d]	1.03 [d]	0.93 [e]	11.38 [d]	12.20 [d]	12.32 [d]
40:60 Grass	3.85 [d]	340.8 [d]	1.09 [d]	0.88 [e]	11.24 [d]	12.77 [d]	12.12 [d]
SEM [b]	0.05	30.92	0.13	0.09	0.92	1.13	0.98

[a] Based on Dry Matter Basis
[b] SEM, Standard Error of difference of the least square means
[c,d,e] Means without common superscript differ $P < 0.05$

Table 5. Expected crude protein compared to actual crude protein of WBG/straw silages

Diet	Expected CP (%)	Actual CP (%)	Difference
20:80 Wheat	7.38	4.39	-2.99
30:70 Wheat	9.70	8.10	-1.60
40:60 Wheat	12.03	7.89	-4.14
20:80 Grass	10.88	10.12	-0.77
30:70 Grass	12.77	10.36	-2.41
40:60 Grass	14.66	10.11	-4.55

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EFFECT OF TRACE MINERAL SUPPLEMENTATION ON FECAL SHEDDING OF *E. COLI* O157:H7 IN CALVES.

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ABSTRACT: Twenty-four early weaned heifers (avg. wt. 217 kg) were used in a 60 d experiment to determine if trace mineral supplementation would alter the rate of shedding of *E. coli* O157:H7. Calves (12/treatment) were allotted to either a control or supplemented diet based on initial liver Cu concentrations. All heifers were individually fed a fixed amount of DM daily. The basal diet was composed of wheat middlings, cottonseed hulls, and corn grain (15% CP and 79% TDN). The supplemented treatment was formulated to provide an additional 399 mg Cu, 1001 mg Zn, and 707 mg Mn/d. The trace minerals were complexed minerals from Zinpro Inc. (Availa-4®). The control diet had no supplemental trace minerals added. All heifers were inoculated with an oral dose of *E. coli* O157:H7. Fecal samples were collected every 18 h for the first three days after dosing and then once daily every three d thereafter to determine *E. coli* O157:H7 concentration. On d 21, liver tissue and venous blood for IBR titers were collected. Trace mineral supplementation did not increase IBR titers (P=0.50) but did increase (P<0.005) Cu concentration in the liver. There were no differences in the rate of fecal shedding of *E. coli* O157:H7 between control and supplemented treatments over the initial 21 d. But, the SEM between treatments often were as great as the mean values. There was a numerical trend for *E. coli* O157:H7 to decrease in concentration during the first 21 d. However, after d 21, fecal *E. coli* O157:H7 concentration increased to a level almost as great as was measured during the first three d after dosing. The results of this study suggest that supplemental trace minerals did not influence the rate of *E. coli* O157:H7 shedding. This may be due to a lack of nutritional stress on the animals, (no differences in IBR titers) or because the control diet provided adequate trace minerals. The study did not explain why fecal shedding rates increased dramatically after d 7 and again at d 21. Further research is needed to look at long term shedding patterns of *E. coli* O157:H7.

Key Words: *E. coli* O157:H7, Beef cattle, Immunity

Introduction

Escherichia coli O157:H7 is a food-born pathogen that can cause significant health risk to consumers. Cattle are a major reservoir of *E.coli* O157:H7 and beef is contaminated during harvesting procedures (Elder et al., 2000; Barkocy-Gallagher., 2001; Rivera-Betancourt et al., 2003). Current post-harvest methods have proven effective in reducing *E.coli* contamination on carcasses (Elder et al., 2000; Barkocy-Gallagher., 2003; Rivera-Betancourt et al., 2003), through a multiple-hurdle intervention system. This system needs to be expanded to decrease the amount of *E.coli* 0157:H7 contaminated cattle during the pre-harvest stage.

E.coli O157:H7 is ubiquitous from the farm to the packing plant (Hancock et al., 1997; Kudva et al., 1997., Rivera-Betancourt et al., 2003). Rice et al. (2003) and Mcgee et al. (2004) found that the introduction of one animal which was shedding at high rates quickly infected other cohorts. Furthermore, Bach et al. (2004) indicated that stress likely increased susceptibility to shedding.

Preharvest nutrition of cattle has been implicated as a preharvest tool that may decrease *E.coli* O157:H7 shedding (Kudva et al., 1997; Berg et al., 2004). Trace mineral and vitamin supplementation play a critical nutritional role by optimizing the immune status of beef cattle. A functional immune system is necessary for an animal to immunologically respond to foreign antigens (Greene et al., 1998). Trace mineral supplementation has increased humoral and cellular immune response in cattle (Ansotegui et al., 1994; Clark et al., 1995).

Results from our laboratory (Choat et al., 2005) showed decreased *E. coli* shedding in MT feeder cattle compared to cattle from other parts of the U.S. The only common management procedure among different groups of cattle was supplementation with increased levels of trace minerals and vitamins prior to shipment to Midwestern feedlots. The objective of this study was to compare fecal shedding of calves dosed with *E. coli* O157:H7 which were either supplemented with trace minerals and vitamins or not supplemented.

Materials and Methods

Twenty-four crossbred heifers (avg. wt. 217 kg) were weaned at approximately 165 d of age. Calves originated from the Montana State University beef herd and had limited access to mineral supplementation prior to weaning. The protocol for the experiment is described in Figure 1. At weaning, heifers were weighed and had fecal, hide, and liver samples collected to determine baseline levels of E. coli O157:H7 shedding and liver trace mineral status. Calves were allotted to either a control treatment (no additional trace minerals) or to a supplemented treatment (diet fortified with trace minerals) based on initial liver Cu concentrations (Table 1). The supplemented diet provided an additional 176mg/d of Cu, 587 mg/d of Zn and 388 mg/d of Mn. The control diet did not have additional trace minerals

added. Diet samples were analyzed, according to AOAC, (2000) procedures for protein and energy and by induction coupled argon plasma methods (Fassell, 1978) for trace minerals.

Heifers were weighed and placed in six pens of four heifers, with three pens per treatment. Heifers were fed to gain 0.68 kg/d and were fed at 0800 daily using individual feeding gates (American Calan). They were placed on their respective diets 30 d prior to dosing with *E.coli* O157:H7 cocktail.

Heifers were inoculated with *E.coli* O157:H7 using the following protocol. Inoculums were prepared by culturing each of five strains of *E.coli* O157:H7 (55AC1, 114AC1, 131AC1, 237AC1, 299AB3) in separate flasks of Luria-Bertani (LB) broth. The cultures were grown for 18 h at 37 C with agitation until culture densities reached 10^9 CFU of *E.coli* O157:H7/ml. Viable cell counts were estimated by spread plate culture of six serial dilutions on LB agar (Brown et al., 1997; Kudva et al., 1997). The five strains were subsequently mixed and cells were obtained by centrifugation and resuspended at 10^9 CFU/ml in sterile saline (PBS). Inoculum was pipetted onto 0.45 kg of ground corn grain and then offered to each heifer at 1200.

Fecal samples were collected at time of dosing and again at 18 h, 32 h, and 50 h post-inoculation. Fecal samples were then collected every three d thereafter until d 21 to measure presence and concentration of fecal shedding of *E.coli* O157:H7. Heifers were weighed off trial on d 21. *E. coli* O157:H7 prevalence in feces was determined by collecting a rectal fecal sample from each heifer followed by shipment in insulated containers containing ice packs for next day delivery to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis of prevalence and most probable number of *E. coli* O157:H7.

Fecal samples were evaluated for prevalence of *E. coli* O157 following enrichment, use of immunomagnetic separation, and plating on ctSMAC and Rainbow agars (Barkocy-Gallagher et al., 2002). Morphologically typical colonies were tested for latex agglutination. Samples positive for latex agglutination were then subjected to most probable number analysis.

On d 7 of the experiment liver tissue and venous blood samples were collected. After this, heifers were injected with a modified live vaccine for infectious bovine rhinotracheitis (IBR) to determine humoral immune response (Ansotegui et al., 1994) due to trace mineral supplementation. Subsequent venous blood samples for IBR titers were collected on d 21 to determine if trace mineral supplementation affected primary and secondary IBR antibody titers

Blood samples were placed on ice and were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analysis of serum for IBR antibody titers was conducted at Colorado State University Diagnostic Laboratory, Fort Collins, CO. Liver biopsy samples were frozen and shipped on dry ice to Michigan State University Diagnostic Lab for Cu, Zn, Mn, and Co analysis by inductively coupled plasma atomic emission spectroscopy techniques.

Statistical Analysis Data were analyzed using the GLM procedures for SAS (SAS Inst. Inc., Cary, NC). Changes in *E.coli* O157:H7 CFU/g of feces from d 0 to d 21 were analyzed by repeated-measures in time. Differences were determined using the LSD procedures of SAS (SAS Inst. , 2003). Changes in liver Cu concentration and IBR titers were analyzed by a simple ANOVA.

Results and Discussion

No differences ($P>0.05$) in initial body weights, liver Cu concentrations (Figure 2) or age was measured at the initiation of the experiment. All heifers were tested for *E.coli* O157:H7 shedding and were negative at d -30 and d 0 of the experiment. Similarly *E.coli* O157:H7 prevalence was not detected on any of the hide swab samples at d 0.

Heifers gained 0.61 kg/d for 50d while on experiment with no differences ($P> 0.10$) measured between treatments. Supplementation with Cu did increase ($P< 0.05$) liver Cu concentration (198 vs. 131 ppm for supplemented and control treatments, respectively; Figure 2). Unexpectedly, the control heifers consumed 223 mg/d of Cu provided in the non-supplemented diet which was approximately four times their requirement. The source of this mineral contamination was not determined but was provided by one of the feedstuffs.

Supplementation did not ($P=0.50$) increase IBR antibody titers compared to the control treatment. This may be due to the level of trace mineral in the control diet which did not cause nutritional stress/deficiency on the heifers.

All heifers were shedding *E.coli* O157:H7 within 18 h of dosing and there was no morbidity observed in any of the heifers post-inoculation. This agrees with other research since cattle are typically asymptomatic to *E.coli* O157:H7 infection (Cray and Moon 1995; Zhao et al., 2003).

Trace mineral supplementation did not ($P=0.71$) cause differences in the rate of *E.coli* O157:H7 shedding. The SEM were often larger than the mean values. Because no differences in shedding were measured, average values were pooled and are presented in Figure 3 to show the pattern of shedding. Following inoculation there was a peak in *E .coli* O157:H7 by 18 h and agrees with research of McGhee et al. (2004) and Cray and Moon (1995). Although fecal shedding declined by days 2 and 3 post inoculation, there was another peak in shedding at d 7. Following d 7 there was a reduction in fecal *E.coli* O157:H7 concentration for the next two wks. This agrees with research reported by Brown et al. (1997), who showed a decrease in the fecal concentration of *E.coli* O157:H7 the first two wks after inoculation. There was another smaller increase in *E.coli* O157:H7 shedding at d 21. These periods of high *E.coli* O157:H7 fecal excretion could indicate the colonization of *E.coli* in the lower gut and more specifically at the recto-anal junction (Grauke et al., 2002; Naylor et al., 2004).

Implications

Copper levels in the liver were increased by supplementation of Cu.. However, in this study, trace mineral supplementation did not change E. coli O157:H7 shedding or increase antibody titers for IBR. Further research is needed to determine long term fecal shedding patterns of *E.coli* O157:H7 since there was not a sustained reduction over 21 d.

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Table 1. Ingredient, nutrient composition and calculated trace mineral intakes of diets fed to heifers

Item	Supplemented	Control
Ingredient, % DM basis		
Wheat Middlings	23.21	23.21
Corn Grain	46.12	46.12
Dried Distillers Grain	8.91	8.91
Corn Cob	8.81	8.81
Soybean Hulls	3.71	3.71
Canola Meal (34%)	2.40	2.85
Nutrient analysis		
CP%	15.0	15.0
TDN%	79	79
NE_m, Mcal·kg^{-1}	1.38	1.38
NE_g, Mca·kg^{-1}	0.95	0.95
Calculated consumption Cu, mg/day	399	223
Zn, mg/day	1001	414
Mn, mg/day	707	319

Figure 1. Timeline for experiment in which heifers were supplemented with trace minerals and dosed with E. coli O157:H7

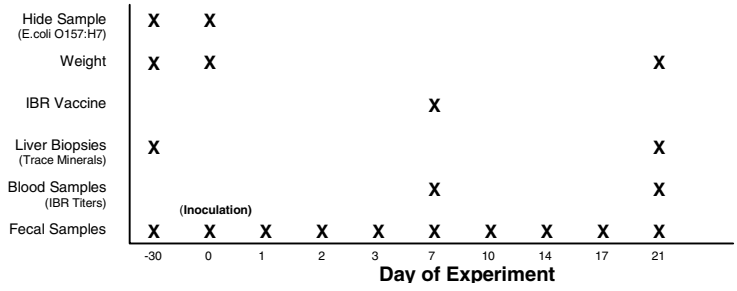
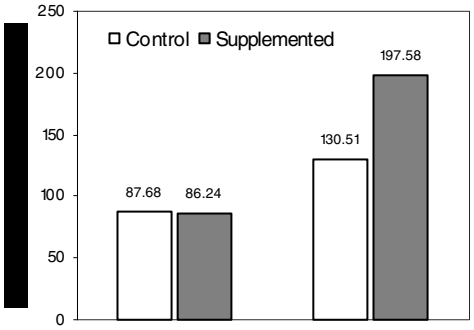
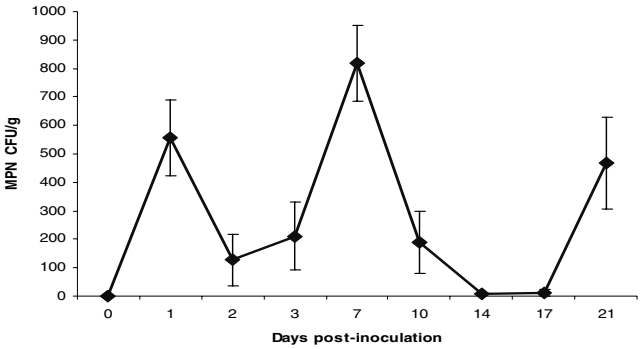


Figure 2. Initial and final liver copper concentrations for heifers supplemented with 176 mg/d copper for 50d



Means differ (P<0.005) for final Cu

Figure 3. Change in average fecal excretion pattern for calves dosed with E. coli O157:H7 with standard errors for each sampling date



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THE USE OF AN EXPERIMENTAL VACCINE IN GESTATING BEEF COWS TO REDUCE THE SHEDDING OF *E. COLI* O157:H7 IN THE NEWBORN CALF[1]

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ABSTRACT: One hundred and thirty seven beef cows in the last trimester of pregnancy were used to determine if vaccinating against *E. coli* O157:H7 would increase antibody titers in the serum and also result in the transfer these antibodies to the neonatal calf. Cows calved within a 30 d period starting the end of March. Seventy-one cows were vaccinated with an experimental vaccine (Ft. Dodge Animal Health) and then co-mingled with 66 non-vaccinated control cows. Cows were vaccinated approximately 30 d prior to parturition. Cow fecal and venous blood samples were collected at trial initiation and again at ~14 d after parturition. Calf feces and serum were collected at ~14 d after parturition and again 60 d later at branding. The serum was analyzed for antibody titers to *E. coli* O157:H7 by an ELISA protocol. The prevalence of *E. coli* O157:H7 in feces was determined by collecting a sample on a RAMS swab and submitting all samples to a commercial laboratory for analysis (Food Safety Net, San Antonio, TX). Initial cow antibody titers to O157:H7 were not different (P=0.50) between treatments but by parturition the antibody titers for O157:H7 in vaccinated cows were ten times higher (P<.001) than for control cows (917 vs. 83). The titers for calves suckling vaccinated cows were similarly higher (P<0.001) than control calves (1485 vs.135) at ~14 d after calving. By 60 d, titer levels were still higher (P<0.001) for calves suckling vaccinated cows but appeared to decline slightly compared to the titers near parturition. Initial fecal O157:H7 concentrations for cows were negative for both treatments and remained low through parturition. There were no differences in fecal O157:H7 at 60 d post partum among calves; less than 5% of all calves were shedding. Results of this experiment suggest that vaccination of gestating cows for *E. coli* O157:H7 resulted in elevated antibody titers compared to control cows and these antibodies were transferred to the calf.

Key Words: E. coli O157:H7, Antibodies, Colostrum, Cattle

Introduction

Escherichia coli O157:H7 is one of several food born pathogens in today's beef industry. *Escherichia coli* is commonly associated with hemorrhagic colitis and has few therapeutic alternatives and poor prognoses for severe sequelae and has led to intensive research targeting elimination or reduction of fecal shedding (Kudva et al., 1999; Lahti et al., 2002). Post-harvest interventions have dealt with controlling food borne pathogens in a "multiple hurdle approach", in order to reduce pathogen contamination in primarily ground beef. More emphasis has been placed on carcasses contaminated by hides and feces; primarily occurring during the slaughter process. The Federal Register (2002) reported: (a) that five multi-state studies showed the apparent prevalence in breeding herds containing one or more infected with *E. coli* O157.H7 were 24%, 61%, 75%, 87% and 100%; (b) three multi-state studies reported the apparent prevalence in feedlots containing one or more cattle infected with *E. coli* O157:H7 was 63%, 100% and 100%; and (c) Elder, et al. (2000) found that 28% of fecal samples from fed-cattle were positive for *E. coli* O157:H7. Similarly, Smith, et al. (2001) reported that the fecal prevalence of *E. coli* O157:H7 in fed cattle was 23%.

As a result, the beef industry has focused more on pre-harvest methods to reduce the incidence of *E. coli* O157:H7 (Elder et al., 2000; Barkocy-Gallagher et al., 2001). Rice et al. (2003) found that the introduction of one animal, which was shedding *E. coli* O157:H7 at high rates quickly infected other cohorts. The beef industry is researching potential interventions at various stages of production. Multiple hurdle interventions have had a positive impact on significantly reducing food born pathogens entering the food supply if the levels of these bacteria allowed into the packing plant were limited (Elder et al., 2000). Recently, Nystrom (August 2004 Agricultural Research magazine, p. 14-15) reported that an experimental vaccine interfered with *E. coli* colonization within the gut of the calf.

The objectives of this research were to determine if an experimental *E. coli* O157:H7 vaccine given to the gestating beef cow would result in transferring antibodies to the newborn calf via colostrum.

Material and Methods

Cow Management. The location of this experiment was the USDA-ARS-LARRL Research Station at Miles City (Ft. Keogh), MT. One hundred thirty seven gestating beef cows in the last trimester of pregnancy were used for this study. Cows were weighed and randomly assigned to the two treatments (Control vs. Vaccinated) approximately 30 d prior to the start of expected calving. The experimental protocol and treatments are described in Table 1. Cows were previously synchronized and artificially inseminated and were expected to calve within a thirty day period starting the end of March. The average weight of the cows approximately 30 d prior to calving was ~567 kg. Seventy-

1 Research partially funded by National Cattlemen's Beef Assoc.

one of the gestating cows were vaccinated with an experimental vaccine, (developed by Fort Dodge Animal Health Laboratories)which was designed to prevent the attachment of *E. coli* O157 to the intestinal wall (Cornick et al., 2002), and then commingled with 66 non-vaccinated control cows. Cows remained in calving pens for the first 15 d and then were placed on native range pasture after parturition.

Sample Collection and Analysis. The cows and calves had 10 ml of whole blood collected from the jugular or tail vein. Blood samples were placed on ice and transported to the laboratory, where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analyses of serum for antibody titers *E. coli* O157:H7 were conducted by Fort Dodge Animal Health Laboratories, Ft. Dodge, Iowa. Serum titers against O157:H7 were analyzed using an ELISA (enzyme-linked immunosorbent assay; Widiasih et al., 2004). Samples were dissolved at 1 mg/ml in 1xPBS (phosphate-buffered saline) solution and diluted to 1:100 with the 1xPBS for a concentration of 10 µg/ml (Cray et al., 1995). Microtiter plates were coated with diluted LPS solution. The samples were placed through a series of similar steps of washing and coating the plates. Results were then read by a spectrophotometer at 405 nm, approximately 10-30 min after the addition of a substrate solution. The results were expressed according to the final dilution factor on the plate. (Fort Dodge Animal Health, 2004)

Recovery of E. coli O157:H7. The prevalence of *E. coli* O157:H7 in feces from cows and newborn calves was determined by collecting a fecal sample via a rectoanal mucosal swab (RAMS; Rice et al., 2003). The swab sample was collected from cows on d-30 (before parturition) and 9-14 days after parturition. The calves were also swabbed at approximately 14 d after parturition and again at ~60 d after birth (branding). Fecal samples were obtained by inserting the sterile foam-tipped applicator approximately 2 to 8 cm (Grauke et al., 2002; Naylor et al., 2004) into the anus, and by using a rapid "in and out" motion, the entire mucosal surface of the rectoanal junction was swabbed (Rice et al. 2003). The swab samples were submitted to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis for *E. coli* O157 (Barkocy-Gallagher et al., 2002).

Statistical Analysis. Data were analyzed using the GLM procedures of SAS (2000). A comparison of control and vaccinated animals for fecal rates were measured by Chi Square analyses. The titer levels were analyzed by one way analysis of variance.

Results and Discussion

The objective of this study was to vaccinate gestating cows with an experimental vaccine to prevent colonization and subsequent shedding of *E. coli* O157:H7 by the cow and the calf.

Initial cow titer levels (prior to vaccination) showed no differences ($P<0.50$) between treatments for O157:H7 antibody. Similarly, there were no differences in fecal shedding rates of O157:H7 on - 30 d. However, at ~ 14 d after calving, the vaccinated cows had eleven times higher titer levels than that of the control group (917 vs. 83). The fecal samples for the second sample again showed no difference between treatment groups.

Initial titer levels of the calves showed ten fold difference ($P<0.001$) between treatments (135 vs. 1485 for Control calves vs. calves which suckled vaccinated cows respectively). Titer levels at branding (~60 d) showed a slight decrease in titer levels, however calves from vaccinated cows were still seven times higher ($P<0.001$) than the control calves.

Fecal samples for the calves were not different with no positive samples detected at 14 d. Similarly, no differences were detected at branding (avg. 3%).

Due to the low number of animals shedding *E. coli* O157:H7 differences were undetectable. This may have been due to the cool spring weather, which could prevent *E. coli* O157:H7 from shedding in greater numbers (Barkocy-Gallagher et al., 2003).

Previous studies have revealed that *E. coli* O157:H7 was pathogenic for neonatal calves (Dean-Nystrom et al,. 1997; Widiasih et al., 2004). These studies showed that calves less than 36 h old developed diarrhea and enterocolitis with attaching and effacing lesions. Other, studies have shown that weaned (ruminating) calves dosed with *E. coli* O157:H7 remained clinically healthy, with no evidence of fever or diarrhea (Zhao et al., 1998), compared to the newborn calf.

Implications

A partial reduction in the shedding of *E. coli* O157:H7 will be useful in controlling the contamination of meat. Results of this experiment showed that vaccinating the gestating cow with an experimental vaccine against *Escherichia coli* O157:H7 resulted in increased both cow and suckling calf antibody titers.

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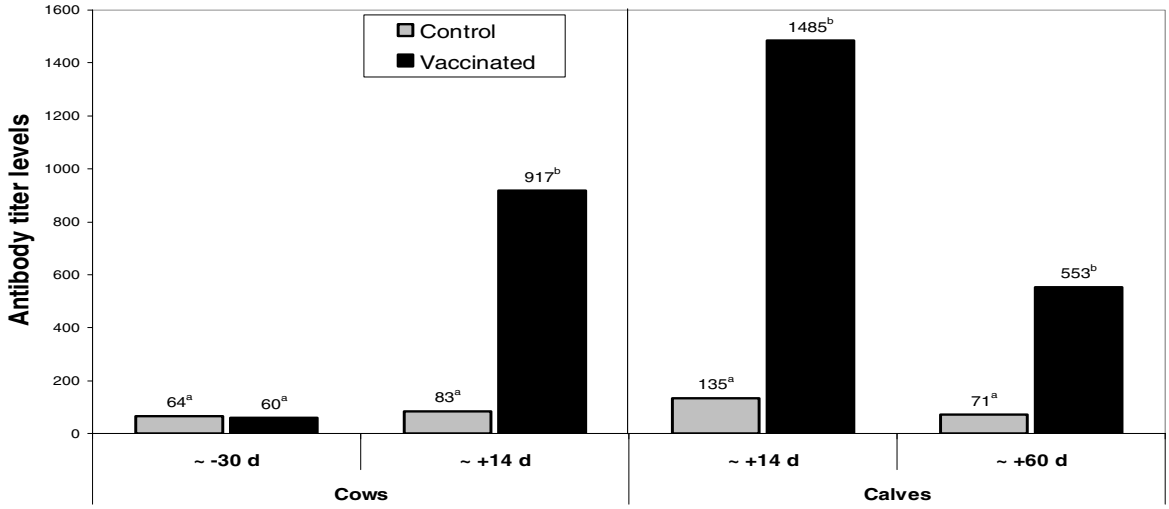
Table 1. Experimental design and samples collected from cows and calves to determine if vaccinating gestating cows with an experimental *E. coli* O157:H7 vaccine would transfer antibodies to the newborn calf via the colostrums

Treatment:	Samples collected:	Samples analyzed for:
66 Pregnant control cows (not vaccinated)	Cows-Blood and fecal samples at start of trial and at calving.	Blood: *E.coli* 0157:H7 antibody titers
71 Pregnant vaccinated cows (vaccinated 30 d and 14d prior to calving)	Calves- Blood and fecal samples within 14 days of calving and again at branding (~ 60 days)	Feces: prevalence of *E.coli* 0157:H7 shedding and enumeration of positive samples

Figure 1. Effect of vaccinating gestating cows with an experimental vaccine against *E. coli* O157:H7 on antibody titers and subsequent titer levels in the neonatal calf.



[a, b] Unlike means within a time period are different (P<0.05)

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RESUMPTION OF OVARIAN CYCLING ACTIVITY AND BREEDING PERFORMANCE OF FIRST-CALF SUCKLED BEEF COWS EXPOSED TO MATURE BULL URINE[1]

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ABSTRACT: The objectives of this experiment was to determine if continuous exposure to bull urine alters resumption of ovarian cycling activity or breeding performance of first-calf suckled beef cows. The null hypotheses were that: 1) interval from exposure to urine to resumption of ovarian cycling activity; 2) proportions of cows cycling at the end of the exposure period; and 3) AI pregnancy rates after estrous synchronization (ES) do not differ between cows exposed to mature bull urine or steer urine. Thirty-eight first-calf suckled beef cows, 4 mature AXH bulls and 4 ten-mo-old AXH steers were used in this study. Cows were stratified by calving date, cow BW, calf BW, calf sex, dystocia score, and BCS, and fitted with a controlled urine delivery device (CUDD) 2 wk before the start of treatments. Cows were assigned randomly to be exposed continuously to urine of bulls (BUE) or steers (SUE) beginning 40 d after calving. Urine was collected from bulls and steers every third d of the experiment. Blood samples were collected from cows starting on d 0 and every third d thereafter until the start of the ES protocol. Likewise, CUDD were filled and refilled on the same schedule. Serum was assayed for progesterone by RIA. A rise in progesterone concentrations of > 0.5 ng/mL in 3 consecutive samples was used to determine resumption of cycling activity. Each cow received a CIDR on d -10 (d 0= d of TAI) and was given $PGF_{2\alpha}$ (PG) 7 d later at CIDR removal. Cows detected in estrus 60 h after PG and were bred by AI 12 h later; cows not detected in estrus by 60 h after PG received GnRH, and TAI at 72 h after PG. Pregnancy rates were determined 35 d after AI by ultrasonography. Neither interval from urine exposure to resumption of cycling activity nor proportions of cows cycling before the breeding season differed between BUE and SUE cows. However, AI pregnancy rate was greater ($P<0.05$) for BUE cows than SUE cows. We conclude that continuous exposure to mature bull urine does not affect resumption of ovarian cycling activity but appeared to improve breeding performance of first-calf suckled beef cows.

Key Words: Breeding Performance, Bull Urine, Postpartum Interval

Introduction

The bull biostimulatory effect reduces postpartum anestrous in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993, 1996). Little is known of the biological mechanism by which the biostimulatory effect initiates the resumption of ovarian cycling activity in postpartum cows. Recent studies indicate that biostimulation is not mediated through social interactions between bulls and cows (Berardinelli et al., 2004), but rather is mediated through the excretory products of bulls (Joshi et al., 2002). These results indicat that bull biostimulation utilizes a pheromone(s) to stimulate resumption of ovarian cycling activity in beef cows.

Pheromones are small airborne chemicals present in the urine, feces or cutaneous glands and perceived by the olfactory or respiratory systems which cause behavioral and endocrine responses in conspecifics (Rekwot et al., 2000). Oronasally administered bull urine to cows on d 7 postpartum increased mean LH and FSH serum concentrations within 80 min after exposure (Baruah and Kanchev, 1993). Joshi et al., (2002) showed that cows exposed to the excretory products of bulls resumed ovarian cycling activity earlier after calving than cows not exposed to bulls. It appears that bulls excrete a pheromone into the urine, feces or cutaneous glands that may initiate a neuro-endocrine endocrine cascade in cows which results in the resumption of ovarian cycling activity.

The objectives of this experiment were to determine if bull urine exposure alters the resumption of ovarian cycling activity before the breeding season and breeding performance of first-calf beef cows. We tested the hypotheses that: 1) the interval from exposure to resumption of cycling activity; 2) proportion of cows cycling before the breeding season; and 3) AI pregnancy rates, did not differ between cows exposed to mature bull urine or cows exposed to steer urine.

Materials and Methods

Animals and Treatments

Thirty-eight two-yr-old Angus x Hereford first-calf suckled beef cows, four Angus x Hereford epididectomized bulls, and four 1-yr-old Angus x Hereford steers were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center, Bozeman. Animal care, handling, and

[1]The research was supported by the Montana Agricultural Experiment Station. Contributing project to Multistate Research Project, W-112, Reproductive Performance in Domestic Ruminants.

protocols used in this experiment were approved by the Montana State University Institutional Animal Care and Use Committee.

Cows and calves had no contact with bulls or steers throughout the experiment and had no contact with their excretory products from calving until the start of treatment. Average calving date was Feb. 9. Two wk before the start of treatment cows were stratified by calving date, cow BW, calf BW, calf sex ratio, dystocia score, and BCS and fitted with a controlled urine delivery device (CUDD). Cows were assigned randomly to exposure to steer urine (SUE) or exposure to bull urine (BUE). The average length of exposure time before the start of estrous synchronization was 64 d.

Lot Areas

Two lots were used for this experiment, designated north and south by their geographic location. Each lot contained four pens (41 m x 18 m) that were identical in east-west configuration, bunk space, aspect, slope, and connection to open-shed shelters. Lots were approximately 0.35 km apart. Animals housed in one lot were not able to see or smell animals housed in the other lot; however, there was a possibility that sounds made by animals in one lot could be heard by animals in the other lot. Cows exposed to bull urine (BUE) were housed in the two eastern-most pens the north lot and cows exposed to steer urine (SUE) were housed in the two western-most pens of the south lot.

Urine exposure

Urine was collected from bulls and steers every 3 d throughout the experiment. Cows were continuously exposed to urine using a Controlled Urine Delivery Device (CUDD). Two wk before the start of treatment CUDD were attached to the mid-line of the dulap on the neck, 7.5 cm anterior to the sternum of cows. Laboratory tests using distilled water as the media, showed that CUDD released fluid into the air over a period of 3 d. Therefore, every third day from the start of exposure each CUDD was filled with urine, inspected for non-functioning parts, and repaired.

Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before the start of the experiment. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 $kg \bullet hd^{-1} \bullet d^{-1}$ cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996). Bulls had ad libitum access to fair quality, chopped barley hay. During collection periods bulls were fed 0.5 kg of cracked barely and good quality, chopped mixed-grass alfalfa hay. Steers were fed a finishing ration that consisted of 70% concentrate and 30% roughage throughout the experiment.

Blood Sampling for Progesterone

Blood samples were collected from each cow by jugular venepuncture at 3-d intervals from the start of the experiment to the start of the breeding season. Serum was assayed for progesterone concentration using a solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated for bovine serum in our laboratory (Custer et al., 1990). Intra- and interassay CV for a serum pool that contained 2.55 ng/mL were 0.4 and 7.4%, respectively; and for a pool that contained 7.49 ng/mL were 11 and 3.4%, respectively. Progesterone concentrations patterns were used to assess the resumption of ovarian cycling activity. A rise in progesterone concentration of > 0.5 ng/ml in three consecutive that was maintained for one or two additional samples provided evidence that cows resumed ovarian cycling activity during the experimental period. The graphic representation of the pattern of progesterone concentrations used to validate this criterion is given in Fernandez et al. (1993) for first-calf suckled beef cows.

Estrous Synchronization, AI, and Pregnancy Diagnosis

Each cow was given exogenous progesterone via a controlled intravaginal drug release (CIDR) device starting on May 18 (d -10). Seven d later (d -3) CUDD and CIDR were removed and cows were given $PGF_{2\alpha}$ (25 mg/hd) intramuscularly. Cows were visually observed for estrus by thrice daily (0730, 1200, 1800 h) after $PGF_{2\alpha}$ injection. Cows that showed estrus within 60 h after $PGF_{2\alpha}$ injection were bred by AI 12 h later. Cows that did not show estrus by 60 h were given GnRH (100 µg/hd) intramuscularly, and bred by AI 72 h after CIDR removal (d 0). Artificial insemination was accomplished using semen from a proven bull and a single AI technician. Pregnancy was determined by transrectal ultrasonography of the uterine contents of each cow 35 d after timed AI.

Statistical Analyses

Calving date, cow BW, calf birth weight, calf sex ratio, dystocia score, and BCS were analyzed by separate 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. Intervals from the start of treatment to resumption of ovarian cycling activity were calculated by assigning the cows the d of the lowest inflection point before the increase of greater than 0.5 ng/mL of progesterone in 3 consecutive samples. Cows not showing a rise in progesterone over three consecutive blood samples were assigned an interval from the start of treatment to $PGF_{2\alpha}$ injection. Interval from the start of treatment to resumption of ovarian cycling activity was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS. The model included treatment. Proportion of cows cycling at the start of estrous synchronization, proportion of cows that exhibited estrus after $PGF_{2\alpha}$ injection, and AI pregnancy rates were analyzed by PROC FREQ of SAS.

Results

Calving date, cow body weight, calf birth weight, calf sex ratio, dystocia score, and body condition score did not differ between cows exposed to bull urine (BUE) and cows exposed to steer urine (SUE).

Cow BW and BCS change from the start of treatment until after ES did not differ (P = 0.22, and 0.98, respectively) between BUE and SUE cows. There was no difference (P = 0.17) in the intervals from treatment to the resumption of ovarian cycling activity between BUE and SUE cows. Similarly, there was no difference in the interval from the start of treatment to resumption of ovarian cycling activity between BUE or SUE cows for cows that resumed or did not resume ovarian cycling activity before $PGF_{2\alpha}$ injection (P = 0.40 and P = 0.75 respectively).

There was no difference (P = 0.25) in the proportion of cows cycling by the end of the exposure period between BUE and SUE cows (Table 1).

Table 1. Number of animals per treatment and percentage of cows exhibiting estrus by 60 h after $PGF_{2\alpha,}$ and TAI and overall AI pregnancy rates for first-calf suckled beef cows exposed to mature bull urine (BUE) or exposed to steer (SUE) before the start of the estrous synchronization protocol

	Treatment			
Item	BUE	SUE	X^2	P value
n	19	19		
% cycling by the end of the exposure period	15.8% [b]	30.6% [b]	1.3	0.25
% in estrus by 60 h after $PGF_{2\alpha}$	80.0% [b]	52.6% [b]	2.9	0.09
% of cycling cows in estrus by 60 h after $PGF_{2\alpha}$	66.7% [b]	33.3% [b]	0.9	0.34
% of anestrous cows in estrus by 60 h after	81.3% [b]	61.5% [b]	1.4	0.24
TAI pregnancy rate	100% [b]	66.7% [b]	1.7	0.19
AI pregnancy rates[a]	89.5% [b]	57.9% [c]	4.9	< 0.05

[a]AI pregnancy rates equal cows bred 12 h after estrus and cows bred at TAI at 35 d after TAI.

[b,c]Percentages within in rows that lack a common superscript differ.

Proportions of cycling and anestrous cows that exhibited estrus by 60 h after $PGF_{2\alpha}$ did not differ (P = 0.34 and 0.24, respectively) between cows BUE and SUE cows (Table 1). Therefore, data were pooled for cycling and anestrous cows that exhibited estrus by 60 h after $PGF_{2\alpha}$ for cows BUE and SUE cows. The proportion of cows that exhibited estrus by 60 h after $PGF_{2\alpha}$ did not differ (P = 0.09; Table 1) between BUE and SUE cows. Timed AI pregnancy rates did not differ (P = 0.19) BUE and SUE cows (Table 1). Therefore, data for cows bred by TAI and cows bred 12 h after estrus were pooled within treatments for AI pregnancy rates. Overall AI pregnancy rate was greater ($P < 0.05$) for BUE cows than for SUE cows (Table 1).

Discussion

The physical presence of bulls decreases the postpartum anestrous interval in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993; 1996). Cows exposed to the excretory products of bulls resumed ovarian cycling activity earlier after calving than cows not exposed to bulls (Joshi et al., 2002). From these data it appears that bulls excrete a pheromone into the urine, feces, or from cutaneous glands that may initiate a neuroendocrine-endocrine cascade which results in the resumption of ovarian cycling activity. Most male to female interactions that alter the reproductive activity of the female are mediated by pheromones excreted in the urine of males (for review, see, Vandenbergh, 1983). Thus, the most likely excretory product to evaluate for pheromonal activity in the biostimulatory effect of bulls is urine.

The objectives of this experiment were to determine if bull urine exposure alters the resumption of ovarian cycling activity before the breeding season and breeding performance of first-calf beef cows. We found that exposing cows to mature bull urine had no effect on the interval to resumption of ovarian cycling activity and did not increase the proportion of cows that started to cycle before the breeding season.

The percentage of SUE cows cycling by the end of the exposure period was comparable to the percentage of cows cycling that were not exposed to bulls in previous years (Berardinelli et al., 2004; Joshi et al., 2002). Only 15% of BUE cows were cycling by the end of the exposure period. This number is quite low considering cows were 92 to 111 days postpartum by the end of the exposure period. These data are contrary to those of Joshi et al. (2002) who reported that more cows exposed to the excretory products of bulls resumed cycling activity than cows exposed to their own excretory products or cows not exposed to bulls or their own excretory products. These results indicate that continuous exposure of postpartum anestrous cows to mature bull urine does not alter the occurrence of resumption of ovarian cycling activity.

In regard to breeding performance, 14 out of 19 BUE cows and only 10 of 19 SUE cows exhibited estrus within 60 h after $PGF_{2\alpha}$. Although this difference is not statistically significant it is not consistent with previous experiments that found no indication for an improvement in response to estrous synchronization between cows exposed to bulls or cows not exposed to bulls (Anderson et al., 2002; Joshi et al., 2002). Overall AI pregnancy rate was 31% higher for BUE than SUE. This result is not consistent with that of Anderson et al. (2002) who

reported that AI pregnancy rates did not differ among; cows exposed to the excretory products of bulls, cows exposed to their own excretory products, cows exposed to the physical presence of bulls, and cows not exposed to bulls or their own excretory products. One difference between Anderson et al. (2002) and the present experiment is the use of CIDR. Progestin was not used by Anderson et al. (2002) and recently, Stevenson et al. (2003) reported that progestin treatment concurrent with a GnRH-based ES protocol improved pregnancy rates in suckled beef cows after AI. Thus, it appears that bull urine exposure in conjunction with an estrous synchronization protocol that included progestin (CIDR), $PGF_{2\alpha}$, GnRH and timed AI improved breeding performance in first-calf beef cows.

In conclusion, continuous exposure of first-calf suckled beef cows to bull urine did not alter the interval from treatment to the resumption of ovarian cycling activity or proportion of cows cycling before the beginning of the breeding season. However, continuous exposure to mature bull urine before an estrous synchronization protocol that included progestin (CIDR), $PGF_{2\alpha}$, and GnRH increased AI pregnancy rate. Therefore, it is possible that bull urine may contain a pheromone(s); continuous exposure to this pheromone(s) did not induce the resumption of ovarian cycling activity. However, continuous exposure to this pheromone(s) before the breeding season may improve the breeding performance of first-calf suckled beef cows.

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EFFECTS OF RUMINAL PROTEIN DEGRADABILITY AND FREQUENCY OF SUPPLEMENTATION ON NITROGEN RETENTION AND APPARENT DIGESTIBILITY IN LAMBS FED LOW-QUALITY FORAGE[1]

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ABSTRACT: Twelve wether lambs (29.9 ± 2.7 kg BW) were used in a replicated completely randomized designed experiment to examine the effects of ruminal protein degradability and supplementation frequency on N retention and digestion of a low-quality forage diet. Wethers were fed mature crested wheatgrass hay (4.2% CP, 59% NDF) for ad libitum consumption plus one of four supplements: 1) a high ruminally degradable protein (RDP) supplement fed daily (RDP-D), 2) the high RDP supplement fed on alternate d (RDP-A), 3) a high ruminally undegradable protein (RUP) supplement fed on an iso-N basis to the RDP supplement on alternate d (RUP-A), or 4) a 50:50 mixture of the RDP and RUP supplements fed on alternate d (MIX-A). Because forage DM intake tended to be greater (P = 0.08), total DM intake was greatest (P = 0.05) for RDP-D lambs. Total tract DM digestibility was unaffected (P = 0.47) by treatment, but increased (P = 0.04) on d of supplementation. Total tract N digestibility was greatest (P = 0.04) on the d of supplementation for lambs supplemented on alternate d, but was lower (P = 0.001) than RDP-D lambs on d following supplementation. Although treatment had no effect (P = 0.44) on urinary N excretion, lambs excreted more (P = 0.02) urinary N on d of supplementation than on the d following supplementation. Alternate d supplemented lambs retained the greatest ($P \leq$ 0.001) amount of N on the d of supplementation, while RDP-D lambs retained more ($P \leq$ 0.001) N on d following supplementation. However, when averaged across the supplementation interval, alternate d supplemented lambs had lower N retention when expressed as a % of N intake (P = 0.006) or N digested (P = 0.004). Although lambs supplemented on alternate d may retain less N overall, their ability to maintain forage intake and digestion suggests that sufficient N is conserved to support ruminal microbial metabolism throughout the alternate d supplementation interval.

Key Words: Protein Degradability, Supplementation Frequency, Lambs

Introduction

Protein supplementation is often necessary to optimize production in ruminants consuming low quality forages. Because such forages are often limiting in the supply of protein, a positive relationship exists between ruminally degradable protein (**RDP**) supplementation and forage utilization. Decreasing the frequency of RDP supplementation to ruminants consuming low quality forages has generally resulted in minimal impacts on nutrient intake or digestion (Bohnert et al., 2002; Ludden et al., 2002a), net flux of nutrients (Krehbiel et al., 1998), or subsequent animal performance (Bohnert et al., 2002; Ludden et al., 2002b). However, consumption of large quantities of RDP on the day of supplementation may result in ruminal ammonia concentrations that greatly exceed the immediate demands of the microbial population. Excess ammonia undergoes ureagenesis in the liver, thereby increasing blood urea concentrations at a time when the propensity for recycling of that N back to the rumen is reduced, resulting in increased urinary N excretion. Alternatively, our hypothesis was that replacing a portion of the supplemental RDP with ruminally undegradable protein (**RUP**) may indirectly stimulate N recycling to the rumen. In addition to moderating ruminal ammonia levels, prolonged deamination of the amino acids contained in supplemental RUP may provide a mechanism whereby blood urea concentrations are increased to coincide with decreased ruminal ammonia concentrations on the day following supplementation. Thus, overall efficiency of N use is enhanced, and forage intake and utilization by the animal is maintained. Therefore, our objective was to examine the effects of alternate-day supplementation with combinations of RDP plus RUP on N retention and the intake and digestion of low quality forage by growing lambs.

Materials and Methods

Animals and Diets

Twelve suffolk wether lambs (29.9 ± 2.7 kg BW) were used in a replicated completely randomized designed experiment. All animal care protocols were approved by the University of Wyoming Animal Care and Use Committee. Wethers were maintained in individual metabolism crates (1.4 × 0.6 m) at a constant room temperature of 20°C under continuous lighting. Wethers had ad libitum access to fresh water and a trace mineralized salt block (Iofix T-M, Morton Salt; Chicago, IL).

Wethers were fed a basal diet of mature crested wheatgrass hay (4.2% CP, 59% NDF, 42% ADF, 61.6% RDP) for ad libitum consumption in two equal portions at 0630 and 1600 daily. Lambs were supplemented at 0600 daily with one of four supplemental protein treatments (n = 3 per treatment): 1) a high RDP supplement (Table 1) formulated to meet estimated RDP requirements assuming a microbial efficiency of 11% of TDN, provided daily (**RDP-D**), 2) the high RDP supplement provided on alternate days (**RDP-A**), 3) a high RUP supplement fed on an

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isonitrogenous basis to the RDP supplement, provided on alternate days (**RUP-A**), or 4) a 50:50 mixture of the RDP and RUP supplements fed on alternate days (**MIX-A**). Supplementation rates for the alternate-day treatments were at twice that of daily supplementation (N basis). The RDP value of the hay was determined by protein fractionation as described by Sniffen et al. (1996).

Table 1. Composition of supplements

	RDP[a]	RUP
Ingredient, % of DM		
Isolated soy-protein[b]	73.1	
Corn gluten meal[c]		75.8
Calcium carbonate	11.7	11.1
Vitamin premix[d]	10.2	8.1
Dried molasses	5.0	5.0
Chemical		
DM, %	95.8	93.9
OM, % of DM	87.9	90.1
CP, % of DM	73.9	54.3
NDF, % of DM	8.0	15.9
ADF, % of DM	6.9	11.3

[a]RDP = ruminally degradable protein, RUP = ruminally undegradable protein.
[b]ARDEX® AF, Archer Daniels Midland Company, Decatur, IL. Assumed to be 100% RDP (% of CP).
[c]RUP source. Assumed to be 60% RUP (% of CP).
[d]Contained 3,628,739 vitamin A 3,628,739 vitamin D3 and 18,144 vitamin E IU/kg.

Sample Collection, and Analysis:

Two experimental periods were each 22 d in duration, with 14 d for diet adaptation followed by 8 d of sample collection. Animals were removed from the metabolism crates for 2 d and allowed to exercise in a dry lot between experimental periods. Wethers were reassigned to treatments in the second period such that no wether received the same treatment as in the first period. Total fecal and urinary output was collected on d 15 through 22 of each period. Urine was collected into plastic collection vessels containing 100 mL of 6 N phosphoric acid to inhibit ammonia volatilization. For each lamb, a 10% aliquot of the daily fecal output was composited within lamb and day of supplementation (**DOS**) versus day of non-supplementation (**NS**), dried in a 55°C oven, and ground (Wiley mill; 1-mm screen). A 10% aliquot of the daily urinary output was composited within lamb and DOS versus NS and frozen at -20°C for later laboratory analysis. Although supplemented daily, feces and urine for lambs fed RDP-D were also composited within DOS versus NS as defined for alternate-day supplemented lambs. Feed and orts were sampled on d 15 to 22 of each collection period and ground (Wiley mill; 1-mm screen) for later laboratory analysis. Feed, orts, and feces were analyzed for DM and ash (AOAC, 1990) and for NDF and ADF content (ANKOM 200 fiber analyzer, ANKOM Technology, Fairport, NY). Feed, orts, feces, and urine were analyzed for N content (LECO model FP-528 Nitrogen determinator, LECO, St. Joseph, MI).

Statistical Analyses:

Data were analyzed using the MIXED procedures of SAS (SAS Inst. Inc, Cary, NC) for a completely randomized design. The model included the effects of treatment, day, period, and all possible interactions. Lamb (treatment × day) was used as the RANDOM statement. There were no period or treatment × period effects ($P \geq 0.05$); thus only treatment, day, and treatment × day effects are reported.

Results and Discussion

No treatment × day interactions ($P \geq 0.20$) were noted for DM, OM, NDF, or ADF intakes or apparent total tract digestibilities. Because forage DM intake tended to be lower ($P = 0.08$), total DM intake was reduced ($P = 0.05$) for RDP-A versus RDP-D lambs (Table 2). Protein supplementation has been shown to increase intake of low-quality forage (Salisbury et al., 2004). However, others (Ferrell et al., 1999; Bohnert et. al., 2002; Ludden et al., 2002a) have reported no difference in forage intake when lambs fed low-quality forage were supplemented with protein of different ruminal degradabilities.

Apparent total tract DM digestibility was unaffected ($P = 0.47$) by treatment, but was greater ($P = 0.04$) on DOS than NS. The greater digestibility on DOS reflects the inclusion of the supplement, which was more digestible than the forage fed. Ludden et al. (2002a) observed no affect of supplemental protein degradability on DM digestibility, but observed a tendency for alternate-day supplementation to increase DM digestibility. Apparent total tract OM, NDF, and ADF digestibilities were not affected ($P \geq 0.24$) by treatment or day of supplementation. These results are similar to others who observed no difference in digestibilities when feeding lambs RDP or RUP on alternate days (Ludden et al., 2002a). Similarly, others have observed minimal effects on digestibility as a result of supplementing RDP versus RUP (Ferrell et al., 1999; Salisbury et al., 2004), or when infusing casein either within the rumen or abomasum (Swanson et al. 2004). This suggests that apparent total tract digestion may not be affected by site of protein digestion. In contrast, Bohnert et al. (2002) reported that apparent NDF digestibility was increased with RUP versus RDP supplements in lambs consuming low-quality meadow hay.

Because forage intake did not differ and supplements were fed on an isonitrogenous basis, total N intake was not affected ($P = 0.11$) by treatment (Table 3). A treatment × day interaction ($P = 0.001$) was detected for apparent total tract N digestibility (% of N intake), wherein alternate-day supplemented lambs exhibited greater total tract N digestibility on DOS than on NS. This suggests that the greater quantity of supplement provided on DOS did not impair protein digestion on that day. However, RDP-D lambs had the greatest ($P = 0.04$) N digestibility when averaged across the supplementation interval. Although lambs supplemented on alternate days had decreased N digestibility, their ability to maintain forage intake and DM digestion suggests that digested N on day of supplementation was sufficiently conserved to support

ruminal microbial metabolism throughout the alternate day supplementation interval.

A treatment × day interaction ($P = 0.04$) in urinary N excretion (g/d) was detected. Due to the greater quantity of N consumed on DOS, alternate-day supplemented lambs excreted more urinary N on DOS than RDP-D lambs. However, urinary N excretion was similar between RDP-D and alternate-day supplemented lambs on NS, in spite of the reduction in N intake on that day. When expressed as a percentage of digested N, urinary N excretion increased from an average of 20.5 to 71.7% on DOS versus NS for alternate-day supplemented lambs. Additionally, within the alternate-day supplemented lambs, increasing the proportion of RUP in the supplement further increased urinary N excretion (% of digested N). This suggests that although alternate-day supplemented lambs were provided with excess N on the day of supplementation, much of this excess N was retained by the animal rather than excreted on that day. This is supported by the work of Bohnert et al. (2002) who observed that plasma urea N in lambs consuming low-quality meadow hay was greatest 24 h after supplementation with RDP or RUP at 3- or 6-d intervals. Thus, urea N appears to remain in the blood until the day following supplementation, and is made available for recycling back to the rumen or is excreted in the urine. Furthermore, our data would suggest that increasing the proportion of RUP in the supplement may result in a greater propensity for the blood urea N to remain until the day following supplementation. Whether this delay is due to prolonged ureagenesis from the amino acids supplied by supplemental RUP, or to alterations in urea filtration by the kidney (Leng and Nolan, 1984) requires further investigation.

A treatment × day interaction ($P = 0.001$) was also observed for N retention (g/d), with alternate-day supplemented lambs retaining more N on DOS than NS. As a percentage of intake, N retention was greatest ($P \leq 0.006$) for alternate-day supplemented lambs on DOS, but decreased ($P = 0.001$) on NS. Treatment had no effect ($P \geq 0.64$) on N retention (% of N digested) on DOS, but was decreased ($P = 0.004$) on NS in alternate-day supplemented lambs. However, when averaged across the supplementation interval, alternate-day supplemented lambs had lower N retention when expressed as a percent of N intake ($P = 0.006$) or N digested ($P = 0.004$). Bohnert et al. (2002) observed no difference in N retention due to protein degradability or supplementation frequency. Ludden et al. (2002a) also observed no effect of protein degradability on N retention; however, they observed an increase in N retention by lambs supplemented on alternate days. Similarly, Collins and Pritchard (1992) observed increased N retention in lambs supplemented with protein every 48 h compared to daily supplementation and this response was further improved in RUP supplemented lambs. These observations would further support the idea that N metabolism is delayed when supplementing protein infrequently, and that provision of amino acids (as RUP) may provide a greater delay in ureagenesis and/or urinary N excretion than additional RDP. Nonetheless, the influence of RUP supplementation on the precise timing of any such delay in N metabolism remains to be elucidated.

Implications

Alternate-day supplementation of ruminally undegradable protein to lambs consuming low-quality forage has little effect on forage intake and digestibility. Although lambs supplemented on alternate days may retain less N overall, their ability to maintain forage intake and digestion suggests that sufficient N is conserved to support ruminal microbial metabolism throughout the alternate-day supplementation interval. Moreover, it may be possible to alter the timing of N recycling to the rumen by altering supplementation frequency and/or the ruminal degradability of the supplemental protein.

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Table 2. Effect of ruminal protein degradability and frequency of supplementation on intake and apparent digestibility.

	TREATMENT[a]					DAY[b]			*P* <[c]	
Item	RDP-D	RDP-A	MIX-A	RUP-A	SEM	DOS	NS	SEM	T	D
DM intake, g/d										
Hay	1153	1023	1078	1052	35.6	1069	1084	25.2	0.08	0.66
Supplement	53	39	57	66	6.70	94	14	4.74	0.06	0.0001
Total	1206[d]	1062[e]	1135[de]	1118[de]	34.5	1163	1098	24.4	0.05	0.07
DM digestibility										
% of intake	46.8	47.4	48.1	46.1	0.95	48.1	46.1	0.67	0.47	0.04
OM intake, g/d	1079	1037	1024	1008	53.1	1047	1027	36.5	0.79	0.70
OM digestibility										
% of intake	49.5	50.9	51.4	48.9	1.00	51.0	49.3	0.69	0.24	0.09
NDF intake, g/d	677	600	635	625	20.8	634	634	14.7	0.09	1.00
NDF digestibility										
% of intake	48.6	49.9	49.9	47.9	1.40	48.7	49.4	0.99	0.68	0.61
ADF intake, g/d	492	436	462	454	14.7	462	460	10.4	0.08	0.93
ADF digestibility										
% of intake	40.8	40.4	41.7	39.2	1.14	40.5	40.5	0.80	0.47	0.99

[a]RDP-D = high ruminally degradable protein (RDP) fed daily; RDP-A = RDP fed on alternate days; RUP = high ruminally undegradable protein (RUP) fed on alternate days; MIX = 50:50 mixture of high RDP and high RUP supplements fed on alternate days.

[b]DOS = day of supplementation; NS = day no supplement was offered. Supplement was offered on both DOS and NS for the RDP-D treatment.

[c]*P* – values for the effects of T = treatment, D = day.

[de]Means with unlike superscripts within treatment are different ($P \leq 0.05$).

Table 3. Interaction of ruminal protein degradability and frequency of supplementation on intake, apparent digestibility and N retention in growing lambs.

	DOS[a]				NS					*P* <[b]		
Item	RDP-D	RDP-A	MIX-A	RUP-A	RDP-D	RDP-A	MIX-A	RUP-A	SE	T	D	T×D
N intake, g/d												
Hay	10.2	9.0	9.5	9.3	10.0	9.1	9.6	9.4	0.52	0.14	0.92	0.98
Supplement	6.1[d]	9.2[c]	11.6[c]	11.4[c]	6.5[c]	0[d]	0[d]	0[d]	1.24	0.46	0.001	0.001
Total	16.3[d]	18.2[cd]	21.1[c]	20.7[cd]	16.5[c]	9.1[d]	9.6[d]	9.4[d]	1.41	0.11	0.001	0.001
N excretion												
Fecal, g/d	7.6	6.5	7.0	6.9	7.6	6.5	6.9	6.7	0.33	0.01	0.73	0.99
Urinary												
g/d	1.8[d]	2.4[cd]	3.0[c]	2.5[cd]	2.3	1.6	1.9	2.1	0.32	0.44	0.02	0.04
% of N intake	11.2	12.7	14.3	12.0	13.9[d]	18.2[cd]	19.1[c]	21.9[c]	1.60	0.04	0.001	0.17
% of digested N	22.6	21.8	21.8	18.0	25.9[e]	66.1[d]	70.3[cd]	78.8[c]	6.79	0.004	0.001	0.001
Total, g/d	9.4	8.9	10.0	9.4	9.9	8.1	8.8	8.8	0.53	0.09	0.09	0.34
Total tract N digestion												
g/d	8.7[d]	11.7[c]	14.2[c]	13.8[c]	8.9[c]	2.6[d]	2.8[d]	2.7[d]	1.28	0.41	0.001	0.001
% of intake	52[d]	60[c]	66[c]	66[c]	54[c]	28[d]	28[d]	29[d]	3.10	0.04	0.001	0.001
N retention												
g/d	6.8[d]	9.4[c]	11.1[c]	11.3[c]	6.6[c]	0.9[d]	0.9[d]	0.7[d]	1.09	0.37	0.001	0.001
% of intake	40[d]	48[cd]	52[c]	54[c]	40[c]	10[d]	9.0[d]	7.0[d]	3.38	0.006	0.001	0.001
% of digestion	77	78	78	82	74[c]	34[d]	30[d]	21[d]	6.46	0.004	0.001	0.001

[a]DOS = day of supplementation; NS = day no supplement was offered. Supplement was offered on both DOS and NS for the RDP-D treatment. RDP-D = high ruminally degradable protein (RDP) fed daily; RDP-A = RDP fed on alternate days; RUP = high ruminally undegradable protein (RUP) fed on alternate days; MIX = 50:50 mixture of high RDP and high RUP supplements fed on alternate days.

[b]*P* – values for the effects of supplementation treatment (T), day (D), and treatment × day interaction (T×D).

[cd]Means with unlike superscripts within day are different ($P \leq 0.05$).

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Effect of Estradiol Cypionate on conception and pregnancy rates of beef cattle synchronized with a modified timed artificial insemination protocol[1, 2]

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Abstract: Induction of ovulation by GnRH during the proestrus period, as occurs in the Ovsynch (OVS) and Cosynch (COS) breeding protocols [GnRH-7d-Prostaglangin $F_{2\alpha}$ (PGF)-2d-GnRH+AI], impair ovarian estradiol (E_2) secretion and may reduce fertility. It is hypothesized that incorporation of a low dose of estradiol administered at the time of the second GnRH injection in the COS would improve conception (CR) and pregnancy rates (PR) in beef cattle. The objective was to determine the effect of incorporating a low dose of estradiol cypionate (ECP), into the COS protocol, on CR and PR in beef cattle. Over the period of two years, 183 cross-bred cows were used for this experiment. All cows received 25 mg PGF (i.m.) 14 d before initiation of the COS protocol. Fourteen days later, on d 0, 100 ug GnRH (i.m.) was administered followed 7 d later by 25 mg PGF (i.m.). On d 9 cows were paired by age, body condition score (BCS), and body weight (BW) and were randomly assigned to receive either GnRH (100 ug) + 0.25 mg ECP (COS-ECP; n = 90) or GnRH (100 ug) + vehicle (CON; n = 93) and were immediately inseminated by a single inseminator. Pregnancy status was determined via ultrasonography 40 and 60 d post-insemination. Data was analyzed by logistic regression. There was no year by treatment interaction; the data was then pooled for further analysis. Conception rates (53.3% COS-ECP vs. 57.0% CON) and pregnancy rates (92.2% COS-ECP vs. 90.3% CON) did not differ between treatments. Days postpartum, age, BCS and BW had no effect on the odds ratio of pregnancy. In summary, the results indicate that the incorporation of ECP into the COS protocol did not improve CR and PR in beef cattle.

Key words: Estradiol Cypionate, Conception Rate, Timed AI

Introduction

Estrus synchronization protocols have been developed as a management practice to shorten the interval from calving to rebreeding, decrease labor costs associated with estrus detection, and increase the implementation of AI. Despite this fact, less than 5% of the beef herds in the U. S. use artificial insemination (AI) per year with less than half of those implementing an estrus synchronization protocol (Wood et al., 2001; Baker et al., 2002).

In an effort to further decrease the labor cost associated with estrus detection and increase reproductive efficiencies, researchers have developed the timed insemination protocols Ovsynch (OVS; Pursley et al., 1995) and Cosynch (COS; Geary and Whittier, 1998), which were designed to synchronize ovulation, allowing timed AI of all cows without estrus detection (GnRH-7d $\rightarrow$ $PGF_{2\alpha}$- 2d$\rightarrow$ GnRH$\rightarrow$0 to 24 h-Timed AI).

A number of studies suggest that induction of ovulation by GnRH during the proestrus period, as occurs in OVS and COS breeding protocols, impairs ovarian estradiol (E_2) secretion and may reduce fertility (Lucy and Stevenson, 1986; Stegner et al., 2002)

Previous research indicated that when estradiol cypionate (ECP®) was incorporated into the OVS (administered at the time of the second dose of GnRH), the first service conception rate of the ECP-treated group tended to improve compared to the control group (68% vs. 57.5%; Ahmadzadeh et al., 2003). The use of the COS protocol reduces the number of times an animal will need to handled.

We hypothesized that incorporation of a small dose of ECP at the time of the second GnRH, in the COS protocol, would improve first service conception (CR) and pregnancy rates (PR) in beef cattle. The objective was to determine the effect of incorporating a low dose of ECP, into the COS protocol, on CR and PR in beef cattle.

Materials and Methods

Animals and Treatments: This study was conducted at the University of Idaho's Nancy M. Cummings Research and Extension Center in Carmen, Idaho. Over the course of two

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years, 183 primiparous and multiparous crossbred beef cattle with normal reproductive health status were used for this experiment. The protocol used in this experiment was approved by the University of Idaho Animal Care and Use Committee. At initiation of the experiment (aprox. 80 days postpartum) body condition scores (BCS) and body weights (BW) were determined. Mean BCS were 6.34 ± 0.90 (mean ± SD; on a scale of 1 to 9, 1 being emaciated and 9 being obese. Body weights averaged 490 kg ± 58. The experimental protocol is depicted in the figure 1. Fourteen days before initiation of the COS protocol all cows received a luteolytic dose of PGF (25 mg; Lutalyse®, Pfizer Animal Health Inc., New York, NY). Fourteen d later, on d 0, 100 ug GnRH (Cystorelin, Merial, Iselin, NJ; i.m.) was administered followed seven d later by an injection of 25 mg PGF (i.m.). On d 9 cows were paired by age, body condition score, and body weight to receive either 0.25 mg ECP (COS-ECP; n = 93) or GnRH (100 ug) + vehicle (CON; n = 90). All cows immediately received AI by a

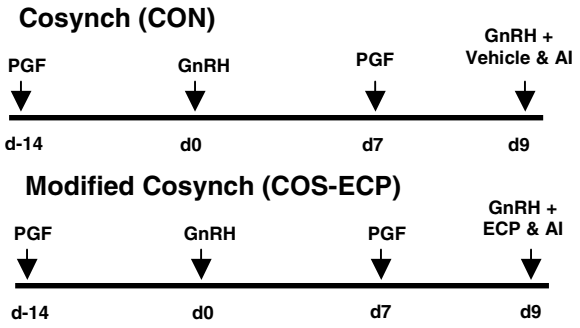

Figure 1. Schematic of the different treatments for CON (n = 93) or COS-ECP (n = 90). All cows received the COS with the exception of ECP or vehicle treatment at AI. ECP treatment consisted of 0.25 mg estradiol cypionate in 1 ml of cotton seed oil; vehicle consisted of 1 ml cottonseed oil.

single inseminator, using semen from four sires stratified across treatment groups. Fourteen d after AI, bulls were placed with cows for a 55-d natural service period. Pregnancy status was determined by ultrasonography (Sonovet Co., Mure, Mitaka-Shi Tokyo Japan) approximately on d 35 and d 70 after AI.

Statistical Analysis: Pregnancy data was analyzed by logistical regression using SAS (SAS Inst. Inc., Gary, NC). The statistical model included year, treatment, age, days postpartum (DPP), BW, and BCS.

Results and Discussion

This experiment was conducted to determine whether incorporation of 0.25 mg of ECP into the Cosynch protocol improved conception and pregnancy rates in beef cattle.

There was no effect of year ($P = 0.60$) on CR and PR; therefore, data was pooled for further analysis. First service CR (defined as the number of animals that were diagnosed pregnant in a treatment group divided by the total number of animals inseminated in that treatment group) was not between treatment groups (53.3% and 57.0% for COS-ECP and CON, respectively; Figure 2). Pregnancy rates (defined as the number of animals that were diagnosed pregnant in a treatment group divided by the total number of animals inseminated and exposed to a bull for 21 days in that treatment group) were not different between treatment groups (COS-ECP 92.2% vs. CON 90.3%). Moreover, PR were not different between treatment groups on d 40 and 70 after insemination. There was no treatment by age, BCS, BW, DPP interaction ($P > 0.05$). The data was then pooled to analyze for main effects. Age, DPP, BW, and BCS ($P > 0.05$) did not affect the odds ratio of CR and PR.

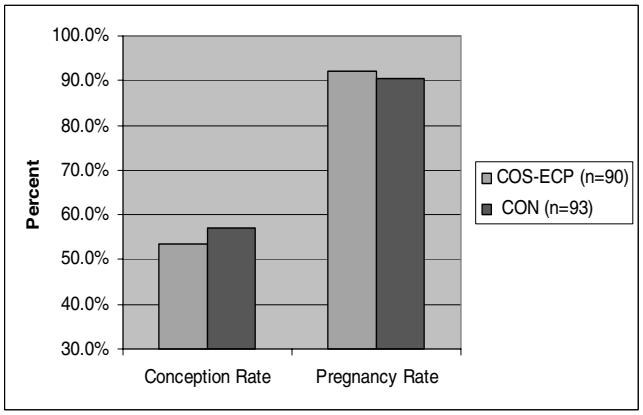

Figure 2. Conception and pregnancy rates of cows in both estradiol cypionate (COS-ECP) and control (CON) treatment groups.

In the current study, incorporation of 0.25 mg of ECP into the COS did not improve the first service AI CR in beef cattle. In contrast, research conducted by Ahmadzedeh et al. (2003) reported that when 0.25 mg ECP was incorporated into OVS an 11% increase in CR was observed. A possible explanation for the difference between these two studies is perhaps the timing of insemination relative to ECP administration. In the study by Ahmadzadeh et al. (2003) all cows were inseminated 6 h after GnRH+ECP treatment, during the time of maximally elevated serum E_2. In contrast, in the present study AI was performed simultaneously with GnRH+ECP administration. Thereby, uterine environment of the ECP-treated cows had not been exposed to an elevated level of E_2 to possibly affect sperm sustained transport and fertility.

Researchers have shown that exogenous E_2 may cause the development of a persistent follicle (Garcia and Salaheddine, 2001). Furthermore, estradiol may inhibit GnRH and/or LH pulses (see Wiltbank et al., 2002 for review). Therefore, it can be argued that the lack of response in CR of the ECP-treated group, in the present study, was due to the development of persistent, non-ovulating follicles. In addition, ECP treatment could have altered the time of ovulation and ovulation rate. However, this may not be the case. Previous research from our laboratory (Sellars, 2002) showed that the rate of ovulation and time of ovulation in cows after GnRH+ECP treatment did not differ from that of the control group when the same dose of ECP was used.

Body condition scores were recorded for each animal at the initiation of the study. There were no interaction between BCS and treatments (P = 0.24). Body condition has been shown to influence pregnancy rates when cattle were inseminated using a timed-AI protocol (Moreira et al., 2000). Animals with low body reserves have a greater probability of suffering from reproductive failure (Morrison et al., 1999; Moreira et al., 2000; Montiel and Ahuja, 2005). A BCS of 5 at calving is important to ensure acceptable postpartum reproduction. In this study there was no association between BCS (P = 0.19) and probability of CR. This is thought to be in part due to the fact that the majority of the cows (> 90%) in this study had a BCS > 5. The limited number of observations in cows with BCS < 5 makes detecting any possible effects of BCS difficult.

There was no interaction between DPP and treatment, and DDP had no association with probability of CR (P > 0.20). Our results were similar to the previous experiments. Hall et al., (2003) found that pregnancy rates were similar for cows that were < 60 d and > 60 DPP at the initiation of COS.

Implications

The results of this study suggest that that incorporation of 0.25 mg of ECP into the Cosynch protocol may not improve CR or PR in beef cattle. Further research into the enhancement of existing timed AI protocols will assist in increasing the economic stability of the beef industry. The findings of this study provide a basis for future research regarding the role of estradiol and its impacts on subsequent fertility.

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EVALUATION OF MILK SOMATIC CELLS AS A SOURCE OF mRNA FOR STUDY OF MAMMARY GLAND LIPOGENESIS IN LACTATING BEEF COWS[a]

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ABSTRACT: Our objective was to compare mRNA levels for acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and stearoyl-CoA desaturase (SCD) extracted from mammary gland and from somatic cell pellets of the milk from each mammary gland. Eighteen primiparous beef cows (BW = 411 ± 24.3 kg.; BCS = 5.25) were fed Foxtail millet hay at 2.13% of BW and either a low-fat control (CON; n = 9) or a cracked high-linoleate (67% 18:2 n-6) safflower seed supplement (LIN; n = 9). Diets were isonitrogenous and isocaloric, and the LIN diet contained 5% of DMI as fat. At slaughter (37 ± 3 d postpartum) mammary tissue was sampled and immediately frozen in liquid N_2 before being stored at -80°C. Milk samples were obtained from the same mammary glands and immediately spun at 1,200 × *g* to pellet somatic cells. Ribonuclease protection assay was used to quantify the mRNA. Data were analyzed for a 2 × 2 factorial experiment to test for dietary, tissue and interactive effects. Dietary, tissue, and interactions were not observed for ACC (*P* = 0.21; 0.91; and 0.45, respectively), FAS (*P* = 0.32; 0.71; and 0.28, respectively), LPL (*P* = 0.09; 0.15; and 0.43, respectively), or SCD (*P* = 0.34; 0.26; and 0.37, respectively). Correlation analysis was performed between mammary tissue and milk somatic cell data within dietary treatment for each mRNA. Within the CON treatment, Pearson correlation coefficients were: ACC, 0.75 (*P* = 0.02); FAS, 0.69 (*P* = 0.04); LPL, 0.69 (*P* = 0.04); and SCD, 0.67 (*P* = 0.05). Within the LIN treatment, Pearson correlation coefficients were: ACC, 0.85 (*P* = 0.004); FAS, 0.75 (*P* = 0.02); LPL, 0.90 (*P* = 0.001); and SCD, 0.73 (*P* = 0.03). We conclude that using milk obtained from lactating beef cows can be used as a source of RNA to study regulation of mammary gland lipogenesis.

Key Words: Mammary gland, mRNA, Milk somatic cells, Lipogenesis

Introduction

Our laboratory has recently observed lower milk output of 10:0, 12:0, and 14:0 by beef cows fed either high-oleate or high-linoleate supplements (Lake et al., 2004). This response may reflect dietary effects on enzymes involved in mammary lipogenesis because the aforementioned fatty acids typically occur by de-novo fatty acid synthesis. Enzymes that play a significant role in mammary lipogenesis are acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL), and stearoyl-CoA desaturase (SCD). Together these four enzymes are responsible for production of new fatty acids (ACC and FAS), uptake of circulating fatty acids (LPL), and desaturation of fatty acids (SCD) in the mammary gland. Because these enzymes are transcriptionally regulated (Munday et al., 2002; Stoeckman et al., 2002; Barber et al., 2003), quantifying their mRNA should provide insight into their dietary regulation.

Sampling mammary tissue, either by dissection or by obtaining a mammary biopsy is required to obtain mRNA directly from the tissue. A biopsy procedure can be costly and potentially injurious to both the cow and technician. However, Boutinaud et al. (2002) used milk somatic cells to study gene expression in the goat mammary gland. We hypothesized that somatic cells obtained from milk in lactating beef cows will provide RNA similar to that extracted from mammary gland tissue. The objective of this study was to evaluate milk somatic cells as a source of mRNA for evaluation of ACC, FAS, LPL and SCD in lactating beef cows fed either a control or high-linoleate dietary supplement.

Materials and Methods

General

Eighteen primiparous beef cows (BW = 411 ± 24.3 kg; BCS = 5.25) were fed Foxtail millet hay at 2.13% of BW and either a low-fat control (n = 9) or a cracked high-linoleate (67%, 18:2 *n-6*) safflower seed supplement (n = 9). Diets were isonitrogenous and isocaloric and the high-linoleate diet contained 5% of DMI as fat (Table 1). Cows were slaughtered on d 37 ± 3 postpartum at which time mammary gland and milk samples were obtained. This study was conducted with approval from the University of Wyoming Animal Care and Use Committee.

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Table 1. Ingredient and nutrient composition of experimental diets

Item	Control	Linoleate
Ingredients, % of DM		
Foxtail millet hay	85.2	89.9
Corn	9.5	-
Safflower seed meal	4.7	-
High-linoleate safflower seeds	-	9.5
Molasses	0.55	0.61
Nutrient composition		
DM, %	90.0	91.0
	----------% of DM-------	
CP	11.6	11.6
Total fatty acids	1.4	8.8
16:0	0.15	0.61
18:0	0.03	0.02
18:1	0.20	0.74
18:2	0.25	6.3
18:3	0.04	0.05

Mammary Tissue Collection and RNA Extraction

Immediately after slaughter, entire mammary glands were excised and tissue samples were obtained from the right and left sides dorsal to the teat area and placed into 25 mL plastic scintillation vials, immersed in liquid N_2, and stored at -80°C. Approximately 60 mg of frozen mammary tissue was weighed into sterile 12 x 75 mm plastic tubes and homogenized with a Tekmar Tissuemizer® (Tekmar Company, Cincinnati, OH). Mammary RNA was extracted using an RNeasy® Lipid Tissue Mini Kit (QIAGEN Inc., Valencia, CA), suspended in 50 μL of sterile water containing 0.1% diethyl pyrocarbonate and stored at -80°C.

Milk Collection and RNA Extraction

Approximately 200 mL of milk was collected from milk pooled within the excised mammary gland, and placed into several sterile 50 mL glass centrifuge tubes, and then centrifuged at 1200 x *g* for 10 min at room temperature. The cellular pellets from each tube were washed in PBS (pH = 7.4) to remove any remaining infranatant. The somatic cell pellet was resuspended in 0.75 mL of Tri Reagent LS® (Molecular Research, Inc., Cincinnati, OH) for total RNA extraction and suspended in 50 μL of sterile water containing 0.1% diethyl pyrocarbonate and stored at -80°C. Concentrations of RNA for all samples were determined using absorbance at λ = 260 nm (A_{260}).

RNA Analysis

Probes of cDNA for ACC, FAS, SCD, LPL, and 18s were generated from bovine mammary RNA as described by Lee et al. (2002). The ribonuclease protection assay (RPA) was used to quantify mRNA according to the procedure published by Lee et al. (2002) modified such that samples were placed in a 95°C water bath for 2 min followed by a 15 h hybridization incubation in a 48°C water bath.

Following hybridization, samples were loaded onto a 10 cm x 8 cm x 1.5 mm thick vertical PAGE apparatus containing a 7 M urea, and 4% acrylamide: bisacrylamide gel (19:1) mixture, adjusted to pH 8.3. All cDNA probes were hybridized simultaneously and standardized for loading using 18s mRNA.

Membrane transfer and autoradiography were conducted according to Lee et al., (2002) except that a 45 min exposure to X-ray film was used (Sterling RX-B®, bio-World, Dublin, OH).

Films were digitized and analyzed using Quantity One® Imaging Software with a Gel Doc 1000® camera and light box (BioRad, Hercules, CA). Band intensities were standardized against the 18s gene probe to compare mRNA abundance.

Statistical Analysis

Data for mRNA represented relative abundance of mRNA expressed as optical density units of each band per optical density units of the 18s band. Data were analyzed as a factorial within a completely randomized design using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). The model included dietary treatment, tissue, and the treatment × tissue interaction. Animal was included in the model using the RANDOM statement. Interactions between treatment and tissue were not observed for ACC (P = 0.44), FAS (P = 0.28), LPL (P = 0.43) or SCD (P = 0.37); therefore, main effects of dietary treatment and of tissue source as fixed effects were reported. Pearson correlation coefficients between mammary and milk mRNA relative abundance within dietary treatments were determined using the CORR procedure of SAS.

Results and Discussion

Each cDNA probe produced a single band with sizes of 501 bp (LPL), 436 bp (ACC), 337 bp (SCD), and 218 bp (FAS; Figure 1)

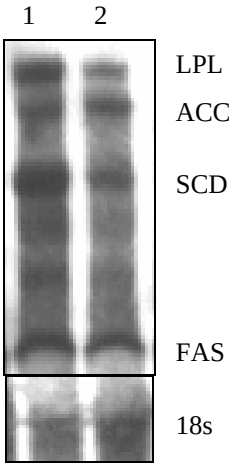

Figure 1. A representative PAGE comparing mRNA from mammary tissue (Lane 1) with milk somatic cells (Lane 2). Bands labeled for each mRNA were: lipoprotein lipase

(LPL), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), fatty acid synthase (FAS), and 18s.

Main Effects of Dietary Treatment

Dietary supplementation of unsaturated fatty acids can have distinct effects on the mammary gland. Griinari et al. (1998) demonstrated that *trans*-octadecenoic acids, a metabolite of ruminal biohydrogenation of unsaturated fatty acids, caused a reduction in fatty acids produced de-novo by 32% and a reduction in overall milk fat yield by 36%. In a study conducted in our laboratory (Lake et al., 2004) high-linoleate and high-oleate supplementation of lactating beef cows resulted in lower milk output of 10:0, 12:0, and 14:0 compared with control cows ($P < 0.001$). These results were supported by a more recent study in our laboratory (Murrieta et al., 2005) in which cows fed a high-linoleate supplement produced lower concentrations of milk fatty acids typically synthesized de novo (10:0, $P < 0.001$; 12:0, $P < 0.001$; 14:0, $P = 0.002$). Therefore, high-linoleate or high-oleate supplements may have affected mammary tissue lipogenic enzymes. Others have also shown that bovine milk fatty acid composition is altered by lipid supplementation strategies (AbuGhazaleh et al., 2003). Few studies, however, have investigated molecular mechanisms involved in these changes.

Between dietary treatments, expression of LPL mRNA tended ($P = 0.09$) to be greater for LIN than for CON. Expression of ACC, FAS, and SCD mRNA were not affected (P = 0.21 to 0.34) by dietary treatment (Table 2).

Table 2. Main effects of dietary treatment on relative abundance of mRNA from milk somatic cells and mammary tissue of cows fed control (CON) or high-linoleate (LIN) diets.

mRNA[a]	CON	LIN	SEM[b]	*P*
ACC	0.02	0.02	0.002	0.21
FAS	1.60	1.84	0.18	0.32
LPL	1.40	1.91	0.21	0.09
SCD	0.72	0.82	0.10	0.34

[a]ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; LPL = lipoprotein lipase; SCD = stearoyl-CoA desaturase.
[b]n = 9 per dietary treatment.

The observation in the current study that only LPL mRNA was affected by dietary fat supplementation suggests that potentially altered lipogenesis in the aforementioned studies may not have been the result of mRNA transcription alone or that changes in typical milk fatty acids were reduced proportionally and not through decreased lipogenesis. If only LPL were affected, the increased activity would have accelerated the uptake of fatty acids from circulating lipoprotein triacylglycerols. The greater influx of fatty acids by the mammary gland of the LIN cows would have blunted ACC activity because of the well accepted inhibitory effect of fatty acids, and more so of fatty acyl-CoA, on ACC activity. Thus, effects directed more towards the enzymes than towards transcription of their mRNA may have occurred. On the other hand, fatty acids taken up by the mammary gland of LIN cows would have diluted the C10 to C14 fatty acids, effectively decreasing their proportions, and thus reducing measured milk output of these fatty acids.

Main Effects of Tissue Type

Relative abundance of each mRNA was similar ($P = 0.15$ to 0.91) for mammary tissue and milk somatic cell RNA (Table 3). Overall, these results were similar to the outcome of studies conducted by Boutinaud et al. (2002). Those authors indicated that exfoliated epithelial cells of goat milk were biochemically similar to alveolar epithelial cells of several species, including cattle. Boutinaud et al. (2002) evaluated RNA in epithelial cells that had been separated from leucocytes in the goat milk. In the current study, total somatic cells were used for the RNA extractions.

Table 3. Main effects of tissue type on mRNA relative abundance of milk somatic cells and mammary tissue

mRNA[a]	Mammary	Milk	SEM[b]	*P*
ACC	0.02	0.02	0.002	0.91
FAS	1.77	1.67	0.18	0.71
LPL	1.87	1.44	0.21	0.15
SCD	0.71	0.83	0.10	0.26

[a]ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; LPL = lipoprotein lipase; SCD = stearoyl-CoA desaturase.
[b]n = 9 per tissue type.

Correlations Between Mammary Tissue and Milk RNA

No interaction between dietary treatment and tissue type was observed in the current study, which allowed us to examine mRNA abundance for each tissue within the CON and LIN treatments (Table 4). Positive correlations between the four mammary tissue and milk somatic cell mRNA were observed within both dietary treatments. Results indicate that milk somatic cells were a reliable source of mRNA for use in analysis of DNA transcription for lipogenic enzymes in mammary tissue. Furthermore, our results suggest that this procedure would be valid for nutritional studies using dietary lipid supplementation, which may alter the fat composition of milk from the lactating mammary gland of beef cows.

Overall, our goal was to evaluate the reliability of beef cow milk somatic cells as a source of mRNA to study mammary gland lipogenic enzymes during lactation, which may be altered by dietary means. Boutinaud et al. (2002) demonstrated a strong relationship between mammary tissue and milk somatic cell RNA for α-S1 casein, α-lactalbumin, and κ-casein RNA and reporting correlation coefficients of 0.99, 0.93, and 0.99 respectively. Boutinaud et al. (2002) concluded that somatic cells obtained from milk accurately reflect gene expression in the lactating mammary gland. An additional advantage of using milk is that repeated sampling in large-scale studies would be possible and far more feasible than if mammary tissue biopsies were to be used. Moreover, the lack of interaction between dietary treatment and tissue

source further strengthens the use of somatic cell RNA to study dietary effects on mammary gland lipogenic enzymes. We conclude that mammary gland lipogenic enzyme transcription can be accurately examined by the analysis of mRNA obtained from milk somatic cells.

Table 4. Correlation coefficients from comparison of mammary tissue and somatic cell mRNA within and across dietary treatment

Item	CON[a]		LIN	
mRNA[b]	r	*P*	r	*P*
ACC	0.76	0.02	0.85	0.003
FAS	0.69	0.04	0.75	0.02
LPL	0.68	0.04	0.90	0.001
SCD	0.73	0.05	0.73	0.03

[a]n = 9 per dietary treatment.
[b]ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; LPL = lipoprotein lipase; SCD = stearoyl-CoA desaturase.

Implications

The use of milk somatic cells as a reliable source of mRNA will provide many advantages over mammary biopsy or dissection. This source of mRNA will allow repetitive sampling and larger studies of feeding influences on mammary gland metabolism. Using milk somatic cells as a source for mRNA will be a valuable tool in evaluating the regulation of lipogenic enzymes in the mammary gland of lactating beef cows using lipid supplementation and likely other dietary treatments.

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EFFECTS OF PRE- AND POSTPARTUM NUTRITION ON REPRODUCTION IN SPRING CALVING COWS AND CALF FEEDLOT PERFORMANCE

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ABSTRACT: Crossbred, spring calving cows (yr 1 n = 136, yr 2 n = 113, yr 3 n = 113) were used in a three year experiment to evaluate the influence of supplemental protein prepartum and grazing sub-irrigated meadow postpartum on pregnancy rates and calf feedlot performance. A 2 X 2 factorial arrangement of treatments was used in a switchback design. During the last trimester of gestation cows were divided into eight pastures (32 ± 6 ha) and grazed dormant upland range (50% TDN, 5.4% CP). The equivalent of 0.45 kg·cow^{-1}· d^{-1} supplement (42% CP) was provided to one-half the cows on a pasture basis three times per wk. For 30 d prior to start of breeding (May 1 – May 31), one-half the cows grazed sub-irrigated meadow (57% TDN, 15.6% CP) while the remainder were fed grass hay (56% TDN, 7.9% CP). Cow weight and body condition score were monitored throughout the year and steer calf performance for the first two years was determined through slaughter. Feeding supplement prepartum improved body condition score pre-calving (5.1 vs. 4.6; $P < 0.001$) and pre-breeding (5.1 vs. 4.9; $P = 0.01$) and increased the percentage of live calves at weaning (97 vs. 90%; $P = 0.03$) but did not affect pregnancy rate (93 vs. 90%; $P = 0.46$). Calves born to dams fed supplement prepartum had similar birth weight (37 vs. 36 kg; $P = 0.29$) and steers had similar 205-d adjusted weaning weight (236 vs. 229; $P = 0.13$). Feedlot DMI (8.4 vs. 8.3 kg; $P = 0.68$) and ADG (1.6 vs. 1.5 kg; $P = 0.32$) were similar but steers born to cows fed supplement had greater hot carcass weight (368 vs. 357 kg; $P = 0.07$) because of greater weight upon entry into the feedlot (210 vs. 201 kg; $P = 0.05$). Allowing cows to graze sub-irrigated meadow improved body condition score pre-breeding (5.2 vs. 4.9; $P < 0.001$) but did not affect pregnancy rate (92 vs. 91%; $P = 0.88$). Grazing sub-irrigated meadow did not affect 205-d adjusted weaning weight (235 vs. 230 kg; $P = 0.35$), feedlot DMI (8.4 vs. 8.3 kg; $P = 0.69$), ADG (1.6 vs. 1.6 kg; $P = 0.62$) or carcass weight (363 vs. 362 kg; $P = 0.91$) of steer calves. Feeding supplemental protein increased percentage of live calves at weaning and carcass weight but not pregnancy rates. Grazing sub-irrigated meadow did not improve pregnancy rates or feedlot performance.

Key Words: Supplementation, Reproduction, Cattle

Introduction

Beef production systems are comprised of a series of segments with potential for complex interactions. Management changes in one segment may influence the entire system.

Body condition score is a good measure of energy reserves (Wagner et al., 1988) and BCS at calving is among the most important factors affecting pregnancy rate (Richards et al., 1986). However, postpartum nutrition may also influence reproduction (Ciccioli et al., 2003).

Objectives of this study were to determine the effects of prepartum protein supplementation and postpartum nutrition and their interaction on the entire production system, especially, cow reproductive performance and calf growth performance through the feedlot.

Materials and Methods

In yr 1, 136 pregnant, MARC II (¼ Angus, ¼ Gelbvieh, ¼ Hereford, ¼ Simmental), spring calving cows age 3 to 5 yr were stratified by age and weaning weight of previous calf then assigned randomly to 1) supplement or no supplement prepartum and 2) sub-irrigated meadow or hay postpartum. In yr 2 cows were switched to the opposite treatment and switched back to their original treatment in yr 3. Cows remained in the experiment unless removed for reproductive failure. In yr 2 and 3 only 113 cows were used because of reduced forage availability caused by drought.

On December 1, cows were divided into eight pastures (32 ± 6 ha) and grazed native upland range at the University of Nebraska, Gudmundsen Sandhills Laboratory, near Whitman, NE. A detailed description of the study site is given by Adams et al. (1998). Either 0 or equivalent of 0.45 kg·cow^{-1}·day^{-1} supplement was provided to cows on a pasture basis, three times per wk, from December 1 to February 28. On a DM basis, supplement ingredients were: 50.0% sunflower meal, 47.9% cottonseed meal, 2.1% urea; and composition was: 42.0% CP and 73.3% TDN.

Cows were managed in a common group during the calving season (Mar 1 to April 30) and fed grass hay in a dry lot. Amount of hay fed was adjusted daily in an effort to satisfy appetite but minimize waste and averaged 14 kg·cow^{-1}·day^{-1} (DM basis). Hay quality was determined by near infrared reflectance spectroscopy at a commercial laboratory (Table 1). Average calving date was March 27.

During the period between calving and start of breeding (May 1 to May 31), half the cows were fed grass hay and half grazed sub-irrigated meadow.

At the beginning of breeding season (June 1) treatment groups were combined and cows grazed upland range as a single group for the remainder of the production cycle. The breeding season lasted 60 d with a 1:20 bull:cow ratio.

Diet quality (Table 1) was estimated from masticate samples obtained from esophageally fistulated cows external to the experiment. Surgical procedures and

management were in accordance with University of Nebraska, Institutional Animal Care and Use Committee guidelines. Surgeries had been performed on all cows at least two years prior to the beginning of the experiment and three animals were used for each diet collection. Cows were withheld from feed for 12 hr before masticate samples were obtained. Cows were fitted with screen bottom bags after removal of the esophageal plug and allowed to graze for 30 minutes in an ungrazed upland pasture or meadow immediately adjacent to ones used in the experiment. Samples were stored frozen at -20º C, freeze dried and analyzed for CP content using a Leco FP-2000 N analyzer (Leco Corp., Henderson, NV) and ADF content using an ANKOM 2000 fiber analyzer (ANKOM Technology, Fairport, NY) from which TDN content was calculated. Masticate samples were obtained from upland range at the beginning of prepartum treatment period and from sub-irrigated meadow at end of postpartum treatment period.

Table 1. Upland and sub-irrigated meadow diet and hay quality (mean ± SD)

Item	Yr 1	Yr 2	Yr 3
Upland range diet			
CP, % DM	6.4 ± 0.6	4.7 ± 1.4	5.1 ± 0.1
TDN, % DM	50.8 ± 5.4	49.0 ± 0.8	50.6 ± 0.8
Sub-irrigated meadow diet			
CP, % DM	15.7 ± 1.9	15.9 ± 1.8	15.1 ± 2.5
TDN, % DM	57.1 ± 1.2	58.6 ± 0.4	55.9 ± 1.0
Hay			
CP, % DM	8.6 ± 1.2	8.7 ± 0.7	6.3 ± 0.6
TDN, % DM	56.0 ± 1.8	54.2 ± 2.1	57.9 ± 1.3

Weight and body condition score (BCS; Wagner et al., 1988) of all cows were recorded at beginning (December 1) and end (February 28) of the prepartum supplementation period, at beginning (May 1) and end (May 30) of the postpartum meadow grazing period, and at weaning (first week of October). Cows were examined for pregnancy via rectal palpation by an experienced veterinarian in October.

In each year, daily milk production was estimated by the weigh-suckle-weigh technique at the start of breeding (68 d after average calving date). Milk production estimates were obtained from one-half of the cows from each treatment combination, selected randomly within age. The afternoon before milk production estimate was made, calves were removed from their dams for 3 hr then allowed to nurse for 15 min. Calves were then separated from their dams for 12 hr. After the 12 hr separation calves were weighed, allowed to nurse until all calves had finished nursing (approximately 15 min), and immediately reweighed. Milk production was the difference in calf weight before and after suckling. Each cow's 12-h milk production was extrapolated to 24-h.

Calves were weighed within 24 to 48 h of birth and at weaning. Between 24 and 48 h of birth, a blood sample was collected in serum separator tubes (Corvac, Sherwood Medical Co. St. Louis, MO) from each calf via coccegeal venipuncture in yr 2 and 3. Serum was harvested by centrifugation at 1500 X g for 15 min and stored at -20º C until analyzed for Immunoglobulin G (**IgG**) concentration by single radial immunodiffusion (Bovine IgG SRID kit; VMRD Inc., Pullman WA). Bull calves were castrated at branding (May).

At weaning, steers (yr 1 n = 61, yr 2 n = 65, yr 3 n = 45) received two doses of infectious bovine rhinotracheitis/ parainfluenza-3 virus/bovine respiratory syncytial virus modified live – bovine virus diarrhea killed vaccine (PRISM 4, Ft. Dodge Animal Health, Overland Park, KS) 14 d apart and with a single dose of vaccine against *Mannheimia* (*Pasteurella*) *haemolytica* type A1 (One Shot, Pfizer Animal Health, Exton, PA). Steers were fed for ad libitum intake of grass hay in a dry lot during a two week preconditioning period before being shipped to a feedlot at the West Central Research and Extension Center in North Platte, NE (167 km). Upon arrival steers were fed grass hay at 2.5% of BW for 7 d. After the 7-d adaptation period, steers were weighed on two consecutive days and implanted with 20 mg estradiol benzoate and 200 mg progesterone (Synovex S, Ft. Dodge Animal Health, Overland Park, KS) and dewormed with moxidectin (Cydectin, Ft. Dodge Animal Health, Overland Park, KS) on the second day. Steers were reimplanted with 24 mg estradiol and 120 mg trenbolone acetate (Revelar S, Intervet, Millsboro, DE) about 100 d prior to slaughter. The starting diet contained 35% alfalfa and steers were adapted over 14 d to a finishing diet that contained 48% dry rolled corn, 40% wet corn gluten feed, 7% alfalfa and 5% supplement (DM basis) by replacing alfalfa with corn. Steers were fed in 8 pens corresponding to the prepartum pasture of their dam until it was visually estimated the average 12th rib back fat of all steers was 1.3 cm. Feedlot performance only includes steers from yr 1 and 2. Feeding of steers in yr 3 is in progress.

Carcass data were obtained via the Cattlemen's Carcass Data Service, West Texas A&M University, Canyon. Hot carcass weight was obtained at harvest. Hot carcass weight was adjusted to a common age by the equation: 430d Adj. HCW = ((HCW – birth weight)/age at slaughter)·430 + birth weight. Average age of steers at slaughter was 430 d. Dressing percentage was calculated using the unshrunk weight obtained at the feedlot prior to shipment to the abattoir. Following a 24-h chill, marbling score, fat thickness at the 12th rib, percentage of KPH, longissimus muscle area, yield grade and quality grade were determined.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model was appropriate for a 2 X 2 factorial arrangement of treatments in a switch back design and included prepartum treatment (supplement vs. no supplement), postpartum treatment (meadow vs. hay), and their interaction as fixed effects. Year was included in the model as a random variable. Prepartum by postpartum treatment interactions did not occur ($P > 0.20$) for any measured variable except milk production. Since cows were fed supplement on a pasture basis, pasture or feedlot pen served as the experimental unit.

Results and Discussion

Cows fed protein supplement prepartum had greater BCS at the end of the supplementation period ($P < 0.001$), at start of postpartum treatment period ($P < 0.001$) and at start of the breeding season ($P = 0.01$) than cows not fed supplement (Figure 1). Feeding supplemental protein did not result in increased pregnancy rates ($P = 0.46$, Table 2). Similarly, cows that grazed sub-irrigated meadow had greater BCS ($P < 0.001$) at start of breeding (Figure 2) but pregnancy rates were not affected ($P = 0.88$). It is likely that pregnancy rates were similar because non-supplemented and hay fed cows were in acceptable body condition at start of breeding. Richards et al. (1986) found a BCS of 5 at calving to be the critical level affecting subsequent reproduction and cows in all treatments were near a BCS of 5 at calving. Results of the present study agree with findings of Morrison et al. (1999) who showed cows that increase condition during late gestation to reach a BCS of 5 at calving have pregnancy rates similar to cows maintained at a BCS of 5 from late gestation to calving.

Cows fed supplement calved three d later ($P = 0.01$) than cows not fed supplement but birth weight was similar ($P = 0.29$). Weaning weight and ADG from birth to weaning were greater for calves born to cows fed supplement and for calves born to cows that grazed sub-irrigated meadow compared to non-supplemented and hay fed cows, respectively. However, actual ($P = 0.33$; data not shown) and 205-d adjusted ($P = 0.13$) weaning weights of steer calves were not different for either treatment.

The percentage of live calves at weaning was greater ($P = 0.03$) for cows fed supplement prepartum but was not different ($P = 0.56$) between cows that grazed meadow or were fed hay. Since only pregnant cows were included in the study each year, differences in percentage of live calves at weaning can not be attributed to failure to conceive. Potentially, failure of passive transfer of immunity could explain differences in weaning rate and weaning weight (Perino et al., 1995). In yr 2 and 3, IgG titers of calves between 24 and 48 h after birth were similar ($P = 0.98$). These results agree with the findings of Perino et al. (1995) who found BCS at calving, ranging from 4 to 7, does not influence IgG titers of calves.

Milk production does not fully explain differences in weaning weight. A prepartum by postpartum treatment interaction ($P = 0.10$) occurred for milk production. In supplement fed cows milk production was 3.6 kg/d greater ($P = 0.05$) for cows fed hay postpartum than for cows that grazed sub-irrigated meadow. In non-supplemented cows, milk production was not affected by postpartum treatment.

Steers born to cows fed supplement were 9 kg heavier ($P = 0.05$) at start of the finishing period than steers born to non-supplemented cows and a weight advantage was maintained for the entire finishing period (Table 3). Feedlot ADG ($P = 0.32$), DMI ($P = 0.68$) and feed efficiency ($P = 0.63$; data not shown) were similar for steers born to supplemented and non-supplemented cows. Steers born to cows fed supplement had 11 kg greater ($P = 0.07$) carcass weight and the carcass weight difference increased to 14 kg ($P = 0.02$) when adjusted to 430-d age. Steers born to cows that grazed sub-irrigated meadow pre-breeding were 8 kg heavier at start of the finishing period but carcass weight and carcass weight adjusted for age was not affected by postpartum treatment. Feedlot ADG, DMI and feed efficiency were similar for steers born to cows that grazed meadow and cows fed hay but steers born to cows fed hay had greater dressing percentage ($P = 0.01$).

Longissimus muscle area tended ($P = 0.13$) to be greater for steers born to cows fed supplement but other carcass characteristics were not influenced by prepartum treatment. Postpartum treatment did not influence carcass characteristics.

One of the most important findings of this experiment was that steers born to cows fed supplement gained 0.04 kg/d more ($P = 0.02$) from birth to slaughter than steers born to cows not fed supplement. Studies that examine the effects of supplement fed to the cow on calf feedlot performance are rare in the literature. However, these data agree with the findings of Ciminski (2002) who also showed improved performance from birth to slaughter in steers born to cows fed supplemental protein.

Implications

Results of this study indicate feeding supplement to spring calving cows grazing dormant forage may have benefits beyond improving reproduction. Feeding supplement did not improve pregnancy rates but increased percentage of live calves at weaning and carcass weight. The increase in carcass weight was observed despite a lack of difference in 205-d adjusted weaning weight. These data demonstrate that changes in management have ramifications beyond the segment in which they occur and may influence the entire production system. In this study, prepartum nutrition had a greater affect on subsequent productivity than did postpartum nutrition.

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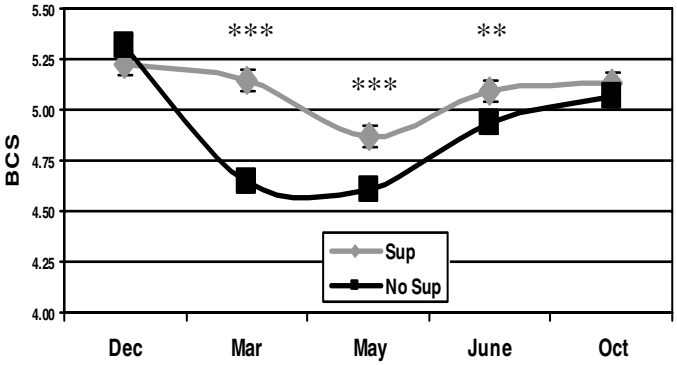

Figure 1. Effects of feeding supplement December 1 to February 28 on cow body condition score (BCS; Scale: 1 = emaciated, 9 = obese).

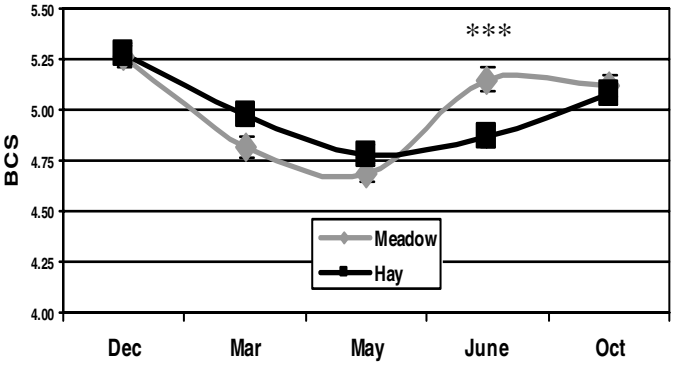

Figure 2. Effects of grazing sub-irrigated meadow or feeding grass hay from May 1 to May 30 on cow body condition score (BCS; Scale: 1 = emaciated, 9 = obese).

Table 2. Reproductive performance and milk production of cows fed 0 or 0.45 kg supplement December 1 to February 28 (prepartum) and allowed to graze sub-irrigated meadow or fed grass hay May 1 to May 31 (postpartum) and calf growth

	Supplement		No Supplement			Effect *P*-value[b]		
Item	Meadow	Hay	Meadow	Hay	SEM[a]	Sup	Mead	S X M
Pregnancy Rate, %	94.8	91.5	89.2	91.3	5.8	0.46	0.88	0.49
Calves weaned, %	95.2	99.0	90.1	89.9	3.7	0.03	0.56	0.51
Julian Calving day	87	88	84	85	2	0.01	0.16	0.80
Milk Production, kg/d	6.3	9.9	4.7	4.1	1.2	0.01	0.24	0.10
Ig G, mg/100ml[c]	3262	3068	3224	3115	600	0.98	0.47	0.84
Calf birth wt, kg	36.3	36.9	35.7	36.4	0.8	0.29	0.20	0.95
Calf wean wt, kg	222	213	213	209	7	0.02	0.01	0.27
ADG birth to wean, kg/d	0.97	0.93	0.92	0.90	0.03	0.002	0.04	0.32
Steer 205d wean wt, kg	241	232	229	230	7	0.13	0.35	0.30

[a]Pooled standard error of treatment means, n = 12 pastures per treatment.
[b]Sup = Prepartum treatment main effect; Mead = postpartum treatment main effect; S X M = prepartum X postpartum treatment interaction.
[c]Immunoglobulin G concentration in calves between 24 to 48 h after birth measured by radial immunodiffusion.

Table 3.Finishing performance and carcass characteristics of steer calves born to cows fed 0 or 0.45 kg supplement December 1 to February 28 (prepartum) and allowed to graze sub-irrigated meadow or fed grass hay May 1 to May 31(postpartum)

	Supplement		No Supplement			Effect *P*-value[b]		
Item	Meadow	Hay	Meadow	Hay	SEM[a]	Sup	Mead	S X M
Finishing period (222 d)								
Start BW, kg	216	205	205	198	5	0.05	0.06	0.63
ADG, kg/d	1.57	1.57	1.53	1.56	0.03	0.32	0.62	0.53
DMI, kg/d	8.48	8.37	8.36	8.32	0.19	0.68	0.69	0.86
Life ADG, kg/d[c]	1.22	1.20	1.16	1.17	0.02	0.03	0.85	0.49
Carcass data								
HCW, kg	371	365	354	359	6	0.07	0.91	0.41
430-d Adj. HCW, kg[d]	370	368	352	358	5	0.02	0.76	0.47
Dressing, %	65.9	66.3	65.4	66.2	0.6	0.14	0.01	0.32
Marbling score[e]	484	481	470	486	14	0.74	0.61	0.49
Longissimus muscle area, cm^2	88.2	86.9	85.7	85.5	2.0	0.13	0.57	0.66
Choice, %	91.3	88.5	92.3	89.6	5.8	0.84	0.59	0.98
Yield Grade	2.90	3.04	2.89	3.02	0.16	0.87	0.29	0.97
Fat thickness at12th rib, cm	1.23	1.34	1.22	1.29	0.13	0.73	0.29	0.79

[a]Pooled standard error of treatment means, n = 8 pens per treatment.
[b]Sup = Prepartum treatment main effect; Mead = postpartum treatment main effect; S X M = prepartum X postpartum treatment interaction.
[c]Average daily gain from birth to shrunk live weight at slaughter.
[d]Hot carcass weight adjusted to a common age by the equation: 430-d Adj. HCW = ((HCW – birth weight)/age at slaughter)·430 + birth weight; mean age of steers at slaughter was 430 d.
[e]Marbling score: 400 = Small[00], 500 = Modest[00].

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COMPARATIVE HEPATOTOXICITY OF METABOLITES ASSOCIATED WITH SNAKEWEED (*GUTIERREZIA* spp.)

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ABSTRACT: Snakeweed (SW) is a noxious range plant toxic to livestock. The objective was to evaluate hepatotoxicity of serum from 7 wethers (76 kg ± 1.03 BW) consuming SW for 7 d on liver slices of rats. Treatments were arranged as a 2 × 2 factorial; serum was collected from hepatic vs. hepatic portal veins of wethers fed SW (30% SW, 69% fescue hay,1% molasses) or a control diet (99% fescue hay, 1% molasses). Serum collected from hepatic and jugular veins on d 6 and 7 was analyzed for metabolic profiles. Liver tissue slices of female Sprague Dawley rats (475 to 500 g) were generated from a Krumdieck Tissue Slicer, plated on titanium mesh screens for 0, 3, 6, and 12 h in sterile six-well tissue culture plates in a 37˚C rotating incubator exposed to a 95% air and 5% CO_2 gas phase. Lactate dehydrogenase (LDH) leakage was measured to evaluate cell death, and cell viability was estimated by potassium and protein content of liver tissue. Serum collected from hepatic or hepatic portal veins of SW and control fed animals were similar ($P > 0.10$) for LDH. Serum components were also similar, except for serum blood urea nitrogen (BUN), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). Serum BUN was higher ($P = 0.05$) for SW fed wethers than control fed wethers. A treatment by vein interaction was observed for serum ALP ($P < 0.01$) and AST ($P = 0.05$). Serum ALP was elevated in hepatic portal veins of SW fed animals ($P < 0.05$). Serum AST levels collected from hepatic portal vein of control fed animals were higher ($P < 0.05$) than SW fed animals. Metabolic constituents of serum collected from wethers on d 5 and 6 were similar between control and SW fed animals ($P > 0.10$) as were constituents between hepatic and hepatic portal veins ($P > 0.05$). Altered BUN, AST, and ALP serum concentrations indicate gastrointestinal, kidney and liver damage in wethers. Finally, serum obtained from hepatic and hepatic portal veins affect rat liver function similarly.

Keywords: Snakeweed, Liver Toxicity, Wethers

Introduction

Snakeweed (SW; *Gutierrezia* spp.) is an unpalatable, noxious weed which may dominate rangelands and retard growth of palatable forage during periods of drought, leaving animals no choice but to consume the plant. Snakeweeds contain a variety of compounds toxic to animals with saponins suspected as the primary toxicant (Shaver et al., 1964; Gardner et al., 1999). Because the liver is the major organ to encounter and metabolize a diverse variety of compounds absorbed from the diet of animals (Fisher et al., 1991), the use of an *in vitro* method to determine the hepatotoxicity of hepatic and hepatic portal vein serum collected from SW fed animals allows for controlled environmental conditions and comparisons of treatment effects on liver preparations from the same animal (Smith et al., 1988).

The objectives of these experiments were to determine if SW at various stages of ruminant digestion/metabolism were affecting liver function utilizing an *in vitro* method for evaluation of toxicity, and to develop a biological assay for the measure of SW hepatotoxicity.

Materials and Methods

Samples of SW foliage, pre-bloom stage, were harvested via hand-clipping of the distal 5 to 10 cm of new plant growth. Samples were collected at the Chihuahuan Desert Range Research Center (CDRRC) located 37 km north of Las Cruces, NM in July 2003. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC).

Seven Rambouillet wethers (76 kg ± 1.03 BW) were surgically catheterized in hepatic and hepatic portal veins. Four wethers consumed a diet of 30% SW/69% Fescue Hay/1% molasses and three wethers consumed a diet of 99% Fescue Hay/1% molasses for 7 d. Crude protein, NDF and ADF for SW and fescue hays were 12.33, 12.34; 35.43, 57.20; and 21.65, 28.79%, respectively.

Blood samples were collected from the hepatic and hepatic portal catheters at 1000 and 1800 on last 2 d of 7 d period, and serum was harvested by centrifugation (4˚C) at 1,500 x g for 25 min and then stored at -20˚C. Because most hepatic catheters lost patency, blood samples also were collected from jugular vein at 1000 and 1800 on last 2 d of 7 d period and processed and stored as described above. Blood samples collected at 1000 and 1800 h were pooled (3 mL from each sample) for each vein of each animal and mixed in William's Media E at

100 µL/mL. A control of William's Media E was also created. (Serum was heat inactivated at 56˚C for 30 min prior to mixing with William's Media E). Serum samples collected from the hepatic portal and jugular veins of wethers at 1800 on d 7 were analyzed for complete metabolic profiles (TriCore Laboratories, Albuquerque, NM).

Serum collected from wethers was cultured for use in biological assay. Nine adult female Sprague-Dawley rats (475-500 g) were used. Rats were euthanized by carbon dioxide inhalation and livers excised and placed in cold Krebs-Ringer Bicarbonate Buffer. Slicing and incubation procedures were conducted as described in Ashley (2001). Liver slices from rats were randomly assigned to four treatments resulting in a particular rat liver having hepatic and portal as well as control and SW samples represented. Treatments were made as described in Hernandez, (2004) and 2.5 mL were aliquoted into each well. Tissue samples were assayed for protein content, intracellular potassium content, and lactate dehydrogenase (LDH) leakage and analysis was conducted as described in Hernandez, (2004). All cells were found viable as indicated by potassium and protein content for all experiments.

Data were analyzed by repeated measures using PROC MIXED (SAS Inst. Inc., Cary, NC), as a 2 x 2 factorial arrangement of vessel by diet (fixed effects) with sheep (random effect). The covariance structure used was compound symmetry. Complete metabolic profiles were analyzed for serum collected on d 7 of trial using PROC GLM of SAS (SAS Inst. Inc., Cary, NC).

Results and Discussion

No interactions were present within complete metabolic profiles with a few exceptions. Also, no treatment or vein effects were present, also with a few exceptions.

Serum total proteins showed no treatment by vein interaction ($P = 0.69$) and no effect of vein ($P = 0.79$). However, SW fed animals had reduced levels ($P = 0.05$) of serum total protein (g/dL) in comparison to control fed animals (5.96 and 7.18 ± 0.4, respectively). Because CP levels were almost identical between the control and SW diets, this difference is more likely due to liver dysfunction as opposed to dietary deficiency.

No difference was observed in serum BUN between veins ($P = 0.46$), although a treatment effect was evident ($P = 0.05$), with levels being higher in SW fed animals than controls (12.25 and 8.42 ± 1.2 mg/dL, respectively). Elevation of BUN levels in serum typically indicates a catabolic state and possibly kidney dysfunction. Because protein levels were similar between the two diets, elevated BUN levels are most likely an indication of kidney dysfunction (Oetting et al., 1990; Edrington et al., 1993).

Serum alkaline phosphatase (ALP) analysis demonstrated a treatment by vein interaction ($P = 0.002$). Elevated ALP indicates cholestasis, but in this case, levels were relatively low. Serum collected from hepatic portal vein of SW fed animals had elevated ALP in comparison to control fed animals ($P < 0.05$; Table 1). No differences in serum ALP occurred in serum collected from jugular vein of SW and control fed animals ($P > 0.10$). Elevated ALP levels in jugular serum were also seen in Oetting et al. (1990) and Edrington et al. (1993). Hepatic portal veins of SW fed wethers had higher ALP levels than control fed wethers (Table 1; $P < 0.05$). The higher concentrations of ALP evident in the hepatic portal veins of SW fed animals is because toxic compounds in SW have not been metabolized by the liver, suggesting damage to the GI tract.

A treatment by vein interaction was also apparent for serum aspartate aminotransferase (AST) levels ($P = 0.05$). No differences between serum AST concentrations occurred between SW and control fed animals for serum collected from jugular veins. Levels of AST from the hepatic portal veins of control fed animals were elevated when compared to SW fed animals ($P < 0.05$; Table 2) which was driven by one animal. This animal possibly had an infection in liver as a result of catheterization surgery and general anesthetic procedures (Brown et al., 1993).

At h 3, no treatment by vessel interaction occurred ($P = 0.88$; Table 2) for LDH leakage and main effects of vessel ($P = 0.69$) and treatment ($P = 0.18$) also resulted in no differences. This indicates that all treatments exhibited similar cell death at this time. Similar results occurred at h 6 and 12 (Table 2).

Implications

No differences in cell death (LDH leakage) of rat liver slices treated with serum collected from control or SW fed animals or in the serum collected from hepatic and hepatic portal veins was evident. It is possible that this occurred because not enough hepatic portal veins were sampled, that serum did not contain toxic components, or that the measure of incorrect indices for cell death in liver slices occurred. It was apparent that SW ingestion caused liver, GI tract, and possibly kidney dysfunction indicated by the elevated levels of serum ALP in hepatic portal veins and elevation of serum BUN in SW fed animals.

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Table 1. Least Square Means of Alkaline Phosphatase (ALK-P;U/L) and Aspartate Aminotransferase (AST; U/L) of Serum Collected on d 6 from Jugular and Hepatic Portal Veins of Wethers Fed Snakeweed and Fescue Hay, a control diet[2].

	Diet		
Vein	Control	Snakeweed	SE[1]
ALK-P			
Jugular	41.7[a]	70.0[a]	19.2
Hepatic Portal	66.0[a]	113.5[b]	10.5
AST			
Jugular	77.8[a]	82.4[a]	28.9
Hepatic Portal	157.7[a]	57.0[b]	33.6

[1]Most conservative standard error reported (n = 3). A treatment by vessel interaction was noted, therefore, data were analyzed within vein.
[2]ALP and AST were determined by TriCore Laboratories, Albuquerque, NM.
[a,b]Row means with different superscripts differ (P < 0.05).

Table 2. Least Square Means of Lactate Dehydrogenase (LDH) Leakage (U LDH/g tissue) of Rat Liver Slices Incubated in Serum Collected from Hepatic and Hepatic Portal Veins of Wethers Fed Snakeweed and Fescue Hay, a control diet on d 5 and 6 at Hours 3, 6, and 12[1]

	Diet[a]		Vein[a]	
	Fescue Hay	Snakeweed	Hepatic	Portal
Profile				
Hour 3	89.9 (25.2)	100.8 (30.2)	94.8 (27.9)	98.3 (30.1)
Hour 6	111.8 (69.8)	129.9 (40.1)	135.5 (60.7)	94.6 (26.3)
Hour 12	134.5 (69.1)	178.5 (110.6)	177.5 (111.8)	121.9 (26.0)

[1]Serum from hepatic and hepatic portal veins of control and SW fed animals was cultured in William's Media E at the 100 µL/mL level. A control was made of William's Media E.
[a]Vessel by treatment interaction did not occur, therefore main effect means were reported (P = 0.88); No treatment effect occurred (P = 0.18); No vessel effect (P = 0.69) occurred. Variances were not equal.
[b](SE)=standard error.

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EFFECTS OF IMPLANT PROGRAMS ON SUBCUTANEOUS AND ABDOMINAL FATTY ACID PROFILES AND SUBCUTANEOUS ADIPOSE LIPOGENIC GENE EXPRESSION IN LONG-FED HOLSTEIN STEERS

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ABSTRACT: Study objectives were to evaluate implant programs effects on adipose gene expression and fatty acid (FA) profiles. Nineteen Holstein steers (initial BW = 182 kg) were randomly assigned to one of four implant treatment regimens including control (no implant; n = 5); 36 mg zeranol (Ralgro) on d 0, 20 mg estradiol benzoate plus 200 mg progesterone (Synovex-S) on d 84 and 168 (RSS; n = 5); Ralgro on d 0, Synovex-S on d 84, and 28 mg estradiol benzoate plus 200 mg trenbolone acetate (Synovex-Plus) on d 168 (RSP; n = 5); and Ralgro on d 0, Synovex-Plus on d 84 and 168 (RPP; n = 4). Steers were individually fed an 86% concentrate steam-flaked corn based diet for 259 d. Subcutaneous adipose biopsies were collected before implants were administered on d –14, and 13 d after the second implant on d 97, 14 d after the third implant on d 182, and at slaughter on d 259. Abdominal fat samples were collected at slaughter for FA analysis. Real Time PCR analysis of s.c. adipose mRNA concentrations for acetyl CoA carboxylase (ACC) and fatty acid synthetase (FAS) indicates implants decreased both ACC and FAS expression ($P < 0.01$) on d 97 compared with controls. Expression of ACC was increased for RPP on d 182 ($P < 0.03$) compared with RSP and increased for the average of RSP and RPP on d 259 ($P < 0.02$) compared with RSS; whereas FAS expression was not affected ($P > 0.16$) during these sampling points. Analysis of FA showed no treatment by tissue interaction for any fatty acid ($P > 0.14$). In addition, no treatment effect on FA profile was observed, however, tissues differed in FA profile ($P < 0.01$). Initial combination implants decreased s.c ACC and FAS gene expression, whereas additional TBA implants increased ACC expression. However, FAS expression and FA profiles of abdominal and s.c. were not affected by treatment. Our results suggest that potential decreases in quality grade as a result of implanting cattle are not related to altered lipogenic gene expression in long-fed Holstein steers.

Keywords: ACC, FAS, Holstein, Implants, Fatty Acid Profiles

Introduction

Anabolic implants are the most effective non-nutritional management tool beef producers have today to improve production. It has been widely accepted in the beef industry that implants improve live performance by increasing ADG, enhancing efficiency, or by improving both simultaneously. However, the use of anabolic implants has caused packing industry concerns about unfavorable effects on carcass quality. The most emphasized detrimental effect is a decrease in quality grade due to decreased marbling and increased maturity scores (Platter et al., 2003)

Implants may directly reduce adipocyte lipogenic gene expression and thereby decrease marbling. Acetyl CoA carboxylase (ACC) and fatty acid synthetase (FAS) are the two main enzymes responsible for fatty acid synthesis. Possible decreases in expression would cause a decrease in de novo fatty acid synthesis and may decrease intramuscular adipose deposition. Estrogen, progesterone, and androgen receptors have been identified in adipocytes from several species and could alter the transcription of several lipogenic genes (Mayes and Watson, 2004). Therefore, implants containing these steroid hormones may have direct genomic regulation on fatty acid synthesis.

Study objectives were to evaluate aggressive implant programs to elucidate the effects of anabolic implants on the expression of ACC and FAS and illuminate the differences caused by implant program on fatty acid profiles of subcutaneous and abdominal adipose depots. Our hypothesis was that more aggressive implant programs would have a profound effect on marbling and carcass adipose depots while simultaneously decreasing ACC and FAS gene expression, and thus, change the fatty acid profile of subcutaneous and abdominal adipose depots.

Material And Methods

Cattle Management. Nineteen Holstein steers (190 ± 22 kg) allowed a 14-d adaptation period and worked up to an 86% concentrate diet (67.5% steam-flaked corn, 14% sudan hay, 6.25% soybean meal, 5% molasses, 4% tallow, 0.75% urea, 2.5% finishing supplement; 87.7% DM, 15.42% CP, 7.91% ADF, 14.0% Ash). After the 14 d adaptation period, steers were processed as described previously (Cheatham et al., 2004). Steers were randomly assigned to one of four treatments including: 1) no implant (CON, n = 5); 2) implanted with 36 mg of zeranol (Ralgro; Schering-Plough Animal Health) on d 0 followed by an implant of 20 mg of estradiol benzoate + 200 mg of progesterone (Synovex-S; Ft. Dodge Animal Health) d 84 and 168 (RSS, n = 5); 3) implanted with Ralgro on d 0 followed by an implant of Synovex-S on d 84 and 28 mg estradiol benzoate + 100 mg trenbolone acetate (Synovex Plus; Ft. Dodge Animal Health) on d 168 (RSP, n = 5); 4) an implant with Ralgro on d 0 followed by an implant with Synovex-Plus on d 84 and 168 (RPP, n = 5). Prior to use and between each animal the implant gun was sterilized by immersion in an anti-microbial disinfectant. After each implant, the ear was inspected to make sure all pellets were inserted and securely in place.

Steers were randomly allocated to individual, partially

shaded, soil-surfaced pens (2.5 x 6 m) with an individual water source and feed bunk. Steers were fed once daily between 0600 and 0700.

Ort samples were collected once/wk and individually weighed and recorded. Ort samples were then pooled and sampled. Feed and ort samples were subjected to DM analysis (oven drying at 55°C until no further weight loss), and feed samples were ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1 mm screen and subjected to CP (% N x 6.25; LECO Corporation, St. Joseph, MI 49085), ADF (ANKOM Technol. Corp., Fairport, NY) and ash (combusted 6-h in muffle furnace at 500°C).

On d –14, 97, and 182, adipose biopsy samples were collected before the morning feeding. Adipose tissue was collected from the area cranial and superior to the hipbone. The area was shaved, antiseptically prepared, and lidocaine hydrocholoride injectable 2% (Vedco Inc., St. Joseph, MO) was administered throughout the biopsy site. Once the area was thoroughly numb, a 3.8 cm incision was made to a depth where adipose tissue was visible. Approximately 1.5 g of adipose tissue was excised and flash frozen in liquid nitrogen. Samples were kept in liquid nitrogen until transported to the University of Arizona were they were stored at –80°C for later analysis. On d 259 steers were humanely slaughtered via exsanguination at the University of Arizona Meat Laboratory (Tucson) and adipose samples were collected.

Fat Extraction. Fat was extracted using a modified Folch method (Folch et al., 1957). Briefly, a 2:1 chloroform:methanol solution was added to tissue (2 g), vortexed for 5 min and centrifuged (400 x g). Supernatant was filtered through a Buchner funnel using a #1 Whattman paper. To filtrate, 0.58% NaCl solution was added, mixed, re-centrifuged (400 x g) and the top layer was removed and discarded. The lower layer was dried under N until less than 8 mL remained, then transferred to a pre-weighed extraction tube and dried down completely.

Fatty Acid Analysis. Fatty acid methyl esters were prepared by the transmethylation procedure described by Christie (1982) with modifications (Chouinard et al., 1999). Briefly, hexane (2 mL, HPLC grade) was added to 40 mg of lipid followed by 40 µL of methyl acetate. After vortexing, 40 µL of methylation reagent (1.75 mL methanol and 0.4 mL of 5.4 mol/L sodium methylate) was added, the mixture was re-vortexed and then allowed to react for 10 min; then 60 µL termination reagent (1 g oxalic acid in 30 mL diethyl ether) and approximately 200 mg of calcium chloride was added and allowed to stand for 60 min. Samples were centrifuged at 2,400 x g at 4°C for 5 min. Following centrifugation, the liquid portion was transferred to a labeled GC vial and stored at –20°C. Fatty acid methyl esters were quantified using a gas chromatograph (Hewlett Packard GC system 6890; Wilmington, DE) equipped with a flame ionization detector and a CP-7489 fused silica capillary column (100 m ± 0.25 mm i.d. with 0.2-mm film thickness; Varian, Walnut Creek, CA). Initial oven temperature (160°C) was held for 42 min then ramped at 5°C/min to 190°C, where it was held for 27 min. Inlet and detector temperatures were maintained at 250°C and the split ratio was 100:1. Hydrogen carrier gas flow rate through the column was 1 mL/min. Hydrogen flow to the detector was 30 mL/min, airflow was 400 mL/min and the nitrogen make-up gas flow was 25 mL/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards (GLC60, GLC68, cis-9, trans-11 and trans-10, cis-12 CLA; Nuchek Prep, Elysian, MN).

Laboratory Analysis of Gene Expression. Adipose tissue (0.5 g; as-is basis) was homogenized (Polytron, Brinkmann Instruments, Inc., Westbury, NY) and total RNA was extracted with TRIzol (Invitrogen; Carlsbad, CA) according to manufacture's guidelines. A chloroform extraction was then performed followed by an isopropyl / 0.8 *M* Na_3 Citrate + 1.2 *M* NaCl precipitation and an ethanol wash. Pelleted RNA was resuspended in 50 µL DEPC H_2O. Sample concentration was analyzed by photospectrometry using a 1:100 dilution. Total RNA was normalized to 0.5-µg concentration and DNase treated (DNase I – Amplification grade, Invitrogen) and cDNA created using Super Script III First-Stand Synthesis System for RT-PCR (Invitrogen) according to product guidelines. Real Time primers were created using published sequences and selected based on visualization on a 1.5% agarose gel (Table 1). Products for PCR were sequenced to ensure primer specificity.

Samples were randomly allocated to 96 well plates and run in triplicate alongside triplicate standard curves of the gene of interest and duplicate standard curves of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A 96 Well Optical Reaction Plate with Barcode (Applied Biosystems, Foster City, CA), BioRad iCycler iQ Optical Tape (Bio-Rad Laboratories, Hercules, CA), and iTaq SYBR Green Supermix with Rox (Bio-Rad Laboratories) were used on every plate to insure continuity.

Genomic Analysis and Technology Core facilities (University of Arizona) were used to run Real Time programs on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Data was visualized and interpreted using ABI PRISM 7000 Sequence Detection System Software (Applied Biosystems). Real Time PCR conditions for GAPDH and FAS were 1 cycle at 95°C for 10 min for denaturation followed by 40 cycles of 95°C for 15 sec and 58°C for one min for annealing. Acetyl CoA carboxylase conditions were identical except for an annealing temperature of 60°C. Disassociation curves were run on every plate to insure single product amplification per primer set. A sample volume of 20 µL was identical for each sample well on every plate.

After each run primer efficiencies were calculated using the equation: Efficiency = $1 - 10^{(-1/\text{ slope of the standard curve})}$ Average of all triplicates across all plates resulted in GAPDH, ACC, and FAS efficiencies of 98%, 97%, and 98%, respectively. Background baselines cycles were 6 and 15 for all plates. Standard deviation and CV values were calculated for cycle threshold values. If an outlier in the triplicate was present, it was removed and duplicate values were used. All values had a CV of lower than 1.0 %. Values for GAPDH were statically analyzed to ensure no difference

due to treatment, thus ensuring a proper choice for an endogenous reference gene. Delta cT values were used for analysis and obtained by the equation:
Δ cT = cT (target gene) – cT (endogenous reference gene)

Statistical Analysis. Gene expression data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for repeated measures. The model included effects for treatment, sampling date and treatment by sampling date interaction as fixed effects. Animal was used as the random effect and sampling date as the repeated factor. Auto regressive of order one was used for covariance structure. Treatment x sampling date interactions were observed for both ACC ($P < 0.02$) and FAS ($P < 0.08$) and data were analyzed within sampling date using the MIXED procedure of SAS. In addition, a difference ($P < 0.09$) was observed in ACC data for d –14 between controls and implanted cattle. Therefore, d -14 was used as a covariant within sampling date. For continuity, FAS was analyzed similarly with d –14 as a covariant. Within sampling date, the model included effects for treatment and covariance. Preplanned contrasts included 1) control vs. the average for implants, 2) RSS vs. the average of RSP and RPP, and 3) RSP vs. RPP. Individual FA data were analyzed using the MIXED procedure of SAS with a model including treatment, tissue (s.c. vs. abdominal) and treatment by tissue interaction. Steer was used as the random effect.

Results

Acetyl CoA carboxylase and FAS data are presented in Table 2 and numerical increases in ΔcT values convey a decrease in gene expression. Subcutaneous ACC and FAS gene expression were decreased by anabolic implants at d 97 ($P < 0.01$) when compared with non-implanted steers. Acetyl CoA carboxylase expression increased on d 182 for RPP compared with RSP ($P < 0.02$) and also increased on d 259 for the average of RSP and RPP compared with RSS ($P < 0.03$). During these same time points FAS did not differ ($P > 0.16$). Fatty acid profiles for subcutaneous and abdominal adipose depots did not exhibit a treatment by tissue effect ($P > 0.14$), or treatment effects, however, sites did markedly differ by fatty acid profiles ($P < 0.01$; data not shown).

Discussion

Live performance and carcass characteristics data was previously described (Cheatham et al., 2004). Briefly, no differences were observed for marbling score ($P > 0.18$), as a result of implanting or implant strategy and thus no difference was observed in quality grade (16 of 19 steers graded Choice or better). Hot carcass weight ($P < 0.01$) and LM area ($P < 0.01$) were increased in implanted steers compared with controls, but no differences ($P > 0.57$) were observed as a result of implant strategy.

Acetyl-CoA carboxylase (Kim, 1997) and FAS (Smith, 1994) are two enzymes responsible for de novo fatty acid synthesis. The production of malonyl-CoA from acetyl-CoA is catalyzed by ACC and this is the rate-limiting enzyme in the fatty acid synthesis pathway. Fatty acid synthetase is a large complex enzyme, which is responsible for directing a thirty-seven step sequential reaction leading to the production of palmitic acid from malonyl-CoA and acetyl-CoA subunits (Smith, 1994).

Changes in ACC and FAS gene expression could alter de novo lipogensis and modify adipose tissue deposition. Researchers have identified adipocyte receptors for estrogen, progesterone, and androgens in several species (Mayes and Watson, 2004). Moreover, fatty acid biosynthesis is primarily regulated at the genomic level (Kim et. al., 1994). Since anabolic implants are composed steroidal hormones, they could have direct effects on genomic expression of ACC and FAS and may explain why implanted animals are generally leaner than controls.

Decreases in subcutaneous ACC and FAS mRNA concentrations on d 97 of implanted steers compared with non-implanted control steers demonstrated an initial reaction of lipogenic genes to hormonal implants. However, these changes were not noted at later time points in the study. This relates well with our carcass data of implant program not having an effect on marbling, KPH or adjusted fat thickness (Cheatham et al., 2004). However, Bruns et al. (2005) data suggested i.m. adipose deposition is sensitive to implants given early in the growing period. These authors reported implanting early decreased i.m. fat content in the LM and decreased marbling scores compared with delayed implanting. Differences in carcass characteristics between our study and Burns et al (2005) may be related to the amount of time on feed. Steers in our study were on feed for 259 d vs. 140 d for cattle fed by Burns et al. (2005). Overall, it is theorized that implanted cattle in our study deposited more muscle and reached physiological maturity later than control steers. However, steers were allowed enough time on feed to deposit sufficient adipose deposition prior to harvest to allow for adequate marbling.

Expression of ACC was increased for RPP on d 182 compared to RSP and increased for the average of RSP and RPP on d 259. This is surprising since trenbolone acetate was hypothesized to decrease lipogenic gene expression. Reasons for discrepancies are not known.

Fatty acid profiles were not affected by treatment ($P > 0.14$). Accordingly, if ACC and FAS were not affected one could extrapolate that de novo synthesis and possibly Δ -9-destaturase activity was not affected and that the majority fatty acids being deposited came mainly from the diet. Differences in fatty acid profiles by site were not a surprise since previous research as shown that variations do exist between profiles of various adipose tissues (Leat, 1978).

Fatty acid profiles of subcutaneous and abdominal adipose depots were not affected by implant treatment, however profiles did markedly differ by site. Gene expression of subcutaneous adipose ACC and FAS were affected by anabolic implants at d 97. Trenbolone acetate implantation caused differences in ACC expression on d 182 for RPP compared to RSP and again for the average of RPP and RSP compared to RSS on d 259.

Implications

These results indicate that lipogenic gene expression is affected by anabolic implants particularly early in the feeding period. Trial data suggests that implanted steers fed to physiological maturity can grade equally as well as non-implanted steers and that implants have little effect on

subcutaneous gene expression or fatty acid profiles. However, when differences in quality grade are noted, as in other studies and in antidotal data form industry, it maybe related to the change in subcutaneous lipogenic genes early in the feeding period.

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Table 1. Real time primer sequences

Primer	Sequence (5'-3')	CDS	Length	EMBL ac. No.
ACCf	GGGAAGGAAGAAGGACTTGG	5699 –5720	166	NM174224 (bovine)
ACCr	CGACCTGGATGGTTCTCTGT	5839 - 5864		
GAPDHf	CATTGACCTTCACTACATGGT	164 - 184	236	AB098985 (bovine)
GAPDHr	ACCCTTCAAGTGAGCCCCAG	381 – 400		
FASf	GGAGGACGCTTTCCGTTACA	15480 - 15499	52	AF285607 (bovine)
FASr	TGACCACTTTGCCGATGTGT	15511 - 15532		

f = forward, r = reverse, CDS = coding sequence, Length = product length, EMBL ac. no. (species in brackets) of the used published nucleic acid sequences

Table 2: Effect of implant program on acetyl CoA carboxylase (ACC) and fatty acid synthetase (FAS) gene expression in subcutaneous adipose tissue

Item/	**Treatment[a]**				**SEM**	**Contrast[b]**		
Biopsy date	**CON**	**RSS**	**RSP**	**RPP**		**1**	**2**	**3**
ACC								
d 97	2.06	4.48	3.30	4.25	0.56	< 0.01	0.27	0.28
d 182	3.20	3.26	5.96	2.35	0.96	0.56	0.42	0.02
d 259	3.62	8.74	4.50	2.91	1.67	0.34	0.03	0.46
FAS								
d 97	0.30	3.97	3.53	4.35	0.97	< 0.01	0.98	0.58
d 182	3.17	2.73	5.06	3.40	1.72	0.75	0.43	0.49
d 259	5.34	6.67	2.38	5.06	1.73	0.67	0.16	0.18

[a]CON = no implant; RSS = Ralgro on d 0, Synovex-S on d 84 and Synovex-S on d 168; RSP = Ralgro on d 0, Synovex-S on d 84 and Synovex-Plus on d 168; RPP = Ralgro on d 0, Synovex-Plus on d 84 and Synovex-Plus on d 168.

[b]Contrast 1 = CON vs. implants; 2 = RSS vs. the average of RSP and RPP; and 3 = RSP vs. RPP.

Expression values based on the equation: ΔcT = cT (target gene) – cT (endogenous reference gene). Numerical increases convey a decrease in gene expression.

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SUPPLEMENTAL METHIONINE AND UTERINE INFECTION ALTERS ESSENTIAL AMINO ACID METABOLISM IN NULLIPAROUS EWES

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ABSTRACT: Nulliparous yearling Rambouillet-cross ewes (n = 21; BW = 61.8 ± 2.7 kg), fitted with chronic indwelling hepatic, portal, and mesenteric vein and mesenteric artery catheters, were used to determine the effects of rumen-protected Met (Mepron M85, Degussa) and uterine infection on splanchnic AA metabolism. Treatments, in a 2 x 2 factorial arrangement, were 1) no Met + intrauterine infusion of saline, 2) no Met + intrauterine infusion of bacteria (9.69 x 10^{10} cfu of *Escherichia coli* and 2.76 x 10^{11} cfu of *Arcanobacterium pyogenes*), 3) 2.4 g/d rumen-protected Met + intrauterine saline, and 4) 2.4 g/d rumen-protected Met + intrauterine bacteria. Methionine treatments were bolused twice daily at feeding (1.3 kg DM/d sugar beet pulp pellets). Intrauterine infusions were initiated 12 h before sampling. Arterial, portal, and hepatic blood samples were simultaneously collected at 0, 4, and 8 h after treatment. Continuous para-aminohippurate (3% wt/vol; 0.57 mL/min; mesenteric vein) infusion was used to estimate vessel plasma flows. No Met × infection (P = 0.14 to 0.98) interactions were observed for essential AA concentrations and fluxes. Infection decreased (P < 0.05) arterial concentrations of Thr and His, and Met treatment decreased (P < 0.04) hepatic-arterial concentration differences of Leu, Ile, Val, and Thr. Treatments did not affect portal and hepatic plasma flows (P = 0.52 to 0.59). Methionine treatment increased (P < 0.06) net hepatic uptake of Met, Leu, Thr, and Phe and increased (P < 0.06) total splanchnic uptake of Met, Leu, Ile, Val, Thr, and Phe, compared with non-Met treatment. The Met × h interaction (P = 0.03) for hepatic flux of Ile was due to an increased net hepatic uptake of Ile for Met treated vs. untreated ewes at h 8. In summary, rumen-protected Met increased the splanchnic uptake of Met, Leu, Ile, Val, Thr, and Phe, and infection decreased arterial concentrations of Thr and His.

Key Words: Essential AA, Splanchnic flux, Ewes

Introduction

Disease results in a catabolic state in which nutrients are partitioned away from tissue deposition and toward support for immune function (Spurlock, 1997). In pigs, chronic lung inflammation decreased plasma concentrations of Trp, Gln, Pro, Gly, Tyr, and total AA (Melchior et al., 2004) indicating an increased requirement of AA. Reeds and Jahoor (2001) suggest an immune response to infection requires specific AA, such as Gln, Arg, aromatic AA, and sulfur AA. Methionine is often the most limiting AA for sheep (Storm and Ørskov, 1984; Nolte et al., 2004). However, limited research is available describing how acute infection alters the demand for Met and other AA. Infection may increase the utilization for essential AA and result in an increased AA requirement. Using uterine infection as a model, our objective was to determine effects of acute infection and dietary Met on the splanchnic metabolism of essential AA.

Materials and Methods

Ewes and surgery. The USDA-ARS, U. S. Sheep Experiment Station Institutional Animal Care and Use Committee reviewed and approved the animal protocols as described herein. Nulliparous yearling Rambouillet-cross ewes (n = 21; 61.8 ± 2.7 kg BW) were surgically fitted with chronic indwelling catheters in the hepatic and portal veins, a mesenteric vein and artery (Huntington et al., 1989), and into the uterine lumen (Williams et al., 1983). Vascular catheter patency was maintained with a heparinized saline solution (100 U/mL). Ewes received 10 mL Penicillin G Procaine i.p. (Agri-Cillin; 300,000 U/mL) at time of surgery and 10 mL s.c. the following morning. Ewes were adapted to a sugar beet-pulp pellet-based diet (Table 1; fed at 1.3 kg DM/d) over a 9-d period.

Experimental. Ewes were randomly assigned to a 2 × 2 factorial arrangement of treatments; factors were two levels (0 vs. 2.4 g/d) of rumen-protected Met (Mepron M85, Degussa Corporation, Kennesaw, GA) bolused twice daily at feeding, and no infection (sterile saline; 9% wt/vol NaCl solution) vs. acute uterine infection (9.69 x 10^{10} cfu of *Escherichia coli* and 2.76 x 10^{11} cfu of *Arcanobacterium pyogenes*) induced 12 h before sampling through 10-mL intrauterine infusions. The bacteria were obtained and stored until use (Seals et al., 2002). To ensure susceptibility to infection, ewes received progesterone (2.0 mg progesterone/2.5 mL canola oil i.m.) injections, twice daily at feeding, beginning 6 d before sampling and continuing throughout the sampling period.

Sampling. Beginning 1 h before sampling, a primed (15 mL) and constant infusion (0.57 mL/min; KDS 220 Multi-Syringe Pump, KD Scientific, Holliston, MA) of para-aminohippurate (**PAH**; 3% wt/vol, pH 7.4; Huntington et al., 1989) was administered into the mesenteric vein for the estimation of venous and arterial plasma flows. Simultaneous arterial, portal, and hepatic blood samples were collected (Monoject syringe, Tyco Healthcare, Mansfield, MA) before feeding (h 0) and at 4 and 8 h after feeding. Blood was transferred (6 mL) immediately to

tubes containing 12.15 mg EDTA (Vacutainer, Franklin Lakes, NJ) and to tubes with no anticoagulant (Corvac Serum Separator, Tyco Healthcare). Blood samples were centrifuged (1,500 × *g*, 30 min, 4°C) and plasma and serum harvested and stored (-20°C). Plasma arterial, portal, and hepatic PAH was determined (Huntington, 1982; Taylor, 2000). Serum AA were measured using gas chromatography (Chen et al., 2002). Arginine, Lys, and Trp were not analyzed.

Table 1. Diet composition

Item	% of DM
Ingredient	
Sugar beet-pulp pellets	99.32
Trace mineral salt [a]	0.62
Vitamin E mix [b]	0.06
Nutrient	
CP	10.68
RDP	4.87

[a] 9.5% NaCl, 0.55% Ca, 0.0007% Cu, 0.002% I, 0.07% Fe, 0.09% Mg, 0.0007% Mn, 0.05% P, 0.12% K, 0.13% S, DM basis; Redmond NTM salt, Redmond, UT.
[b] 36,287 IU Vitamin E/kg.

Calculations and statistics. Portal and hepatic vein, and arterial plasma flow rates and AA flux across the splanchnic tissues were calculated (Krehbiel et al., 1992). A positive flux indicates a net release and a negative flux indicates a net uptake of AA. Data were analyzed as repeated measures (covariance structure = autoregressive order one) using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included Met, infection, h, Met × infection, Met × h, infection × h, and Met × infection × h with ewe as the experimental unit. Data are presented as least squares means, and differences were considered significant when $P < 0.06$.

Results

No Met × infection × h interactions ($P = 0.08$ to 0.97) were observed. A rumen-protected Met × infection interaction ($P = 0.06$) for arterial plasma flow indicated that, without uterine infection, 2.4 g/d rumen-protected Met increased arterial plasma flow, compared with 0 g/d (Table 2). However, with infection, arterial plasma flow decreased when rumen-protected Met was fed (Table 2). Also, hepatic plasma flow tended ($P = 0.10$) to increase in uninfected ewes, but decreased in infected ewes in response to rumen-protected Met.

The interaction for rumen-protected Met × h was significant ($P = 0.03$) for the hepatic flux of Ile with a greater hepatic uptake occurring at h 8 in ewes receiving 2.4 g/d rumen-protected Met relative to 0 g/d (-2.342 vs. 1.582 mmol/h for 2.4 g/d vs. 0 g/d rumen-protected Met, respectively; SE = 1.00). Rumen-protected Met increased net hepatic uptake ($P < 0.06$) of Met, Leu, Thr, and Phe and increased ($P < 0.06$) net splanchnic tissue uptake of Met, Leu, Ile, Val, Thr, and Phe (Table 2). Regardless of infection status, hepatic-arterial (**H-A**) differences of Leu, Ile, Val, Thr, and Phe were more negative ($P < 0.06$) for ewes fed 2.4 g/d rumen-protected Met, compared with 0 g/d. All bacteria-treated, but no saline-treated, ewes developed infections. Acute infection decreased ($P < 0.05$) arterial concentrations of Thr and His.

The rumen-protected Met × infection interactions were not significant ($P = 0.14$ to 0.98) for arterial AA concentrations, portal-arterial (**P-A),** H-A**,** and hepatic-portal (**H-P**) AA concentration differences, and portal-drained viscera (**PDV**), hepatic, and total splanchnic fluxes of essential AA. Neither rumen-protected Met nor acute uterine infection affected ($P = 0.07$ to 0.90) arterial concentrations of Leu, Ile, Val, and Phe, or P-A, H-P and PDV flux of all essential AA measured.

Discussion

Rumen-protected Met and acute infection altered the metabolism of essential AA in ewe lambs. Specifically, rumen-protected Met resulted in a greater hepatic and splanchnic tissue uptake of Met, Leu, Thr, and Phe, and acute uterine infection decreased arterial Thr and His.

Arterial Met changed ($P = 0.05$) with time (data not shown) as previously reported (Rémond et al. 2003), in which AA concentrations were highest during a meal and lowest between meals. Based on the venous-arterial differences, rumen-protected Met-treated ewes released more essential AA from the PDV than did untreated ewes. Because of the increased supply, hepatic and total splanchnic tissues removed (negative flux), as opposed to released (positive flux), much of the essential AA. In particular, the hepatic and total splanchnic tissue removed Met, Leu, Thr, and Phe, and Met, Leu, Ile, Val, Thr, and Phe, respectively. These results are in contrast to previous findings (Bach et al., 2003), in which rumen-protected Met fed to dairy cows increased splanchnic tissue net release of Met, His, and Thr, but had no effect on hepatic AA flux. However, similar to the current study, increased metabolizable protein supply to the small intestines resulted in greater portal release and hepatic uptake of α-amino N (Krehbiel et al., 1998; Ferrell et al., 2001) and AA (Raggio et al., 2004). Furthermore, hepatic removal of essential AA, in response to AA infusions into the mesenteric vein, is greatest for Met, followed by Phe, Val, Leu, Lys, and Ile (Wray-Cahen et al., 1997).

As in the current study, plasma Thr decreased in mice undergoing septic shock (Komarov and Reddy, 1998). It was suggested that this response was because of increased gluconeogenesis commonly associated with sepsis. Although a decrease in arterial Thr was observed, Meinz et al. (1998) concluded that glycogenolysis accounts for the majority of hepatic glucose production in dogs challenged with an acute intraportal endotoxin infusion. Therefore, as with His, Thr may be used to support an inflammatory response to sepsis. For example, Thr and Ser are important components of protein kinase C which is required for macrophage activation (Clark et al., 2003). Histamine is formed from the decarboxylation of histidine in response to inflammation. Klausz et al. (2004) reported lower tumor necrosis factor-α, interlukin-6, and antibody production in histidine decarboxylase knock-out mice infected with *Helicobacter pylori*.

Implications

Increasing methionine supply in the diet through a rumen-protected source increases hepatic and splanchnic tissue uptake of methionine, leucine, threonine, and phenylalanine indicating the importance of methionine in the metabolism of essential amino acids. Acute infection may increase the threonine and histidine requirement for nulliparous ewe lambs.

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Table 2. Arterial concentration, venous-arterial differences, plasma flow, portal drained viscera (PDV) flux, hepatic flux, and total splanchnic flux of essential AA in ewes receiving rumen-protected Met and acute uterine infection.

	Treatment[a]							
	0 g/d Met		2.4 g/d Met			*P*-value[b]		
Item[c]	No Infection	Infection	No Infection	Infection	SEM	M	I	M x I
n	5	4	6	6				
Arterial, mM								
Met	0.017	0.014	0.021	0.138	0.003	0.67	0.13	0.59
Leu	0.079	0.067	0.063	0.063	0.008	0.24	0.45	0.48
Ile	0.083	0.066	0.077	0.061	0.011	0.57	0.14	0.95
Val	0.355	0.301	0.334	0.302	0.051	0.84	0.40	0.82
Thr	0.079	0.051	0.094	0.054	0.016	0.57	0.05	0.69
Phe	0.076	0.075	0.093	0.081	0.013	0.37	0.61	0.69
His	0.178	0.076	0.152	0.095	0.032	0.90	0.02	0.48
P-A difference, mM								
Met	0.004	0.005	0.004	0.005	0.002	0.86	0.81	0.98
Leu	0.015	0.009	0.008	0.010	0.004	0.37	0.66	0.32
Ile	0.016	0.013	0.014	0.021	0.008	0.70	0.75	0.50
Val	0.036	0.018	0.036	0.031	0.024	0.77	0.62	0.78
Thr	0.010	0.008	0.018	0.005	0.007	0.69	0.25	0.42
Phe	0.022	0.012	0.015	0.017	0.007	0.90	0.58	0.37
His	0.016	0.017	-0.003	0.029	0.020	0.84	0.39	0.43
H-A difference, mM								
Met	0.003	0.002	-0.003	0.000	0.002	0.07	0.74	0.35
Leu	0.017	0.008	-0.006	0.000	0.006	0.02	0.82	0.18
Ile	0.018	0.013	-0.008	0.003	0.009	0.04	0.70	0.32
Val	0.044	0.030	-0.039	-0.014	0.031	0.04	0.83	0.49
Thr	0.011	0.007	-0.015	-0.006	0.009	0.04	0.78	0.47
Phe	0.009	-0.001	-0.017	-0.007	0.009	0.06	0.98	0.23
His	-0.004	0.015	-0.051	0.004	0.027	0.25	0.14	0.46
H-P difference, mM								
Met	-0.002	-0.002	-0.005	-0.004	0.002	0.10	0.68	0.63
Leu	0.002	-0.002	-0.012	-0.006	0.006	0.11	0.89	0.35
Ile	0.006	-0.002	-0.012	-0.010	0.006	0.07	0.65	0.43
Val	0.019	-0.004	-0.043	-0.028	0.033	0.14	0.88	0.50
Thr	0.003	-0.004	-0.016	-0.006	0.008	0.12	0.80	0.20
Phe	-0.014	-0.012	-0.020	-0.020	0.008	0.33	0.86	0.86
His	-0.016	-0.003	-0.023	-0.021	0.017	0.38	0.61	0.69
Plasma flow, L/h								
Arterial	33.60	41.26	37.55	23.04	5.63	0.53	0.20	0.06
Portal	105.92	119.26	106.69	104.94	10.90	0.59	0.52	0.48
Hepatic	128.05	152.91	140.75	127.97	11.35	0.58	0.58	0.10
PDV flux, mmol/h								
Met	0.488	0.536	0.454	0.372	0.209	0.63	0.93	0.75
Leu	1.763	1.082	0.921	0.962	0.575	0.40	0.57	0.53
Ile	1.747	1.482	1.828	1.375	0.790	0.99	0.65	0.90
Val	4.163	2.009	4.728	3.010	2.996	0.79	0.52	0.94
Thr	1.211	0.868	2.307	0.337	0.946	0.76	0.23	0.39
Phe	2.443	1.358	1.611	1.885	0.865	0.86	0.64	0.43
His	1.742	1.903	-0.585	2.868	2.310	0.77	0.43	0.47
Hepatic flux, mmol/h								
Met	0.072	-0.084	-0.735	-0.438	0.240	0.02	0.74	0.30
Leu	1.065	0.183	-1.302	-0.913	0.884	0.05	0.75	0.42
Ile	1.510	0.243	-1.670	-0.945	1.310	0.08	0.81	0.38
Val	4.479	0.666	-6.776	-4.502	4.967	0.08	0.86	0.48
Thr	1.118	-0.224	-2.842	-0.910	1.229	0.05	0.78	0.15
Phe	-0.779	-1.197	-3.052	-2.671	0.991	0.06	0.98	0.65
His	-0.199	0.161	-5.338	-2.783	2.616	0.11	0.54	0.65
Total splanchnic flux, mmol/h								
Met	0.634	0.308	-0.383	-0.055	0.358	0.05	1.00	0.32
Leu	3.126	1.227	-0.797	0.008	0.966	0.01	0.53	0.14
Ile	3.411	1.891	-0.956	0.362	1.253	0.02	0.93	0.22
Val	9.090	4.321	-5.155	-2.019	4.743	0.03	0.85	0.36
Thr	2.389	0.947	-1.868	-0.764	1.634	0.06	0.91	0.39
Phe	2.062	0.015	-2.400	-0.868	1.372	0.05	0.84	0.17
His	1.210	2.056	-7.258	0.073	3.917	0.20	0.30	0.41

[a]Infection = acute uterine infection caused by inoculation of bacteria (9.69×10^{10} cfu of *Escherichia coli* and 2.76×10^{11} cfu of *Arcanobacterium pyogenes*); no infection = non-infection, sterile saline infusion.

[b]Significance level for Met (M) and infection (I) main effects, and for Met × infection (M × I) interaction.

[c]P-A = portal-arterial concentration difference; H-A = hepatic-arterial concentration difference; H-P = hepatic-portal concentration difference.

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INFLUENCE OF ENERGY SUPPLEMENTATION AND MONENSIN ON FORAGE DIGESTIBILITY AND INTAKE

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ABSTRACT: It may be desirable to reduce forage intake by grazing beef cattle during forage shortages such as drought. The effect of supplementing corn grain with or without monensin on diet digestibility and forage intake by beef cows grazing summer range was studied. The hypothesis was that including monensin in a grain-based supplement would decrease forage intake and increase diet digestibility. This should allow cows to perform adequately while conserving forage. Crossbred cows (n=36, mean BW = 573 kg) were blocked by weight and body condition into one of three treatments: control, receiving no supplement; corn supplement receiving 0.91 kg of cracked corn; and corn plus monensin, receiving 350 mg of monensin in 0.91 kg of cracked corn. Cows were fed supplement individually on a daily basis. The rumen evacuation technique was used to collect diet samples. Diet digestibility was estimated using indigestible acid detergent fiber as an internal maker. Fecal output was estimated using chromic oxide from sustained release boluses as an external marker. Forage intake was calculated from fecal output and diet digestibility. Diet and fecal sampling were conducted in 2 periods (July and September). Data were analyzed in a randomized complete block design with period as a repeated measure in the mixed procedure of SAS. Diet organic matter digestibility (OMD) and forage intake expressed as a percentage of body weight both displayed treatment by period interactions ($P < 0.001$). In period one, OMD was lower ($P < 0.001$) for corn-supplemented cows than the control or monensin supplemented cows. In period 2, OMD was different ($P < 0.001$) among all treatments, with greatest OMD for monensin-supplemented cows and least for control cows. Forage intake in period one was greatest ($P < 0.001$) for control cows and least for monensin-supplemented cows, with corn-supplemented cows intermediate and similar to both other treatments. Forage intake did not differ ($P > 0.05$) among treatments in period 2. Monensin decreased intake while maintaining digestibility when forage quality was higher in June, but was not effective at reducing intake when forage quality was lower in September

Key Words: Beef Cattle, Monensin, Forage Intake

Introduction

Restricted nutrient intake is probably the major factor that limits production by grazing animals (Hodgson, 1982). With drought, the amount of available forage is limited. The economic impact of low forage availability causes a need for grazing livestock to optimally utilize the forage that is available. Simultaneously improving feed efficiency and reducing forage intake would allow maintenance of cow performance while extending limited forage during drought.

Monensin was approved by the U.S. FDA in the mid-1970's as a feed additive (Edrington et al., 2003), and much is known about the effect of monensin on ruminal fermentation. Supplementation with grain typically decreases forage digestibility (Branine and Galyean, 1990), but use of monensin enhanced forage digestibility by cattle grazing wheat pasture (Branine and Galyean, 1990) or native, Northern Great Plains rangeland (Ward et al., 1990). Monensin has also been shown to decrease feed intake across a variety of diets (NRC, 1996).

The effects of supplemental grain and monensin on forage digestibility and intake were evaluated in beef cows grazing native range in mid-summer and early fall. It was hypothesized that monensin would increase forage digestibility and decrease forage intake.

Materials and Methods

The study was conducted at the Cedar Mountain Research Site near Cedar City in southwest UT during July and September of 2003. Crossbred, mixed aged, lactating beef cows (n = 36, mean BW = 591 kg) grazing native, high-elevation forested rangeland were assigned by weight and body condition score (on a scale of 1-9) to one of three treatment groups. Treatments consisted of a control that received no supplement, a corn supplement treatment that was fed 0.91 kg hd^{-1} d^{-1} (as-fed basis) of cracked corn, and a corn plus monensin supplement treatment that was fed 0.91 kg hd^{-1} d^{-1} (as-fed) of cracked corn with 385 mg of monensin kg^{-1} of corn. Each cow in this treatment received 350 mg of monensin d^{-1}. All animals were kept on the same pasture, but were gathered and separated individually for feeding of supplement once daily at approximately 1000.

Two experimental periods were conducted that lasted 24 d each. Period one was in July (mid-summer) and period two was in September (early fall). Each consisted of a 14-day adaptation period followed by a 10-day sampling period.

Six cows fitted with ruminal cannulas were used to collect diet samples in the evacuated rumen. These six cows were introduced to the herd of 30 intact cows and allocated so two were in each treatment group. Diet samples were collected twice per sampling period on day 15 and 24 before feeding supplements. Diet samples were frozen after collection, freeze dried, ground (1-mm screen) and composited for the two cows in each treatment group and sampling period to determine dietary DM and OM

(AOAC, 1996); crude protein by the combustion method (AOAC, 1996) using a N analyzer (Leco, St. Joseph, MI); and NDF and ADF in an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY; Table 1).

Intake was estimated from fecal output and digestibility. Digestibility was estimated using indigestible acid detergent fiber as an internal marker (Olson and Sunvold, 1991). Sustained release boluses of chromic oxide (Captec®, Auckland, NZ) were used as an external marker to estimate fecal output as described by Adams et al (1991). On day 8, boluses were inserted into each cow. Fecal grab samples were collected daily from day 15 to 24 immediately after cows consumed supplement. The fecal grab samples were stored frozen after collection. Fecal samples were oven-dried at 60° C and ground (1-mm screen). Fecal samples were prepared for chromium analysis according to Williams et al. (1962) and chromium concentration was determined by inductively coupled plasma emission spectroscopy using a Thermo Jarrell Ash Iris Advantage (Franklin, Massachusetts). Fecal samples were composited by cow to determine IADF, NDF, OM, and DM.

A randomized complete block design was used in the Mixed procedure of SAS (SAS Institute, Cary N.C.) to analyze responses of OM and NDF digestibility of the diet, total and forage intake, and digestible organic matter intake to treatments. Treatments were arranged in a 3 by 2 factorial with 3 supplementation treatments and 2 sampling periods. Cows were experimental units and were designated as random effects. Sampling periods were designated as repeated measures. Means were separated using protected LSD.

Results and Discussion

All dependent variables displayed a treatment by time interaction ($P \leq 0.05$). Organic matter digestibility (OMD) differed across treatments during both period 1 and 2, as well as across periods (Table 2). Organic matter digestibility was continuously higher in period one compared with period 2. During period one, control cows and cows that received the monensin supplement had similar dietary OMD, but OMD was lower for cows receiving the corn supplement ($P < 0.001$). Period two OMD differed ($P < 0.001$) between all treatments. Monensin-supplemented cows had the highest OMD with corn-supplemented cows intermediate and control cows had the lowest OMD. Branine and Galyean (1990) suggested increased DM disappearance was probably more attributable to monensin than to supplemental grain.

Neutral detergent fiber digestibility of the diet for control cows and cows receiving monensin in period one did not differ ($P = 0.89$, Table 2), but digestibility of NDF by corn-supplemented cows was lower than control and monensin treatments ($P = 0.0002$). In period two, all treatments differed ($P < 0.001$) in NDF digestibility. Monensin-supplemented cows had the highest NDF digestibility with corn–supplemented cows intermediate and control cows had the lowest NDF digestibility. In period one, corn depressed digestion of the fiber in the forage, displaying a typical negative-associative effect of a starch-based supplement on fiber digestion. However, monensin overcame that negative effect. Results for period two were not as straight-forward. Corn supplementation was associated with an increase in fiber digestion, which was not expected. Monensin also caused an increase in fiber digestion, which was expected.

In period one, both total ($P = 0.02$) and forage ($P = 0.01$) organic matter intake were reduced by monensin compared to the control treatment, but intake by the corn-supplemented cows was intermediate and not different ($P \geq 0.11$) from the other treatments. During period 2, total and forage OMI were similar ($P > 0.41$) across all treatments.

In period one, digestible organic matter intake did not differ ($P > 0.47$) among treatments, but in period two, cows receiving monensin had higher DOMI ($P = 0.01$) than control cows, but neither differed ($P \geq 0.11$) from the corn-supplemented cows (Table 2). The sustained DOMI and reduced forage intake in period one means forage utilization was reduced while maintaining nutrient intake. Thus, cow performance should not suffer because of lower forage intake. In period 2, digestibility was improved by monensin which lead to higher DOMI. This did not meet the goal of reducing forage intake, but indicates monensin contributes to improved use of nutrients from the forage. Other literature states that addition of monensin in the diet would have a positive effect of increased digestibility (Branine and Galyean, 1990) and reduced intake (NRC, 1996), but this study indicates that it does not always reduce forage intake. Differences in dietary intake, forage quality, supplementation of nutrients, and amount of monensin may cause variable intake responses. Branine and Galyean (1990) indicated that the level at which monensin was given had no influence on intake. However, more research evaluating the level of monensin with different feed types appears needed to more fully characterize factors influencing monensin efficacy.

Implications

The hypothesis that monensin would increase forage digestibility was partially supported by our data. In period two, it increased both OM and NDF digestibility over both other treatments. However, in period one, it did not improve forage digestion over the control, although it did overcome the depression in both OM and NDF digestion that was caused by corn supplementation. Total and forage intake in period one were lowered by monensin compared to the control, but DOMI was similar. Total and forage intake were similar between all treatments during the second period, but DOMI was increased by monensin because monensin increased the digestibility of OM. The use of monensin on this moderate to low-quality diet improved efficiency of forage utilization, either by improving digestion or decreasing forage intake. The corn only supplement treatment was not effective at improving efficiency of forage utilization because it decreased digestion without a significant influence on forage intake.

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Table 1. Nutrient concentrations in diets selected by ruminally fistulated cattle during each sampling period

Item	Period 1[a]	Period 2
DM%	93.86	95.04
	-------- % of DM --------	
OM%	92.43	91.70
	-------- % of OM --------	
NDF	75.29	78.91
ADF	44.50	50.27
CP	9.05	9.40

[a] Period 1 was July 8-31, 2003 and period 2 was September 3-27, 2003.

Table 2. Least squares mean ±SE responses of digestibility and intake to supplementation with corn grain or monensin plus corn grain

Item	Period	Control[a]	Corn	Monensin
OMD[b], %	1	53.88±0.889^{d}	47.96±0.851^{c}	52.40±0.889^{d}
	2	31.45±0.851^{c}	38.59±0.851^{d}	47.75±0.851^{e}
NDF Digestibility, %	1	58.1±1.12^{d}	51.8±1.12^{c}	57.9±1.07^{d}
	2	38.2±1.03^{c}	47.0±1.03^{d}	54.7±1.12^{e}
Total OMI, %BW	1	3.19±0.332^{d}	2.58±0.315^{cd}	2.14±0.300^{c}
	2	1.24±0.287	1.24±0.287	1.58±0.300
Forage OMI, %BW	1	3.18±0.334^{d}	2.44±0.317^{cd}	2.00±0.302^{c}
	2	1.20±0.302	1.15±0.289	1.44±0.302
DOMI, %BW	1	1.24±0.143	1.28±0.120	1.17±0.114
	2	0.40±0.109^{c}	0.56±0.109^{cd}	0.82±0.114^{d}

[a] Control received no supplement, Corn received 0.91 kg cracked corn hd^{-1} d^{-1}, and Monensin received 350 mg monensin mixed in 0.91 kg cracked corn hd^{-1} d^{-1}.

[b] OMD = organic matter digestibility, OMI = organic matter intake, DOMI = digestible organic matter intake.

[c,d,e] Within rows, means lacking a common superscript differ ($P < 0.05$).

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The effect of bloat on ingestive behavior patterns of steers grazing wheat forage.

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ABSTRACT: The effect of bloat status and time of day on grazing behavior of 6 bloated and 5 non-bloated rumen cannulated steers was examined on continuously stocked wheat forage swards maintained at 8 to 9 cm sward surface height. Temporal grazing behavior dynamics over 24 h periods were recorded automatically using jaw movement sensors to measure temporal patterns of grazing time, ruminating and idling behavior. The number of jaw movements, grazing and ruminating bouts continuously recorded and stored in an onboard data logger. Overall, total grazing time and ruminating time were similar between bloated and non-bloated steers. In contrast, idling time was greater for bloated ($P < 0.001$) than for non-bloated steers (10.9 vs. 7.9 h/24h). During the 24 h grazing periods, total jaw movements were lower ($P < 0.01$) for bloated than for non-bloated steers (52653 vs. 65872 jaw movements), but was no difference in ruminating bouts (267 vs. 313 bouts). Grazing bouts tended to be greater ($P = 0.15$) for non-bloated than bloated steers. Grazing patterns of non-bloated steers showed a quadratic effect ($P < 0.05$) of with time of day (Fig. 1a). Grazing time decreased during three periods (1 to 6 am, 9 to 10 am, and 7 to 9 pm,). Grazing activity increased from 6 to 7 am with maximum grazing activity between 2 to 5 pm, and intermediated activity levels between 10 am to 12 pm. Similar grazing behavior patterns were displayed by bloated steers. The incidence bloat x grazing time interactions were significant ($P < 0.01$) for grazing activity, suggesting that grazing time decreased with bloat incidence between 8 to 10 pm, and 6 to 8 am, but grazing activity increased sharply between 10 and 11 pm. Ruminating activity (Fig. 1b) was similar to and followed grazing activity periods. Ruminating activity in non-bloated steers peak occurred between 2 to 3 and 6 to 7 pm. Ruminating activity was higher ($P < 0.01$) for bloated than for non-bloated between 8 and 9 pm. It was concluded that the bloated steers reduced grazing activity and jaw movements. Limited effect of bloat on ruminating and activity bouts may or not be biologically accurate. Increased idling time in bloated steers suggests bloat induced malaise suppresses grazing and ruminating activities.

Key Words: Bloat, Grazing behavior, Steer, Wheat forage

Introduction

Studies of grazing behavior of ruminants show that there is a diurnal pattern of grazing activities (Champion et al., 1994). The diurnal pattern of grazing activities as day length changes during the yr, i.e. evening grazing always begins 3 to 4 h before sunset at pasture and in confinement (O'Connell et al., 1989). Ruminating generally occurs at night and during the intervals between the grazing bouts during the day (Phillips and Leaver, 1986). There is limited information on the effect of frothy bloat on diurnal ingestive behavior patterns or conversely the role of ingestive behavior in frothy bloat dynamics. Rutter et al. (2004) suggested that incidence of bloat could influence grazing activities in heifers grazing forages.

Frothy bloat is a ruminal dysfunction resulting from the accumulation of excess gas and stable foam production (Majak et al., 1995). When the cardia is covered with foam, eructation is inhibited (Dougherty, 1953), and associated intraruminal pressures increase up to 70 mmHg or 9.34 kPa (Lippke et al., 1972) to the point eructation ceases. Animal respiration rate increases and breathing becomes labored. In severe cases, bloat induced pulmonary and/or cardiac result in death. Sub-lethal increases in ruminal pressure and suppression of eructation may interrupt grazing behavior patterns. Rutter (2000) hypothesized it is possible that sheep and cattle show a partial preference for clover because rapid ruminal degradation supports rapid ingestion of large quantities of clover even though this frequently leads to bloat. Our hypothesis for grazing behavior of steers on wheat forage reflects association of rapid ingestion of rapidly fermentable forage with

satiety that may also at times induce bloat, however, bloated steers would alter their grazing patterns because bloat induced malaise as severity of frothy bloat increases. An experiment was therefore conducted to examine the effect of time of day and bloat status on ingestive behavior of yearling steers grazing wheat forage.

Materials and Methods

Research was conducted on continuously cropped wheat pasture comprised of two 4.5 ha paddocks in Wilbarger County, Texas (33° 57' N, 99° 26' W). Nine Ruminally cannulated steers (Angus x Hereford x Brangus; primarily *Bos taurus*; 320 ± 20 kg) grazed per paddock at a forage allowance of 18 kg DM/(100 kg BW·d^{-1}). Cattle were adapted to grazing wheat for 45 d and to being fitted with behavior monitors and harnesses for 14 d prior to initiation of the behavior trial. The effect of bloat status and time of day on grazing behavior of 6 bloated and 5 non-bloated rumen cannulated steers was examined on wheat forage swards maintained at 8 to 9 cm sward surface height for 14 d (from February 14 to 28 2005). Temporal grazing and ruminating behavior dynamics for each pair of steers (bloated vs. non-bloated steers) over 24 h periods were recorded automatically using jaw movement sensors (Ultra Sound Advice; IGER, North Wyke, Okehampton, Devon, U.K.) to measure time specific grazing, ruminating and idling behavior (Rutter, 2000). The number of jaw movements, grazing and ruminating bouts continuously recorded and stored in an onboard data logger. Periods during with no jaw movements were classified as 'other activities'.

Statistical analysis. The total number of min grazing, ruminating and in other activities, the total number of grazing jaw movements, the total number of boli regurgitated during rumination, and the temporal patterns of each activity by 1 h time steps within 24h were analyzed by analysis of variance using the MIXED procedure of SAS (SAS Inst., Cary, NC). Mean separation was performed using least significant differences when the F statistic was significant ($P < 0.05$). The statistical model consisted of bloat status (bloated vs. non-bloated steers), time of day, and associated interactions. Data are presented as mean values, together with the standard error of the mean (SEM).

Results and Discussion

Overall, while total grazing time and ruminating time were similar between bloated and non-bloated steers, however, there was a tendency ($P < 0.27$) for bloated steers to graze and ruminate less that non-bloated steers (Table 1). In contrast, idling time was greater for bloated ($P < 0.001$) than for non-bloated steers (10.9 vs. 7.9 h/24h). During the 24 h grazing periods, total jaw movements were lower ($P < 0.01$) for bloated than for non-bloated steers (52653 vs. 65872 jaw movements), but was no difference in ruminating bouts (267 vs. 313 bouts). The number of grazing bouts tended to be greater ($P = 0.15$) for non-bloated than bloated steers. This is the first report of bloat decreasing grazing activity, but increasing idling time in wheat pasture steers. Majak et al. (2003) reported that ruminal movements usually increase in the early stages of bloat, but decrease and even completely cease when ruminal distension is extreme. Results from previous review show that bloated animals had high intra-rumen and blood pressures and decreased oxygen utilization (Colvin and Backus, 1988) which support a decrease in grazing activity and increase in idling activity in response similar to that observed in this experiment.

The 24 h temporal patterns of grazing activity by the yearling steers are shown in Figures 1 and 2. Grazing patterns of non-bloated steers exhibit a quadratic response ($P < 0.05$) with time of day (Figure 1a). Grazing time increased from 6 to 7 am (sun rise) with maximum grazing activity between 2 to 5 pm (before sun set), and intermediated activity levels between 10 am to 12 pm. Similar diurnal grazing patterns were observed in bloated steers. However, the occurrence of bloat x grazing time interactions ($P < 0.01$) for grazing activity suggests that grazing time decreased with bloat between 8 to 10 pm and 6 to 8 am, but grazing activity increased sharply between 10 and 11 pm. The consistency in diurnal pattern of grazing activities over the time is similar to that observed in grazing dairy cows (Gibb et al., 1998).

Ruminating activity (Fig. 1b) was similar to and followed grazing activity periods. Ruminating activity in non-bloated steers peak occurred between 2 to 3 and 6 to 7 pm, but was greater ($P < 0.01$) for bloated than for non-bloated between 8 and 9 pm. The prehension (Figure 2b) and boli (Figure 2c) activities were similar to and followed grazing patterns, but mastication time (Figure 2a) was consistently lower ($P < 0.01$) for bloated than for non-

bloated. It is appears that the steers attempted to maximize intake before sunset (between 2 to 5 pm) and spent much of that time ruminating (Figure 2b), but bloated steers had occasionally limited activities of ruminating, mastication and boli activities.

Animal susceptibility to bloat is related to the clearance of small feed particles from the rumen. Frequent bloaters have a slower clearance than non-bloaters (Majack et al. 2003). The present study shows that total grazing time was generally similar between bloated and non-bloated steers, but grazing activities (ruminating, mastication and boli) were concomitantly lower for bloated than for non-bloated steers, suggesting that ingested material may be more resistant to rapid fermentation which may affect clearance rate from the rumen. Waghorn and Barry (1987) reported that in order to have a high probability of flowing out of the rumen through the reticulo-omasal orifice, the critical particle size must be breakdown to less than 2 mm in cattle. Most of this particle size reduction occurs through the action of the teeth in the processes of chewing during eating and chewing during rumination. Particle size reduction rate may have been less in bloated steers increased idling time.

Implications

Although overall grazing time only tended to be affected by mild bloat status, it had a quadratic effect on the proportion of diurnal grazing patterns (grazing, ruminating, mastication, prehension, and boli activities) and jaw movements, resulting in increased idling time. Limited effect of bloat on ruminating and activity bouts may or not be biologically accurate. Increased idling time in bloated steers suggests bloat induced malaise suppresses diurnal grazing patterns.

Acknowledgements

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Table 1. The effect of physiological state (non-bloated vs. bloated steers) on grazing time, ruminating, idling time, jaw movements and bouts by steers grazing winter wheat forage[1]

Item	Bloated	Non-bloated	SEM
n	5	6	
Grazing activities			
Grazing time, 24 h	9.6	11.4	1.01
Ruminating, h	3.2	4.5	0.74
Idling, h	10.9[a]	7.8[b]	0.57
Others, min	26	30	1.4
Jaw movements	52,653[b]	65,872[a]	2557
Total bouts	458	561	50.5
Ruminating bouts	267	313	58.4
Grazing bouts	82	150	29.3

[1]Grazing activities were recorded automatically using mouth sensors to measure their temporal patterns of grazing time, ruminating and idling behavior over 24h. n = number of animals.

[a vs.b]Numbers within a row with different superscripts are different ($P < 0.05$). SEM = Standard error of the mean.

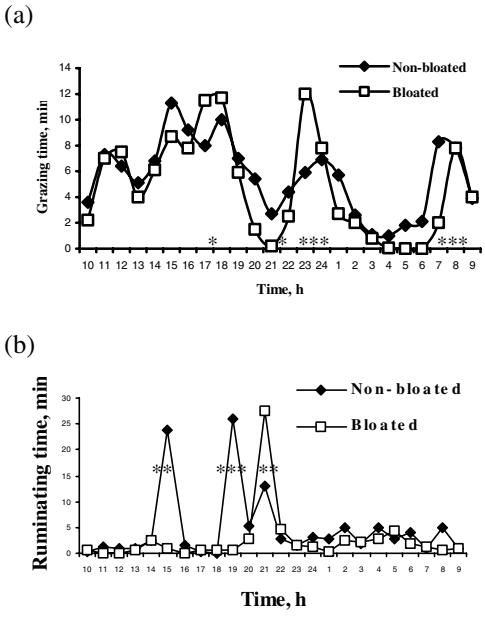

Figure 1. Effect of physiological state (bloated vs. non-bloated) and time of day on grazing activity (a) and ruminating time (b) by rumen cannulated steers grazing wheat forage. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

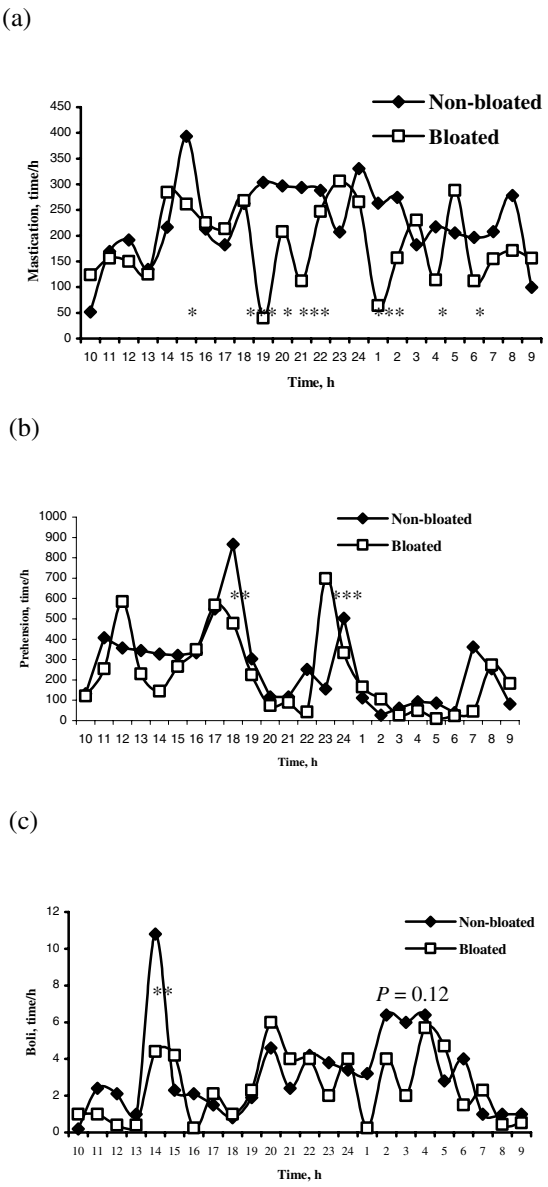

Figure 2. Effect of physiological state (bloated vs. non-bloated) and time of day on mastication (a), prehension (b), and boli (c) activities by rumen cannulated steers grazing wheat forage. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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RELATIONSHIP BETWEEN SEXUAL BEHAVIOR CLASSIFICATIONS OF RAMS AND LAMBS SIRED IN A COMPETITIVE BREEDING ENVIRONMENT[1,2]

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ABSTRACT: The objective for this study was to determine the relationship between three sexual classes of rams and lambs sired in a competitive breeding environment. Fifteen 2- to 3-yr-old white-faced rams classified as female-oriented, with high (**HPFOR**) or low (**LPFOR**) sexual performance, or male-oriented (**MOR**) were used in a multiple-sire breeding arrangement. Five groups of approximately 200 ewes each were exposed for 21 d to three rams per group consisting of one ram from each of the classes. Rams were initially blocked for sexual class, and those with similar genetic relationships were assigned to different pens. Genomic DNA was prepared from blood collected from rams, ewes, and lambs. Up to four microsatellite markers were used to assign sire parentage within pens. Sexual partner preference tests (**PREFT**) used to identify MOR did not accurately predict sexual performance during competitive breeding. In contrast to only mounting and servicing males in PREFT before breeding, MOR sired 480 lambs from 330 ewes. Serving capacity tests (**SCT**) predicted sexual performance of HPFOR and LPFOR. The HPFOR impregnated more ewes (499 vs 258; $P < 0.05$) and sired more lambs (756 vs 357; $P < 0.05$) than LPFOR, respectively. The LPFOR and MOR did not differ for ewes impregnated or lambs sired. We conclude 1) that the PREFT used to classify MOR did not predict their breeding performance in competitive breeding; 2) that SCT did predict that HPFOR rams would impregnate more ewes than LPFOR and sire more lambs than either LPFOR or MOR; and 3) under conditions of this study, LPFOR and MOR did not have an adverse impact on the overall breeding outcome. Combined, LPFOR and MOR sired as many lambs as HPFOR, thus requiring twice as many rams to obtain equivalent breeding results as HPFOR. Therefore, we suggest that SCT should be used to select HPFOR and reduce the number of rams with marginal sexual performance.

KEY WORDS: Competitive Mating, Ewes Lambing, Lambs Born, Paternal Typing, Sexual Behavior

Introduction

Most mature rams readily court, mount, and mate estrual ewes, but intensity of sexual behavior varies from asexuality to high sexual activity (Price, 1987). After extensive serving capacity tests at U.S. Sheep Experiment Station, 15 to 20% of 18 to 24-mo-old rams showed no interest in estrual ewes. Approximately 50% of rams that are not interested in estrual ewes are classified as male-oriented. At 8 to 10 mo of age, in a different population of rams, Price et al. (1988) found a similar incidence of asexual rams (18.5%; n = 54), and, of 44 sexually active rams, 68.2% were female-oriented, 22.7% showed no statistically significant gender preference, and 9.1% were male-oriented. Even though different ram classes are recognized, it is less clear what effect rams with different classifications have on flock fertility. In single-sire mating pens, 83 to 93% of 30 estrus-synchronized ewes lambed after a 9-d exposure to high performing rams compared to 21 to 48% of ewes lambing that were exposed to low performing rams for 9 d (Perkins et al., 1992). However, it is unknown what influence male-oriented rams have in a competitive breeding environment with high and low performance rams. The present study was designed to determine the relationship among three sexual classes of rams (i.e., high sexual performance female-oriented [**HPFOR**], low sexual performance female-oriented [**LPFOR**], or male-oriented rams [**MOR**]) and lambs sired in a competitive breeding environment to determine whether the male-oriented ram test is valid.

Materials and Methods

General. Before breeding, ewes were herded on native rangelands and given free access to water until time of breeding. During confinement, all sheep were fed to meet their nutrient requirements (NRC, 1985)

Sexual performance tests. Rams were white-faced, 2- to 3-yr-old, and selected after two types of sexual performance tests: 1) series of nine, 30-min serving capacity tests (**SCT)** in which rams were observed with three unrestrained, estrual teaser ewes; and 2) series of three 30-min sexual partner preference tests (**PREFT)** in which rams were observed with two restrained estrual ewes and two restrained rams. Rams were classified as HPFOR or LPFOR based on average ejaculations with ewes and as MOR if they were exclusively sexually active with rams during the initial SCT and PREFT. Rams were given one

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[2]Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, USU or the authors and does not imply its approval to the exclusion of other products that may also be suitable.

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more 30-min PREFT within 2 wk after breeding. Mounts and ejaculations were recorded during all tests. Teaser ewes were ovariectomized and estrus-induced (Stellflug and Berardinelli, 2002). Ewes were considered to be in estrus when they would stand to be mounted by a ram.

Semen collection and evaluation. An electorejaculator (Ideal Instruments Inc. Chicago, IL) was used to collect semen. Sperm was evaluated for morphology and motility with a phase contrast microscope (Nikon Instruments Co., Inc. Melville, NY). Normal and motile sperm were estimated to the nearest 10%, followed with a forward progressive movement score (1 to 4) using a modification of Terrill's (1937) system. Metabolic activity of sperm was evaluated with a resazurin reduction assay (Wang, et al., 1998).

Breeding management. Rams classified as HPFOR (n = 5), LPFOR (n = 5), and MOR (n = 5) were used in a multiple-sire breeding arrangement. Five flocks of approximately 200 ewes each (996 total) were used. One ram of each of the three classes was kept with each flock of ewes for 21 d in 1.5-ha plus. Rams with known genetic relationships were assigned to different pens. Black-faced clean-up rams were introduced to the ewes after the study.

Blood collection and paternal typing. Blood from rams, white-faced lambs, and their dams was collected in vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ), stored at 4 ºC, and processed within 10 d after collection. Genomic DNA was extracted from blood using a standard salt-precipitation method (Miller et al., 1988). Eight microsatellite markers were used in parentage testing of lambs: *McM111* (Smith et al., 1995a) for Pens 1, 3, 4, and 5; *McM136* (Hulme et al., 1995) for Pen 2; *McM137* (Smith et al., 1995b) for Pen 1; *OarFCB304* (Buchanan and Crawford, 1993) for Pen 3; *McM63* (Smith et al., 1995b) for Pen 4; *OarCP34* (Ede et al., 1995) for Pen 5; *BMS460* (Stone et al., 1995) for Pens 2, 3, and 5; and *RM004* (Kossarek et al., 1993) for Pens 3 and 5. Microsatellite genotyping was according to Cockett et al. (1996). Sire of each lamb was determined as the ram that could exclusively provide the remaining microsatellite allele after accounting for the dam's allele. If a sire could not be unequivocally assigned after examination of genotypes for up to four microsatellites, the sire was designated as ambiguous for that lamb. Of 1,757 white-faced lambs, 1,593 lambs could be assigned unambigously to one of three rams in their dam's breeding pen. A range of five to 11% of lambs sired in each breeding pen was excluded from the data set because their sires could not be identified.

Statistical analyses. The GLM procedures of SAS (SAS Inst. Inc., Cary, NC) were used to analyze number of ewes lambing, percentage normal sperm, forward progressive movement of sperm, and resazurin score for each ram class. Mixed model analyses of SAS were used to analyze number of lambs born from each ram class and SCT scores for HPFOR and LPFOR. The Genmod procedures of SAS with Poisson link were used to analyze mounts and ejaculations on ewes and rams recorded during PREFT. The GLM and Mixed models consisted of ram class and breeding pen as main effects and ram class x breeding pen interaction as error term. Breeding pen was not significant, but it was left in models to account for variation. Levene's test (Milliken and Johnson, 1984) was used to test for homogeneity of variance. Variances for ewes lambing were homogeneous. Data, which required log transformation, were transformed back to original units, and a SE for original units was estimated by subtracting least squares means (LSM) from the upper confidence limit and dividing by two. The Genmod models consisted of ram class and preference test number as main effects and ram class x preference test number interaction as error term. If main effects were significant ($P < 0.05$), Fisher's protected LSD was used as a postanalysis test to detect difference between individual means.

Results

Of 884 ewes with lambs that had assigned sires, 178 ewes had single lambs, 408 had multiples sired with a single ram, and 298 had multiples sired with more than one ram. Number of ewes lambing after exposure with the three classes of rams varied ($P < 0.05$) according to ram class but not among breeding pens. The HPFOR impregnated 46% of ewes (N = 499), compared with 24% (N = 258) and 30% (N = 330) for LPFOR and MOR, respectively (Table 1).

Number of lambs sired for each ram class differed ($P < 0.02$) among ram classes but not among breeding pens. The HPFOR sired a greater ($P < 0.05$) number of lambs (756 ± 84; LSM ± SE) than either LPFOR (357 ± 84) or MOR (480 ± 84). Pooled standard errors were used to depict variation for data presented in Table 1. Percentage of ewes producing one, two, or three lambs from each ram class was similar to percentages for total ewes impregnated.

Sperm morphology, progressive forward motility, and resazurin score did not differ among ram classes. Five HPFOR had 3.2 ± 0.2 ejaculations per 30-min test, compared with 0.4 ± 0.2 ejaculations for five LPFOR in nine SCT. Five MOR rams did not have any mounts or ejaculations during nine SCT. During three PREFT before breeding, HPFOR averaged 11.5 ± 1.5 mounts and 3.4 ± 0.4 ejaculations on estrual ewes, compared with 5.7 ± 3.1 mounts and 0.9 ± 0.4 ejaculations for LPFOR. The MOR averaged 20.9 ± 7.1 mounts and 0.6 ± 0.3 ejaculations on males and no mounts on estrual females in PREFT before breeding. In the one PREFT given after breeding, all HPFOR mated ewes with an average of 9.2 ± 1.3 mounts and 3.0 ± 0.5 ejaculations, compared with only three of five LPFOR mating ewes with an average of 3.0 ± 2.0 mounts and 0.2 ± 0.2 ejaculations. After breeding, only three of five MOR exhibited sexual activity. One of three sexually active MOR mounted ewes 16 times without ejaculating and did not mount rams. A second MOR mounted ewes eight times and rams 53 times but did not ejaculate. The third MOR mounted ewes twice and ejaculated once, whereas he mounted rams 40 times without ejaculating.

Discussion

Individual PREFT did not predict breeding performance of MOR, whereas SCT did predict breeding performance of HPFOR and LPFOR. Sexual activity of all five MOR to impregnate ewes and sire lambs differed from the lack of sexual activity in 4.5 h of SCT, and sexual

activity toward only male partners in 1.5 h of PREFT before the breeding period.

Data are not available to explain the inability of PREFT to predict the actual breeding performance of MOR. Some possibilities for the inability of the ram test to predict the breeding performance are restraint of teasers, lack of competition, and ability to acclimate to the test environment. Restraint of estrual ewes, as used in PREFT, does not have a major impact on sexual performance in preference tests (Price, et al., 1993), but restraint of teaser males may result in different behavioral effects than restraint of ewes. Restrained males may divert attention from ewes similar to influence of anestrous ewes in SCT (Zenchak et al., 1988). Lack of competition in the tests and slow acclimation of rams to test conditions may lead to lower sexual activity than normal. Sexual activity was enhanced when rams were exposed to a recently mated ram (Maina and Katz, 1997), and this influence with competition may improve acclimation of some rams that was less for HPFOR than the MOR.

The competitive, natural breeding experience did not alter the predominately male mounting of two of three MOR rams that exhibited sexual activity during the PREFT after breeding. This observation indicates that breeding experience did not completely change sexual partner preference behavior in all MOR. However, the fact remains that PREFT did not accurately predict breeding performance of MOR during competitive breeding, and SCT predicted increased breeding performance of HPFOR compared to both LPFOR and MOR. The HPFOR impregnated approximately twice as many ewes as LPFOR and sired nearly as many lambs as LPFOR and HPFOR combined.

Results of the present study are in agreement with a previous report using single-sire mating that indicated HPFOR service more ewes and produce more lambs than LPFOR rams (Perkins et al., 1992). The present study also agrees with reports that sexual behavior of rams influences flock fertility (Kilgour, 1993), but contrasts with others (Kilgour and Wilkins, 1980) where little difference was found. One key difference among the studies relates to number of ewes that were in estrus daily (i.e., high sexual performance only manifests itself under conditions providing a sufficient breeding challenge). Greater breeding success of HPFOR compared with LPFOR and MOR rams probably related to their greater sexual motivation as documented with more mounts and ejaculations in sexual performance tests rather than general dominance. This concept is supported with results from a study specifically designed to determine differences in competitiveness for food and estrual ewes between high and low performance rams (Erhard et al., 1998).

With paternal typing data, we were able to document a large number of ewes that gave birth to multiple lambs sired by more than one ram. This agrees with observations that rams do not necessarily lose their opportunity to service ewes when they are not among the first to find and breed estrual ewes because ewes remain receptive for many hours even when frequently bred (Parsons and Hunter, 1967). We also found that different classes of rams impregnated a similar percentage of ewes that gave birth to singles, twins, and triplets. There was no indication that any of the three classes of rams was able to disproportionately select and impregnate the more prolific ewes. Thus, the greater number of lambs sired by HPFOR, compared to LPFOR and MOR was primarily the result of the greater number of ewes HPFOR impregnated. Rams selected for this study represented extremes of a continuum regarding serving capacity test results. Thus, we cannot conclude that average-scoring rams would be inferior to HPFOR rams under these same conditions.

The combination of HPFOR, LPFOR, and MOR in the same breeding pen did not adversely impact overall reproductive success during the breeding period. Together, LPFOR and MOR sired 81 more lambs than HPFOR, but this required twice as many rams to obtain approximately equivalent breeding results to the HPFOR in a competitive environment.

Implications

Individual sexual partner preference tests did not predict breeding performance of male-oriented rams. However, serving capacity tests did predict breeding performance of high and low sexual performance female-oriented rams. Exclusively male-oriented rams, as identified in sexual partner preference tests before the breeding trial, bred ewes under competitive conditions, sired as many lambs as low sexual performance rams, but did not sire as many lambs as high performance rams. Serving capacity test results have a close relationship to breeding performance. Under the conditions of this study, low performance and male-oriented rams did not have an adverse impact on the overall breeding outcome. Together, the low performance and male-oriented rams bred as many ewes as a single high performance ram. Therefore, we suggest that serving capacity tests should be used to select high performance rams and reduce the number of rams with marginal sexual performance.

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Table 1. Reproductive performance for ewes giving birth to lambs sired from high (HPFOR) or low (LPFOR) sexual performance female-oriented rams or male-oriented rams in a competitive mating environment replicated in five breeding pens with approximately 200 ewes per pen.

	Ram Classification [a]			
	HPFOR	LPFOR	MOR	Total
Ewes Lambed	499 ± 57[b]	258 ± 57[c]	330 ± 57[bc]	1,087
Lambs Sired	756 ± 84[b]	357 ± 84[c]	480 ± 84[c]	1,593

[a] Rams were classified as HPFOR, LPFOR, or MOR with nine serving capacity and three preference tests before breeding.
[bc] Values within rows (least squares means ± pooled SE) with uncommon superscripts differ ($P < 0.05$).

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DEVELOPMENT OF A MULTIPLE TRAIT SELECTION INDEX FOR FEEDLOT TRAITS IN BEEF CATTLE INCLUDING FEED EFFICIENCY

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ABSTRACT: A multiple trait index including residual feed intake was constructed to improve net feedlot income in market progeny. The selection objective was defined as $H = v_1E_1 + v_2E_2 + v_3E_3$. where aggregate genetic merit (H) was a linear function of daily DMI (E_1, kg/d), ADG (E_2, kg/d), and slaughter weight (E_3, kg) in market progeny. Regression of steer (n = 426) net income on traits in the objective yielded the vector of economic weights (**v**) with elements v_1 = \$-21.49, v_2 = \$183.73, and v_3 = \$-0.27. The selection criterion was defined as $I = b_1X_1 + b_2X_2 + b_3X_3$ where index value (I) was a linear function of residual feed intake (X_1, kg/d), ADG (X_2, kg/d), and adjusted 365-d weight (X_3, kg) of bulls tested between weaning and yearling. Residual feed intake was defined as the difference between actual intake and that predicted by phenotypic regression (R^2 = 0.69, residual SD = 0.58 kg/d) of daily DMI on ADG, metabolic mid-test weight, and on-test gain in ultrasound subcutaneous fat depth and longissimus muscle area. The matrix of genetic covariances of traits in the criterion with those in the objective (**G**) was estimated from recent reports in the literature and the matrix of phenotypic (co)variances among traits in the criterion (**P**) was estimated from a group of yearling Angus bulls with test data. Selection index weights were obtained from the solution $\mathbf{b} = \mathbf{P^{-1}Gv}$ with elements: b_1 = -10.12, b_2 = 24.79, and b_3 = -0.09. Index values of Angus bulls (n = 99) adjusted to a mean of 100 (SD = 7.81) ranged from 80.1 to 115.7. Phenotypic correlation estimates for index values with bull daily DMI, ADG, 365-d weight, and residual feed intake were -0.22, 0.53, 0.01, and -0.74, respectively. The phenotypic correlation estimate for index value with end of test scrotal circumference (0.16) was low but favorable. Therefore, higher index values were obtained for bulls with lower daily dry matter consumption, lower residual feed intake, higher ADG, and slightly larger scrotal circumference.

Key Words: Beef Cattle, Feed Efficiency, Selection

Introduction

Beef cattle selection programs have traditionally focused on output traits related to reproductive rate, growth performance and(or) carcass composition. Recently, there has been a renewed interest in genetic characterization of traits related to inputs of beef production, mainly those of feed, which account for the largest proportion of non-fixed costs. Breed differences and genetic parameters for measures of feed efficiency have been well characterized in most cases (Koots et al., 1994a). Several recent studies (e.g. Archer et al., 1999; Arthur et al., 2001a,b) have been devoted to residual feed intake (**RFI**), defined as the difference between actual intake and that predicted by phenotypic regression of intake on body weight and growth rate (Koch et al., 1963), and in some cases also on various measures of body composition (e.g., Basarab et al., 2003). There is a lack of research, however, investigating potential implementation of multiple trait selection programs including RFI. One potential reason for this is the relative lack of estimates of covariances of RFI with other economically important traits (Archer et al., 1999), as well as the costs associated with collection of individual feed intake data (Archer et al., 2004).

With adequate centralized data collection systems typical of beef postweaning test centers and decreasing cost of hardware, feed intake measurement can be more cost effective. The challenge remains to develop multiple trait selection models which include feed efficiency. The objectives of this study, therefore, were to propose a three-trait index of yearling bull test phenotypic data with the objective to increase feedlot profitability of market progeny, and to estimate the associations of the index with other traits commonly measured in bull tests.

Materials and Methods

Charolais and crossbred, Charolais-sired steers (n = 426) produced from 1999 to 2002 at the Agriculture and Agri-Food Canada Onefour Research Substation were fed following weaning (200 d of age) until prior to harvest (455 d of age) at the Lethbridge Research Centre feedlot which is equipped with hardware to record daily feed intake (GrowSafe Systems, Ltd., Airdrie, AB). Individual daily feed intake was measured during the final 112-d portion of the feeding period wherein steers consumed a diet consisting of 75% rolled barley, 20% barley silage, and 5% supplement. Further details regarding the experimental protocol can be found in Crews et al. (2003). Revenue per steer in the feedlot sector was calculated by multiplying final live weight by a ten-year average price (\$1.59/kg) for fed steers weighing approximately 575 kg. Cost estimates included total feed costs plus average live weaned calf price (\$2.03/kg) multiplied by adjusted 205-d weaning weight. Total feed costs were estimated as total DMI during the 112-d feeding period adjusted to a feeding period of length equal to harvest age minus 205 d. Net feedlot revenue (**NFR**) was then defined as revenue minus costs.

A multiple trait linear regression model (SAS Ver. 8.3, SAS Inst., Inc., Cary, NC) and stepwise procedures were used to identify key component traits in the steers that were related to NFR, and to estimate partial regression

coefficients for the final model which were then equated to the marginal economic value of the component traits. Steer traits in the final model included daily DMI (**SFI**), ADG (**SDG**), and final live weight (**SWT**).

Data from Angus bulls which had completed a postweaning test at the Beef Development Center in Millican, TX were also used in this study. This bull test facility was equipped with a feed intake recording system (GrowSafe Systems, Ltd., Airdrie, AB) that was used to measure individual daily feed intake of bulls during an 84-d period. Feed intake data were converted to daily DMI (**BFI**) using DM analysis of composited feed ingredient samples. For this study, data from Angus bulls (n = 99) in test 1 as described by Lancaster et al. (2005) were used. Additional raw data available on bulls included serially-measured (14-d) live weight, adjusted 365-d weight (**BYW**), end-of-test scrotal circumference (**BSC**), and ultrasound measures of subcutaneous fat depth, and longissimus muscle area at the start and end of the test. Gain in ultrasound fat (**FGN**) and muscle area (**MGN**) were computed from start- and end-of-test measurements. Linear regression of serial live weight on test day was used to compute ADG (**BDG**), and mid-test live weight$^{0.75}$ (**BMW**). Residual feed intake of bulls (**BRFI**) was defined as the difference between actual DM intake and that predicted by phenotypic regression (R^2 = 0.69, residual SD = 0.58 kg/d) of BFI on BDG, BMW, FGN, and MGN (Arthur et al., 2001a,b; Basarab et al., 2003). The BRFI phenotype was therefore phenotypically independent of the traits in the prediction.

Using multiple trait selection index methods (e.g., Cameron, 1997), economic weights for SFI, SDG, and SFW in the selection objective and appropriate phenotypic and genetic (co)variance matrices were used to compute weighting factors for BRFI, BDG, and BYW in the selection criterion. Briefly, the selection objective was defined as $H = v_1E_1 + v_2E_2 + v_3E_3$, where aggregate genetic merit (**H**) was a linear function of SFI (E_1), SDG (E_2), and SFW (E_3) breeding values with economic weights from regression described above. The selection criterion was defined as $I = b_1X_1 + b_2X_2 + b_3X_3$ where index value (**I**) was a linear function of BRFI (X_1), BDG (X_2), and BYW (X_3) with appropriate weighting factors from the solution to the selection index equations, $\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\mathbf{v}$. In these equations, **P** is a symmetric (3 × 3) matrix of phenotypic (co)variances among traits in the criterion measured on bulls. The (3 × 3) matrix **G** contained genetic covariances of bull traits in the criterion with steer traits in the objective. Post-multiplication of the matrix product $\mathbf{P}^{-1}\mathbf{G}$ by the vector **v** containing economic weights for steer traits in the objective yields the vector **b** containing phenotypic weights for bull traits in the criterion. Genetic covariances in **G** were derived from the recent literature (e.g., Koots et al., 1994a,b; Archer et al., 1999; Arthur et al., 2001a; Crews et al., 2003) because experimental data to estimate parameters among traits measured on bulls and their market progeny were not available. Phenotypic (co)variances in **P** were estimated from the Angus bull data described previously. Matrix inversions and algebra for the selection index methods were computed using OCTAVE, an interpreted matrix language component of the Linux (Red Hat Ver. 8.0, Research Triangle Park, NC) operating system. Index values (I) were computed for all Angus bulls and adjusted to a mean of 100. Simple correlations of the adjusted index value with component traits and BSC were computed to quantify their phenotypic associations.

Results and Discussion

Table 1 contains summary statistics for the relevant traits measured on Angus bulls during postweaning test.

Table 1. Summary statistics for traits measured on Angus bulls (n = 99) during postweaning test

Trait[a]	Mean	Minimum	Maximum	SD
BFI, kg/d	8.40	6.31	11.58	1.06
BDG, kg/d	1.40	0.75	2.32	0.26
BMW, kg	94.1	77.7	110.7	7.13
BYW, kg	438.9	330.4	542.5	42.32
FGN, mm	0.30	-0.69	0.76	0.26
RGN, cm^2	25.7	4.6	51.8	8.86
BRFI, kg/d	0.00	-1.58	1.33	0.58
BSC, cm	35.0	30.0	45.0	2.60

[a] BFI = daily DMI, BDG = ADG, BMW = metabolic mid-test weight, BYW = adjusted 365-d weight, FGN = on-test gain in ultrasound fat depth, RGN = on-test gain in ultrasound longissimus muscle area, BRFI = residual feed intake, BSC = end-of-test scrotal circumference.

As noted previously, BRFI was computed so as to be phenotypically independent of body size, ADG, and body composition as measured by gains in ultrasound fat depth and longissimus muscle area. The correlation (data not reported in tabular form) of BFI with MGN (0.42) was higher than that with FGN (0.18). These results suggest that adjustment of residual feed intake for body composition in bulls may depend more on adjustment for muscle deposition than fat deposition, although the RFI estimation model R^2 was minimally increased by the addition of body composition measurements compared to a more traditional base RFI model including only weight and growth rate. Conversely, Basarab et al. (2003) showed that base RFI was more related to gain in empty body fat than to gain in empty body protein in steers. It may be that restricted fat deposition in bulls on test accounts for part of the apparent discrepancy between these results.

The regression of steer net revenue on SFI, SDG, and SWT yielded the economic weights in the vector **v** with elements v_1 = -21.49, v_2 = 183.73, and v_3 = -0.27. Basarab et al. (2003) calculated a difference of $45.60 in 120-d feeding costs between steers with the highest (i.e., least efficient) and lowest (i.e., most efficient) RFI values. The marginal value of SFI, therefore, would be negative which is supported by the present study. The higher economic value for SDG apparently reflects the impact of weight gain during steer finishing relative to feed intake and slaughter weight, but also the relatively low variability of the trait. In the presence of measures of DMI and ADG, the marginal economic value of SWT was lower.

Phenotypic covariances in the matrix **P** can be derived from values in Table 1 for bull traits in the selection criterion. It should be noted, however, that if data and

pedigree structure were sufficient, an index of EPD could be constructed based on bull EPD expressed on the appropriate market progeny scale and economic values in **v** (Cameron, 1997). Due to the lack of suitable data to obtain EPD among the Angus bulls in this study and because the index was to be used as a marketing tool for the bull test, we extend the criterion to include traits on bulls with the objective to improve aggregate genetic merit of their progeny. The inverse of **P**, used to solve for the index weights in **b** was obtained as:

$$\mathbf{P}^{-1} = \begin{bmatrix} 2.933 & -0.244 & 0.003 \\ & 21.853 & -0.074 \\ \text{Symm.} & & 0.001 \end{bmatrix}$$

with elements in rows (and columns) 1, 2, and 3 corresponding to BRI, BDG, and BYW, respectively. Diagonal elements of **P** were phenotypic variances, with symmetric off-diagonals equal to covariances. Table 2 summarizes genetic parameters for bull and steer traits relevant to the index.

Table 2. Phenotypic variance, heritability (h^2) and additive genetic SD (σ_G) for bull[a] and steer[b] traits in the index

Trait	Var(P)[c]	h^2	σ_G
BRFI	0.342	0.39	0.365
BDG	0.066	0.28	0.136
BYW	1791.2	0.33	24.31
SFI	0.996	0.51	0.713
SDG	0.060	0.46	0.166
SFW	3783.9	0.40	38.90

[a] Bull traits: BRFI = residual feed intake, BDG = ADG, BYW = 365-d weight.
[b] Steer traits: SFI = daily DMI, SDG = ADG, SFW = final live weight.
[c] Phenotypic variance.

Phenotypic variances in Table 2 were obtained from the Angus bull measurements. Heritabilities assumed for the bull traits were from Arthur et al. (2001a) who studied Angus bulls in Australia, with the exception of BYW, which was not reported in that study. The heritability of 0.33 assumed for BYW was the weighted average estimate reported by Koots et al. (1994a). Heritability estimates for steer traits were extracted from Crews et al. (2003) who reported on the same steers used in this study to derive the economic weights.

The non-symmetric matrix **G** relates traits in the criterion with traits in the objective and in this case, consists of elements equal to the genetic covariances of BRFI, BDG, and BYW with SFI, SDG, and SFW. Because the traits in the objective and criterion are dissimilar, each element of **G** is defined as

$$g_{ij} = \Gamma_{ij} h_i h_j \sigma_i \sigma_j$$

where g_{ij} is the element in the i^{th} row and j^{th} column of **G**, Γ is the genetic correlation, h is the square root of heritability, and σ is phenotypic SD (Cameron, 1997). Subscripts i and j refer to trait i in the criterion and trait j in the objective, respectively.

Genetic correlations of bull traits with steer traits were derived from literature sources (Table 3) and then the nine elements of **G** were computed using heritability and phenotypic SD estimates reported in Table 2.

Table 3. Genetic correlations of bull traits with steer traits

Bull trait (i)[a]	Steer trait (j)[b]	Γ_{ij}[c]
BRFI	SFI	0.60
	SDG	0.00
	SFW	0.00
BDG	SFI	0.50
	SDG	0.75
	SFW	0.70
BYW	SFI	0.50
	SDG	0.50
	SFW	0.75

[a] Bull traits: BRFI = residual feed intake, BDG = ADG, BYW = 365-d weight.
[b] Steer traits: SFI = daily DMI, SDG = ADG, SFW = final live weight.
[c] Genetic correlation.

Genetic correlations involving BRFI were generally similar to those in Arthur et al. (2001a) where RFI had a positive genetic correlation with feed intake, but near zero genetic correlations with ADG and live weight. The remaining genetic correlations were derived based on: 1) observing that similar postweaning traits measured in bulls and steers (e.g., BDG and SDG) have been reported to have genetic correlations of approximately 0.75 (e.g., Devitt and Wilton, 2001; Crews et al., 2004), and 2) dissimilar trait pairs (e.g., BYW and SFI) may likely have genetic correlations of magnitude equal to that if they had been measured on the same animals (Koots et al., 1994b), but should be reduced by the fact they were measured in animals of different gender and under different management schemes (Crews et al., 2004). For example, the genetic correlation between BYW and SFI was assumed to be 0.50 which is the product of the genetic correlation between yearling weight and feed intake (0.76; Koots et al., 1994b) and the genetic correlation of bull yearling weight with steer yearling weight (0.66; Devitt and Wilton, 2001), or 0.76 × 0.66 = 0.50. A similar procedure was used for the remaining trait pairs to obtain assumed genetic correlations in Table 3.

Solution to the equations $\mathbf{P^{-1}Gv}$ yielded the vector of selection criterion or index weights (**b**) with individual elements b_1 = -10.12, b_2 = 24.79, and b_3 = -0.09. The elements in **b** were used to calculate index values, defined as I = -10.12(BRFI) + 24.79(BDG) + -0.09(BYW). Because producers consigning bulls to the test preferred to report index values similar to more traditional ratios, 100 was added to the index value as a deviation from the index mean, adjusting the index mean to 100. The mean, minimum, maximum, and SD of the adjusted index were 100, 80.1, 115.7, and 7.81, respectively. The index weight was negative for BRFI, positive for BDG, and near zero for BYW. The result that a negative weight was obtained for BRFI is consistent with the desired selection trend to decrease feed intake. The increases in efficiency gained through decreasing BRFI is expected to also result in a

negative genetic trend for feed intake in steer progeny. Both of these would be considered favorable. Also intuitive was the positive weight on BDG. The relative contribution of BYW in this index was relatively low which may reflect the smaller economic value of SFW in the objective, but may also reflect that measurement of BYW adds little more information to the index in the presence of BRFI and BDG. From Cameron (1997), the contribution of BYW to selection response in the objective as

$$1-\sqrt{1-\frac{b_3^2}{(\mathbf{b}'\mathbf{P}\mathbf{b})P_{3,3}^{-1}}}$$

where b_3 is the element of **b** corresponding to BYW and $P_{3,3}^{-1}$ is the third diagonal element of $\mathbf{P^{-1}}$, gives 0.09, indicating that removal of BYW from the criterion would reduce the accuracy of the criterion by 9%. Comparatively, removal of BRFI from the criterion would reduce accuracy of the criterion by 34% which was more than for BYW or BDG (27%). This result emphasizes the importance of including RFI in the selection criterion.

To further characterize the index, correlations with bull traits are reported in Table 4.

Table 4. Phenotypic correlations of the index with bull traits

Bull trait[a]	r_P[b]	*P*-value
BRFI	-0.74	<0.001
BFI	-0.22	0.029
BDG	0.53	<0.001
BYW	0.01	0.897
BSC	0.16	0.121

[a] BRFI = residual feed intake, BFI = daily DMI, BDG = ADG, BYW = 365-d weight, BSC = end-of-test scrotal circumference.
[b] Phenotypic correlation.

Phenotypic correlation estimates ($P < 0.03$) of index value with BRFI, BFI, and BDG were -0.74, -0.22, 0.53, respectively. These results suggest that higher index values were obtained for bulls with lower daily DMI, lower residual feed intake, and higher ADG. The correlation of index value with 365-d scrotal circumference was low (0.16) indicating only a marginal ($P < 0.13$) association although the sign of the correlation was favorable. The mean BSC of bulls with index values below100 (34.74 cm) was similar ($P < 0.30$) to that of bulls with index values above 100 (35.26 cm). There was essentially no association ($r = 0.01$; $P > 0.89$) between index value and BYW.

Implications

The design of selection programs should not only consider the multiple traits that are economically important to beef production, but also that profitability is a function of both revenues and costs. The recent increase of available individual feed intake data from traditional bull tests can be used to develop index selection tools that consider growth and saleable weight outputs, but also feed inputs. The index proposed here is an example multiple trait selection tool that is expected to increase growth rate and decrease feed intake during the feedlot phase of market progeny from centrally tested sires with feed efficiency phenotypes.

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PREDICTION OF REPRODUCTIVE TRAITS IN BRANGUS HEIFERS USING A SNP AND THE TRANSLATED PRODUCT OF THE IGF-I GENE

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ABSTRACT: Objectives were to evaluate associations of reproductive traits with IGF-I genotypes of a SNP in the 5'-untranslated region (5'-UTR) and serum concentrations in Brangus heifers. Blood samples were collected post-weaning in the autumn of 1997 to 2002 in spring-born heifer progeny (n = 190) of 14 sires. Flouroprobe SNP detection was conducted with DNA extracted from white blood cells and RIA was used to determine serum concentrations of IGF-I. Heifers were developed on forage and observed for estrus morning and evening for 90 d the ensuing spring. Heifers were then estrous synchronized, AI if observed in estrus, and then exposed to bulls for another 90 d. Allele and gene frequencies were estimated with Chi-square analyses. Associations were tested in prediction analyses with fixed effects of year, genotype, and age of dam and covariates of age, weight, and concentration of IGF-I. Sire served as a random term in the model. Frequencies ($\chi^2 = 88.3$, $P < 0.01$) for C and T alleles and CC, CT, TT genotypes were 0.79, 0.21, 0.6, 0.38, and 0.02, respectively. Heifers of the CT genotype had greater ($P < 0.05$) serum concentrations of IGF-I than heifers of the CC or TT genotypes (194.8 > 167.0 = 164.2 ± 6.6 ng/mL). Only 62.2% of heifers were observed in estrus when synchronized and only 30% of these heifers became pregnant from AI at 13.5 ± 0.01 mo of age and 365.1 ± 4.8 kg of BW, whereas 92.7% became pregnant by 17 mo of age. Genotype predicted ($P < 0.02$) estrus observed at synchronization. A greater ($P < 0.01$) percentage of heifers with CC genotype expressed this estrus than heifers of the CT or TT genotypes (i.e., 66.3% > 33.7% > 0%). Post-weaning serum concentration of IGF-I was 11.5% greater ($P < 0.05$) in heifers observed in estrus versus heifers not observed in estrus at synchronization, but these values were not significant predictors of reproductive traits. The CC genotype of a SNP in the 5'-UTR of IGF-I appeared to be associated with a response to estrus synchronization in Brangus heifers 13.5 mo of age; however, this single locus association should be cautiously interpreted until evaluated for linkage disequilibrium.

KEY WORDS: Brangus, IGF-I, SNP

Introduction

Beef heifers that calve by 2 yr of age have greater lifetime production than those that calve later (Wiltbank et al., 1985). However, *Bos indicus*-influenced cattle usually have later onset of puberty than *Bos taurus* cattle. This may be partly due to differences in growth rate and frame size, and (or) concentrations of metabolic hormones. Echternkamp and co-workers (2000) determined cows selected for twinning had greater pituitary responsiveness to GnRH hormone and greater serum concentrations of IGF-I relative to unselected controls. A QTL proposed to regulate ovulation/twinning in these cattle exists on BTA5 and the IGF-I gene underlies this QTL (Kappes et al., 2000; Lien et al., 2000). A SNP located in the 5'-untranslated region (**5'-UTR**) of the IGF-I gene was associated with reproductive traits in Angus cattle divergently selected for serum concentrations of IGF-I (Ge et al., 1997; Yilmaz et al., 1999). Cumulatively, these results suggest regions of BTA5 may be useful as a tool to predict reproductive competency in heifers. Objectives were to evaluate association of IGF-I genotypes of a SNP in the 5'-UTR and serum concentrations with reproductive traits in Brangus heifers.

Material and Methods

From 1997 to 2002, DNA samples were collected from spring-born Brangus heifers. Heifers were progeny of 14 Brangus sires, born and weaned at the Chihuahuan Desert Rangeland Research Center in New Mexico.

Heifers were observed for behavioral estrus twice daily at 0700 and 1900 for 1 h beginning in the spring of the year following weaning. Observation of standing estrus was used to calculate age of first observed estrus. Heifers were estrually synchronized later that spring using a progestogen-based protocol comprised of either Syncro-mate-B® (Merial Inc., Woodbridge, N.J.) or feeding melengestrol acetate (MGA; 0.5 mg•animal^{-1}•d^{-1}). Heifers observed in standing estrus upon synchronization were recorded as expressing estrus at synchronization and AI. Fifteen days after estrus synchronization, heifers were placed with bulls for 90 d. Transrectal palpation was used to determine if heifers were pregnant after their first breeding season. Calving records were used to determine if pregnancy resulted from AI or natural service.

Weaning weight, yearling weight, and BW at time of GnRH challenge and synchronization were collected and used in analyses. Adjusted 205- and 365-d weaning weights were calculated using Breed Improvement Federation age of dam adjustments (2004; **AOD**). Postweaning ADG was calculated by subtracting BW collected at time of GnRH challenge from BW recorded at synchronization and divided by number of days between measurements

Two blood samples were collected via jugular venipuncture postweaning. One sample was used to determine serum concentrations of IGF-I (Berrie et al., 1995) and intra- and interassay CV for IGF-I RIA were 14% and 18%, respectively. Genomic DNA was extracted by saturate salt procedure or commercial kits (NucleoSpin Blood Mini Kit, Clontech Laboratories, Inc., Palo Alto, CA; QIA DNA Blood Mini Kit, Qiagen, Valencia, CA). Probes and primers for Cytosine/Thymine (**C/T**) SNP in the IGF-I gene were designed by Applied Biosystems (Foster City, CA) from a 600 bp DNA sequence (Lien et al., 2000; Accession# AF210383S1) located within the 5'-UTR of exon I of IGF-I upstream and downstream of the C/T SNP (74 cM; Ge et al., 1997). Reagents for real time PCR were a Taqman universal master mix, assay mix with probes and primers, RNA/DNA free water and 1.5 µL of DNA diluted to a concentration of 15 ng/µL and assayed in duplicate.

Analyses were performed using SAS (Version 8.1, SAS Inst. Inc., Cary, NC). Heifer served as experimental unit and sires with greater than 2 progeny were included in analyses. Levene's and Shapiro Wilk's test and residuals tested for homogeneity of sample variances and normality of residuals. Least squares means for Julian birthday, growth and reproductive traits, and serum concentrations of IGF-I were evaluated in a mixed model using fixed effects year, AOD, covariates of Julian birthday and BW, and random effect sire. Birth weight and BW at synchronization were used as covariates in evaluation of growth traits, serum concentrations of IGF-I, and reproductive traits.

Chi-square analysis for homogeneity of frequency was determined for categorical IGF-I genotypes and dichotomous reproductive traits first observed estrus, estrus at synchronization, pregnant during first breeding season, pregnant by AI, and calved by 2 yr of age.

Serum concentrations of IGF-I were predicted in a mixed model using fixed effects year, AOD, and genotype of the IGF-I gene, covariates Julian birthday, BW at time of GnRH challenge, and LHAUC, and random effect sire. Where appropriate, means were separated by genotype using LSMEANS and PDIFF procedures of PROC MIXED. Estrus at synchronization was predicted in PROC GENMOD using fixed effects year, AOD, and genotype, covariates Julian birthday, BW at synchronization, LHAUC, and serum concentrations of IGF-I, and random effect sire. PROC GENMOD DESCENDING was used to model occurrence of the dichotomous response variable, REPEATED option was used with SUBJECT statement identified as sire, and distribution was labeled as BINOMIAL. Sire was used as a repeated term and the SUBJECT term would account for within subject heterogeneity. To determine if sire was significant, model was analyzed in PROC MIXED with and without the random term sire. Difference between -2REML log likelihood from model with and without random term and a Chi-square distribution with 1 df was used to determine probability of likelihood ratio statistic. This statistic tests the hypothesis that no variability is present among Brangus sires. If sire is significant, variability among sires is a source of variation to explain variation observed in the dichotomous trait.

Results and Discussion

Average data of birth for Brangus (n = 190) heifers was 58.4 ± 3.8 d. Adjusted 205- and 365-d weights were 264.6 and 356.9 ± 4.3 kg, respectively, and heifers gained 0.93 ± 0.04 kg/d from time of GnRH challenge to synchronization.

Of Brangus heifers that expressed first observed estrus (Table 1), heifers were 11.8 ± 0.11 mo of age. Heifers weighed 365.1 ± 4.8 kg at time of synchronization and 23.7 ± 0.09 mo at calving. The SNP in the 5'-UTR of the IGF-I gene produced 2 alleles and allelic frequency for the C allele was greater (χ^2 = 30.0, P = 0.01) than T allele and frequency of the CC genotype was greater (χ^2 = 88.3, P = 0.01) than CT or TT genotypes. A greater percentage (χ^2 = 7.3, P = 0.03; Table 2) of Brangus heifers with the CC genotype expressed estrus at synchronization than CT genotype. No differences were detected among genotypes for the remaining dichotomous reproductive traits in Brangus heifers (Table 2).

Year, AOD, and genotype were significant (P = 0.01) sources of variation to predict serum concentrations of IGF-I. Brangus heifers with the CT genotype had greater (P < 0.01) serum concentrations of IGF-I than heifers with homozygous genotypes, which were similar (Table 3). Genotype and serum concentrations of IGF-I were significant ($P \leq 0.02$) sources of variation in occurrence of estrus at synchronization in Brangus heifers (62%). Heifers that expressed estrus had greater serum concentrations of IGF-I than heifers not expressing estrus (P = 0.02; 194.0 > 171.7 ± 8.9 ng/mL), but heifers with CC genotype had lower (P = 0.01) serum concentrations of IGF-I than heifers with CT genotype (172 < 215 ± 7.5 ng/mL); no heifers expressing estrus at synchronization had the TT genotype.

A relationship between hypothalamo-pituitary secretion of LH and hepatic secretion of IGF-I can be inferred due to presence of a QTL for increased ovulation rate on BTA5 (Kappes et al., 2000; Lien et al., 2000). Twinning cattle have been evaluated to have greater serum IGF-I and expressed estrus earlier than non-twinning control cattle (Echternkamp, 2000). The IGF-I gene lies within this QTL and a C/T SNP was identified in the 5'-UTR of exon I (Ge et al., 1997). Genotype of the SNP was a significant predictor of estrus at synchronization in Brangus heifers and heifers with CC genotype may be advantageous in production systems

requiring early age of puberty and *Bos indicus* trait heat tolerance. Prediction of reproductive traits with genotypes, therefore, appears to be more applicable in *Bos indicus*-influenced cattle because these cattle mature later than British-*Bos taurus* cattle such as Angus (Lopez et al., 2005).

A curvilinear rather than linear response and a threshold preceding onset of puberty was observed in serum concentrations of IGF-I in heifers in other studies that evaluated serum concentrations of IGF-I relative to onset of puberty (Garcia et al., 2003). Lopez and co-workers (2005) determined serum concentrations of IGF-I were greater in Brangus than Angus heifers, but Brangus heifers were ~40 d older at onset of puberty. The current study and Lopez et al. (2005) were performed on an age-constant basis and evaluating puberty in this manner may conceal changes in serum concentrations IGF-I and other metabolic profiles in *Bos indicus*-influenced cattle preceding puberty

Serum concentrations of IGF-I were significant sources of variation of estrus at synchronization, and genotype was a significant predictor of serum concentrations of IGF-I. Including both variables in prediction models for estrus at synchronization was not appropriate even though goodness of fit criteria indicated model validity. Therefore, alternative but more appropriate models to explain variation analyzed genotype and serum concentrations of IGF-I separately. These results indicated sire and serum concentrations of IGF-I were not significant sources of variation, but genotype continued to be significant for predicting variation observed in Brangus heifers that expressed estrus at synchronization.

Even though data were limited, estimates of age at first observed estrus were similar to those observed in Lopez et al. (2005). Visually observed estrus is a difficult trait to accurately detect in *Bos indicus* cattle (Bo et al., 2003). While modern electronic technologies have improved detection of behavioral estrus, Lopez et al. (2005) failed to detect estrus 33% of the time using an electronic detection system that coincided with luteal phase increase in progesterone. Also, *Bos taurus*-influenced heifers are more likely to express nonpuberal estrus (Rutter and Randel, 1986). Perhaps *Bos indicus*-influence in Brangus heifers in the current study was enough to impair accurate detection of behavioral estrus.

The IGF-I gene should be tested for linkage disequilibrium with other markers and (or) genes located within the QTL on BTA5. Linkage disequilibrium suggests nonindependence of alleles at different loci and allele linkage rather than the gene itself may be responsible for observed response in a quantitative trait (Pritchard and Przeworski, 2001).

The CC genotype of a SNP in the 5'-UTR of IGF-I appeared to be associated with a response to estrus synchronization in Brangus heifers 13.5 mo of age; however, this single locus association should be cautiously interpreted until evaluated for linkage disequilibrium.

Implications

In Brangus heifers, genetic technologies to predict reproductive traits may be useful as these *Bos indicus*-influenced animals will struggle to meet breeding and calving goals established by British-*Bos taurus* counterparts. Genomic selection based on genotype of the IGF-I gene, specifically the CC genotype, may be feasible to improve reproductive traits in *Bos indicus*-influenced cattle

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Table 1. Percentage of Brangus heifers[a] expressing the dichotomous reproductive traits. Chi-square results indicate homogeneity of occurrence of traits.

Trait	n	Percentage	χ^2	*P*-value
First observed estrus	162	35.8	13.1	0.01
Estrus at synchronization	143	62.2	8.6	0.01
Pregnant during first breeding season	128	85.2	63.3	0.01
Pregnant by AI	109	29.4	18.6	0.01
Calved by 2 yr of age	109	92.7	79.3	0.01

[a]Results for dichotomous reproductive traits are reported for a proportion of Brangus heifers used (n = 190).

Table 2. Percentages of genotypes associated with each dichotomous reproductive trait in Brangus heifers[a]. Chi-square results indicate homogeneity of genotype for the C/T SNP in the IGF-I gene.

Trait	n	CC	CT	TT	χ^2	*P*-value
First observed estrus	155	61.8	36.4	1.8	0.1	0.94
Estrus at synchronization	137	66.3	33.7	0	7.3	0.03
Pregnant during first breeding season	124	57.1	41.9	1.0	4.4	0.11
Pregnant by AI	105	66.7	33.3	0	1.5	0.47
Calved by 2 yr of age	105	59.2	39.8	1.0	2.7	0.26

[a]Results for dichotomous reproductive traits are reported for a proportion of Brangus heifers used (n = 190).

Table 3. LSMEANS for serum concentrations of IGF-I and ages for the continuous reproductive traits age at first observed estrus and age at first calving by genotype for Brangus heifers.

	TT		CT		CC	
Trait	Mean	SE	Mean	SE	Mean	SE
Serum IGF-I, ng/mL	165.2[b]	5.6	195.9[c]	6.6	166.7	28.3
Age at first observed estrus, mo[a]	11.9	0.1	11.5	0.2	11.9	0.6
Age at first calving, mo[a]	23.7[c]	0.1	23.6[c]	0.1	25.6[b]	0.8

[a]Results for continuous reproductive traits are reported for a proportion of Brangus heifers expressing first observed estrus (36%) and calved by 2 yr of age (93%).
[b,c]Within a row, means without a common superscript letter differ, $P < 0.05$.

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SIRE AND DAM EFFECTS ON DISTRIBUTION PATTERNS OF COWS GRAZING MOUNTAINOUS RANGELAND[1]

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ABSTRACT: A preliminary study was conducted to determine the influence of sire and dam pedigree on the grazing distribution patterns of individual cows (n = 157). Cows were sired by Angus, Charolais, Piedmontese, and Salers bulls, and the dams of these cows were Herefords, Tarentaise and Hereford x Tarentaise crosses. Cow locations were recorded by horseback observers at 0630 to 0900 for 5 yr during the summer on foothill rangeland in northern Montana. The slope, horizontal and vertical distances from water were recorded for each cow at each location. These data were summarized by averaging the observations adjusted for pasture, year, and lactation status. Sire within breed had a significant effect ($P \leq 0.05$) on horizontal and vertical distance from water, but neither sire nor dam breeds were important factors in terrain use. Sire groups within a breed varied by as much as 625 ± 18 to 715 ± 37 m in horizontal distance from water and 50 ± 2 to 65 ± 4 m in vertical distance from water. The regression of observed vertical distance from water of daughters on dams was significant ($P = 0.01$). Across five dam breeds, an increase of 1 m of vertical distance traveled to water by the dam corresponded to a 0.22 ± 0.07 m increase in vertical distance traveled by the daughter. The regression of daughter on dam was not significant for slope use or observed horizontal distance from water. The observed differences among sire groups in terrain use suggest that cattle grazing patterns in mountainous rangeland may be inherited. The relationships between dams and daughters may be inherited or a reflection of learned behavior. Further evaluations of the genetic and environmental parameters associated with terrain use and grazing distribution of beef cattle appear warranted.

Key Words: Behavior, Genetic, Beef Cattle

Introduction

Many concerns with livestock grazing on mountainous rangeland are the result of uneven distribution patterns (Bailey et al., 1996). Cattle often congregate in portions of extensive pastures and graze forage to excessive levels while other areas receive little use (Pinchak et al., 1991). Concentrated grazing, especially in riparian zones, may reduce vegetative cover and increase soil erosion (Blackburn, 1984; Kauffman et al., 1983). If cattle spend more time grazing upland slopes farther from water, condition and function of riparian areas can be improved and wildlife habitat can be managed more effectively. The problem is determining the most efficient and cost effective method to modify grazing patterns and prevent animals from overusing preferred areas within pastures (Bailey, 2004).

Selecting cattle with desirable grazing patterns and culling cattle with undesirable grazing patterns has been suggested as a tool for improving distribution (Roath and Krueger 1982; Howery et al., 1996). Culling cows with undesirable grazing patterns is the most obvious application of this proposed technique, but more progress could be made if grazing distribution was used as a trait for sire selection. The objective herein was to determine if the sire and dam of a cow affected her grazing distribution patterns on foothill rangeland. If differences among sire groups are detected, this result would provide evidence that grazing distribution may be inherited. Observed relationships between daughters and dams would suggest that terrain use is inherited or a reflection of learned behavior.

Materials and Methods

Study Site. Research was conducted at the Thackeray Ranch located in the Bear's Paw Mountains approximately 25 km south of Havre, Montana. Lower elevations with gentle slopes were dominated by Kentucky bluegrass (*Poa pratensis* L.), and steep slopes (> 20º) were dominated by rough fescue (*Festuca scabrella* Torr.). Kentucky blue grass, rough fescue, bluebunch wheatgrass (*Pseudoregnaria spicata* [Pursh] A Love), and Idaho fescue (*Festuca idahoensis* Elmer) were dominant in the majority of areas in each pasture. Standing crop of grasses during the study averaged 1280 kg/ha. Grasses composed 59 to 86% of the total herbaceous standing crop during most years. Areas of trees and shrubs were limited and only 1 to 5 ha in size. Seven pastures were used in the study, and their size varied from 63 to 337 ha. Slopes within pastures were variable (0 to 107%), and vertical relief varied within pastures by 70 to 110 m. Average distance to water within pastures was at least 325 m and in one pasture it was 890 m.

Cattle. Cattle used in this study resulted from a crossbreeding experiment (Boss et al. 2001). Angus,

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Charolais, Piedmontese, and Salers sires were mated to Hereford, ¾ Hereford x ¼ Tarentaise , ½ Hereford x ½ Tarentaise, ¼ Hereford x ¾ Tarentaise and Tarentaise dams (Kress et al. 1996) to produce: Angus-, Charolais-, Piedmontese-, and Salers-sired cows. The above matings result 20 different combinations of cow breed. Cow age varied from 2 to 4 yr. Cows began calving in early March and ended in late April.

Observations. Locations of cows were recorded during the summers from 1997 to 2001 by horseback observers (Bailey et al., 2001a). Cows were observed two to four times per week in two pastures during the summer grazing season. Topographic maps of each pasture were subdivided into 1 to 7 ha units based on slope, elevation, aspect and distance to water. Observers were trained to recognize the boundaries of all subunits within each pasture. Two to four observers on horseback rode pastures during a 1.5 to 2.5 h period during the early morning (0600 to 0900 hours) and attempted to record the location of every cow in the pasture. Observers rode close enough to each cow to observe her identification number from a plastic ear tag or a firebrand on the animal's hip. Observers recorded the activity and the pasture unit in which the animal was located. Most cattle were grazing during the morning observation period. The goal of these observations was to obtain a scan sample of individual animal locations of the entire herd. Ideally, scan samples should be instantaneous (Lehner, 1979). However, individually identifying and observing 40 to 160 animals instantaneously on extensive foothill rangeland pastures was not feasible. Observers recorded about 81% of the animals in the herd during an observation period.

To describe the terrain use in a pasture, average slope and distance to water (horizontal and vertical) were calculated for each pasture subunit. For each cow, all location data collected in a pasture during a grazing season (4 to 6 wk period) were pooled and used to determine the average slope, horizontal and vertical distance to water of observed cow locations in a pasture (Bailey et al., 2001a).

Statistical Analysis. In order to combine terrain use of individual cows that grazed as many as seven pastures over 5 yr, a statistical model that included year, pasture, and lactation status (dry or lactating) as fixed effects was fit to location data for each cow. The residuals from this model were used to calculate least-squares means of slope use, horizontal distance to water and vertical distance to water for individual cows. Residuals allowed us to adjust terrain use attributes for differences in grazing patterns that resulted from year to year variation in water and forage production, differences in terrain and forage production among pastures, and accounted for lactation status in a single value for each cow. Non-lactating cows typically use steeper slopes and travel farther horizontally and vertically from water than lactating cows (Bailey et al., 2001a). These adjusted values were used as dependent variables for subsequent analyses. Positive values indicate terrain use that was greater than average, while negative values reflected below average terrain use.

Cows used in the analyses were from Hereford, Tarentaise, or Hereford x Tarentaise cross dams that had been observed for at least 1 year in pastures at the study site. Terrain use of the dams was calculated and adjusted using the same methods as the daughters. In addition, cows sired by bulls with only one or two daughters were also excluded from the study. A total of 157 cows were used in the analyses.

To evaluate the effect of sire on terrain use of cows, we used a mixed model that included sire breed, dam breed, and the birth year of the cow as fixed effects and sire within breed as a random effect (Littell et al., 1996). A separate fixed effect model that included sire breed, sire within breed, dam breed and cow birth year was used to calculate least-squares means of sire groups for visual comparison purposes only. This fixed effect model was not used to evaluate if terrain use was affected by the cow's sire.

To evaluate the relationship between daughters and dams for terrain use, we used a model that included sire breed, dam breed and birth year of the daughter as fixed effects and the least-squares mean of the terrain use attribute for the daughter's dam (residual adjusted for year, pasture and lactation status) as a covariate. This dam terrain use covariate was nested within dam breed.

Results and Discussion

Effect of Sire Breed. Sire breed did not affect terrain use in this study. In previous research (Bailey et al., 2001b, VanWagoner et al., submitted), the horizontal distance from water of observed cows differed among sire breeds. Cows from Piedmontese and Charolais bulls traveled farther horizontally from water than cows from Angus bulls. Cows from Piedmontese sires traveled farther vertically from water than cows from Angus sires (Bailey et al., 2001b). In this study, least-squares means of sire groups followed the same trends as these previous studies for horizontal and vertical distance to water (Table 1), but with the limited number of sires within breed in the error term (17 df) the differences were not significant.

Although this study does not show any effect of sire breeds on terrain use, it is not directly comparable to the previous studies of Bailey et al. (2001b) and VanWagoner et al. (submitted). In the previous studies, more animals were included in the analyses. Cows from sires with two or less daughters and cows whose dams were not observed were not excluded from previous analyses. In addition, variation between cows within sire breeds was used as the error term to test for differences between sire breeds in previous studies (Bailey et al. 2001b; VanWagoner et al., submitted), and in this study the variation between sire groups within sire breed was used as the error term.

Effect of Dam Breed. Dam breed did not affect terrain use in this study. Bailey et al. (2001a) reported that Tarentaise cows traveled farther vertically from water than Hereford cows. Terrain use of cows that were ½ Hereford and ½ Tarentaise were not different from either Herefords or Tarentaise. With only half of the genotype coming from the dam and the limited number of cows in

the study, it is not surprising that dam breed was not an important factor in terrain use in this study.

Table 1. Least-squares means for terrain use of sire breeds using values adjusted for pasture, year and lactation status (residuals)[1,2].

Sire breed	n[3]	Horizontal distance to water, m	Vertical distance to water, m	Slope use, %
Angus	43	-11.8	-1.3	-0.1
	(5)	(12.5)	(1.4)	(0.3)
Charolais	30	22.1	0.2	-0.5
	(5)	(14.2)	(1.6)	(0.3)
Piedmontese	45	20.9	2.7	0.2
	(3)	(14.4)	(1.6)	(0.3)
Salers	39	6.9	0.4	0.0
	(7)	(12.6)	(1.4)	(0.3)

[1] Positive values represent terrain use greater than the average of all observations, while negative numbers reflect terrain use less than the observed average.
[2] Standard errors are listed below in parentheses.
[3] Number of cows observed from each sire breed are listed above, and the number of sires within each sire breed are listed below in parentheses.

Sire within breed affected (P = 0.05) the observed horizontal distance from water (Figure 1). There was a large variation among sires within breed in the least squares means for horizontal distance from water, except for Angus where five of the six groups were below average (negative) for observed cows. Sire within breed also influenced (P = 0.03) the vertical distance that cows were observed from water (Figure 2). Sire within breed did not affect slope use.

The variation among sires within breed observed in this study suggests that terrain use may be inherited. Unfortunately the size of this data set is limited, and limited number of sires and number of daughters from each sire makes it difficult data to estimate genetic parameters from this herd. These cows were developed as part of a crossbreeding study, and the large number of breeds evaluated also restricts its use for genetic parameter estimation. However, grazing distribution observations from this cow herd are unique. We are not aware of other research where grazing patterns of a cow herd have been recorded on an individual basis. Despite its limitations, this study supports the hypothesis that cattle grazing distribution is at least partially inherited.

Relationship between Daughters and Dams. For vertical distance from water, there was a relationship between daughters and dams (P = 0.006). Dams that traveled farther vertically from water and used higher elevations had daughters that also used higher terrain (Table 2). There was no relationship between daughters and dams for horizontal distance from water and slope use.

Similarity of daughters and dams can result from inheritance and learned behaviors. Howery et al. (1996) found that different cows grazed in different areas within extensive mountain pastures during summer and they used the same areas during the next four summers. These authors (Howery et al., 1998) then studied the habitat preferences of offspring from these cows. When evaluated after weaning at 2 and 3 years of age, daughters preferred the areas that their mothers preferred. This study also included a cross-fostering experiment where female calves from cows that preferred one area of the habitat (drainage) were reared by unrelated cows (foster mothers) that preferred a different habitat (adjacent drainage). Cross-fostered offspring preferred the areas that the cow they were reared with (foster mother) spent more time in. Experiences that occur early in life appear to affect grazing distribution patterns later in life.

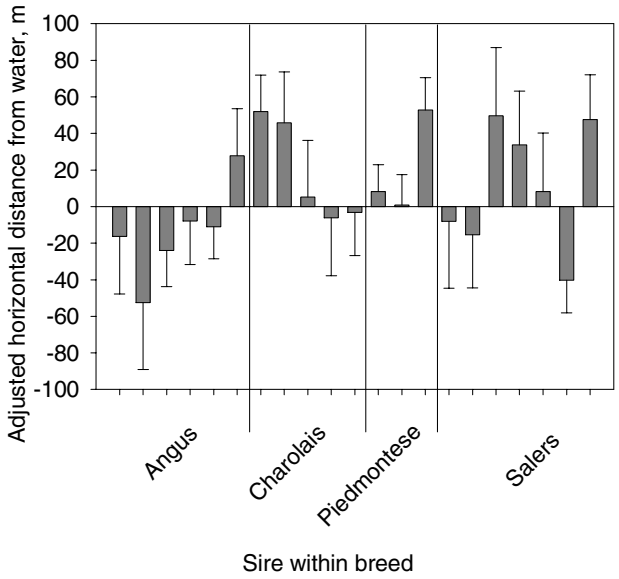

Figure 1. Adjusted least-square means of daughters of sires within breed for observed horizontal distance from water. Values were adjusted for pasture, year and lactation status (residuals). Positive values indicate that daughters of a sire were observed farther from water than average of all observed cows, while negative values indicate that a sire's daughters were observed closer horizontally from water than average. Sire within breed affected (P = 0.05) horizontal distance from water using a mixed model, but the least-squares means and standard errors shown here were calculated from a fixed model.

Implications

The variation between groups of daughters sired by different bulls within breeds for horizontal and vertical distance from water suggests that terrain use of mountainous rangeland may be inherited in beef cattle. The relationship between daughters and dams for the vertical distance from water and corresponding use of higher elevations may reflect inheritance and learned behavior. Further evaluations of the genetic and environmental parameters associated with terrain use and grazing distribution of beef cattle appear warranted.

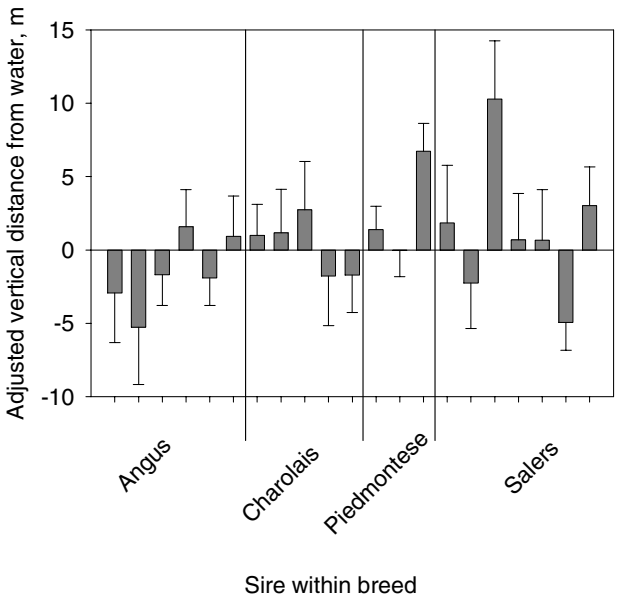

Figure 2. Adjusted least-square means of daughters of sires within breed for observed vertical distance from water. Values were adjusted for pasture, year and lactation status (residuals). Positive values indicate that daughters of a sire were observed farther vertically from water than average of all observed cows, while negative values indicate that a sire's daughters were observed closer average. Sire within breed affected ($P = 0.03$) vertical distance from water using a mixed model, but the least-squares means and standard errors shown here were calculated from a fixed model.

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Table 2. Regression of daughter on dam within dam breed for the vertical distance from water using values adjusted for pasture, year and lactation status (residuals).

Dam breed	Estimated coefficient	SE	P-value
Hereford	0.387	0.176	0.05
¾ Hereford ¼ Tarentaise	0.155	0.124	0.21
½ Hereford ½ Tarentaise	0.183	0.161	0.26
¾ Tarentaise ¼ Hereford	0.556	0.192	0.004
Tarentaise	0.439	0.309	0.16

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Weaning Weight Heritability Estimates In Different Environments

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ABSTRACT: Heritability estimates for weaning weight have been published for many years. Research has shown beef cattle weaning weight heritability estimates differ depending on the sex of the individual. Differences in heritability estimates for dairy cattle production traits also depend on levels of production of the herd. The purpose of this study was to estimate heritability for weaning weight recorded under two environments and to identify whether or not a single heritability estimate is appropriate for the differing environments experienced by cows from year to year. Data used in this analysis was obtained from the Red Angus Association of America and consisted of 93,740 cow weight observations and 41,693 calf weaning weight observations. A dam's change in weight from one year to the next was used to classify each calf's weaning weight into one of two environmental groups. Least square, cow age solutions were obtained from a cow weight (n = 93,740) analysis using a repeated measures, animal model. Cow age (1 to 18) solutions were used to determine the expected change in cow weight from one year to the next. If a cow met or exceeded this expected weight change, their calf's weaning weight associated with the end of this time frame was classified as a good weight, or their observation occurred in a better than average environment. If not, their calf's corresponding weight was classified as having occurred in a poorer than average environment. Classified calf weight observations (N = 41,693; n_{good} = 27,693; n_{bad} = 14,000) were used to estimate variance components as two distinct traits in a multivariate, multiple component, animal model. Heritability estimates of 0.20 ± 0.02, 0.20 ± 0.03, 0.27 ± 0.02, and 0.29 ± 0.02 were obtained for weaning weight good direct, bad direct, good maternal and bad maternal respectively. Since the weaning weights were classified according to the dam's performance, a larger difference in maternal heritability was expected. The small difference between the two may be attributed to incomplete identification of the calf's environment.

Key words: Beef Cattle, Heritability, Weaning Weight, Environment

Introduction

Beef cattle heritability estimates have been available to producers and researchers alike for many years. These estimates, while widely accepted, may not be fully appropriate in changing environments experienced by cows. Drought conditions and the rising cost of feed have placed an enormous burden on today's modern beef cow. As a result, beef females have an almost an equal probability of being asked to raise a calf in restrictive environment as in a non-restrictive one.

Many studies have been published that demonstrate genotype by environment interactions for the different types of livestock. In beef cattle, one of the classic studies reporting these types of interactions transported Hereford cattle from Montana to Florida and vice versa to study the effects on reproductive, birth, weaning, and post-weaning traits (Butts et al., 1971; Koger et al., 1979 Burns et al., 1979; Pahnish et al., 1983; Pahnish et al., 1985). Those studies indicated that G x E interactions do exist, and play a significant role in beef cattle production. In dairy cattle, heterogeneity of variance components for production traits recorded in differing environmental conditions has been reported. DeVeer and VanVleck (1987) showed increases in heritability for first lactation milk yield as production levels increase. Different heritability estimates were also reported between registered and non-registered Holsteins by Wade and VanVleck (1989).

Other studies have shown no difference between environmental conditions and heritability. For instance, Carabaño and others (1990) looked at genotype by environment interactions for milk and fat production across regions of the United States and found no differences among sire and residual variances.

The purpose of this study was to estimate heritability of weaning weights recorded in restrictive and nonrestrictive environments and to identify whether or not a single heritability estimate is appropriate for the differing environmental conditions experienced by cows from year to year.

Materials and Methods

Two analyses were performed. Least square means for cow weight by cow age were used to classify cows into one of two environments. If a cow's weight change from one year to the next met or exceeded their expected weight change as calculated from the least square mean age solutions, their calf's weaning weight observation corresponding to the end of this time period was classified as an observation in a good environment. Otherwise, their calf's observation was classified as occurring in a poor environment. The classified weaning weights were then analyzed in a separate analysis to obtain heritability estimates

for weaning weight observations recorded in two environments.

Cow Weight Analysis

Cow weight (n = 93,740) observations obtained from the Red Angus Association of America (**RAAA**) were analyzed using a single trait, repeated measures, animal model. A three generation pedigree consisting of animal ID, sire ID and Dam ID was built from the final data file used in the analysis. This pedigree consisted of 67,594 individuals and their parents, and was used to build the inverse of the numerator relationship matrix. Fixed effects included in the analysis were contemporary group, year of measure (1981 to 2004), and 18 levels of cowage in years of age (1 to 18). Cow weight contemporary group included the weaning contemporary group of the calf and the breed composition of the dam. Random effects included a direct animal effect and a permanent environmental effect. The cow weight model used in the analysis is represented in matrix form below as

$$y = Xb + Zu + Wp + e$$

where **X**, **Z** and **W** were incidence matrices relating observations in **y** to fixed (**b**) random (**u**) and permanent environmental effects (**p**), and **e** was a vector of random residual errors. Random effects in the model were assumed to have means equal to zero and variances represented below.

$$\text{var}\begin{bmatrix} u \\ p \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_u^2 & 0 & 0 \\ 0 & I_D\sigma_p^2 & 0 \\ 0 & 0 & I_N\sigma_e^2 \end{bmatrix}$$

In this set of equations, **A** was Wright's numerator relationship matrix, $\mathbf{I_D}$ and $\mathbf{I_N}$ are identity matrices with the length of the number of females with mature weight observations.

The cow weight linear model presented above was implemented and analyzed using various tools found in the Animal Breeders Toolkit (Golden et al., 1992).

Weaning Weight Analysis

The final weaning weight file used in the analysis contained 41,693 records (N = 41,693; n_{good} = 27,693; n_{bad} = 14,000). From this final file, a 2 generation pedigree consisting of 75,254 individuals with their corresponding sires and dams was built. Weaning weight observations were analyzed using a bivariate multiple component full animal model that contained the fixed effects of calf weaning contemporary group, and a sex by age of dam interaction for both traits. The model used in the analysis is represented in matrix form below.

$$y=Xb + Zu + e=\begin{bmatrix} y_{w_g} \\ y_{w_b} \end{bmatrix}=\begin{bmatrix} X_{w_g} & 0 \\ 0 & X_{w_b} \end{bmatrix}\begin{bmatrix} b_{w_g} \\ b_{w_b} \end{bmatrix}$$
$$+\begin{bmatrix} Z_{w_g} & Z_{m_g} & 0 & 0 \\ 0 & 0 & Z_{w_b} & Z_{m_b} \end{bmatrix}\begin{bmatrix} u_{w_g} \\ u_{m_g} \\ u_{w_b} \\ u_{m_b} \end{bmatrix}+\begin{bmatrix} e_{w_g} \\ e_{w_b} \end{bmatrix}$$

In the equations above, **X** and **Z** were design matrices that related the fixed effects in **b** and random effects in **u** ($\mathbf{u_d}$ as weaning weight direct and $\mathbf{u_m}$ as its maternal component) to the observations in **y**, and **e**, a vector of random residual error terms. The subscripts in the model equation above denote the observations and model terms that are relative to weaning weight records occurring in good environments ($\mathbf{w_g}$) versus poor environments ($\mathbf{w_b}$). Similar to the cow weight analysis, the random effects were assumed to have means of zero and variances represented below.

$$\text{var}\begin{bmatrix} u_{w_g} \\ u_{m_g} \\ u_{w_b} \\ u_{m_b} \end{bmatrix} = \begin{bmatrix} \sigma^2_{w_g}A & \sigma_{w_g,m_g}A & \sigma_{w_g,w_b}A & \sigma_{w_g,m_b}A \\ \sigma_{m_g,w_g}A & \sigma^2_{m_g}A & \sigma_{m_g,w_b}A & \sigma_{m_g,m_b}A \\ \sigma_{w_b,w_g}A & \sigma_{w_b,m_g}A & \sigma^2_{w_b}A & \sigma_{w_b,m_b}A \\ \sigma_{m_b,w_g}A & \sigma_{m_b,m_g}A & \sigma_{m_b,w_b}A & \sigma^2_{m_g}A \end{bmatrix}$$

and

$$\text{var}\begin{bmatrix} e_{w_g} \\ e_{w_b} \end{bmatrix} = \begin{bmatrix} \sigma^2_{e_{w_g}}I_g & 0 \\ 0 & \sigma^2_{e_{w_b}}I_b \end{bmatrix}$$

In the above equations, **A** denotes Wright's numerator relationship matrix. $\mathbf{I_m}$ and $\mathbf{I_b}$ are identity matrices with the length of the number of good weaning observations and the number of poor observations respectively. Residual covariances were set to 0 because no individuals had both good and poor observations recorded.

Using the linear model above, weaning weight variance and covariance estimates were obtained from average information REML using ASREML (Gilmour et al., 2002).

Results and Discussion

Cow Weight Analysis

Yearling weights of each individual with a recorded cow weight were included in the analysis to obtain an expected weight change from 1 to 2 years of age. This was done so calf weaning observations from 2 year old dams could be classified into a good or poor environment and subsequently included in the weaning weight analysis. The summary statistics for cow weight observations are presented in Table 1.

Table 1. Cow weight summary statistics

	N	Mean	Min[a]	Max[a]	SD
Cow Weight	41,693	487.52	194.1	904.9	112.8

[a] Min = Minimum Value, Max = Maximum Value

The summary statistics above represent the raw cow weights that are included in the analysis, across the ages from 1 year to 18 years. The minimum weight (194.1 kg) is a yearling weight that qualified for inclusion based on the RAAA data validation filters.

The least square solutions for expected cow weight change are presented in Table 2. For example, if a cow's weight change from 4 to 5 years of age was greater than 20.06 kg the corresponding calf's weaning weight observation for the cow at 5 years of age was classified as occurring in a good environment. According to the weight change solutions, cows initially lost a small amount of weight from 1 to 2 years of age. They then gained weight up to 7 years of age at which point they experienced weight loss throughout the remainder of their life.

Weaning Weight Analysis

Classification of weaning weight observations resulted in 27,693 good observations and 14,000 poor observations (Table 3).

Table 3. Summary Statistics for weaning weight observations

	N	Mean	Min[a]	Max[a]	SD
Overall[b]	41,693	244.73	107.9	433.2	35.17
Good[c]	27,693	244.15	107.9	433.2	35.41
Poor[c]	14,000	245.88	112.5	400.5	34.66

[a] Min = Minimum Value, Max = Maximum Value
[b] Both good and poor weaning weight observations combined
[c] Good and poor observations separated.

These simple summary statistics show virtually no difference in weaning weight observations between the overall mean, good and bad weaning weight classifications.

Heritability (diagonal), covariance (above diagonal) and genetic correlation (below diagonal) estimates are shown in Table 4. No significant differences were seen between the direct and maternal heritability for both traits. However, we also observed no differences between the maternal estimates of heritability in each environment. The lack of differences may be due to the inability to appropriately account for the dam's change in weight. Error resulting from the estimation of the least square cow age solutions may have inappropriately classified calf weaning weight observations.

Looking at genetic correlations between the traits, weaning weight maternal appears to be more strongly tied to its direct counterpart in good environments than in bad environments. This is probably due to the fact that in poorer environments, the dam doesn't expend as much energy in milk as she would in better a better environment. This would result in the calf relying less on his or her dam to provide these nutrients,. There appears to be no differences between the correlation between the direct good effect, poor maternal effect and vice versa. A cow's calf's weaning observation may be classed differently one year to the next. Classifying weaning weights in this manner resulted in approximately the same number of cows moving from good to poor environments as there are cows moving from poor to good environments in one year to the next.

Implications

No differences were found for heritability estimates obtained for the two weaning weight traits. There may be no difference in variability due to genetics under those environmental conditions. Alternatively, this approach for classifying environments may not fully account environmental differences, and therefore may not classify the weaning weights properly. Future studies are currently in progress evaluating different methodologies to accounting for the dam's environment.

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Table 2. Cow least square expected age change solutions.

Age Range	Expected Weight Change
1 to 2	-1.26
2 to 3	16.31
3 to 4	35.78
4 to 5	20.06
5 to 6	10.15
6 to 7	5.66
7 to 8	-0.53
8 to 9	-1.59
9 to 10	-4.72
10 to 11	-4.47
11 to 12	-6.91
12 to 13	-9.61
13 to 14	-6.10
14 to 15	-0.34
15 to 16	1.87
16 to 17	14.05
17 to 18	-2.89

Table 4. Estimates of additive genetic covariances, heritability and genetic correlations among both weaning weight traits.

WWg_d[a]	**0.20 ± 0.021**	-80.8 ± 21.71	231.9 ± 24.05	-51.1 ± 23.82
WWg_m[a]	-0.29 ± 0.06	**0.27 ± 0.018**	-58.0 ± 24.24	332.0 ± 20.83
WWb_d[a]	0.97 ± 0.04	-0.21 ± 0.08	**0.20 ± 0.027**	-32.4 ± 27.29
WWb_m[a]	-0.18 ± 0.08	0.99 ± 0.02	-0.11 ± 0.09	**0.29 ± 0.024**

[a] Heritability (±SE) are indicated by bold type on the diagonal, genetic correlations (±SE) are below the diagonal, and additive genetic covariances (±SE) are above the diagonal.

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ECONOMIC EVALAUTION OF GENETIC DIFFERENCES AMONG ANGUS BULLS[1,2,3]

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ABSTRACT: Expected progeny differences (EPD) have been used as a basis for selection to improve individual traits. However, producers need strategies to aid in efficient multiple-trait selection. The Angus Sire Alliance progeny test breeding program was initiated to identify bulls that produce profitable progeny and to market those sires based upon the research results. Therefore, our objective was to develop a multiple-trait selection index to improve profit derived from Angus terminal sires and then to rank the sires based on results of a multi-trait genetic evaluation for profit. Angus sires were randomly mated to commercial Angus females in a designed progeny testing program. Data were from calves born in 1997 – 2000. Birth weight, weaning weight, post-weaning ADG, marbling score, yield grade, and DMI were phenotypes considered to determine differences in profitability among terminal sires. A multiple trait genetic evaluation was conducted to obtain BLUP of EPD for these traits. Relative economic values (REV) were calculated by approximating partial derivatives of profit with respect to the phenotypes using a bio-economic simulation model. Sire differences in profit per progeny were then estimated as the sum of the products of each trait EPD and its respective REV. There was a range of $37.62 profit per progeny between the highest and lowest ranking sires that were evaluated.

Key Words: Breeding objective, Selection index, Beef cattle

Introduction

Widespread use of EPD to identify candidates for selection has centered primarily on growth as shown by published genetic trends (AAA, 2002). Single trait selection for growth or composition traits could lead to undesirable correlated responses (MacNeil et al., 1984; Bullock et al., 1993; Meyer, 1993). Reproductive inefficiencies may result either from genetic covariation (MacNeil et al., 1984; Splan et al., 1998) or from an imbalance between nutrient requirements and availability (Buttram and Willham, 1989; Fiss and Wilton, 1992). Although Hazel (1943) proposed a multiple-trait selection procedure that weighted each trait by its relative effect on profit, producers have not had readily available tools to quantify the economic importance of various phenotypes or rank candidates for selection based on expected differences in profit derived from progeny. Thus, our objective was to determine potential genetic differences in profitability of progeny from Angus sires and to provide an economic evaluation of genetic differences among those sires.

Materials and Methods

Cattle Management

Sires being evaluated were mated with cows to create steer progeny consistent with the protocol recommended by the American Angus Association for evaluation of carcass traits in sire progeny testing programs. Angus breeders throughout the United States nominated bulls to be evaluated in the Angus Sire Alliance. At Circle A Angus Ranches in Huntsville, Iberia, and Stockton, Missouri, commercial Angus females were randomly mated to test candidates and reference sires using artificial insemination. Test and non-test sires were also used for natural-service matings. Calves were born each year from late-December to early-April over no more than 112 days in any given calving season. Progeny information was collected beginning at birth and included birth, weaning, backgrounding, and yearling weights. Calves were weaned at an average of 192 days.

Steers born in 1997 were backgrounded for 104 d before being transported to Supreme Cattle Feeders, Inc., Liberal, Kansas. Steers born in 1998, 1999, 2000 and 2001 were backgrounded for 95, 108, 131 and 92 d, respectively, before transport to Platte Valley Feeders, Kearney, Nebraska. Contemporary groups of steers were assigned to feedyard pens before transport. A contemporary group was

[1] Research conducted under a cooperative agreement between USDA, ARS and the Montana Agric. Exp. Sta. USDA, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer. All agency services are available without discrimination.

[2] Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Sta. or the authors nor imply its approval to exclusion of other products that may be suitable.

[3] We thank the management and staff of Circle A Ranches for their interest and assistance in conducting the study.

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defined as steers that had been together since birth and given an equal opportunity to perform. Fat thickness, LM area, and marbling of the steers born in 1998-2000 were determined by ultrasonography at approximately one year of age. Steers remained in their assigned pens until harvest.

Daily feed intake of individual animals was measured in contemporary groups of 96 steers using a Calan Broadbent Feeding System. Steers were trained and acclimated to the feeding system. After seven days, all steers were trained to eat through their assigned feeding door. Thus, initial weights were taken at this time and daily feed intake was recorded from this day to the end of the feeding period. A stepwise series of five finishing diets that were identical to the series of diets fed to the remaining test cattle were used throughout the finishing period. The afternoon before harvest, steers were weighed and then transported overnight to the packing plant for harvest and collection of carcass data.

Harvest date was determined to target a contemporary group to average 1.3 cm fat depth at the 12-13 rib and/or to avoid discounts for under- and over-weight carcasses. Regression of age on ultrasound fat depth was used to assist with determining harvest date. All steers from a contemporary group were harvested on the same day. The 1997 born steers were harvested at National Packing, Liberal, KS, on April 22, May 7, and June 1, 1998. The 1998 born steers were harvested on May 12 and 26, 1999, at Conagra Beef, Grand Island, NE. The 1999 born steers were harvested on April 19, May 17, and May 24, 2000, at Conagra Beef, Grand Island, NE. The steers born in 2000, were harvested on May 2, and May 9, 2001, at IBP, Lexington, NE. The 2001 born steers were harvested on April 16, April 23 and May 7, 2002, at Conagra Beef, Grand Island, NE. Carcass data were collected by experienced personnel from the University of Florida and USDA Grading Service and included: harvest date, hot carcass weight, marbling score, fat depth, LM area, and percentage kidney, pelvic and heart fat.

Genetic Evaluation

A 6-trait animal model was used to estimate genetic differences among sires evaluated in the Angus Sire Alliance and other animals that existed in the Circle A performance and carcass database. While cows used in the Angus Sire Alliance are “commercial Angus”, most had pedigree and performance data. Expected progeny differences were computed for birth weight, direct and maternal weaning weight direct, post-weaning ADG, marbling score, yield grade, and daily DMI of 35,648 animals. For birth weight, the model included fixed effects of birth contemporary group, sex, and age of dam and a random direct genetic effect. For weaning weight, the model included weaning contemporary group and sex fixed effects, a weaning age covariate, and random direct and maternal genetic effects, and a random permanent environmental effect of the dam. For postweaning ADG, the model included a fixed contemporary group effect and a random direct genetic effect. For daily DMI, the model included a covariate of on-test age, fixed effect of contemporary group, and a random direct genetic effect. For marbling score and yield grade, the model included fixed effects of harvest contemporary group and a harvest age covariate with a random direct genetic effect. Postweaning ADG was derived using estimates of final live weight from dressed weight of steers (assuming a 62% dressed weight; Boggs and Merkel, 1984) and weaning weight. Daily intake was converted to a 100% DM basis.

Genetic parameters used for the analysis are provided in Table 1. Genetic and environmental parameters were compiled from various sources including estimation from these data using MTDFREML programs developed by Boldman et al. (1993) and estimates reported by other researchers including AAABG Genetic Parameters (http://www.gparm.csiro.au), MacNeil et al. (1984), Wilson et al. (1993) and Bertrand (personal communication).

Contemporary groups were genetically tied through repeated records of dam and sire progeny over years. Most cows and several sires did not have progeny with feed intake observations. However, the genetic model used to calculate feed intake EPD relied on the existing relationships of intake with post-weaning gain, weaning weight with post-weaning gain, and the numerator relationship matrix to compute intake EPD for all animals. Similarly, all animals also have EPD for carcass traits.

Economic Simulation

Expected income and expense for a production system over a planning horizon were required. Cull cow price estimates were determined from USDA Market News ten year average from Sioux Falls and the Food and Agricultural Policy Research Institute ten year forecast for utility cows. Feeder steer price estimates were determined from ten year average Oklahoma City price estimates and forecasted ten year average, based on USDA Market News reports. Carcass quality grade, yield grade, and off-grade price estimates were based on National Carcass Premiums and Discounts for Slaughter Steers and Heifers as reported by USDA Market News service. Backgrounding and feeding cost estimates were based on ten year average Kansas State University Extension Monthly Performance, Cost of Gain, and Breakeven Prices.

Relative economic value (REV) was defined as the marginal change in expected profit per progeny from increasing a particular phenotype by one unit. Bio-economic simulations were performed using a modified version of the computer software described by MacNeil et al. (1994) with biological parameters derived from average performance of the steers (Table 2). The principle modification of the model described by MacNeil et al. (1994) was stochastic generation of phenotypes at harvest. Thus, in the simulations steers were valued based on a multivariate normal distribution of marbling, yield grade and carcass weight. There were 76 production and economic variables used in simulating performance of straightbred Angus. To calculate REV, profit generated in the initial simulation was compared to profit from a simulation in which one phenotype was perturbed by one unit. This derivation is an approximation of the differential of profit with respect to each phenotype. Sire differences in profit were estimated by computing the product of sire EPD

and the REV for each trait and summing across all traits to produce index values. The product of the REV and corresponding trait genetic SD indicated the relative magnitude of the economic weights.

Results and Discussion

Phenotypic characterization of progeny sired by the bulls evaluated is shown in Table 2. After editing and pooling data across years, there were 9,982 steers and heifers evaluated with weaning weight records from the progeny test program. Weaning weight ranged from 90.7 to 362.0 kg. Fat depth ranged from 2.5 to 35.6 mm and marbling score from Practically Devoid[10] to Abundant[80]. Hot carcass weight ranged from 215.9 to 450.4 kg and calculated USDA Yield Grade ranged from 0.5 to 6.4.

Phenotypes of the 381 steers from which intake data were collected are shown in Table 3. Feed consumed per kg gain ranged from 3.9 to 14.4 kg and combinations of post-weaning ADG and feed intake varied widely.

Ranges in EPD for the 363 sires evaluated are presented in Table 4. These EPD were calculated only from data produced in this study and are not to be confused with other EPD published by the American Angus Association. These ranges indicate considerable variation in genotypes among bulls for all of the economically important traits.

The REV estimated by simulation and their magnitudes relative to genetic variation in corresponding phenotypes are shown in Table 4. Based on these data, weaning weight was the single most important selection criterion followed by DMI. Postweaning gain and yield grade were somewhat less important than DMI, and marbling score and birth weight were least important to selection among Angus terminal sires for use on mature cows in a straightbred production system. Relative importance of EPD to the selection process may vary with production system, as illustrated by comparing these results with those of MacNeil (2005), wherein DMI was markedly less important in the South African situation than in the U.S. situation using Angus terminal sires for crossbreeding.

Of the 363 sires tested, there was a range of $37.62 in predicted profit per progeny between the highest and lowest ranked bulls. Thus, if the highest and lowest indexing bulls were used in a production system similar to the one described in the economic simulation, a difference in profitability of $37.62 per calf would be expected.

The breeding objective developed here typically rewards sires whose progeny exhibited more growth, less feed consumption, and had greater carcass yield. Differences in marbling and birth weight of progeny between sires in the first and tenth deciles of predicted profitability were relatively minor. However, selection index does reward sires appropriately when they have unusual genetic profiles. For example, the sire ranked third had EPD for birth weight, marbling score and yield grade in the respective first deciles and EPD for weaning weight in the seventy-second percentile of all sires evaluated using this terminal sire index.

Implications

If these bulls provide a sample of genetic variability that exists within the Angus breed, it is evident that wide differences exist in profit potential. Using an approach such as the one described here, added value can be attached to certain herd sire prospects if the genetics of the prospect can be accurately described. Commercial cattlemen should be encouraged to use a comprehensive approach to genetically alter profit rather than single trait selection or ad hoc independent culling levels.

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Table 1. Genetic parameter estimates used in prediction of EPD for Angus bulls evaluated in the Angus Sire Alliance[1]

Phenotypes	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1. Birth weight	0.41							
2. Weaning weight direct	0.48	0.23						
3. Weaning weight maternal		-0.26	0.27					
4. Weaning permanent environment				0.05				
5. Postweaning daily gain		0.26			0.36			
6. Daily dry matter intake					0.52	0.41		
7. Marbling score		-0.07					0.26	
8. USDA Yield Grade							0.15	0.22

[1] Estimates of heritability are on the diagonal and estimates of genetic correlation are below the diagonal

Table 2. Phenotypic characterization of steer progeny sired by Angus bulls evaluated in the Angus Sire Alliance

Phenotype	Mean	SD	Minimum	Maximum
Birth weight, kg	35.4	5.0	22.7	68.0
Weaning weight, kg	209.6	39.5	90.7	362.0
Weaning age, d	194.0	25.0	108.0	277.0
Postweaning gain[a], kg/d	1.32	0.18	0.50	2.04
Estimated slaughter weight[b], kg	549.3	53.5	348.4	726.2
Slaughter age, d	445	21	364	503
Hot carcass weight, kg	340.7	33.1	215.9	450.4
Fat depth, cm	1.42	0.46	0.25	3.56
Marbling score[c]	5.8	1.0	2.1	10.8
LM area, cm^2	76.1	9.0	40.0	117.4
USDA Yield Grade	3.4	0.7	0.5	6.4

[a] (Slaughter weight - weaning weight) / days
[b] Carcass weight/0.62
[c] 4.0 = $Slight^{00}$; 5.0 = $Small^{00}$; etc.

Table 3. Phenotypic characterization of steer progeny sired by Angus bulls that were individually fed and evaluated in the Angus Sire Alliance

Phenotype	Mean	SD	Minimum	Maximum
On-test BW., kg	381.0	38.1	233.1	507.1
Off-test BW., kg	562.9	54.9	381.0	756.6
Postweaning gain, kg/d	1.63	0.27	0.93	2.27
Dry matter intake, kg/d	9.2	1.0	5.4	11.9
Feed conversion, kg dry matter/kg gain	5.7	0.8	3.9	14.4

Table 4. Ranges in EPD of sires (n = 363) evaluated in the Angus Sire Alliance and relative economic values (REV) for economically important phenotypes[1]

Phenotype	Minimum EPD	Maximum EPD	REV	Genetic SD	Relative REV
Birth weight, kg	-2.3	4.3	-1.86	3.20	5.94
Weaning weight, kg	-2.7	24.0	0.90	18.94	17.04
Postweaning gain, kg/d	-0.11	0.07	104.29	0.11	11.26
Dry matter intake, kg/d	-0.27	0.37	-22.05	0.64	14.11
Marbling score	-0.35	0.90	13.54	0.51	6.90
USDA Yield Grade	-0.31	0.26	-35.28	0.33	11.58

[1]EPD are calculated only from data in this study and are not to be confused with EPD from American Angus Association.

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SEGREGATION OF DISPOSITION SCORES AMONG FAMILIES OF *BOS INDICUS-BOS TAURUS* CROSSES

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ABSTRACT: A population of 11 F_2 Nellore-Angus full sib families was established in 2002 through embryo transfer from four F_1 sires and 11 F_1 dams; four half sib families were also created with the same sires bred to F_1 Brahman-Hereford and Brahman-Angus dams. Calves were spring- and fall-born beginning in spring 2003. This population was established to find QTL associated with cow productivity, feed intake and efficiency in steers, and disposition in steers and females. Calves are scored at approximately 8 mo of age by four evaluators. Components of temperament (aggressiveness, flightiness, nervousness, gregariousness) and overall temperament were each scored on a 1 to 9 scale. Two animals were placed in an alley with two evaluators at each end approximately 20 m apart. After one to two minutes, one animal was allowed back into the holding pen, and the remaining animal was kept in the alley, scored, and released into a different pen. Approximately 15 animals at a time were placed in the holding pen immediately prior to evaluation to minimize handling before evaluation. Disposition scores were averaged across evaluator before analyses. This report includes 212 animals from three calf crops. Data were analyzed through GLM procedures with independent effects of sex, birth season within year, family, family x sex and the regression on sequence number within pen-season-year combination. Disposition components were highly correlated (r of .78 to .98). Although all disposition traits were analyzed, only overall disposition is discussed here. Sex and season within year did not affect overall disposition; however, evaluation sequence (P = .002), family (P = .001) and family x sex (P = .06) did account for differences. The regression on sequence number ranged from .01 to .26 of a score per evaluation sequence across the seven pen-season-year combinations. Family least squares means ranged from 3.4 to 6.4 (where 1 is completely docile, and 9 is crazy). In 10 families females were numerically higher than males, whereas in five families males were numerically higher, but most differences were not significant. This cattle population appears to illustrate genetic differences for temperament.

Key Words: Cattle, Disposition, Genetic differences

Introduction

It has been reported for several many years that there are underlying genetic differences for disposition (temperament) in cattle in a several different breeds (Shrode and Hammack, 1971; Stricklin et al., 1980; Hearnshaw and Morris, 1984; Le Neindre et al., 1995) with heritability estimates ranging from approximately .20 to approximately .50 across studies. Furthermore, differences in disposition where females have less desirable scores than male contemporaries have also been reported in several cattle populations (Shrode and Hammack; Stricklin et al., 1980; Voisinet et al., 1997; Gauly et al., 2001). The objectives of this paper are to present preliminary results on family differences for disposition from a project designed to identify QTL for cow productivity and steer feedlot and carcass traits.

Materials and Methods

Cattle in this study are produced for the "McGregor Genomics Project" where the objectives are to map QTL associated with (1) cow reproduction/productivity traits, (2) feed intake and efficiency in steers, and (3) study disposition (temperament) effects upon these productivity traits. The population is based on full-sib families of F_2 Angus-NelloreNellore-Angus produced through embryo transfer for spring- and fall-born calves. Four F_1 sires and 11 F_1 donor dams have been utilized. The goal is to produce 40 progeny per full-sib family. Additionally, the four sires are also being used to produce additional half-sib families through AI from F_1 Brahman-Angus and Brahman-Hereford dams for spring-born calves. Families coded as 70 through 83 are full-sib families, and families 95 through 98 are half-sib families. Recipient cows that are approximately one-half Brahman and one-half British are used to produce the embryo transfer calves.

All calves are evaluated for disposition score four weeks after weaning at approximately 8 mo of age. Steers are also scored as yearlings by a single evaluator, and they are assigned a subjective chute score for temperament immediately prior to harvest. Females will be assigned a subjective temperament score annually as caretakers obtain calf birth weights. Four evaluators assign disposition scores post-weaning, where two evaluators are located at each end of an alley that is approximately 4 m wide and 23 m long, and the pair of evaluators are approximately 20 m apart. Animals are kept adjacent to the evaluation alley in a holding pen with two calves at a time brought into the alley. After approximately two minutes, one animal is cut back into the holding pen, and the animal remaining in the alley is then scored and released into another holding pen. Each animal is scored on a 1 to 9 scale for aggressiveness, nervousness, flightiness, gregariousness and overall disposition where higher scores represent undesirable characteristics. Aggressiveness refers to the animal's

willingness to hit evaluators (1 = non-aggressive). Nervousness refers to animals pacing, running, shaking, vocalizing, etc. (1 = completely calm). Flightiness refers to the animal's attempt to get away from handlers (1 = totally quiet). Gregariousness refers to an animal's desire to get back to the group from which it came (1 = totally willing to be separated). Overall disposition is scored as a separate trait (not an average of other component traits) where 1 = completely docile and 9 = crazy.

In the first group to be scored (spring-born 2003), all animals were kept as one group in the holding pen before evaluation, and evaluators noticed that some animals were brought into the alley many times before they were actually scored. There was a significant correlation (r = .49) between sequence number and overall disposition score. Consequently, with the Fall-born 2003 calves, smaller groups of approximately 15 animals were kept in the holding pen immediately prior to evaluation to minimize the number of times an individual entered the evaluation alley before being scored. With this group of animals the correlation between sequence number and overall disposition score was not significant (r = -.04), and this approach continues to be used with subsequent groups. The summary of the five disposition traits is presented in Table 1.

The mean disposition scores from the four evaluators were used in statistical analyses. Although all component traits were analyzed, overall disposition score is the focus of this paper, as these traits are all highly correlated. Mean disposition score was studied through analysis of variance (PROC GLM in SAS) with a model that included independent effects of sex, family, family × sex, season-birth year combination, and the regression on sequence number within pen. Family was considered a random effect, and the family × sex term was used as the error term to test for family differences. Simple linear correlation analysis was also utilized to study relationships among the five disposition component traits.

Results and Discussion

Simple linear correlations among the five component disposition traits across all animals are presented in Table 2. Although these traits are highly related, the correlations involving aggressiveness with the other traits seem to be lower than correlations involving other pairs of traits.

Effects of calf sex (P = .16) and season of birth (P = .84) did not influence overall disposition score. However, family (P = .06), family × sex (P = .06) and the regression on sequence number within evaluation pen (P < .001) were important sources of variation for overall disposition score. The coefficients for regression of disposition score on sequence (for each season-year-pen combination) were .05 ± .01 (spring-2003-1), .26 ± .09 (fall-2003-1), .09 ± .04 (fall-2003-2), .03 ± .02 (fall-2003-3), .08 ± .06 (spring-2004-1), .05 ± .02 (spring-2004-2), .03 ± .01 (spring-2004-3), and .01 ± .01 (spring-2004-4).

Least squares means for sex and family are presented in Table 3. Due to most families having small numbers of progeny at this point, many are not statistically different. However, there does seem to be substantial underlying genetic differences both within and between some families in this population that could provide for mapping of disposition QTL. For example, families 74 and 83, which showed the most difference in disposition score, are by the same bull (Sire 437J). This sire was also used to produce families 75, 81 and 97. The sire 297J was used to produce families 70, 71, and 95, while sire 432H was used to produce families 72, 73, and 96, and sire 551G was used to produce families 76, 77, 80, and 98. How informative these parents are for QTL mapping of disposition is yet to be determined, but they appear promising at this point. A divergent cross between *Bos indicus* x *Bos taurus* cattle was chosen as the resource population to maximize heterozygosity of QTL within the population. Angus are well known for relatively quiet disposition, while Nellore are generally thought to be more nervous. Therefore, in crosses between these breeds genes affecting disposition should be segregating. Hiendleder et al. (2003) have mapped three QTL for temperament in German dairy cattle, and Schmutz et al. (2001) have reported six potential QTL for behavioral traits in Canadian beef cattle; Brodkin et al. (2002) have identified two QTL for aggressiveness in mice.

Least squares means for family × sex combinations are presented in Table 4. At this point in time there are considerably more male calves that have been produced overall and within many families, which could simply be sampling variation, but it is curious, and this will be monitored as the study progresses. The only family which had a statistically significant difference between sexes was family 77. The investigation of the family × sex interaction comes from previous studies where in *Bos indicus-Bos taurus* crosses there has been increased birth weight and gestation length when *Bos indicus* influence is inherited solely through the sire, and the increased birth weight has been highly exaggerated in male calves, even when produced through embryo transfer (Amen et al., 2004). As a result, genotypes that are homozygous for Angus and Nellore alleles of origin as well as alternative heterozygotes (NA vs. AN, *Bos indicus* allele from sire or dam, respectively) will be investigated for several traits in this project including disposition. Furthermore, Voisinet et al. (1997) speculated that sex differences in temperament might be more pronounced in some breeds than others. Also, there have been some reports where *Bos indicus* crossbred cattle have had less desirable disposition scores than purebred contemporaries (Bonsma 1975a,b as cited by Grandin and Deesing, 2005; Riley et al., 2004). These types of reports make the study of underlying genetic mechanisms associated with disposition in cattle both more intriguing, and potentially more complicated.

Summary

Several previous reports have well-established that disposition (temperament) in cattle has a moderate degree of heritability within breeds, and that significant differences between breeds also exist. Furthermore, it has been reported in many studies that female cattle have less desirable (more excitable) disposition scores as compared to their male contemporaries, but the difference in

disposition between sexes has been speculated to vary across populations. Although results from this project are preliminary, it appears that this resource population of *Bos indicus-Bos taurus* should be useful to map QTL for disposition in cattle.

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Table 1. Summary of disposition scores averaged across four evaluators

Trait	Mean	SD	CV	Range
Aggressiveness	2.8	1.61	.58	1.0 – 7.3
Nervousness	4.2	1.76	.42	1.0 – 7.0
Flightiness	4.0	1.86	.47	1.0 – 7.5
Gregariousness	4.0	1.75	.44	1.0 – 7.3
Overall	3.8	1.79	.47	1.0 – 7.5

Table 2. Simple linear correlations between disposition component traits.

Trait	NERV	FLIGHT	GREG	Overall
AGGR	.824	.846	.793	.890
NERV		.968	.945	.976
GREG			.943	.974
				.955

Table 3. Least squares means for overall disposition score by sex and family

Effect		n	Score
Calf sex			
Female		78	4.8 ± 0.39
Male		134	4.4 ± 0.35
Family	Sire		
70	297J	17	4.2 ± 0.64[b]
71	297J	18	3.6 ± 0.64[b]
72	432H	20	4.9 ± 0.62[ab]
73	432H	8	4.6 ± 1.01[ab]
74	437J	7	6.4 ± 0.92[a]
75	437J	11	4.1 ± 0.75[b]
76	551G	7	3.4 ± 1.00[b]
77	551G	12	4.9 ± 0.85[ab]
80	551G	17	4.8 ± 0.67[ab]
81	437J	25	6.3 ± 0.59[a]
83	437J	5	3.2 ± 1.01[b]
95	297J	18	4.4 ± 0.65[ab]
96	432H	24	3.9 ± 0.62[b]
97	437J	8	5.7 ± 0.91[ab]
98	551G	15	5.1 ± 0.79[ab]

[a,b]Least squares means with different superscripts differ ($P < .05$).

Table 4. Least squares means (LSM) and standard errors for overall disposition score by family × sex combinations

Family-sex	n	LSM	SE
70 – F	7	4.2	0.63
70 – M	10	4.3	0.62
71 – F	8	3.9	0.66
71 – M	10	3.3	0.57
72 – F	10	5.3	0.58
72 – M	10	4.6	0.59
73 – F	2	4.2	1.22
73 – M	6	5.0	0.72
74 – F	3	6.7	0.95
74 – M	4	6.0	0.89
75 – F	5	4.9	0.77
75 – M	6	3.4	0.71
76– F	2	2.6	1.21
76 – M	5	4.1	0.77
77 – F	5	6.7	0.87
77 – M	7	3.2	0.71
80 – F	7	4.8	0.65
80 – M	10	4.7	0.63
81 – F	7	6.7	0.66
81 – M	18	5.9	0.45
83 – F	2	3.6	1.12
83 – M	3	2.9	0.95
95 – F	9	4.4	0.62
95 – M	9	4.3	0.62
96 – F	6	3.3	0.70
96 – M	18	4.5	0.49
97 – F	2	5.5	1.13
97 – M	6	5.8	0.70
98 – F	3	5.6	0.98
98 – M	12	4.5	0.56

ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS OF POSTWEANING GROWTH TRAITS IN LIMOUSIN CATTLE IN SAMALAYUCA, MEXICO.

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ABSTRACT: Data came from a herd of Limousin cattle located at the north of México. Progeny (n=17 and n=24) calves from heifers and mature cows, respectively of 41 of a total of 51dams involving inheritance of Limousin mated to Limousin sires (n=9) were used. The objective was to estimate heritability direct, and phenotypic parameters for weight at 365-d **YWT**, total feed consumption **TFC**, average daily gain **ADG**, and feed per unit of gain **FUG**, during 112 d test. Separate analysis for each trait used least squares mixed model SAS (1989). The analytical model included**:** year of birth, age of dam, sex of the calf, with date of birth as a covariate to adjust a common age as fixed effects**;** sire and residual as random. Mean weight to initiate the 112 d performance test was 232.14 kg. Mean **YWT** was 364.76 kg. Mean value of **TFC** per animal during 112 d test was 841.56 kg. Mean weight of gain per animal at the end of 112 test was 159.13 kg. Mean weight gain at the end of 112 d test ranged from 122.08 to 183.04 kg. The **ADG** was 1.351 kg. The **ADG** ranged from 1.09 to 1.642 kg. Mean value of **FUG** was 7.514 kg. The **FUG** ranged from 6.4 to 8.8 kg. The estimated heritability values (h^2=0.33±.04, h^2=0.39±.05, h^2 =0.44±.06, and h^2=0.37± .04) corresponded to weight at 365-d, total feed intake, average daily gain and feed efficiency, respectively.

Key Words: Genetic Parameters, Growth Traits, Postweaning

Introduction

Differences between breeds are primarily attributable to average effects of genes present at diverse frequencies in different breeds. Breed differences are usually greatest for traits which have responded to many generations of selection toward different goals (e.g., growth rate in postweaning in beef cattle as economically important trait). Intrapopulation (i.e., within breed or composite) variation is virtually restored generation after genration by the Menelian process; while variation between populations, accruing slowly as a result of different selection goals, genetic drift (associated with inbreeding), can only be exploited rarely. Intrapoulation response to selection is dependent on heritability and selection pressure applied. Response in other traits, not subject, to direct selection, are dependent on the genetic correlations and heritability of direct and correlated traits (Cundiff, 1986). The relative importance of direct and maternal additive genetic effects for growth should be considered when beef breeders design their breeding plans. Comprehensive characterization of germplasm resources available to beef breeders requires the evaluation of all economically important traits in beef cattle production (Thallman, et al., 1999). Thus, our objective was to estimate heritability direct, and phenotypic parameters for weight at 365d, total feed consumption **TFC**, average daily gain **ADG**, and feed per unit of gain **FUG**, during 112 d test.

Materials and Methods

Data came from a herd of Limousin **L** cattle located in Samalayuca, at the north of México. Cows were maintained in a rangeland of desert brush characterized by Larrea tridentata, Prosopis juliflora, Mimosa biuncífera, Ephedra californica, and Atriplex canescence; Grasses: Hilaria melangueri, Sporobolus airoides, Aristida trífida, and Distichlis spicata. Calving was in (April, May, and June). At birth all calves were identified, dehorned (paste), and vaccinated against viral scours. Because a six five year drought period. Cows received a feed supplement during the critical periods of drought. Calves were weighed at weaning, being adapted to the concentrate diet (70% TDN) during a 10 d period. Feed was offered ad-libitum, and water available 24 hr a day. No preferential management was practiced during the performance test. All of the animals were identified (tag), and weighed individually at the end of 112 d of study.

Statistical procedure

Each set of data was analyzed separately for each trait by using least squares mixed model. The analytical model included: year of birth, age of dam, sex of the calf, with date of birth as a covariate to adjust a common age as fixed effects; sire and residual as random components.

Results and Discussion

Least squares means for age and weight for postweaning growth traits in this study are presented in Table 1. Mean weight at 365-d was 364.76 kg. Weight at 365-d ranged from 268 to 430 kg. Mean weight to initiate the 112 d performance test was 232.14 kg. Weight to initiate the performance test ranged from 166 to 334 kg. Gregory et al. (1993) evaluated the mean weight at 365-d of 375 kg (n=254) progeny of 320 Limousin dams mated to sires involving inheritance of Limousin. Table 1, also shows that mean values of total feed consumption per animal during 112 d test was 841.56 kg. Mean value of total feed consumption per animal during 112 d test ranged from 716.78 to 1097 kg. The overall mean weight of gain per animal at the end of 112 test was 159.13 kg (Table 1.) Mean weight of gain per animal at the end of 112 d test ranged from 122.08 to 183.04 kg. The average daily gain per animal during 112 d test was 1.351 kg. The average daily gain during 112 d test ranged from 1.09 to 1.642 kg. Mean values of feed efficiency per unit of gain was 7.514 kg are presented in (Table 1.) Mean values of

feed efficiency per unit of gain ranged from 6.4 to 8.8 kg. The estimated heritability values of weight at 368 d, total feed consumption, average daily gain, and feed efficiency were (h^2=0.33± .04, h^2=0.39± .05, h^2 =0.44± .06, and (h^2 =0.37± .04) respectively, are presented in Table 2.

Weight at 368-d

The estimates of heritability value for weight at 368 d in this study was (h^2=0.33± .04). Gregory et al. (1993) estimates a heritability value (h^2=0.32±.04) for weight at 365d Van Vleck et al. (1999) estimated genetic parameters of heritability (h^2=0.32±.04) direct for weight at 368 d. The estimates of heritability values for weight at 368-d of these authors , was derived as an average, using 6 animal models with and without inbreeding coefficients (F) for animals and dams and with and without relationships matrix (A and A=I) for sire models. Gregory (1992) based in data of an experiment involving inheritance of composite and purebreds beef cattle reported estimates of heritability and phenotypic deviations values (h^2=0.26 and h^2=0.43**;** σ_p=30.05 and σ_p= 28.96 kg**;** h^2=0.27 and h^2=0.43**;** σ_p= 37.50 and σ_p= 34.59 kg) for 368-day weight in females and females, respectively. Van Vleck et al. (1999) conducted an experiment to estimate genetic parameters resulting from various analytical models for (BWT, n=4,155), 205-d weight (WWT, n= 3,884), and 365-d weight (YWT, n= 3,476). Data consisted of records for Line 1 involving inheritance of Hereford cattle selected for postweaning growth from 1934 to 1989 at ARS-USDA, Miles City, MT. They developed lines with the first calves in Line 1. Two sons of Advance Domino 13th, Advance Domino 20 th, and Advance Domino 54 th were the primary foundation sires of Line 1 (MacNeil et al., 1992). The authors compared twelve models. They oustanding that Models **3 and 9** best fit the data for estimation of variances and covariances for direct, maternal genetic, and permanent environment effects. **Model 3** included fixed effects of year, sex, age of dam; coevariates for birth day**;** and random animal genetic, random animal genetic, maternal genetic effects with covariance between direct and maternal genetic effects, maternal permanent environmental effects, and residual effects. **Model 9** was a sire and dam model but with relationships to account for direct and maternal genetic effects; dams also were included as uncorrelated random effects. Heritability estimates (h^2=0.30) for **YWT** was similar regardless of whether inbreeding effects were in the model. Other models resulted in changes in ranking for predicted breeding values and for estimates of direct and maternal heritability. Heritability estimates of direct effects were smallest with sire and sire-maternal grandsire models (Van Vleck et al. (1999). Sire models have lower estimates of heritability for direct effects than full animal or sire and dam models, especially for BWT and WWT. The author indicates that full animal and sire-dam models provide similar estimates. Selection among sires and lower sire variances could explain this (Van Vleck et al. (1999).

Feed intake

The estimates of heritability value for feed intake in this study was (h^2 =0.39± .05). Koch et al. (1968), reported heriability values (h^2 =0.64, and h^2 =.46) for feed intake. The authors based their estimates from data (n=1324, and n=480) involving different breeds. Willis et al. (1970) has summarized 8 estimates of heritability (h^2 =0.44). Koots et. al. 1994 and Green, 1999 have reported estimates of heritability values (h^2 =0.34). The authors based the average heritability value for feed intake weighted by number of observations in twenty one studies. The estimates of heritability value at this study (h^2 =0.39± .05) is quite similar to the estimates of heritabilities values (h^2 =0.44 , h^2 =0.46, and h^2 =0.34) for (Willis et al.,1970; Koch et al., 1968; Koots et. al. 1994 and Green, 1999), respectively. Nevertheless our estimates (h^2 =0.39) for feed intake differs considerable if comapred to the estimated heritability value (h^2 =0.64) reported by Koch et al. (1968) for the same trait.

Average daily gain

For average daily gain during the 112 d test, the estimates of heritability value in this study was (h^2 =0.44± .06). This estimated value for this genetic parameter of heritability it is in agreement to the reported value for average daily gain by Koch et al. (1968) based in (n=1324) observations. They reported a heritability value (h^2 =0.44) for this trait. Willis et al. (1970) based in a summary of 56 reports of estimates of heritability for daily gain weight expressed as a weighted average value (h^2 =0.52) is quite different compared to our estimates of heritability (h^2 =0.44±.06) for average daily gain

Feed per unit of gain

The estimates of heritability for feed consumption per unit of gain, at this study was (h^2 =0.37±.04). Koch et al. (1968) estimated a heritability value (h^2 =0.36) for the this trait. The estimated heritability value (h^2 =0.68) reported by Langlet et al. (1967) is considerable different and higher, if compared to the estimated value in this study. Nevertheless (Koots et. al. 1994 and Green, 1999, and Willis et al. 1970) based in (n=25 and n=15) studies, estimated heritabilities values (h^2 =0.34 and h^2 =0.40) expressed as weighted means. The estimates of heritability (h^2 =0.37±.04) for feed per unit of gain in this study is quite similar to the the reported values (h^2 =0.34 and h^2 =0.40) for (Koots et. al. 1994 and Green, 1999, and Willis et al. 1970). The estimates of heritability for feed per unit of gain shown more variation than the genetic estimates for feed intake. Findings of some researches shown a higher heritability values for feed per unit of gain. Willis et al (1970) oustanding that the variation in results, suggest to be no surprising because of the used feed systems in the programs of performance tests. The use of different concentrate- forage ratios conduct to make assumptions concerning how to calculate the feed per unit of gain in a uniform basis (i. e., dry matter, total nutrients digestible or metabolizable energy). It could result in lost of accuracy, and as a consequence, more environmental variation.

Implications

Based on the parameters presented and cited in this study, suggests that selection can be effective in changing economically important traits as postweaning growth traits of cattle. Within breeds, phenotypic variation is highly heritable for growth traits. Direct selection would be an effective method for improving preweaning and postweaning growth rate. An equilibrium among growth traits and adaptation it is important for efficient beef production. The relative importance of direct and maternal additive genetic effects for growth should be considered when beef breeders formulate their breeding plans.

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Table 1. Least squares means for yearling weight, total feed consumption, total weight gain, average daily gain, and feed per unit of gain calves from Limousin dams mated to Limousin sires, during a 112 d period test in feedlot.

Trait	Mean	Range
Initial weight(kg)	232.14	166-334
Yearling weight (kg)	364.76	268-430
Total feed consumption per animal (kg)	841.56	716.78-1097
Weight gain per animal (kg)	159.13	122.08-183.04
Daily gain per animal (kg)	1.35	1.09-1.64
Feed per unit of gain (kg)	7.51	6.40-9.80

Table 2.Estimates of heritability of weight at 368 d, total feed consumption, average daily gain and feed efficiency in calves from Limousin dams mated to Limousin sires, during 112 d period in feedlot.

Trait	Heritability h^2
Yearling weight	.33± .04
Total feed consumption	.39± .05
Daily gain	.44± .06
Feed per unit of gain	.37± .04

GENETIC PARAMETERS FOR BIRTH WEIGHT AND CALVING DIFFICULTY IN THE PROGENY OF HOLSTEIN HEIFERS MATED AI BY JERSEY SIRES.

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ABSTRACT: Genetic parameters for birth weight BW and calving difficulty CD were estimated in the progeny of 53 Holstein heifers mated AI to Jersey sires (n=7), under a grazing system, and heat stress conditions, in a dairy herd located in a desertic region at the north west of Mexico. Data was analyzed by using least squares The analytical model included**:** dam, sex of the calf, and season of parturition as fixed effects**;** sire, sire x season parturition interaction and the residual as random. The average age of heifers and weight at first mating AFM were 17.39±2.03 month and 345 kg, respectively. Overall mean BW was 31.82±1.59. BW ranged 32.04 ±1.60 to 30.44 ±1.52 kg in male and female calves, respectively. Male calves were 5.25% heavier (P<0.05) than female calves. The average values of number of services per conception SPC and gestation length GL were 1.37± 0.07 and 270.71±9.40 and weight**;** 268.68± 5.37 and 272.59± 5.45 d for female and male calves, respectively. GL of Jersey x Holstein heifers was 12.41 d (4.36%) shorter than Holstein calves. Estimates of heritability values for BW and CD were (h^2 = 0.30±0.04 and h^2 = 0.05±0.03), respectively. Eight births (15.68%) required to be assisted. These results which are based in limited numbers of observations suggest the advantage of involving inheritance of Jersey sires to be mated to Holstein heifers to reduce calving difficulty because birth weight is the main cause of dystocia in young cattle.

Key Words: Heritability, Birth Weight, Calving Difficulty

Introduction

There exists breeds differerences in their physiological response and adaptation to thermal environments (Young, 1985). Jersey cows are more resistant to high ambient temperatures than Friesian cows (Harris et al., 1960; West et al., 1990; Legates et al., 1991); nevertheless little is known concerning the performance of the progeny of Holstein heifers mated to Jersey sires under heat stress conditions. McDowell, (1972) indicated that dairy cows are normally well adapted to relatively cool conditions, but are sensitive to high ambient temperatures during summer. The most serious problem in most parts of the world is not to keep adult farm animals warm during winter, but keep them cool during the summer (McDowell, 1972).

Birth weight is important in the growth pattern. Calves with heavier birth weights have higher than average postnatal survival if born with excessive problems and have superior subsequent growth. Nevertheless, BW may be associated with difficult births and high death rates of calves at or near birth. The importance of this relationship varies with breed or cross and is usually more severe than in mature cows.

Calving difficulty which trends to increase with BW of the calf results in increased calf mortality and lowered rebreeding performance of the cow (Laster et al., 1973; Smith et al., 1976b). However sire breeds that produce calves with heavier BW (and more CD) also produce calves with increased growth rate, leaner carcass composition and improved age-and weight-constant feed efficiency (Koch et al., 1976a).

Hahn (1994) oustanding that the pervasive nature of weather and climate and the difficulties in adequately predicting their impact on beef and dairy cattle often lead to inadequate management strategies, resulting in a situation as coping as the need arises. Heat stress extreme can cause adverse effects especially when combined with factors (e.g., precipitation, poor nutrition wind, and high humidity in heat). The objective of the study was to estimate the heritability value of **BW** and **CD** in the progeny of 51 Holstein heifers mated artificially to Jersey sires, under grazing and heat stress conditions.

Materials and Methods

Data came from a dairy herd of Holstein, located in a desertic region at the northwestern of Mexico. The data available were on observations on **BW** in the progeny of 51 Holstein heifers mated artificially to Jersey sires (n=7). Heifers were maintained on irrigated cool season (rye grass) or warm season pastures and fed (grass and alfalfa) or silage during the winter, and bermuda grass in summer and fed (grass and alfalfa). The AFM was 17.23 ± 2.03 month. Calving was in the spring (March and April). At birth all calves were identified, weighed, dehorned (paste), and vaccinated against viral scours. The traits analyzed in this study were birth weight **BW**, services per conception **SPC**, and gestation length **GL**.

Statistical analyses

Least squares were used to estimate variance components. Each trait was analyzed separately SAS version 6.07 (SAS, 1992). The analytical model included: dam, sex of the calf, and season of parturition as fixed effects; sire, sire x season parturition interaction and the residual as random components.

Results and Discussion

Descriptive statistics and their standard errors for **BW, SPC**, and **GL** are presented in Table 1. Averaged **BW** weight was 31.82±1.59; 32.04 ±1.60 and 30.44 ±1.52 kg for male and female calves, respectively. The average age of heifers at first mating was 17.39±2.03 month.

The number of services per conception was 1.37± 0.07. The average **GL** was 270.71±9.40**;** 268.68± 5.37 and 272.59± 5.45 d for female and male calves respectively. Gestation length of Jersey x Holstein heifers was 12.41 d (4.36%) shorter than Holstein calves. Estimates of heritability values for BW and CD were (h^2 = 0.30±0.04 and h^2 = 0.05±0.03), respectively. Male calves were 5.25% heavier (P<0.05) than female calves**;** eight births (15.68%) required to be assisted. Gregory et al. (1993) estimates genetic parameters for several traits for nine pure breeds and three composite populations from records on females that produced calves as two-yr-old (2,942 females by 438 sires). Least squares analyses showed that for calf BW males was 7.66 % heavier than females (87.1 versus 80.9 lb) respectively.

Pollak (1975) and Menissier (1976) reported that correlations between BW and CD ranging from 0.04 to 0.21, in both heifers and cows, may underestimate the real amount of association. Phillipson (1976c) reported a non linear relationship between GL and stillbirth incidence SBI in Friesian heifers; stillbirth frecuency increased after long (> 278 d) as well as short (< 267 d) gestation periods. This phenomenon may result in the low estimated linear correlations (r= 0.02 to 0.06) between SBI and GL reported in Friesian and Flekvieh heifers and cows (Phillipson, 1976a) and it may also account for the lack of difference in averge GL between live and stillborn calves sometimes observed. Witt et al. (1964) and Anderson et al. (1976) suggested that the phenotypic relationship between GL and CD is mediated by BW. Burfening et al. (1978c) found that the association between GL and CD is not significant after adjustment for BW introduced as a covariate.

Meijering et al. (1986) for a study of a six year period analyzed 250 (pure breed) lactating cows of Holstein **HF**, Dutch Red White **DRW** and Dutch Friesian **DF** breeds and four groups of sires: 33 low-risk sires **L,** 32 high risk sires **H,** 23 low-risk sires L^* and 25 high risk sires H^***.** F_1 heifers were mated at 14-15 months of age. The authors reported that during rearing, body weights and sizes were measured at three month intervals. At mating, the F_1 females within each sire line (**L** or **H**) were randomly assigned to low-risk (L^*sires) or high risk (H^* sires). A total of 23 ($L\_^*$) sires (6 H, 9 DRW, 8 DF) and 25 ($H\_^*$) sires (8 HF, 9 DRW, 9 DF) were used to sire the F_1 generation. Dams were weighed and measured 10 days after calving date. The authors reported that BW, sire group, breed, sex, maternal grandshire group, sire group and season of birth did not interact significantly (P>0.10). The contrast between sire groups was 2.68± kg. Moreover sex of calf and breed-by-sex interaction were significant sources of variation. BW increased significantly with parity of dam, while differences among sexes were larger in the HF and DF breeds (4.08±0.60 and 3.338±0.56 kg), respectively than in DRW (1.80±0.58 kg). Except for absence of significant breed by sex interaction and breed specific differences in single measurements, parameter estimates for calf size corresponded well with those observed for birth weight (Meijering, 1986).

Koch and Clark (1955), Thompson (1980), Whillham (1963), and Meyer (1990) as cited by Mohiudin (1993) reviewed reports of genetic parameters of growth traits in beef cattle published worldwide. Heritability estimates for birth weight (weighed by number of observations) averaged were (h^2=0.46 and h^2=0.26) for males and females calves, across sexes, respectively. Direct and maternal heritabilities averaged (h^2= 0.30 and h^2= 0.10), respectively; while maternal permanent environmental variance as proportion of the phenotypic variance averaged (p^2= 0.03) for birth weight.

Crossbred Holstein x Jersey displayed lower values for heat stress indicators on hot days July and August (maximum temperature ≥ 44 °C), suggesting higher tolerance capabilities. This may be due mainly to their smaller body size compared to Holstein and do have a greater surface to volume ratio (12.1 m^2/m^3 than Holstein. A greater surface to volume gives the Jerseys a slight thermal loss by having a larger area for evaporation to dissipate a volume specific rate of metabolic heat production.

Estimates of phenotypic correlations among BW and AFM and length of parturition were (r_P= 0.20±0.04, and (r_P=0.21± 0.06), respectively. A negative phenotypic correlation (r_P=-0.16±0.05) was found between AFM and length of parturition. These results which are based in limited numbers of observations suggest the advantage of using Jersey sires to be mated to Holstein heifers to reduce calving difficulty because birtha weight is the main cause of dystocia in young cattle.

Ferrell (1993) reported that BW lower than optimum are associated with reduced energy reserves, lowered thermoregulatory capability, and increased calf deaths at or near birth. Lower BW are also related to low rates of growth after birth and decreased mature size. Conversely, BW greater than optimum are associated with greater calving difficulty, calf losses at birth and increased difficulties with rebreeding the cow. The fetal genotype determines the maximum potential for fetal growth. Nevertheless, it may be argued that the fetus rarely expresses its full genetic potential for growth (Ferrell, 1993). The author suggests that maternal nutrition, number of fetuses, and environmental temperature may be cause further limitation of fetal growth, because these factors are most apparent during the latter stages of gestation when fetal growing rate and nutrients needs are the greatest.

Hahn (1994) oustanding that the pervasive nature of weather and climate and the difficulties in adequately predicting their impact on beef and dairy cattle often lead to inadequate management strategies resulting in a situation as coping as the need arises. Heat stress extreme can cause adverse effects especially when combined with factors (e.g., precipitation, poor nutrition wind, and high humidity in heat).

Implications

Producers must consider birth weight and calving difficulty as important traits in their breeding programs.These results which are based in limited numbers of observations suggest the advantage of using sires Jersey to be mated to Holstein heifers under field conditions to reduce calving difficulty because birth weight is the main cause of dystocia in young cattle. Because hot weather can strongly affect bioenergetics, with adverse effects on the performance and well being livestock, suggest an additional advantage of involving inheritance of Jersey sires to be mated to Holstein-heifers.

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Table 1. Average means and their standard errors for birth weight of male and female calves, services per conception and gestation length of Holstein heifers mated artificially to Jersey sires.

Birth weight kg (female and male calves) ±SE	Birth weight female calves kg ±SE	Birth weight male calves kg ± SE	Services Per conception ±SE	Gestation length (female and male calves) ±SE	Gestation length (female calves) ±SE	Gestation length (male calves) ±SE
31.82±1.59	30.44±1.52	32.04±1.60	1.37±1.07	270.71±9.40	268.68±68	272.59±5.45

Table 2. Heritability estimates of birth weight and phenotypic correlations among birth weight and age at first mating and length of parturition; and age at first mating and length of parturition of Holstein heifers mated artificially to Jersey sires.

Traits	Heritability ± SE
Birth weight	$h^2 = 0.30 \pm 0.04$
Calving difficulty	$h^2 = 0.05 \pm 0.04$
	Phenotypic correlations
Birth weight/ age at first mating	$r_P = 0.20 \pm 0.04$
Birth weight/ length of parturition	$r_P = 0.21 \pm 0.06$
Age at first mating/ length of parturition	$r_P = -0.16$

[a] h^2=Heritability, estimated through half paternal sibs; r_P = Phenotypic correlations.

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REPRODUCTION TRAITS AND CALF PERFORMANCE OF FIRST CALF HEIFERS SIRED BY BULLS WITH HIGH MARBLING OR HIGH RETAIL PRODUCT EPD

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ABSTRACT: A group of mature Angus cows from the Bair Ranch near Martinsdale, MT were artificially inseminated from 1999 through 2001 to Angus bulls with either high EPD for marbling (**MB**) or high EPD for retail product (**RP**). Heifer calves from these matings were retained as replacements in the herd. These females were artificially inseminated to a single sire that was also used as the clean-up sire. This study was initiated to evaluate the impact of different selection criteria on reproduction within a herd of western range cattle. Total pregnancy (AI and natural service) of heifers as yearlings (**BRED**), re-breeding pregnancy rate of 2-yr-old dams (**REBRED**), pre-breeding body weight (**COWWT**), and weaning weight of first calves (**WWT**) was analyzed. Reproductive traits were analyzed by categorical modeling with the model including year and sire type. Cow and calf weights were analyzed by general linear modeling. The model for COWWT included year and type (MB or RP), and WWT included calf age as a covariate, year, sex, and dam type. No differences in COWWT due to type were found (P=0.2). Year was significant (P<0.001) with those heifers born in 2001 being heavier than the other years. Significant calf age effects were found for WWT (P<0.01), but type and sex were not significant sources of variation (P>0.3). Each day of age of calves increased WWT by 0.75 kg. Statistical analysis showed calves weaned by dams born in 2001 were not as heavy as calves weaned in other years, though overall year affect was not significant. Year was a significant source of variation for BRED and REBRED (P<0.05), but type was not. Probabilities for non-pregnant heifers were high in 2001 (32%) compared with other years (15%). Conception to AI was at approximately 35% in 2001 heifers compared with 50% in other years. However, this trend was reversed in REBRED with 2001 born heifers averaging 5% non-pregnant compared with 20% for the other years.

Key Words: Beef Cattle, Selection, Marbling, Retail Product, Reproduction

Introduction

In recent years, consumer preference for lean meat has influenced production in the beef cattle market. Use of EPD for sire selection for carcass traits in slaughter animals has proven effective. Many sire summaries report EPD for marbling and retail product as indicators of fat deposition and lean meat production. However, questions have arisen about the affects of this selection on subsequent production within the cow herd. This study was initiated to look at differences in cow production when selection was based on different criteria for fat deposition. Cow weights, first calf weaning weights, and reproductive traits for daughters sired by bulls with high EPD for marbling or high EPD for retail product were evaluated.

Materials and Methods

From 1999 through 2001, mature Angus cows from the Bair Ranch near Martinsdale, MT were artificially inseminated using bulls rated high in marbling score EPD (**MB**) or high in retail product EPD (**RP**). Some bulls were used across years with new sires added each year. Further details on sire selection can be found in Davis et al. (2002). The cows were synchronized prior to AI. Heifer calves were retained as replacement heifers for three years.

Heifers were shipped from the ranch at 45 d post-weaning for development in a feedlot. Heifers were weighed (**COWWT**) and heat synchronized at approximately 12 mo of age. Synchronization protocols differed in years, with heifers either receiving the same treatment, or randomized within treatment by sire. A single sire was collected for AI breeding, and used as the clean-up sire after AI breeding was complete. Pregnancy was determined by rectal ultra-sound techniques at 30 d and 60 d intervals after AI. Pregnancy was coded 0 = not pregnant, 1 = pregnant by AI, and 2 = pregnant by natural service. Calf birth date the following year was used to verify AI or natural service breeding. Re-breeding rates (**REBRED**) were determined by rectal palpation following weaning of the first calf. Pregnancy was coded as 0 for non-pregnant and 1 for pregnant. Calf weaning weights (**WWT**) on first calves were collected in the fall when calves averaged 213 d of age.

Weight data from 133 MB and 121 RP replacement heifers and 156 calves were analyzed using general linear modeling procedures of SAS (SAS Inst., Inc., Cary, NC). The model for COWWT included year and sire type (MB or RP), and for WWT included calf age as a covariate, year, sex, and dam's sire type. Sire within sire type was used as the error term for type. All two-way interactions were tested and dropped if they were found non-significant. Pregnancy rates were analyzed using the categorical modeling procedure of SAS with year and sire type in the model.

Results

The year by sire type interaction was significant (P=0.07) for COWWT (Table 1). Heifers born in 2001 were heavier than those born in either 2000 or 2002. No differences existed for COWWT due to sire type (MB or RP). Vieselmeyer et al. (1996) reported no differences in gains or final weights when heifers finished for slaughter were sired by high marbling EPD Angus bulls versus low marbling EPD Angus bulls.

Age at weaning was significant for WWT with each additional day of age resulting in an increase of .75 kg. No other effects were significant (P>0.1) though calves weaned by heifers born in 2001 were not as heavy as in other years (Table 2).

Analysis of breeding data showed no difference due to MB or RP sires. However, significant year effects (P<0.05) for both pregnancies as yearling heifers and while raising their first calf were present (Figures 1 and 2). Yearling heifers born in 2001 failed to become pregnant 32% of the time compared with 16% and 14% non-pregnant heifers for 2000 and 2002, respectively. However, this was reversed the following year when those heifers born in 2001 became pregnant 95% of the time compared with others years when rebreeding pregnancy rates ranged from 80% to 86%.

Body condition score, a measure of fat deposition and energy reserves in cattle, has been used to improve reproduction for many years (Lemenger, 1987: Lusby, 1987). Selection of sires for marbling or retail product does not necessarily relate to differences in replacement cow body condition. Gwartney et al. (1996) reported it was possible to use marbling EPD as a selection criteria without subsequently increasing fat deposition in other areas.

Slaughter steers from this study were followed through meat processing plants in order to obtain carcass characteristics data. Davis et al. (2002) reported no differences in carcass traits between sires selected for high marbling or high retail product EPD, except in marbling score and quality grade. Gwartney et al. (1996) reported no differences in steer carcasses when fed to a common day endpoint except in marbling score for a group of steers sired by bulls with high or low EPD for marbling. The same was true for heifer carcasses from high EPD marbling sires. They had more marbling and lighter carcasses when fed to common day endpoints. When steers and heifers were fed to a constant fat endpoint, the carcasses from low marbling EPD sires were heavier and had more subcutaneous fat than those animals from the high EPD marbling sires. While we have no heifer carcass information, we can assume the heifers deposited fat in the same manner as their steer half-sibs.

Year affects in this study were the primary source of variation in all traits reported. Heifers born in 2001 were significantly heavier, had low pregnancy rates as yearlings, and weaned smaller calves than heifers born in other years. Smith et al. (1989) reported favorable correlations between heifer growth and reproductive traits for a group of Hereford and Angus females. The genetic correlation of yearling weight and age of puberty or age of first calving were -0.14 and 1.32, respectively, but the standard errors of the estimates were 0.44 and 5.97, respectively. Fiss and Wilton (1992) reported no significant associations between cow weight and first-service pregnancy rates in heifers of four different rotational breeding systems though they observed decreasing pregnancy rates when cow weights increased.

Implications

Differences in heifers sired by MB or RP bulls for reproductive and production traits in this study were not important. Selection for lean meat or higher quality grade should not have a negative impact on reproduction of replacement females.

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Table 1. Least Squares Means and SE for Cow Weight (kg) at Breeding as Yearlings by Year and Sire Type EPD

Year * Type[a]	LSM ± SE[b]
MB 2000	375 ± 7.8[a]
RP 2000	361 ± 7.2[a]
MB 2001	400 ± 5.9[b]
RP 2001	415 ± 7.5[c]
MB 2002	359 ± 12.4[a]
RP 2002	372 ± 7.9[a]

[a]MB = High marbling EPD sires, RP = high retail product EPD sires
[b]Means in the same column without a common superscript differ (P<0.1)

Table 2. Least Squares Means and SE for First Calf Weaning Weight (kg)

Dam Year Born	LSM ± SE[a]
2000	214 ± 4.3[a]
2001	203 ± 4.2[b]
2002	209 ± 5.4[a]

[a]Means in the same column without a common superscript differ

Figure 1. Yearling Heifer Pregnancy by Sire Type and Pregnancy Status

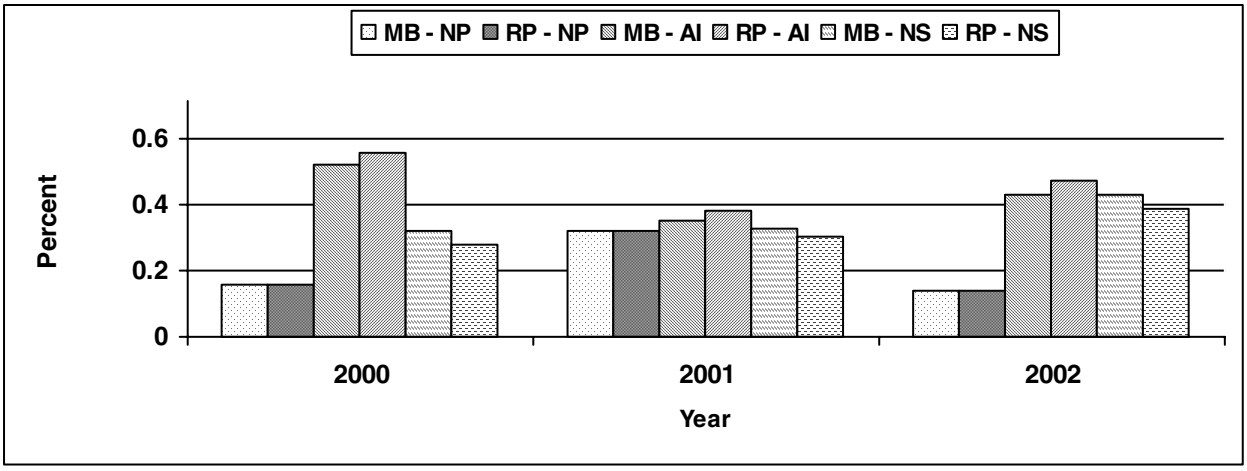
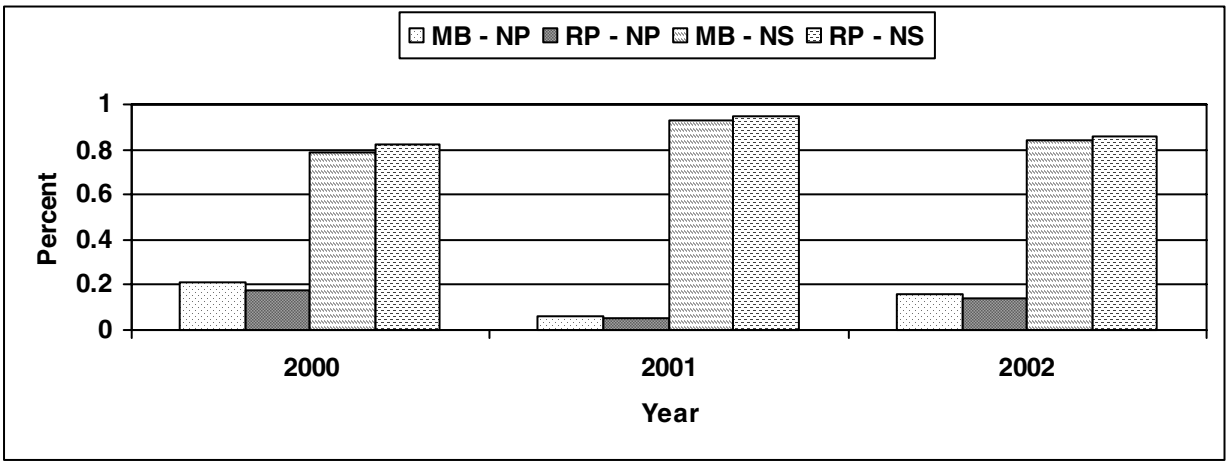


Figure 2. Dam Pregnancy at Second Breeding by Sire Type and Pregnancy Status



MB = High Marbling EPD Sires, RP = High Retail Product EPD Sires
NP = Not Pregnant, AI = Pregnancy by AI Breeding, NS = Pregnant by Natural Service

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Using Additional Phenotypic Information in Data Poor Analyses

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ABSTRACT: A problem faced when performing genetic evaluations is accounting for base genetic differences between animals of different breed compositions. Currently, two approaches exist that allow for the incorporation of breed differences into genetic analyses. One method is a Bayesian approach fitting both breed additive genetic and heterosis effects. Animals are grouped according to breed percentage and analyzed using heterogeneous variance components estimated from the base breed. A second method groups individuals according to known genetic differences based on respective expected progeny difference (EPD) from their original breed association. To successfully use this last method, a sufficient number of animals with external EPD are needed. The current genetic evaluation of the North American Limousin Foundation includes sires registered with both the American Angus Association and the Red Angus Association of America. These Angus animals are divided into six groups, high medium and low for each breed based upon their respective external association EPD. This approach yielded groups solutions which were not reflective of the differences between Limousin and Angus breeds as reported in scientific literature. The high, medium and low Angus group solutions for marbling score were -0.59,-0.055 and -0.068 respectively compared to -0.011 for Limousin. The objective of this study was to evaluate the addition of phantom animals into genetic evaluation to more appropriately discriminate breed differences. Phenotypic differences between Limousin and Angus bred animals reported from Meat Animal Research Center (MARC) were used to create foundation breed differences. Three thousand animals, 500 in each group, were added to the data set with phenotypic observations matching phenotypic differences found at the MARC. The addition of these phantom animals resulted in estimates of breed differences shown in the MARC research. New genetic group solutions were 0.73, 0.37 and 0.0007 for high, medium and low Angus animals compared to -0.026 for Limousin.

Key Words: genetic evaluation, groups, beef cattle

Introduction

Improvement and advancements in accounting for genetic groups in genetic evaluations has been an area of interest particularly in the beef cattle as crossbreeding has become more and more prevalent. In the past twenty years several approaches have been proposed to perform genetic evaluations for genetically diverse data sets to account for genetic differences between animals of different breeds. The so-called Q-P transformation (Quaas and Pollak, 1981) simplified the computation necessary to account for groups, making the genetic grouping methods more usable. Westell et al. (1988) outlined a set of rules for calculating coefficients of group effects in the mixed model equations. Golden et al. (1994) proposed a method of using external expected progeny difference **(EPD)** as additive genetic information to account for differences in genetic evaluations with multiple breeds. This method is satisfactory when sufficient numbers of progeny from the outside animals exist. The North American Limousin Foundation (NALF) has their carcass EPD calculated using Golden's method of grouping; however, with relatively limited carcass data from foreign registered animals, this method may underestimate breed differences.

The objective of this study was to investigate the effect of adding external, or "phantom", animals to more appropriately represent breed differences. In this study the term phantom animal is used to describe an animal generated to exhibit a group average phenotype.

Materials and Methods

The North American Limousin carcass data set used in the 2004 genetic evaluation was used for this study. The data set included 50,845 individuals with some carcass data and a pedigree of 90,078

Table 1. EPD ranges of Angus and Red Angus Sires														
Group	XSWT		REA		UREA		BF		MS		UBF		UIMF	
A1	-35	0	-0.57	-0.01	-0.49	-0.09	-0.08	-0.01	-0.6	0.02	-0.82	-0.1	-1.25	-0.06
A2	0	6	-0.01	0.13	-0.09	0.04	-0.01	0.01	0.02	0.13	-0.1	0.07	-0.06	0.17
A3	6	41	0.13	0.87	0.04	0.8	0.01	0.1	0.13	0.76	0.07	1.02	0.17	1.17
R1	-25	-4	-0.62	-0.14	N/A	N/A	-0.05	-0.01	-0.2	-0.04	N/A	N/A	N/A	N/A
R2	-4	1	-0.14	-0.01	N/A	N/A	-0.01	0	-0.04	0.04	N/A	N/A	N/A	N/A
R3	1	21	0	0.58	N/A	N/A	0	0.03	0.04	0.64	N/A	N/A	N/A	N/A

A1=Angus low, A2= Angus medium, A3=Angus high R1=Red Angus low, R2=Red Angus medium, R3=Red Angus high.
XSWT=Carcass weight, REA=Rib-eye area, UREA=ultrasound rib-eye area, BF=backfat, MS=marbling score, UBF=ultrasound backfat, UIMF=ultrasound intramuscular fat

individuals. Of the individuals with carcass data 4,442 also appeared as parents. The pedigree contained 2,869 purebred Angus and 46 purebred Red Angus sires. Of these Angus and Red Angus sires, 262 and 3 Angus and Red Angus, respectively, were sires of Limousin cross animals. The remainder were included as they increased genetic relationships between outside individuals. A total of 1,135 purebred Angus animals who shared weaning contemporary groups with Limousin animals were included in the data set for better comparisons with Angus sires.

Carcass traits to be analyzed included carcass weight (**XSWT**), back fat (**BF**), marbling score (**MS**), rib-eye area (**REA**), ultrasound back fat (**UBF**), ultrasound rib-eye area (**UREA**) and ultrasound intramuscular fat (**UIMF**). Two separate models were used to analyze the data. Model one included weaning weight (**WWT**), weaning weight maternal (**WWM**), BF, MS, UBF and UIMF. Model two included WWT, WWM, XSWT, REA and UREA. The Animal Breeder's Tool Kit (Golden et al., 1992) was used to perform analyses in two separate models. Multiple trait animal models (Henderson and Quaas, 1976) were used for both the four trait and five trait models. The fixed effects in model one included age of dam and weaning, carcass and ultrasound contemporary group for each associated measure. Outcross percent and weaning, carcass and ultrasound age were fit as covariates for each trait. Additive genetic effects were included for each of the traits in the model. Model two included the same fixed effects, covariates and random effects as model one for each of the appropriate traits. Each of the models were fit with the same data set with and without additional animals to assess the effectiveness of fixing the group difference.

Contemporary groups were formed based on data supplied by NALF. Weaning contemporary groups are defined by a NALF supplied weaning contemporary group combined with and weaning work group, weaning management group, sex, and creep fed status. Carcass contemporary groups were formed using the weaning contemporary group combined with date of slaughter. Ultrasound contemporary groups were formed using the yearling contemporary group in conjunction with sex, ultrasound management group, scan date and scan technician. A total of 4,958 weaning contemporary groups were formed for the analysis. Of these, 701 contained Angus or Red Angus animals. Carcass and Ultrasound contemporary groups numbered 1,334 and 5,016 respectively. Angus or Red Angus animals were represented in 79 and 286 carcass and ultrasound contemporary groups. Heterosis effects were estimated as a linear function of outcross for each animal. Outcross was calculated for each animal based upon the breed fractions of parents of the animal.

External animals were grouped according to published EPD from the American Angus Association and the Red Angus Association of America. The external animals were split into three groups, high, medium, and low, for each association based upon their EPD for a particular trait. The minimum and maximum EPD of each group for American Angus and American Red Angus animals is shown in Table 1. By design each group had nearly identical numbers of foreign individuals represented. In total, the external sires accounted for a total of 402 Angus sired progeny within the data set. American Angus sired progeny numbered 356 and Red Angus sire progeny numbered 46. Angus and Red Angus

Table 2. Average Phenotypic values							
GRP	XSWT	REA	UREA	BF	MS	UBF	UIMF
A1	815.3	12.5	12.5	0.7	7.4	0.7	4.6
A2	753.6	11.5	11.5	0.6	6.7	0.6	3.6
A3	690.2	10.4	10.4	0.5	6.0	0.5	2.7
R1	815.3	12.5	12.5	0.7	7.4	0.7	4.6
R2	753.6	11.5	11.5	0.6	6.7	0.6	3.6
R3	690.2	10.4	10.4	0.5	6.0	0.5	2.7

A1=Angus low, A2= Angus medium, A3=Angus high
R1=Red Angus low, R2=Red Angus medium, R3=Red Angus high
XSWT=Carcass weight, REA=Rib-eye area, UREA=ultrasound rib-eye area, BF=backfat, MS=marbling score, UBF=ultrasound backfat, UIMF=ultrasound intramuscular fat

Table 3. Genetic groups solutions of Limousin, Angus and Red Angus before and after the addition of phantom animals

	Carcass Wt				REA				BF				MS			
	AA Before	AA After	RA Before	RA After	AA Before	AA After	RA Before	RA After	AA Before	AA After	RA Before	RA After	AA Before	AA After	RA Before	RA After
L	12.373	-30.6	-1.808	-25.7	0.16	-2.18	0.0482	-1.98	-0.002	0.078	0.007	0.172	-0.136	0.029	0.3363	0.184
M	-29.84	33.11	-41.14	32.91	-0.05	-1.09	0.0032	-1.1	-0.008	0.208	-0.009	0.202	-0.11	0.758	0.313	0.759
H	-4.461	94.75	-1.53	89.94	0.028	-0.03	-0.005	-0.24	-0.018	0.318	0.012	0.229	-0.117	1.468	0.351	1.314
	LL Before	LL After			LL Before	LL After			LL Before	LL After			LL Before	LL After		
	-9.42	-8.71			-0.04	0.006			0.005	0.002			-0.02	-0.05		

L=Low genetic group, M=Medium genetic group H=High genetic group.
AA=Angus, RA=Red Angus, LL=Limousin. REA=Rib-eye area, BF=Backfat, MS=Marbling score

dams are not included in the EPD grouping because of lack of EPD information from their respective Associations

Data from the Meat Animal Research Center, Clay Center Nebraska, has shown significant differences in carcass trait performance between Angus and Limousin. The Germplasm Evaluation Report 22 (Cundiff et al, 2004) included both Angus and Limousin breeds. To develop the phantom animal observations, phenotypic differences from MARC were used as breed averages. The phenotypic values for the phantom animals are presented in Table 2. Phenotypic values for the high and low breed groups were calculated so that each group average was above or below the medium group by 33%.

A total of 3,000 phantom Angus animals and 500 phantom Limousin were simulated. The Limousin animals were added into the analysis to set the base of the Limousin animals to the phenotypic performance levels found at MARC. All of the phantom animals were placed into contemporary groups based upon group designation, high, medium or low, each group had identical phenotypic observations. Parentage of phantoms was coded as unknown. Because of the unknown parentage, the phenotypic values of the phantom animals set the base differences between the breeds.

Results and Discussion

Group solutions from the two analyses (with or without additional phantom animals) are presented in Table 3. Ultrasound group solutions are not included given that NALF does not report any ultrasound EPD. The addition of phantom animals yielded results nearly identical in magnitude to the differences between the breeds as reported by the MARC.

A preliminary analysis investigated the addition of 600 phantom external animals (100 per group). That approach did not produce sufficient breed differences, so the previous method described was used. The current approach also included a base addition of 500 phantom Limousin animals in the analysis. These 500 Limousin animals had phenotypic observations equivalent to those reported by the MARC for Limousin breed animals.

A trait of particular concern in the Limousin breed is marbling score. Without phantom animals, Limousin cross animals with Angus parentage showed little difference in genetic level when compared to purebred Limousin. Prior to the addition of the phantoms, the medium Angus group solution for marbling score was -0.11 and Limousin solution was -0.02, both negative and similar in magnitude. Several studies have shown on average Angus marble better than Limousin. This difference was not apparent prior to the addition of the phantom animals. After the addition, the marbling group solutions were -0.04 and 0.758 for medium Limousin and Angus

respectively. A difference of 0.798 between medium Limousin and Angus marbling scores more appropriately describes the difference in genetic potential between the two breeds. The EPD on crossbred animals were significantly different between the two methods. A rank correlation statistic was calculated for all Limousin sires comparing marbling score EPD with or without the phantom animals. The rank correlation between the two analysis was 0.8685, displaying little re-ranking of sires. Because of this high rank correlation this method of setting group differences has been adopted into NALF carcass evaluation.

Implications

This study has shown that in multi-breed data sets without sufficient amounts of crossbred data the addition of phantom animals can force differences between breeds to a desired value. A potential drawback to this method is accuracy bias. Fixing the group difference through phantom observations does not fairly account for high and low accuracy external EPD. Breeder reaction to this method has been favorable, however.

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Genetic Parameter Estimates for Docility in Limousin Cattle

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ABSTRACT: The objectives of this study were to compare the current method used for genetic evaluation of docility to a model including not only direct, additive effects but maternal effects as well and to determine the relative importance of maternal effects on docility. Docility scores are obtained at weaning where animals are evaluated on aggressiveness in the processing chute. Scores 1 through 6 represent a continuum from docile to aggressive, respectively. Data used in this study came from the North American Limousin Foundation (NALF). Observations used in the analyses were from contemporary groups of 10 or greater resulting in 23,453 observations and a two generation pedigree of 56,521 animals. Docility was analyzed using a single trait multiple component animal model that included fixed effects of contemporary group, sex, age of dam; and random direct genetic, maternal genetic, maternal permanent environmental; and residual effects. Direct and maternal heritabilities for docility were .37±.03 and .04±.01, respectively. Permanent environment was calculated as explaining .04±.01 of the phenotypic variance. The genetic correlation between the direct and maternal effect was -.55±.08. Docility was also analyzed using a single trait single component model equivalent to the current method. Direct heritability for docility was .66±.02 with the single component model. The original method was compared with the theorized model containing maternal and permanent environmental components. Log likelihood ratio tests showed that the method with a maternal component is a better representation of the data. The addition of a maternal component to the model for the genetic evaluation will improve the accuracy of the current docility EPD. This study shows that a maternal model is more appropriate when calculating docility EPD in Limousin cattle and that maternal effects, while small, are an important factor influencing calf docility score.

Key words: Temperament, Heritability, Maternal Effects

Introduction

Beef producers should consider temperament an important part of their selection criterion when producing or purchasing cattle. An aggressive animal can cause problems during handling which puts the animal's welfare and the stockperson's safety at risk (Grandin, 1989).

Some studies have investigated the association between temperament and productivity. Drugociu et al. (1977) reported that dairy cows with calmer temperaments had 25 to 30% increases in milk production. Cattle in the feedlot with calm temperaments had higher average daily gains when compared to cattle with excitable temperaments (Voisinet et al. 1997). Factors that induce dark cutting beef carcasses include, but are not limited to genetics, disposition and handling practices (Scanga et al. 1998). In general, these studies indicate an unfavorable relationship between temperament and productivity and safety making docility an economically relevant trait.

Traits in beef cattle such as birth weight and weaning weight have long been known to be influenced by maternal effects (Willham, 1972). Docility is heritable and some breeds have produced EPD rankings of bulls' additive genetic effects for this attribute. Sufficient data has accumulated to investigate the role of maternal effects on calf docility. The objectives of this study were to estimate the direct and maternal genetic variability of docility and the genetic relationship between direct and maternal effects in Limousin cattle; and to compare that model to one including only additive effects.

Materials and Methods

Description of Data

Docility scores, pedigrees, and other pertinent performance information were obtained from the North American Limousin Foundation (NALF). Individuals within contemporary groups with docility observations on 10 or more animals for a total 23,453 animals were used in this analysis. Two generations of pedigree information was assembled for these animals resulting in a total pedigree size of 56,521. Seven percent (1,614) of the 23,453 individuals with docility scores contributed to the maternal component of the study as they went on to become dams.

Docility scores were obtained at weaning and were determined based on aggressiveness while in the chute. Sores of 1 or 2 are designated as docile or restless, are considered to possess a mild disposition, and are gentle to handle with little trouble (NALF, 2000). These represent highly acceptable behavior. A score of 3 is assigned if the animal is nervous, impatient or exhibits a moderate amount of struggle. These animals are considered to be manageable but exit the chute briskly. Animals scored as 4, 5 or 6 (flighty to very aggressive) are jumpy, out of control and may exhibit attack behavior when handled alone. The NALF implemented docility EPD's in 1991 to provide breeders with the opportunity to minimize the percentage of animals which possess genetics for unacceptable behavior.

The Models

Docility scores were analyzed and variance components estimated with the following animal models:

Direct: $\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_D\mathbf{u}_D + \mathbf{e}$

Direct + Maternal: $\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_D\mathbf{u}_D + \mathbf{Z}_M\mathbf{u}_M + \mathbf{Z}_C\mathbf{u}_C + \mathbf{e}$

where

y is a N × 1 vector of docility observations;

b is the vector of fixed effects (discussed below);

$\mathbf{u}_D$, and $\mathbf{u}_M$ are vectors of direct and maternal genetic effects, respectively;

$\mathbf{u}_C$ is a vector of permanent environmental effects;

X, $\mathbf{Z}_D$, $\mathbf{Z}_M$ and $\mathbf{Z}_C$ are the incidence matrices that relate observations to their respective fixed and random effects.

The (co)variance structure of the random effects in the two models

$$\mathrm{V}\begin{bmatrix} u_D \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2{}_D & 0 \\ 0 & I\sigma^2{}_e \end{bmatrix}$$

$$\mathrm{V}\begin{bmatrix} u_D \\ u_M \\ u_C \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2{}_D & A\sigma_{DM} & 0 & 0 \\ A\sigma_{DM} & A\sigma^2{}_M & 0 & 0 \\ 0 & 0 & I\sigma^2{}_C & 0 \\ 0 & 0 & 0 & I\sigma^2{}_e \end{bmatrix}$$

where

A is the numerator relationship matrix;

σ^2_D is the additive direct genetic variance;

σ^2_M is the additive maternal genetic variance;

σ_{DM} is the genetic covariance between additive direct and maternal effects;

σ^2_C is the maternal permanent environmental variance;

σ^2_e is the remaining environmental (residual) variance.

Heritability (h^2) estimates were derived from the variance components as follows:

$h^2_D = \sigma^2_D / (\sigma^2_D + \sigma^2_M + \sigma_{DM} + \sigma^2_C + \sigma^2_e)$

$h^2_M = \sigma^2_M / (\sigma^2_D + \sigma^2_M + \sigma_{DM} + \sigma^2_C + \sigma^2_e)$

$C^2 = \sigma^2_C / (\sigma^2_D + \sigma^2_M + \sigma_{DM} + \sigma^2_C + \sigma^2_e)$

Estimation of Variance Components

The fixed effects included contemporary group, sex and age of dam. The 1,333 contemporary groups were formed by combining herd, year, season of birth, weaning date, NALF weaning contemporary group, and creep designation. The dependant variable docility was scored from 1 to 6 and was standardized at -1.05, 0.14, 1.13, 2.14, 2.74, and 3.2, respectively.

Variance Components were estimated with ASREML (Gilmour et al., 2002) which fits linear mixed models using Residual Maximum Likelihood (REML). This program was used to obtain the solutions to the mixed-model equations and the log-likelihood values. The maximum number of iterations was set at 20 for both models, but convergence criterion was met at the eighth iteration for the direct model as well as the direct-maternal model. Convergence is presumed when the REML log-likelihood changes less than 0.002 from the current iteration number and the individual variance parameter estimate changes less than 1% (Gilmour et al., 2002).

Results and Discussion

The performance of an individual is determined by its genetic makeup and environmental conditions. Maternal effects can play a part in determining a calf's behavior particularly prior to weaning. Results of this study suggest that maternal effects may be important for docility. Estimates of heritability and direct-maternal correlations for docility are shown in Table 1.

Table 1. Parameters for docility estimated with the program ASREML

Model	Parameter	Estimate (SE)
Direct	h^2_D	0.66 ±0.02
Direct + Maternal	h^2_D	0.37 ±0.03
	h^2_M	0.04 ±0.01
	C^2	0.04 ±0.01
	r_{DM}	-0.55±0.08

The phenotypic variance was 0.4371. The estimate of direct heritability for docility was moderate in the multi-component model, as was expected but higher than the 0.22 in Limousin reported by Le Neindre et al. (1995). Other heritability estimates of cattle temperament also indicate that it is a moderately heritable trait (Shrode and Hammack, 1971; Stricklin et al., 1980; Fordyce et al., 1988). The lower maternal heritability compared to a higher direct heritability would indicate a greater genetic influence from genes in the individual than that of its dam (Splan et al., 2002). To our knowledge there are no previous estimates for maternal heritability of docility. A negative direct-maternal genetic covariance was estimated. Other studies have also reported large negative correlations between direct and maternal genetic variances for a variety of traits. Phocas and Laloë (2003) reported direct and maternal genetic correlation for calving difficulty in beef cattle to be between -0.36 and -0.34, respectively. In swine, Ferraz and Johnson (1993) estimated the direct and maternal genetic correlation to be -0.34 for average daily gain. The mean direct and maternal genetic correlation representing three breeds and three traits for lamb weights was -0.4 (Tosh and Kemp, 1994). According to Robinson (1995) negative direct maternal correlations could be the result of inappropriate models by heterogeneous herd variance, sire x herd, or sire x year interactions. To clarify, the negative correlation could possibly be explained by sires that are being ranked differently based on different facilities, and handling practices within different herds which are ultimately affecting the mean score of their offspring. Lee and Pollack (1997) reported that fitting a sire x year interaction in an animal model analysis of Simmental field data found that the sire x year interaction explained 62% of the negative covariance between direct and maternal genetic effects for weaning weight in Simmental cattle. If the negative direct-maternal genetic correlation is an artifact of the data structure, the typical interpretation that animals which have superior genes for docility tend to have inferior maternal genes for docility, would not be appropriate.

Log likelihood ratio tests were used to compare the current method of estimating variance components with only additive genetic effects, to the hypothesized model including maternal effects (Table 2). The likelihood ratio test indicated that the model with direct and maternal

effects was more appropriate than the model with only additive variance. The direct model with maternal effects is a more complete model and should be used in the future when calculating EPD for docility in Limousin cattle.

Table 2. Log-Likelihoods from two models using ASREML

Model	Log-Likelihood
Direct	-2864.99
Direct + Maternal	-2848.40

Implications

Producers selecting cattle for more docile dispositions may decrease risk of accident for handlers, wear on facilities, and simultaneously increase the animal's welfare. Another advantage from selecting cattle with calmer temperaments would be increased production (e.g. increased milk production, higher average daily gain, and subsequent meat quality). This study indicates the need for further research to determine if the large negative relationship between direct and maternal genetic effects for docility represent a true genetic relationship or are an artifact of data structure. Information obtained from this study would indicate that selection for cattle with a more favorable docility score would be effective in producing cattle with more acceptable disposition. The maternal effect has shown to play a role in determining temperament up until the time the calf leaves the cows side at weaning. The addition of a maternal component to the analysis will allow for more appropriate identification of superior docility genetics. The addition of information provided by the direct and maternal genetic correlation improves the accuracy of the docility EPD. The log-likelihood ratio test shows that a maternal model should be used when calculating EPD for docility in Limousin cattle.

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VARIABILITY IN ECONOMIC VALUE IS DEPENDENT UPON HERD AVERAGE STAYABILITY

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ABSTRACT: Expected progeny differences (EPD) such as calving ease, stayability, and heifer pregnancy are calculated with threshold models in many beef cattle genetic evaluations. The application of these EPD for selection decisions result in a response that is dependent upon the average level of performance in the cow herd. The objective of this study was to quantify the economic value of selection decisions on stayability to 6 years of age given various cow herd phenotypic means for stayability. A 1000 cow herd representing females with average EPD of active dams in the Red Angus Association of America (RAAA) was simulated. The average EPD of these dams were .7, 27, 46, 14, 4, 9, 4, 9, 4 for birth weight, weaning weight, yearling weight, milk, calving ease (CE) direct, heifer pregnancy, CE total maternal, stayability, and maintenance energy requirements, respectively. Nineteen herds, each with a different level of phenotypic stayability ranging from 20 to 95% in 5% increments, were generated. Fourteen sire types were also generated representing the current range in active sire stayability EPD in the RAAA sire summary (ranging from -2 to 21). All other EPD were identical to those in the base cow herd. Simulated matings were made between the 16 herds and each of the 14 sire types resulting in complete replacement of the cow herd with offspring. The net income for each scenario was calculated based on weaning weight, price per unit weaning weight, and sale of all surplus calves at weaning. Change in net income from the base herd (stayability EPD = 9) was calculated for all combinations and ranged from a low of $-3872.16 to $2801.76 for phenotypic herd stayability of 95% to a high range of $-35938.13 to $38652.32 for a herd with phenotypic stayability of 20%. Coefficients for the regression of change in net income on sire stayability EPD were calculated for each level of phenotypic stayability, tested for equality, and found significantly different (P<.0001). The economic value of stayability depends upon phenotypic herd level of stayability.
Key Words: stayability, expected progeny difference, beef cattle

Introduction

A variety of EPD for U.S. beef cattle genetic evaluation are produced using threshold model technology and include traits like stayability, heifer pregnancy, and calving difficulty. Unlike the interpretation of traditional weight EPD, the interpretation of EPD from threshold models are dependent upon the level of performance within the herd. A consistent change on the underlying genetic scale for a threshold trait may result in unequal levels of change on the observed scale.

Many beef breed associations have begun to release selection indexes to provide beef producers with a selection tool that combines EPD with economic information to make more profitable selection decisions. Genetic improvement in female reproductive traits has been shown to have a greater impact on profitability than any other class of traits at the cow/calf level of the beef industry (Ponzoni and Newman, 1989; Melton, 1995).

Given the importance of female reproduction and the desire to use new reproductive EPD in economic selection indexes, the varying phenotypic response to selection on the reproductive EPD should be examined for effects on profitability of the cow/calf system. The objective of this study was to determine the economic value of genetic change in stayability under different levels of average phenotypic stayability in the cow herd. In this study, phenotypic stayability represents the proportion of replacement heifers remaining in the cow herd after 6 years.

Materials and Methods

Model Parameters

The decision support tool (ert.agsci.colostate.edu) outlined by Brigham et al. (2004) was parameterized to represent the average EPD of active cows in the spring 2005 genetic evaluation of the Red Angus Association of America registry (**RAAA**). The EPD for these dams were .7, 27, 46, 14, 4, 9, 4, 9, 4 for birth weight, weaning weight, yearling weight, milk, calving ease (CE) direct, heifer pregnancy, CE total maternal, stayability, and maintenance energy requirements, respectively.

Briefly, the web-based, decision support tool is a deterministic computer model that requires basic production, management, genetic, and economic information on the cow/calf operation (e.g. information presented in Table 1). Based on user inputs, the model generates a base cow herd age distribution, calf weaning weights by age of dam class, and other performance measures such as reproductive rate, and dystocia rate. The model assumes that all open cows and cows of a user-specified maximum age are sold. A fixed cost of $25 is assigned to an incidence of dystocia and a $5 per calf cost is assumed for non-feed calf expense. The user supplies a value for non-feed cow costs. Revenue is generated through the sale of weaned calves, and cull cows and heifers. The sale price of these animals is input by the user.

The EPD of potential replacement sire(s) are then used to derive an updated cow age structure accounting for base cow herd stayability EPD and new animal performance levels at herd equilibrium. In this process, the total number of cows is modified to equal the feed

resources used by the base herd. The model produces output enabling a producer to compare production and economic performance of the new herd derived from use of potential sires to that of the base herd.

Average phenotypic performance and values of economic inputs for the model as used in this study are shown in Table 1. For this study, the base cow herd consisted of 1000 cows with the EPD previously described.

Table 1. Production, management, and economic assumptions for the simulated base herd.

Model Parameter	Value
Heifer calving rate	95%
Mature cow weight	1200
Calf survival	95%
Heifer calving difficulty	22%
Mature cow	
Calf birth weight	80
Calf weaning weight	550
Maximum age	12
Cows per bulls	30
Fixed cow costs	$25
Cattle values	
Cull cow price	$55/cwt
Calf price	$100/cwt
Replacement heifer price	$1000/head

The average phenotypic stayability for the cow herd was varied from 20% to 95% in 5% increments on the observed scale for a total of 16 different herd stayability levels. Again, phenotypic stayability is defined as the proportion of replacements heifers entering the herd that remain in the herd through six years of age. Phenotypic stayability levels were generated through changes in cow herd conception rates. Each of the 16 different herds was sequentially mated to 1 of 14 different sire types representing the range of active sire stayability EPD in the current RAAA genetic evaluation. The stayability EPD of these bulls ranged from -2 to 21 and included bulls of -2, 0, 2, 4, 6, 8, 9, 10, 12, 14, 16, 18, 20, and 21 stayability EPD. The bull with the stayability EPD of 9 represents no change in the base herd. The EPD for all other traits were identical to those of the base cow herd. Each sire was mated to the base cow herd until replacements were generated in sufficient numbers to replace the entire cow herd. This is analogous to the average cow in the herd having the same stayability EPD as the sire.

Change in net income from the base herd was calculated for each sire within each phenotypic stayability level. This resulted in 266 different net change in income values—14 sires within each of 19 herd stayability levels.

Statistical Analysis

Within each phenotypic herd stayability level a general linear model was used to calculate the regression of change in net income on sire stayability EPD representing the change in profit per change in stayability EPD. The amount of variability in change in net income explained by change in stayability was also calculated. Regression coefficients were tested for equality across herd stayability levels.

To evaluate a possible nonlinear relationship between change in net income and stayability EPD, an additional model was evaluated that included both linear and quadratic effects of sire stayability EPD.

Results and Discussion

The linear regression coefficients are listed in Table 2 and represent the value of a unit change in stayability EPD for each herd phenotypic stayability level. In all scenarios, genetic improvement in stayability increased net income as evidenced by positive regression coefficients. Throughout the scenarios, the percentage of variability of change in net income explained by linear changes in stayability exceeded 98%. All coefficients were significantly different (P<.01). In subsequent analysis, quadratic effects were also found significant (P<.01), but given the high degree of variability explained by the linear regression, the quadratic effects were not examined further.

Table 2. Coefficients for the regression on change in net income on sire stayability EPD[a] under various herd phenotypic stayability levels.

Herd Phenotypic Stayability[b]	Regression coefficient ($/Stay EPD)	Intercept ($)	R^2
20	3245.00	-29303.13	>.9999
25	3088.90	-28075.42	.9999
30	2907.72	-26590.08	.9996
35	2715.99	-24974.20	.9993
40	2518.81	-23278.86	.9990
45	2316.22	-21509.39	.9985
50	2113.62	-19717.14	.9980
55	1915.67	-17947.46	.9976
60	1715.48	-16141.40	.9971
65	1514.62	-14314.49	.9964
70	1314.23	-12478.36	.9957
75	1115.03	-10640.31	.9949
80	930.80	-8929.00	.9939
85	720.42	-6960.33	.9924
90	491.13	-4794.16	.9899
95	285.47	-2825.77	.9860

[a]Sire stayability EPD ranged from -2 to +21.
[b]Herd phenotypic stayability represents the proportion of females replacements remaining in the herd after 6 years.

Genetic improvement of stayability had a more profound influence on herd profitability when overall base herd stayability was low, compared to base herds with high stayability (Table 2 and Figure 1). Throughout the range tested, the value of similar changes in sire stayability EPD increased with decreasing herd phenotypic stayability level. At the extreme low end of stayability, 20%, the difference in value of genetic improvement in stayability was over ten times greater than the value of stayability improvement at the other extreme of 95%.

A portion of the value in improving stayability was due to increases in the number of mature cows and a

corresponding increase in calf weaning weight, all else constant. Figure 2 shows changes in mature cow numbers associated with changes in the stayability EPD of the sire used in each herd. At 20% herd stayability, use of a sire with a +21 stayability EPD shifted herd age structure and resulted in 60 more mature age cows.

The values reported in this study could be used in the development of an economic selection index including stayability EPD, or in the development of strategies to evaluate risk associated with selection decisions for stayability EPD. Previous research has shown that minor changes in economic weights are likely to have minor effects on the efficiency of index selection (Smith, 1983), but larger changes in economic weights can produce considerable loss in selection efficiency especially if the changes are in traits that dominate an index. With importance of reproduction relative to production and carcass traits (MacNeil and Newman, 1994; Ponzoni and Newman, 1989) reproductive traits have the potential to dominate indexes for cow/calf production. Given the change in economic importance of stayability EPD in herds with differing cow fertility levels, the optimum delivery of economic selection indexes to the industry would account for different levels of herd performance. Alternatively, even though influence of stayability EPD on profitability spanned a wide range in this study, the values of improved stayability were always positive. Smith (1983) also indicated that the largest losses in selection index efficiency occurred when the direction of selection was reversed for important traits, a situation that did not occur here. In all scenarios, change in net income, or profitability, increased with selection of sires with superior stayability EPD.

Implications

Genetic improvement in cow herd stayability resulted in improved profitability of the beef cow enterprise irrespective of the current herd stayability level. Economic value of genetic improvement of stayability increases with decreasing herd stayability. Given the changing economic importance of genetic improvement of stayability, development of decision support tools for improving profitability through genetic change, should include flexibility for herd specific performance information.

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Figure 1. Change in net income ($, change) from a base cow herd of 1000 females with differing levels of phenotypic stayability (PHEN) when mated to sires of various stayability EPD (STAY) where phenotypic stayability is the percent of replacement females reaching age 6 in the herd.

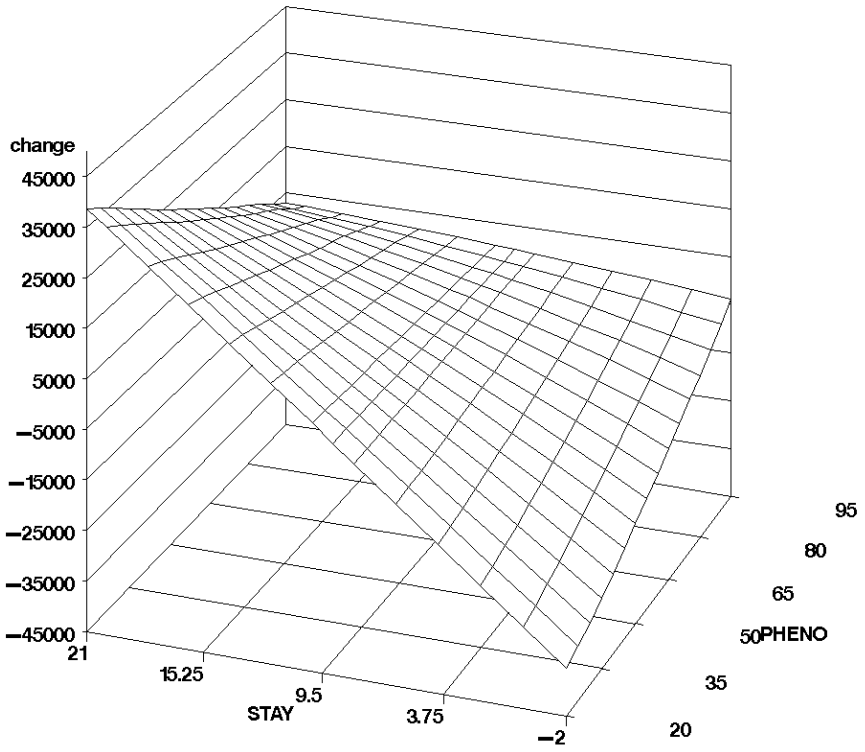
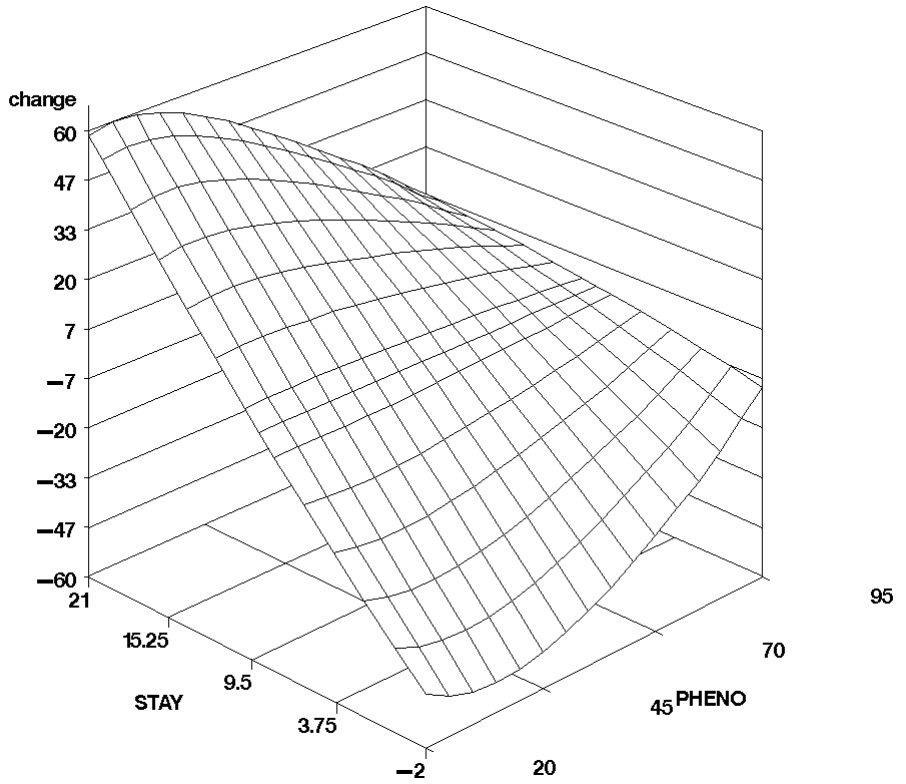


Figure 2. Change in number of mature cows (change), aged five years and greater, from the 1000 animal base cow herd resulting from the use of sires of various stayability EPD (STAY) in herds with different levels of phenotypic stayability (PHEN) where phenotypic stayability is the percent of replacement females reaching age 6 in the herd.



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CHARACTERIZATION OF THE MONTANA LINE 4 INBRED HEREFORD HERD

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ABSTRACT: Records for 1971 calves produced in the Line 4 Hereford herd at the Northern Agricultural Research Center (NARC) in Havre, Montana from 1976 to 2004 were analyzed to determine the current status of this closed herd. From 1976 to 1995, selection in this line was performed using an index of adjusted yearling weight minus 3.2 times adjusted birth weight. Since 1995, selection has been based on single trait selection for scrotal circumference. Records available across the majority of years included birth weight, weaning weight, weaning wither height, and weaning condition score. The average inbreeding coefficient for calves born in 2004 was 36.4%. Genetic parameters for all traits were analyzed using a univariate model. Estimates of direct heritability (and associated s.e.) were 0.51 (0.09), 0.21 (0.07), 0.28 (0.11), and 0.24 (0.09) for birth weight, weaning weight, weaning wither height, and weaning condition score, respectively. Estimates of maternal heritability were 0.08 (0.04), 0.09 (0.06), 0.13 (0.07), and 0.09 (0.07), respectively. The estimate of the direct-maternal genetic correlation was non-significant for most traits with estimates of 0.15 (0.26), -0.12 (0.35), -0.52 (0.22), and -0.48 (0.29), respectively. The proportion of variance due to maternal permanent environmental effects was only significant for weaning weight and weaning condition score with estimates of 0.30 (0.05) and 0.14 (0.04), respectively. Birth weight direct breeding value (DBV), maternal breeding value (MBV), and phenotypic trends were estimated to be -0.005, -0.004, and -0.034 kg/yr, respectively. Weaning weight trends were estimated to be +0.289, +0.082, and +0.276 kg/yr, respectively. Weaning wither height trends were +0.025, +0.010, and -0.130 cm/yr, respectively. Weaning condition score trends were +0.0061, - 0.0002, and +0.0040 score/yr. Trends for these traits are relatively flat for the period of time studied.

Keywords: Birth weight, Genetic trends, Weaning weight

Introduction

Highly inbred strains or lines of animals are relatively common in model species, but are infrequent in livestock populations. This is partially due to the longer generation interval of livestock relative to model species and partially due to the lack of production associated with inbred animals, making justification for maintaining such lines difficult. However, inbred lines of livestock can be valuable genetic resources for researchers.

The purpose of this manuscript is to characterize the current status of the Line 4 inbred Hereford herd, a closed population of cattle that has been maintained by Montana State University's Northern Agricultural Research Center in Havre, Montana since 1962.

Materials and Methods

Measurements were collected for 1971 calves born from 1976 to 2004 in Montana State University's Line 4 Hereford herd. Traits included in the dataset were birth weight (**BW**), weaning weight (**WW**), weaning wither height (**WH**), and weaning body condition score (**CS**) which was recorded as the average of two independent measurements made by two different observers.

From 1976 to 1994, sire selection in this herd was based on the "Dickerson Index" (Dickerson et al., 1974) which incorporated adjusted birth weight (**BW**) and adjusted yearling weight (**YW**):

$$I = \text{Adj. YW} - 3.2\ (\text{Adj. BW})$$

Adjustments for BW were sex of calf and age of dam and YW was adjusted for sex of calf, age of dam, and age at measurement.

Beginning in 1995, sire selection was based on adjusted yearling scrotal circumference (**SC**) with adjustments based on age of dam and age at measure. This selection continues through the present.

The average inbreeding coefficient of the herd is estimated at 36.4% for the 2004 calf crop and is increasing at an average of 0.7% per year. The animal with the largest inbreeding coefficient was estimated to be 50.5% inbred, but did not appear as a parent in this pedigree.

Genetic parameters and breeding values to calculate genetic trends for each trait were estimated using univariate models as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_a a} + \mathbf{Z_m m} + \mathbf{Z_d d} + \mathbf{e}$$

where:

- **y** is a vector of observed measures;
- **β** is a vector of fixed effects including year, sex, age of dam, and weaning date (excluded in the analysis of BW) and linear and quadratic covariates of individual inbreeding, date of birth, and weaning age (excluded in the analysis of BW);
- **a** is a vector of direct genetic effects;
- **m** is a vector of maternal genetic effects;
- **d** is a vector of maternal permanent environmental effects;
- **e** is a vector of random error effects;

X is a known incidence matrix associating fixed effects with records in **y**; and

$\mathbf{Z_a}$, $\mathbf{Z_m}$, **and** $\mathbf{Z_d}$ are known incidence matrices associating random effects with records in **y** with zero columns associated with animals in the pedigree that do not have records.

Furthermore,

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

$$\mathrm{Var}\begin{bmatrix}\mathbf{a}\\ \mathbf{m}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{A}\sigma_{am} & 0 & 0\\ \mathbf{A}\sigma_{am} & \mathbf{A}\sigma_m^2 & 0 & 0\\ 0 & 0 & \mathbf{I}\sigma_d^2 & 0\\ 0 & 0 & 0 & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where **A** is the numerator relationship matrix of all 7103 animals included in the pedigree, including those with no records, **I** are the identity matrices of the proper order, σ_a^2 is the variance due to additive genetic effects of the cow, σ_m^2 is the variance due to maternal genetic effects, σ_d^2 is the variance due to permanent environmental effects of the dam, and σ_e^2 is the variance due to random error.

Genetic parameters were estimated using the multiple-trait derivative-free REML program (**MTDFREML**) of Boldman et al. (1995) modified by Dodenhoff et al. (1998) for calculation of standard errors of estimates of genetic parameters for certain models.

Results and Discussion

Birth weight

Genetic parameters (and associated s.e.) for BW were estimated to be 0.51 (0.09), 0.08 (0.04), and 0.15 (0.26) for direct heritability, maternal heritability, and the direct-maternal genetic correlation, respectively. These estimates are in agreement with those previously reported for other populations (i.e., Dodenhoff et al., 1998; Ferreira et al., 1999; MacNeil, 2003).

The regression of the effect of inbreeding on BW was estimated to be -6.03 F – 50.51 F^2 indicating that as the level of inbreeding is increasing in this herd, birth weight can be expected to decrease. At the current inbreeding level, animals can be expected to be 8.9 kg lighter than if they were not inbred.

The genetic trends for both direct (**DBV**) and maternal breeding values (**MBV**) of BW are shown in Figure 1. For almost all years, MBV were estimated to be larger than DBV. Birth weight appears to have remained fairly constant in this herd since the herd was developed. The genetic trend for DBV is a decrease of 0.005 kg/yr and for MBV is a decrease of 0.004 kg/yr from 1962 to 2004. The phenotypic trend was a decrease of 0.034 kg/yr. Calculating the genetic trend within selection protocol, the trends from 1976 through 1995 when index selection was occurring were -0.074 and -0.034 kg/yr for DBV and MBV, respectively. For 1996 through present, when single trait selection based on scrotal circumference was practiced, the trends are +0.047 and +0.015 kg/yr, respectively. It appears that once index selection, which sought to decrease BW, ceased, BW began to increase genetically.

Weaning weight

Genetic parameters for WW were estimated to be 0.21 (0.07), 0.09 (0.06), -0.12 (0.35), and 0.30 (0.05) for direct heritability, maternal heritability, the direct-maternal genetic correlation, and the proportion of variance attributed to maternal permanent environment, respectively. These estimates are in general agreement with estimates previously reported for other populations (i.e., Arthur et al., 2001; Koch et al., 2004) although the direct-maternal genetic correlation estimated here is smaller in magnitude than most estimates in literature (i.e., Ferreira et al., 1998; Iwaisaki et al., 2005).

The regression of the effect of inbreeding on WW was estimated to be -109.61 F – 556.57 F^2 indicating that, like BW, as the level of inbreeding is increasing, weaning weight can be expected to decrease. At the current level of inbreeding, weaning weight could be expected to be 113.6 kg lighter than if animals were not inbred.

The genetic trend of WW is shown in Figure 2. Over the entire history of this herd, WW DBV have been increasing, on average, 0.289 kg/yr while MBV have been increasing 0.082 kg/yr. Phenotypically, WW has been increasing 0.276 kg/yr. During index selection, trends for WW were increasing with values of 0.397 and 0.156 kg/yr for DBV and MBV, respectively. Since single trait selection for SC was initiated, WW trends have slowed down with increases of 0.254 and 0.008 kg/yr, respectively.

Weaning Wither Height

Genetic parameters for WH were estimated to be 0.28 (0.11), 0.13 (0.07), and -0.52 (0.22) for direct heritability, maternal heritability, and the direct-maternal genetic correlation, respectively. Few analyses of WH are reported in literature, but this heritability estimate is less than is reported for weaning hip height by Vargas et al., 2000, but similar to that estimated by Rodriguez-Almeida et al., 1995.

The regression of the effect of inbreeding on WH was estimated to be – 10.35 F – 47.51 F^2. Like earlier discussed traits, this indicates that height is decreasing as inbreeding is increasing. At the current rate of inbreeding in this herd, WH can be expected to be 10 cm less than non-inbred animals.

Genetic trends for WH are shown in Figure 3. The genetic trend of WH is increasing at 0.025 and 0.010 cm/yr for DBV and MBV, respectively. Phenotypically, WH is decreasing at 0.130 cm/yr. During index selection, WH was genetically increasing at a rate of 0.020 and 0.018 cm/yr for DBV and MBV, respectively. After the selection protocol changed to SC selection, the DBV trend reversed to an estimated -0.028 while the MBV doubled to an estimated +0.040 cm/yr.

Weaning Body Condition Score

Genetic parameters for CS were estimated to be 0.24 (0.09), 0.09 (0.07), -0.48 (0.29), and 0.14 (0.04) for direct heritability, maternal heritability, the direct-maternal

genetic correlation, and the proportion of variance attributed to maternal permanent environment. There is little literature concerning weaning condition score, but this estimate of heritability is slightly larger than those reported in literature for mature cows (i.e., Mialon et al., 2000; Nephawe et al., 2004).

The regression of the effect of inbreeding on CS was estimated to be -2.59 F – 13.96 F^2, which like the other traits reported, indicates that CS is decreasing as inbreeding is increasing. At the current level of inbreeding in this herd, CS should be expected to be 2.8 score units less than a non-inbred animal.

Genetic trends for CS are shown in Figure 4 and are not biologically significant. Genetic trends for CS were +0.0061 and –0.0002 score units/yr for DBV and MBV, respectively, and the phenotypic trend was +0.0040 score units/yr. During index selection, genetic trends were +0.0078 and +0.0007 score units/yr, respectively. During scrotal circumference selection, genetic trends were +0.0067 and -0.0007 score units/yr.

Implications

For all traits considered, selection protocol had an effect on the genetic trends with trends changing when selection changed. Furthermore, inbreeding has been shown to cause decreased performance in all four of the traits analyzed. However, due to the change in selection schemes through the history of this herd, the Line 4 Hereford herd has remained relatively constant over the past 43 years.

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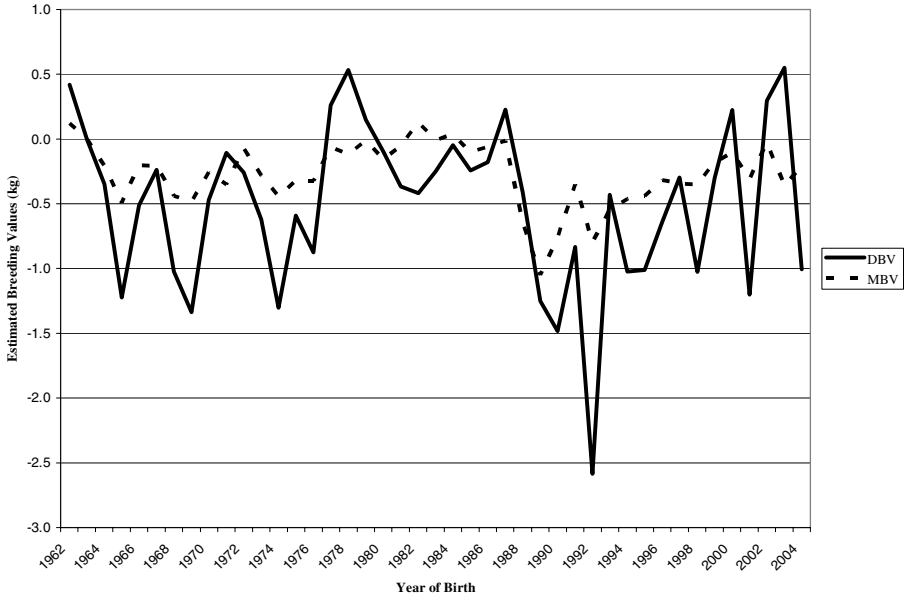

Figure 1. Genetic trends for direct (DBV) and maternal breeding values (MBV) of Birth Weight by Year of Birth

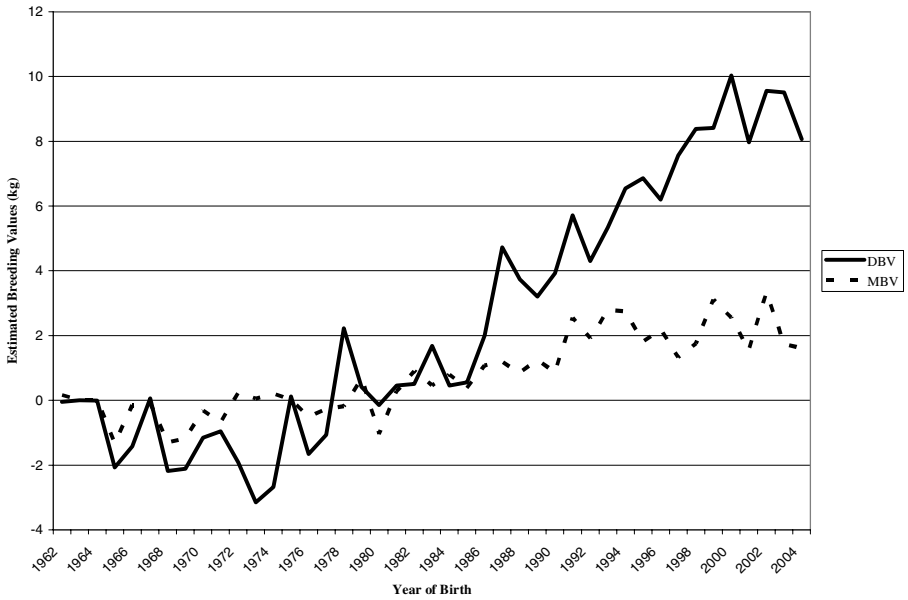

Figure 2. Genetic trends for direct (DBV) and maternal breeding values (MBV) of Weaning Weight by Year of Birth

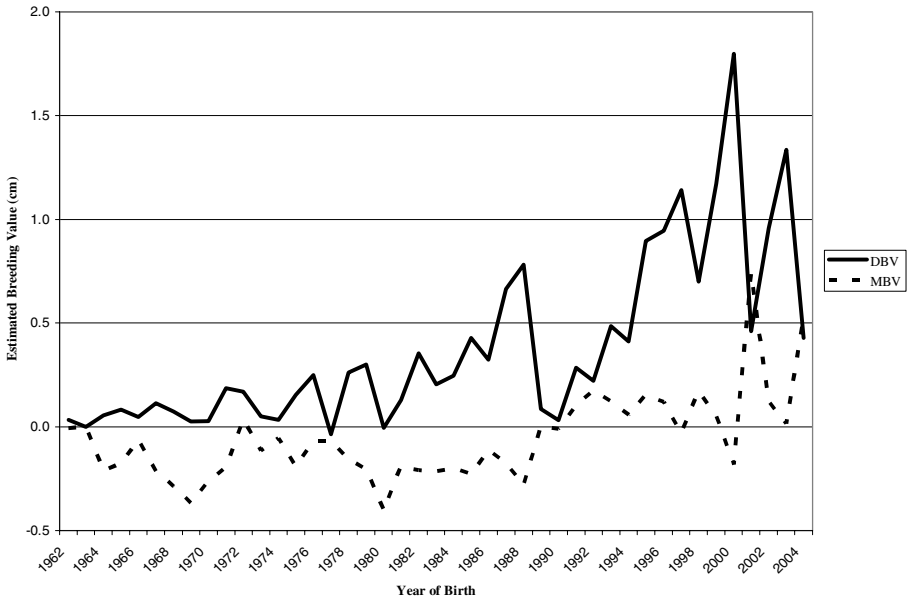

Figure 3. Genetic trends for direct (DBV) and maternal breeding values (MBV) of Weaning Wither Height by Year of Birth

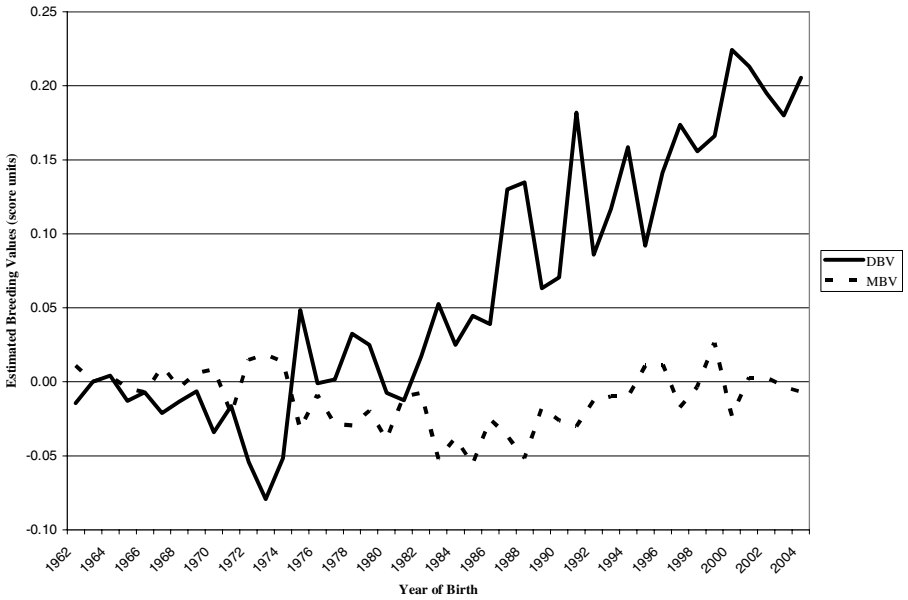

Figure 4. Genetic trends for direct (DBV) and maternal breeding values (MBV) of Weaning Condition Score by Year of Birth

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INFLUENCE OF INCORRECT ESTIMATES OF INBREEDING ON THE GENETIC ANALYSIS OF MATURE COW WEIGHTS IN A POPULATION OF INBRED HEREFORD CATTLE

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ABSTRACT: Data of 9051 weights measured on 642 cows in the Line 4 inbred Hereford herd at the Northern Agricultural Research Center in Havre, Montana from 1976 to 1997 were analyzed. Age of cow ranged from 18 mo to 15 yr. Previous reports described the 1995 calf crop from this line to have an average inbreeding coefficient of 20.7%. After pedigree reconstruction, the average inbreeding coefficient of the 1995 calf crop was determined to be 33.8% and increasing at a rate of 0.3% per year from 1976 to 1995. A maximum of six weigh dates per year were analyzed as part of this study in a univariate repeatability model. Estimates of genetic parameters were not significantly different from the previously reported estimates that included reduced estimates of inbreeding. Estimates of genetic parameters (and associated s.e.) from this analysis were 0.69 (0.13), -0.63 (0.19), 0.09 (0.05), and 0.13 (0.08) for direct heritability, direct-maternal genetic correlation, maternal heritability, and proportion of variance due to direct permanent environmental effects, respectively. Genetic trends for direct breeding values were small, but three times greater (in absolute value) than had been previously reported with an estimate of -0.19 kg/yr. Average direct breeding values (**DBV**) by year of birth were generally smaller with the correct estimates of inbreeding, with differences from the previous estimates ranging from -2.4 to +0.4 kg. Average maternal breeding values (**MBV**) by year of birth were larger, with differences from the previous estimates ranging from 0.7 to 1.3 kg compared to the previous estimates. Spearman rank correlations comparing the new and old estimates were 0.98 and 0.98 for DBV and MBV, respectively. Incorrect estimates of inbreeding have little effect on genetic trends and relative ranking, but do alter the actual breeding values reported for animals.

Keywords: Inbreeding, Mature Weight, Selection

Introduction

Researchers conducting genetic evaluations seek to produce the most trustworthy genetic predictions for use by producers in animal selection. Including pedigrees of sufficient size to accurately estimate relationships is important. Inclusion of these pedigrees for accurate estimates of inbreeding may also be important because incorrect estimates of inbreeding coefficients in models for genetic evaluation may affect the parameter estimates and estimated breeding values calculated in these evaluations. The objective of this research was to determine the effect that correct estimates of inbreeding using pedigrees with more generations included would have compared to analyses using information with fewer generations.

Materials and Methods

The Line 4 Hereford herd at the Northern Agricultural Research Center (**NARC**) in Havre, Montana has been maintained as a closed herd since 1963 and descended from the Miles City Line 1 Hereford herd. From 1976 through 1995, sires were selected in this herd based on the "Dickerson Index" (Dickerson et al., 1974) which incorporated adjusted birth weight (**BW**) and adjusted yearling weight (**YW**). Adjustments for BW were sex of calf and age of dam and YW was adjusted for sex of calf, age of dam, and age at measurement. The index was:

$$I = \text{Adj. YW} - 3.2\ (\text{Adj. BW})$$

Data analyzed for this study included 9051 cow weight records measured over multiple seasons and years on 642 cows born and/or used as dams during the 22 years of the index selection. Weights included were measured a maximum of six times per year: prior to calving, after calving, at two different milk test dates, prior to breeding, and after breeding. Cows averaged 14.1 records in their lifetime and 3.8 records a year. A cow's earliest weight was taken in the fall of her yearling year, when she was approximately 18 mo old. The oldest cow was weighed at 15 yr of age.

Genetic parameters and breeding values to calculate genetic trends were estimated using an analysis including all seasons analyzed together in one univariate, repeatability model as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_a a} + \mathbf{Z_m m} + \mathbf{Z_c c} + \mathbf{e}$$

where:

- **y** is a vector of observed cow weights;
- **β** is a vector of fixed effects including age of cow, year, and the two-way subclass of weigh code and season as well as linear and quadratic covariates of individual inbreeding and weigh date;
- **a** is a vector of direct genetic effects;
- **m** is a vector of maternal genetic effects;
- **c** is a vector of direct permanent environmental effects;
- **e** is a vector of random error effects;

X is a known incidence matrix associating fixed effects with records in **y**; and

Z_a, Z_m, and Z_c are known incidence matrices associating random effects with records in **y** with zero columns associated with animals in the pedigree that do not have records.

Furthermore,

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

$$\text{Var}\begin{bmatrix}\mathbf{a}\\ \mathbf{m}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{A}\sigma_{am} & 0 & 0\\ \mathbf{A}\sigma_{am} & \mathbf{A}\sigma_m^2 & 0 & 0\\ 0 & 0 & \mathbf{I}\sigma_c^2 & 0\\ 0 & 0 & 0 & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where **A** is the numerator relationship matrix of all 7103 animals included in the pedigree, including those with no records, **I** are the identity matrices of the proper order, σ_a^2 is the variance due to additive genetic effects of the cow, σ_m^2 is the variance due to maternal genetic effects, σ_c^2 is the variance due to permanent environmental effects of the cow, and σ_e^2 is the variance due to random error.

Genetic parameters were estimated using the multiple-trait derivative-free REML program (**MTDFREML**) of Boldman et al. (1995) modified by Dodenhoff et al. (1998) for calculation of standard errors of estimates of genetic parameters for certain models.

Previously reported estimates of genetic parameters for mature weight in this dataset were analyzed and reported by Rumph et al. (2004). At that time, the pedigree file included animals from the time the herd was developed at NARC in the early 1960's and through the animals in the current study. This resulted in 2079 animals, and did not include the Miles City Line 1 Hereford ancestors of this line. Exclusion of these animals was hypothesized to result in the underestimation of the current level of inbreeding in the Line 4 herd, so reconstruction of the pedigree commenced.

Pedigree information was traced back twenty generations from the 1973, 1974, and 1975 calf crops or as far back as allowable if twenty generations were not possible. These calving years were chosen because they were the first years in which all calves born were progeny of NARC born animals. Additionally, inclusion of calves from three years would minimize the probability of losing an animal due to not having descendents born in the included years. Any pedigree information after 1975 was assumed to be correct.

Results and Discussion

Reconstruction of the pedigree resulted in the inclusion of a total of 7103 animals in the pedigree and increased the estimate of the average inbreeding coefficient of the 1995 calf crop from 20.7% (Rumph et al., 2004) to 33.8%. The animal with the largest inbreeding coefficient was estimated to be 50.5% inbred, but did not appear as a parent in this pedigree. Both the previously reported and the current estimates of inbreeding trends are shown in Figure 1. The average inbreeding coefficient is increasing by 0.7% per year when considering animals born from 1962 (the point at which the animals were first moved from Miles City to NARC) to present, compared to the estimate of 0.3% when considering the previous pedigree. The trend is 0.3% when only considering the years involved in the index selection with the reconstructed pedigree, but 0.4% when considering the abridged pedigree. Using both pedigrees, animals born in 1961 and 1963 had average inbreeding coefficients of 0 due to Miles City bulls being mated to grade Hereford cows at Havre whose pedigrees could not be traced.

The regression of the effect of inbreeding on mature weight was estimated to be $-281.4F + 232.5F^2$ compared to estimates of $-235.1F + 162.0F^2$ using the previous pedigree indicating that at the current level of inbreeding in this herd, inbreeding has a larger effect on mature weight than previously reported (Rumph et al., 2004). At higher levels of inbreeding than are currently in the NARC herd (> 65.7%) the reverse would have been true and inbreeding would have a lesser effect than previously reported.

Genetic parameters estimated in the current analysis using the reconstructed pedigree were not statistically different than those reported previously (Rumph et al., 2004). Estimates (and associated s.e.) were 0.69 (0.13), -0.63 (0.19), 0.09 (0.05), and 0.13 (0.08) for direct heritability, direct-maternal genetic correlation, maternal heritability, and proportion of variance due to direct permanent environmental effects, respectively. These are in agreement with other estimates from other populations of beef cattle (i.e., Brinks et al., 1962; Benyshek and Marlow, 1973; Rumph et al., 2002).

The genetic trends for direct breeding values (**DBV**) using both pedigrees are shown in Figure 2 and the difference between the two analyses is shown in Figure 3. As time went on, the difference between the two DBV increased in absolute value with the reconstructed pedigree producing smaller estimates of DBV, in general. Trends for maternal breeding values (**MBV**) are shown in Figure 4 with the difference between the two analyses shown in Figure 5. There was no apparent increase in the difference in MBV as time went on, but the general trend was inexplicably similar to the trend seen in the actual MBV, although the magnitude is smaller for the differences than for the actual MBV.

Spearman rank correlations of the current and previously estimated DBV and MBV were performed using the CORR procedure of SAS (SAS Inst., Inc., Cary, NC). Spearman rank correlation between the old and new estimates of DBV was 0.98 ($P < 0.01$) and between the old and new estimates of MBV was 0.98 ($P < 0.01$). Indicating that although DBV and MBV are changing, the rank of the animals is not.

Implications

Fitting the correct level of inbreeding in statistical models does not significantly alter the parameter estimates

or the ranking of animals analyzed. However, it does effect the actual estimates of the breeding values and therefore inbreeding should be accounted for as accurately as possible to increase the trustworthiness of genetic evaluations.

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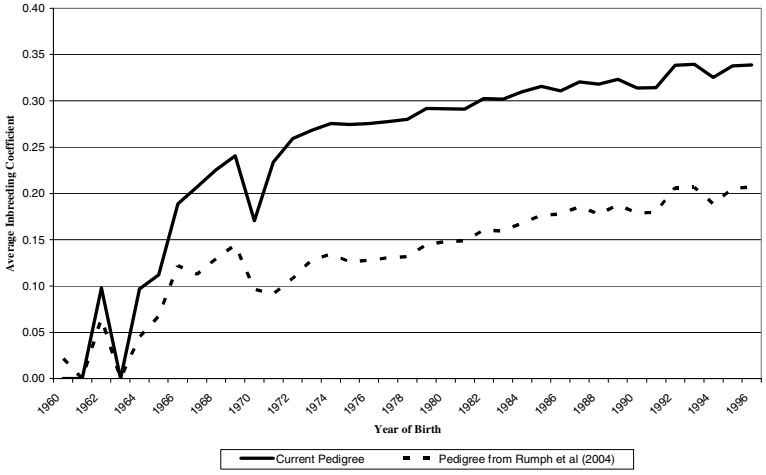

Figure 1. Comparison of Estimated Inbreeding with Two Different Pedigrees

Figure 2. Average Direct Breeding Value (DBV) by Year of Birth

Figure 3. Difference Between Estimates of Direct Breeding Values Using the Reconstructed Pedigree vs. the Pedigree reported in Rumph et al. (2004)

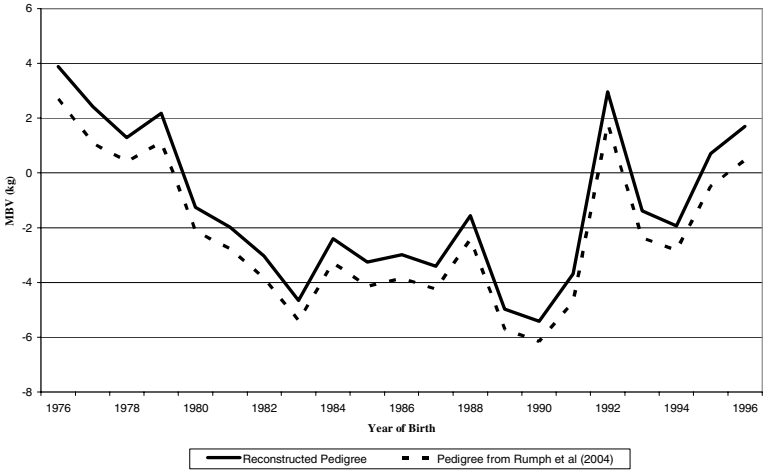

Figure 4. Average Maternal Breeding Value (MBV) by Year of Birth

Figure 5. Difference Between Estimates of Maternal Breeding Values Using the Reconstructed Pedigree vs. the Pedigree reported in Rumph et al.

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AGE-OF-DAM ADJUSTMENT FACTORS FOR SCROTAL CIRCUMFERENCE IN THE MONTANA LINE 4 HEREFORD HERD

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ABSTRACT: The Montana Line 4 Hereford herd at the Northern Agricultural Research Center in Havre, Montana has been a closed herd since 1962 and currently has an average estimated inbreeding coefficient for 2004 born calves of 36.4%. Beginning in 1995, single trait selection based on an index of scrotal circumference was initiated. This index includes an additive age-of-dam adjustment and linear regression adjustment to 365-d of age. Age-of-dam adjustment factors are recalculated every five yr in order to accurately characterize this effect. Scrotal circumference data on 573 bull calves born from 1983 to 2003 were analyzed to compute current scrotal circumference age-of-dam adjustment factors for this population. Effects were computed using MTDFREML. The model used was a univariate model with year and age-of-dam included as fixed effects. Inbreeding of the individual and age at measure were included as linear and quadratic covariates. Heritability (and associated s.e.) for scrotal circumference was estimated to be 0.33 (0.12). Age-of-dam adjustment factors were calculated to be 1.79, 0.95, 0.11, 0.00, 0.30 cm for 2-, 3-, 4-, and 10-yr-old dams, respectively, adjusted to a mature dam base of 5 to 9 yr of age. These adjustment factors are larger for 2-, 3-, and 4-yr-old dams than had been estimated with earlier data. Older dams, however, were estimated to have the same effect as in previous analyses. It appears that selection for scrotal circumference in the Montana Line 4 herd is resulting in age-of-dam effects that are changing with time, particularly with younger dams.

Keywords: Age-of-dam adjustment factors, Scrotal circumference, Selection

Introduction

Previous research has indicated that when a trait is increasing, such as mature weight, the age-of-dam adjustment factors for correlated traits may change with time (Northcutt et al., 1994). Scrotal circumference is often selected by producers as an indicator trait for serving capacity and/or age at puberty of daughters. However, research concerning the change of scrotal circumference age-of-dam adjustment factors when selection for scrotal circumference is occurring is limited.

The purpose of this project was to recalculate the age-of-dam adjustment factors for scrotal circumference in a herd undergoing single trait selection for scrotal circumference to determine if the adjustments are changing with time.

Materials and Methods

The Line 4 inbred Hereford herd maintained by Montana State University's Northern Agricultural Research Center in Havre, Montana was established in 1962 and has remained closed since 1963. This herd is descended from the Miles City Line 1 Hereford herd. The current inbreeding in this herd is 36.4% for the 2004 calf crop and has been increasing, on average, at a rate of 0.7%.

Beginning in 1996, single trait phenotypic selection for adjusted yearling scrotal circumference (**SC**) was used for bulls. Scrotal circumference was adjusted for age at measure and age of dam (**AOD**) with AOD adjustments being recalculated approximately every 5 years. Current AOD adjustment factors, calculated in 2000 using animals born through 1998 are shown in Table 1. Sons of heifer calves had the largest adjustment of 1.60 cm when compared to sons out of 5- to 10-yr-old dams. Adjustments decreased with age through 4 yr old dams and then increase for older dams.

Table 1. Current Age-of-Dam Adjustment Factors for Scrotal Circumference

Age-of-Dam (yr)	Adjustment (cm)
2	1.60
3	0.76
4	-0.15
5-10	0.00
11	0.30

Data in the current analysis included yearling SC measurements on 573 bulls from 1983 to present. Genetic parameters and fixed effects to calculate current adjustment factors were estimated using a univariate analysis as follows:

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

where:

y is a vector of observed scrotal circumferences;
β is a vector of fixed effects including year and age-of-dam as categorical effects and individual inbreeding and age at measure as linear and quadratic covariates;
a is a vector of direct genetic effects;
e is a vector of random error effects;

X is a known incidence matrix associating fixed effects with records in **y**; and

$\mathbf{Z_a}$ is a known incidence matrix associating random genetic effects with records in **y** with zero columns associated with animals in the pedigree that do not have records.

Furthermore,

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

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

where **A** is the numerator relationship matrix of all 7103 animals included in the pedigree, including those with no records, **I** is the identity matrices of the proper order, σ_a^2 is the variance due to additive genetic effects of the animal, and σ_e^2 is the variance due to random error.

Genetic parameters were estimated using the multiple-trait derivative-free REML program (**MTDFREML**) of Boldman et al. (1995) modified by Dodenhoff et al. (1998) for calculation of standard errors of estimates of genetic parameters for certain models.

Results and Discussion

Heritability (and associated s.e.) was estimated to be 0.33 (0.12) which agrees with most literature estimates (i.e., Smith et al., 1989; Keeton et al., 1996; Martinez-Velazquez et al., 2003), but is less than the estimate of 0.53 reported by Kriese et al. (1991) and the estimate of 0.71 reported by Evans et al. (1999).

The regression of the effect of inbreeding on scrotal circumference was estimated to be $-9.93\ F + 5.47\ F^2$ indicating that inbreeding is having an effect on scrotal circumference and that with the current level of inbreeding in this herd, animals can be expected to have SC that are 2.89 cm smaller than comparable bulls that are not inbred.

Estimates of AOD adjustment factors calculated using this data are shown in Table 2. Age classifications vary from those previously used because adjusting to a mature cow base of 5 to 9 yr of age and classifying old cows as 10 yr of age and older was a better fit to the data. Estimates for 10-yr-old cows were more similar to older cows than the 5- to 9-yr-old group that it had been included in for the previous analyses.

Table 2. Recalculated Age-of-Dam Adjustment Factors for Scrotal Circumference

Age-of-Dam (yr)	Adjustment (cm)
2	1.79
3	0.95
4	0.11
5-9	0.00
10	0.30

Adjustments for all age groups, except the old category increased in value from the adjustments that are currently being used. Sons of heifer calves will have 1.79 cm added to their SC measure prior to selection and sons of three-yr-old dams will have 0.95 cm added to their SC measure. These adjustment factors will encourage selection of bulls out of younger dams, particularly 2-yr-old dams. This can be expected to decrease the generation interval of the herd, potentially increasing the amount of genetic progress achieved in this herd per year.

These values are smaller than those reported by Kriese et al. (1991) who estimated Hereford SC AOD adjustments to be 0.7, 0.3, 0.2, 0.2, and 0.3 cm for sons of 2-, 3-, 4-, 5-, and 8-yr-old and older cows adjusted to a 6- to 8-yr-old mature cow basis. These are also less than estimates reported by Bourdon and Brinks (1986) of 0.8, 0.2, and 0.1 cm for sons of 2-, 3-, and 4-yr-old cows. Neither of these two populations were significantly inbred. The level of inbreeding in the Line 4 Hereford herd would explain the differences between the current estimates in this herd and those reported in literature.

Implications

Selection for scrotal circumference results in age-of-dam adjustment factors that change with time. If using age-of-dam adjustments when selecting for SC, these values should be recalculated frequently to insure that proper values are being used.

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MILK YIELD OF BEEF HEIFERS FROM THREE CALVING SYSTEMS

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ABSTRACT: In a 2-yr study, first-calf heifers from three calving systems were used to study milk yield throughout a 190-d lactation. Calving occurred in late January to late February (Feb calving), mid-March to mid-April (Apr calving), and mid-May to mid-June (Jun calving). Milk production was measured by weigh-suckle-weigh technique on seven dates for each herd. Average days in milk at measurement were 20-, 38-, 55-, 88-, 125-, 163- and 190-d. Milk yield for the entire lactation period was calculated as area under the curve by trapezoidal summation. Total milk yield over the 190-d lactation exhibited a calving system x year interaction ($P < 0.001$). Total yield did not differ between heifers in Feb and Apr systems, yet both differed from Jun. In 2002, Jun heifers produced more ($P < 0.05$), whereas in 2003 they produced less ($P < 0.01$) milk than heifers in other calving systems. The ADG of calves for the three systems related well to the total milk yields. The Feb calves showed an increased ($P < 0.05$) rate of gain in 2003 compared to 2002 and Jun calves grew more slowly ($P < 0.01$) in 2003 than 2002. Milk yield at peak lactation was affected ($P < 0.001$) by calving system and showed a calving system x year interaction ($P < 0.01$). In 2002, peak milk yield did not differ between Feb and Apr heifers, and both were less ($P < 0.01$) than Jun heifers. In 2003 peak milk yield in Apr and Jun heifers did not differ and both tended to be greater ($P < 0.10$) than Feb heifers. Day of peak lactation differed among calving systems ($P < 0.001$) with heifers in the Feb system peaking later ($P < 0.01$) than those heifers from other systems. Average date of peak lactation was May 4 for the Feb system, May 31 for the Apr system, and July 19 for the Jun system. Date of calving affects milk yield of heifers and ADG of their calves while grazing Northern Great Plains rangelands.

Key Words: Milk yield, beef cows, rangeland, calving date

Introduction

Milk yield of the dam is a major determinant of growth rate in beef calves (Totusek et al., 1973). Forage quality within rangeland systems can affect growth rate of calves through influences on the milk yield of dams and the quality of the forage portion of a calf's diet (Grings et al., 1996). Adjusting calving time for beef cows from late winter through late spring impacts the quality of forage available for milk production and growth of calves in the Northern Great Plains. Previous research at this location has shown decreased weaning weights in calves from June compared to February and April systems (Grings et al., 2003). This study evaluated the milk yield of first-calf heifers born and raised within three calving systems and the impact on growth of their calves.

Materials and Methods

Study Site

This study was conducted at the Fort Keogh Livestock and Range Research Laboratory near Miles City, MT (46° 22' N 105° 5' W). The potential natural vegetation is a grama-needlegrass-wheatgrass (*Bouteloua-Hesperostipa-Pascopyron*) mixed grass dominant. Climate is continental and semi-arid. Average annual rainfall in this area is 338 mm with 60% received during the 150-d, mid-April to mid-September growing season. Average daily temperatures range from -10 C in January to 24 C in July. Precipitation patterns for 2002 and 2003 are presented in Figure 1.

Herd Management

In a 2-yr study, first-calf heifers from three calving systems were used to study milk yield throughout a 190-d lactation. Heifers and their calves were from the same calving system, with calving dates of late January to late February (Feb calving), mid-March to mid-April (Apr calving), and mid-May to mid-June (Jun calving). First-calf heifers were sired predominately by composite bulls (1/2 Red Angus, ¼ Charolais, ¼ Tarentaise) with dams being crossbred cows of varied genetic background, including some combinations of Hereford, Limousin, Charolais, and composite breeding. In 2002, calves were sired by bulls that were at least one-fourth composite breeding, whereas in 2003 calves were sired by Angus bulls. Breeding was from about April 6 to May 9, June 6 to July 9, and August 6 to September 9 (exact dates vary by year). Each calving herd was managed separately throughout the year, with inputs appropriate for the specific calving season. Quantity and quality of hay and supplements were provided based upon forage and weather conditions, physiological state of the cows, and available harvested feed resources within a year. During the period of milk production measurements heifers were maintained primarily on native rangeland. However, supplemental feed was provided to Feb heifers

[1] This research was conducted under cooperative agreement between USDA-ARS Northern Plains Area, and the MT Agric. Exp. Sta. USDA-ARS, NPA is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

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through the third milk yield measurement and to the Apr heifers through the first milk yield measurement. No supplemental feed was provided to the Jun heifers during lactation.

After calving, heifers selected for milk production measures from each calving system (2002 n = 20, 2003 n = 24 per calving system) were managed in 3 groups (one for each system) which were moved to new pastures as dictated by forage availability.

Animal Data Collection

Milk production was measured by weigh-suckle-weigh technique. Average days in milk at yield measures were 20-, 38-, 55-, 88-, 125-, 163- and 190-d. Calves were separated from their dams for 8 hr, allowed to suckle until full, and separated again for 12 hr. Calves were then weighed, allowed to suckle until full and re-weighed. Milk yield was calculated as the difference between the pre- and post-suckling weight. Milk yield was multiplied by two to obtain 24-h milk production estimates for calculation of total yield.

Average daily gain from birth to weaning was calculated by subtracting birth weight from weaning weight and dividing by the day of age at weaning.

Diet Quality

Diet quality during grazing periods was estimated from esophageal extrusa. Extrusa samples were collected within a week of milk yield measures for each herd. Extrusa samples were collected using three to six adult esophageally cannulated cows in each pasture during a 30-45 min period. Cows had previous grazing experience in all pastures and were familiar with the vegetation types being grazed. Before each extrusa sample collection, cows were penned overnight with access to water. Extrusa samples were lyophilized, ground to pass a 1-mm screen and stored until analysis for DM, OM (both AOAC, 1990), CP, and in vitro OM digestibility. Extrusa samples were not collected at the second milk yield period in 2002 or the first and second milk yield periods for 2003 for the Feb calving system because snow cover precluded grazing at that time.

Samples for CP determinations were placed in a roller grinder for 12 h (Mortenson, 2003). Nitrogen was determined by combustion techniques in a C-N (CE Elantech, Inc., Lakewood, NJ) analyzer. Nitrogen was multiplied by 6.25 to obtain CP and these values were expressed on an OM basis. In vitro OM digestibility was determined by the method of Tilley and Terry (1963).

Statistical analysis

Milk yield for the entire lactation period was calculated as area under the curve by trapezoidal summation using Graph Pad Prism software (GraphPad Software, Inc., San Diego, CA). Yield and day of peak milk production were also calculated using this software.

A total of 12 milk production values were not used due to obvious weighing errors during the weigh-suckle-weigh procedure. If these weighing errors were at the final milk measure, total yield values were not included in the data analysis (six occurrences; four in Feb in 2002, two Jun in 2003). Total milk yield data were not used from two Feb calves in 2003 that were less than 165 d of age at weaning.

Data were analyzed using Proc Mixed procedures in SAS (SAS Institute, Cary, NC). Terms in the model included calving system, year, and the year by calving system interaction as fixed class effects and calf sex and day of age at final milk yield measurement (weaning) as covariates. Year effects were included as fixed effects to evaluate the impact of measured environmental differences among years.

Pregnancy was diagnosed by transrectal ultrasonography in the fall. Proportion of cows pregnant was tested using CATMOD procedures in SAS using a model that included calving system, year, and the calving system by year interaction.

Results

Precipitation patterns differed between the two years of the study (Figure 1), resulting in varied extrusa quality between the two years. Although year effects were not significant for milk and calf gain measures, there were calving system x year interactions for all measures except day of peak lactation.

Total milk yield over the 190-d lactation exhibited a calving system x year interaction ($P < 0.001$; Table 1). Total yield did not differ between heifers in Feb and Apr systems, yet both differed from Jun. However, this relationship was affected by year. In 2002, Jun heifers produced more ($P < 0.05$), whereas in 2003 they produced less ($P < 0.01$) milk than heifers in other calving systems.

While yield for heifers in the Apr system did not differ between years, it varied between years for both the Feb and Jun systems. Heifers in the Feb system produced more milk in 2003 than in 2002, whereas the opposite was true for the Jun heifers. The lowered milk yield in 2003 for the Jun heifers is related to the lowered extrusa quality observed in that year (Figure 2). The extrusa quality curves observed for 2003 may be the more typical for this Northern Great Plains environment.

The ADG of calves for the three systems (Table 1; calving system x year interaction, $P < 0.001$) related well to the total milk yields. Feb calves showed an increased ($P< 0.05$) rate of gain in 2003 compared to 2002 and Jun calves grew more slowly ($P < 0.01$) in 2003 than 2002, both relationships following the trend in milk yield. Calves in the Apr system grew faster in 2003 compared to 2002. Milk yield was numerically, but not statistically greater for the Apr heifers in 2003 compared to 2002.

Milk yield at peak lactation was affected ($P < 0.001$; Table 1) by calving system and showed a calving system x year interaction ($P < 0.01$). In 2002, peak milk yield did not differ between Feb and Apr heifers and both were less ($P < 0.01$) than Jun heifers. In 2003 peak milk yield in Apr and Jun heifers did not differ and both were greater ($P < 0.01$) than Feb heifers.

Day of peak lactation differed among calving systems ($P < 0.001$) with heifers in the Feb system peaking later ($P < 0.01$) than those heifers from other systems. Average calendar day of peak lactation was May 4 for the Feb system, May 31 for the Apr system, and July 19 for the Jun system. Day of peak did not differ among years even

though extrusa quality patterns differed between years (Figure 2).

Proportion of heifers pregnant was not affected (P > 0.10) by calving system, year, or the calving system x year interaction. Proportion of heifers pregnant averaged 0.81 (± 0.06) for Feb, 0.89 (± 0.05) for Apr, and 0.82 (± 0.06) for Jun calving systems.

Implications

Season of calving and its associated management affects time and amount of milk yield in heifers, corresponding to varied weight gains in their calves. Understanding the impacts of calving date on amounts and patterns of milk production can aid in developing management systems to best match nutrient needs of cow-calf pairs in different calving systems.

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Table 1. Least squares means of total milk yield, yield at peak lactation, day of peak lactation, and calf ADG measured in 2002 and 2003 for first-calf heifers from three calving systems

	Calving system				Probability [1] value for an effect of:		
	Feb	Apr	Jun	SEM	Calving system	Year	Calving system x year
Total yield, kg				19.4	0.21	0.37	0.001
2002	1093 ^{aA}	1053 ^{a}	1238 ^{bC}				
2003	1215 ^{cB}	1161 ^{c}	897 ^{dD}				
Yield at peak, kg				0.2	0.001	0.48	0.002
2002	8.3 ^{c}	8.3 ^{cA}	11.3 ^{dC}				
2003	8.3 ^{e}	9.5 ^{fB}	9.2 ^{efD}				
Day of peak	88 ^{c}	61 ^{d}	51 ^{d}	3.4	0.001	0.56	0.27
Calf ADG, kg/d				0.008	0.001	0.20	0.001
2002	0.95 ^{aE}	0.88 ^{bC}	0.91 ^{abC}				
2003	1.01 ^{aeF}	0.95 ^{afD}	0.71 ^{bD}				

[1] All *P* values for the covariate, calf sex, in milk variable models were > 0.60. For calf sex in ADG model *P* < 0.01 All *P* values for the covariate, age at final milk measure were > 0.10.

^{ab} Means in rows with differing superscripts differ (*P* < 0.05)

^{cd} Means in rows with differing superscripts differ (*P* < 0.01)

^{ef} Means in rows with differing superscripts tend to differ (*P* < 0.10)

^{AB} Means in different years with differing superscripts tend to differ (*P* < 0.10)

^{CD} Means in different years with differing superscripts differ (*P* < 0.01)

^{EF} Means in different years with differing superscripts differ (*P* < 0.05)

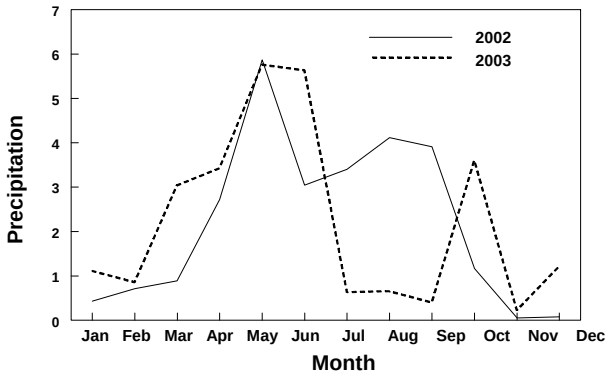

Figure 1. Precipitation during 2002 and 2003 at Miles City MT (NOAA 2002, 2003)

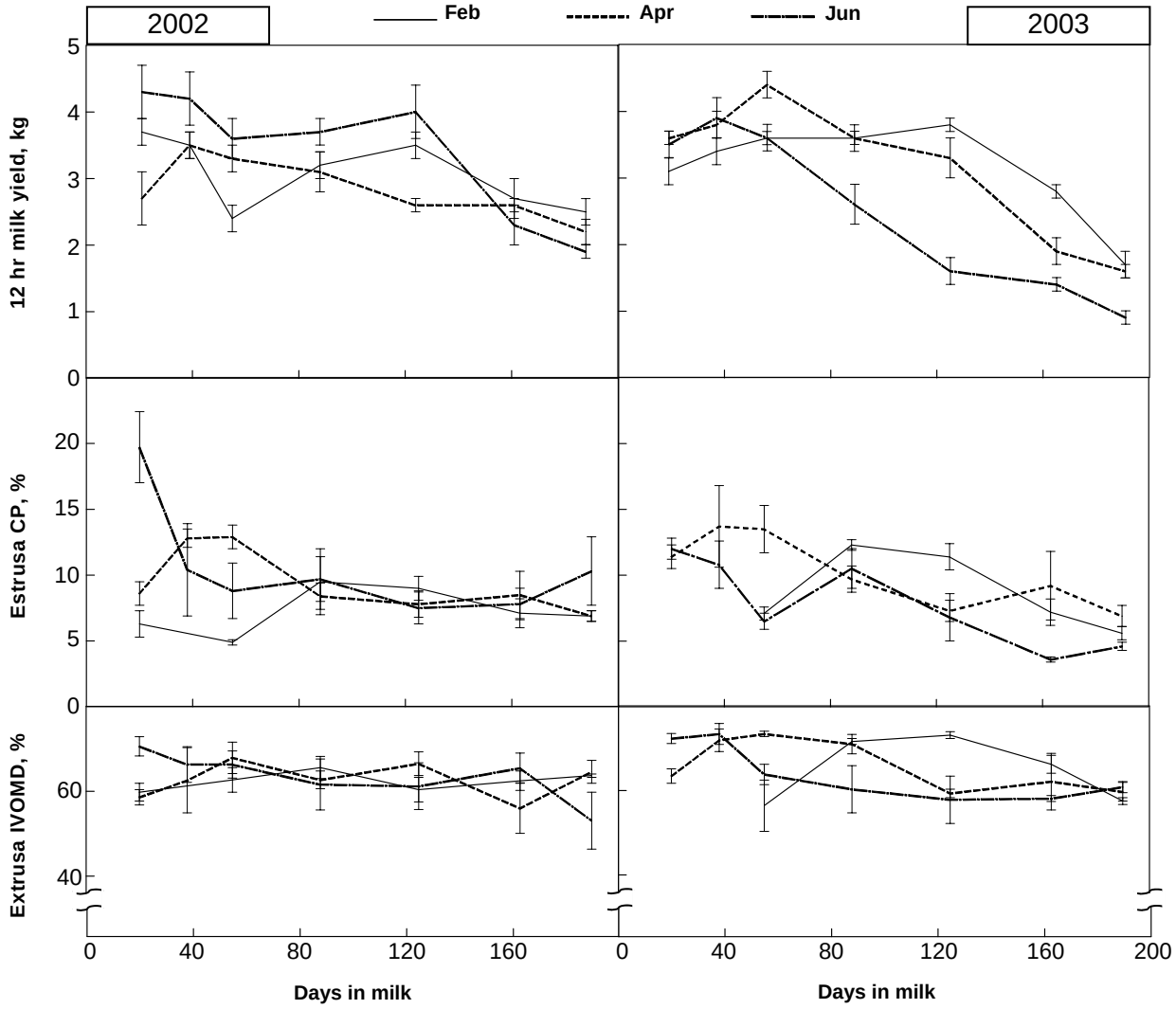

Figure 2. Twelve hour milk yield, estrusa % CP and IVOMD ± SE in 2002 and 2003 for heifers from three calving systems (Feb, Apr, Jun). In addition to range forage, supplemental feed was provided to Feb heifers through the third milk yield measurement and to the Apr heifers through the first milk yield measurement. Supplemental feed was not provided to the Jun heifers during lactation. No diet samples were collected at the second milk yield period in 2002 or the first and second milk yield periods for 2003 for the Feb calving system because snow cover precluded grazing at that time.

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GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING HOGS CONSUMING 'DUN' OR 'WHITE' FIELD PEAS

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ABSTRACT: Sixty commercial cross barrows and gilts (initial BW = 74.5 ± 16.4 kg) were used in a completely randomized design experiment to examine the effects of two varieties of field peas on growth performance and carcass characteristics of finishing hogs. Hogs were weighed, randomly assigned to pens (10 pigs per pen), and fed one of three finishing diets. Diets were corn and soybean meal (Control); and Control with Forager 'dun' field peas (Dun) or Carnival 'white' field peas (White) incorporated at 16% of the diet at the expense of corn and SBM. Diets were formulated to be isonitrogenous and meet NRC recommendations for a 75 kg finishing hog. Initial and final BW was determined from an average of weights taken on two consecutive days. Following an average of 60 d on feed, hogs were slaughtered at a commercial abattoir. No differences among dietary treatments were detected for initial BW (P = 0.92), final BW (P = 0.89), total gain (P = 0.39), ADG (P = 0.63), or ADFI (P = 0.21). Feed efficiency was greater (P = 0.01) for hogs fed White but did not differ (P = 0.62) between hogs fed Control or Dun diets. No differences among dietary treatments were noted for HCW (P = 0.86), USDA muscling score (P = 0.12), last rib fat depth (P = 0.74), loin muscle area (P = 0.95), carcass length (P = 0.57), and standardized fat-free lean (P = 0.43). Tenth-rib fat depth was less (P = 0.01) for hogs fed Dun compared with hogs fed Control or White diets. Estimated percent lean was greater (P = 0.03) for hogs fed White or Dun diets compared with hogs fed the Control diet. Feeding value of Dun and White field peas was similar when included at 16% of finishing hog diets. Partial substitution of field peas for corn and soybean meal does not have a negative effect on finishing hog performance or carcass characteristics.

Key Words: 'Dun' peas, 'White' peas, Swine

Introduction

Field pea (*Pisum sativum* L.) production in the past decade has increased throughout the Northern United States (NASS, 2002). In North Dakota alone, production has increased from 5,600 ha in 1994 to 55,800 ha in 2003 (NDASS, 2003 as cited by Reed et al., 2004a,b). With energy content similar to that of corn and CP of about 23% (NRC, 1998), field peas have great potential for feedstuff applications. Field peas have replaced as much as 36% of corn and soybean meal in swine diets without affects on growth performance (Stefanyshyn-Cote et al., 1998; Szabo et al., 2001; Stein et al., 2004).

Forager and Carnival are two different varieties of field pea produced in Wyoming. Carnival is classified as a 'White' pea, and is readily marketable. Forager, with its darker seed coat, is considered a 'Dun' pea, which is subject to a discount because it falls into the miscellaneous market class. Field peas with darker seed coats are presumed to be less nutritious. Therefore, our objective was to determine if feeding Dun field peas caused a negative influence on growth performance and carcass charictaristics in hogs compared with feeding White peas. Our hypothesis was that inclusion of cracked Dun or White field peas to a swine finishing diet would not adversely affect rate of gain, feed efficiency and carcass characteristics.

Materials and Methods

Animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Commercial cross barrows and gilts (n = 60, initial BW = 74.5 ±16.35 kg) were weighed on two consecutive days. Animals were then assigned to one of six pens (10 animals per pen) located at the University of Wyoming Livestock Center and fed one of three diets: a corn-soybean based control (Control); and Control with either 'Dun' Forager peas (Dun) or 'White' Carnival peas (White) incorporated as 16% of the diet at the expense of soybean meal. Hogs were weighed on two consecutive days following 60 ± 3 d of consuming the respective diets.

Diets

Diets were formulated to meet the nutrient requirements of a 75 kg finishing hog (NRC, 1998). Diets were also formulated to provide similar quantities of energy, CP, lysine, Ca, and P (Table 1). All hogs had ad *libitum* access to feed and water throughout the experiment.

Table 1. Ingredient and nutrient composition of diets fed to finishing hogs, % as-fed basis

	Treatment		
Item	Control	Dun	White
Corn	77.8	66.3	67.6
Soybean meal	18.5	13.8	12.6
'Dun' peas	-	16.1	-
'White' peas	-	-	16.1
Dicalcium phosphate	1.2	1.5	1.5
Calcium carbonate	1.0	0.8	0.8
Mineral premix	1.0	1.0	1.0
Salt	0.3	0.3	0.3
Vitamin premix	0.2	0.2	0.2
Lysine	0.2	0.1	0.1
DE, kcal/kg	3391.5	3369.0	3368.0
ME, kcal/kg	3255.9	3221.9	3222.8
CP	15.1	15.2	15.1
Lysine	0.913	0.913	0.913
Ca	0.735	0.736	0.735
P	0.641	0.642	0.642

Growth Performance

Hogs were weighed regularly to determine ADFI, ADG, and G:F. Initial and final BW were each taken as the average of BW collected on two consecutive days. Daily feed intake was determined by dividing total feed by days on feed for each pen, with proper adjustments for removal of two Control hogs and one Dun-fed hog due to health concerns not associated with treatment.

Carcass Characteristics

Following the finishing period, hogs were individually tattooed and shipped to a small commercial abattoir. Hot carcass weight was determined by dividing cold carcass weight 0.9855 (NPPC, 2001). Back fat over the loin muscle was measured at the 10th and last rib. Loin muscle area at the 10th rib was measured by bisecting the left LM at the 10th rib using a 20 dot-per-inch loin-eye grid. Carcass length was measured from the anterior point of the aitch bone (pubic symphasis of the pelvis) to the anterior edge of the first rib on the right side. Muscling score (1 = thin, 2 = average, 3 = thick) was assessed visually. Standardized fat free lean and estimated percent lean were calculated according to the formulas of the NPPC (2001). Quality attributes, such as PSE conditions were also noted where applicable.

Statistical Analysis

Data were analyzed as a one-way ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC) with treatment as the main effect and pen serving as the experimental unit. Following a preliminary F-test (α = 0.05), means were separated using the LSMEANS option.

Results and Discussion

Growth Performance

No differences among dietary treatments were detected in initial (P = 0.92) or final (P = 0.89) BW, total gain (P = 0.39), and ADG (P = 0.63; Table 2). Gain-to-feed ratio was greater (P = 0.01) for hogs consuming White but did not differ (P = 0.62) between hogs fed Control or Dun diets. Therefore, hogs fed diets with either Dun or White peas replacing portions of dietary corn and soybean meal performed at least as well as hogs fed typical corn and soybean meal-based diets. In agreement with our findings, Stein et al. (2004) reported that pig performance was not impacted by feeding diets with up to 36% peas. Similarly, Thacker and Racz (2001) did not observe any performance differences between pigs fed diets that contained either soybean meal or peas. Szabo et al. (2001) likewise reported no differences in ADG or feed efficiency among finishing pigs fed peas as partial substitution for soybean meal. Hogs consuming peas as the sole supplemental protein source had similar ADG, ADFI, and G:F to hogs fed soybean meal as the protein source (Shelton et al., 2001). Thus, peas are a viable protein supplement for swine. Although further investigation into increased G:F when feeding White peas is warranted, our data indicate that replacing a portion of corn and soybean meal with Dun peas will yield similar performance of finishing hogs fed conventional corn-soybean meal diets.

Table 2. Growth performance of pigs fed finishing diets based on corn and soybean meal, or with inclusion of 16% 'Dun' or 'White' field peas

	Treatment				
Item	Control	Dun	White	SE[a]	*P*
Initial BW, kg	74.0	74.7	74.9	1.5	0.92
Final BW, kg	120.9	120.7	122.2	2.4	0.89
Total gain, kg	422.1	435.8	473.5	23.1	0.39
Days on feed	59.0	60.5	61.0	-	-
ADG	0.80	0.76	0.78	0.03	0.63
ADFI	2.96	2.80	2.60	0.11	0.22
Feed-to-gain ratio[b]	3.72[d]	3.68[d]	3.35[e]	0.04	0.02
Feed efficiency[c]	0.27[d]	0.27[d]	0.30[e]	0.004	0.02

[a]n = 2/treatment
[b]kg consumed/kg gain
[c]kg gained/kg consumed
[d,e]Means within a row lacking a common superscript differ ($P < 0.05$).

Carcass Characteristics

No differences among treatments were detected in HCW (P= 0.86), USDA muscling score (P = 0.44), fat depth opposite the last rib (P = 0.74), loin muscle area (P = 0.95), carcass length (P = 0.57), and standardized fat free lean (P = 0.43; Table 3). Fat depth over the 10th rib was less (P = 0.01) for hogs consuming Dun compared with hogs consuming Control or White diets. Estimated percent lean meat was higher (P = 0.03) among hogs consuming Dun or White peas compared with hogs consuming the Control diet.

Our findings are similar to Thacker and Racz (2004), who found no differences among carcass traits for pigs fed pea-based diets versus those fed traditional diets. Stein et al. (2004) did not detect differences for backfat thickness or estimated lean yield, and loin lean depth. Likewise, Szabo et al. (2001) reported no differences in lean meat percentage among finishing hogs fed diets with peas as partial substitution for soybean meal. Conversely, Shelton et al. (2001) found that inclusion of field peas as the only supplemental protein source (to the total exclusion of soybean meal) caused an increase in back fat, and a decrease in estimated lean muscle meat. These studies would seem to infer that peas should only partially substitute for soybean meal in finishing hog diets.

The findings of our study indicate that hogs fed White or Dun field peas as a partial substitute for corn and soybean meal produce carcasses comparable to hogs fed conventional corn-soybean meal diets. Moreover, carcasses from hogs fed Dun peas were similar to carcasses from hogs fed White peas. The possibility to favor decreased fat deposition with the inclusion of dietary field peas warrants further investigation.

Table 3. Carcass characteristics of pigs fed finishing diets based on corn and soybean meal, or with inclusion of 16% 'Dun' or 'White' field peas

	Treatment				
Item	Control	Dun	White	SE[a]	*P*
HCW, Kg	91.1	90.3	92.0	2.1	0.86
Loin area, cm^2	43.0	43.4	44.2	2.8	0.95
Tenth-rib fat, cm	2.6[f]	2.0[g]	2.3[f]	0.07	0.02
Last-rib fat, cm	2.3	2.2	2.2	0.1	0.74
Carcass length[b], cm	83.2	82.3	83.7	0.9	0.57
Muscling score[c]	2.5	2.6	2.7	0.4	0.12
Fat free lean[d]	45.3	47.4	47.0	1.1	0.43
Percent lean[e]	49.8[f]	52.7[f]	51.2[fg]	0.4	0.03

[a]n = 2/treatment
[b]measured from anterior edge of first rib to anterior point of aitch bone (pubic symphasis)
[c]1 = thin, 2 = average, 3 = thick
[d]Standardized fat-free lean (NPPC, 2001) = 8.588 + (HCW * 0.465) - (10th rib fat depth*21.896) + (Loin muscle area * 3.005)
[e]Estimated percent lean (NPPC, 2001) = (Standardized fat-free lean/HCW)*100
[f,g]Means within a row lacking a common superscript differ ($P < 0.05$)

Implications

Field peas can be fed to finishing hogs at 16% of the total diet. Hogs fed Dun field peas had similar growth performance and carcass characteristics as hogs fed White peas. In light of discounted market prices, Dun peas may serve as an economically viable alternative protein supplement.

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Effect of Protein Level on Feedlot Performance and Carcass Characteristics of Texas Rambouillet Feeder Lambs

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ABSTRACT

One hundred twenty Rambouillet feeder lambs with a mean body weight of 36.7 kg were used to determine the effects of feeding low (12.7% CP) versus high (15.5% CP) protein rations on feedlot performance and carcass characteristics. The lambs were blocked by weight and sex, and randomly assigned to either the low or high protein treatments. Feed and water was offered *ad libitum*. Lambs were weighed four times during the trial. The lambs were harvested when the average pen weight reached 56.8 kg. A difference ($P < 0.05$) in days on feed (DOF) was observed with the high protein treatment requiring five fewer days. No difference ($P > 0.05$) in mean weight gain, average daily gain (ADG), feed/gain efficiency, carcass characteristics, and cost/profit were observed. Numerical trends in decreased feed costs and increases in carcass value for the high protein treatment resulted in an increase of $1.49 more profit per lamb.

INTRODUCTION

With wool and mohair subsidies being terminated in the early 1990s, many sheep producers in the Edward's Plateau region of Texas began to turn their focus from primarily wool production to the production of red meat. Now, with the production of red meat becoming a major focus of sheep producers, they have begun to place their lambs in feedlots because of the added advantages in growth and gain. When lambs are placed in feedlots, they are fed high concentrate rations, which increases their plane of nutrition. Increasing lambs' plane of nutrition has a major impact on their rate of gain and therefore makes them more profitable to the producer. Lambs placed in a feedlot also reach their market endpoints sooner than their contemporaries fed forages. With feedlot lambs reaching their market endpoints quicker, they are less likely to receive the discounted mature grade.

Unfortunately, many Texas-fed lambs are, by market standards, considered too fat (Jackson et al., 1997). The USDA yield grade scale is from 1 to 5, with 1 being very lean and 5 being very fat. The lamb industry prefers lambs with United States Department of Agriculture (USDA) yield grades of 1 and 2.

Through the years, numerous cattle nutrition studies have been conducted to find out how the plane of nutrition can be manipulated to affect feedlot performance and carcass characteristics. However, limited data exists regarding the effects of feedlot nutrition on Rambouillet feeder lambs. This is of great importance because differences can exist in the nutrient requirements of different breeds of cattle as indicated by Prior et al. (1977), and it is also believed to hold true for other species of livestock.

The specific objectives of this study were: to determine the effect of protein level on feedlot performance of Texas Rambouillet feeder lambs, and to determine the effect of protein level on carcass composition of Texas Rambouillet feeder lambs.

MATERIALS AND METHODS

Animals and Feeding

This research was conducted at the Angelo State University Management, Instruction, and Research Center located in Tom Green County, north of San Angelo, Texas. A total of one hundred twenty Rambouillet wether and ewe lambs averaging 36.7 kg were blocked by weight and sex and randomly assigned to one of two treatments. The treatments were offered *ad libitum* and consisted of a high energy feedlot ration with a low (12.7% CP) or high (15.5% CP) level of protein (Table 1). The high protein ration was formulated by replacing 1 percent of the milo in the low protein ration with 1 percent of feed grade urea. Rations were formulated to meet or exceed NRC (1985) requirements for finishing lambs. At the initiation of the trial, lambs were fed a series of four rations that gradually increased in fermentable grains for an average of three days each until the final ration was reached. Lambs had *ad libitum* access to clean fresh water and were kept in 30 pens with 4 lambs per pen and 15 pens per treatment. Each pen measured 3.048 m by 9.144 m.

Upon arrival, the lambs were weighed, tagged, shorn, vaccinated against Enterotoxemia, and treated with an anthelmintic. The lambs were placed on *ad libitum* feed and water for two weeks prior to the beginning of the feeding trial so they could regain any shrink and become accustomed to feed.

Data collection

Lambs were weighed on days 0, 31, 59, and 87 to determine the feedlot performance for each treatment. Feed refusals were removed and weighed each time a new ration was placed in the feeders so feed efficiency could be calculated. Lambs were harvested at Ranchers' Lamb of Texas, Inc. located east of San Angelo, Texas, as their average pen weight was 56.8 kg. A single fat depth measurement was taken over the ribeye between the 12th and 13th rib. Yield grades were calculated by the equation: Yield Grade = 0.4 + (10 X Adjusted fat thickness, inches; USDA, 1992). Dressing percent was also calculated by taking the hot carcass weight (HCW) and dividing it by the live weight and multiplying it by 100.

Statistical Analysis

This trial was a randomized complete block design with a pen of four lambs being the experimental unit. The General Linear Model procedure of SAS (SAS Inst. Inc, Cary, NC) was used to determine the effects of protein and energy level on feedlot performance and carcass characteristics. Analysis of variance and Fisher's protected LSD test were used to determine statistical significance at a predetermined alpha level of 0.05.

RESULTS AND DISCUSSION

Feed Analysis

Chemical analysis of low and high protein feed treatments was conducted by Dairy One Inc., Ithaca, NY and revealed both treatments had elevated levels of CP from what we originally calculated from book values. However, our initial goal of having a 3 percent difference in CP level was not affected by the increases in nutrient value and was accomplished with the addition of urea. The use of urea allowed us to increase the nitrogen content of the feed without greatly increasing the cost of the ration. Furthermore, the use of urea enabled us to make only minor changes to the low protein ration formula to yield the high protein ration. Theoretically, the addition of urea provided an increase in ammonia in the rumen and this, along with carbon skeletons from grains, provided the vital substrates the rumen microbial population needed to grow and multiply. This increase in microbial population provided more microbial protein to flow out of the rumen and to the small intestine for breakdown and absorption. Furthermore, an increase in microbial population should have allowed for more complete fermentation and breakdown of feedstuffs (Owens and Zinn, 1993).

Table 1. Ingredients and nutrient composition of dietary treatments (on an "as fed" basis) fed to Rambouillet feeder lambs.

	Treatments							
Ingredients, %	Low, # 1	High, # 1	Low, # 2	High, # 2	Low, # 3	High, # 3	Low, # 4	High, # 4
Milo	36	35	50	49	62	61	73	72
SBH	22	22	10.5	10.5	19	19	13	13
CSM	6.5	6.5	4.5	4.5	3.0	3.0	2.0	2.0
Alfalfa	30	30	29.5	29.5	10.5	10.5	6.5	6.5
Molasses	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Urea	0	1.0	0	1.0	0	1.0	0	1.0
Nutrient analysis, %								
TDN	67	68	70	68	71	72	67	70
ADF	25.1	28.0	18.0	22.0	11.5	9.9	20.6	14.4
NDF	35.8	32.6	27.0	33.8	19.0	16.2	31.7	19.9
CP	13.7	17.7	14.5	17.1	13.0	15.9	12.7	15.5

[a]Step-up and final rations for both low and high protein treatments.

Table 2. Least squares means of body weights, gains, and days on feed for Rambouillet lambs fed low and high protein rations.

	Treatments		
Item	Low	High	SE[b]
n	60	60	
Initial BW, kg	36.7	36.7	0.45
Final BW, kg	57.8	57.3	0.62
Gain, kg	21.1	20.7	0.53
ADG[c], kg/d	0.27	0.28	0.01
DOF[d]	80.9[e]	75.3[f]	1.66

[a]Low or high protein ration.
[b]SE = most conservative standard error of the mean.
[c]Mean average daily gain.
[d]Days on feed.
[e,f]Means in row with different superscripts differ $P < 0.05$.

Performance Data

Table 2 displays the least squares means of body weights, gains, and days on feed (DOF) for Rambouillet lambs fed low and high proteins rations. A difference ($P < 0.05$) in (DOF) was observed with the high protein treatment requiring five fewer days on feed. No differences ($P > 0.05$) were observed for mean initial and final weight as expected since lambs were blocked by weight at the start of the trial and harvested at a predetermined pen average weight. These findings are in agreement with that of Braman et al. (1973) who found that increasing CP levels in both steer and lamb rations with urea did not improve ADG, and when fed at 2 and 3 percent, ADG actually decreased. However, Fluharty and McClure (1997) saw increases in ADG ($P < 0.01$) and final weight ($P < 0.05$) in growing lambs when they increased the recommended NRC protein requirement by

25%.

Table 3 illustrates least squares means of intake and feed efficiency for pens of Rambouillet feeder lambs fed low and high protein rations. No differences ($P > 0.05$) were observed for dry matter intake and feed/gain efficiency. This coincides with findings in lambs (Neary et al., 1995) fed soybean meal and fish meal, and steers (Haskins et al., 1967; Milton et al., 1997) fed soybean meal and urea. In contrast, Fluharty and McClure (1997) illustrated increases ($P < 0.01$) in dry matter intake, but observed no differences in feed efficiency when the protein level was increased in lamb rations. Although, no significant differences were seen in feed/gain efficiency, numerical differences were observed with the lambs on the high protein treatment showing a 5 percent improvement. This is likely due to the high protein ration providing more ammonia for microbial synthesis, thus increasing the microbial population and providing more efficient use of feed. Murchinson (1985) observed using cottonseed meal as the primary source of protein in a 14.5 percent CP feedlot ration did not provide enough ammonia in the rumen to meet the lower level of the optimum range for rumen ammonia concentration. Therefore, the addition of urea should meet the needs for optimum rumen function.

Carcass Data

Table 4 represents carcass characteristics of Rambouillet feeder lambs fed low and high protein feedlot rations. No differences ($P > 0.05$) were revealed in carcass characteristics measured. However, we observed trends of numerical decreases in fat thickness and hot carcass weight leads to the decrease in dressing percent and thus a decrease in carcass fat. Our findings are also in agreement with Kemp et al. (1976) in lambs and Braman et al. (1973) in steers. Lofgreen (1978) also reported genetically similar animals had similar levels of carcass fat when harvested at equal weights.

Economic Data

Table 5 illustrates least squares means of economic data for Rambouillet feeder lambs fed low and high protein rations. No differences ($P > 0.05$) in costs and profit between treatments were observed. The price per 909 kg of high protein ration was $4.96 more expensive than the low protein. However, trends in numerical decreases in total feed cost and cost of gain, and the numerical increases in lamb value resulted in the high protein treatment yielding an increase of $1.49 more profit per lamb than the low protein treatment.

Implications

The results from this trial indicate feeding a high protein feedlot ration to Texas Rambouillet feeder lambs will consistently decrease DOF. A decrease in DOF for feeder lambs has the potential to decrease the incidence of lambs receiving a mature grade at slaughter. Over time, a decrease in DOF may also allow feedlots to increase the total number of lamb turnover for a given period of time. Furthermore, decreases in DOF coupled with numerical trends in increases in feed/gain efficiency and decreases in cost of gain and total feed cost has the potential not only to increase profit, but also reduce environmental pollution.

Table 3. Least squares means of intake and feed efficiency for pens of Rambouillet feeder lambs fed low and high protein rations.[a]

	Treatments		
Item	Low	High	SE[c]
n	15	15	
Intake, kg	1.6	1.6	0.06
Efficiency, kg feed/kg gain	6.2	5.9	0.19

[a]Randomized complete block design with pen as the experimental unit (4 lambs/pen).
[b]Low or high protein ration.
[c]SE = Most conservative standard error of the least square mean.

Table 4. Least squares means of fat depth, calculated yield grade, hot carcass weight, and dressing percent for Rambouillet feeder lambs fed low and high protein rations.

	Treatments		
Item	Low	High	SE[b]
n	60	60	
Fat depth[c], cm	0.73	0.70	0.02
CYG[d]	2.6	2.5	0.09
Hot carcass weight, kg	30.7	30.3	0.32
Dressing Percent[e]	53.2	52.9	0.26

[a]Low or high protein ration.
[b]Most conservative standard error of the least square mean.
[c]Fat depth measurement at the twelfth rib.
[d]Calculated yield grade = 0.4 + (10 X adjusted fat thickness, in inches).
[e]Dressing percent = hot carcass weight/live weight.

Table 5. Least squares means of economic data (U.S. dollars) for pens of Rambouillet feeder lambs fed low and high protein rations.

	Treatments		
	Low	High	SE[b]
Purchase price[c], $	84.35	84.35	
Carcass value[d], $	114.45	115.21	
Lamb value[e], $	30.10	30.86	.442
Total feed cost[f], $	15.50	14.77	.456
Cost of Gain kg[g], $	0.74	0.72	.022
Profit[h], $	14.60	16.09	.786

[a]Low or high protein ration.
[b]Most conservative standard error of the least square mean.
[c]Average price of lambs at the beginning of the trial.
[d]Average value based on carcass sell price.
[e]Lamb value = Carcass value – Purchase price.
[f]Average cost of ration/lamb.
[g]Cost of ration/weight gain in kg.
[h]Profit = (Carcass value) – (purchase price + cost of gain).

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TIMING OF PUBERTY, ANESTRUS, AND RETURN TO CYCLICITY IN FEMALE OFFSPRING OF EWES TREATED WITH PROPYLTHIOURACIL AND MELATONIN DURING PREGNANCY

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ABSTRACT: Twenty four Rambouillet ewe lambs were used to examine seasonal cyclic responses after maternal thyroid suppression (propylthiouracil, PTU) and melatonin treatment during gestation. Beginning January 2 (first day of treatment at 76.8 ± 4.7 d of gestation), ewes received 0 or 40 mg PTU/kg BW daily (gavage) for 15 d. After 15 d, the 40 mg dosage was decreased to 20 mg/kg BW for 20 d, and melatonin was given (i.m., 5 mg/d) for 30 d. Five and 19 female lambs were produced by PTU-treated and control ewes, respectively. Lambs were weaned at 60 d of age and fed alfalfa hay ad libitum and ground corn at 0.3 kg/animal daily until 7 mo of age. Alfalfa hay was fed at 2 kg/d for the remainder of the experiment. Blood samples were collected from ewe lambs two or three times weekly beginning in mid-August and continuing for 15 mo. Serum progesterone (P4) was measured to determine date of puberty, date of anestrus, and date of return to cyclicity. At birth, control ewe lambs (5.3 ± 0.3 kg) weighed more (P = 0.05) than did those from PTU-treated dams (4.6 ± 0.3 kg). This weight difference tended to be present (P = 0.08) at weaning and at monthly weigh periods after weaning. Control ewe lambs reached puberty on October 17 (± 12 d) while those from PTU-treated ewes were pubertal on November 4 (± 12 d, P = 0.17). Female offspring from control and PTU-treated ewes were 206 and 227 (± 12) d of age at puberty, respectively (P = 0.13). Control ewe lambs became anestrus on December 4 (± 13 d) compared with November 27 (± 13 d, P = 0.61) for those from PTU-treated ewes. Female offspring from control and PTU-treated ewes were 255 and 249 (± 13) d of age at the beginning of anestrus, respectively (P = 0.71). Control ewe lambs began to cycle again on June 26 ± 13 d at 458 ± 14 d of age, while those from PTU-treated ewes began on July 6 ± 13 d at 471 ± 14 d of age (P > 0.40). Maternal thyroid suppression and melatonin during gestation did not influence seasonal cyclicity of female offspring.

Key Words: Sheep, Puberty, Thyroid

Introduction

The practice of breeding spring born ewe lambs is a management strategy that sheep producers can utilize to increase total lifetime productivity from the ewe flock. Exposure to long days of spring and summer, followed by short days in autumn induces photoperiodic cues to stimulate the ewe lamb's first estrus (Foster et al., 1985; Herbosa et al., 1994). Karsch et al. (1984) suggested that onset of anestrus results from a change in sensitivity of estradiol feedback on LH. Thyroxine (T4) has been indicated to play a role in seasonal reproduction in mature ewes. Thyroidectomy of ewes before the end of the breeding season lengthened the period of cyclicity throughout the anestrous season (Karsch et al., 1995) as did treatment of nonpregnant ewes with propylthiouracil (**PTU**, Hernandez et al., 2003). The thyroid gland is therefore involved in seasonal anestrus. However, Wells et al. (2003) demonstrated that administration of PTU to ewe lambs did not hasten puberty or improve pregnancy rates. The objective of this study was to determine if maternal treatment with PTU and melatonin during gestation would influence timing of reproductive events of female offspring.

Materials and Methods

Treatments imposed on eight mature Rambouillet ewes were previously reported by Gifford et al. (2004). Briefly, ewes received (gavage) 40 mg PTU/kg BW/d for 15 d (beginning on Jan 2, 77 ± 5 d of gestation) then 20 mg/kg BW for an additional 20 d. Melatonin was given (i.m. injections at 5 mg/d) for 30 d. The PTU and melatonin dosages were based on previous studies in our laboratory by Hernandez et al. (2003) and Perez-Equia et al. (1994), respectively. The female offspring (n = 5) produced by PTU-treated ewes were subsequently managed as described in detail by Knight et al. (2004). Briefly, these five ewe lambs and 19 contemporary control ewe lambs were weaned at 60 d of age, retained in the flock, and bled two to three times weekly for 15 mo. Weights were recorded monthly. Serum progesterone (P4) was determined by RIA (Schneider and Hallford, 1996) and values were used to determine onset of puberty (≥ 1 ng/mL), onset of anestrus (≤ 1 ng/mL), and onset of subsequent cyclicity (≥ 1 ng/mL).

Growth patterns and onset of puberty were described previously (Knight et al., 2004). Onset of anestrus and resumption of cyclicity were determined as well as ewe age at these two reproductive events. These variables were subjected to analyses of variance for a completely random design. All analyses were computed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

Results and Discussion

As reported by Knight et al. (2004), actual weaning weight was similar (P = 0.26) between groups; but when 60-d weaning weights were adjusted to a single ewe

lamb, mature ewe basis (Scott, 1977) control lambs (27.3 ± 1.4 kg) tended to be heavier (P = 0.08) than lambs produced by PTU-treated dams (24.4 ± 1.4 kg). At approximately 7 mo. of age (142 d after weaning), control ewe lambs weighed 54.9 ± 1.8 kg while those from treated ewes weighed 50.2 ± 1.8 kg (P = 0.03). At the time of anestrus, control ewe lambs (63.9 ± 2.0 kg) weighed more (P = 0.05) than did those from PTU-treated dams (59.1 ± 2.0 kg). At the time of return to cycling, control ewe lambs weighed 72 (± 2.9 kg) compared to 66. 4 (± 2.9 kg, P = 0.10) for the PTU-treated lambs.

Ewe lambs in this study had a very uniform time of birth (March 22 and 24 for treated and control females, respectively). Average date of puberty for controls was October 17 compared with November 4 (SE = 12 d, P = 0.17) for female offspring of treated ewes. Control lambs were 206 ± 12 d of age at puberty compared with 227 ± 12 d (P = 0.13) for ewe lambs produced by PTU-treated dams. This difference likely reflects the difference in BW between the two groups rather than an effect of maternal PTU treatment. Shirley et al. (2001) also noted that heavier ewe lambs reached puberty at 200 d of age compared with 214 d of age (P = 0.06) for ewe lambs classified as light.

Control ewe lambs became anestrus on December 4 (± 13 d) compared with November 27 (± 13 d, P = 0.61) for those from PTU-treated ewes. Female offspring from control and PTU-treated ewes were 255 and 249 (± 13) d of age at the beginning of anestrus, respectively (P = 0.71). After only a few reproductive cycles, the young female offspring become anovulatory and no longer exhibit estrous behavior. This termination of the ewe lamb's first breeding season occurs even before 1 yr of age due to the reversal in sensitivity to estradiol negative feedback (Foster, 1988). Control ewe lambs began to cycle again on June 26 (± 13 d) while those from PTU-treated ewes began on July 6 (± 13 d, P = 0.48). Control ewe lambs were 458 (± 14) d of age and PTU-treated ewe lambs were 471 (± 14, P = 0.42) d of age upon returning to cyclicity.

Implications

Maternal thyroid suppression and melatonin during gestation resulted in lower weights of female offspring which also tended to delay onset of puberty. However, this did not influence seasonal cyclicity of female offspring.

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SERUM THYROXINE AND TRIIODOTHYRONINE IN PREWEANING RAMBOUILLET LAMBS

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ABSTRACT: Rambouillet lambs (n = 86; 23 singles, 63 twins) were used to examine relationships between serum concentrations of triiodothyronine (T3) and thyroxine (T4) during the preweaning period and actual weaning weight. Lambs (48 females, 38 males) were born in the spring (avg. birth date = March 23 , range March 14 to April 3; avg. birth weight = 5.1 ± 0.8 kg) and were allowed free access to creep feed (14% CP) from d 10 after lambing until weaning at approximately 60 d of age. Serum was collected from each lamb at 14 and 28 d of age and at weaning and T3 and T4 were quantified. Male lambs were intact for the 14- and 28-d sampling periods and were castrated immediately after sampling on d 28. Effects of lamb gender, type of birth, and day of sampling on thyroid hormone concentrations were examined (no two or three way interactions, $P > 0.10$). Neither lamb gender nor type of birth affected ($P > 0.13$) serum T3 or T4 during the preweaning period although single-born lambs tended to have greater ($P = 0.13$) serum T4 (93 ± 4 ng/mL) than did twin-born lambs (87 ± 2 ng/mL). Serum T3 values decreased ($P < 0.0001$) during the preweaning period (3.0 > 2.3 > 1.7 ± 0.05 ng/mL on d 14, d 28, and at weaning, respectively). Likewise, serum T4 concentrations also declined ($P < 0.0001$) over the preweaning period (103 > 90 > 78 ± 2 ng/mL on d 14, d 28, and at weaning, respectively). Correlation coefficients ($P < 0.0001$) between serum T3 and actual weaning weight were 0.46, 0.52, and 0.43 on d 14 and 28 and at weaning, respectively. For serum T4, correlation coefficients with actual weaning weight on the same respective dates were 0.43 ($P < 0.0001$), 0.41 ($P < 0.0001$), and 0.20 ($P = 0.07$). Serum T3 and T4 concentrations decline during the preweaning period, are unaffected by gender or type of birth, and concentrations on d 14 and 28 are positively related to actual weaning weight.

Key words: Sheep, Thyroxine, Triiodothyronine

Introduction

Thyroid hormones are important endocrine factors in normal growth and development. The principal thyroid hormones are thyroxine (**T4**) and triiodothyronine (**T3**). The thyroid gland produces both T4 and T3 but most circulating T3 results from deiodination of T4 by type 1 deiodinase which is found primarily in the liver and also in the kidney, thyroid, and brain (Griffin and Ojeda, 2000). Well known effects of thyroid hormone deficiency in early life include cretinism and growth retardation. Numerous attempts have been made to manipulate thyroid hormone production in order to improve livestock performance. Early work by Andrews and Bullard (1940) showed that partial thyroidectomy resulted in increased gains in beef steers. However, Andrews et al. (1947) observed no improvement in growth of lambs fed the thyroid antagonist thiouracil. Treatment of 7-mo-old ewe lambs with exogenous T3 resulted in decreased growth rates (Hinrichs and Hallford, 1987). Wells et al. (2003) treated ewe lambs with propylthiouracil for 28 d and reported decreased BW beginning 3 wk after treatment ended. Attempts to improve performance by supplementation or inhibition of thyroid hormones have therefore met with limited success. The objective of this study was to determine if preweaning thyroid hormone profiles in lambs are influenced by gender or type of birth and to examine relationships between these profiles and weaning weight.

Materials and Methods

Management. Rambouillet lambs born during spring (avg. birth date = March 23, range March 14 to April 3) at the West Sheep Unit on the main campus at New Mexico State University were used to examine serum T3 and T4 profiles during the preweaning period. A total of 86 lambs consisting of 23 single-born and 63 twin-born animals were included in the data set. The average birth weight of all animals was 5.1 ± 0.8 kg. Forty-eight lambs were female and 38 were male. At lambing, ewes and offspring were separated from the flock. Lambs were individually identified (ear tag), and birth weight, type of birth, and lamb vigor were recorded. On the second day after birth, lambs were docked and treated (i.m.) with 1 mg of Se and 68 USP units of vitamin E (BO-SE, Schering-Plough Animal Health; Union, NJ). Lambs and dams were then placed in groups of five to six ewes with lambs for 8 to 10 d before being mixed with the larger flock. Beginning at approximately 10 d of age, lambs were allowed access to a creep feeding pen in which alfalfa hay was available free choice. Cracked corn was also available in limited amounts until lambs were 28 d of age at which time lambs were immunized against *Clostridium perfringens* types C and D and *Clostridium tetani* (Bar Vac CD/T; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). Also at 28 d of age, male lambs were castrated using elastrator bands. After immunization, the amount of cracked corn available was increased to approximately 0.25 kg/lamb

daily until lambs were weaned at an average age of 60 d.

Serum Collection and Analysis. Blood samples were collected by jugular venipuncture from all lambs at 14 and 28 d of age and at weaning. Samples were collected into 10-mL vacuum serum separator tubes (Corvac, Sherwood-Davis and Geck, St. Louis, MO), which were allowed to stand at room temperature for 30 min before centrifugation at 4° C for 15 min at 1000 x g. Serum was then transferred to plastic vials and stored frozen until analyzed.

Thyroxine and T3 were quantified by RIA using components of commercial kits supplied by Diagnostic Products Corporation, Inc. (Los Angeles, CA). Both the T4 and T3 kits were validated for use in ruminant serum in our laboratory by Richards et al. (1999) and Wells et al. (2003), respectively. Within and between assay coefficients of variation were 12% or less for both hormone assays.

Statistical Analysis. One of the primary aims of this study was to determine if preweaning T3 and T4 concentrations were influenced by lamb gender, type of birth, age of lamb (time after birth). These factors were examined by subjecting hormone values to split plot analyses of variance for repeated measures on animals as described by Gill and Hafs (1971). Effects of gender, type of birth, and gender by type of birth interaction were included in the main plot and tested using animal within the gender by type of birth as the error term. Effects of time after lambing and its two- and three-way interactions with gender and type of birth were included in the subplot and tested with residual error. Because no two- or three- way interactions were detected ($P > 0.10$), main effects of gender, type of birth, and time after lambing are reported. When significant time after lambing responses were observed, means were separated using the predicted difference method of SAS (SAS Inst., Inc., Cary, NC). All analyses were computed using GLM of SAS. To examine possible relationships between serum T3 and T4 concentration and growth characteristics of lambs, correlation coefficients were determined using the correlation procedure of SAS.

Results and Discussion

When serum T3 data were pooled across type of birth and time after lambing, values in females (2.3 ± 0.06 ng/mL) were similar ($P = 0.45$) to those observed in males (2.4 ± 0.06 ng/mL). Likewise, female and male lambs had similar ($P = 0.62$) concentrations of serum T4 during the preweaning period (91 and 89 ± 3 ng/mL), respectively.

Serum T3 before weaning measured 2.3 ± 0.07 ng/mL in both single- and twin-born lambs ($P = 0.19$). Although the difference was not large, single-born lambs had serum T4 value of 93 ± 4 ng/mL which tended to differ ($P = 0.13$) from the 87 ± 2 ng/mL value for twin-born animals. The data demonstrated that gender of lamb and type of birth are not important factors affecting serum T3 and T4 profiles during the preweaning period.

However, time after birth (age of lamb) appeared important in affecting T3 and T4 concentrations. Serum T3 values on day 14, 28, and at weaning were 3.0, 2.3, and 1.7 (± 0.05) ng/mL, respectively ($P < 0.001$). Likewise, serum T4 measured 103, 90, and 78 (± 2) ng/mL on d 14, 28, and at weaning respectively ($P < 0.001$). These responses demonstrate a sizable decline in both thyroid hormones during the preweaning period.

Relationships between serum thyroid hormones on d 14, 28, and at weaning with actual weaning weights of lambs were also examined. Correlation coefficients between serum T3 and weaning weight were 0.46 ($P < 0.001$), 0.52 ($P < 0.001$), and 0.22 ($P = 0.04$) for T3 on d 14, 28, and at weaning, respectively. Correlation coefficients between serum T4 on the same days and weaning weight were 0.43 ($P < 0.001$), 0.41 ($P < 0.001$), and 0.20 ($P = 0.07$), respectively. These positive relationships between thyroid hormones and the economically important trait of weaning weight warrant further study.

Implications

The similarities in serum thyroxine and triiodothyronine concentrations in male and female lambs and in single- and twin-born lambs demonstrate that these metabolic hormones are relatively unaffected by these factors. However, both hormones appear to decline steadily as the lamb approaches weaning and gains weight. The positive associations between the thyroid hormones and weaning weight merit further investigation aimed at determining predictive capabilities of these relationships.

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FERTILITY, PROLIFICACY AND BIRTH WEIGHTS IN CROSSBREDS FROM PELIBUEY SHEEP IN TWO CONSECUTIVE YEARS

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ABSTRACT: The purpose of this study was to evaluate the fertility, prolificacy, and birth weight in two crossbreds hair sheep obtained from laparoscopic artificial insemination (LAI) in the northwest of Mexico in two consecutive years. In year one (Y1), thirty six Pelibuey ewes were randomly distributed in three groups to be inseminated with three breeds (Katahdin [K], n=11; Dorper [D], n=13; Pelibuey [P], n=12). In year two (Y2), forty three Pelibuey ewes were randomly distributed in three groups using the same breeds (K, n=14; D, n=15; P, n=14). Prolificacy and birth weight were analyzed using analysis of variance and fertility using chi-square test. Fertility in both years was similar (P>0.05) for the three groups in the two years (Y1: K=36.4%, D=69.2%, P=66.6%; Y2: K=57%, D=53%, P=50%). Prolificacy, measured as average number of kids per doe (kpd), was also similar (P>0.05) in the three groups for the two years (Y1: K=3.72 ± 1.26 kpd, D= 2.13 ± 1.13 kpd, P= 2.77 ± 0.97 kpd; Y2: K=2.38 ± 0.24 kpd, D=2.27 ± 0.28 kpd, P=2 ± 0.27 kpd). Average birth weight was also similar (P>0.05) for the three crossbreds in the two years evaluated (Y1: K=2.75 ± 0.21 kg, D=2.91 ± 0.19 kg, P=2.99 ± 0.15 kg; Y2: K=2.47 ± 0.29 kg, D=2.72 ± 0.27 kg, P=2.57 ± 0.29 kg). These results suggest that crosses of KxP and DxP had similar performance than Pelibuey purebred, although more information is needed to evaluate the convenience of introducing sheep hair breeds using LAI in northwestern Mexico.

Key Words: Reproduction Traits, Hair Sheep, Laparoscopic Insemination

Introduction

Consumption of sheep meat has been traditional in many states of south and central Mexico. Sheep industry for meat production has rapidly increased in states located in the northwest of Mexico during the last five years. Pelibuey hair sheep has been the preferred breed due to their adaptation to arid and hot conditions (Molina-Ramírez et al., 2000). However, producers are demanding easy care breeds to produce high quality lambs for improved productivity and competitiveness. Improvements in efficiency of sheep production could be achieved by reducing labor and management costs, which trend to be higher than those required for beef production. Hair sheep ewes are hardy and highly fertile and lambs are vigorous, but hair sheep generally are small and grow slowly (Simm, 1998). Thus, use of Dorper and Katahdin breeds will require crossing with Pelibuey to attempt to capture the best characteristics of both types in this zone. Traits contributing to reproduction efficiency include seasonality, fertility, prolificacy, maternal ability and lamb vigor. These traits could be combined to be improved thorough the use of Artificial Insemination (AI). Techniques for AI using frozen semen for sheep has not been wide spread because of limited success in being able to achieve high fertility when thawed semen is deposited in the vagina or into the uterous via the cervix (El-Gaafary et al., 1987; Gourley and Riese, 1990). However, laparoscopic has evolved as one of the least invasive techniques depositing frozen-thawed semen in the uterous of the sheep and the fertility may reach up to 70% (Hill et al., 1998; McKelvey et al., 1985). Therefore, the introduction of hair sheep breeds in order to obtain heterosis for growth traits using laparoscopic AI may be an appropriate tool to improve sheep industry for meat production (Simm, 1998). The objective of this study was to compare fertility and prolificacy of Pelibuey ewes inseminated by laparoscopic method with frozen semen of Dorper, Katahdin and Pelibuey breeds, as well as birth weights of their offspring in an arid region of northwestern Mexico in two consecutive years.

Materials and Methods

The study was carried on at the sheep experimental unit of the CBTa No. 41, located in Mexicali valley, state of Baja California, in the northwest of Mexico. This is a desert region with high temperatures during summer and moderate winter temperatures. In year one (Y1), thirty six Pelibuey sheep ewes were randomly assigned into three groups to be inseminated with three breeds (Katahdin [K], n=11; Dorper [D], n=13; Pelibuey [P], n=12). In year two (Y2), forty three Pelibuey ewes were randomly distributed in three groups using the same breeds (K, n=14; D, n=15; P, n=14). All ewes were multiparous and they spent the first three months after the AI grazing in rye-grass pasture and later on were fed in a pen with a mixed diet containing 50% of alfalfa hay, 25% of Sudan hay and 25% of wheat straw. In both years estrus were synchronized with intravaginal sponges containing 40 mg of FGA (fluorogestone acetate; Intervet International BV, Boxmeer, Holland) for 10 days and eCG (Equine

Chorionic Gonadotropin, 500 UI;) was given in a single im injection 2 days before sponges removal. Heat detection was carried out 24 h after sponge's removal. Twenty-four hours before surgery both feed and water were withheld from all ewes. For the LAI, the ewes were placed on her back in a laparoscopy cradle and the posterior abdominal region in the area of the pubis was surgically prepared by removing the hair and disinfecting the skin. The ewe was given an injection of local anesthetic approximately 5 inches anterior of the udder and 2 inches on either side of the midline. The cradle was then placed in its surgical position with the posterior of the ewe lifted to an approximate 40 degree angle. Two small incisions were made in the skin at the site where the anesthetic was injected to facilitate puncturing the abdominal wall with a trocar. Once the abdominal wall was punctured, the trocars were removed from the cannulas and an endoscope and a manipulating probe were placed through the cannulas into the abdominal cavity. A manipulating probe was used to bring the uterus into the proper position for AI. Once the uterus is in the correct position, the probe is removed and replace by the inseminating gun containing the semen. The technician then punctures the uterine horn half way between the uterine bifurcation and the utero-tubal junction. The semen is injected directly into the lumen of the uterus, and the same procedure is repeated on the other uterine horn. Technician was the same in both years. Fertility was statistically analyzed by chi-square test and prolificacy and birth weight were analyzed using analysis of variance (Steel and Torrie, 1980) thorough SAS (SAS, 1998).

Results and Discussion

Fertility. Results of fertility in both years was similar (P>0.05) for the three groups in the two years as is shown in Table 1. In average, fertility was 58.3% during Y1 and 53.5% for Y2. The overall average for both years was 55.7%.

Table 1. Fertility of LAI in Pelibuey ewes during two consecutive years in the three groups.

Group	Number of animals	Calved animals	Fertility (%)
Y1			
Katahdin	11	4	36.4[a]
Pelibuey	12	8	66.6[a]
Dorper	13	9	69.2[a]
Average	36	21	58.3 [a]
Y2			
Katahdin	14	8	57.2[a]
Pelibuey	14	7	50.0[a]
Dorper	15	8	53.3[a]
Average	43	23	53.5 [a]

[a] Percentages with the same superscript by year do not differ (P>0.05).

These results are similar to those reported in the literature. Maxwell and Hewitt (1986) reported 55.6% of fertility in a study using frozen semen; in other study, Gourley and Riese (1990) obtained 49% of fertility using the same technique. Perkins et al. (1996) reported 51% of fertility depositing the semen in one uterine horn and 55% of fertility depositing the semen in both uterine horns.

Prolificacy and birth weights. Prolificacy, measured as average number of kids per doe (kpd), for the three crossbred groups is presented in Table 2. This table also shows the average of birth weight for the same crossbred groups.

Table 2. Average of prolificacy and birth weights during two consecutive years in the three groups.

Group	Prolificacy	Birth weight
Y1		
Katahdin	3.72 ± 1.26 [a]	2.75 ± 0.21 [a]
Pelibuey	2.77 ± 0.97 [a]	2.99 ± 0.15 [a]
Dorper	2.13 ± 1.13 [a]	2.91 ± 0.19 [a]
Average	2.87 ± 1.12 [a]	2.88 ± 0.18 [a]
Y2		
Katahdin	2.38 ± 0.24 [a]	2.47 ± 0.29 [a]
Pelibuey	2.00 ± 0.27 [a]	2.57 ± 0.29 [a]
Dorper	2.27 ± 0.28 [a]	2.72 ± 0.27 [a]
Average	2.22 ± 0.26 [a]	2.59 ± 0.28 [a]

[a] Averages with the same superscript do not differ (P>0.05).

Average prolificacy was similar (P>0.05) in the three groups for the two years analyzed. Also, overall averages were very close each other. Average birth weight (BW) was also similar (P>0.05) for the three crossbreds in the two years evaluated. Again, overall averages for prolificacy and birth weights were similar (P>0.05). These averages agree with those reported in the literature for hair sheep in different environments (desert, tropical and subtropical areas). Combellas (1980) reported 2.6 kg of BW in tropical sheep breeds. Using Pelibuey and its crosses, several authors have reported average BW in a range between 1.5 to 3.33 kg (Carrillo y Segura, 1993; Perón et al., 1991; Rodríguez et al., 1999). In general, prolificacy of hair sheep has been reported as low. Combellas (1980) and Perón et al. (1991) summarized several studies in tropical and subtropical areas using hair sheep based on Pelibuey and the averages ranged between 1.2 and 1.6 as number of lambs per parturition. Prolificacy observed in the present study was slightly higher than those reported in the literature, however, this study did not indicate a heterosis effect from using Katahdin and Dorper.

Implications

The results of the current study are consistent with those reported in Mexico and other countries, and

further indicate that LAI can be a useful reproduction strategy to genetically improve sheep in the Northwest region of Mexico. However, more research is needed in order to support it.

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DEVELOPMENT OF AN ASSAY TO DETERMINE SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) IN THE PRION GENE FOR THE DIAGNOSIS OF GENETIC SUSCEPTIBILITY TO SCRAPIE IN SHEEP.

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ABSTRACT: The objective of this study was to develop a reliable assay to genotype sheep for scrapie susceptibility and then use the information for breeding purposes. The sheep prion gene contains two SNPs that may mediate resistance to developing scrapie, one at codon 136, alanine (A) or valine (V), and another at codon 171, arginine (R) or glutamine (Q). The R allele is thought to confer resistance to scrapie, with the $AA_{136}RR_{171}$ genotype the most resistant to scrapie and QR_{171} only rarely infected. The Assays by Design protocol was used for development of probes and primers for codon 136 and Primer Express® for codon 171 (Applied Biosystems; Foster City, CA). To perform the analysis, genomic DNA was isolated from blood or muscle using commercially available kits. For validation, 70 SNP determinations for each codon were conducted without prior knowledge of genotype and compared to commercial testing (GeneCheck, Inc.; Ft. Collins, CO). Error rate determined by comparisons to the reported genotype was less than 1%. To date, 935 samples from blood (n = 818) and muscle (n = 117) have been tested for both codons with 928 successful determinations and only 7 samples (<1% of total samples) that could not be determined. Genotypes of the animals tested were AA QQ (n = 102; 11.0%), AV QQ (n = 28; 3.0%), AA QR (n = 396; 42.7%), AV QR (n = 54; 5.8%), and AA RR (n = 348; 37.5%). Thus, 86% of the sheep tested (n = 798) contained R at codon 171 and were substantially scrapie-resistant. This new SNP genotyping assay is accurate, relatively easy to perform, and will be useful in the study of many aspects of the scrapie disease in sheep, especially its lateral transmission. *Supported in part by Hatch Proj. ND1705 and by USDA, ARS, ADRU.*

Key Words: Scrapie, Genotype, Prion, Sheep

Introduction

Scrapie is a fatal and incurable neurological disease of sheep that belongs to a family of prion diseases known as transmissible spongiform encephalophies (TSE). Other well-known members of the family include bovine spongiform encephalophy (BSE), variant Creuzfeldt-Jakob disease (vCJD, the disease associated with BSE transmission to humans), and chronic wasting disease of cervids. Although scrapie does not appear to be transmitted to humans, concerns for the effects of TSEs on human welfare and for the loss of millions of dollars to sheep producers from this debilitating disease have prompted a call for worldwide scrapie eradication by 2010 (USDA, 2003; NIAA, 2004).

Genotyping sheep for genes that appear to confer resistance to contracting scrapie has been used for several years in an attempt to eradicate scrapie through selective breeding. In 2003, there were three commercial laboratories approved by the USDA Animal Plant Health Inspection Service (APHIS) to do the official scrapie genotyping: GenMark (DeForest, WI); GeneCheck, Inc. (Ft. Collins, CO) and GeneSeek, Inc. (Lincoln, NE) and now there are several more that are approved. Their tests are reliable (most claim 99% reliability) and accurate, but relatively expensive.

Real time PCR methodology for detecting single nucleotide polymorphisms in genes, particularly those in humans, has been developed recently and widely used. This methodology might also detect the polymorphisms at codon 136 and codon 171 that affect susceptibility to scrapie in sheep. Sheep that have at least one R at codon 171 are less likely to be infected and when infected, they survive longer than those who have Q or histidine (H). Commercial testing does not distinguish between Q, H, or lysine (K). Additionally, having a V at codon 136 increases susceptibility to contracting scrapie and also appears to affect survival time; sheep that are AV at codon 136 live longer those that are VV (Hunter et al., 1994, 1996; Baylis et al., 2002). Thus, the objective of the present study was to develop a SNP genotyping assay that would reliably test for A/V at codon 136 and Q/R at codon 171 of the sheep prion gene.

Materials and Methods

Development of probes and primers

Fourteen or more complete cDNA or protein sequences of the sheep prion gene were selected from the NCBI and EMBL databases and compared for homology. Variations in codon 136 (amino acids corresponding to nucleic acids 406-408 of the prion mRNA) and codon 171 (amino acids corresponding to nucleic acids 511-513 of the prion mRNA) were noted and codon bias for the amino acids corresponding to A, V, Q, R, H, and K in the sheep prion gene were determined. For codon 136, GCC coded for A and GUC coded for V; for codon 171, CAG coded for Q, CGG coded for R, CAU coded for H, and AAG coded for K. The nucleic acid sequence from base pair (bp) 351 through 570 of the prion cDNA of GenBank Accession Number AJ000736 was selected as the template for

preparation of SNP probes (Bossers et al., 1996). After a series of experiments to optimize PCR temperatures, the probes and primers designed for codon 171 using Primer Express® (Applied Biosystems) performed successfully, but not those for codon 136. Thus, the cDNA template sequence from bp 351 through 509 was submitted to Applied Biosystems for the Assays-by-Design℠ Service and was successfully optimized. The NCBI Blast for specificity of each amplicon was highly specific. Fluorescent probes and unlabeled primers for codon 171 were purchased from Applied Biosystems. For codon 136, the fluorescent probes and unlabeled primers were included in a 40X master mix. The sequences of probes and primers were disclosed with the order, but their exact concentration in the 40X master mix was not.

Isolation of DNA

Commercially available kits were used for DNA isolation from blood or muscle. Although several kits were tested for isolation from blood, the one ultimately selected was the column-based GenElute blood genomic DNA kit from Sigma-Aldrich (Bellefonte, PA). Blood samples were collected using K_3 EDTA anticoagulant. Improper mixing caused some blood samples to be clotted. These clotted samples were homogenized briefly with a polytron and then processed successfully with the GenElute DNA isolation kit. DNA from muscle was isolated by homogenizing the sample with a polytron in DNAzol using the protocol from Molecular Research Center, Inc. (Cincinnati, OH). Samples that did not run well in the PCR reaction (often from the blood isolation) were ethanol precipitated and concentrated before a second PCR run.

SNP assay using Real Time PCR

The PCR protocols and solutions from Applied Biosystems were used, but the reaction was scaled down to a 12.5-µl total reaction volume per sample per well of the 96-well PCR plate. This volume contained 2.5 µl of the DNA sample combined with Taqman Universal PCR Mastermix (Cat. No. 4324020), primers (900 nM, final dilution), and the two fluorescent probes (200 nM each, final dilution). The concentration of DNA in the sample could vary widely for this assay and still give satisfactory results. The PCR reaction was conducted exactly as for gene expression assays and"read" by comparing the relative exponential increase in each fluorescent probe for each individual sample. Controls of known genotypes (GeneCheck) were included on each 96-well plate and compared to unknown samples. For both codons, a typical PCR run on the ABI 7000 was 2 min at 50°C, 10 min at 95°C, and then 40 cycles of 95°C for 15 sec and 62°C for 1 min.

Genotyping of sheep

First, the ABI 7000 profile for known homozygous and heterozygous genotypes was determined. Then, 20 samples were run without prior knowledge of genotype and all were correctly identified at both codons. Finally, 50 more SNP determinations for each codon were compared to commercial testing; only 1 sample had an error at one codon (136), giving an error rate for 70 samples of less than 1%.

Results and Discussion

The cDNA sequences used as probes and primers for each codon in the SNP Real Time PCR reaction, as well as their Primer Express® software-estimated melting temperature (**Tm**), GC content, length, and the fluorescent labels on the probes are shown in Table 1. For a SNP assay (Allelic Discrimination), the primers ideally should have a Tm of 58-60°C and the probes a Tm of 65-67°C, preferably only having a 1°C difference in Tm between the two probes. Amplicon size should be between 50-150 bases. Primer Express® has many of the other criteria for probe development built into their selection protocol. However, there are numerous selections of probe/primer combinations obtained for each SNP determination desired and other requirements that need user selection for the best performance. Even after all these points are considered, the first choice of probes and primers may not be the best one. Because the original selection for codon 136 probes and primers did not perform well, the sequence was sent to the Assays-by-Design℠ Service of Applied Biosystems, which apparently has a "better" algorithm for probe and primer selections.

Proper DNA isolation is an important factor in a successful SNP determination. However, the DNA concentration and the A_{260}/A_{280} ratio of the DNA could vary widely and still give a reliable test. Additionally, heparin should not be used as a coagulant in the collection of blood samples because it inhibits the PCR reaction (Johnson et al., 2004).

The patterns of results from the real time SNP assays for known homozygous and heterozygous alleles for codon 136 are shown in Figure 1 (A-C) and for codon 171 are shown in Figure 1 (D-F). These patterns were used as the standard for identifying homozygous or heterozygous alleles. In some cases, the homozygous identification was based on whether one allele was dominant, or always on top of the other allele (Fig. 1; A, C, and D). Both alleles would amplify nearly equally if heterozygous (Fig. 1; B and E). For homozygous RR, the Q allele did not amplify at all (Fig. 1; F). If the patterns of the samples were not similar to those of the known genotype controls, or if the amplification was inadequate for either or both alleles, the assay was redone and often the DNA was concentrated by ethanol precipitation or re-isolated before repeating the assay.

The results of the genotyping of 928 sheep are shown in Table 2. To date, 928 out of 935 samples from blood (n = 818) and muscle (n = 117) have been tested successfully for both codons. Only 7 samples (<1% of total samples) could not be determined. These samples may have had extremely low DNA concentrations or were otherwise poor samples that did not run well in the PCR reaction. If another sample could have been taken, likely the problem(s) would have been resolved. A DNA sample can potentially be isolated from any nucleated cell in the body; thus, most problems can be overcome with a new DNA sample.

Of the 928 sheep tested, 86% of the sheep (n = 798) contained R at codon 171 (Table 2). Of these, the most abundant genotypes were AA QR (42.7%), and AA

RR (37.5%). Least abundant genotypes were AV QQ (3.0%), AV QR (5.8%), and AA QQ (11.0%). There was no VV QQ genotype in this group of animals, which is not surprising because very few sheep (less than 2%) of this genotype exist in the U.S. (USDA, 2003; DeSilva et al., 2003; O'Rourke et al., 1996).

In the future, we will develop a SNP assay for determining the polymorphisms at codon 154. We have already begun development of probes to distinguish H and K at codon 171 from Q or R. These genotypes represent very small numbers of animals, but may contribute to atypical results in the SNP genotyping assay.

Implications

Real time PCR methodology for detecting SNPs in the sheep prion gene is an efficient, reliable, and accurate method of genotyping sheep for the genes that control susceptibility to contracting scrapie or contribute to the incubation times for progression of the disease.

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Table 1. Nucleotide sequences of the Taqman Probes and Primers used in the SNP genotyping assay for codon 136 and codon 171 of the sheep prion gene

	Codon 136			
	Nucleotide sequence	Tm	%GC	Length
Probe A[1]	5'-(6FAM)-CTGCTCATGGCACTT-(MGBNFQ)-3'	66.7	53.3	15 bp
Probe V[1]	5'-(VIC)-CCTGCTCATGACACTT-(MGBNFQ)-3'	67.7	50.0	16 bp
Fwd Primer	5'-GCCTTGGTGGCTACATGCT-3'	57.4	57.9	19 bp
Rev Primer	5'-CGGTCCTCATAGTCATTGCCAAAAT-3'	63.1	44.0	25 bp
	Codon 171			
Probe R[1]	5'-(6FAM)-CAGTGGATCGGTATAGT-(MGBNFQ)-3'	65.9	47.1	17 bp
Probe Q[1]	5'-(VIC)-CAGTGGATCAGTATAGT-(MGBNFQ)-3'	65.0	41.2	17 bp
Fwd Primer	5'-GTTACCCCAACCAAGTGTACTACAGA -3'	58.5	46.2	26 bp
Rev Primer	5'-TGTTGACACAGTCATGCACAAAGT-3'	59.2	41.7	24 bp

[1]Probes are labeled with a fluorescent reporter dye at the 5' end, either 6FAM™ or VIC™, and a quencher at the 3'end; MGBNFQ = minor groove binding non-fluorescent quencher

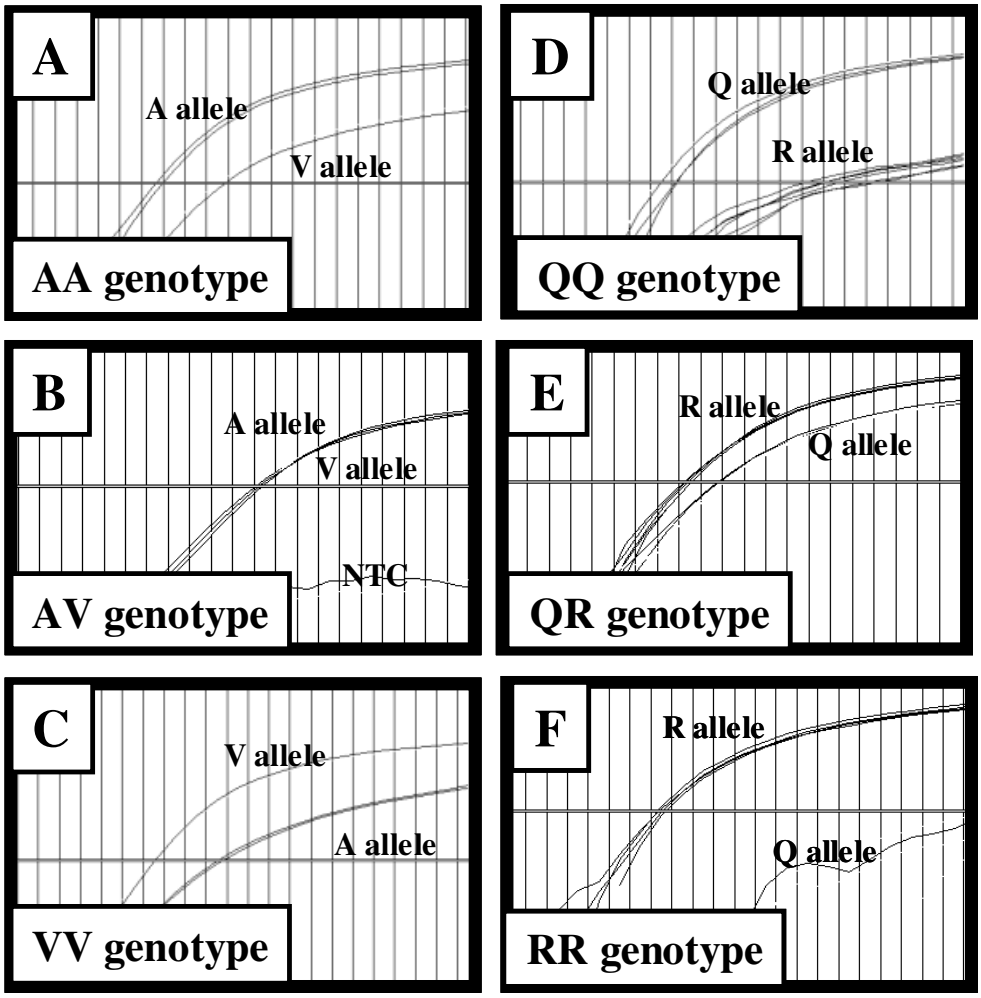

Figure 1. Patterns of results from the real time PCR SNP assays for known homozygous and heterozygous alleles of the sheep prion gene at codon 136 (A-C) and at codon 171 (D-F). For codon 136, **(A)** represents the pattern for homozygous AA; **(B)** represents the pattern for heterozygous AV; and **(C)** represents the pattern for homozygous VV. For codon 171, **(D)** represents the pattern for homozygous QQ; **(E)** represents the pattern for heterozygous QR; and **(F)** represents the pattern for homozygous RR. **NTC** = no template control, only shown in **(B)**, but used in all the assays.

Table 2. Distribution of genotypes in sheep tested for SNPs at codon 136 and 171 of the prion gene.

Genotype	Number	Percent
AA RR	348	37.5%
AA QR	396	42.7%
AA QQ	102	11.0%
AV QR	54	5.8%
AV QQ	28	3.0%
Total	928	100%

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RELATIONSHIPS AMONG GROWTH TRAITS, SERUM HORMONE PROFILES, AND DATE OF PUBERTY IN RAMBOUILLET EWE LAMBS

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ABSTRACT: Twenty five Rambouillet ewe lambs (eight singles, 17 twins) were used to examine growth traits, pubertal responses, and serum concentrations of triiodothyronine (T3), thyroxine (T4), growth hormone (GH), and IGF-1. Ewe lambs were born in spring (avg birth date = March 23, range March 14 to April 2), weaned at approximately 60 d (21.1 ± 4.5 kg), and fed a pelleted diet (14% CP) for 84 d then ad libitum alfalfa hay for the remainder of the study. Animals had continuous access to water, salt, and shade. Weights were recorded at weaning and at 28-d intervals thereafter. Serum samples were collected 14 and 28 d after birth and at weaning, and three times weekly after weaning beginning on Aug 25. Serum T3 and T4 were determined on all preweaning samples and weekly after weaning. Progesterone (P4) was measured in all postweaning samples. Day of puberty was considered as the day on which serum P4 reached 1.0 ng/mL. Average date of puberty was Oct 18 (range Sept 8 to Nov 17). On Sept 14, serum samples were collected from each ewe before and at 30 and 60 min after i.v. administration of 10 ug GHRH. Four ewes (16%) failed to reach puberty by Dec 31. Eight animals reached puberty before (early group) and 13 after (late group) Oct 18. Ewes that reached puberty early (26.6 ± 1.1 kg) weighed more ($P = 0.01$) at weaning (adjusted) than did late puberty ewes (22.9 ± 0.8 kg) and the correlation coefficient between weaning weight and date of puberty was -0.53 ($P = 0.02$). Early puberty ewes continued to be heavier ($P < 0.05$) than late puberty animals throughout the experiment. At 28 d of age, ewes that were heavier at weaning had serum T3 of 2.59 compared with 2.02 ± 0.13 ng/mL for lighter weight ewes. Serum T4 declined over the preweaning period with values of 109 = 96 > 72 (± 6) ng/mL on d 14, 28 and at weaning ($P < 0.01$). Date of puberty and BW were negatively correlated ($P < 0.06$) until ewes were approximately 6 mo of age. Correlation coefficients between weaning weight and serum T4 on d 14 and 28 were 0.52 ($P = 0.01$) and 0.53 ($P = 0.01$), respectively. The correlation coefficient between serum T4 on d 14 and date of puberty was -0.45 ($P = 0.04$). Postweaning serum T3 and T4 and area under the curve (AUC) for GH after GHRH were similar ($P > 0.20$) in the two puberty groups. However, AUC for IGF-1 after GHRH was greater ($P = 0.06$) in ewes that reached puberty early compared with late puberty ewes. The correlation coefficient between date of puberty and IGF-1 AUC was -0.48 ($P = 0.03$). Serum IGF-1 is related to date of puberty and relationships of preweaning T3 and T4 to date of puberty and growth characteristics warrant further study.
Key words: Sheep, Thyroid, Growth Hormone, IGF-1

Introduction

Sheep are seasonal breeding animals that breed in the fall during times of decreasing day length and lamb in the spring. The ability of the ewe lamb to reach puberty is determined by several factors including age, body size, nutrition, season of birth, and photoperiod (Yellon and Foster, 1986). Lambs born in the spring have the chance to reach puberty in the fall of that year. However, lambs born in the fall will not reach puberty until the fall of the following year. Ewe lambs that grow faster generally reach puberty earlier than slower growing animals (Land, 1978; Quirke, 1979; Little et al., 1981). Female lambs must reach approximately 60% of mature BW before attaining puberty. Kennedy and Mitra (1963) stated that timing of puberty is based on energetic status of the animal. Foster (1994) showed that severely undernourished lambs did not achieve puberty until the fall of the following year. Metabolic hormone signals may also play a role in determination of onset of puberty. Foster and Nagtanni (1999) suggested that hormones regulating body growth, such as IGF-1 and leptin, may be indicators of sufficient body size to begin reproduction. Shirley et al. (2001) found that heavier ewes tended to produce more growth hormone (GH) after a GHRH challenge and attain puberty earlier than lighter weight ewes. Webster et al. (1991) suggested also that thyroxine (**T4**) may be an indicator of onset of puberty because T4 rises gradually from low concentrations just before onset of the breeding season to peak concentrations just before the transition to anestrus. Therefore, the objective of this study was to examine the relationship among IGF-1, triiodothyronine (**T3**), T4, and GH and onset of puberty in ewe lambs.

Materials and Methods

Animals. Twenty five spring born Rambouillet ewe lambs (8 singles, 17 twins) were used to examine growth traits, pubertal responses, and serum concentrations of T3, T4, GH, and IGF-1. All procedures and facilities used in the experiment were approved by the New Mexico State University Institutional Animal Care and Use Committee. Ewe lambs were weaned at approximately 60 d (21.1 ± 4.5 kg), and fed a pellet diet (14% CP) for 84 d then ad libitum alfalfa hay for the remainder of the study. Lambs were vaccinated at 30 and 60 d of age against enterotoxemia and tetanus and maintained at the West Sheep Unit on the main campus at New Mexico State University in a single pen (4 x 12 m) with continuous access to water, salt and shade. Weights were recorded at 28-d intervals throughout the experiment.

Blood Collection and GHRH Challenge. Before weaning, blood samples were collected by jugular venipuncture from all lambs at 14 and 28 d of age and at weaning. After weaning, samples were obtained three times weekly from August 25 (before onset of puberty) through December 31. On September 14, each ewe received an i.v. injection containing 10 ug of synthetic bovine GHRH (Sigma G-0644) suspended in 2 mL of 0.9 % saline. Blood samples were collected from each animal before and at 30 and 60 min after GHRH administration. All samples were collected into 10-mL vacuum serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) which were allowed to stand at room temperature for 30 min before centrifugation at 4° C for 15 min at 1000 x g. Serum was then transferred to plastic vials and stored frozen until analyzed.

Hormone Analysis. Serum samples collected during the preweaning period and weekly samples obtained after weaning were analyzed for concentrations of T3 and T4 by RIA as described by Wells et al. (2003) and Richards et al. (1999), respectively. All samples collected after weaning were quantified for progesterone by RIA as described by Schneider and Hallford (1996). Progesterone values in the postweaning samples were used to estimate date of onset of puberty. Specifically, puberty was considered the date on which serum progesterone reached 1.0 ng/mL. The T3, T4, and progesterone assays used components of commercial antibody-coated tube kits (Diagnostic Products Corp., Los Angeles, CA). Serum collected during the GHRH challenge were quantified for GH (Hoefler and Hallford, 1987) and IGF-1 (Berrie et al., 1995). Within and between assay coefficients of variation were 15% or less for all assays.

Statistical Analysis. One of the objectives of this experiment was to examine relationships of both pre- and postweaning serum T3 and T4 profiles with date of puberty. Ewes that reached puberty before (n = 8) October 18 were considered early and those that attained puberty after October 18 (n = 13) were considered late in terms of date of puberty. Heavy lambs (n = 11) were those having an actual weaning weight greater than 21.1 kg (avg for all ewes) and light ewes (n = 10 ewes) weighed less than 21.1 kg. Effects of puberty and weight groups on serum T3 and T4 during the preweaning period were subjected to split plot analysis of variance for repeated measures as described by Gill and Hafs (1971). Effects of puberty and weaning weight group and their interaction were included in the main plot and day of sampling and the two and three way interactions with day were included in the subplot. Effects of puberty and weight group and their interaction were tested using animal within puberty group by weaning group as the error term. Effects of day and its two and three way interactions were tested with residual error. Areas under the GH and IGF-1 curves after GHRH challenge were determined by the trapezoiodal summation method. Simple correlation coefficients between hormone profiles and date of puberty were also determined. All statistical analyses were computed using GLM and correlation procedures of SAS (SAS Inst. In., Cary, NC).

Results and Discussion

The average date of birth for the ewes was March 23 (range March 14 to April 2). Average date of puberty was October 18 with a range of September 8 to November 17. Eight animals reached puberty before October 18 (early group) and 13 after (late group). Four ewes (16%) failed to reach puberty by December 31. Lamb birth weights averaged 5.2 ± 0.6 kg. Ewes in the early puberty group (5.5 ± 0.2 kg) had a higher birth weight than did those in the late puberty group (5.0 ± 0.1 kg). Ewes that reached puberty early (26.6 ± 1.1 kg) weighed more (P = 0.01) at weaning (adjusted) than did late puberty ewes (22.9 ± 0.8 kg) and the correlation coefficient between weaning weight and date of puberty was -0.53 (P = 0.02). Early puberty ewes continued to be heavier (P < 0.05) than late puberty animals throughout the experiment. Date of puberty and BW were negatively correlated (P < 0.06) until ewes were approximately 6 mo of age.

In addition to early and late puberty groups, ewes were also classified into weight groups as described previously based on their actual weaning weight. Preweaning T3 and T4 levels on d 14 and 28 after birth and at weaning were then examined in terms of the puberty and weight classifications. For serum T3, the only interaction that was considered important was weaning weight group by day after birth (P = 0.01). Effects of puberty group were, therefore, pooled across weaning weight groups and days. Ewes in the early puberty group had a serum T3 value of 2.36 ± 0.16 ng/mL compared with 2.33 ± 0.08 ng/mL for those in the late puberty group. On d 14 after birth and at weaning, ewes in the light and heavy groups had similar (P > 0.15) serum T3 concentrations. However at 28 d of age, ewes with heavier actual weaning weights had a serum T3 value of 2.59 ng/mL compared with 2.02 (± 0.13) ng/mL for those with lighter weaning weights (P = 0.01). Within the light weight group, serum T3 values were lower (P < 0.01) at weaning than on d 14 or 28 (2.93 = 2.02 > 1.75 ± 0.12 ng/mL on d

14, 28 and at weaning, respectively). Likewise in ewes classified as heavy, T3 on d 14, 28 and at weaning were 3.21 > 2.59 > 1.69 (± 0.13) ng/mL, respectively. The correlation coefficients between actual weaning weight and T3 on d 14 and 28 were 0.54 (P = 0.01) and 0.72 (P < 0.001).

Neither two- nor three-way interactions were detected (P > 0.10) for preweaning T4 concentrations; therefore, effects of weight and puberty groups and day were pooled. Ewes classified as attaining puberty early had a serum T4 value of 96 ± 9 ng/mL compared with 89 ± 5 ng/mL for late puberty ewes (P = 0.53). Animals classified as having lighter weaning weights had serum T4 concentrations of 89 ± 9 ng/mL compared with 96 ± 5 ng/mL for the heavy ewes (P = 0.45). Serum T4 declined over the preweaning period with values of 109 = 96 > 72 (± 6) ng/mL on d 14, 28 and at weaning (P < 0.01). Correlation coefficients between weaning weight and serum T4 on d 14 and 28 were 0.52 (P = 0.01) and 0.53 (P = 0.01), respectively. The correlation coefficient between serum T4 on d 14 and date of puberty was -0.45 (P = 0.04).

When examining postweaning serum T3 and T4 responses in puberty and weaning weight groups, neither two- nor three-way interactions were detected (P > 0.10). Ewes reaching puberty early had similar (P > 0.19) T3 (1.41 and 1.41 ± 0.08 ng/mL, respectively) and T4 (64 and 58 ± 3.5 ng/mL, respectively) values compared with those attaining puberty later. Likewise, T3 (1.36 and 1.46 ± 0.08 ng/mL) and T4 (63 and 59 ± 3.4 ng/mL) concentrations were also similar (P > 0.30) in light and heavy weaning weight groups. Area under the curve (AUC) for GH after GHRH were similar (P > 0.60) in the two puberty and weaning weight groups. Likewise, weaning weight group did not affect (P = 0.73) IGF-1 AUC. However, AUC for IGF-1 after GHRH was greater (P = 0.06) in ewes that reached puberty early (14795 ± 1083 units) compared with late puberty ewes (12282 ± 609 units). The correlation coefficient between date of puberty and IGF-1 AUC was -0.48 (P = 0.03).

Implications

Preweaning concentrations of serum thyroxine and triiodothyronine appear to be related to growth patterns in lambs. This relationship likely extends to date of puberty. The combination of serum thyroid hormones and insulin-like growth factor-1 as tools for examining onset of puberty in ewe lambs warrants further study.

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EFFECT OF CHROMIUM METHIONINE SUPPLEMENTATION ON KIDNEY FUNCTION INDICATORS, LIVER AND KIDNEY WEIGHT IN FINISHING HAIR LAMBS

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ABSTRACT: To determine the effect of chromium methionine supplementation on kidney function indicators, liver and kidney weight in finishing hair lambs were conducted two experiments. Exp.1. Twelve Pelibuey Tabasco lambs (19.54 ± 0.86 kg), placed individually in metabolic crates, in an complete randomized experiment design were assigned to receive during 56 days one of three treatments: 1) Corn-soybean meal diet containing 14.6 % CP and 2.99 Mcal/kg of DE (Control); 2) Diet similar to control supplemented with 0.4 ppm of chromium from chromium methionine (Microplex ®; Zinpro Co.); and 3) Diet similar to control supplemented with 0.8 ppm of Cr. Animals were weighed on days 1 and 56, blood and urine samples were taken each 14 days. Sera creatinine, and urine pH, density, protein and glucose analyses were performed. In day 56, the lams were slaughter, kidney and liver were collected and fresh weighed, and samples were taken to histological determinations. Exp 2. Twelve Pelibuey Tabasco lambs (18.14 ± 1.5 kg), were used in an experiment with similar procedure that in experiment 1, animals were assigned to receive diets containing 0.0, 1.0 or 2.0 ppm of chromium. In exp. 1, the animals fed 0.8 ppm of Cr tended ($P = 0.08$) to be higher ADG, and has heavier carcass ($P = 0.05$) than control. Cr supplementation not affected ($P > 0.10$) cretinine concentration. Urine pH, density, protein and glucose were not altered ($P > 0.10$) by treatments. Liver and Kidney weights were similar ($P > 0.10$) across treatments. Histological changes were not observed. Exp. 2. Body weight, gain and carcass were not affected ($P > 0.10$) by treatments. In the complete experiments (day 56), creatinine was not affected ($P > 0.79$) by treatments. Urine pH, density, protein and glucose, liver and kidney weights were not altered ($P > 0.10$) by treatments. Histological changes were not observed. It is concluded that supplementing up to 2 ppm of chromium in diets for finishing hair lambs, there are not risk of kidney or liver damage.

Key Words: Chromium, Kidney Function, Lambs

Introduction

The chromium is an essential mineral in human and animal nutrition (Schachter et al., 2001). The chromium supplementation in the diet of sheep reduces carcass fat, blood-cholesterol (Kitchalong et al., 1995) and blood triglycerides (Uyanik, 2001); increases rib eye area and improves growth performance (Dominguez et al., 2001). The use of trivalent chromium both in the human as animal nutrition, has been extended in the last decades, however there are some interrogates about its toxicity (Anderson et al., 1997; Jeejeebhoy, 1999). Anderson et al. (1997) adding 100 mg of Cr/kg of diet (Cr-picolinate and $CrCl_3$) not found effect on body weight, organs weight, blood-N nor blood-creatinine, as well in histology of kidney and liver in rats. Giving to rats thousand times the dosage suggest to human has been not observed toxicity signs (Dattilo and Miguel, 2003), while that Wasser et al. (1997) report kidney failure in a woman that intake daily 0.6 mg of Cr from Cr-picolinate during six weeks. Gentry et al. (1999) observed that kidney weight was increased and liver weight diminished in sheep fed chromium. NRC has been proposed as a research line for animal nutrition, evaluate the possible chromium toxicity and determine the optimum levels to be safety included in the diets (NRC, 1997). This research was conducted to determine the effect of chromium methionine supplementation on kidney function indicators, liver and kidney weight in finishing hair lambs.

Material and Methods

This research was conducted in the facilities of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autonoma de Sinaloa, in Culiacan, Sinaloa, Mexico.

Experiment 1. Twelve Pelibuey Tabasco lambs (males; 19.54 ± 0.86 kg) were used in a 56 days experiment. Lams were weighed, dewormed (Iberfull® ; Laboratorios Aranda), injected with vitamins A, D, and E (Vitafluid® ; Laboratorios Virbac). The animals were placed individually in metabolic crates (0.6 x 1.2 m). As a preventive medication against respiratory diseases, the animals receiving during three continues days, an injection with 50 mg of Enrofloxacin/head/day (Aquinase® 10%; Forth Doge Lab). In agreement to a completely randomized design (Hicks, 1973), were assigned the follows treatments: 1) Feed a soybean meal corn-based diet containing 14.6 % CP and 2.99 Mcal of DE/kg for 14 days then switched to a finishing diet 13.5 % CP and 3.1 Mcal of DE/kg offered during 42 days (Control); 2) Diets similar to control but supplemented with 0.4 ppm of chromium (Cr-0.4) from chromium methionine (Microplex®; Zinpro Co.); and 3) Diets similar to control but supplemented with 0.8 ppm of chromium (Cr-0.8) from chromium methionine. Animals were weighed on days 1 and 56. Blood samples by jugular punction using vacutainer (Vecton Dickinson) were taken

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in days 1, 14, 28, 42, and 56. Sera was obtained by centrifugation, and cretinine concentration was performed by colorimetric methods using specific kit (CR524 Randox Lab) and an spectrophotometer (Technicon RA-1000). Morning (0700 h) urine samples were collected in plastic bottles during days 1, 14, 28, 42, and 56, and density, pH, protein and glucose analyses were performed using Multistics (Bayer Lab) and were read in a Clinitek Status apparatus (Bayer Healt Care). On day 57, lambs were sacrificed in an slaughterhouse. Hot carcass weight was obtained, after that kidney and liver were removed, fresh weighed. Kidney and liver tissue samples were taken, immerses in formaldehyde 10 % solution in to the glass sealed bottles. In the laboratory were paraffin imbibed, and 5 µ slices were stained with hematoxiline and eosine (Prophet et al., 1995), and optic microscopic observation was performed.

Experiment 2. Twelve Pelibuey Tabasco lambs (males; 18.15 ± 0.43 kg) were used in a 56 days experiment, using a similar procedures that in experiment 1, the treatments consisted 1) Diet similar to control of experiment 1; 2) Diet similar to control supplemented with 1.0 ppm of chromium (Cr-1) from chromium methionine (Microplex®; Zinpro Co.); and 3) Diets similar to control but supplemented with 2.0 ppm of chromium (Cr-2) from chromium methionine.

Statistical Analysis. The data of both experiments were analyzed as a completely randomized design (Hicks, 1973), using each animal as the experimental unit. General AOV/AOCV procedure of Statistix® 8 (Analytical Software, Tallahassee, FL) was used to perform the analyses, Tukey test was used to compare the means when F-test indicated statistical differences ($P < 0.05$).

Results and Discussion

The effect of chromium supplementation on growth performance of hair sheep in the experiment 1, are shown in table 1.

Table 1. Effect chromium supplementation on growth performance of hair sheep (exp. 1).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	0.4	0.8		
Lambs, n	4	4	4		
Body weight, kg					
Initial	19.67	19.38	19.56	0.25	0.89
Ending	29.94	30.44	32.44	0.54	0.13
ADG g/d	177 [b]	191 [ab]	222 [a]	8.68	0.08
Feed/gain	4.42	4.21	3.82	0.12	0.11
Carcass kg	17.9[b]	18.1[ab]	19.6[a]	0.33	0.05

[1] Standard error of the mean

In despite that the low number of replicates, is not enough to considerate the experiment as a growth performance trial, the fact that the animals in all treatments gains weight, is an indicator that they were not damaged by chromium supplementation. The tendency ($P = 0.08$) to increase ADG in lambs receiving 0.8 ppm Cr-diet, is in agreement with observed in bovines (Kegley et al., 1997; Barajas and Almeida, 1999; Barajas et al., 1999). The increment ($P < 0.05$) in carcass weight observed with Cr-0.8 is in concordance with results of Dominguez et al. (2001) in sheep, and by Pollard et al. (1999) in steers.

The influence of chromium supplementation level on serum creatinine is shown in table 2. Chromium supplementation not affected ($P > 0.45$) creatinine level, this results is similar to found by Anderson et al. (1997) supplementing 0.1 ppm of Cr in a diet for rats.

Table 2. Effect of chromium supplementation on sera creatinine concentration in lambs (exp. 1).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	0.4	0.8		
Lambs, n	4	4	4		
Creatinine, mg/dL					
Day 1	0.89	0.90	0.92	0.01	0.79
Day 14	0.84	0.88	0.81	0.02	0.45
Day 28	0.87	0.88	0.84	0.01	0.66
Day 42	0.88	0.85	0.86	0.01	0.67
Day 56	0.93	0.90	0.88	0.03	0.81

[1] Standard error of the mean.

The effects of chromium supplementation on urine values, kidney weight and liver weight observed in experiment 1, are presented in table 3.

Table 3. Effects of chromium supplementation on urine values, kidney weight and liver weight observed in (exp. 1).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	0.4	0.8		
Lambs, n	4	4	4		
Urine					
Density	1.02	1.02	1.02	0.01	0.28
pH	8.62	8.87	9.00	0.11	0.39
Protein, mg/dL	14.1	9.4	7.8	1.94	0.43
Kidney wt., g	86.2	86.0	93	1.81	0.24
Liver wt., g	658	612	666	19.1	0.51

[1] Standard error of the mean

Chromium supplementation up to 0.8 ppm has not effect ($P > 0.24$) on urine density, urine pH, protein in urine, kidney weight, and liver weight. Glucose was not detected in urine samples. Histological changes were not observed. These results joint with the lack of effect on serum creatinine level, permits affirm that chromium supplementation from chromium methionine in levels up to 0.8 ppm of Cr in the diet, not affect the kidney function.

The effect of supplementation with 1 and 2 ppm of Cr on growth performance is shown in table 4. Chromium supplementation has not effect ($P > 0.58$) on growth performance and carcass weight of lambs. In others experiments supplementing diets with chromium picolinate, has been not observed effects on performance of lambs (Kitchalong et al., 1995; Forbes et al., 1998;

Gentry et al., 1999). Barajas et al. (1999) adding chromium methionine to bull calves diets, observed a quadratic effect of Cr, increasing growth response with 0.4 ppm of Cr, while 1.0 ppm of Cr was similar to observed in not supplemented animals.

Table 4. Effect of supplementation with 1 and 2 ppm of Cr on growth performance of lambs (exp. 2).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	1.0	2.0		
Lambs, n	4	4	4		
Body weight, kg					
Initial	17.75	18.63	18.06	0.43	0.74
Ending	31.84	30.70	30.40	0.69	0.62
ADG g/d	249	228	223	10.1	0.58
Feed/gain	3.88	4.05	4.00	0.20	0.68
Carcass kg	18.4	18.9	17.8	0.37	0.59

[1] Standard error of the mean.

The influence of 1.0 and 2.0 ppm of supplementary chromium in the diet on serum creatinine is shown in table 5.

Table 5. Effect of 1.0 and 2.0 ppm chromium supplementation on serum concentration of creatinine in lambs (exp. 2).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	1.0	2.0		
Lambs, n	4	4	4		
Creatinine, mg/dL					
Day 1	0.75[b]	0.84[ab]	0.90[a]	0.02	0.02
Day 14	0.77	0.98	0.79	0.04	0.09
Day 28	0.71	0.77	0.80	0.02	0.09
Day 42	0.85	0.94	0.85	0.02	0.08
Day 56	0.83	0.73	0.73	0.06	0.79

[1] Standard error of the mean

In day 1, animals assigned to 2.0 ppm of Cr treatments, shown higher (P = 0.02) serum creatinine level, this fact is not attributable to chromium; however its possible influence on ulterior results was discarded (P > 0.40) after performed covariance analyses. In days 14 and 42, Cr-1 tended to increase (P < 0.09) creatinine, while Cr-2 tended to increased (P = 0.09) in day 28. After 56 days experiment, there are not effect (P = 0.79) of treatments on creatinine concentration. Almeida and Barajas (2002) observed a diminishing on creatinine level in calves fed diets containing 1.0 ppm of Cr, from Cr-methionine during 28 days, they interpreted its results as evidence that Cr improves the well fare of stressed calves. In the present experiment, the absence of effect of 1.0 ppm and 2.0 ppm of Cr on creatinine levels is considered as evidence that glomerular filtration capacity was not altered.

The effects of 1.0 and 2.0 ppm chromium supplementation on urine values, kidney weight and liver weight found in experiment 2, are shown in table 6.

Table 6. Effects of supplementation with 1.0 and 2.0 ppm of chromium on urine values, kidney weight, and liver weight (exp. 2).

Items	Treatments, Cr ppm			SEM[1]	*P*
	0	1.0	2.0		
Lambs, n	4	4	4		
Urine					
Density	1.01	1.02	1.02	0.01	0.57
pH	8.87	8.24	8.87	0.19	0.31
Protein, mg/dL	26.6	17.5	17.2	5.22	0.75
Kidney wt., g	106	99	104	2.69	0.65
Liver wt., g	669	649	611	0.02	0.44

[1] Standard error of the mean

Chromium supplementation up to 2.0 ppm has not effect (P > 0.31) on urine density, urine pH, protein in urine, kidney weight, and liver weight. Gentry et al. (1999) observed that kidney weight was increased and liver weight diminished in sheep fed 0.4 ppm of Cr diets.

Glucose was not detected in urine samples. Histological changes were not observed on kidney and liver tissue samples. The results od this research, permits affirm that 2.0 ppm chromium supplementation, using chromium methionine as source of Cr, not affect the kidney function or liver weight

Implications

The significance of the findings in this research, is that chromium methionine supplemented at conventionally used levels from 0.2 to 1.0 ppm of Cr, not represent any toxicity risk for lambs.

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COMPARATIVE HEPATOTOXICITY OF PLANT EXTRACTS, PURE COMPOUNDS, AND GASTROINTESTINAL FLUIDS ASSOCIATED WITH SNAKEWEED (*GUTIERREZIA* spp.)

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ABSTRACT: Snakeweed (SW) is a noxious range plant that has long been toxic to livestock. The primary toxicant in SW is thought to be a class of compounds called saponins, although other compounds may be involved. Two experiments were conducted to evaluate the hepatotoxicity of SW plant extracts and pure compounds (exp. 1) and rumen and abomasal fluids collected from SW (30% SW/70% hachita blue grama hay) and control (100% hachita blue grama hay) fed ewes (exp. 2). Liver tissue slices of Female Sprague Dawley rats (475-500 g) were generated from a Krumdieck Tissue Slicer, plated on titanium mesh screens for 0, 3, 6, and 12 h in sterile six-well tissue culture plates in a 37˚C rotating incubator exposed to a 95% air/5% CO_2 gas phase. Cell death was evaluated by measuring lactate dehydrogenase leakage into the media and cell viability was measured by potassium and protein content of liver tissue. In exp. 1, ethanol, ether, and hexane extracts of SW and known compounds were dissolved in dimethyl sulfoxide (DMSO), cultured in William's Media E and liver slices were incubated in respective treatments. Results demonstrated that DMSO caused equal or more cell death than treatments ($P < 0.05$). Subsequent studies were conducted without DMSO. In exp. 2, rumen and abomasum fluid were cultured in William's Media E. Rumen fluid collected from control fed animals caused more cell death than rumen fluid from SW fed animals ($P < 0.05$), and both rumen treatments caused more cell death than fluid collected from the abomasum of SW and control fed animals ($P < 0.05$). In conclusion, results demonstrated that DMSO cannot be utilized as a solvent in plant compounds in *in vitro* culture systems. Rumen fluid collected from control animals is more toxic to liver cells than from SW animals and abomasum fluids indicating that rumen microflora could be altering compounds in SW or microflora could be altered by toxic compounds in SW.

Keywords: *Gutierrezia* spp., Krumdieck Tissue Slicer, *In vitro* tissue culture

Introduction

Snakeweed (SW; *Gutierrezia* spp.) is an unpalatable, noxious weed which may dominate rangelands and retard growth of palatable forage during periods of drought, leaving animals no choice but to consume the plant. Snakeweeds contain a variety of compounds toxic to animals with saponins suspected as the primary toxicant (Shaver et al., 1964; Gardner et al., 1999). Because the liver is the major organ to encounter and metabolize a diverse variety of compounds absorbed from the diet of animals (Fisher et al., 1991), the use of an *in vitro* method to determine the hepatotoxicity of SW plant extracts and ruminal and abomasal fluids collected from SW fed animals allows for controlled environmental conditions and comparisons of treatment effects on liver preparations from the same animal (Smith et al., 1988).

The objectives of these experiments were to determine if SW at various stages of ruminant consumption/digestion were affecting liver function utilizing an *in vitro* method for evaluation of toxicity, and to develop a biological assay for the measure of SW hepatotoxicity.

Materials and Methods

Snakeweed Collection: Samples of SW foliage, pre-bloom stage, were harvested via hand-clipping of the distal 5 to 10 cm of new plant growth. Samples were collected at the Chihuahuan Desert Range Research Center (CDRRC) located 37 km of north of Las Cruces, NM in July of 1999 and 2003. Plant extractions were conducted as described in Ashley, (2001).

Biological Assay: Rats used with these studies were euthanized by carbon dioxide inhalation and their livers were excised and placed in cold Krebs-Ringer Bicarbonate Buffer (# K-4002). Slicing procedures were conducted as described in Ashley, (2001).

After tissue slicing was completed, slices were drained into a sterile container. Each slice was placed on a tissue well insert containing titanium mesh screens and placed in sterile six-well tissue culture plates. Treatments

were made as described in Hernandez, (2004) and 2.5 mL were aliquoted into each well.

Tissue samples were assayed for protein content, intracellular potassium content, and lactate dehydrogenase (LDH) leakage. Analysis was conducted as described in Hernandez, (2004). All cells were found viable as indicated by potassium and protein content for all experiments. All treatments created for cell culture were sterile filtered using a .22µm filter in a sterile hood prior to culture. All animal procedures were approved by the Institutional Animal Care and Use Committee at NMSU.

Experiment 1: Twenty-one adult female Sprague-Dawley rats (475-500g) were used. Animals were fed Lab Diet 5001 Rodent diet (PMI Nutrition International, Brentwood, MO), water ad libitum, and exposed to a 12-h light/dark cycle.

Seven different treatments were created at three concentrations in order to compare cell death caused by SW extracts to cell death caused by pure compounds; a control (DMSO and William's Media E), 12.5 µg/mL, 100 µg/mL, and 200 µg/ml for each compound being used. Three different extraction fractions of snakeweed were utilized (ethanol, ether, and hexane fractions), as well as four pure compounds, related to plant extracts, were purchased from Sigma Chemical Co., St. Louis, MO; saponin from the quillaja bark (# S-4521; Sigma Chemical Co., St. Louis, MO), quercetin(# Q-0125; Sigma Chemical Co., St. Louis, MO), α-pinene (# 27-439,9; Sigma Chemical Co. St. Louis, Mo), and β-myrcene (# M-0382; Sigma Chemical Co., St. Louis, MO).

Three animals (n = 3) were used for each compound, except for hexane in which two animals (n = 2) were used due to an unexpected death. Six-well plates were loaded (2 plates/treatment) with slices (8 slices/2 plates). For each rat, two slices per time period for each treatment were used as sub-samples and averaged to represent the experimental unit. Of the eight wells used to incubate the liver tissue slices, two represented a 3 h incubation period, two a 6 h incubation period, two a 12 h incubation, and two a 24 h incubation period.

Experiment 2: Four ewes were fed, with two ewes (n = 2) being fed a diet of grass hay and two ewes (n = 2) being fed a diet of 30% SW, 70% grass hay (Table 1). Ewes were fed respective diets for 7 d and then humanely euthanized and ruminal and abomasal fluids collected. Once collected, fluids were centrifuged at 10,793 x g for (Labofuge 400; Heraeus Instruments, South Plainfield, NJ) for 30 min and placed in a freezer (-80˚C) for storage.

Four treatments were created, rumen SW, rumen control, abomasum SW, and abomasum control and mixed at 200 µL/mL in William's Media E. A control was created consisting of William's Media E.

Three adult female Sprague-Dawley rats (475-500g) were used in this experiment. Three rats were assigned to each treatment. A total of 15 sterile six-well tissue culture plates (five plates/rat) were loaded with slices (six slices/one plate). For each rat, two slices per time period for each treatment were used as sub-samples and averaged to represent the experimental unit. Of the six wells used to incubate the liver tissue slices, two represented a 3-h incubation period, two represented a 6-h incubation period, and two represented a 12-h incubation.

Statistical Analysis

Data for Exp. 1 were analyzed by repeated measures analysis using the mixed procedures (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC). The covariant structure utilized was compound symmetry (CS). Intracellular potassium, protein, and LDH levels were analyzed by time within treatment at each concentration level, with rat liver serving as the experimental unit in the whole plot and liver slice serving as the experimental unit in the subplot. Treatment type served as the error term between rats and treatment concentration served as the error term within rats.

Data for Exp. 2 was analyzed utilizing the mixed procedure of SAS (SAS Inst., Cary, NC). The experimental design was a randomized complete block with rat as the block and liver slice as the experimental unit. Tissue weights were all expressed in grams, protein concentrations as mg protein/g of wet tissue weight, intracellular potassium contents as mg potassium/g of wet tissue weight, and LDH enzyme leakage as units (U) of leakage/g of wet tissue weight. Only pre-planned comparisons were made using pairwise contrasts (SAS Inst., Cary, NC).

Results and Discussion

Experiment 1: The purpose of this experiment was to compare LDH leakage of SW plant extracts and pure compounds thought to be in SW of rat liver slices. Results indicated that DMSO control caused equal or more cell death than any of the plant extracts or pure compounds to rat liver slices ($P < 0.05$). Therefore, it was concluded that the presence of DMSO in control and treatments masked the effects of the treatments themselves.

Experiment 2: A treatment by hour interaction was not detected for LDH leakage, potassium, or protein concentrations of rat liver slices ($P > 0.10$; Table 2). Even though data are presented by time, only overall means were statistically tested. Abomasum control and abomasum SW had equal amounts of cell death in rat livers (Table 2), whereas, rumen SW and rumen control had different amounts of LDH leakage, with rumen control having elevated levels compared with rumen SW. Both ruminal treatments exhibited substantially higher amounts of LDH leakage than either abomasal treatment (Table 2). Even though a particular mechanism is not implied by our data, a potential explanation could be that the acidic pH of the abomasum has altered compounds from the rumen by acid hydrolysis creating compounds that have the ability to protect abomasal cells against potential toxicants.

Results of exp. 2 indicated that the rumen content causes more cell death than the abomasum content of a sheep. Also, it is apparent that rumen fluid collected from SW fed animals resulted in a lower amount of LDH leakage in rat liver slices in comparison to control

fed animals. It is possible that the rumen could potentially be altering compounds in SW to a less toxic form possibly by SW altering microflora population (Hall et al., 1991). It is also possible that a shift in the pH of the rumen as a result of SW ingestion from a neutral to an acidic environment could be making rumen contents less toxic (Edrington et al., 1993). Further investigation into how rumen fluid alters SW in a sheep should be conducted.

Implications

Due to the masking effect of DMSO found in Exp. 1 of all treatments as well as control, it was concluded that DMSO is not adequate as a solvent when using rat liver slices. A suitable solvent must be found in order to repeat this experiment so that comparisons can be made between plant extracts and pure compounds thought to be in SW.

Because the rumen fluid causes more LDH leakage than the abomasum, and rumen fluid from control fed animals cause more LDH leakage than fluid collected from SW fed animals, it is possible that the rumen microflora could be altering compounds in SW such that these compounds are less toxic or that a shift in the pH of the rumen to a more acidic environment could be altering the rumen contents of SW fed animals to a less toxic form. More investigation into how rumen microflora are possibly altering compounds in SW should be conducted. Potential acid hydrolysis of compounds in the diet could be occurring in the abomasum as a result of the shift to an acidic environment, therefore altering compounds in SW.

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Table 1. Nutrient Composition of Feedstuffs used in Experiment 2.

	Experiment 2[a,b,e]	
Item (%)	Snakeweed	Hachita Blue Grama Hay[c]
DM	93.4	93.2
Ca	0.87	n/a
P	0.18	n/a
Mg	0.13	n/a
K	1.75	n/a
Na	0.009	n/a
CP	12.33	11.4
NDF	35.43	74.7
ADF	21.65	42.4

[a]Feed analysis was performed in the laboratories of the Department of Animal and Range Sciences, New Mexico State University and the Forage Testing Laboratory, Ithaca, New York.
[b]Dry matter basis.
[c]Feed analysis as reported by Stow (2003) was conducted by New Mexico Department of Agriculture, Division of Agricultural and Environmental Services, Office of the State Chemist and utilized for experiment 1.
[e]n/a=not available.Two ewes (n = 2) were fed a control diet of grass hay.

Table 2. Least Square Means of Lactate Dehydrogenase (LDH) Leakage (U LDH/g tissue), Potassium Content (mg potassium/g tissue), and Protein Content (mg protein/g tissue) of Rat Liver Slices Incubated in Ruminal and Abomasal Fluid Collected from Ewes consuming Snakeweed and Hachita Blue Grama Hay as a control[2]

	Treatments[3]			
LDH	AC	ASW	RC	RSW
Hour				
3	11.1(1.2)	18.8(2.1)	231.1(24.2)	145.8(35.7)
6	12.7(1.7)	10.0(1.4)	308.0(61.9)	129.0(18.2)
12	34.3(12.0)	31.6(3.8)	239.8(40.2)	242.8(41.3)
Overall Means	19.4(4.3)[a]	20.1(3.6)[a]	259.6(25.8)[b]	172.6(21.0)[b,c]
Potassium				
Hour				
3	0.07(0.009)	0.06(0.003)	0.08(0.02)	0.06(0.01)
6	0.07(0.008)	0.04(0.009)	0.10(0.02)	0.05(0.006)
12	0.05(0.02)	0.03(0.003)	0.05(0.009)	0.09(0.03)
Overall Means	0.06(0.007)[a]	0.05(0.004)[a]	0.08(0.01)[a]	0.07(0.01)[a]
Protein				
Hour				
3	0.07(0.007)	0.08(0.003)	0.08(0.003)	0.08(0.02)
6	0.06(0.005)	0.07(0.005)	0.07(0.008)	0.08(0.008)
12	0.04(0.008)	0.04(0.005)	0.04(0.002)	0.07(0.01)
Overall Means	0.06(0.005)[a]	0.06(0.005)[a]	0.07(0.005)[b]	0.08(0.007)[b]

[1]Rumen fluid control (RC), rumen fluid SW (RSW), abomasum fluid control (AC) and abomasum fluid SW (ASW) were mixed with William's Media at one level: 200 µL/mL. A control was made of William's Media E and implemented within every treatment.
[2]Treatment by hour interaction was not observed ($P < 0.10$). Even though means are reported by time, only treatment means were statistically tested.
[3](SE)=standard error (n = 6; 3 rats and 2 slices). Variances were not equal.
[a-c]Row means with different superscripts differ ($P < 0.05$).

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Breeding Suffolk Rams to Western White Face Ewes on New Mexico Rangelands Increases Weaning Weight of Lambs

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ABSTRACT: The objective of the two-year study at the New Mexico State University Corona Range and Livestock Research Center was to determine the efficacy of breeding Suffolk rams to Western whiteface ewes while under range conditions. Approximately 120 ewes in 2003 and 2004, respectively, were divided among four similar pastures. Pastures were randomly assigned to receive either Suffolk rams or Rambouillet rams. Three rams were assigned to each pasture for a 34 d breeding season. One week before expected lambing approximately half of the ewes from each pasture were randomly selected to be shed lambed in order to estimate lambs born per ewe and birth weight of lambs. Birth weights (d 0 is the onset of lambing) were collected from only the shed lambs and within 24 h of parturition ewes and lambs were returned to their respective pastures. Body weights at marking (ear tagging and docking; approx. d 55) and weaning (approx. d 150) were recorded and analyzed from the entire lamb crop. Birth weights were similar ($P<0.05$) between sire groups (measured only on the shed lambed lambs) for both years. Suffolk sired lambs were heavier at d 55 in 2003 and 2004 ($P<0.10$ and $P<0.05$, respectively). The crossbred lambs were also heavier at weaning ($P<0.05$) in 2004 but no difference was detected between breeds in 2003 ($P>0.10$). In both years, the increased weight gain of crossbreds compared to straightbreds occurred between birth and marking, and the weight advantage was maintained through weaning. No differences were detected in lamb survivability between sire groups in either year. In conclusion, sheep producers that decide to use Suffolk rams on western white face ewes can expect crossbred lambs to be 8.5 % heavier at weaning than straight bred lambs.

Keywords: sheep, Suffolk, western white face, crossbreeding

Introduction

Many sheep producers in the Western United States use a terminal sire on Western white face (Rambouillet base) ewes to increase lamb productivity. This production system was developed by ranchers to utilize sheep breeds specialized to produce desirable lambs and yet maintain highly marketable fleeces of finewool flocks while grazing range pasture. The two year study at the New Mexico State University Corona Range and Livestock Research Center was conducted to mimic the production system which has been used New Mexico for many years. Even though terminal crossbreeding is widely accepted as a common practice within the sheep community, little research has been reported to support the specific crossbreeding system. A dated report by Neville et al. (1958) showed 2.32 and 1.3 kg improvements of Suffolk sired crossbred lambs 120 day weights over Hampshire, Oxford, and Shropshire sired crosses, which were all bred to Western whiteface ewes in 1955 and 1956, respectively. Suffolk rams have, more recently, been proven to be an optimum terminal sire because of their superiority to increase gain in crossbred lambs, which results in the production of larger leaner carcasses (Makarechian et al., 1978; Neville et al., 1958;Sidwell and Miller, 1971).

The US lamb industry is in need of lambs that possess larger leaner carcasses, both qualities in which the Suffolk breed excels. Purcell (1998) reported that the US lamb industry has not provided enough incentives to the sheep breeders to promote improvements in slaughter lamb carcass traits. Waldron (2002) stated sheep breeders/raisers need to take it upon themselves to genetically improve lamb as a marketable product in order to increase consumption of American lamb. Therefore, this study was conducted to prove that crossbred lambs from Western white face ewes and Suffolk rams will grow faster, increase herd productivity, and provide sheep producers with information regarding this management strategy.

Material and Methods

In 2003 and 2004, approximately 120 Western white face ewes (each year) were divided equally into four pastures. Each pasture consists of approximately 550 acres and was comparable in forage production. Suffolk or Rambouillet sires were randomly assigned to each pasture (three rams per pasture). Each pasture contained approximately 30 ewes and was exposed to the rams for 34 days.

One week prior to the onset of lambing (day 0 is onset of lambing) half the ewes from each pasture were randomly selected to be penned and lambed in confinement. Birth weights were recorded and lambs were ear tagged for identification purposes. Within 24 h post parturition, these ewes and lambs were returned to their original pastures. Birth weights and type of birth were recorded from the confinement lambed group. On day 55, all the lambs were ear tagged, docked, males castrated, and body weights recorded. On day 150, all lambs were weaned and body weights were recorded. Sire influence on lamb survivability was measured by the presence or absence of shed lambs at marking and weaning.

Data were analyzed as a completely random design and pasture within sire group was used to test the effect or sire group (PROC GLM of SAS; SAS Inst., Inc. Cary, NC). Survivability data were analyzed using Chi Square (SAS Inst., Inc. Cary, NC).

Results and Discussion

Birth weight of lambs that were lambed in confinement (n=69 and n=67 in 2003 and 2004, respectively) was similar between sire groups (P=0.12 and *P=0.31* for 2003 and 2004, respectively; Table 1 and 2).

Body weights taken on day 55 for all lambs (n=136 and n=133 for 2003 and 2004, respectively) were heavier for Suffolk sired lambs over Rambouillet sired lambs in 2003 and 2004 (*P=0.06* and *P=0.08*, respectively). The Suffolk sires markedly improved growth rate at a time when maternal influence is thought to be the greatest. However, weaning weight of lambs (n=128) taken on day 150 was similar (*P=0.17*) between breeds for in 2003 (Table 1). Although, in 2004, lambs (n=122) were heavier (*P=0.05*) at day 150 for the Suffolk sired lambs compared to Rambouillet sired lambs. The difference in 2003 and 2004 may be attributed to the quality and quantity of forage available since moisture totals for the years varied widely. Recorded rainfall for the years of 2003 and 2004 was 16.2 and 43.4 cm, respectively (average = 40.3 cm). The straight bred lambs would have been expected to gain better in the dryer environment than the crossbred lambs because the Rambouillet breed is known for its hardiness in western range land conditions. Neale (1943) mated Hampshire rams to Rambouillet ewes while under range conditions and the crossbred lambs marked heavier weights than the straight bred Rambouillet lambs. But in contrast to our current study, Neale's straight bred Rambouillet lambs weaned the largest lambs in poor range conditions and were comparable to the crossbred lambs on fair range conditions.

No difference of survivability between breeds at day 55 (*P=0.91* and *P=0.95* for 2003 and 2004, respectively) was detected between sire on the survivability of the lambs. At day 150, survivability of the two sired groups was also similar (*P=0.63* and *P=0.44* for 2003 and 2004, respectively). No difference (*P>0.10*) was found between shed and pasture born lambs for marking and weaning weights, or lamb survivability when compared to in both 2003 and 2004.

In conclusion, Suffolk sired lambs were similar at birth to the straight bred lambs, gained weight faster than straight bred lambs from birth to day 55, and maintained a heavier body weight for another 95 days to weaning. This study has shown an 8.5% advantage in weaning weights when breeding Suffolk sires to Western white face ewes under New Mexico range conditions. This is useful to the New Mexico sheep producers considering use of a terminal cross on their Western white face flock.

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TABLE 1. Body weights (kg) of lambs born in spring 2003 to Western white face ewes grazing native rangelands and sired by Suffolk or Rambouillet rams[ab]

	Sire Group			
Age of lambs	Rambouillet	Suffolk	SE[e]	p-value
Birth[c]	4.7	5.1	0.16	0.12
Day 55[d]	14.0	16.0	0.67	0.06
Day 150[d]	37.0	40.2	1.42	0.17

[a] 120 ewes were divided into four equal pastures and each pasture was randomly assigned a treatment (breed of sire). Pasture was used as the experimental unit.
[a] Three rams were allotted to each pasture for 34 days.
[c] Birth weights (day 0) were only taken from the shed born lambs, and half of each pasture was shed lambed.
[c] On day 55 lambs were ear tagged, docked, and males castrated. Lambs were weaned on day 150.
[e] Standard error (n=33, n=68, and n=63, respectively; lambs per sire group)

TABLE 2. Body weights (kg) of lambs born in spring 2004 to Western white face ewes grazing native rangelands and sired by Suffolk or Rambouillet rams[ab]

	Sire Group			
Age of lambs	Rambouillet	Suffolk	SE[e]	p-value
Birth[c]	5.1	5.4	0.22	0.31
Day 55[d]	22.6	26.3	1.12	0.08
Day 150[d]	41.0	45.9	1.24	0.05

[a] 120 ewes were divided into four equal pastures and each pasture was randomly assigned a treatment (breed of sire). Pasture was used as the experimental unit.
[b] Three rams were allotted to each pasture for 34 days.
[c] Birth weights (day 0) were only taken from the shed born lambs, and half of each pasture was shed lambed.
[d] On day 55 lambs were ear tagged, docked, and males castrated. Lambs were weaned on day 150.
[e] Standard error (n=27, n=66, and n=60, respectively; lambs per sire group)

TABLE 3. Sire effect on percent lamb survival from birth to day 55 and from birth to day 150 in 2003 and 2004[ab]

	Sire Group		
2003 Survivability	Rambouillet	Suffolk	p-values
Birth to day 55	90.9	91.6	0.91
Birth to day 150	81.8	86.1	0.63
2004 Survivability			
Birth to day 55	81.8	82.4	0.95
Birth to day 150	78.8	70.6	0.44

[a] Lamb survivability was measured only on the lambs born in confinement.
[b] Survivability was measured by the presence or absence of lambs at marking and weaning.

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PERFORMANCE OF HOLSTEIN CALVES AT WEANING ACORDING ITS BIRTH SEASON IN MEXICALI VALLEY

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ABSTRACT: The objective of this study was to evaluate the effect of calving season on calf birth (CBW), weight at 30 d (W30), weight at 60 d (W60), and daily weight gain from birth to 60 d of age (DWG) of Holstein calves in a dairy herd located in a desert region of Baja California, México. Calving season was grouped in summer (n=50), autumn (n=91), and winter (n=59). Calves consumed 2 L of colostrum at 6 and 12 h of age and then 4 L of whole milk until 60 d of age. Calf birth weight was recorded and also calves weights each 10 d until 60 d of age. Calf starter was offered to all calves from the first week of age. Calves were maintained in the same pen during the raising. Statistical analyses were performed using linear models through analysis of variance in SAS. CBW was higher (P <.05) in calves born in autumn and winter (31.67 ± 0.78 and 33.61 ± 0.91 kg, respectively) than those born in summer (28.76 ± 1.03 kg). W30 was higher (P <.05) in calves born in summer and autumn (43.85 ± 0.56 and 42.83 ± 0.40 kg, respectively) than in winter (40.92 ± 0.51 kg). W60 was higher (P <.05) in those calves born in summer (64.75 ± 1.08 kg) than those born of autumn (62.22 ± 0.78 kg) and winter (58.65 ± 0.99 kg), while those calves born in autumn were heavier (P <.05) than those of winter. DWG at 60 d of age was similar (P> .05) in calves born in summer and autumn (0.533 ± 0.01 and 0.506 ± 0.01 kg), and both were higher (P <.05) than calves born in winter (0.451 ± 0.01 kg). These results indicate that even though calves born in summer have lighter weights, they have heavier weights at 30 and 60 d age.

Key Words: Weaning Weigth, Calf Birth Weigth, México.

Introduction

The recently born calves are highly susceptible to a great number of diseases that can have an effect in the mortality and morbidity, and on their behavior before the weaning (Quigley, 1997; Joslin et al., 2002). In order to reduce the morbidity and the mortality, calves should be fed with colostrum of high quality in the first hours of life (Roblee et al., 2003). At weaning, the rumen of the calf will be appropriately developed to use grain and forages (Joslin et al., 2002). The systems of dairy calves feeding have the purpose of facilitating the ruminal development in order to rapidly avoid the use of milk and or milk or substitutes, introducing the calves to a started solid diets and forages, to contribute to reduce feeding costs (Medina, 1994). The early offer of starter concentrate to dairy calves in the first week of life lead to stimulate the development of the rumen and, finally, to a early weaning (Joslin et al., 2002). For several decades has been a great interest to enhance the systems of production of milk in Mexico; this is due to a low milk production and the increasing milk demand of milk from human consumption. One way to increase milk production is thorough the application of system of nursing of calves using reduced quantities of whole milk or commercial substitutes of milk for a short time of nursing (De Peters et al., 1986; Plaza and Fernandez, 1991). The productivity of dairy cattle decreases notably when undergoes adverse climatic conditions. The dry period is a stage characterized by a limited attention for the dairy cow, and dry cows exposed to hot conditions may deliver calves of low weigth, which in turn come from small placentas (Lewis et al., 1984). The objective of the present study was to evaluate the performance of Holstein calves according its birth season.

Materials and Methods

The study was carried out at the Experimental Dairy Herd Unit of the Instituto de Ciencias Agrícolas of the Universidad Autónoma de Baja California. Two hundred Holstein calves were used (95 females and 105 males) from 2 to 60 days of age. Calves considered born from August 1997 to March 202. Calves were assigned to a season according their birth date as follows: summer 50 calves (22 females and 28 males); autumn 91 calves (46 females and 45 males) and winter with 59 calves (27 females and 32 males). Calves were separated from their dam at birth and isolated in a breeding room consuming 2 L of colostrum at 6 and 12 h of age; subsequently, they were fed 4 L of whole milk using a nursing bottle in two doses of 2 L each (am and pm); until 60 d of age. Calf birth weight was recorded and also calves weights each 10 d until 60 d of age. Calf starter (21 % PC) was offered to all calves from the first week of age. Calves were maintained in the same pen during the raising. The information were subject to analysis of variance using a completely randomized design with repeated measures analyzed by means of variance analysis, the analyses were carried out using the procedure GLM (General Line Models) of the statistical program SAS (1991).

Results and Discussion

Live weights of calves from birth to 60 d (weaning) are indicated in the Table 1. Birth weight was higher (P <.05) in calves that were born in autumn and winter (31.67 ± 0.78 and 33.61 ± 0.91 kg, respectively) than those calves born in summer (28.76 ± 1.03 kg). At 10 d of age, live weigth was higher (P <.05) in calves that were born in summer (35.81 ± 0.26 kg) that those born in winter (34.84 ± 0.23 kg), while calves born in autumn (35.28 ± 0.18 kg) were similar to calves born in summer (P> .05). At 30 and 40 d of age, calves that were born in summer (43.85 ± 0.56 and 49.59 ± 0.71 kg) and autumn (42.83 ± 0.40 and 48.35 ± 0.51 kg) had similar live weight (P> .05), but they were higher (P <.05) than calves that were born in winter (40.92 ± 0.51 and 45.64 ± 0.65 kg). Live weigth at 60 d (weaning) was higher (P<0.05) in calves that were born in summer (64.75 ± 1.08 kg) that calves born in autumn (62.22 ± 0.78 kg) and winter (58.65 ± 0.99 kg).

Birth weights of calves born in autumn and winter were higher (P<0.05) than those born in summer, representing these differences 10.1% in autumn and 16.86% in winter, respectively. Collier et al. (1982) compared birth weights of calves from cows subjected to shade in late gestation period with cows without shade in the same period of time, finding a difference of 3.1 kg (8%) in favor of the shaded-group. Wolfenson et al. (1988) found 2.6 kg (6%) more in birth weight of calves from cows under a cooling system based on spray and fans during their dry period over cows subjected just to a shade. These results agree with the birth weights observed in the present study comparing cool seasons with summer season. Live weights at 60 d in summer were higher (P <.05) in 2.53 and 6.1 kg than those weights born in autumn and winter respectively; also, calves born in autumn had higher (P <.05) live weights at 60 d than those calves born in winter. Weaning weight registered during summer and autumn in this study were higher than those reported by Franklin et al. (2003) of 60.5 kg and Robblee et al. (2003), however, Quigley et al. (2002) reported higher weaning weights to the ones presented. Regarding the low birth weights observed during summer and higher birth weights obtained in autumn and winter, it is possible that a compensatory gain was occurred starting from day 10 of age in calves that were born in summer and at 50 d of age for calves born in autumn, which suggest that research considering compensatory growth after birth in dairy calves is needed.

Table 2 shows the results on the daily weight gain (DWG) of the calves from birth to 60 d of age. At 30 d of age, DWG was higher (P <.05) in calves that were born in summer (0.394 ± 0.01 kg) that those born in winter (0.297 ± 0.01), but no differences (P>0.05) to those born in autumn (0.361 ± 0.01 kg). However, at 40, 50 and 60 d of age, DWG was higher (P <.05) in calves that were born in summer and autumn that in winter. DWG at 60 d was 0.533 ± 0.01 in summer and 0.506 ± 0.01 kg in autumn; both DWG were higher (P<0.05) than DWG observed in winter (0.451 ± 0.01 kg).

Implications

Live weight of calves was higher in calves that born during summer. Daily weight was higher in calves that were born in summer and autumn. There is a marked effect from birth season on the performance of the calves in this arid region of northwestern Mexico.

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Table 1. Calves growth from birth to weaning (60 d) according to birth season.

	BIRTH SEASON		
VARIABLES	SUMMER	AUTUMN	WINTER
No. of Animals	50	91	59
Weigth (kg):	Verage ± EE	Average ± EE	Average ± EE
Birth	28.76 ± 1.03[b]	31.67 ± 0.78[a]	33.61 ± 0.91[a]
10 d	35.81 ± 0.26[a]	35.29 ± 0.18[ab]	34.84 ± 0.23[b]
20 d	39.59 ± 0.45[a]	38.82 ± 0.32[a]	37.60 ± 0.40[ab]
30 d	43.85 ± 0.56[a]	42.83 ± 0.40[a]	40.92 ± 0.51[b]
40 d	49.59 ± 0.71[a]	48.35 ± 0.51[a]	45.64 ± 0.65[b]
50 d	56.74 ± 0.90[a]	54.56 ± 0.65[b]	51.59 ± 0.82[c]
60 d	64.75 ± 1.08[a]	62.22 ± 0.78[b]	58.65 ± 0.99[c]

[a, b, c] Different ltters in rows indicate significant difference (P<.05).

Table 2. Daily weigth gain in four periods of age according from birth season.

	BIRTH SEASON		
VARIABLES	SUMMER	AUTUMN	WINTER
No. of Animales	50	91	59
Weigth (kg):	Average ± EE	Average ± EE	Average ± EE
30 d	0.394 ± 0.01[a]	0.361 ± 0.01[ab]	0.297 ± 0.017[b]
40 d	0.430 ± 0.01[a]	0.410 ± 0.01[a]	0.346 ± 0.01[b]
50 d	0.483 ± 0.01[a]	0.453 ± 0.01[a]	0.398 ± 0.01[b]
60 d	0.533 ± 0.01[a]	0.506 ± 0.01[a]	0.451 ± 0.01[b]

[a, b,] Different letters in rows indicate significant difference (P<.05).

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PRODUCTIVITY AND PROFITABILITY OF PASTURE-BASED VERSUS INTENSIVE VALUE ADDITION STRATEGIES FOR CULLED BEEF COWS

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ABSTRACT: Forty-eight culled beef cows were assigned to compare short-term (49 d) grazing vs. confinement strategies for the cull cow enterprise. Treatments were intensive confinement feeding (**FDL**), blue grama pasture + mineral supplement (**NON**); NON + 926 g/d of a 38% CP supplement (**PRO**), or NON + 1726 g/d of a 20% CP supplement (**ENR**). Cow BW and condition score responses were evaluated. Initial value, treatment costs, and sales value were recorded. Cow initial body condition and BW were used as covariates and orthogonal contrasts were used to compare FDL vs. pasture-based treatments, NON vs. PRO and ENR, and PRO vs. ENR. Over the 49-d period, FDL resulted in greater BW gain than pasture treatments ($P < 0.01$) and supplementation tended to improve gain ($P = .19$), with little difference among supplements ($P = .98$). Weight gains were 98, 4, 10 and 10 (SE = 3.8) kg/cow for FDL, NON, ENR and PRO. Final condition scores were 6.0, 4.3, 4.5 and 4.5 (SE = .04) for FDL, NON, ENR and PRO, respectively. Final condition was greater in FDL than pastured cows ($P < .01$), was greater in supplemented vs. NON ($P < .01$), and was similar among PRO and ENR ($P = .84$). Production responses resulted in differences in gross revenue, with FDL generating the most revenue ($P < .01$), NON tending to receive less than PRO and ENR ($P = .14$) and ENR tending to receive more than PRO ($P = .12$). Due to total cost differences, net returns did not follow gross revenue. Net returns ($/cow) were 52, 26, 15, and 15 (SE = 7.9) for FDL, NON, ENR and PRO. Net returns to FDL were greatest ($P < .01$); returns to NON were numerically higher than supplemented groups ($P = .23$), while PRO and ENR were similar ($P = .99$). Productivity and profits are maximized with intensive management of culled cows, while cost management is critical for profitability of low-input strategies.

Key words: cull cows, supplementation

Introduction

Producers can add value to culled cows by increasing weight, increasing cow body condition to upgrade market classification; and/or control timing of marketing to capture seasonal price highs. Although several reports are available evaluating intensive feeding of culled cows (Sawyer et al., 2004; Schnell et al., 1997), few examine grazing programs for the cull cow enterprise. Little et al. (2002) reported on such an enterprise, but used assumed performance values over 180 d in an economic model. The objectives of this study were to compare grazing-based vs. confinement strategies for the cull cow enterprise and their effects on performance and profitability.

Materials and Methods

The study was conducted at the New Mexico State University Agricultural Science Center at Tucumcari (35º 12' 0.5" N, 103º 41' 12.0" W; elev. 1247 m). In summer 2001, a 10.9 ha pasture was established to 'Hachita' blue grama (*Bouteloua gracilis*). Following establishment, the pasture was divided into nine 1.2 ha paddocks with watering points.

Forty-eight culled range cows of predominantly British breeding were procured from local cooperators. Cows were delivered a minimum of ten days prior to initiation of the study and were placed on a common native rangeland pasture to equalize gastrointestinal fill. Cows were fasted overnight, weighed, checked for pregnancy via rectal palpation, treated with a topical anthelmintic, and assigned a body condition score. Body condition scores were assigned on a 9-point scale (1 = emaciated, 9 = obese). Cows were stratified by origin and randomly assigned to one of 12 experimental groups (4 cows per group).

Experimental groups were randomly assigned to receive one of 3 pasture-based treatments or a confinement feeding treatment (3 groups/treatment) for a 49-d period. The confinement feeding treatment (**FDL**) was considered a positive control. Cows in FDL resided in 5 X 15 m soil surfaced pens with covered wooden feed bunks. Beginning d 0, FDL cows were adapted from a 30% roughage diet to a 10% roughage diet over a 21-d period. All FDL diets were formulated for 13.7% CP, 2.2 Mcal/kg NE_m, 1.5 Mcal/kg NE_g, to meet or exceed requirements for minerals, and to contain 33 mg/kg monensin. Average DMI for FDL was 13.9 kg/d as-fed. This feeding strategy has been demonstrated to be efficacious for intensive feeding of culled beef cows (Sawyer et al., 2004).

Pasture-based treatments consisted of a negative control (**NON**), in which cows received no supplemental feed; a high protein supplement based on cottonseed meal (**PRO**; 38% CP, Hi-Pro #38) fed at 925 $g \bullet cow^{-1} \bullet d^{-1}$; or an energy supplement (**ENR**; 20% CP, Hi-Pro #1301) fed at 1796 $g \bullet cow^{-1} \bullet d^{-1}$. Supplements were fed on a prorated basis 3 times/week, and PRO and ENR were fed to supply equivalent amounts of crude protein. Cows on all pasture-

based treatments had ad libitum access to a salt-mineral mix containing 1620 mg/kg monensin (Hi-Pro #1820, Hi-Pro Feeds, Friona, TX) which was consumed at 60 g•cow^{-1}•d^{-1}.

Cows were fasted overnight without water and weighed on d 0, 14, 28, 42, and 49. Body condition scores were assessed on d 0, 28, and 49. Body weight changes were calculated from shrunk weight to shrunk weight. Average daily gain was computed as BW change divided by days between weights.

On weigh days, forage mass was estimated by clipping to ground level at two uniformly spaced locations in each paddock. Each sample was placed in a separate bag and dried for 48 hr at 65°C to determine herbage DM mass. Within paddock means were recorded and analyzed statistically as described below. Within paddock samples were composited by dry weight and analyzed for nutrient composition (Ward Laboratories, Inc., Kearney, NE).

Initial cow value on d 0 was determined based on the market price of cows at the nearest auction market (Clayton, NM) according to the USDA-AMS market report. A pricing rubric was constructed to assign prices to cows based on body condition score and weight. Within weight class, cows with BCS less than 4.5 and greater than 4.5 were respectively categorized as "lean" and "boners," and priced. Cows were marketed on d 49 at Dalhart, TX, and final valuation was established by actual selling price.

Feed costs were: PRO, $30.95/100 kg ($280.80/ton); ENR, $25.35/100 kg ($230.00/ton); and mineral supplement, $66.14/100 kg ($600/ton). Feedlot rations were charged $14.25/100 kg ($129.28/ton). Grazing fees were assessed at $0.46•hd^{-1}•d^{-1}, reflecting a $14.00/AUM lease rate. Yardage was charged to FDL cows at a rate of $0.32•hd^{-1}•d^{-1}. Carriage costs from weaning until initiation of the study, freight charges and commission were not included, as they apply equally to all cows.

Forage mass data were evaluated by sampling date using the General Linear Models procedures of SAS v.9 (SAS Institute, Cary, NC). Paddock served as the experimental unit and the supplemental feeding treatment assigned to the paddock as an effect in the model. Cow performance and economic data were analyzed using the General Linear Models procedures of SAS v.9 Pen or pasture was considered the experimental unit for all response variables. Body weight on d 0 and BCS on d 0 were included as covariates for all responses. Orthogonal contrasts were used to compare treatments: FDL vs. pasture-based treatments; PRO and ENR vs. NON; and PRO vs. ENR.

Results and Discussion

Standing crop values for each sampling date are shown in Table 1, and forage quality assessments in Table 2. Initial (d 0) and final (d 49) forage mass was similar for all treatments (P > .26). Mean standing crop was lowest on d 42, and mean forage allowance on this date was 608 kg/cow. It is unlikely that performance responses of grazing cows in this study were limited due to forage availability, although forage quality estimates are below estimated nutrient requirements.

Performance responses of cows to different management strategies are shown in Table 3. The use of initial BW as a covariate resulted in scaling of all initial BW values to a constant 409 kg. Average daily gain during the first 14 d of treatments was positive for all treatments (different than zero, P < 0.03). Cows fed in confinement had greater ADG than cows grazing pasture (P = 0.04), but ADG was similar in cows fed pasture supplements compared with NON (P = 0.51). The high ADG across treatments may be indicative of greater intake and thus increased gut fill during the initial realimentation period. Although cows were shrunk before weighing, the high amount of available forage in pastures and ad libitum access to feed for FDL cows probably contributed to high apparent gains. Other studies have shown high rates of initial gain in realimented cull cows (Swingle et al., 1979) and high variation in initial responses (Sawyer et al., 2004).

Gain response to treatments followed similar patterns in subsequent periods. Cows receiving FDL treatment consistently outperformed those grazing pasture (P < 0.01 in all periods). During d 15 through 28, gains were substantially reduced for all pasture treatments. From d 29 through 42, gains of cows receiving ENR and NON were less than zero (P < 0.04), while those receiving PRO were similar to zero (P = 0.36), so that supplemented cows on average had ADG similar to NON (P = 0.26). The cumulative effects of treatments (overall ADG) resulted in significant and expected advantages to the FDL treatment compared with grazing treatments (P < 0.01) and a tendency for supplemented cows to gain more than unsupplemented cows on pasture (P = 0.19).

The measured protein content of available forage suggests a protein deficiency existed for grazing animals, and some response to protein in the supplements was realized. The TDN value of the available forage (especially relative to the FDL diet) might have limited gain despite high availability; however, supplying additional energy did not improve ADG. This suggests that protein was the primary limitation and that a greater amount of protein supply might have enhanced gain, but that increased supplemental energy could not be utilized due to the protein limitation (Wallace and Parker, 1992).

Body condition score has a large impact on the value of slaughter cows (Apple, 1999). Adjusted initial BCS of cows used in this study was 3.67. Condition of all cows increased during the first 28 d (Table 3), and the magnitude of increase was consistent with observed BW gain. On d 28, FDL cows had greater BCS than cows on pasture treatments (P < 0.01). All cows on pasture-based treatments achieved similar BCS by d 28 (P > 0.32). At the end of the feeding period, cows consuming FDL had further increased BCS, and had greater BCS than cows receiving pasture-based treatments. Cows that received a supplement on pasture (ENR and PRO) had higher final BCS than those that did not receive a supplement (NON; P < 0.01). Condition score changes are consistent with observed BW changes (NRC, 1996) and with responses of cull cows to moderate (Swingle et al., 1979) or high-energy diets (Schnell et al., 1997; Sawyer et al, 2004).

Responses to treatments were mixed. Cows fed intensively performed as predicted, based on previous work

(Sawyer et al., 2004) and exhibited high rates of BW gain and a relatively large increase in body condition. Cows fed supplements on pasture (ENR and PRO) exhibited similar total BW gain (10.1 ± 3.8 vs. 10.3 ± 3.7 kg, respectively, P = 0.98). Cows receiving NON gained only 4.0 ± 3.4 kg during the trial. While contrasts comparing supplemented cows to NON failed to separate these treatments, mean gains for both ENR and PRO were greater than zero (P = 0.03), while BW gain for NON was not different from zero (P = 0.30). This distinction allows the conclusion that cows responded to supplementation, however, the level of supplementation was inadequate in relation to the quality of the basal diet to encourage high rates of production.

The NRC (2000) Level 1 simulation model was used to evaluate this conclusion. Model parameters were set to best reflect the conditions of the trial. Microbial efficiency was set at 11% rather than 13%. The 'on pasture' module was not utilized. Values from the forage analysis were used to reflect diet composition and formulated values from the feed manufacturer were used to represent the supplements. Total DMI predicted by the model was used to estimate forage intake. With these estimates, the model predicted performance (days to change 1 condition score) of pasture cows closely (PRO, 62 d predicted vs. 58 d observed; ENR, 53 d vs. 58 d; NON, 597 d predicted, maintenance observed). However, the model indicated that rate of gain was constrained by energy intake. This is contrary to the observed results, in which ENR cows gained at the same rate as PRO cows, but may have resulted from differential impacts of supplements on forage intake. When FDL cows were modeled, performance was substantially overpredicted. Using observed intake values and diets, the model predicted 9 d to change 1 condition score, compared with an observed rate of change of 1 score increase per 21 d. This error may be due to diet conditions outside the range of the data used to develop cow performance models.

Enterprise budgets for each treatment group are shown in Table 4. By design, feed costs were significantly affected by treatments (P < .01). Feed cost disparity between pasture-based and FDL treatments was partially offset by difference in assessed grazing fees vs. yardage charges. Cow initial value was included as a cost to represent initial investment in the cull cow enterprise. Initial estimates of cow value were similar for all treatments, but represent a significant proportion of total enterprise costs for any treatment. Because cow values were initially similar, differences in total cost were driven by feed cost.

Sales prices differed among treatments. Cows receiving FDL had a sales price ($/kg) 7.3% greater than those on pasture-based programs (P < 0.01). Sales prices were similar for cows that received supplement compared to NON (P = 0.43) and were higher for ENR compared to PRO (P = 0.06). Higher price for FDL cows is expected due to differences in final BCS and BW (Apple, 1999). Differences between ENR and PRO are more difficult to explain, as cows from these treatments were similar in final weight and BCS. Other factors which may have influenced price such as sale order, color, and age of cows (Troxel et al., 2002) were not recorded.

Selling price and weight differences among treatments resulted in differences in gross sale revenue. Due to higher price and greater BW, cows receiving FDL had greater sale value than cows on pasture-based treatments (P < 0.01). Supplemented cows tended to have greater value than NON (P = 0.14); the lack of strong difference is a result of the similarity in mean sale revenue between NON and PRO. Cows receiving ENR tended to have a greater sale value than those receiving PRO (P = 0.12), and the difference in revenue was driven entirely by the differences observed in sale price.

Net returns did not follow performance observations. Cows receiving FDL treatment generated the greatest net return (P < 0.01); however, cows receiving NON had numerically greater net returns than cows supplemented on pasture (P = 0.23). Despite differences in total costs, cows receiving PRO or ENR produced similar net returns (P = 0.98). Net returns to total costs were 12.3 %, 7.9 %, 4.1 %, or 4.3 % for FDL, NON, ENR and PRO, respectively. These increases in cow value are the result of increased BW, BCS changes, and/or market seasonality. Net returns to cow-calf operations in the Southwest average about 2% annually (Mathis and Sawyer, 2001). Cull cow sales represent about 13% of gross revenue, it is apparent that an increase in net value of the cull cow enterprise could increase operational net returns by as much as 100%. Following this logic, treatments in this study might be used to increase ranch profits by 80%, 51%, 27%, or 28% for FDL, NON, ENR and PRO, respectively.

Conclusions

There is high potential to add value to cull cows by capitalizing on market seasonality and market classification changes. Currently available nutritional models may adequately represent cull cow performance, and allow projections to be made for cull cow enterprises. Producers with capacity to take advantage of intensive production strategies may maximize profits. Extensive strategies that focus on minimum inputs and lower costs will sacrifice returns and potentially increase risk in the enterprise due to lower rates of production, however, even systems with relatively low rates of production have the potential to dramatically improve ranch profitability.

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Table 1. Forage mass (kg DM/ha) in pastures grazed by cull cows receiving different supplemental feeds.

Day	NON	ENR	PRO	SE[a]	P-value[b]
0	3493	3359	4160	512	0.55
14	2896	2308	3014	204	0.13
28	2394	2759	2806	206	0.39
42	1938	2004	2072	226	0.92
49	2532	1926	2238	221	0.27

[a]n = 3
[b]Probability of treatment effect

Table 2. Nutrient composition (% DM) of forages grazed by cull cows receiving different supplemental feeds.

Item	NON	ENR	PRO
Day 0			
CP	6.0	6.5	6.2
ADF	44.7	44.9	45.8
TDN	53	52.8	51.8
Day 49			
CP	4.8	5.5	5.4
ADF	46.6	45.6	48.2
TDN	50.9	52	49.1

Table 3. Production measures for cull cows fed in confinement or receiving different supplemental feeds on pasture.

Item	FDL[a]	NON	ENR	PRO	SE[b]	C1[c]	C2	C3
BW, kg								
Day 0	408.7	408.7	408.7	408.7	--	--	--	--
Day 49	507[x]	413	419	419	4	<0.01	0.19	0.98
ADG, kg /d								
Day 0-14	2.53	1.10	1.31	1.59	0.46	0.04	0.51	0.69
Day 15-28	1.87	0.28	0.49	-0.11	0.30	<0.01	0.80	0.22
Day 29-42	1.78	-0.97	-0.78	-0.30	0.32	<0.01	0.26	0.34
Day 43-49	1.95	-0.28	-0.71	-1.03	0.46	<0.01	0.28	0.65
Day 0-49	2.05	0.09	0.21	0.21	0.08	<0.01	0.19	0.98
Body Condition Score (1-9 scale)								
Day 0	3.67	3.67	3.67	3.67	--	--	--	--
Day 28	5.05	4.37	4.64	4.38	0.16	<0.01	0.48	0.32
Day 49	6.03	4.33	4.50	4.51	0.04	<0.01	<0.01	0.84

[a] FDL = cows fed feedlot ration in confinement, NON = cows on pasture, mineral supplement only; ENR = cows on pasture, fed 20% CP supplement at 1796 g/d; PRO = cows on pasture, fed 38% CP supplement at 925 g/d
[b]Most conservative estimate of error, n = 3
[c]C1 = FDL vs. pasture supplements, C2 = NON vs. PRO + ENR, C3 = ENR vs. PRO

Table 4. Economic measures for cull cows fed in confinement or receiving different supplemental feeds on pasture.

Item	FDL[a]	NON	ENR	PRO	SE[b]	C1[c]	C2	C3
Costs								
Cow initial price, $/45.45 kg	34.70	34.37	34.64	34.48	0.19	0.35	0.41	0.59
Cow initial cost, $/cow	313.59	310.31	313.69	311.76	2.07	0.47	0.33	0.55
Grazing/Yardage, $/cow	15.68	22.57	22.57	22.57	--	--	--	--
Feed Expenses, $/cow	97.22	1.86	29.09	14.84	1.89	<0.01	<0.01	<0.01
Total Costs, $/cow	426.49	334.74	365.35	349.17	2.58	<0.01	<0.01	<0.01
Revenues								
Sales price, $/45.45 kg	42.82	39.54	40.98	39.14	0.57	<0.01	0.43	0.06
Sales value, $/cow	478.97	361.05	380.27	364.31	6.20	<0.01	0.14	0.12
Net Return, $/cow	52.48	26.31	14.91	15.14	7.84	<0.01	0.23	0.99

[a]FDL = cows fed feedlot ration in confinement, NON = cows on pasture, mineral supplement only; ENR = cows on pasture, fed 20% CP supplement at 1796 g/d; PRO = cows on pasture, fed 38% CP supplement at 925 g/d
[b]Most conservative estimate of error, n = 3
[c]C1 = FDL vs. pasture supplements, C2 = NON vs. PRO + ENR, C3 = ENR vs. PRO

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IDAHO DAIRY BEEF QUALITY ASSURANCE SURVEY

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ABSTRACT: The major and most recognized product produced by dairies is milk. However, one product from dairies that is often overlooked, or disregarded, is beef. Non-fed beef is represented by cull cows and bulls that go directly from dairies to the packer without going through the feedlot phase of the beef industry. Reports estimate non-fed beef accounting for 20% of all beef produced in the United States. Approximately 75% of non-fed beef results from the slaughter of cows. Dairy cows represent one-third of domestic beef production and one-half of cow beef produced in the United States. In Idaho, there are 435,000 lactating dairy cows. According to 2004 Idaho DHIA records, 30 to 33% of these cows left the herd and were destined for beef markets. This results in 130,500 to 143,550 Idaho dairy cows being slaughtered and sold as beef and beef products. In 2004, a mail-in survey was conducted to establish a baseline level of awareness and knowledge related to dairy beef quality assurance issues. A 30-question survey was mailed to every (n = 759) registered Idaho dairy. Two-hundred and eighty (36.9%) dairies participated in the survey. Eighty-nine percent of respondents indicated they followed beef quality assurance recommendations for animal care. In cows, the neck region was used by 68% of dairies for i.m. injections and by 80% of dairies for s.c. injections. In calves, the values were 61% and 78%, respectively. Of the individuals administering injections at the dairies, 74% indicated they had been trained for the job. The primary language of those administering injections was English (73%) followed by Spanish (24%). Survey participants rated the importance of beef quality assurance in the dairy industry at 2.6 on a five-point scale (0 = low; 4 = high). Using the same scale, participants rated the impact of dairy animals on the beef industry at 2.5. Respondents cited publications, periodic bulletins/newsletters, and workshops as their three preferred formats for obtaining beef quality assurance information.

Key Words: Beef Quality Assurance, Dairy Cattle, Survey

Introduction

Today's consumer expects each food product they buy to be safe, wholesome, high quality, and consistent with regard to each of these areas. Consumers have various choices for protein sources in the marketplace today. To maintain consumer demand for beef, the beef industry has found it necessary to address and eliminate quality and consistency shortfalls.

The major and most recognized product produced by dairies is milk. One product from dairies that is often overlooked, or disregarded, is beef. Non-fed beef is represented by market animals (cull cows and bulls) that go directly from the producer (dairy or beef) to the packer without going through the feedlot phase of the beef industry. In 1994, it was estimated (Smith et al., 1994a, 1994b) that non-fed beef accounts for approximately 20% of all the beef produced in the United States. Approximately 75% of non-fed beef is derived from the slaughter of cows (cull dairy and beef cows). Dairy cows represent 33% of domestic beef production and 50% of the cow beef produced in the United States. In Idaho there are 435,000 lactating dairy cows. According to 2004 Idaho Dairy Herd Improvement Association (**DHIA**) records, 30 to 33% of these cows left the herd and were destined for beef markets. This results in 130,500 to 143,550 Idaho dairy cows being slaughtered and sold as beef and beef products. The objective of this study is to assess the awareness, knowledge, and understanding of beef quality assurance (**BQA**) principles and practices on Idaho dairies.

Materials and Methods

The survey was designed to gather information about BQA practices on Idaho dairies. A draft questionnaire was reviewed by university personnel and feedback was incorporated into the final version. The final survey consisted of 30 questions and was mailed to all dairy operations in the state of Idaho (n = 759). Questions were a mix of open- and closed-ended questions with multiple choices where applicable. An initial survey, cover letter, and postage-paid return envelope were mailed to dairies by first class postage on July 1, 2004. A postcard reminder was sent 30 days after the initial mailing to dairies that had not responded to the initial questionnaire. At 30 days subsequent to the reminder-postcard, a second survey form, cover letter, and postage-paid return envelope were mailed to dairies that had not responded to the initial questionnaire form. All data were entered into a spreadsheet and analyzed with SAS (SAS Inst., Inc., Cary, NC). Dairies were categorized based on current herd size. Herd size categories were small (less than 201 cows), medium (201 to 1000 cows), and large (more than 1000 cows). The analysis examined frequencies using PROC SURVEYMEANS of SAS and then tested for statistical difference by dairy size using PROC GENMOD of SAS. Differences among groups were tested using the least significant differences. Some participants chose not to answer all the questions, thus, the reported percentage was the percentage response to the individual question. Some questions allowed several answers and, thus, data might not add to 100%.

Results and Discussion

Of the 759 surveys that were mailed to Idaho's registered dairy producers, 280 were returned for a response rate of 36.9%. Responses came from 33 of Idaho's 44 counties. The vast majority of individuals completing and returning the survey were dairy owners (93.4%) followed by dairy managers (5.1%). Beef quality assurance definitions, practices, recommendations, etc. were not included on the survey or on correspondence materials. Dairy producers' knowledge and perceptions of BQA came from previous exposure to the issue. When asked if BQA recommendations were used during animal care activities, 89% of respondents indicated that BQA recommendations were followed on their dairies.

A number of questions were included on the survey to gain an understanding of dairies' injection procedures and practices. Tables 1 to 4 include the locations for routine i.m. and s.c. injections for cows and calves. Results in Table 1 show that the percentage of large dairies using the neck for i.m. injections in cows is greater than the percentage of medium or small dairies. Overall, the neck region was used by 68% of dairies for i.m. injections and by 80% of dairies for s.c. injections in cows. In calves, the values were 61% and 78%, respectively. The use of the neck region by Idaho dairies for i.m. and s.c. injections is greater than the percentages reported for Pennsylvania dairies (Tozer et al., 2004)

The survey asked if those individuals giving injections were trained for the job. Results are shown in Table 5. The percentage of trained workers on large dairies is greater than the percentage of trained workers on small dairies. This may be due in part to the greater resources of large dairies and the ability of large dairies to send some workers to trainings while others remain at work. Training methods varied. Of the respondents that identified a training method, 19.8% received training from a veterinarian, 16.8% received training from the owner of the dairy, 9.9% received on-the-job training (experience), 4.5% received training in a school or BQA event and 3.5% received training from other sources. Forty-five percent of respondents cited no specific method of training.

To ascertain dairy producers' views on their role and responsibilities to the beef industry, the survey included questions focused on the importance of BQA on dairies and the impact dairy animals have on the beef industry. Survey respondents rated each of these items as being moderately important. Using a five-point scale (0 = low; 4 = high), dairy producers rated the importance of BQA on dairies at 2.6. Similarly, the impact that dairy animals have on the beef industry was rated at 2.5 by dairy producers. While these numbers are encouraging, there still is an opportunity to share information with dairy producers that outlines BQA practices and procedures, shows how BQA practices can be implemented on dairies, and shows how the implementation of BQA practices can lead to higher quality beef and beef products.

One of the overall goals of the project is to use survey results to develop and deliver educational programs aimed at the understanding and adoption of BQA practices, procedures, and recommendations by dairies. In the current technological age, many would assume that dairy producers would prefer educational materials to be delivered via digital formats (web sites, CD-ROM, etc.). However, survey respondents cited publications, periodic bulletins/newsletters, and workshops as their three preferred formats for obtaining BQA information. Websites and CD-ROM ranked 5th and 7th, respectively.

Language is often a barrier when considering educational materials for dairy workers. Survey participants indicated that the primary language of those administering injections was English (73%) followed by Spanish (24%). A snapshot of many dairies would show that the primary language for a majority of workers would be Spanish. Since BQA includes more than giving injections, and since numerous tasks on a dairy are performed by individuals that speak Spanish, individuals providing educational opportunities for dairy workers should be prepared to bridge the language gap.

Implications

Even though there is room for improvement, Idaho dairies seem to have implemented BQA procedures and practices. Efforts should be directed toward increasing the dairies' awareness of the value of BQA programs and the value of producing consistent, high quality beef and beef products. Beef quality assurance training materials should be prepared in English and Spanish and delivered via preferred formats.

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Table 1. Location of routine intramuscular injections in cows

Dairy Size[a]	Neck (%)	Shoulder (%)	Upper rear leg (%)	Side or ribs (%)	Lower rear leg (%)	Tail-head (%)
Small (n = 139)	64.0[c]	10.0	31.7	3.6	7.9	18.7
Medium (n = 74)	64.9[c]	14.9	29.7	2.7	14.9	13.5
Large (n = 54)	87.0[b]	11.1	18.5	0	13.0	22.2

[a]Small = < 200 cows; Medium = 201 to 1,000 cows; Large = > 1,000 cows.
[bc]Row values with different superscripts differ (P < 0.05).

Table 2. Location of routine subcutaneous injections in cows

Dairy Size[a]	Neck (%)	Shoulder (%)	Upper rear leg (%)	Side or ribs (%)	Lower rear leg (%)	Tail-head (%)
Small (n = 139)	75.5	12.9	6.5	5.8	2.9	12.2
Medium (n = 74)	79.7	13.5	5.4	10.8	2.7	6.8
Large (n = 54)	87.0	7.4	5.6	0	1.9	20.4

[a]Small = < 200 cows; Medium = 201 to 1,000 cows; Large = > 1,000 cows.

Table 3. Location of routine intramuscular injections in calves

Dairy Size[a]	Neck (%)	Shoulder (%)	Upper rear leg (%)	Side or ribs (%)	Lower rear leg (%)	Tail-head (%)
Small (n = 139)	59.0	9.4	29.5	0.7	7.2	10.8
Medium (n = 74)	63.5	9.5	25.7	0	8.1	6.8
Large (n = 54)	61.1	5.6	20.4	0	11.1	7.4

[a]Small = < 200 cows; Medium = 201 to 1,000 cows; Large = > 1,000 cows.

Table 4. Location of routine subcutaneous injections in calves

Dairy Size[a]	Neck (%)	Shoulder (%)	Upper rear leg (%)	Side or ribs (%)	Lower rear leg (%)	Tail-head (%)
Small (n = 139)	77.7	13.7	5.0	3.6	0.7	5.8
Medium (n = 74)	81.1	12.2	5.4	9.4	1.4	1.4
Large (n = 54)	74.1	5.6	9.3	0	3.7	3.7

[a]Small = < 200 cows; Medium = 201 to 1,000 cows; Large = > 1,000 cows.

Table 5. Percentage of dairy workers trained to give injections

Dairy Size[a]	Workers Trained (%)
Small (n = 139)	75.8[c]
Medium (n = 74)	85.7[bc]
Large (n = 54)	92.3[b]

[a]Small = < 200 cows; Medium = 201 to 1,000 cows; Large = > 1,000 cows.
[bc]Row values with different superscripts differ (P < 0.05).

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A SURVEY OF COW-CALF PRODUCERS IN OREGON AND NEVADA – PRODUCTION PRACTICES

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ABSTRACT: In 2004, we sent a cow-calf production survey to 1,400 individuals in NV (NV Cattleman's Association members and associate members; University of NV – Reno, Cooperative Extension Service livestock mailing list) and 2,090 individuals in OR (OR Cattleman's Association mailing list). Objectives were to better understand current cow-calf management practices and enhance Extension beef programs in the Intermountain West. A total of 462 surveys were returned (NV=91; OR=371). Herd sizes varied widely, with 25, 36, 24, and 13% of respondents listing 0-50, 51-200, 201-600, and greater than 600 hd, respectively (2% didn't provide a herd size). Fifty-two percent of the survey respondents always cull open cows. In addition, of the 48% (222 respondents) that claimed to not cull all open cows, the top 3 reasons for keeping an open cow were young cows (51%), if the cow had been a good producer in the past (25%), and if the cow lost her calf through no fault of her own (23%). The most frequent culling rate for cows was 10% or less (43%), while 54% of respondents claimed annual cow death loss to be less than 0.5%. An overwhelming majority of producers (74%) raised their own replacement heifers, with 49% rating their heifer replacement program as excellent and 42% claiming to need improvement. The most common annual cow cost was $251-300 (22%), with both $201-250 and $301-350 being listed by 18% of producers. The annual return on investment was listed as positive, breakeven, and negative by 64, 16, and 5% of respondents, respectively. Only 25% of producers conduct complete breeding soundness exams on their bulls each year and 40% don't test their bulls at all. Nevertheless, the average pregnancy rate was listed as greater than 91% by 70% of the respondents. This survey provides information on current cow-calf production practices in NV and OR and will assist in developing cow-calf Extension programs.

Key words: Intermountain, Cow, Management, Survey, West

Introduction

The diversity of the climate and topography in Nevada and Oregon can be a challenge to cow-calf producers. Consequently, an understanding of current production practices by cow-calf producers is necessary to develop effective Extension programs that help improve the economic efficiency of beef production in the Intermountain West.

This survey was developed to obtain information on current cow-calf management practices. This will allow Extension personnel to better understand beef production in the Intermountain West and develop Extension beef programs that address current needs and/or deficiencies in production practices.

Materials and Methods

We developed a survey and sent it to cow-calf producers throughout Nevada and Oregon. The survey posed 22 questions related to cow management, female replacement programs, bull management, and annual cow-herd economics. In Nevada, the survey was mailed to 1,400 individuals who were members of the Nevada Cattleman's Association and/or the University of Nevada-Reno Cooperative Extension Service Livestock mailing list. In Oregon, 2,090 surveys were mailed to the members of the Oregon Cattleman's Association mailing list. The Nevada and Oregon mailings included a postage-paid return form that allowed the survey respondents to mail the completed surveys back at no cost and in complete anonymity.

As of March 1, 2005, the cutoff date for this report, 462 surveys had been returned (Nevada = 91; Oregon = 371). Information from each response was entered into a Microsoft® Access 2002 (Microsoft Corporation, Redmond, Washington) database to facilitate data summary and analysis.

Survey Results

General Respondent Information

Surveys for both states listed western, central and eastern as location choices. If no response was given we placed the survey in a "general" category. In Nevada, 45, 15, 39, and 1% of respondents listed western, central, eastern and general as the location of their operation (data not shown), while in Oregon, 24, 16, 57, and 3% of respondents listed western, central, eastern and general as the location of their operation (data not shown).

The average herd size varied widely, with the most common herd being 50 head or less, which was reported by 25% of respondents (Table 1). The least common herd size, greater than 1000 head, was noted by 6% of respondents. Approximately 2% of the returned surveys did not list a herd size.

We listed seven categories for type of beef operation, and respondents were asked to check all that applied. Many respondents listed multiple operation types, indicating the diverse nature of beef operations in the Intermountain West. Categories that we provided on the survey, with the proportion of respondents listing each in

parenthesis, were: registered seed stock producer (16%), commercial producer (66%), outside year round operator (13%), irrigated/improved pasture operator (57%), common allotment range operation (16%), desert range (low input-tough) operator (11%), and desert range (moderate input–more balanced) operator (27%).

The two most frequently listed calf weaning weights were over 272 kg and 250-272 kg, which were each selected by 27% of cow-calf producers. The next two most common weaning weights were 227-250 kg and 204-227 kg by 19 and 16% of producers, respectively. Six percent of respondents listed an average weaning weight of 182-204 kg while 1% listed less than 182 kg as their average weaning weight. We took the mid-weight from each weaning weight range provided on the survey, multiplied that number by the number of respondents in that respective category, and then averaged the estimated weights per category to get an overall average weaning weight for survey respondents. That weight was 241 kg.

Culling Practices

The most common cow culling rate was 0-10% which was noted 43% of the time followed by 10-15% which was listed by 28% of respondents. The remaining three culling rates, 16-20%, 21-25%, and > 25%, were selected by 8, 1, and 0.2% of producers, respectively. In addition, approximately 1% of respondents stated that their annual cow culling rate depended on ranch finances and drought conditions while 10% stated that multiple strategies were used in determining culling rate. Two percent of returned surveys did not list a selection for cow culling rate.

The proportion of respondents that always cull open cows was 52% (240 of 462). The reasons given by the remaining 48% of respondents for not culling open cows are listed in Table 2. It should be pointed out that many of the survey respondents listed multiple reasons for not culling open cows; therefore, the percentages listed in Table 2 were calculated as the number of responses listed for each category divided by the 222 respondents that claimed to not always cull open cows. The majority of responses (51%) listed a young cow as the primary reason to not cull. The next two most frequently stated reasons for not culling an open cow were past performance (25%) and if it was "not the cows fault" that she was open (23%). The remaining reasons for not culling an open cow were listed less than 10% of the time.

Cow Replacement Programs

The response for cow death loss was 54, 27, 13, and 4% of survey respondents stating that annual death loss was 0-0.5, 0.5-1.0, 1.0-1.5, and > 1.5%, respectively. Three percent of respondents didn't respond with an estimate.

Approximately 42% of cow-calf producers stated that they replace a constant percentage of their cow herd each year. This was followed by 24% stating that they replace all culls and 12% retaining more cows when cow prices are low. Other considerations listed for determining the annual cow replacement rate included selling more cows when cash was short (5%), keeping more cows when cow prices are high (4%), and replacing a constant dollar value of cows each year (0.4%). Also, 9% of the respondents chose more than one of the reasons listed above and 4% didn't provide any response.

The most common replacement strategy listed by cow-calf producers was raising their own replacement heifers (74% of respondents). The next most frequently listed replacement strategy was purchasing pregnant heifers at 5%. Purchasing pregnant cows was listed by 2% of respondents and purchasing both pregnant cows and heifers was listed by 1% of producers. The remaining responses involved some combination of the variables above (data not shown).

Heifer replacement programs were rated as excellent by 49% of respondents while 42% felt that they could use improvement. Only 1% of the survey responses listed their heifer development program as poor. Seven percent of cow-calf producers didn't provide a response in relation to their heifer development program.

Annual Cow Cost/Economics

The reported annual cow cost for survey respondents is listed in Table 3. The most common response (22%) was $251-$300 per year. However, there was a wide range in reported cow cost with 70% of all cow-calf producers listing costs between $200 and $400. Almost 14% of producers either didn't know or didn't provide an estimate of their annual cow cost.

The proportion of respondents that provided an estimated annual return on investment (**ROI**) was 25, 24, 15, 16, and 5% for a ROI of 0-5%, 5-10%, > 10%, breakeven, and negative, respectively. Similar to annual cow cost, approximately 15% of cow-calf producers didn't know or didn't provide an estimate of their annual ROI.

Bull Management

Most survey respondents (32%) purchase their bulls via private treaty and only slightly fewer (27%) purchase bulls from a bull sale. Similarly, 16% listed both private treaty and bull sales as sources of seed stock. Therefore, 75% of the survey respondents purchase their bull battery from either a bull sale or by private treaty. Only 5% of producers raise their own bulls and 1% purchase bulls from a sale barn. The percentage of surveys that did not provide a type of bull purchase was 5%. The remaining responses involved some combination of the variables above (data not shown).

Yearling bulls were preferred for purchase by 34% of cow-calf producers followed by 2-yr olds at 18%. Also, approximately 14% of respondents purchase both yearlings and 2-yr olds. Surprisingly, 34% of producers chose not to respond. Table 4 provides a breakdown of the price that survey respondents pay for bulls.

The responses to our questions regarding annual testing of bulls are provided in Table 5. Briefly, 40% of respondents don't do any form of testing while 25% have complete breeding soundness exams performed. Only 8% of cow-calf producers test for trichomoniasis and 12% have semen tests conducted.

The average age that most cow-calf producers dispose of their bulls was 5 yr (43% of respondents). The next most frequent disposal age was 6 yr or older, which was selected by 26% of producers. This was followed by 4 (16%) and 3 (4%) yr old bulls. Four percent of respondents didn't list a bull disposal age. The remaining respondents selected multiple ages (data not shown).

The most frequently listed cow-to-bull ratio was 20-25:1, which was noted by 46% of cow-calf producers. The next most common ratios were 15-20:1 (28%) and greater than 25:1 (14%). Approximately 12% of respondents didn't provide a cow:bull ratio.

Cow Reproduction

Spring calving was listed by 68% of producers and fall calving was selected by 6% of respondents. Interestingly, 21% stated that they had both spring and fall calving cows. About 5% of producers did not respond.

Length of breeding season information is provided in Table 6. Approximately 75% of the cow-calf producers use a breeding period of 90 d or less. Consequently, 23% of respondents listed a breeding season greater than 90 d. Only 2% of the surveys didn't offer a length of breeding season.

Pregnancy rate is one of the most important performance variables for a cow herd. Seventy percent of survey respondents said their herd's pregnancy rate was greater than 91%, with 19% saying their rate was 86-90%. The remaining responses were 0.2% for < 70% pregnancy rate, 0.4% for 71-80%, 4% for 81-85% and 6% declined to provide a pregnancy rate.

Table 1. Average herd size of survey respondents[a]

Herd Size, hd	Percentage
0-50	25.1
51-100	19.5
101-200	16.2
201-300	10.0
301-400	6.3
401-600	8.0
601-1000	6.5
>1000	6.1
No selection on survey	2.4

[a] 462 total respondents

Table 2. Reasons for keeping open cows[a]

Reason	Percentage
Young cow	51.4
Past performance	25.2
Climate/drought	5.9
Sentimental	4.1
Not cows fault	23.4
Rebreed and sell	3.2
Economics	6.8
Cow will eventually rebreed	5.4
Foothill abortion	2.7
Genetic base	8.6
Use in Embryo transfer program	2.3

[a] 222 respondents; multiple selections by most respondents

Table 3. Annual cow cost of survey respondents[a]

Cow cost, $	Percentage
100-200	10.8
201-250	17.7
251-300	22.3
301-350	18.0
351-400	12.3
> 400	5.2
No selection on survey	13.6

[a] 462 respondents

Table 4. Price paid for bulls by survey respondents[a]

Purchase price, $	Percentage
≤ 1,000	4.5
1,001-1,300	9.7
1,301-1,600	14.7
1,601-1,900	8.2
1,901-2,200	13.6
2,201-2,500	14.7
2,501-3,000	13.6
> 3,000	14.5
Multiple prices chosen	0.6
No selection on survey	5.6

[a] 462 total respondents

Table 5. Bull testing practices of survey respondents[a]

Type of test	Percentage
Trichomoniasis	8.0
Semen	12.1
Trichomoniasis + semen	9.3
Breeding soundness exam	25.1
No testing	39.8
No selection on survey	5.6

[a] 462 total respondents

Table 6. Length of breeding season listed by survey respondents[a]

Breeding season, d	Percentage
≤ 45	5.2
45-60	29.2
60-90	40.9
90-120	14.3
>120	8.2
No selection on survey	2.2

[a] 462 total respondents

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IMPACT OF PRECONDITIONING DURATION ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS AND PROFITABILITY OF NEW MEXICO RANCH TO RAIL STEERS

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ABSTRACT: The duration from weaning to feedlot entry has been reported to be an important factor in feedlot performance of cattle. The objective of this study was to evaluate preconditioning duration on feedlot performance, carcass characteristics and profitability of 834 steers in the New Mexico Ranch to Rail Program from 2001 to 2004. Steers were classified into one of four categories, based on the number of days preconditioned prior to entry into the feedlot. Preconditioning categories were 0 to 20 d, 21 to 40 d, 41 to 60 d, and 61 d or more. Initial calf market, carcass value grid, and unit feed values were standardized across years to remove market variation. Ultrasonography was employed to assign a marketing date for estimated maximum profit for each steer. Net income per head increased as preconditioning duration increased, with minimal improvement above 41 to 60 d (quadratic, $P < 0.05$). Net income was -$41.66, -$20.02, $2.23 and $4.00 per head, for steers preconditioned for 0 to 20 d, 21 to 40 d, 41 to 60 d, and 61 d or more, respectively. Steers preconditioned 41 to 60 d had the highest ADG (quadratic, $P < 0.05$) of 1.48 kg/d, followed by steers preconditioned 21 to 40 d (1.44 kg/d ADG), 61 d or more (1.35 kg/d ADG), and 0 to 22 d (1.34 kg/d ADG). Steers preconditioned 41 to 60 d spent fewer days on feed (quadratic, $P < 0.05$) and had the lowest total cost of gain (quadratic, $P < 0.05$). Marbling score increased (linear, $P < 0.05$) as preconditioning duration increased, while fat thickness and calculated yield grade peaked (quadratic, $P < 0.05$) at 41 to 60 d. This data indicates that among steers entered in the New Mexico Ranch to Rail program optimum preconditioning duration was achieved when steers were preconditioned for 41 to 60 d.

Key Words: Preconditioning, Feedlot, Steers

Introduction

The New Mexico Ranch to Rail program was established in 2000 to educate beef producers about factors that influence calf value beyond weaning, and to provide performance, carcass and financial data from their cattle. The duration from weaning to feedlot entry has historically varied greatly among steers in the program and is an important factor in preconditioning calves for the feedlot. During preconditioning, calves are introduced to new feedstuffs and become acclimated to new environments. The cost effectiveness and subsequent performance impact of preconditioning has been identified as an arena needing further research (Cole, 1985; Cravey, 1996). A commonly recommended preconditioning duration is 45 d (McNeil, 2001). However, the length of preconditioning is often determined by economic and management factors including the costs associated with feeding calves and the availability of feed resources. The objective of this study was to evaluate the impact preconditioning duration had on feedlot performance, carcass characteristics and profitability of 834 steers enrolled in the New Mexico Ranch to Rail Program from 2001 to 2004.

Materials and Methods

Performance, carcass and financial data from 834 steers entered in the New Mexico Ranch to Rail program from 2001 to 2004 were used to evaluate the impact of preconditioning duration on feedlot performance and profit. The preconditioning period duration (number of days from weaning until entry into the feedlot) was determined from standardized background information surveys completed by the owner of each animal. Management of calves between weaning and feedlot entry was not standardized across years or sources of origin. Steers were classified into one of four categories, based on preconditioning duration prior to entry into the feedlot. Preconditioning duration categories were 0 to 20 d, 21 to 40 d, 41 to 60 d, and 61 d or more. All steers in the 0 to 20 d through the 41 to 60 d categories were spring-born calf-feds. The majority of steers in the 61 d or more category were yearlings ranging from 66 to 396 d between weaning and feedlot entry.

Steers were received on a single day in mid-November each year. Upon arrival, all steers were weighed, tagged, processed and assigned frame and muscle scores. Ultrasonography was utilized to assign steers to marketing groups based on estimated marketing date for predicted maximum profit (Brethour, 2000). Hot carcass weight, longissimus muscle area, fat thickness, yield grade and USDA quality grade were collected upon slaughter.

Initial calf value market, carcass value grid, and unit feed values were standardized across years to remove market variation. Body weight, frame score, and muscle score on receiving day were used to assign an initial value to each steer. Unit feed values were standardized to $173.04 per metric ton of dry feed ($157.12/ton), which was the average unit feed cost of steers in the program from 2001 to 2004. A standardized carcass value grid was constructed using weekly USDA premium and discount reports from March to August across all four years of the program (Table 1).

The effect of preconditioning duration on net income, total medicine cost, total feed cost, total cost of gain, average daily gain, days on feed, number of treatments, hot carcass weight, fat thickness, longissimus muscle area, yield grade, and carcass value was evaluated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), using individual steer as the experimental unit.

Table 1. Standardized premiums and discounts for quality grade, yield grade and carcass weight for steers in the New Mexico Ranch to Rail program from 2000 through 2004[a].

Item	Premium/Discount ($/45.4 kg carcass)
Base	$120.38
Quality Grade	
Prime	$6.30
2/3 Choice	$2.05
Choice	$0.00
Select	-$10.42
Standard	-$18.08
Dark Cutter	-$27.56
Hardbones	-$24.37
Yield Grade	
1	$2.95
2	$1.51
3	$0.00
4	-$12.58
5	-$18.37
Carcass Weight	
<500	-$23.40
500-550	-$15.65
550-600	-$3.47
600-900	$0.00
900-950	-$7.11
950-1000	-$17.30

Results and Discussion

Initial weight was highest among steers preconditioned 21 to 40 d and 61 d or more and lower for steers preconditioned 0 to 20 d, and 41 to 60 d (cubic, $P < 0.01$, Table 2). The cubic nature of this relationship is difficult to explain and likely results from differences in origin and other management factors not measured. Peak ADG (quadratic, $P < 0.05$) and fewest days on feed were achieved among steers preconditioned 41 to 60 d. The relationship between ADG and number of days on feed were expected because steers that gain weight faster are likely to become market ready earlier. The increase in ADG to the 41 to 60 d category is possibly due, in part, to an improvement in gain during the initial months of the feeding period. In a review of studies by Cole (1985) where calves were preconditioned on pasture for 21 d or more and compared to non-weaned controls from the same farm-of-origin, ADG during the first 30 to 45 d in the feedlot was greater among preconditioned calves. However, by 100 d in the feedlot control and preconditioned calves had similar ADG. The decline in rate of gain and increase in number of days on feed beyond the 41 to 60 d category is in contrast to the work of Schoonmaker et al. (2002) who found that older yearling-fed steers that were on pasture for 183 d between weaning and feedlot entry gained 0.2 kg/d more and were on feed for 27 d fewer than their calf-fed counterparts that were placed in the feedlot 14 d postweaning.

Steers in the 41 to 60 d category had the peak number of treatments per hd for sickness (quadratic, $P < 0.01$) and subsequent medicine cost/hd (quadratic, $P < 0.01$) followed by a substantial decline from the highest to the lowest level at 61 d or more of preconditioning. The initial rise in treatments/hd and medicine cost up to 41 to 60 d is in contrast to earlier work (Cole, 1985; Lofgreen, 1988; Cravey, 1996) that reported lower treatment rates and medicine costs/hd for preconditioned calves compared to calves that entered the feedlot immediately after weaning. Moreover, the relationship between medicine cost and both ADG and days on feed is difficult to explain and contrasts previous reports comparing performance of healthy steers to sick steers (Mathis, 2002).

Exhibiting the same pattern (cubic, $P < 0.01$) as initial weight, hot carcass weight was higher among steers preconditioned 21 to 40 d and 61 d or more and lower among steers in the 0 to 20 d and 41 to 60 d classifications. Differences in rank of hot carcass weight and initial weight partially result from the quadratic ($P < 0.01$) effect observed for backfat, with the highest backfat among steers preconditioned 41 to 60 d, and lowest for steers preconditioned 0 to 20 d. Longissimus muscle area was similar among all preconditioning classifications. The quadratic nature of backfat and lack of difference in longissimus muscle area is reflected in the quadratic ($P < 0.01$) response of calculated yield grade. Peak calculated yield grade was achieved among steers in the 41 to 60 d preconditioning classification. The most notable incremental difference in backfat and calculated yield grade were between the 0 to 20 d and 21 to 40 d classifications. Marbling increased linearly ($P < 0.01$) as preconditioning duration increased. The magnitude of the numerical increase in marbling score between steers preconditioned for 21 to 40 d and 41 to 60 d compared to steers in the 61 d or more category, is likely related to both the morbidity advantage and enhanced marbling potential among the older cattle (Wertz et al., 2002) in the 61 d or more category. There was no difference in carcass unit value ($P = 0.21$).

Total feed cost/hd was cubic ($P < 0.05$), increasing from the 0 to 20 to the 21 to 40 d classification, then declining to 41 to 60 d and dramatically rising to the highest total feed cost among steers preconditioned for 61 d or more. Medicine cost/hd and total feed cost/hd followed divergent patterns, the result of which is the quadratic pattern ($P = 0.05$) in total cost of gain. Medicine cost is an important factor in total cost of gain, and ultimately profitability; however, in this case medicine cost was not reflected by total cost of gain. Net income/hd increased as preconditioning duration increased, with minimal incremental change among steers preconditioned longer than 41 to 60 d (quadratic, $P < 0.01$).

Implications

This data indicates that preconditioning durations in excess of 41 days yield increased profitability. When financial risk associated with time is considered, optimum preconditioning duration was achieved when steers were preconditioned 41 to 60 days.

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Table 2: Impact of preconditioning duration on feedlot performance, carcass characteristics and profitability of New Mexico Ranch to Rail steers.

	Preconditioning Duration[a]					Contrasts[b]		
Item	0 to 20 d	21 to 40 d	41 to 60 d	61 d +	SEM[c]	L	Q	C
Number of Steers	137	260	286	131				
Performance								
In weight, kg	264	277	261	276	3.94	0.09	0.60	<0.01
Days on feed	196	191	187	199	3.57	0.08	0.01	0.84
ADG, kg/d	1.34	1.44	1.48	1.35	0.039	0.24	<0.01	0.58
Number of treatments	0.19	0.28	0.32	0.20	0.050	0.39	0.01	0.85
Number of railers[d]	2	3	2	1	-	-	-	-
Number of deads	1	3	2	4	-	-	-	-
Carcass								
HCW, kg[e]	340	355	345	354	3.93	0.06	0.25	<0.01
Marbling score[f]	467	480	480	495	6.49	<0.01	0.19	0.32
LM area, cm^2	88.4	89.0	87.7	88.4	0.97	0.95	0.52	0.12
Backfat, cm	1.09	1.17	1.22	1.17	0.038	0.81	0.01	0.79
Calculated yield grade	2.46	2.62	2.66	2.63	0.061	0.30	<0.01	0.49
Financial								
Medicine cost, $	6.06	8.27	9.05	5.43	1.49	0.23	0.05	0.76
Cost of gain, $/45.4 kg	66.00	56.00	57.00	60.00	4.20	0.96	0.05	0.27
Total feed cost, $	302.41	314.32	294.06	336.33	7.11	<0.01	0.17	<0.01
Carcass value, $/45.4 kg	111.77	113.21	114.91	114.63	1.95	0.44	0.21	0.81
Net income $	-41.66	-20.02	2.23	4.00	14.84	0.09	0.03	0.75

[a]Steers were classified into one of four categories, based on the number of days preconditioned prior to entry into the feedlot. Preconditioning categories were 0 to 20 d, 21 to 40 d, 41 to 60 d, and 61 d or more.
[b]L = Linear, Q = Quadratic, C = Cubic.
[c]Standard error of the mean (n = 131).
[d]Railer = Steer sold prior to finishing due to illness or injury.
[e]Hot carcass weight.
[f]Marbling score: Small 00 = 500.

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TRACKING CATTLE FROM THE RANCH TO THE PACKER: THE MONTANA BEEF NETWORK AND NATIONAL ID PILOT PROJECT

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ABSTRACT: The Montana Beef Network (MBN) was initiated in 1999 as a cooperative effort with the MT Stockgrowers Association with three objectives: 1) Beef Quality Assurance education, 2) self-certification that calves followed a defined health protocol prior to sale and 3) traceability of calves from the ranch to the packing plant with the return of carcass data to the producer. A fourth objective was added during 2004 in cooperation with the MT Department of Livestock to develop ranch premise registration methodology and evaluation of new protocols for traceability of calves using hot iron brands, radio frequency identification tags (RFID), retinal scanning and plastic ear tags for disease traceback. Currently ~1,200 producers are BQA certified and more than 90,000 calves have been tagged with RFID tags or plastic ear tags since 1999. The rate of return on carcass data from the packing plant to the rancher has ranged from a low of 27% in 2002 to a high of 72% in 2004. Incomplete data return continues to be due to individuals who do not want to share feedlot data and calves being sorted into groups which are not RFID. Individual carcass data were collected on approximately 17,000 animals. Average carcass weight was 783 lb, a REA of 12.8 sq in, a back fat of .51 in, calculated YG of 3.1 and a REA/carcass weight ratio of 1.64. Survey data suggest that calves which were BQA-certified received an average $12/head additional compared to non-certified calves. Approximately 55% of producers responded in 2003 that some form of national animal ID should to be implemented with no agreement as to whether it should be voluntary (31%) or mandatory (30%). With regard to a national disease traceback ID program, the questions that require answers include: 1) where did this animal originate from (state, ranch); 2) who has owned it (cow calf, stocker, feedlot, packer), 3) what other animals was it exposed to (eg. cograzing on public lands, mixing at auction markets, mixing prior to transportation to other states), and 4) can traceback be accomplished within 48 h of a disease confirmation? The ID pilot project is designed to address these questions.
Key Words: Traceability, Cattle, Animal identification

Introduction

What is the national need for this research and extension effort? The beef industry is becoming more consumer-focused and specific quality and consistency targets are being established in all segments of the industry. To satisfy customer concerns over food safety and quality and return additional revenue to cattle producers, a systems network must be in place to ensure that a quality and consistent product is being produced. Central to this networking approach is the exchange of information from the producer to the end user (feedlot, packing plant) and from the end user to the producer.

Food safety is an increasingly important issue in the food industry. It is a top five issue of concern for consumers eating beef. A systems approach utilizing individual animal identification and source and process verification will provide the information necessary to track the safety and quality of beef produced in MT and other states. Although specific requirements and implications from the cow-calf producer to the retailer are presently unknown, the passage of the country of origin labeling (COOL) legislation may require some form of source verification and information transfer. Even though the COOL legislation, scheduled to be implemented in Oct. 2006, specifically prevents the Secretary of Agriculture from requiring an animal identification program, legislation has been introduced into the US Congress by Congressmen Peterson (D-MN) and Osborne (R-NE) to implement animal identification and tracking within 90 d of passage of these bills.

The Montana Beef Network

The Montana Beef Network has three primary objectives; 1) educational programs aimed at meeting beef quality assurance standards, production and marketing goals and providing additional educational programs through interactive video conferencing, 2) voluntary certification of feeder calves that have met defined management protocols and 3) information feedback from the feedlot and packing plant to the cow-calf producer showing if the feeder calves met industry requirements for quality, consistency, safety and red meat yield.

Funding was used to develop and publish training manuals and present over 200 Beef Quality Assurance educational programs in Montana so producers could certify that calves were vaccinated using a standard health management protocol. The training has been presented to over 2000 producers in the state and more than 1100 are certified through the Montana Beef Quality Assurance program. Beef Quality Assurance education is provided to MT producers through an interactive web site (mbn.montana.edu), a textbook, or a CD. The course can be taught either in a self-study format or by county agents or beef specialists. Additional projects completed under Objective 1 include:

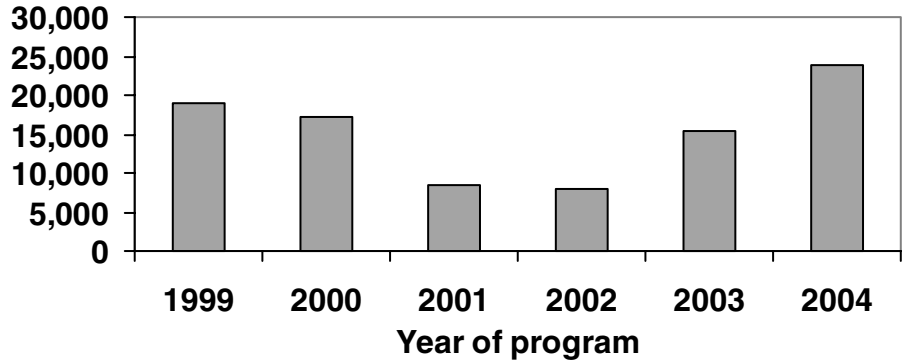
1) initiation of a state-wide audit of ranchers to determine value-added practices related to breeding, health management, nutrition and marketing

2) one- to two-day short courses were held each year, in which issues pertinent to the beef industry were presented. This program is called Montana Beef University.

3) fourteen interactive television short courses aimed at carcass evaluation, genetic management, opportunities for backgrounding calves and marketing options and beef safety and drought management.

The cattle certification and tracking component of the project (Objectives 2 and 3) has used electronic identification (RFID) tags for enrolling producers and to secure the feedlot and carcass data on the calves certified in the program. Approximately 18,000 calves were certified during the first year, 17,000 the second year, 8,000 the third year, and 24,000 the sixth year (91,732 animals total, Figure 1). The difficulty in convincing all the various segments of the beef industry to cooperate has resulted in a less than desirable rate of return of carcass data. This difficulty has caused some producers to drop out of the program until data recovery is improved. Changes were made to the program in 2002 to improve rate of data return through the hiring of an individual to work closely with the feedlots to ensure the collection of carcass data. The data captured throughout the process is synthesized, presented, and explained to the producer to provide information on how they might modify their breeding and (or) management practices to improve the quality of their product.

Figure 1. Number of cattle enrolled in the Montana Beef Network from 1999-2004



During the winter of 2005, a cooperative project with Certified Angus Beef and Colorado State University was implemented to begin to summarize carcass data for the years of 1999 through 2003. The following table (Table 1) summarizes part of these data.

Table 1. Summary of carcass data collected from 1999-2003 for Montana calves enrolled in the Montana Beef Network (Rolfe, et al., 2005, unpublished)

Year	No. of observations for each yr	Yield Grades	Avg. distribution of YG (No. observations)	Quality Grades	Avg. distribution of QG (No. observations)	Selected carcass traits	Mean (Range)
1999	2841	1	4.33 (460)	Avg. Ch or better	35.13 (5998)	Carcass wt, lb	784 (344-1160)
2000	4990	2	33.33 (3543)	Choice -	31.45 (5369)	REA, sq. in	12.82 (7.4-18.4)
2001	1806	3	52.24 (5554)	Select	31.76 (5422)	Backfat, in	.51 (.10-1.52)
2002	2479	4	9.69 (1030)	Standard	1.02 (175)	YG	3.1 (0-6.81)
2003	5190	5	.41 (44)			REA/carcass wt	1.64 (.91-3.34)

Data on 17,306 carcasses were collected. The average carcass weight for calves was 355 kg (784 lb) with a range of 156 to 526 kg (344-1160 lb) whereas the average YG was 3.1 with a range of 0 to 6.81. While the REA was very acceptable (12.8 sq. in), the ratio of REA to carcass weight (1.64) appears to suggest the need for slightly more muscling in the calves.

Duffey et al. (2004, unpublished) surveyed ranchers in MT to determine how the carcass data was utilized. Table 2 summarizes how the information was used as well as selected rancher responses. The responses suggest that the data were used for information only (37%) or that producers were using the information to change bull genetics (37%). It also appears that ranchers used the information to make culling selections (21%).

Table 2. Summary of how BQA and non-BQA certified producers utilize carcass data results (Duffey et al., 2004, unpublished)

How do you use carcass information?	BQA certified producers	Non-BQA certified producers
Information only	36	37
To cull cows	22	19
To change bull genetics	37	38
Other, please specify[a]	5	5

[a] to market calves, not consistent enough to make management decisions, year to year comparison, ID the marbling cows, feedlot management for grid marketing, we need help.

From an extension programming focus, the survey by Duffey et al. (2004, unpublished) suggested that the three subject areas that need attention include marketing, nutrition and health and consumer issues related to safety, source verification and nutritional value.

Montana Pilot Project for National Animal Identification

Leann Saunders from IMI Global, Inc. suggested that the drivers for a national identification and traceability program include 1) protecting our nation's livestock herds -- preparedness for disease and bioterrorism, 2) promoting consumer confidence -- to assure export-market access and to deliver on brand promises and 3) adding value as a benefit of supply-chain management -- improving ability to capture and evaluate critical information that will improve profitability. Smith and Saunders at the International Livestock Congress in Houston (ILC, 2005) reported that traceability is a truly daunting task because we live in a world with over four billion livestock animals. Traceability of a food consists of development of "an information trail that follows the food product's physical trail. Internationally, the U.S. is lagging behind many countries in developing traceability systems for food -- in general -- and for livestock, poultry and their products -- especially." Table 3 compares several traceability programs in the world.

Table 3. Mandatory vs. voluntary traceability in global beef supply chains

Country or region	Mandatory or voluntary program
EU and Japan:	Mandatory, farm-of-origin to retail, all beef.
Australia and Brazil:	Mandatory only for exported beef, but plans for general and mandatory traceability.
Canada:	Mandatory for animals moving away from farm-of-origin.
Argentina:	Mandatory only for exported beef and for domestic beef produced in regions where animal diseases still persist.
USA:	Voluntary at present

Souza-Monteiro & Caswell (2004) http://www.umass.edu/resec/workingpapers.htm.

While the present debate on National Animal ID has been focused on foreign animal disease traceback, Saunders and Smith (2005, International Livestock Congress, Houston) suggested that there are other reasons to consider animal ID and especially traceability. Table 4 summarizes their presentation on this subject.

Table 4. Identification, traceability and verification across the entire complex of the livestock and meat industries (Saunders and Smith, 2005, International Livestock Congress, Houston)

- Ascertain origin and ownership to deter theft and misrepresentation
- Surveillance, control and eradication of foreign animal diseases
- Biosecurity protection of the national livestock population
- Compliance with requirements of our international customers
- Compliance with Country of Origin Labeling requirements
- Facilitate value-added and value-based marketing
- Isolate food safety problems

The Montana Pilot Project will demonstrate an individual animal traceability system by tagging cattle in various management scenarios, collecting data at a variety of cattle movement or change of ownership points, and testing the ability of the system to provide 48-hour traceback at any point in the production chain. Unique characteristics of Montana and its livestock industry offer broad and varied opportunities to implement and test a national identification system. For example, MT is a brand law state with a 545-mile border with Canada, seven tribal reservations, and nearly 35 mil ac of public lands. Montana's livestock industry consists mainly of seed-stock and cow-calf producers of a number of different breeds and is the sixth largest cow/calf producing state in the United States. Operations are both large and small with 40% of operations with 100-499 head and 51% with over 500 head.

With few feedlots and only small packing facilities in the state, a large majority of Montana's 1.5 mil calves are shipped out of state for finishing and processing. It has been estimated that approximately 65% of these calves are shipped to feedlots in NE. There are 14 livestock markets in Montana and they are the second most common method for selling calves in the state behind order buyers. Approximately 90% of all cows are sold through auction. The objectives of this project are to 1) establish premise registration for cattle and sheep producers and 2) track interstate movement of cattle and sheep. Table 5 outlines proposed tracking treatments for beef cattle which will utilize RFID, hot iron brands, retinal scanning and plastic ear tags as methods of animal identification.

Table 5. Examples of disease traceability treatments for cattle which have an original premise location of Montana

Scenario	*Original Premise and Management Description*	*Marketing method from ranch*	*Feedlot Phase*	*Packing Plant*
1	Calves born, raised and weaned on ranch.	Shipped directly from ranch to feedlot	Calves finished in feedlot in NE	Calves harvested in CO
2	Calves born, raised and weaned on ranch	Calves shipped to OK to graze wheat pastures	Calves finished in feedlot in IA	Calves harvested in NE
3	Calves born on a ranch, cow-calf pairs commingled on federal grazing land, weaned with several other owners' cattle	Calves marketed though an auction barn. (Calves may be further commingled with calves from other owners).	Calves finished in feedlot in Midwest	Calves harvested
4	Calves raised on Indian Reservation	Calves sold through an auction market	Calves finished	Calves harvested
5	Tracking of roping calves	Calves from MX, transported to MT and moved between MT, SD and WY	?	?
6	Tracking of cull cows	Majority of cull cows sold through auction barns	Cows sent directly to harvest or to feedlot for finishing	Cows harvested
7	Calves purchased and raised in MT	Summer Stocker program in South Dakota	Yearlings transported to feedlot in NE	Yearlings harvested in NE

As the national animal ID program develops, there will be at least three questions raised in regard to numerous systems of traceability. These questions are 1) what should be the depth (e.g. how far back and/or forward the relevant information is tracked), 2) what should be the breadth (e.g. the amount of information collected) and 3) how precise (the degree of assurance with which a tracing system can pinpoint a particular food product's movement or characteristics) should specific traceability systems be?

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Tri-National National Animal Identification System (NAIS) Project Synopsis

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ABSTRACT: The Tri-National Livestock Identification project involves three contiguous Western states (Arizona, Colorado, and New Mexico), three sovereign Native American nations (the Navajo Nation, the Hopi Tribe, and the Ute Mountain Ute Tribe), and two Mexican states (Chihuahua and Sonora Mexico). All participating jurisdictions are brand states that are using existing brand inspection infrastructure to carrying out livestock movement recordings. A third-party software company maintains the consortium's jurisdictional databases; interfaces with the NAIS premises allocator, premises repository and animal identification repository; and distributes and records animal identification and animal movements. The project is divided into five basic components: 1) outreach; 2) premises registration for all major livestock species and non-producer participants; 3) livestock identifier application; 4) recording of GPS coordinates with livestock movement from farm of origin to livestock markets, change of ownership, feedlots and packing plants; and 5) livestock traceability exercises. Cost data is being collected to establish an economic evaluation. Additionally, pre- and post-project surveys of producers' attitudes toward animal identification are being assessed. All jurisdictions are working with Cooperative Extension and others to facilitate outreach efforts. During outreach meetings the third party database provider facilitates producer premises registration via the Internet. Animal identification including RFID and biometrics are being utilized and integrated into the Consortium's databases. Traceability exercises will evaluate the projects ability to meet the 48-hour trace back goal.

KEYWORDS: Animal Identification; Traceability

Introduction

State Veterinarians from Arizona, Colorado, and New Mexico along with animal health officials from the Indian Nations of Navajo and Hopi tribes formed the Tri-National Consortium to oversee NAIS efforts in their region. In addition to the states and Indian Nations the consortium is also working with the Mexican states of Chihuahua and Sonora, completing the *Tri-National* effort. Membership in the Consortium remains open to all neighboring states and all Native American nations located within the region. The Consortium is focused on premises registration and education for the upcoming year with a select group of producers, in each region, tagging animals for tracking projects.

Specific goals are:

1. Development and initiation of communications, outreach and training programs;
2. Use of shared premises registration and animal ID databases for capturing locally needed information in addition to USAIP required information;
3. Implementation of premises registration throughout the region;
4. Conversion and utilization of premises registration and animal ID data from existing animal health surveillance and disease eradication programs;
5. Incorporate and utilization of GPS coordinates in combination with other identifiers for enhanced physical access to registered premises;
6. Early implementation and testing of animal identification across multiple species, including bovine, equine, ovine, caprine, and cervidae;
7. Incorporation of existing human resource infrastructures into local planning and implementation efforts, particularly the use of livestock (brand) inspectors and range enforcement officers to facilitate recording of animal tracking events;
8. Definition and testing of alternative ID device dissemination and tracking methodologies that minimize the requirements for technology at the ranch/farm;
9. Integration with and linkages to the livestock brand inspection and veterinary inspection processes of each of the member jurisdictions and demonstration of use of these systems to augment the auditability of the animal ID system;
10. Integration and electronic communication with other state or regional systems performing like functions in other areas of the country, especially in running trace back trials;
11. Exploration of alternative forms of animal identification, especially biometrics;
12. Investigation of the possible uses of smart card technology for streamlining premises identification at points of transfer and in transportation;
13. Integration and electronic communication with the Mexican national ID system in conjunction with our Mexican affiliate states, Chihuahua and Sonora, to facilitate tracking of animal movements across international borders.

Procedure

A primary objective of this project is to facilitate premises registration. The mechanism for this has been developed in conjunction with the USDA premises allocator and premises repository. Guidelines for defining premises were

developed based on guidelines set forth by USDA (USDA, 2004). The specific definition of "premises" will be determined by each State's or Tribe's animal health authority. Registration of premises can be done via phone, mail-in applications, or web-based interface.

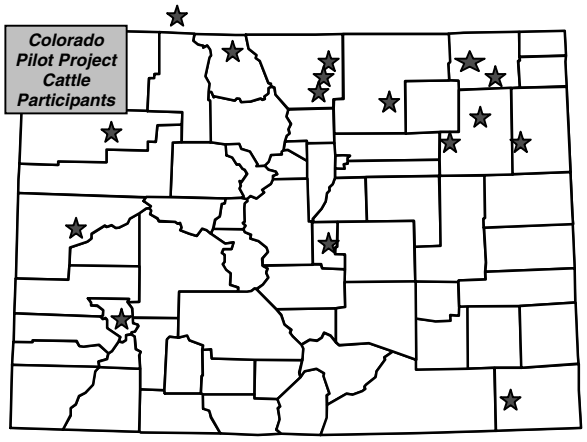
The Consortium will use a single regional database for premises registration and livestock tracking within the United States. This will be used to address both shared problems and issues unique to each state and nation and develop the links with Mexican members to track livestock movements across the U.S.-Mexican border. Research Management Systems USA, Inc. (RMS), headquartered in Fort Collins, Colorado, will provide the Consortium with the web site and database, training, project management and implementation services. The implementation project will use the existing LivestockTrust™ Animal Identification and Tracking system of RMS to provide the relevant database and reporting capabilities. In addition, the necessary software applications have been developed to enable the collection of animal movement data by livestock inspectors (brand inspectors) in the field and securely report these movements to the LivestockTrust database. The database system will be USDA certified for compliance with USAIP/NAIS standards and will interface with the USDA premises allocator, premises repository and AID repository.

Colorado has designated 16 cattle operations to serve as test herds for the Colorado portion of the Pilot Project. These herds were selected to represent large and small-scale operations; seedstock and cow-calf; as well as range, pasture and dairy businesses. The producers were assisted in applying the RFID eartags and tracking movements of cattle by brand inspectors and personnel from RMS. Notification of movement of cattle was the responsibility of both the owner and the brand inspector assigned to each herd.

Results

There are currently over 1000 cattle tagged and being tracked for movement in Colorado. Figure 1 represents the locations of the 16 herds participating in the Pilot Project.

Figure 1. Locations (indicated by the stars) of Colorado cattle producers participating in the Colorado portion of the Tri-National Livestock Consortium Pilot Project.

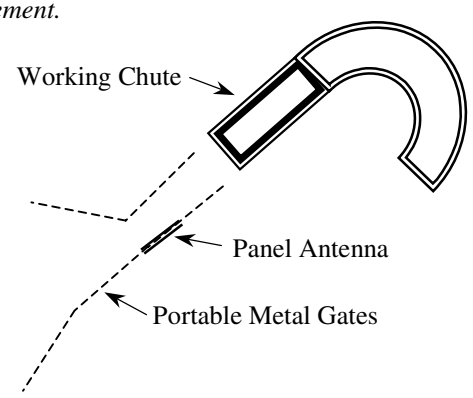


Descriptions of example tagging and reading evaluations with the cooperating herds follow. These examples are not intended to be stringent evaluations of products or facilities, rather as observations and field experiences.

Example 1. *Field trial was at Stull Ranch, Sterling, CO on September 25, 2004.*

Weanling calves were processed and tagged with RFID tags. Two State Brand Inspectors were applying tags and acting as primary recorders of calf information and EID number. Portable metal gates were placed in front of the working chute to direct calves past a single panel antenna, attached directly to the gate at approximately a 45º angle, tilting toward the direction of cattle movement, cord coming out the top. The widest point at panel placement was around 30 inches. Set-up time was about 35 to 40 min to place portable gates, panel antenna, run cable/cords, turn on computer/reader and obtain a successful read. The panel reader captured 19 of the 32 calves tagged. While 60% is typical or to be expected for this type of set-up it is not acceptable for field use, as it will require additional handling to read all of the tags missed. Steps will be taken in future applications to increase this percentage by the additional of a second panel and buffering "noise" through the reduction of vibration/movement of the portable gates.
Personnel: 3 people from Pilot Project, plus 5 Ranch Personnel.
Time: 2 hours **60% Read**

Figure 2. Top view of processing area and panel antenna placement.



Example 2: *Field trial was held at Kettle Ranch in Westcliffe, CO October 4th, 2004.*

A portal antenna was set-up at the end of the working chute to read cattle that were being tagged with RFIDs. Brand Inspectors were present to tag animals and RMS was present to trial the portal antenna. The antenna was placed at the end of the working chute. Portal antenna was strapped and zip tied into location against 2 x 4's running along the top and the bottom of the antenna. The portal antenna was attached to a Y-Tex reader, connected to a Panasonic Toughbook by serial port, com1. LivestockTrust was the software used to capture tags. Set-up time from evaluation of facilities to determine best placement of antenna to the first successful read was approximately 40

minutes. Brand Inspectors also read every tag before placing it in the animal's ear. This was accomplished by the use of a Bluetooth handheld wand, Tablet PC and LivestockTrust.
Personnel: 5 people from the Pilot Project, plus 10 Ranch Personnel.
Time: 6 hours **100% Read**

Example 3: *Field Testing at Sterling Livestock, Sterling, CO October 12th, 2004*

Calves were run through working chute to read RFID tags. A single panel antenna was placed on the right side of the chute to read tags as all calves were tagged in the right ear. Panel was mounted to ¾" plywood and fastened to the metal runs with straps and rope, pitched towards animal movement. Calves were moved through the chute in groups of 7-15 animals per group. One calf was caught to make certain RFID tag was read, panel antennas read all other animals. In addition to the 34 calves one cow and bull were tagged. The cow and bull were read with the handheld Bluetooth wand. Every animal at the salebarn from Niccoli Ranch with an RFID was read.
Personnel: 2 people from the Pilot Project and 2 Salebarn Personnel.
Time: 1.5 hours **100% Read**

Example 4: *Field Testing at Mountain Ute –Dolores, CO (San Juan National Forest) October 14th, 2004*

The Mountain Ute Tribe has a solid loading and weighing facility; however, there were very few areas ideal for setting up a portal or panel antenna to read RFID tags. A swing gate after the weigh scale was used to squeeze calves down to single file. A panel reader was attached to the outside fence line prior to the portal reader. Laptop computer and RFID reader was run off of a power inverter plugged into the cigarette lighter outlet on a vehicle. Either this set-up did not supply enough power or not in a constant fashion and therefore blew the vehicle fuse and the power inverter fuse. National forest had a small generator that we were able to use and powered both the laptop and reader successfully. Calves were originally going to be moved through the read area 10 at a time after they moved off the scale, but do to the power problem calves were weighed and moved back around through the read area. While all attempts were made to keep calves calm and moving through the portal antenna one at a time, there were several occasions of multiple calves moving through the portal antenna. It was also very easy for calves to bump, move and rattle the antennas resulting in tuning issues. The multiple animals and tuning problems resulted in less than 100% read rate. There were 219 calves that moved through the read area and 183 were read. To obtain an 83% read on calves not verified to have RFID tags on all animals and all RFID tags to be functional should be considered a success.
Personnel: 3 people from the Pilot Project and 6 Ranch Personnel.
Time: 4 hours **83% Read**

Example 5: *Field Testing at Schramm Feedlot Tagging – Yuma, CO October, 19th 2004*

Yuma, CO COOP producers purchased 550 RFID tags for calves going into a feedlot. Around 500 calves were tagged. Birth date and visual identification (VID) were recorded during tagging. A Bluetooth handheld wand was used to scan tags. All tags scanned successfully, but Bluetooth disconnected two times resulting in lost RFID and two animals were not stored before the next animals was read, resulting in lost RFID read.
Personnel: 1 person from Pilot Project and 7 Feedlot Personnel.
Time: 5.5 hours **99% Read**

Example 6: *Field Testing at Stull Ranch – Sterling, CO November 4th, 2004*

Commercial calves being shipped to local feedlot were scanned twice before movement to feedlot. After calves were weighed and sorted a narrow run was created with portable metal gates within an alleyway. A swinging gate was used to funnel calves down and ropes were used to keep the portable metal gates from moving to far apart. One panel antenna was securely fastened to outside wooden rails and the second panel antenna was attached to one of the portable metal gates. HyperTerminal tune reads were around 16,126 suggesting a successful placement of antennas and solid reads.

Cattle were pushed through the narrowed alley by 3 people (1 on Horseback) and 3 other people helped keep the calves moving single file through the narrow run. Calves were moved through in groups of 10 to 15. Movement of the calves through the narrowed alley was sporadic with groups of three to five moving at a time. Provided the first calf did not balk at the sight of the panel, calves would move freely past the panel antennas. The first group of calves moving through was startled by the sound of the reader and capture computer, but turning off the read tone on both hardware pieces relieved this problem. Sunlight reflecting off the panel antennas seemed to be the most logical explanation for the calves stopping at the sight of the panels. This was further demonstrated when the panel antennas were moved to the load out chute, where the panels did not reflect sunlight, and the calves walked past them freely.

Panels and reader were moved to load out chute. Panel antennas were attached to the solid metal load out chute with self tapping metal screws. HyperTerminal tune reading suggested that the metal was providing a great deal of interference and after disconnecting one of the antennas a better tune reading was obtained. While the reading was still high 32,105 successful reads were easily obtained half way across the load out chute. For this reason the left panel antenna was connected to the reader and the right panel remained unconnected. RFID tags were in the left ear of all calves, so leaving the left antenna suggested the best chance for successful read of tags.

Calves were moved through the chute and past the panels with relative ease. RFID antennas and reader did not slow time to load. Due to the metal interference it was necessary to wait an extra 5-10 minutes before starting to load the cattle.

Personnel: 1 person from Pilot Project, plus 5 Ranch Personnel.

Time: 3.5 hours **100% Read and ~80% Read**

Implications

Experiences thus far with implementation of animal identification and traceback have enabled producers, regulators and educators to become familiar with opportunities and obstacles associated with this program. Use of technology will enhance data capture and management; however there will be a steep learning curve for widespread application in diverse production settings.

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THE NORTHWEST PILOT PROJECT: FINDING REAL-WORLD SOLUTIONS TO ANIMAL IDENTIFICATION

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ABSTRACT: The Northwest Pilot Project (NWPP) is a USDA-supported, producer-driven coalition in California, Hawaii, Idaho, Nevada, Oregon, Utah, and Washington formed to develop solutions for the implementation of the National Animal Identification System (NAIS) in the region. The NWPP is using applied production systems to test multiple identification methods to track livestock across state and national borders and from the herd of origin through harvest. In addition to helping shape the NAIS to be workable and practical for the region, the NWPP provides the opportunity for producers to gain experience with different identification systems, while developing and demonstrating multiple methods for meeting the USDA goal of 48-h traceability. Initiated by state beef cattle associations of the region, the NWPP coalition also involves dairy, sheep and bison industries, state departments of agriculture, state veterinarians, brand departments, and university extension services. The NWPP is also working with the states to help build uniformity in premises definitions and registration processes, while providing actual production scenarios to assist in the development of state identification programs and databases. To meet the enrollment goal of 27,000 animals, producers are asked to contribute 100 to 200 head of livestock and record the movements of those animals in the temporary NWPP database. The NWPP is also working with auction markets, feedlots, and packers in the region to track project cattle as they continue through the production chain. The NWPP will audit the movement records of enrolled livestock from birth until harvest to identify problems and find solutions for tracking livestock through the diversity of production systems in the region. Ultimately, workable, functional, and cost-effective solutions for producers in the Northwest will be identified.

Key Words: Animal Identification, Northwest Pilot Project, National Animal Identification System

Introduction

A year after a single dairy cow in Washington State tested positive for bovine spongiform encephalopathy (**BSE**), the U.S. beef industry is in a transition phase unrivaled throughout most of its history, as "animal ID," "source verification," and "traceability" become household words. As the U.S. works to regain access to export markets, foreign and domestic consumers continue to place increased pressure on the American beef industry to design and implement an animal tracking system that will provide them with additional confidence in the safety of our product (USDA, 2004b).

Mark Gustafson, senior vice-president of international sales for Swift and Co. (Greeley, CO), recently stated that, "to compete in a global market, the United States needs a compatible trace program. By virtue of being classified as a low-risk country for animal disease, the United States has thus far been able to avoid creating a tracking system" (Gustafson, 2004). In addition to marketing implications, the need for a national identification system to help protect animal agriculture from domestic and foreign disease threats has become critical (USDA, 2004a). Recognizing these needs, the USDA is working diligently on the development of a national identification and traceability program for livestock.

Agriculture Secretary Ann M. Veneman announced the framework for implementing a National Animal Identification System (**NAIS**) on April 27, 2004 (Gray, 2004; USDA, 2004a). As the development of the NAIS progressed, livestock industries in the Pacific Northwest recognized the need to become thoroughly involved with the development of the system to ensure that it would be practical and cost-effective, while not increasing producer liability or violating the confidentiality of production data.

Accordingly, the cattle industries of the Northwest saw an opportunity to come together and help USDA address the unique challenges of designing and implementing the NAIS in the region. The resulting effort, known as the Northwest Pilot Project (**NWPP**), has gained tremendous support and momentum from both within the region and from USDA, as they work to find positive solutions for NAIS implementation (Northwest Pilot Project, 2004).

Materials and Methods

Working in the states of California, Hawaii, Idaho, Nevada, Oregon, Utah, and Washington, the NWPP is using applied production systems to test multiple identification methods in order to develop solutions for the NAIS that are workable, reasonable, functional, and cost-effective for producers in the Northwest. The NWPP is tracking livestock enrolled in the project across state and national borders and from herds of origin through harvest, ultimately demonstrating multiple methods for meeting the USDA goal that all animals and premises that have had direct contact with a foreign or domestic animal disease of concern be identified within 48-h after discovery (USDA,

2004b). Further, the NWPP will provide feedback to USDA on how to address issues unique to the region, such as: 1) incorporation of existing brand laws, brand infrastructure, and current animal health regulations into the NAIS; 2) dependence on public lands grazing, and resulting issues of common grazing allotments, lack of handling facilities, and pastures that cross state boundaries; and 3) a commerce system that involves extensive interstate movements of livestock across industry segments. The NWPP is helping to populate and test state premises identification systems, and is working with other states in an attempt to build commonality between premises definitions and registration processes across the region.

The project coalition includes the region's beef and dairy cattle associations, state departments of agriculture and state veterinarians, brand boards, sheep and bison industries, BSE testing laboratories at Washington State University and University of California-Davis, university extension services, and the Alberta Cattle Association. The industry associations are conducting outreach programs to provide current information regarding the NWPP and the NAIS to producers.

The NWPP received funding from the USDA for one year beginning November 1, 2004, and lasting until October 31, 2005. In order to accomplish the stated objectives, however, the NWPP anticipates that the project will continue for multiple years, depending on the development of the NAIS at state and national levels.

In order to achieve an adequate cross-section of the various production systems in the Northwest, the NWPP plans to enroll a minimum of 125 cow/calf producers, 6 auction markets, 5 order buyers, 14 feedlots, and 5 packing plants in the project during the first year. These industry participants will provide at least 27,000 head of beef and dairy cattle, sheep, and bison to the project. The enrolled livestock are being classified according to the production scenarios listed in Table 1 in order to meet the enrollment goals shown. Within the general breakdown of production scenarios, the NWPP is also targeting five classifications of identification technologies, including: 1) brand only, 2) visual tags only, 3) electronic identification only, 4) visual tags and electronic identification, or 5) brands, visual tags, and electronic identification. The NWPP is technology-neutral and will not endorse any particular technology, vendor, or management system.

As enrolled livestock are identified and moved between premises, participants are recording these transactions in the temporary database system created by the NWPP, which is only accessible to participants via the project website. The NWPP anticipates an average of five transactions per enrolled animal, for a total of at least 135,000 entries into the database during the first year of the project. The entries will then be audited and used in disease traceback simulations to identify problems and challenges, develop solutions, and determine which methods of identification will sufficiently meet the 48-h traceability goal.

The NWPP is seeking producer participants whose animals are born, fed, and harvested within the region encompassed by the NWPP and are willing to enroll 100 to 200 head of livestock in the program. Participants are covering the initial hardware and implementation costs for their choice of identification systems. They are also choosing between group/lot and individual identification for enrolled animals, and they are entering the livestock movement information into the project database via the internet. For participants choosing individual animal identification, the NWPP is reimbursing them for database entries at the rate of $0.75/head for each transaction. For group/lot entries, each participant is receiving $40.00/entry. Participants have agreed to target a 75% success rate in tracking enrolled animals from birth to harvest. The NWPP is providing database training for participants to become acquainted with the data entry process for the project, as well as the information requirements of the NAIS in general.

In order to participate in the NWPP, feedlots, auction markets, and packers had to demonstrate a willingness to take part in the collection, sharing, and transmittal of data as project animals move through their facilities. They also had to demonstrate diversity in the types of livestock transactions that occur at their operations to ensure that a meaningful industry cross-section could be achieved through the project. These entities are also helping to cover the initial hardware and implementation costs of their choice of identification systems and devices.

Given the breadth and diversity of producers and production systems that are becoming involved in the NWPP, there are numerous opportunities for third-party technology vendors to participate in the program. While the NWPP will not endorse any particular identification technology, individual producer participants are determining which products and vendors they utilize, and involve them in the project. The NWPP does specify, however, that all proposed technology shall meet the standards and specifications determined by USDA Animal and Plant Health Inspection Service as the NAIS is developed.

Results and Discussion

As of March 2005, NWPP outreach efforts have reached over 2,500 cattle producers and other industry representatives in the seven states involved in the project. NWPP representatives have presented the project at the National Cattlemen's Beef Association Cattlemen's College, 2005 Intermountain Cow Symposium, Spokane Agriculture Exposition, California State University-Chico Beef Day, Western Rancher's Beef Profitability Conference, Western Livestock Investigators Association Conference, and numerous other state and local cattle association conventions and meetings. The project has also been featured by media sources, such as the Northern Ag Network, www.meatingplace.com, www.loostales.com, *Western Livestock Journal, Houston Chronicle, New York Times, Seattle Post-Intelligencer*, *Capital Press, Oregon Beef Producer, Ag Weekly, Progressive Rancher, Cascade Cattleman, Idaho Cattle Association Line Rider,* National Public Radio, and in a variety of associated press articles.

Enrollment of livestock in the NWPP as of March 2005 exceeded 18,000 head of beef and dairy cattle, bison, and sheep. Current participants entered over 1,600

transactions into the NWPP database by early March. Based on those entries and feedback from producers during outreach efforts, the NWPP has identified several issues for which the project will continue to seek solutions. One such issue is the need to investigate the possibility of utilizing group/lot identification for cattle as an alternative to individual identification, particularly in situations where producers retain ownership and their animals stay in one group throughout the production chain. The NWPP will also investigate using group/lot identification at the cow/calf segment, which may be able to be converted to individual identification at other segments of the production chain, such as an auction market or feedlot.

As NWPP participants enroll livestock in the NWPP and work toward registering their operations in the premises identification system, many issues surrounding premises have arisen that the project is addressing. One such issue is the need for USDA premises number allocation to involve the use of location descriptions other than a valid 911 emergency street address, such as global positioning system coordinates. Alternative location descriptions have become critical, given the difficulty of obtaining premises numbers experienced by NWPP participants in rural areas. Further, in determining how to define livestock production systems for premises identification, there is also a need for producers to have the flexibility to choose how to define their premises, based on typical movement patterns, management systems, and their willingness to accept risk. In scenarios presented by NWPP participants, there is also a need for flexibility in the premises registration system to accommodate temporary grazing situations, such as corn stalks or wheat stubble that vary annually by location and lessee. Many NWPP participants also feel that the premises identification system should allow flexibility so that a premises can be registered by the lessee of a property instead of only by the owner. Overall, the NWPP will continue to work toward the coordination of premises identification efforts between states to avoid disparate regulatory processes for producers operating in multiple states or with operations spanning across state boundaries.

The NWPP will also continue to address situations where there is a lack of handling and processing facilities that allow for the reading of individual livestock identification numbers and, in such cases, to investigate such possibilities as allowing the receiver of animals to apply the identification or to read and report the identification of livestock for the seller or shipper.

Summary

The NWPP will continue to identify issues affecting animal identification in the region. Additional participants will be sought, information will be provided to producers, and feedback will be presented to the USDA in order to promote the development of a workable, practical, and cost-effective solution for the implementation of the NAIS in the Pacific Northwest.

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Table 1. Production scenarios and enrollment goals during year 1 of the Northwest Pilot Project

Species	Production scenario	No. of head
Beef/bison -- Single state	Cow/calf direct to harvest	2,300
	Cow/calf to feedlot to harvest	4,700
	Cow/calf to grass pasture to feedlot to harvest	4,600
Beef/bison -- Multiple states	Cow/calf direct to harvest	2,700
	Cow/calf to feedlot to harvest	4,100
	Cow/calf to grass pasture to feedlot to harvest	4,200
Beef – Imported	Import direct to harvest (fed cattle)	200
	Import direct to harvest (feeder cattle)	200
Dairy	Calf yard to feedlot to harvest (bull calves)	1,200
	Calf yard to heifer feedlot to milk herd	1,000
	Direct to harvest (non-fed cattle)	1,100
Sheep	Lambs to pasture to harvest	700
Total		**27,000**

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DEVELOPING BEEF QUALITY ASSURANCE MATERIALS FOR YOUTH

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ABSTRACT: 4-H and FFA livestock programs provide a unique educational experience for youth development. In Utah, 4-H and FFA youth livestock projects produce livestock including beef, pork and lamb with a commercial market value of more than $1 million per year. Youth must realize that they are not just raising a project for the county fair; they are in the business of producing a food product for the consumer. This study focuses on three objectives. 1) Help youth understand and have an increased awareness of the commercial livestock industry; 2) teach principles of Beef Quality Assurance (BQA) and how these principles relate to youth livestock projects; and 3) provide 4-H and FFA leaders and teachers with materials to enable them to teach Beef Quality Assurance. Economic and production data on live animals and corresponding carcass information were collected. Digital photographs and video footage of live animals and carcasses were taken to provide FFA instructors, county agents and youth leaders with visual teaching aids. BQA resources produced from this project include. 1) A teaching manual, *From the Farm to the Table, Teaching Youth about Carcass Quality;* 2) a 19 minute DVD video production titled, *Youth Beef Quality, Producing a Quality Product for the Consumer;* 3) a second 26 minute DVD produced by Utah State University Extension and Fort Dodge, titled *Realizing the Impact of Injection Site Lesions;* 4) historical carcass data and comparisons of this data to industry standards and 5) a supplemental material CD containing 53 illustrated calf data sheets categorized by quality grade. 4-H and FFA beef project leaders and parents can use these materials to teach their youth about live animal and carcass evaluation and BQA and how they can produce a better beef product for the consumer.

Keywords: BQA, Youth Beef Project, Carcass Data

Introduction

Beef Quality Assurance is a program that ensures that beef cattle are maintained in a manner which will result in a safe and wholesome beef product for the consumer. Youth livestock programs such as 4-H and FFA involve thousands of youth nationwide. These programs provide youth with a unique opportunity to use live animals to develop valuable life-long skills. When youth are provided with correct information, they usually make correct choices and improve the quality of their livestock project. To make those decisions accurately, youth must first be provided with the correct information. With the increasing popularity of junior livestock shows, product safety and quality is every bit as important as it is in the commercial industry. Today, more than ever before, 4-H and FFA students need to realize they are not just raising a livestock project for the county fair, they are in the business of producing food. 4-H and FFA youth must take every precaution to ensure a high quality product that the consumer will find safe and wholesome. This project developed educational materials that will enhance the educational aspects of 4-H and FFA junior livestock projects.

The objectives of this study were: 1) Increase youth awareness of the commercial livestock industry, the impact junior livestock shows have on that industry and how his or her steer project and product compares to industry benchmarks as determined by national beef quality audits. 2) Teach youth principles of Beef Quality Assurance (BQA) and animal livestock evaluation from the perspective of the wholesale and retail trades. These principles will benefit them as future producers and/or consumers. 3) Provide 4-H and FFA leaders with materials that will enable them to teach youth about carcass quality and other BQA principles that will assist in a greater understanding of the beef industry.

Materials and Methods

Live animal, carcass, and economic data on 4-H and FFA steer projects were collected from the Box Elder County Fair and Junior Livestock Show held August 25–28, 2004.

We used a digital camera for all still photography. A Nikon D70 with a 18 – 70 mm lens, and a 6.1 megapixel image processor with high speed 512 mb storage disk provided ample image processing speed and data storage. A Canon ZR50 Digital Video Camcorder including *Firewire*™ hardware to transfer digital video data to a computer hard drive was used to develop video footage for the DVD, "Youth Beef Quality, Producing a Quality Product for the Consumer." Many digital cameras come equipped today with USB 2.0 hardware which performs basically the same functions as *Firewire.* We used Adobe Premier Software for video editing and compilation and production. A 120 GB external hard drive was necessary to handle the large amount of video storage space required.

4-H and FFA youth exhibiting beef projects are required to register and weigh their beef animal with the livestock show committee in March and care for their animal for a

minimum of 150 days. Beef projects were consigned to the livestock show on August 25. No project was accepted under 1,075 lbs net weight. The average market weight was 1,245 lbs live. A standard shrink of 3% was applied to all show steers.

Data collected on the steers included: 1) A photograph of the rear and side view of each live animal (Figure 1) was taken as the exhibitor and project exited the judging contest; 2) an ultrasound scan including a digital image (Figure 2) of the ribeye area and backfat thickness taken by a certified ultrasound technician; 3) live weight and breed type which were gathered at the livestock show; and 4) after harvesting the animals, a photograph of a cross section of the ribeye between the 12^{th} and 13^{th} rib was taken (Figure 3). Collecting accurate carcass measurements in a large beef packing plant is difficult because the measurements usually need to be made while the carcass is moving on the rail. Limited storage area and poor lighting also make it difficult to collect carcass measurements (Trenkle et al., 1999).

Because of limited processing area, it has become almost impossible to have commercial processors rail aside carcasses for closer examination. For this reason, we had to incorporate data gathered from ultrasounds with the limited data gathered from the processor.

Carcasses were measured for quality and yield grade, carcass yield, hot carcass weight, percent kidney, pelvic and heart fat, ribeye area, backfat thickness and marbling score. Carcass data were compared to data acquired from the ultrasound scan and the placings in the livestock show.

Percent Retail Cuts was estimated using the following formula: 51.34 - (5.78 x Fat) - (0.0093 x HCW) - (0.462 x KPH) + (0.74 x REA), where Fat = Backfat thickness determined in inches; HCW = Hot Carcass Weight; KPH = Kidney, Pelvic, and Heart fat; and REA = Ribeye Area. Yield grade was determined with the formula: Yield Grade = 2.5 + (2.5 x Fat) + (0.0038 x HCW) + (0.2 x KPH) - (0.32 x REA).

Results and Discussion

Objective 1 – Help youth gain an understanding of the livestock industry.

In Utah, 4-H and FFA youth livestock projects produce market livestock including beef, pork and lamb for the consumer valuing more than $1 million per year (Holmgren and Reid, 2001). Recent county and state junior livestock show research indicates that many youth are trained in fitting and showing their steers but do not associate their project with the overall beef or consumer retail industry. Many know little about careers available to them in the livestock industry (Holmgren and Reid, 2001).

In 1991, the beef industry conducted its first ever National Beef Quality Audit (NBQA) and since that time, the industry is showing signs of improvement. For example, the 2000 audit reports that the incidence of injection site lesions in top sirloin butts has dropped significantly from 11% in 1995 to 2%. Losses to the industry from challenges to carcass quality have decreased from $277.81 to $104.92 per carcass (NCBAQA-2000).

Educational BQA resources included a teaching manual, *From the Farm to the Table, Teaching Youth About Carcass Quality*. Some of the material contained in this manual has been gathered from reliable beef production resources around the country. A series of 17 color sheets are included in the manual which have live animal photos, ultrasound images, actual ribeye photos and measured carcass data for each individual animal. These sheets can be used to teach youth about quality and yield grading. Additional calf sheets are included on a CD, along with other multimedia materials to assist with teaching. A 19 minute DVD video production titled, *Youth Beef Quality, Producing a Quality Product for the Consumer* were developed to help youth understand their relationship as beef producers to the consumer who purchases this product. It stresses that youth who are raising project beef for the retail market improve the quality of their beef product by reducing the frequency of producer-caused carcass quality problems and ensure that best management practices are followed. Specifically, it discusses:

1. Daily Care and Management
2. Prevention
3. Handling
4. Carcass Quality
5. Health Care

A second 26 minute DVD, *Realizing the Impact of Injection-site Lesions*, produced by Utah State University Extension was included with the educational materials. This video discusses the impact of injection site lesions and emphasizes the economic losses to the industry that occur from improperly injecting vaccinations, vitamins, and other drugs into the animal.

Objective 2 – Teach youth principles of Beef Quality Assurance and how these principles relate to 4-H and FFA projects.

Traditionally, packers discount prices paid for carcasses outside the hot carcass weight range of 550 to 950 lbs (McKenna et al., 2002). Recent trends in the junior livestock industry show that 4-H and FFA beef project carcass weights continue to increase but remain within acceptable National Beef Quality Audit – 2000 standards as shown in Table 1. According to the National Beef Quality Audit – 2000, 4.6% of carcasses had hot carcass weights outside of the 550 to 950 lbs range.

One objective of the NBQA was to increase the percentage of cattle making it into the USDA Prime and the upper part of the Choice grade (Busby et al., 2001). Table 2 illustrates

a comparison between Iowa State University 4-H beef carcass trait averages for carcasses grading Choice or better (Strohbehn, 2001) and similar carcass trait averages at the Box Elder County Junior Livestock Show. Both comparisons demonstrate that since the mid 1980s there has been a consistent improvement in the percentage of carcasses grading Choice or higher.

Objective 3 — 4-H and FFA leaders will have materials that will enable them to teach youth about carcass quality and other BQA principles that will assist in a greater understanding of the beef industry.

A CD containing supplemental material included 53 calf data sheets which have rear and side view live animal photos, ultrasound images, actual ribeye photos and measured carcass data for each individual animal. These sheets can be used to teach youth about quality and yield grading and how much variability can exist between different carcasses with the same quality or yield grade.

Implications

Often in our youth livestock programs, youth get so involved in competitive aspects of the project that they lose sight of the fact that the livestock they produce is eventually destined to the consumer. 4-H and FFA beef project leaders and parents can use these materials to teach their youth about Beef Quality Assurance and how they can produce a better beef product for the consumer.

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Figure 1. Side and rear view photograph of steer.

Figure 2. Ultrasound image of rib eye taken between the 12th and 13th rib of the above steer.

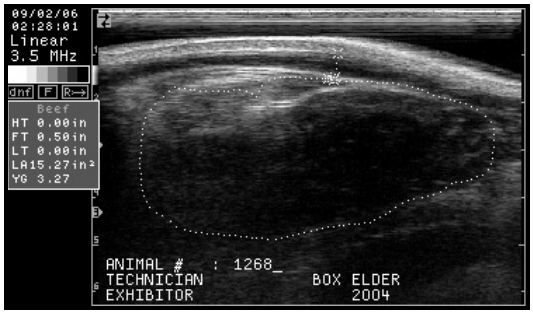

Figure 3. Digital photograph of the same ribeye muscle.

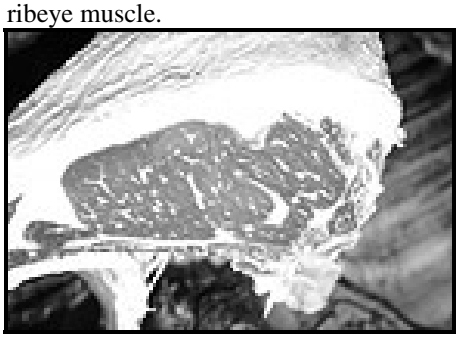

Table 1. Average production data from 1974 to 2004 in 5 year increments at the Box Elder County Fair

Period	1974–78	1979–83	1984–88	1989–93	1994–98	1999–03	2004–
# Steers	508	362	473	502	488	502	106
Live Weight	1,011	1,095	1,156	1,162	1,177	1,225	1,243
Hot Weight	621	674	700	713	719	743	738
% > 700 lb HW	7.24%	31.88%	52.48%	59.45%	62.70%	75.00%	91.00%

Table 2. Percent grading choice or better at Box Elder Junior Livestock (BEJL) Show as compared to Iowa State University 4-H Beef Carcass Summary.

	BEJL	Iowa 4-H
1975 – 1978	58.74%	69.05%
1979 – 1982	44.00%	75.71%
1983 – 1986	31.57%	53.95%
1997 – 2000	43.78%	74.20%
2001 – 2004	59.58%	73.93%

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Vaccination on the ranch as an intervention strategy to reduce the probability of detecting *E. coli* O157:H7 associated with commercial feedlot cattle

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ABSTRACT: A clinical trial was conducted to test the effect of vaccinating freshly weaned Montana calves against *Escherichia coli* on the probability to detect *E. coli* O157:H7 (EC) in feces or on RAMS. Two cow/calf sources (1 Central MT; 1 Southeast MT), two feedlots (1 Central NE; 1 Western NE), and five packing plants (2 CO, 1 KS, 2 NE) participated in the study. Steer and heifer calves (N=1001) were weaned during the months of September and October 2003 and systematically allocated to treatment so that one-third of the calves would receive vaccine (VAC; vaccinated at weaning, 21 d post weaning, and 80-100 d prior to harvest) and two-thirds would not (NOVAC). Following weaning and a backgrounding period at the ranch of origin, calves were transported to NE feedlots. During finishing, the distribution of treatments within a pen was maintained as 1/3 VAC calves and 2/3 NOVAC calves. Each calf was sampled four times for 1 pre-treatment period (d 0; weaning; fecal), 2 interim periods (21 d post weaning; 80-100 d prior to harvest; fecal), and 1 test-period sampling (harvest; Rectoanal Mucosa Swab, RAMS). In total, 3595 individual fecal or RAMS samples were collected from 1003 calves. The odds for a VAC animal to shed EC were compared to that of NOVAC cattle accounting for repeated measures, feedlot, pen and the time of marketing. Overall pre-treatment probability of detecting EC averaged 0.40%. The probability of detecting EC for samples two, three, and four averaged 0.0, 0.40, and 1.24%, respectively, and were not different (OR=0.82; P>0.10) between vaccination treatments. The highest probability for detecting EC was observed at harvest for both NOVAC (1.37%) and VAC (1.00%) cattle, and were not different (OR=0.67, P>0.10). Because of the low probability of detecting EC shedding throughout the entire production period, this study could not determine the effect of vaccination on the ranch as a pre-harvest food safety intervention strategy.

Key Words: Cattle, *E. coli* O157:H7, Vaccination

Introduction

Human exposure to *Escherichia coli* O157:H7 can cause severe diarrhea (hemorrhagic colitis), and in a small percentage of cases, hemolytic-uremic syndrome (HUS). Beef cattle populations are important reservoirs for *Escherichia coli* O157:H7 and this pathogen causes important economic losses to the beef industry. Historically, the beef industry has focused their efforts to control this pathogen at the post-harvest stage of beef production. However, management practices aimed at controlling food borne pathogens prior to harvest have been suggested as potential pre-harvest food safety interventions to reduce the prevalence of *E. coli* O157:H7 in the feces and on the hides of beef feedlot cattle; including diet change, feeding direct-fed microbial products, sodium chlorate, antibiotics, water trough treatment, and vaccines (Callaway et al., 2004).

In an earlier study we found that vaccinating feedlot cattle against Type III secretory proteins of *Escherichia coli* O157:H7 reduced the probability for cattle to shed the organism in feces by 59% (Potter et al., 2004). In that study, cattle were vaccinated three times at three-week intervals with the first dose of vaccine given when cattle would normally enter the feedlot for the finishing phase of beef production. The use of a three-dose vaccination protocol is of practical importance because feedlot operators may be challenged to comply with the need to repeatedly vaccinate cattle. However, if vaccination could be implemented into pre-existing pre-conditioning programs at the ranch of origin, one or two doses of vaccine could be given to cattle before they are ever sent to a feedlot for the finishing phase of production.

Our objective was to evaluate the effectiveness of vaccinating calves against Type III secretory proteins of *E. coli* O157:H7 at the ranch (1 dose given at weaning and a 2nd dose given 14-21 d post weaning) and at the feedlot (reimplanting) on the probability to detect *E. coli* O157:H7 at the rectoanal juncture in cattle at harvest.

Materials and Methods

Source of Cattle

Spring born steer and heifer (n=1001) calves were weaned, and subsequently enrolled for study, starting September 29, and ending October 15, 2003. Calves originated from two sources in Montana. Source 1 enrolled 438 steers and source 2 enrolled 290 steers and 298 heifers. Calves were pre-conditioned at the ranch of origin for an average of 45 and 105 d for sources 1 and 2 respectively. At weaning, cattle were weighed, individual fecal samples collected, and were allocated to treatment.

Treatments

The vaccine (2ml/dose; Bioniche Life Sciences) was administered subcutaneously in the neck using an 18 ga x 5/8-inch needle. The vaccine contained supernatant proteins prepared from *E. coli* O157:H7 as previously described (Li et al., 2000), and formulated with the adjuvant VSA3 such that the protein concentration was 50µg/dose. Vaccination treatments included vaccination and no vaccination. Cattle were systematically allocated to treatment so that one-third of the calves would receive vaccine (VAC) and two-thirds would not (NOVAC). For VAC cattle, the first dose of vaccine was given at weaning, a second dose given 14-21 d later following the pre-conditioning protocol already in place at each respective ranch, and a third dose of vaccine given at reimplant time during the finishing phase of beef production (80-100 d prior to harvest).

Each animal was aseptically sampled by rectal fecal grab at weaning, at the end of the preconditioning period at the ranch of origin, and again at reimplant time, resulting in one pretreatment sample and two interim samplings. At harvest, each animal was aseptically sampled by swabbing the rectoanal juncture using Rectoanal Mucosal Swabs (RAMS) prior to the bunging bench at commercial beef packing plants.

Cattle were marketed at the discretion of the respective feedlot manager where the cattle were finished. Because we did not want to interfere with the normal cattle marketing activities at each feedlot, cattle were harvested at five different packing plants (2 CO, 1 KS, 2 NE).

Microbiological measurements

Laboratory personnel were blinded to treatments. Fecal samples were collected directly from the rectum of each animal and shipped by overnight delivery to the UNL *E. coli* lab and analyzed for presence of *E. coli* O157:H7 using procedures previously described (Smith et al., 2001) with modifications. Briefly, ten-gram fecal samples were incubated for 6 hr in Gram Negative (GN) broth containing vancomycin, cefixime, and cefsoludin. An aliquot of culture material was then subjected to immunomagnetic bead separation and plated onto sorbitol-MacConkey agar containing cefixime and tellurite (CT-SMAC). After 18-24 hr incubation, three non-sorbitol-fermenting colonies were picked and subcultured onto CT-SMAC to ensure purity then were subcultured onto MacConkey and Flourocult agars. After 18-24 hr incubation, lactose-fermenting colonies that yielded a negative MUG (4-methylumbelliferyl-ß-D-glucuronide) reaction were streaked for isolation on blood agar. After an overnight incubation, one colony per isolate on blood agar was tested for *E. coli* O157 and H7 antigens by latex agglutination. Isolates that were positive for both the O157 and H7 antigens were tested in a 5-primer-pair multiplex polymerase chain reaction (PCR) assay that detected genes for E. coli O157, H7, Shigatoxins 1 and 2, and intimin. Detection of genes for O157, H7, and at least one other target in the assay was considered to be confirmation of an isolate as *E. coli* O157:H7.

RAMS samples were collected directly from the rectoanal juncture of each animal at harvest, placed in 3 ml of TSB in a 19 ml Falcon tube, and taken directly to the UNL *E. coli* lab and analyzed for presence of *E. coli* O157:H7 using procedures previously described (Rice et al., 2003). Briefly, each sample was vortexed for 20 sec and 1 ml of vortexed suspension was transferred to a 5 ml tube. An aliquot of the vortexed solution (100 µl) was pipetted into a tube containing 900 µl of PBS buffer, and mixed well. This dilution was repeated once more. An aliquot (100 µl) of both the 10 and 100 x dilutions were then plated on CTVM SMAC and aseptically spread using the standard spread plate method. Plates were then incubated for 18-24 hr. After 18-24 hr incubation, RAMS samples were treated the same as fecal samples in order to determine presence of *E. coli* O157:H7.

Statistical Analysis

The effect of vaccine was tested by modeling the probability of detecting *E. coli* O157:H7 from feces or RAMS. Treatment differences were considered significant at $\alpha \leq 0.05$. Pre-treatment *E. coli* O157:H7 data were analyzed using a generalized linear mixed model (GLMM) with a logit link function accounting for vaccination treatment and source. Interim *E. coli* O157:H7 data were analyzed using a GLMM with a logit link function accounting for vaccination, source, and sex as fixed effects in the model and repeated measures within pen as random effects. Final *E. coli* O157:H7 data were analyzed using a GLMM with a logit link function accounting for vaccination, source, and sex as fixed effects in the model and sale period and pen as random effects.

Results

We collected a total of 3595 individual fecal or RAMS samples from the 1001 calves housed in 9 commercial feedlot pens during this study. One entire pen of cattle (n=72 hd) was not sampled at harvest because we were not notified that the feedlot had marketed that pen of cattle. Portions of the 1001 cattle original enrolled in the study were held back from the feedlot for various reasons. Additionally, interim fecal samples were missed at each feedlot due to cattle being held in sick pens or not located in the pen of interest on the day of sampling. In total, 901 individual RAMS samples were collected from 8 pens of cattle from 2 sources and 2 commercial feedlots at harvest. Cattle on this study were harvested at five different commercial beef packing plants (2 CO, 1 KS, 2 NE).

There were no factors that explained the probability to detect *E. coli* O157:H7 in the feces of cattle. The pre-treatment probability of detecting *E. coli* O157:H7 in the feces of calves was not different (P=0.62) between vaccination treatments and averaged 0.40% at weaning. The odds of detecting *E. coli* O157:H7 in the feces of calves at source 2 (0.71%) were 3.63 times

greater than detecting *E. coli* O157:H7 in the feces of calves at source 1 (0.00%; P=0.053).

The probability of detecting *E. coli* O157:H7 for samples two, three, and four averaged 0.00, 0.40, and 1.24%, respectively, and the probability to detect *E. coli* O157:H7 was not different between vaccination treatment (OR=0.82; P>0.10). During test periods two, three, and four, the probability to detect *E. coli* O157:H7 for source 1 was 0.00, 0.34, and 0.49%, respectively. For source 2, the probability to detect *E. coli* O157:H7 for test periods two, three, and four was 0.00, 0.43, and 1.87%, respectively. The probability to detect *E. coli* O157:H7 from the feces of calves was not different (OR=3.75; P=0.07) between sources for test periods two, three, and four.

The highest probability to detect *E. coli* O157:H7 was observed at harvest for both NOVAC (1.37%) and VAC (1.00%) cattle. There were no differences in the probability to detect *E. coli* O157:H7 at harvest by treatment (OR=0.68, P=0.60). Additionally, the probability to detect *E. coli* O157:H7 by source (OR=4.20; P=0.28) and gender (OR=0.45; P=0.56) was not different at harvest.

Discussion

We have demonstrated that vaccinating feedlot cattle against Type III secretory proteins of *E. coli* O157:H7 reduced the probability for cattle to shed the organism in feces by 59% (Potter et al., 2004). In a follow up study we showed dose response and herd immunity effects in response to vaccinating feedlot cattle against *E. coli* O157:H7 (Peterson et al., 2005). The current study was designed to evaluate the effects of vaccinating cattle at the ranch and at the feedlot under a commercial management environment.

We chose to use the individual animal as the experimental unit and co-mingled VAC and NOVAC cattle within pen. Research has shown when the majority of cattle are vaccinated within a pen vaccinated pen mates confer protection to non vaccinated cattle within the same pen (Peterson et al., 2005). We tried to off-set any herd immunity effects by only vaccinating 1/3 of the animals enrolled in the trial and maintaining 1/3 to 2/3 ratio of VAC to NOVAC cattle within a pen throughout the production period. However, due to the low probability to detect *E. coli* O157:H7 in the feces it is reasonable to suggest that we may not have completely limited the effects of herd immunity in this trial.

Lab personnel were blinded to treatment and we systematically allocated cattle to treatment within source and day of weaning. Lab personnel had no knowledge concerning which treatment each sample was collected from. Additionally, when enrolling cattle for the study, a systematic treatment allocation scheme was used so that every third animal through the chute at processing would be assigned to the VAC treatment. This allocation scheme should eliminate selection bias between treatments. If selection bias was to occur, it would usually occur before the study begins. A selection bias would be expected if steers selected for VAC and NOVAC treatments originated from a different management background, and therefore different opportunities for exposure. In our study all cattle were allocated to treatment within source and day of weaning, resulting in an equal distribution of source and day of weaning to vaccination treatment.

Research indicates that *E. coli* O157:H7 infection occurs in cattle before weaning, prior to entry to feedlots (Leigreid et al., 1999). More specifically, *E. coli* O157:H7 is widely dispersed at low prevalence in prefeedlot, weaned calves (Dunn et al., 2004). In our study, *E. coli* O157:H7 was isolated from feces at weaning from only one of the two sources that participated in the project, and the overall prevalence of *E. coli* O157:H7 in the feces of freshly weaned calves was very low (0.40%). However, it should be noted that the majority of the samples were collected during the winter months. Research has documented the seasonal patterns associated with *E. coli* O157:H7 infection in cattle (Garber et al., 1999; Van Donkersgoed et al., 1999; Smith et al., 2005) and similar seasonal patterns for human cases of the illness (Mead et al., 1999; Wallace et al., 2000; Chapman et al., 2001). Nevertheless, the probability to detect *E. coli* O157:H7 in feces of these freshly weaned Montana calves was low.

The observed low probability to detect *E. coli* O157:H7 observed at weaning persisted throughout the entire production period. The probability of detecting *E. coli* O157:H7 in feces of cattle on this study never reached 1.0%.

We collected RAMS samples at harvest for two primary reasons. First, it has been suggested that RAMS provide a superior sampling method when compared to collecting individual fecal samples (Rice et al., 2003) because *E. coli* O157:H7 colonizes in cattle 3-5 cm proximal to the rectoanal juncture (Naylor et al., 2003). Secondly, we wanted to collect our final sample at the point of harvest without causing considerable disruption to normal packing plant operations. Although we were able to collect RAMS samples at chain speed in each of the packing plants the cattle in this study were harvested at, recent paired testing research conducted at the University of Nebraska suggests that fecal samples are a more sensitive sampling technique compared with RAMS under the conditions this study was conducted (Moxley, 2005).

The probability to detect *E. coli* O157:H7 associated with cattle was the highest at slaughter. Other research has documented similar results (Potter et al., 2004; Peterson et al., 2005). However, the cattle on this study were sent to slaughter primarily during the summer months, when one would expect higher levels of *E. coli* O157:H7 infection in cattle (Garber et al., 1999; Van Donkersgoed et al., 1999; Smith et al., 2005).

The probability to detect *E. coli* O157:H7 in VAC was numerically lower at harvest (1.00%) compared with the probability to detect *E. coli* O157:H7 associated with NOVAC cattle (1.37%). Although not statistically significant, it would be hard to rule out vaccination as an intervention strategy from the results of this experiment because the probability of detecting *E. coli* O157:H7

associated with cattle was so uncharacteristically low. The low sensitivity of RAMS compared with feces (Moxley, 2005) may help explain the low probability to detect *E. coli* O157:H7 at harvest associated with these Montana cattle. The results of paired testing comparing RAMS and feces at harvest suggest that fecal samples are more sensitive (Moxley, 2005). Given the results of that study, it is reasonable to suggest that the probability to detect *E. coli* O157:H7 at harvest would have been higher had we collected individual fecal samples instead of RAMS When the probability to detect *E. coli* O157:H7 in the feces is high, research has shown vaccine to be 59% and 70% effective compared with non vaccinated cattle (Potter et al., 2004; Peterson et al., 2005).

Implications

Because of the low prevalence of *E. coli* O157:H7 shedding throughout the entire production period, this study could not determine the effect of vaccination as a pre-harvest food safety intervention strategy. Additional research should evaluate pre-harvest interventions with methodology to detect colonization in the individual animal or assign treatments to the pen.

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EFFECTS OF FEEDING ENDOPHYTE-INFECTED TALL FESCUE SEED TO SHEEP EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI* O157:H7[1]

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ABSTRACT: *Escherichia coli* O157:H7 is one of the most common agents of foodborne illness in humans. Objectives of this experiment were to determine the effects of short-term exposure to endophyte-infected tall fescue seed on fecal shedding and intestinal concentrations of *E. coli* O157:H7 in sheep. Twelve ewes (mean BW = 45 kg) were blocked by body weight and breed, and fed a diet containing either endophyte-infected (E+) or endophyte-free (E-) tall fescue seed for 7 d. Each diet consisted of 50% (DM basis) tall fescue seed. Ewes were experimentally inoculated with *E. coli* O157:H7 on d 2 of feeding treatment and fecal shedding of inoculated pathogens was monitored daily. On d 7, ewes were euthanized, tissues and contents sampled from the ileum, cecum, and rectum for quantification and qualification of the challenge strain of *E. coli* O157:H7, and urine collected to determine total ergot alkaloid concentrations. Ewes fed E+ seed had lower ($P < 0.05$) DMI than E- ewes (0.8 and 1.6 kg/d DMI for E+ and E- ewes, respectively); consequently, E+ ewes lost 0.3 kg/d and E- ewes gained 0.2 kg/d during the 7 d ($P = 0.06$). Concentrations of urinary ergot alkaloids were increased ($P < 0.001$) in ewes fed E+ (67.3 ng/g creatinine) than E- ewes (5.3 ng/g creatinine). Fecal shedding of *E. coli* O157:H7 was increased ($P < 0.06$) in E+ ewes [5.4 cfu ($\log_{10}$)/g feces] compared with E- ewes [4.5 cfu ($\log_{10}$)/g feces]. Population of *E. coli* O157:H7 in luminal contents from the ileum, cecum, and rectum did not differ ($P > 0.10$) between treatments. Treatment did not influence ($P > 0.10$) the occurrence of *E. coli* O157:H7 in cecal or rectal tissues; however, ileum tissues from E+ ewes tended ($P = 0.12$) to have an increased incidence of *E. coli* O157:H7. We conclude that short-term consumption of endophyte-infected tall fescue seed will decrease DMI and subsequent ADG, and may increase fecal shedding of *E. coli* O157:H7 in sheep. Further research is needed to determine if the observed increase in pathogen shedding is caused by ingestion of endophyte-infected tall fescue or alterations in nutrition; specifically, reduced dry matter intake.

Key Words: *Escherichia coli* O157:H7, Tall Fescue, Sheep

Introduction

Escherichia coli O157:H7 is one of the most common agents of foodborne illness in humans and has been isolated from ruminants at all stages of production (Elder et al., 2000). Annually, pathogenic bacteria-related illnesses cost an estimated $2.9 to 6.7 billion (Buzby et al., 1996).

A majority of the research investigating *E. coli* O157:H7 shedding has been conducted on confined, grain-fed cattle. A survey of 100 feedyards throughout the U.S. reported a 1.8% incidence of *E. coli* O157:H7 shedding (APHIS, 1995). Incidence of the pathogenic shedding of grazing animals is more limited. Laegreid et al. (1999) isolated *E. coli* O157:H7 from 13 of 15 cow-calf herds. Incidence of fecal shedding within positive herds ranged from 1.7 to 20% of individual animals shedding *E. coli* O157:H7 in their feces.

Stress may predispose animals to metabolic disease, making stressed animals more susceptible to opportunistic bacteria such as *E. coli* O157:H7 (Hillman et al., 1982). Fitzgerald et al. (2003) reported that the incidence of fecal shedding of *E. coli* and *Salmonella* shedding tended to be increased by production stressors (i.e., milking status and lactation phase) in dairy cows. It is well documented that cattle grazing endophyte-infected (E+) tall fescue have increased body temperature during summer months, reduced milk production and reproductive performance, and decreased growth rate (Hoveland et al., 1983; Paterson et al., 1995). The maladies associated with ingestion of E+ tall fescue appear to be associated with the alkaloids produced by the fungus *Neotyphodium coenophialum* (Hill et al., 1994; Glenn et al., 1996).

We recently reported that fecal shedding of *E. coli* O157:H7 in naturally-infected cattle tended to be reduced in calves and was decreased in cows grazing E+ tall fescue (*Festuca arundinacea* Schreb.) compared with cows and calves grazing E- tall fescue during the summer months (Looper et al., 2003). Further, steers previously grazing E+ tall fescue, and then confined and fed common bermudagrass [*Cynodon dactylon* (L.) Pers.] hay shed less *E. coli* O157:H7 than steers continuously grazing common bermudagrass in the summer (Looper et al., 2005). However, *in vitro* studies utilizing three ergot alkaloids commonly found in E+ tall fescue did not affect growth of *E. coli* O157:H7 (Looper et al., 2004). Therefore, the objectives of this experiment were to determine the effects of short-term exposure to E+ tall fescue seed on fecal

[1]Names are necessary to report factually on available data; however, the USDA does not guarantee or warrant the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that also may be suitable.

shedding and intestinal concentrations of *E. coli* O157:H7 in sheep.

Materials and Methods

Twelve non-lactating hair-type sheep (n = 6 each of Katadhin and St. Croix; mean BW = 45 kg; mean age 2.7 y) were blocked by body weight and breed, and housed indoors in individual pens. Ewes were acclimated to the diet (corn substituted for fescue seed) for 7 d prior to the initiation of the experiment. On d 0 of the experiment, ewes were fed a diet containing either endophyte-infected (E+) or endophyte-free (E-) tall fescue seed for 7 d (Table 1). Ewes were offered the diet at 3.5% BW, and orts were weighed daily to calculate DMI. Concentrations of ergovaline in diets were determined by HPLC (Moubarak et al., 1996) and were 748 and 112 ppb for the E+ and E- seed diets, respectively. Ewes were experimentally inoculated with *E. coli* O157:H7 on d 2 of feeding treatment and fecal shedding of inoculated pathogens was monitored daily. On d 7, ewes were weighed and euthanized. Tissues and contents were sampled from the ileum, cecum, and rectum for quantification and qualification of the challenge strain of *E. coli* O157:H7, and urine collected to determine total ergot alkaloid concentrations via immunoassay (Hill et al., 2000). The Animal Care and Use Committee of the USDA-ARS, Food and Feed Safety Research Laboratory approved care, use, and handling of experimental animals.

Bacterial cultures. *Escherichia coli* O157:H7 strain BDMS T4169 (ATCC 700728) was obtained from the American Type Culture Collection (Manassas, VA) and was cultivated in anoxic TSB medium at 37ºC. This strain was made resistant to novobiocin and nalidixic acid (20 and 25 µg/mL, respectively) via successive cultivation in tryptic soy broth (TSB) containing up to 20 µg/mL of novobiocin and 25 µg/mL nalidixic acid. Overnight cultures (1,000 mL) were harvested by centrifugation (7,500 x g, 10 min) and the cell pellets were re-suspended in TSB medium (150 mL total volume). Sheep were individually inoculated with 10 mL of TSB containing *E. coli* O157:H7 (4×10^{11} cfu) via oral gavage. Fecal samples were collected 3 d prior to dosing and screened for the presence of wild-type *E. coli* O157:H7 and generic *E. coli* resistance to novobiocin and nalidixic acid. The day of dosing (d 2) and on each of the subsequent 5 d, fecal samples were collected and fecal shedding of inoculated *E. coli* O157:H7 were qualitatively and quantitatively enumerated daily.

Bacterial Enumeration. Ten to 15 g of fecal material were collected from each ewe daily. From each composited fecal sample, 1 g of fecal material was serially diluted (10-fold increments) in sterile PBS and plated on MacConkey's agar, each agar supplemented with novobiocin (20 µg/mL) and nalidixic acid (25 µg/mL). Plates were incubated 24 h at 37ºC and colonies that grew on agar plates directly counted (quantitative enumeration). To qualitatively confirm the presence of inoculated *E. coli* O157:H7, daily fecal samples, intestinal contents, and epithelial tissue samples were incubated (24 h, 37ºC) in 20 mL GN Hajna with novobiocin/naladixic acid and streaked on agar plates as above. Plates showing colony growth were judged to be positive for the inoculated bacteria (qualitative enumeration).

Statistical analyses. Daily fecal shedding and dry matter intake were analyzed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with a compound symmetry covariance structure. Treatment, day, and ewe were included in the model. Effects of treatment on ADG, urinary concentrations of total ergot alkaloid, and bacterial counts from luminal contents (quantitative) were determined by the MIXED procedure of SAS. Chi-square analysis, using the FREQ procedure of SAS, was used to determine influence of treatment on qualitative bacterial enumeration of epithelial tissue samples. Least squares means were compared using the PDIFF statement of SAS when protected by a significant ($P < 0.05$) treatment effect.

Results and Discussion

Ewes fed E+ seed had lower ($P < 0.05$) DMI than E- ewes (0.8 and 1.6 kg/d DMI for E+ and E- ewes, respectively); consequently, E+ ewes lost 0.3 kg/d and E- ewes gained 0.2 kg/d during the 7 d ($P = 0.06$). It is well established that consumption of E+ tall fescue will reduce DMI and weight gains in ruminants (Aldrich et al., 1993; Paterson et al., 1995). Urinary concentration of total ergot alkaloids was increased ($P < 0.001$) in ewes fed E+ (67.3 ng/g creatinine) compared with E- ewes (5.3 ng/g creatinine). Stuedemann et al. (1998) found concentrations of urinary ergot alkaloids increased within 2 d of steers initiating E+ tall fescue grazing.

Table 1. Composition of endophyte-infected or endophyte-free tall fescue seed diets fed to sheep experimentally infected with *E. coli* O157:H7

Item	DM basis
Fescue seed[1]	50.0
Cottonseed hulls	15.0
Corn	11.1
Corn gluten	8.3
Soyhulls	6.4
Molasses	6.6
Vitamins (A, D, and E)	0.1
Limestone	1.4
Dicalcium	0.9
Salt	0.2

[1]Either endophyte-infected or endophyte-free tall fescue seed.

Fecal samples collected prior to inoculation with *E. coli* O157:H7 were negative for wild-type *E. coli* strains. Fecal shedding data of *E. coli* O157:H7 during the 5-d collection are shown in Figure 1. There was no treatment x day interaction ($P > 0.10$); however, overall mean shedding of *E. coli* O157:H7 was increased ($P < 0.06$) in E+ ewes [5.4 cfu ($\log_{10}$)/g feces] compared with E- ewes [4.5 cfu ($\log_{10}$)/g feces]. These data contradict our previous work that suggested consumption of E+ tall fescue reduced fecal shedding of *E. coli* 157:H7 in naturally infected cattle (Looper et al., 2003, 2005). Differences in fecal shedding

patterns between experimentally inoculated animals and naturally infected animals may explain variations among these studies. Shedding of *E. coli* O157:H7 in naturally infected animals is very sporadic, with an animal testing positive at one sample collection and negative at the subsequent collection (Callaway et al., 2004). Length of exposure to the toxic effects of E+ tall fescue also may attribute to differences between studies. Currently, sheep were exposed to a diet containing E+ seed for 7 d while cattle utilized in our previous studies (Looper et al., 2003, 2005) were exposed to E+ tall fescue from 87 d to approximately 2 y. It is possible that animals may become acclimated to the toxic effects of fescue after prolonged exposure (Spiers et al., 2005). Differences in diets between the current sheep experiment (total mixed ration) and previous cattle studies (forage grazing) may have contributed to differences in fecal shedding. Sudden alterations in diet, specifically a shift from concentrate to forage feeding may decrease pathogen shedding (Callaway et al., 2003). Further, possible differences in the pattern of fecal shedding between cattle and sheep also may have influenced results within studies.

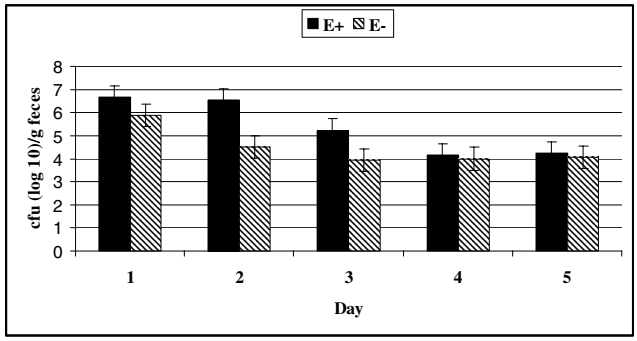

Figure 1. Fecal shedding [cfu (log_{10})/g feces] of *E. coli* O157:H7 in sheep experimentally infected with *E. coli* O157:H7 and fed diets of endophyte-infected (E+) or endophyte-free (E-) tall fescue seed; treatment effect ($P < 0.06$; standard error = 0.49); treatment x day interaction ($P > 0.10$).

Luminal contents from the ileum, cecum, and rectum contained similar ($P > 0.10$) populations of *E. coli* O157:H7 between treatments (Table 2). Tissue samples (after a 24-h enrichment) from the cecum and rectum had a similar ($P > 0.10$) occurrence of *E. coli* O157:H7; however, ileum tissues from E+ ewes tended ($P = 0.12$) to have an increased incidence of *E. coli* O157:H7 (Table 2).

Stress may predispose animals to metabolic disease, making animals more susceptible to opportunistic bacteria such as *E. coli* O157:H7 (Hillman et al., 1982). Common production stressors such as milking status (lactating or non-lactating) and lactation phase ($\leq$ 60 or > 60 d in milk) influenced pathogenic shedding in dairy cows (Fitzgerald et al., 2003). Lactating dairy cows shed more *E. coli* O157:H7 in their feces than non-lactating cows, and cows less than 60 d in milk shed more *Salmonella* than cows > 60 d in milk (Fitzgerald et al., 2003). Cattle grazing E+ tall fescue have increased body temperature during summer months, reduced milk production and reproductive performance, and decreased growth rate (Hoveland et al., 1983; Paterson et al., 1995). Sheep consuming E+ seed diets in the current experiment had decreased DMI and subsequent weight loss that are typical of fescue toxicosis. Further, there was a 12-fold increase in urinary alkaloid concentrations in E+ ewes compared with E- ewes. Collectively, these responses indicate sheep consuming E+ tall fescue seed were experiencing some degree of fescue toxicosis, and the stress associated with this toxicosis may have increased fecal shedding of *E. coli* O157:H7.

Table 2. Luminal contents [cfu (log_{10})/g feces] and tissue samples (number of ewes positive) of the gastrointestinal tract positive for *E. coli* O157:H7 in sheep experimentally infected with *E. coli* O157:H7 and fed diets of endophyte-infected (E+) or endophyte-free (E-) tall fescue seed

Item	E+	E-	SEM
Luminal contents			
Ileum	2.10	2.75	0.61
Cecum	2.27	3.06	0.57
Rectum	2.64	2.09	0.73
Tissue samples			
Ileum	6/6[a]	4/6[b]	--
Cecum	5/6[a]	6/6[a]	--
Rectum	6/6[a]	5/6[a]	--

[a,b]Numbers in a row with no superscript in common differ ($P = 0.12$).

Further research is needed to determine if the observed increase in pathogen shedding is caused by ingestion of E+ tall fescue or alterations in nutrition; specifically, reduced dry matter intake. Animal species, environmental conditions, type of diet, and length of exposure to the toxic effects of E+ tall fescue all may influence the association between consumption of E+ tall fescue and pathogenic shedding from livestock.

Implications

Short-term consumption of endophyte-infected tall fescue seed decreased DMI and subsequent ADG, and increased urinary concentrations of total ergot alkaloids in sheep. Fecal shedding of *E. coli* O157:H7 was increased in sheep consuming endophyte-infected tall fescue seed. Management strategies that prevent livestock from grazing endophyte-infected tall fescue and/or alleviate stressors associated with consumption of endophyte-infected tall fescue prior to harvest may be necessary to reduce fecal shedding of pathogenic bacteria from livestock.

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VACCINATION AS AN INTERVENTION STRATEGY FOR REDUCTION OF *ESCHERICHIA COLI* 0157:H7 IN CATTLE FECES[1]

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ABSTRACT: Three hundred sixty-seven recently weaned steers were used in a growing (45 d) and finishing (189 d) experiment to determine if vaccination with an experimental *E. coli* O157:H7 vaccine would reduce fecal shedding and elevate antibody titers. Treatments (Trt.) compared were: Group 1) received two doses of the *E. coli* vaccine in the growing phase only; Group 2) received two doses of the *E. coli* vaccine during the growing phase and a third dose on d 100 of the finishing phase; Group 3) received two doses of the *E. coli* vaccine; one on d 21 of the finishing phase and a second dose on d 100 of the finishing phase. Treatment group 4 served as the control (no vaccination). On d 0 and 45 of the growing phase and d 21, 100 and 162 of the finishing phase, fecal grab and venous blood samples were collected. Fecal samples were analyzed for *E. coli* O157:H7 by Food Safety Net Services. Blood samples were sent to Fort Dodge Laboratories for analysis of antibody titers against *E. coli* O157:H7. Prevalence of fecal *E. coli* O157:H7 was not different (P>0.05) at any sampling period among treatment groups. The initial and final prevalence rates during the feedlot phase for Trt. 1 were one and 10%; Trt. 2 were 3 and 8%; Trt. 3 were 3 and 17% and Trt. 4 (Control) were 5 and 8%, respectively. Serum titers did show an elevated immune response. During the 45 d growing phase, the vaccine increased (P<0.001) blood titers from an average of 62 to 2,217. During the finishing period vaccinated calves had higher titers compared to control calves (avg. of Trt 1, 2, 3 vs. Trt 4 (P<0.05). Vaccination during the 45 d growing phase and then again on d 100 of the finishing phase (Trt. 2) resulted in the greatest (P=0.012) pre-slaughter titer level, but did not affect (P>0.05) *E. coli* O157:H7 prevalence. At harvest, Trt 1. steer's titre levels had returned to near pre-vaccination levels indicating that the immune levels did decline over time. Although an immune response was generated by this vaccine, the limited number of animals shedding *E. coli* O157:H7 warrants additional research in calves with higher levels of shedding.

Key words: *E. coli* O157:H7; Cattle; Vaccination

Introduction

During the past few years, significant investment [1]dollars have been allocated towards research investigating intervention strategies to reduce contamination of beef carcasses with *E. coli* O157:H7. Both pre- and post-harvest methods have been evaluated. Post-harvest interventions have dealt with controlling foodborne pathogens in a "Multiple Hurdle Approach" including washing cattle prior to harvest followed by pre-evisceration wash with water and an organic acid, steam vacuuming, hot water wash and proper carcass chilling. Recently more emphasis has been placed on attacking pathogens at their source. These "pre-harvest" methods investigated have included dietary changes, direct fed microbials, water treatments and the addition of antibiotics to rations prior to slaughter. The direct fed microbials are currently the only products commercially available claiming to reduce *E. coli* O157:H7 in live cattle. Many of the attempted pre-harvest interventions however, have met with challenges including ethics, low overall effectiveness and/or production feasibility.

Initial experiments have developed hypothesis towards the possibility of vaccination against enterohemorrhagic *E. coli* (McKee et al. 1995, Dean-Nystrom et al. 1998 and Cornick et al. 2002). These experiments revealed the basic mechanisms by which the pathogenic *E. coli* attach to the epithelial cells of the gastrointestinal tract. Immunity was first used to protect livestock (pigs; Dean-Nystrom et al., 2002) from asymptomatic infection with enterohemorrhagic *E. coli*. Two vaccines have been developed and are currently awaiting government approval for use in beef cattle. One of these vaccines has been successfully tested (Potter et al. 2004; Peterson et al. 2005).

The objective of this study was to evaluate the effectiveness of vaccinating live cattle prior to harvest, as a method of pre-harvest reduction in the shedding of *E. coli* O157:H7.

Materials and Methods

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Three hundred sixty-seven steers were transferred from a 45 d pre-conditioning experiment where 183 of the 367 steers had received vaccination against *E. coli* O157:H7 on d 0 and 21 of the 45 d pre-conditioning period.

Pre-conditioning Experiment Background. Three hundred eighty-nine heifers and three hundred sixty-seven steers were selected from a single herd in central MT. The selected herd was composed of Angus and Simmental genetics and primarily calves in February and March. The calves selected were removed randomly from their mothers (weaned) over a three week period in October of 2003 with approximately one third of the calves being weaned each week. On the day of weaning, calves were transported from pasture to a receiving yard consisting of six, 150 hd pens and an appropriate cattle handling facility. Upon arrival at the receiving yard, calves were sorted by gender (steers vs. heifers), placed in holding pens and then processed separately. At processing all calves received an individual electronic identification tag, and an individual panel tag each with a unique number and a single dose of: 1) Nasalgen IP (Schering Plough); 2) Pyramid 4 + Presponse (Fort Dodge Animal Health); 3) Vision 7 + Somnus (Intervet); and 4) Cydectin pour-on (Fort Dodge Animal Health). The Pyramid 4 + Presponse and Vision 7 + Somnus were administered subcutaneously on the right side of the animal in the lateral neck and boosted on d 21.

A systematic randomization scheme was utilized so that every other animal through the chute would be given a dose of the experimental *Escherichia coli* vaccination (E-vac) designed to prevent the attachment of *Escherichia coli* O157:H7 to the intestinal wall of cattle. The *E. coli* vaccine was administered sub-cutaneously in the left neck at a dose of 2 ml / head and was boosted with a second dose on d 21. Following processing on d 0, calves were systematically allocated to pens as they exited the chute; resulting in pens with a final disposition of approximately 50% E-vac and 50% control within gender. Calves were fed a grass hay based diet combined with a protein supplement that contained additional minerals and vitamins The response variables measured were initial weight (d 0), final weight (d 45), average daily weight gain, blood antibody titers (d 0 and 45) and individual fecal prevalence of *E. coli* O157:H7 (d 0 and 45).

Finishing Phase Experimental Design. Only steers (n = 367) from the pre-conditioning experiment were utilized in the finishing experiment. Prior to shipping from the pre-conditioning facility to the feedlot, steers were individually weighed (feedlot initial weight), treated again for internal and external parasites with Cydectin pour-on, boosted with Pyramid 4 + Presponse and randomly sorted four ways. The randomization for the sort was conducted by sorting steers only (in a database) by previously vaccinates and previously controls. All sorted steers were then assigned a random number using Microsoft Excel's random number generator. Steers which were previously controls were sorted by random number in ascending order and steers from previously vaccinates were sorted by random number in ascending order. The first 92 steers from the previously vaccinated group were assigned into finishing group 1; the second 92 steers from the previously vaccinated group were assigned into finishing group 2; the first 91 steers from the previously control group were assigned into finishing group 3 and; the remaining 92 steers from the previously control group were assigned into finishing group 4. Four feedlot pens were utilized and pens were assigned by alternating pen numbers 1 – 4 throughout the list of steers in each finishing group until all steers were assigned to a pen. The pen allotment was designed to provide for equal finishing group representation in each pen.

At shipping to the feedlot each of the four sorts were placed on a separate truck and upon feedlot arrival each truck was placed in a separate pen as was previously assigned in the randomization. All steers were fed a similar high energy ration throughout the experiment, were individually weighed on d 0, 21, 100 and prior to harvest on d 162 and given a Synovex Choice implant on d 21 and 100.

Treatments were assigned to each finishing group as follows (Table 1.): Group 1 received two doses of the *E. coli* vaccine in the pre-conditioning phase only; Group 2 received two doses of the *E. coli* vaccine during the pre-conditioning phase and a third on d 100 of the finishing phase; Group 3 received two doses of the *E. coli* vaccine one on d 21 and one on d 100 of the finishing phase; and Group 4 never received any *E. coli* vaccine (Control).

Sample Collection. On d 0 and 45 of the pre-conditioning experiment and on d 21, 100 and 162 of the finishing phase a fecal grab and venous blood sample was collected from all calves. The fecal samples were collected aseptically via rectal palpation using a new OB glove for each animal and then placing approximately 50 g of feces into a new screw top cup (Fisher Scientific). Cups were labeled with barcodes and then placed in shipping containers with ice packs to prevent temperature abuse. Samples were shipped overnight to Food Safety Net Services (San Antonio, TX) for analysis.

Recovery of E. coli O157:H7. Samples were analyzed for the presence of *E. coli* O157 using the previously described (Barkocy-Gallagher et al., 2002) MARC MRU method. The method involved enrichment in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.), immunomagnetic separation, and selective plating.

Antibody Titre Analysis. Ten milliliters of whole blood were collected from the jugular or tail vein. Blood samples were placed on ice and transported to the Montana State nutrition laboratory, where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analysis of serum for antibody titres *E. coli* O157:H7 were conducted by Fort Dodge Animal Health Laboratories, Ft. Dodge, Iowa. Serum titres against *E. coli* O157:H7 were analyzed using an ELISA (enzyme-linked immunosorbent assay; Widiasih et al. 2004). Samples were dissolved at 1mg/ml in 1xPBS (phosphate buffered saline) solution and diluted to 1:100 with the 1xPBS for a concentration of 10µg/ml (Cray et

al. 1995). Microtiter plates were coated with diluted LPS solution. The samples were placed through a series of similar steps of washing and coating the plates. Results were then read by a spectrophotometer at 405 nm, approximately 10 – 30 min after the addition of a substrate solution. The results are expressed according to the final dilution factor on the plate. (Fort Dodge Animal Health, 2004).

Statistical Analysis. The odds of a vaccinated animal shedding *E. coli* O157:H7 was compared to that of unvaccinated control cattle, accounting for repeated measures and pen using the GENMOD procedure of SAS (SAS Inst., Inc., Cary, NC). Odds ratios (OR) and their 95% confidence limits are reported. Antibody titre data was evaluated using the MIXED procedure of SAS accounting for repeated measures and pen. Feedlot performance was evaluated using the MIXED procedure of SAS accounting for pen.

Results and Discussion

E. coli O157:H7. In total, *E. coli* O157:H7 was recovered from 101 of 1,835 (5.5%) fecal samples. Table 1. shows the odds ratios (odds of a vaccinated animal shedding *E. coli* O157:H7 compared with controls), the 95% confidence limits and the probability that differences were due to vaccination. The fifth sampling period was the only period used in the overall analysis due to it being the only period following all vaccination as was designed. Results presented in Figure 1 show the prevalence of control cattle that were at or below the levels of all vaccinated groups resulting in unfavorable odds ratios relative to vaccine efficacy. The high level of variation in the 95% confidence limits of the odds ratios indicates that more post vaccination samplings would have been beneficial. These design flaw conclusions are substantiated by the results reported by Peterson et al., (2005) where four post treatment test periods were used to evaluate vaccine efficacy and resulted in a significant reduction in shedding. Moreover, point in time estimates of *E. coli* O157:H7 may misrepresent the true prevalence of *E. coli* O157:H7 associated with cattle (Smith et al., 2005). Additionally, in a similar experimental design where 75% of cattle in a pen received vaccination, Peterson et al. (2005) reported that unvaccinated cattle commingled with vaccinated cattle were less likely to shed *E. coli* O157:H7 than cattle in pens not receiving vaccine (external controls). Therefore, the low probability to detect *E. coli* O157:H7 in the current experiment may be a result of herd immunity. Never the less these data suggest (P = 0.2742) that the vaccine was unsuccessful in reducing the prevalence of *E. coli* O157:H7 in the feces of cattle under the conditions of this experiment.

Table 1. Experimental design and odds of detecting E. coli O157:H7 in cattle feces at harvest with 95% confidence limits by group.

	Treatment Groups			
Day of Trial / Phase	**1**	**2**	**3**	**4**
- 45 / Pre-Conditioning	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control	Sampled / Control
- 24 / Pre-Conditioning	Vaccinated	Vaccinated	Control	Control
0 / Finishing	Sampled	Sampled	Sampled	Sampled
			Feedlot Arrival	
21 / Finishing	Sampled / Control	Sampled / Control	Sampled / Vaccinated	Sampled / Control
100 / Finishing	Sampled / Control	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control
183 / Finishing	Sampled	Sampled	Sampled	Sampled
			Harvest	

		95% Confidence Limits		
	Odds Ratio	**Lower**	**Upper**	**P-value**
Treatment Group				
1	1.29	0.46	3.61	0.2742
2	1.02	0.34	3.05	
3	2.37	0.92	6.13	
4	1.00	1.00	1.00	

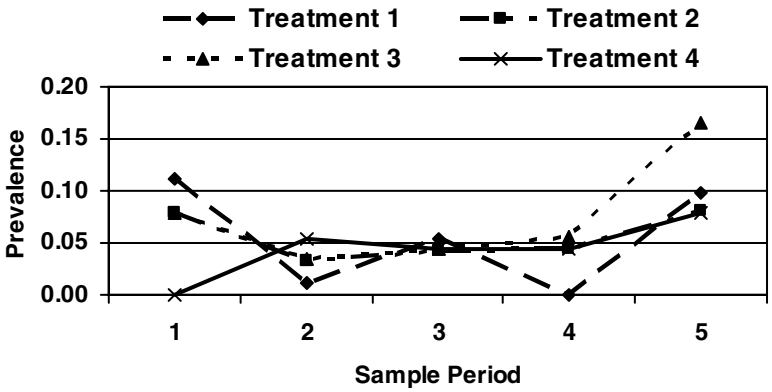

Figure 1. The proportion of steers shedding *E. coli* O157:H7 by sample period and treatment group. Where: Treatment 1 = vaccination during pre-conditioning only, Treatment 2 = vaccination during pre-conditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

Antibody Titers. Venous blood samples were collected on d 0 and 45 of the pre-conditioning experiment and on d 21, 100 and 162 of the finishing phase. Blood titer levels were elevated after vaccination ($P < 0.0001$; Figure 2). There were no differences in pre-vaccination blood titer levels ($P = 0.25$). Vaccination during the pre-conditioning phase (groups 1 and 2) did increase blood titers ($P < 0.0001$) compared with controls (groups 3 and 4). The blood titer levels of steers that were vaccinated only during the pre-conditioning phase (group 1) returned to approximately pre-vaccination levels by the fourth sampling period indicating that effects of vaccination may have a defined effectiveness. The group that maintained the highest overall immune response were those vaccinated during pre-conditioning and again on d 100 of the finishing phase (Group 2). The steady decline of titer levels by Groups 1 and 2 following test period 2 indicate that the feedlot dose may have been delayed in Group 2 and may have been more beneficial at d 21. This observation if substantiated could prove beneficial to the overall acceptance of the vaccine in production systems if vaccination during pre-conditioning and again upon feedlot entry proved to be the most efficacious, however further experiments are required to confirm these observations. The Group 3 steers were only vaccinated on d 21 and 100 of the finishing phase and overall did not respond as well as those steers vaccinated during the pre-conditioning phase. At harvest all vaccinated groups continued to have higher ($P \leq 0.02$) titer levels compared with controls, but these effects on shedding of *E. coli* O157:H7 were limited due to low prevalence.

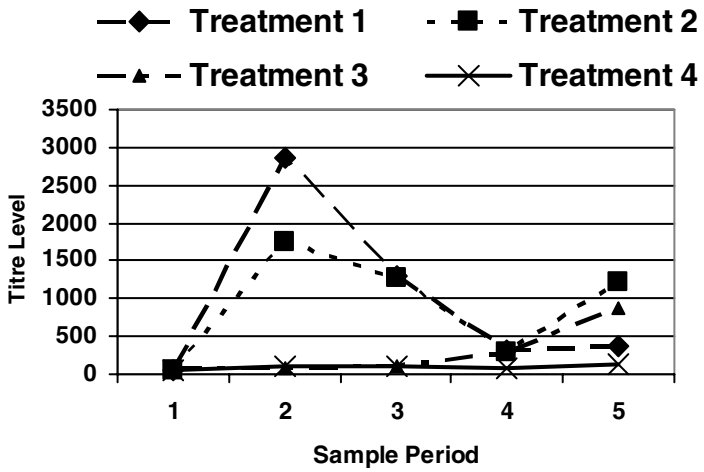

Figure 2. *E. coli* O157:H7 antibody titre levels by sample period and treatment group (Vaccination effect - $P = 0.0001$; Interaction effect – $P = 0.0001$). Where: Treatment 1 = vaccination during pre-conditioning only, Treatment 2 = vaccination during pre-conditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

Feedlot Performance. Least squares means for finishing performance measures are presented in Table 2. There were no differences (P > 0.10) in finishing performance for steers receiving the *E. coli* vaccine in three different regimes. These data suggest vaccinating cattle against Type III secretory proteins of enterohemorrhagic *Escherichia coli* will have no considerable impact on finishing performance.

Table 2. Effects of Vaccination against *E. coli* O157:H7 on finishing performance of steers

	Treatment Group [a]					
Item	1	2	3	4	SEM[b]	VAC[c]
Steers	92	92	91	92		
Initial BW, kg	274	264	270	273	3.28	0.16
Final BW, kg	525	511	512	520	5.38	0.19
Daily gain, kg	1.55	1.52	1.49	1.53	0.02	0.48

[a]1=vaccination during pre-conditioning only; 2=vaccination during pre-conditioning and on d 100 of finishing; 3=vaccination on d 21 and 100 of the finishing phase; 4=no vaccination (control)
[b]Standard error of least squares means
[c]Main effect of vaccination treatment

Implications

In conclusion an effect of vaccination on Escherichia coli O157:H7 shedding was not detected, however, elevated blood titres against *Escherichia coli* O157:H7 indicate that an immune response was generated. The limited number of animals found positive for *Escherichia coli* O157:H7 in this experiment coupled with limited post vaccination test periods and an elevated immune system warrant additional research of this vaccine. The blood titer levels may be beneficial in the development of further experiments targeting practical use of this vaccine.

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PREVALENCE OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN BEEF CATTLE

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ABSTRACT: The beef safety concerns have increased recently due to tracing a large number of human illness outbreaks to consumption of Shiga toxin-producing *Escherichia coli* (STEC)-contaminated beef products. These illnesses include diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. The objective was to assess STEC prevalence in beef cattle under different production conditions. Fecal samples from 970 cattle (321 in feedlots, 240 grazing irrigated pastures, and 409 grazing rangeland forages) from 14 beef operations in California were tested in the summer and fall of 2004. STEC prevalence rates ranged from 3.7 to 7.5% in feedlot cattle, from 1.3 to 3.8% in cattle grazing irrigated pastures, and from 1.3 to 15.0% in those grazing rangeland forages. No effects on prevalence rates of STEC were found for the season, time on feed (11 to 91 d vs 110 to 290 d), or age (cows vs calves). The prevalence rate, however, was higher for cattle on the range than for those on pasture (4.6 vs 1.7%). The STEC isolates belonged to 21 serotypes (O1:H2, O26:HUT, O86:H19, O125:H2, O125:H19, O125:H27, O125:H28, O125:HUT, O127:H2, O127:H28, O128:H2, O128:H20, O153:HUT, O158:H28, O158:HUT, O166:H2, O166:H20, OUT:H2, OUT:H19, OUT:H20, and OUT:HUT). Of these, 2 (*E. coli* O1:H2 and OUT:H2) are known to cause human illnesses and 16 (*E. coli* O1:H2, O26:HUT, O86:H19, O125:H19, O125:H27, O125:H28, O125:HUT, O127:H2, O127:H28, O128:H2, O128:H20, O158:H28, O158:HUT, O166:H2, O166:H20, and OUT:H20) have not been reported previously in beef or dairy cattle. Interestingly, *E. coli* O157 isolates were not found in the cattle tested.

Key words: Food Safety, *Escherichia coli*, Beef Cattle

Introduction

The safety concerns with foods of bovine origin began with the first two reported outbreaks of human illnesses in 1982. In these outbreaks (Riley et al., 1983), human infections with Shiga toxin-producing *Escherichia coli* (STEC) were traced to contaminated ground beef in Michigan and Oregon. Worldwide, the number of outbreaks and sporadic cases of STEC infections due to consumption of STEC-contaminated ground (CDC, 2003), roast (CDC, 2003), or smoked (Germani et al., 1997) beef has been on the rise. Other beef products such as sausage (CDC, 1995), steak (CDC, 2003), tri-tip (CDC, 2003), and veal (CDC, 2003) were also implicated in human illnesses that included (Paton and Paton, 2000) mild diarrhea, bloody diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS). These illnesses can lead to death of individuals with compromised immune systems. The increased number of outbreaks and the severity of human illnesses due to consumption of undercooked beef emphasized its role as an important vehicle of STEC transmission.

Because beef cattle were found to harbor a wide range of STEC serotypes at high rates, they are considered reservoirs of these foodborne pathogens (Hancock et al., 1997). STEC from beef cattle included O157 and over 200 non-O157 serotypes (WHO, 1998). Prevalence of STEC in beef cattle varied among production systems. With regard to *E. coli* O157, the prevalence rates ranged from 0.3 to 19.7% in feedlot cattle (Hancock et al., 1994; □i□ek et al., 1999) and from 0.7 to 27.3% in grazing cattle (Hancock et al., 1994; Ezawa et al., 2004). Higher prevalence rates were reported for non-O157 STEC and ranged from 4.6 to 55.9% in feedlot cattle (Gioffré et al., 2002; Padola et al., 2004) and from 4.7 to 44.8% in grazing cattle (Thran et al., 2001; Geue et al., 2002).

Of the large number of STEC strains from beef cattle, 44 serotypes (i.e., O2:H7, O2:H29, O5:H$^-$ [non-motile], O6:H$^-$, O8:H2, O8:H21, O22:H8, O25:H2, O26:H11, O26:H$^-$, O49:H$^-$, O55:H$^-$, O91:H10, O91:H21, O98:H$^-$, O103:H2, O103:H$^-$, O105:H18, O105ac:H18, O111:H8, O111:H$^-$, O113:H21, O118:H16, O119:H6, O121:H19, O128ab:H2, O145:H28, O145:H$^-$, O153:H25, O157:H7, O157:H$^-$, O161:H$^-$, O163:H19, O165:H25, O165:H$^-$, O171:H$^-$, O174:H21, O174:H$^-$, OX3:H2, OX3:H21, OX3:H$^-$, OUT [an untypeable O antigen]:H2, OUT:H25, and OUT:H$^-$) are known to cause HUS in humans (WHO, 1998). Additional serotypes (e.g., O22:H$^-$, O26:H2, O26:H32, O39:H8, O77:H18, O82:H8, O84:H2, O91:H14, O91:HUT [an untypeable H antigen], O103:H25, O104:H7, OUT:H18, and OUT:H21) of beef cattle origin are also known to cause other human illnesses (WHO, 1998). With a few exceptions, data on prevalence of STEC in US beef cattle have been limited to *E. coli* O157. Because of this limitation and the health risks associated with non-O157 STEC, this study was designed to assess prevalence of O157 and non-O157 STEC in US beef cattle under various production conditions. Another objective was to determine effects of season and animal factors on STEC prevalence.

Materials and Methods

Study Population and Geographic Area. A total of 14 beef operations in California were enrolled in this study and included four feedlots ranging from 13,000 to 46,000 cattle and 10 cow/calf operations, four of which were on irrigated pastures (i.e., ranging from 60 to 1,350 cows per herd) and six were on rangelands (i.e., ranging from 65 to 225 cows per herd). In these beef operations, fecal samples

were collected from 970 cattle, with 321 from feedlot cattle (i.e., steers ranging from 4 to 15 mo old), 240 from cows and calves grazing irrigated pastures, and 409 from cows and calves grazing rangeland forages. The samples were collected in the summer (i.e., June and July) and fall (i.e., September and October) of 2004. A standardized questionnaire was administered to the farm or ranch owner or manager to collect data on herd composition and management.

Fecal Sampling. In the feedlots, fresh fecal samples were collected from 161 steers that had been present for the shortest period of time (i.e., ranging from 11 to 91 d) and from 160 cattle that had been present for the longest period of time (i.e., ranging from 110 to 290 d). In the cow/calf operations, the fecal samples were collected from 160 cows and 80 calves on pasture and from 199 cows and 210 calves on the range. Each fecal sample was placed in a sterile Whirl-pak bag and the bags were shipped on ice to our laboratory for analysis. Sample processing began at # 24 h after collection.

Enrichment and Initial Selection of STEC Isolates. Initial selection of *E. coli* isolates was conducted by adding 1 g of fresh feces from each animal to 50 mL of brain heart infusion (BHI) medium. This medium contained several antibiotics (i.e., cefixime at 50 :g/L, novobiocin at 20 mg/L, potassium tellurite at 2.5 mg/L, and vancomycin at 40 mg/L. The diluted feces were immediately incubated at 37EC for 12 h with continuous shaking to allow for antibiotic selection and toxin induction. At the end of the incubation time, the enriched fecal samples were serially diluted to 10^{-7} in BHI medium, plated in duplicate onto sorbitol-MacConkey (SMAC) agar, and incubated at 37°C for 18 h. At the end of this incubation time, sorbitol-fermenting (i.e., pink colonies) and non-sorbitol fermenting (i.e., white colonies) bacteria on SMAC plates were subcultured to 4-methylumbelliferyl-∃-D-glucuronide (MUG) MacConkey (MMUG) agar grid plates. Ten (i.e., sorbitol positive or negative) or less (i.e., when unavailable) colonies from each category were randomly selected and transferred to the MMUG plates. The MMUG plates were incubated at 37°C for 18 h and observed on a UV light box. Results were recorded for MUG positive or MUG negative (i.e., blue fluorescence or no fluorescence under UV light, respectively). When available, two colonies from each of the potential four biochemical categories (i.e., sorbitol positive/MUG positive, sorbitol positive/MUG negative, sorbitol negative/MUG positive, and sorbitol negative/MUG negative) were selected at random. These potential *E. coli* isolates were transferred from the MMUG plates to 5-mL tubes containing 2 mL of tryptic soy broth (TSB) and incubated at 37°C for 6 h with continuous shaking. At the end of the incubation time, the culture was diluted with equal volume (i.e., 2 mL) of sterile glycerol, mixed well, and stored at - 80°C. At that time, the same isolates from the MMUG plates were subjected to biochemical testing for *E. coli*.

Screening for Potential STEC Isolates. The enriched fecal samples were screened for STEC. This was accomplished by using the VTEC (i.e., verotoxin-producing *E. coli*)-Screen kit (Denka Seiken Co., Ltd., Tokyo, Japan). A total of 5 mL of enriched culture and 100 :L of polymyxin solution (Denka Seiken Co., Ltd., Tokyo, Japan) were incubated at 37EC for 30 min with continuous shaking to ensure optimal extraction of Shiga toxins from the periplasmic space. The mixture was then centrifuged at 900 ×*g* for 20 min and the supernatant was removed to test for Shiga toxins in 96-well V-bottom microtiter plates. In the microtiter plates, the culture supernatant (25 :L) was mixed with equal volume of the supplied diluent (i.e., phosphate buffered saline and 0.08% sodium azide). An equal volume of latex particles sensitized with rabbit polyclonal anti-Shiga toxin 1 (Stx1) and anti-Shiga toxin 2 (Stx2) immunoglobulin G antibody was mixed in the appropriate wells. The plates were mixed, covered, incubated at room temperature, and examined for latex agglutination after 18 h. The positive and negative control toxins supplied with the kit were run with each assay. A positive result was recorded when agglutination in the sample well was two levels above the control well.

Identification of E. coli Isolates with Shiga Toxin Potential. The isolates that were selected based on sorbitol fermentation and ∃-glucuronidase activity (i.e., maximum eight for each fecal sample) were tested for *E. coli* biochemically by using the API 20E Identification System (bioMérieux Vitek, Inc., Hazelwood, MO). Only isolates that were confirmed as *E. coli* were stored at - 80°C.

Identification of STEC Isolates. *E. coli* isolates were grown in 5 mL BHI at 37°C for 12 h with continuous shaking. At the end of the incubation time, the cultures were subjected to the same agglutination assay (i.e., VTEC-Screen kit) as described previously for the enriched fecal samples.

Verocytotoxicity of STEC Isolates. The STEC isolates were tested for cytotoxicity (Thran et al., 2001).

Identification of Shiga Toxins. The VTEC-Reversed Passive Latex Agglutination (VTEC-RPLA) assay (Denka Seiken Co., Ltd., Tokyo, Japan) was used according to the manufacturer's instructions after growing the isolates in BHI at 37°C for 12 h.

Expression of the Enterohemolysin gene. Enterohemolysin (Hly) is a toxin that is responsible for enterocyte damage and lysis of a wide range of human cell types, especially erythrocytes (Saunders et al., 1999). The enterohemolysin gene (*hly*A) was reported (Saunders et al., 1999) to be present in all *E. coli* O157:H7 strains and in about half of non-O157:H7 pathogenic STEC strains. Because of this critical role, expression of *hly*A was evaluated (Bettelheim, 1995) by using standard sheep blood agar that was based on TSB agar containing 5% defibrinated sheep blood.

Serotyping of STEC Isolates. The STEC isolates were serotyped for the O and H antigens by the slide agglutination method using rabbit antisera (Denka Seiken Co., Ltd., Tokyo, Japan).

Statistical Analysis. A significant difference ($P <$ 0.10) in the prevalence of STEC between or among levels or categories was determined by using an exact conditional scores test (Mehta and Patel, 2000).

Results and Discussion

The STEC were prevalent in only two of the four feedlots (i.e., Feedlots A and B) at 4.9 and 7.5%, respectively. The prevalence rates of STEC were slightly higher ($P > 0.10$) in cattle that were on feed for the shortest time (i.e., 5.0 vs 1.2%) and there was no difference ($P > 0.10$) in prevalence rates between the summer and fall prevalence (i.e., averaging 3.1%). The STEC were prevalent in only two of the four cow/calf operations (i.e., Operation A and B) at 2.5 and 3.8%, respectively. The prevalence rates were not different ($P > 0.10$) between cows and calves and averaged 2.2%. Interestingly, STEC were prevalent in the summer at a slightly lower ($P > 0.10$) rate than in the fall. The STEC were prevalent in all cow/calf operations at different rates (i.e., ranging from 1.3 to 15.0%). Similar to the cattle grazing irrigated pastures, no differences ($P > 0.10$) were found between cows and calves in STEC prevalence (i.e., averaging 4.2%). The prevalence rates also were not different ($P > 0.10$) between the summer and fall. No differences ($P > 0.10$) in prevalence rates were found between cows and calves (i.e., averaging 3.4%) or between the two seasons. However, prevalence rate of STEC was higher ($P < 0.10$) for cattle grazing rangeland forages than for those grazing irrigated pastures (i.e., 4.2 vs 2.1%).

A total of five STEC serotypes were found in feedlot cattle during the summer (i.e., O125:H19, O153:HUT, and OUT:HUT) and fall (i.e., O86:H19, OUT:H20, and OUT:HUT). The STEC isolates had different biochemical characteristics (i.e., sorbitol fermentation and ∃-glucuronidase activity). Of the serotypes detected, *E. coli* O86:H19, O125:H19, and OUT:H20 have not been reported previously in beef or dairy cattle. None of the STEC detected are known to cause human illnesses (WHO, 1998). However, these STEC isolates expressed various virulence genes. For example, all isolates produced ∀-Hly and expressed stx_1 (3), stx_2 (4), or both genes (3).

A total of five STEC serotypes with different biochemical characteristics were found in cattle grazing irrigated pastures during the summer (i.e., O26:HUT) and fall (i.e., O1:H2, O125:H2, O125:HUT, and OUT:HUT). The diversity of STEC isolates was greater in cows (i.e., O1:H2, O125:H2, O125:HUT, and OUT:HUT) than in calves (i.e., O26:HUT and OUT:HUT). Of these serotypes, only *E. coli* O1:H2 is known to cause human illnesses such as mild diarrhea, bloody diarrhea, and HC (WHO, 1998). This *E. coli* serotype and two other serotypes (i.e., *E. coli* O26:HUT and O125:HUT), however, have not been isolated previously from beef or dairy cattle. Testing the STEC isolates for Shiga toxins revealed that one produced Stx1, one produced Stx2, and six produced both toxins. Interestingly, all the isolates from cows produced both toxins whereas the isolates from calves produced either toxin. It is worth noting that all the STEC isolates produced ∀-Hly and were lethal to Vero cells.

A total of 16 STEC serotypes with different biochemical characteristics were found in cattle grazing rangeland forages during the summer (i.e., O1:H2, O125:H2, O125:H27, O125:H28, O127:H2, O127:H28, O158:H28, O166:H2, OUT:H2, and OUT:HUT) and fall (i.e., O125:H2, O125:H28, O125:HUT, O127:H28, O128:H20, O158:HUT, O166:H20, OUT:H2, OUT:H19, and OUT:HUT). Similar to that observed with the cattle grazing irrigated pastures , a greater diversity of STEC isolates was found in cows (i.e., O1:H2, O125:H2, O125:H27, O125:H28, O125:HUT, O127:H2, O127:H28, O128:H2, O128:H20, O158:H28, O158:HUT, O166:H2, O166:H20, and OUT:HUT) than in calves (i.e., O125:H2, O125:H28, O158:HUT, OUT:H2, OUT:H19, and OUT:HUT) grazing rangeland forages. Of these serotypes, 12 (i.e., *E. coli* O1:H2, O125:H27, O125:H28, O125:HUT, O127:H2, O127:H28, O128:H2, O128:H20, O158:H28, O158:HUT, O166:H2, and O166:H20) have not been reported previously in beef or dairy cattle. The O128:H2 serotype, however, has been isolated from beef products (Brooks et al., 2001). The *E. coli* OUT:H2 and O1:H2 serotypes are known to cause HUS and other human illnesses, respectively (WHO, 1998). *E. coli* OUT:H2 has been detected in beef cattle in Australia (Hornitzky et al., 2002), Brazil (Leomil et al., 2003), Canada (Schurman et al., 2000), India (Khan et al., 2002), and the UK (Jenkins et al., 2002). Testing the 29 isolates for Shiga toxins revealed that 9 produced Stx1, 2 produced Stx2, and 18 produced both toxins. These isolates also produced ∀-Hly and were lethal to Vero cells.

Implications

Fecal testing of 970 beef cattle in 4 large feedlots and 10 cow/calf operations in California over the summer and fall revealed prevalence rates of STEC that ranged from 3.7 to 7.5% in feedlot cattle, from 1.3 to 3.8% in cattle grazing irrigated pastures, and from 1.3 to 15.0% in those grazing rangeland forages. No effects on prevalence rates of STEC were found for the season (i.e., summer vs fall), time on feed (i.e., shortest vs longest in the feedlot), and age (i.e., cow vs calf). However, a higher prevalence rate was found for cattle grazing rangeland forages than for those grazing irrigated pastures (i.e., 4.6 vs 1.7%). A total of 21 STEC serotypes were detected in beef cattle feces, of which two (i.e., *E. coli* OUT:H2 and O1:H2) are known to cause HUS and other illnesses. Of these serotypes, 16 (i.e., *E. coli* O1:H2, O26:HUT, O86:H19, O125:H19, O125:H27, O125:H28, O125:HUT, O127:H2, O127:H28, O128:H2, O128:H20, O158:H28, O158:HUT, O166:H2, O166:H20, and OUT:H20) have not been reported in beef or dairy cattle. Only the *E. coli* O128:H2 serotype, however, has been isolated from beef products.

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EFFECTS OF FEED INTAKE, STRESS OR EXOGENOUS HORMONES ON *SALMONELLA* ENTERICA SEROVAR CHOLERAESUIS IN PIGS

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ABSTRACT: Two experiments were conducted to examine the role of feed restriction (FR), stress (STR), or exogenous hormones on *Salmonella* colonization in pigs. Thirty pigs (avg. BW = 13.5 kg) were randomly assigned to pen (2 pigs/pen) and treatment: control (CON, 3 pens); FR (4 pens); STR (daily co-mingling, 4 pens) and FR+STR (4 pens). On d 1, all pigs were inoculated with *S. enterica* serovar choleraesuis and treatments initiated. Fecal samples, feed intake, scour and activity scores were collected daily and on d 6 pigs euthanized and gut tissue and luminal contents collected along with ileo-cecal lymph nodes, spleen and liver tissue. Treatment had no effect ($P > 0.10$) on daily fecal shedding of *Salmonella* averaging 2.26, 1.98. 1.74 and 1.89 CFU ($\log_{10}$/g feces) across the experimental period for CON, FR, STR and FR+STR treatments, respectively. Compared to CON, STR decreased ($P < 0.01$) *Salmonella* in cecal (1.85 vs 3.79) and tended to decrease ($P < 0.10$) counts in colon (1.80 vs 2.98) and ileal (4.0 vs 5.33) contents. The number of *Salmonella* positive lymph nodes was doubled ($P < 0.05$) in all treatments compared to CON, while FR and FR+STR tended to increase the number of positive stomach tissue samples. In a second experiment, 28 pigs (avg. BW = 10.6 kg) were assigned to treatment (7 pigs/treatment): CON; melatonin (MEL, 1.3 mg/kg BW); serotonin (SER, 0.5 mg/kg BW); or tryptophan (TRP, 0.2 g/kg BW). Pigs were dosed daily for 9 days, inoculated with Choleraesuis on d 6, and euthanized on d 9 for sample collection as described above. Treatment had no affect ($P > 0.10$) on daily fecal shedding. The SER treatment numerically increased ($P = 0.15$) populations of *Salmonella* in the colon contents and increased ($P < 0.01$) the number of *Salmonella* positive spleen samples. These results support the hypothesis that stress and/or hormones may play a role in the colonization and pathogenicity of *Salmonella* in swine.

Key Words: *Salmonella*, pigs, stress, hormones

Introduction

Foodborne *Salmonella* infections in the United States are estimated to cost approximately $2.3 billion annually. Of these human cases of Salmonellosis, 6-9% are associated with the consumption of pork or pork products (Frenzen et al., 1999). *Salmonella* has been isolated throughout all stages of the pork production cycle and has received considerable attention, not only from a food safety standpoint, but additionally, because *Salmonella* can cause clinical infection in swine. *Salmonella* positive pigs are thought to arise from one of two general factors, inputs (pigs, feed, rodents, etc.) and activities within the swine production process (mixing of animals, transport, housing, and other management factors). Although, modern pork production systems are designed to keep stress to a minimum, it is still an inherent part of the process. Stressors commonly encountered by pigs include: transport, handling, co-mingling, breakdown of social groupings, food deprivation, temperature fluctuations, and noise. Additionally, early weaning ($\leq$ 21 d of age) which has gained in popularity, results in an immature digestive tract (Shields et al., 1980) and perhaps more importantly, a decrease in immune system function (Blecha et al., 1983).

It is well known that stress affects the immune system (Khansari et al., 1990) and it is generally believed that stress will increase the number of pigs shedding *Salmonella* and increase their susceptibility to *Salmonella* infections. Physiological responses to stress involve a neuroendocrine response that facilitates adaptation to the external environment and is in part regulated by the hypothalamic-pituitary-adrenocortical axis, resulting in increased levels of glucocorticoids and catecholamines. The immunosuppressive effect of elevated glucocorticoids is the basis for their use in treatment of organ transplant recipients and patients with autoimmune diseases. The catecholamines, in addition to having effects on gut motility and smooth muscle contraction, have been shown to stimulate growth of *E. coli* (Burton et al., 2002) and *Salmonella* (Rahman et al., 2000) *in vitro*. Norepinephrine, present throughout the small intestine, is hypothesized to play a role in the growth of *E. coli* (Lyte, et al., 1996) and may have a similar effect on other gram-negative bacteria such as *Salmonella*.

Melatonin, a pineal hormone most often associated with daylength, has been associated with immune function (Wuliji et al., 2003) and may also have an effect on cortisol production and secretion. Cortisol production was inhibited by melatonin *in vitro* (Torres-Farfan et al., 2003) although others reported no effect of elevated melatonin, as a result of increased daylength, on plasma cortisol (Griffith and Minton, 1992). More recently, the gastro-intestinal (GIT) tract has been shown to be a rich source of melatonin (Huether 1993), producing considerably more than the pineal gland. While pineal melatonin is produced in response to the photoenvironment, GIT melatonin appears to be more dependent on nutritional factors, such as the amount and

composition of ingested food and availability of nutrients (Bubenik et al., 1996). Interestingly, while *Salmonella* in pigs has been extensively researched, nobody to our knowledge has examined the role of hormones on GIT populations of *Salmonella*.

Materials and Methods

Experiment I. To determine the effects of stress on *Salmonella*, thirty crossbred weanling pigs (approx. 21 d of age) were purchased from the Texas Department of Criminal Justice and transported to our facilities at the Food and Feed Safety Research Unit in College Station, TX. Immediately upon arrival, piglets were weighed, ear-tagged, rectally swabbed and randomly assigned to pens (2 pigs/pen) and treatment. Rectal swabs were plated on brilliant green agar (BGA_{NN}) containing novobiocin (25 µg/mL) and naladixic acid (20 µg/mL) to screen for the presence of *Salmonella* prior to experimental infection. Pigs were given *ad libitum* access to pig starter and water and allowed to adjust to their new environment for one week. Following the adjustment period, all pigs were experimentally infected with 5 mL of 4.8×10^9 *Salmonella* enterica serovar choleraesuis via oral gavage and the following treatments intitiated and continued daily throughout the experimental period: Control (3 pens) = *ad libitum* feed and water; Feed restricted (FR; 4 pens) = intake restricted to 50% of control intake; Stress (STR; 4 pens) = co-mingling of pigs within treatment, one pig moved to new pen daily; and FR+STR (4 pens). Fecal swabs were collected and feed intake and diarrhea and activity scores recorded daily. Five days post-infection, all pigs were humanely euthanized and tissue and luminal contents collected from the stomach, ileum, cecum, colon and rectum for isolation of the challenge strain. Additionally, tissue samples from the tonsils, ileocecal lymph nodes, spleen, liver and lung were collected for qualification of *Salmonella*. Body weights were recorded at the beginning and end of the study.

Experiment II. Twenty-eight weaned pigs were used to determine if exogenous hormones or a hormone precursor influences *Salmonella* populations. Pigs were purchased, handled and housed as above. Following the adjustment period, pigs were randomly assigned to one of the following treatments (7 pigs/treatment) administered daily (via oral gavage or fed) for 9 d: contol (gelatin capsule only); melatonin (MEL; 1.3 mg/kg BW); serotonin (SER; 0.5 mg/kg BW); or tryptophan (TRP; 0.2 g/kg BW fed in small amount of peanut butter). On d 6 all pigs were experimentally infected (4.5×10^{10} cfu/mL in 5 mL) as above. Fecal swabs and activity and scour scores were obtained daily and on d 10 (5 d post-inoculation) pigs euthanized and samples collected as described previously.

Salmonella culture. Daily fecal swabs (considered to contain 0.1 g fecal material) and luminal contents (1 g) were serially diluted, plated on BGA_{NN} agar and incubated overnight (24 h, 37° C). Tissue samples were enriched in tetrathionate broth (24 h, 37° C) before plating on BGA_{NN} for qualitative determination of the challenge strain.

Statistical Analyses. Daily fecal shedding, scour and activity scores, and feed intake were analyzed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Chi-square analysis using the FREQ procedure of SAS, was used to determine influence of treatment on qualitative bacterial enumeration of tissue samples. *Salmonella* populations in luminal contents and BW were subjected to analysis of variance appropriate for a completely randomized design. Least square means were compared using Duncan's mean separation statement when significant ($P < 0.05$) differences detected. Individual pigs were used as the experimental unit except when analyzing scour and activity scores, in which case pen was used.

Results

Experiment I. Daily fecal shedding of the challenge strain of *Salmonella* showed a significant day effect, but no treatment effect or treatment x day interaction ($P > 0.20$). Average counts (CFU $\log_{10}$) averaged across 4 days are presented in Table 1. *Salmonella* populations in the luminal contents collected from the cecum were lower in the STR treatment ($P < 0.01$) and tended to be lower in the colon ($P = 0.06$) and ileum ($P = 0.07$). No differences were observed in rectal contents ($P > 0.20$; Table 1). The number of lymph nodes *Salmonella*-positive was increased ($P < 0.05$) in all treatments compared to control animals while the FR and FR+STR treatments tended ($P < 0.10$) to increase the number of positive stomach tissue samples. No differences ($P > 0.20$) were detected for tissue samples from the cecum, colon, ileum, liver or spleen (Table 1). Scour and activity scores showed a day effect ($P < 0.05$) but no treatment or treatment x day interaction. Treatment effect on scour score approached significance ($P = 0.11$) with STR and FR+STR treatments reducing the severity of scours compared to control and FR pigs. The STR pigs gained more ($P < 0.05$) BW over the course of the study compared to the other treatments, however these pigs were significantly heavier at the start of the study which may have affected their subsequent performance.

Experiment II. Data from the second experiment are presented in Table 2 below. No differences in fecal shedding of *Salmonella* over the experimental period were observed ($P > 0.20$) although a day effect ($P < 0.05$) was noted. Populations of the challenge strain from luminal contents were similar in the ileum, cecum, colon and rectum, although colon counts in the SER treated pigs were numerically higher ($P = 0.15$). The number of *Salmonella* positive tissues after enrichment were similar ($P > 0.05$) for all tissues examined with the exception of the spleen ($P < 0.05$) in which the SER treatment had a higher number of positive tissues compared to all other treatments. Scour and activity scores were not influenced by treatment ($P > 0.05$). Body weight change was similar across treatments with all pigs gaining weight over the course of the experimental period.

Discussion

Salmonella is a major problem for the swine industry throughout the pork production process, producing disease in pigs and constituting a serious food safety concern. Associated costs represent a significant loss of potential profit to the swine industry. *Salmonella enterica* serovar choleraesuis is a host-adapted serotype, rarely isolated from species other than swine. Suckling pigs can become infected by *Salmonella* (Schwartz, 1991) and Anderson and co-workers demonstrated that weaned pigs are a good model for experimental infection with Choleraesuis (Anderson et al., 1998). Stress has been shown in pigs to stimulate recurrent shedding by *Salmonella* carriers (Schwartz 1991).

We conducted an experiment to examine the role of stress, administered as restricted feed intake and co-mingling, on *Salmonella* in weaned pigs. Minimal effects of our treatments were noted and surprisingly, the stress treatments tended to decrease populations of the challenge strain of *Salmonella*. Possibly because the pigs were adapted to their surroundings for one week prior to initiation of treatments, the stress we implemented was not severe enough to produce either a hormone or immune response sufficient to increase pathogen populations.

The gut has been reported to produce significant quantities of melatonin (Bubenik et al., 1996) which we hypothesize may play a role in population dynamics of the foodborne pathogens *E. coli* O157:H7 and *Salmonella*. Reduced food intake has been reported to increase MEL, SER and TRP concentrations in the gut (Bubenik et al., 1992) of mice. Additionally, gut MEL concentrations in pigs increased following refeeding after a 30 hr fasting (Bubenik et al., 1996). This research led us to speculate that perhaps the effects observed in the first experiment in the FR treatment, although mild, were perhaps due to changes in gut MEL or a similar compound due to the reduced feed intake. Only the SER treatment resulted in any differences among the treatments and they were minimal. Possibly increasing the hormone dose or length of time treatments were applied would have produced more of an affect on *Salmonella* populations.

Implications

To respond to the challenge of providing a safe pork product for the consumer, improve swine health, and maintain a safe environment, a thorough understanding of *Salmonella* epidemiology and host-interactions is crucial. Investigating *Salmonella* population dynamics in response to stress and determining the role of hormones on *Salmonella*, may possibly result in treatment or management recommendations that will decrease the negative impact of this important pathogen.

Table 1. Daily fecal shedding and luminal content populations of *Salmonella* (CFU $\log_{10}$), *Salmonella* positive tissue samples, scour scores and BW change in weaned pigs infected with *Salmonella* and stressed with feed restriction (FR), co-mingling (STR) or a combination of both (FR+STR).

	Treatment			
	Control	FR	STR	FR+STR
Daily shedding	2.26	1.98	1.74	1.89
Luminal contents				
Ileum	5.33	4.74	4	4.15
Cecum	3.79[c]	2.72[cd]	1.85[d]	3.73[c]
Colon	2.98	1.6	1.8	2.17
Rectum	2.31	1.56	1.6	2.01
Tissue enrichments[a]				
Stomach	5/6	8/8	5/8	8/8
Cecum	5/6	7/8	8/8	8/8
Ileum	5/6	8/8	8/8	8/8
Colon	4/6	6/8	7/8	3/8
LN[a]	4/6[c]	8/8[d]	8/8[d]	8/8[d]
Spleen	4/6	7/8	6/8	5/8
Liver	6/6	7/8	6/8	5/8
Scours score[b]	1.08	0.81	0.44	0.44
BW change, kg	3.4[d]	4.0[d]	5.3[c]	3.6[c]

[a]Number positive/total samples. LN = ileo-cecal lymph nodes.
[b]Scour score: 0 = normal, 3 = severe scours.
[cd]Row values with different superscripts differ ($P < 0.05$).

Table 2. Daily fecal shedding and luminal content populations of *Salmonella* (CFU $\log_{10}$), *Salmonella* positive tissue samples, scour scores and BW change in weaned pigs infected with *Salmonella* and administered melatonin (MEL), serotonin (SER) or tryptophan (TRP).

	Treatment			
	Control	MEL	SER	TRP
Daily shedding	1.38	1.25	1.56	1.42
Luminal contents				
Ileum	2.81	3.76	3.59	2.76
Cecum	1.0	1.86	1.86	1.76
Colon	1.0	1.54	2.26	1.40
Rectum	1.14	1.0	1.0	1.29
Tissue enrichments[a]				
Cecum	3/7	5/7	5/7	6/7
Ileum	7/7	7/7	7/7	7/7
Colon	3/7	3/7	3/7	4/7
LN	6/7	6/7	7/7	7/7
Spleen	2/7[c]	1/7[c]	6/7[d]	1/7[c]
Liver	4/7	5/7	5/7	3/7
Scours score[b]	0	0.09	0.39	0.21
BW change, kg	6.1	5.6	4.8	5.2

[a]Number positive/total samples. LN = ileo-cecal lymph nodes.
[b]Scour score: 0 = normal, 3 = severe scours.
[cd]Row values with different superscripts differ ($P < 0.05$).

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SALMONELLA PERSISTANCE ON A SOUTHWESTERN UNITED STATES DAIRY

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ABSTRACT: Dairy cattle are reservoirs for *Salmonella* and mature cattle typically appear asymptomatic while shedding this pathogen into the environment. However, in specific geographic regions, *Salmonella* outbreaks have been reported in lactating animals resulting in lost milk production and cow mortality. Dairy cattle on a single farm (2000 hd dairy; southwestern U.S.), were identified and sampled upon entering the milking string immediately following freshening. Thirty head were randomly selected from a group scheduled to calve within a 14 day period. Fecal samples were collected from the same cows, via rectal palpation, and cultured for *Salmonella* monthly from February to October 2004. Due to culling, only 26 of the original group remained by October. Individual feed ingredients, total mixed ration (TMR), water, and soil were also sampled monthly. *Salmonella* was isolated from dairy cattle each month with prevalence ranging from 19 to 96%, averaging 53% over the 9 month period. Although the cattle did not break with *Salmonella* as in previous years, the highest prevalence (96%) was observed in August when *Salmonella* is typically a problem for this area's dairy producers. At least one individual feed ingredient tested positive on four occasions. A portion of the water samples collected each month tested positive, with all samples negative in May and August. Although not all TMR samples were positive for *Salmonella*, at least one positive sample was collected each month and generally, most samples tested positive throughout the 10-month period. However, a positive TMR sample did not always correlate to a feed ingredient or water sample testing positive. Numerous soil samples collected tested positive for *Salmonella* with the greatest incidence observed in August through October. Results indicate that control of foodborne pathogens on the farm is a complex task with numerous routes of infection and environments for persistence.

Key Words: *Salmonella,* dairy cattle

Introduction

In the United States, *Salmonella* accounts for an estimated 1.3 million cases on foodborne infections and 553 deaths annually (Mead et al., 1999). Recently, a multidrug resistant strain of *Salmonella* Newport was implicated in a number of illnesses and one death in the Eastern United States, as a result of consuming ground beef from dairy cattle (Zansky et al., 2002). Dairy cattle are natural reservoirs for *Salmonella*, often appearing asymptomatic while shedding this pathogen into the environment (Richardson 1975; McDonough 1986; Edrington et al., 2004 a,b). The National Animal Health Monitoring System Dairy '96 study reported 5.4% of milk cows shed *Salmonella* and 27.5% of dairy operations had at least one cow shedding *Salmonella* (Wells et al., 1998).

While *Salmonella* has been isolated from all ages of dairy cattle and at all stages of production, it is typically pathogenic only in calves. Previously, we reported a great deal of variation in fecal shedding of *Salmonella* in healthy, lactating dairy cattle, not only among farms within a small geographic area, but also within a farm from season to season (Edrington et al., 2004a). Interestingly, some of these same farms have reported a seasonal outbreak of salmonellosis within the milking animals, resulting in financial losses due to decreased milk production and cow mortality. Of the four farms monitored previously, one in particular had an increased incidence of *Salmonella* shedding, even in the winter when the incidence of *Salmonella* typically decreases (Edrington et al., 2004a). Preliminary examination of *Salmonella* isolates obtained during an outbreak in 2003 from healthy and diarrhetic cattle on this farm, showed similar percentages of fecal shedding, but did not explain why the *Salmonella* was pathogenic in some animals (Edrington, unpublished data). Therefore, the current study was undertaken in an attempt to determine why *Salmonella*, shed year around by lactating dairy cattle, become pathogenic only during a single specific time of the year.

Materials and Methods

Mature multiparous Holstein dairy cattle on a single farm (approx. 2000 head) in the southwestern United States were sampled monthly over a 9 month period (February to October 2004). Cattle were maintained in drylot pens with shade available and fed a TMR, typical of dairy farms in this part of the United States. Thirty head were randomly selected from a group scheduled to freshen within a 14 day period. Four animals were culled from the sample population for various reasons, resulting in 26 of the original group remaining in October. At the time of sampling, animals were restrained in self-locking head stanchions, and utilizing a sterile palpation sleeve, approximately 30 g of feces were retrieved from the rectum. Fecal samples were placed on ice and shipped to the USDA-ARS laboratory

in College Station, TX for *Salmonella* culture and isolation described below.

At each monthly collection, samples of individual feed ingredients, TMR, water, and soil were collected as detailed below. Individual feed ingredients were sampled from the commodity bins and multiple TMR samples were collected from the feed apron after thorough mixing. Samples of TMR were collected representing each pen where study cows were housed. Water samples were collected from the trough of each pen through April, after which, water was collected from the pen troughs and also directly from the water spouts supplying each trough. On March 23rd, all drinking water was chlorinated on the farm as a means of *Salmonella* control. Soil samples were collected from several locations within each pen, under shade structures and in areas of full sun exposure.

Salmonella Culture and Isolation. *Salmonella* was cultured by enriching approximately 10 g of fecal material in 90 mL tetrathionate broth (24 h, 37° C). Following incubation, 200 µL of the above enrichment was added to 5 ml Rappaport-Vassiliadis R10 broth and incubated an additional 24 h at 42° C before spread plating on brilliant green agar supplemented with novobiocin (25 µg/mL). Colonies exhibiting typical *Salmonella* morphology were confirmed biochemically using lysine and triple sugar iron agars. *Salmonella*-positive samples were further confirmed by slide agglutination with SM-O antiserum poly A-I and V-I and group C1 factors. *Salmonella* isolates were stored as glycerol stocks (10% v/v) in tryptic soy broth at -80° C. All media and agar were from Difco laboratories (Detroit, MI).

Results and Discussion

The percentage of dairy cattle shedding *Salmonella* in their feces each month is presented in Figure 1. The incidence of cattle shedding *Salmonella* in the feces ranged from a low of 19% in October to a high of 96% in August, with a mean average over the 9 month sampling period of 53%. Others have likewise shown a high degree of variation in fecal shedding of *Salmonella* in dairy cattle (Wells et al., 1998, 2001; Huston et al., 2002a) reporting ranges from 1 to 97% positive.

August is typically the time of year when dairymen in this area experience salmonellosis in their mature lactating animals. Severe scours, decreased milk production and in some instances mortality have been reported, but interestingly, this occurs only in the late summer and only in this specific geographic location. We saw the highest incidence of cows shedding *Salmonella* in August, however the dairy did not experience the "break" in salmonellosis as seen in previous years. Previously we reported examination of *Salmonella* shedding on this farm, comparing sampling time (season) to other farms within the same geographic area (Edrington et al., 2004b). The incidence of cows shedding *Salmonella* was much higher on this farm compared to others in the area and similar shedding levels were recorded in the month of August in two successive years as in the present study. Interestingly, this farm was the only farm identified with an appreciable number of cattle shedding *Salmonella* in the winter (Edrington et al., 2004b). In the current study, the second highest prevalence was observed when the sampling began, in February, a month we did not expect to find a high percentage of cattle shedding *Salmonella*. Seasonal patterns have been reported for *Salmonella* with the greatest incidence typically observed in the warmer summer months (Huston et al. 2002b; Edrington et al., 2004b). With the exception of August, in which we recorded the highest percentage of shedders, June and July had relatively few shedders compared to the other months.

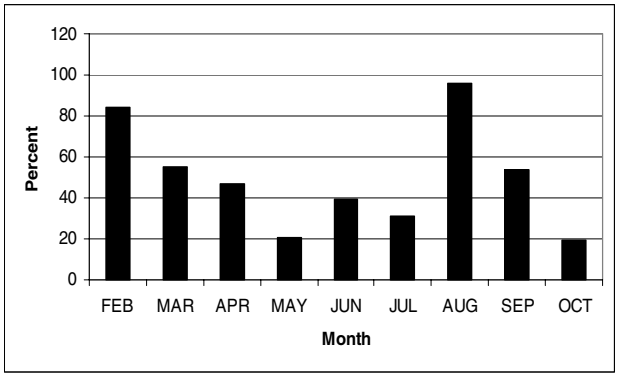

Figure 1. Percentage of lactating dairy cows shedding *Salmonella* in feces by month.

Salmonella was isolated from individual feed ingredients throughout the sampling period (Table 1). No one single ingredient was more predisposed to testing positive for *Salmonella* and only steam-flaked corn and distiller's grains tested negative every time. It is unknown if the ingredients were contaminated with *Salmonella* prior to delivery to the farm or at some time point while in storage prior to feeding, with any number of potential routes of contamination possible (rodents, birds, insects, farm workers, machinery, etc.). Due to changes in feedstuff usage on the farm, not all ingredients were sampled an equal number of times, however this data demonstrates that feedstuffs are a potential source of *Salmonella* contamination and/or persistence on the farm. Others have likewise reported feedstuffs as a potential source of animal infection (Jones et al., 1982; Anderson et al., 1997; Kabagambe et al., 2000).

Table 1. Incidence of *Salmonella* contamination in individual feed ingredients (February – October 2004)

Feed Ingredient	n[a]	month[b]
Dry hay	1/9	Apr
Corn silage	1/5	Oct
Steam-flaked corn	0/8	
Ground corn	1/4	Jul
Hominy	1/2	Jun
Distiller's grains	0/4	
Whole cottonseed	2/8	Apr, Jun
Beet pulp (pellet)	1/6	Jun

[a]Number of samples positive/total number of samples over the entire sampling period (Feb – Oct).
[b]Month positive feed ingredient was recorded.

Samples of TMR had a high incidence of *Salmonella* contamination, with *Salmonella* being isolated from more than half the samples collected each month (Table 2). The one exception was July when only one positive TMR sample was cultured out of 16 collected. A *Salmonella*-positive TMR sample did not always correlate to a positive feed ingredient as positive TMR samples were collected every month while positive feed ingredients were collected in only 4 of the 9 months. Water was added to the TMR, however this does not explain the high number of TMR samples testing positive.

Water samples collected either from pen troughs or from lines feeding pen troughs were collected throughout the study period and are presented in Table 2. As might be expected, a trough sample was much more likely to contain *Salmonella,* with 38% of the samples collected testing positive. Only one water line sample was found positive. Interestingly, in August when 96% of the cows tested positive for *Salmonella*, all water samples were negative, including those collected from the troughs. Chlorination of the water began in March, after sample collection, and we speculate that this is why water line samples were almost uniformly negative. Unfortunately, water samples collected in March were lost. However, we speculate that while chlorination was by no means 100% effective in eliminating *Salmonella*, it did appear to decrease the number of positive samples compared to the month of February prior to chlorination. Others have reported similar results (Kabagambe et al., 2000). Continual recontamination from cattle drinking and defecating in the water troughs, not to mention other potential sources of contamination (birds, etc.) may have overwhelmed the ability of chlorine to reduce *Salmonella* in the troughs.

Table 2. Monthly *Salmonella* incidence in TMR and water samples[a]

		Water[b]	
	TMR	Trough	Line
Month	n	n	n
Feb	8/9	6/9	ns
Mar	10/11	ns	ns
Apr	8/8	2/8	ns
May	5/8	0/4	0/4
Jun	12/13	3/4	0/5
Jul	1/16	3/8	0/8
Aug	13/16	0/8	0/8
Sep	12/16	4/8	0/8
Oct	12/12	5/11	1/12
TOTAL	81/107	23/60	1/45

[a]Number positive/total number of samples.
[b]Water samples collected either from pen water troughs or directly from water line supplying troughs. ns = no sample.

Soil samples were collected monthly from each pen housing cattle during the experimental period from areas of full sun and also under the shade structures (Table 3). Location of sampling did not affect the incidence of a *Salmonella* positive soil sample (41 vs 38% positive for sun and shade samples respectively, when averaged over the collection period). All soil samples were negative (sun and shade) in only two months, May and July.

Table 3. Monthly incidence of *Salmonella* in pen soil samples

	Soil[a]	
Month	Full-sun	Shaded
Feb	3/3	1/1
Mar	1/6	2/6
Apr	1/4	1/4
May	0/4	0/4
Jun	1/6	2/5
Jul	0/8	0/8
Aug	7/8	5/8
Sep	4/8	5/8
Oct	5/7	3/6
TOTAL	22/54	19/50

[a]Soil samples collected from areas of full-sun or under shade structures. Number positive/total number of samples

While a number of studies have examined factors associated with pathogen shedding on dairy operations, most have been shown to have limited effects on *Salmonella* prevalence (Kabagambe et al., 2000; Fitzgerald et al., 2003; Edrington et al., 2004a). Similarly, as the current research demonstrates, a number of avenues for infection, re-infection or persistence of *Salmonella* exist within the farm environment. Large herd size and intensive management have been suggested as providing an environment conducive to *Salmonella* shedding and chronic dairy herd infection (Huston et al., 2002b) and once introduced, *Salmonella* will self-perpetuate and recirculate among cows and calves if left unchecked (Bender 1994).

Herein we present preliminary sampling data from a single southwestern dairy farm with a high incidence of *Salmonella* isolation from both cattle and the environment. Fortunately for the dairy producer, the cattle did not "break" with *Salmonella* as in previous years, which may or may not have yielded insight into the problem as the data presented here is examined. The analysis of serogroups and serotypes from this years isolates is underway and may yield important information. Additionally, *Salmonella* isolates collected from healthy and sick cattle during an outbreak the previous year is ongoing and collectively, may provide insight into what "triggers" the pathogenicity of these *Salmonella* isolates.

Implications

Results of this study highlight the complexity of *Salmonella* control at the farm level due to the numerous environments favorable for persistence and growth of this pathogen. Additionally, seasonal shedding patterns were

not observed in this study, suggesting methods to control *Salmonella* in order to be effective need to be employed year around.

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EFFECTS OF ESTRADIOL (E2) AND LINSEED MEAL (LSM) ON LIVER WEIGHT, PROTEIN CONCENTRATION, AND DNA CONCENTRATION IN (OVX) EWES[1]

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ABSTRACT: Flaxseed contains secoisolariciresinol diglycoside (SDG), a phytoestrogen (PE) proposed to have both estrogenic and anti-estrogenic properties. The objective of the current study was to determine the estrogenic properties of SDG on liver characteristics in OVX ewes. The OVX ewes (n = 48) were fed a PE-free diet for four wks (d –28 to d 0) after OVX to ensure the absence of any circulating endogenous E2 or dietary PE. On d 0, OVX ewes were assigned randomly to a control group (PE-free diet; CON; n=12) or a 12.5% LSM for 1, 7, or 14 d (n = 12/group). Diets were based on beet pulp and formulated to provide similar amounts of energy (2.7 Mcal/kg) and CP (13.6%). On the last day of LSM feeding, OVX ewes were implanted with a subcutaneous E2 implant (100 mg) for 0, 6, or 24 h. At necropsy, livers were weighed and samples frozen for dry weight, DNA, and protein determination. To determine cell size, DNA:protein ratio was calculated. Liver wts were expressed as % of empty body wt (live wt minus blood and digesta wt). Further, within a day liver wts, DNA and protein concentrations were divided by the average 0 h E2 mean to determine % change from 0 h E2 exposure. There was no effect of E2 or days fed LSM on % liver dry wt. Liver wt tended to increase in response to E2 (P = 0.08; 1.46, 1.47, 1.56 ± 0.04% for 0, 6, and 24 h E2 exposure, respectively). Similarly, liver wts at 6 and 24 h post E2 tended to be heavier (P = 0.06) and increased liver wt by 1.7 and 7.6 ± 0.03%, respectively. In contrast, ewes fed 14 d LSM had a decrease (P < 0.05) in liver wt compared to the 0 day LSM ewes (1.42 vs 1.50 ± 0.04%). Likewise, liver wt as a % of 0 h E2 exposure decreased (P < 0.01) with increased days fed LSM (12.1, 4.6, 0.8, and -4.5 ± 3.0% for 0, 1, 7, and 14 d LSM, respectively). While there was no affect of E2 or LSM on cell size (DNA:protein), protein or DNA concentration, there was an increase (P < 0.01) in liver protein % change from 0 h E2 exposure within a day of LSM (26.2, -5.8, -13.3, and 5.14 ± 8.71% for 0, 1, 7, and 14 d LSM, respectively). The ability of LSM to alter liver function warrants further investigation.

Key Words: Estrogen, Linseed Meal, Sheep

Introduction

Flaxseed (*Linum unisatissimum*) has a history of food use in Europe and Asia. In the United States, it was not until the 1990s that flaxseed was incorporated into some brands of breakfast cereal, breads and other bakery products (Carter, 1993). This mass movement of flaxseed into our diets has occurred due to the potential health benefits of omega-3 fatty acid-rich foods, of which flaxseed is a prominent source. Consumption of flaxseed and flaxseed meal has been reported to have anticancer, antiviral and bactericidal activity, anti-inflammatory effect, ion reduction, laxative uses, and reduction of atherogenic risks (Bierenbaum et al., 1993; Craig, 1999; Cunnane et al., 1995; Jenab and Thompson, 1996; Jenkins et al., 1999; Kurzer and Xu, 1997; Serraino and Thompson, 1991, 1992; Thompson et al., 1996a,b). Flaxseed consists of oil (~36%), protein (~24%) and fiber (~32%; Collins et al., 2003). Not only is flax high in omega-3 fatty acids, but flaxseed contains the highest known levels of lignans of any cereal grain, and more specifically, secoisolariciresinol diglycoside (**SDG**). While lignans have been reported as phytoestrogens (Wilcox et al., 1990), SDG has also been reported as anti-carcinogenic (Jenab and Thompson, 1996; Thompson et al., 1996a,b), anti-estrogenic (Collins et al., 1997; Kurzer et al., 1995), and anti-aromatase (the enzyme needed to convert certain androgens to estrogens; Wang et al., 1994). However, few studies have investigated how the phytoestrogen contained in flaxseed may potentially hinder or aid the growth and health of livestock. As flaxseed oil is being extruded, an omega-3 fatty acid free, byproduct would be a potential feedstuff for livestock. Because the SDG is contained in the hull and not the oil of the seed, linseed meal (**LSM**) may possess growth promoting qualities, similar to estrogen implants. Estrogen has been shown to influence growth hormone and insulin-like growth factor-1 synthesis (Griffin and Mader, 1997). We hypothesized that feeding LSM to OVX ewes would show similar responses as estrogen treatment. Therefore, the objective of the current study was to determine if feeding LSM and/or implanting estradiol-17β (E_2) to ovariectomized (**OVX**) ewes would enhance liver cellularity and/or function.

Materials and Methods

Animals and treatments

All animal procedures were approved by the NDSU animal care and use committee. Forty-eight ewes

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were OVX and fed a phytoestrogen-free diet at least 28 days before treatments began to eliminate any circulating endogenous estrogen or dietary phytoestrogens from their system. During this adaptation period, ewes were kept in pens of 9 to 11 ewes per pen and fed according to their maintenance requirements determined by their metabolic body weight. Ewes were weighed every two weeks and rations were adjusted accordingly to account for weight gain or loss within pens. Before beginning treatment, ewes were randomly assigned to treatment groups and moved to individual pens. Ewes assigned to a control group (n=12) were fed the phytoestrogen-free diet (81.25% beet pulp, 17% dried distillers grain, and 1.75% sunflower meal) throughout the course of the experiment. The remaining 36 OVX ewes were fed the treatment diet which was formulated to contain 12.5% linseed meal and 87.5% beet pulp. Control and treatment diets were isonitrogenous and isocaloric. Ewes assigned to LSM treatment groups (n = 36) were fed LSM for 1, 7, or 14 d (n = 12 per experimental day). Within each length of LSM treatment, ewes were randomly assigned to receive subcutaneous E_2 implants (Silastic implant; Dow Corning, Midland, MI) containing 100 mg for 0, 6, or 24 h (n = 4 ewes per group within each length of LSM feeding) prior to slaughter.

Tissue collection

On the day of slaughter, ewes were stunned via captive bolt and exsanguinated. The blood and digesta were collected and weighed. Following evisceration, the gallbladder was removed from the liver, and the liver weighed. A sample was taken from the left lobe of the liver. Samples were snap-frozen in liquid nitrogen and stored at -80° C for later analysis of DNA and protein concentration.

To calculate empty body weight, digesta and blood weights were subtracted from ewe live weight. Liver weights were expressed as a percentage of the ewe's empty body weight.

Laboratory analyses

Liver DNA concentration was determined using the diphenylamine procedure as described in Johnson et al. (1997). Liver protein concentration was determined using Coomassie Brilliant Blue as described in Bradford (1976), with bovine serum albumin (Fraction V, Sigma Chemical) as the standard (Johnson et al., 1997). Changes in DNA concentration was used as an index of hyperplasia and DNA:protein ratios were used as an index of hypertrophy. DNA and protein concentrations were also multiplied by the weight of the liver to derive total DNA and protein content.

Statistical Analysis

Data were analyzed using GLM procedures in SAS. Data were analyzed as a completely random design arranged as a 3 × 4 factorial. Linseed meal treatments and E_2 implant treatments and their interactions were included in the model. When there was no interaction, it was removed from the model. Reported means are least squared means. Treatments are considered different if $P < 0.05$ unless otherwise stated. When significance was detected, means were separated.

Results and Discussion

There was no effect of LSM or E_2 on ewe weights at the beginning or end of the experiment which averaged 51.8 ± 1.1 kg. At necropsy, there was no effect of LSM or E_2 on liver weights, however, when based as a percentage of empty body weight, liver weights tended to increase ($P = 0.08$) in response to 24 h E_2 exposure and decrease ($P = 0.09$) by 14 d LSM feeding (Table 1).

In order to determine if E_2 exposure was influencing liver cellular characteristics within a LSM feeding period, liver weight as well as liver DNA and protein concentrations were calculated as a percentage change from the 0 hr E2 exposure average. While there were no differences in liver dry weight percentage, DNA or protein concentrations, total DNA or protein, or the DNA:protein ratio (Table 1), when expressed as a percentage of the 0 h E_2 exposure average, liver wt decreased ($P < 0.01$) with increased days fed LSM (12.1, 4.6, 0.8, and -4.5 ± 3.0% for 0, 1, 7, and 14 d LSM, respectively). Further, an increase ($P < 0.01$) in liver proteins was determined with increased d fed LSM (26.2, -5.8, -13.3, and 5.14 ± 8.71% for 0, 1, 7, and 14 d LSM, respectively). An increase in protein production from the liver may result in increased growth factor production aiding in metabolism and/or growth of the animal after longer exposure to LSM feeding. Currently, the impacts of LSM, and more specifically, SDG's effects, on the growth and feed efficiency of domestic livestock are still unknown. These results warrant further studies into the mechanisms of SDG in the growing animal.

Implications

Estrogenic implants have been shown to promote growth and improve performance through increased feed efficiency (Griffin and Mader, 1997). If the estrogenic activity of phytoestrogens were able to mimic the growth promoting properties of estrogenic implants or have a synergistic effect when used in combination with estrogenic implants, phytoestrogen containing crops and forages could prove to be valuable feedstuffs for livestock producers wanting to improve growth and increase feed efficiency.

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Table 1. Effects of feeding OVX ewes LSM and E_2 on liver characteristics.

	Days Fed LSM					Hours Post E2				*P* Value	
	0	1	7	14	Pooled SEM	0	6	24	Pooled SEM	Flax	E2
Beginning ewe wt, kg	51.7	52.0	52.8	52.2	2.2	52.0	52.4	52.0	1.9	0.97	0.93
End ewe wt, kg	51.5	51.9	52.2	50.7	2.1	51.4	52.1	51.1	1.3	0.96	0.90
Liver wt, g	648.1	668.8	685.3	613.7	26.6	633.1	643.2	685.6	23.1	0.29	0.26
Liver wt/ empty body wt	1.50[a]	1.52[a]	1.55[a]	1.42[b]	0.04	1.46[a]	1.47[a]	1.56[b]	0.03	0.09	0.08
Liver dry wt, %	32.10	31.29	30.81	32.03	0.38	31.56	31.21	31.90	0.34	0.06	0.31
Liver DNA, mg/g	2.42	2.16	2.25	2.80	0.23	2.41	2.39	2.42	0.20	0.17	0.99
Total Liver DNA, mg	1.58	1.43	1.53	1.71	0.17	1.52	1.51	1.66	0.14	0.60	0.68
Liver Protein, mg/g	7.29	6.76	7.33	7.88	0.65	7.25	7.44	7.26	0.57	0.65	0.96
Total Liver Protein, mg	4.82	4.46	4.92	4.82	0.44	4.56	4.78	4.93	0.38	0.87	0.77
DNA:Protein	0.34	0.33	0.32	0.35	0.02	0.35	0.32	0.33	0.02	0.78	0.60

[a,b]Means ± Pooled SEM within a row and main effect differ; P<0.05.

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RELATIONSHIPS BETWEEN CALF SERUM METABOLITES AT FEEDLOT ENTRY AND SUBSEQUENT CARCASS CHARACTERISTCS IN BRANGUS-CROSSBRED CALVES

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ABSTRACT: Our aim was to determine the relationship between serum metabolites of Brangus-crossbred calves (n = 85; BW = 324 ± 9 kg) at feedlot entry with their subsequent carcass characteristics. Calves were weighed, and blood samples collected 2 d after arrival at the feedlot. Serum samples were analyzed for lactate dehydrogenase (LDH) activities, hemoglobin, protein, creatinine, prolactin, triiodothyronine, thyroxine, cortisol, testosterone, and IGF-I. After harvest, longissimus muscle and ribfat thickness were measured, and USDA quality and yield grades were assigned to each carcass. Sixty-eight percent of carcasses were assigned Choice grade. Carcass distributions among yield grade were 18, 52, and 26%, for yield grade 1, 2, and 3, respectively. Serum reverse LDH (rLDH) activity was lower (P = 0.07) in calves with Choice carcasses than calves with Select carcasses. Thyroxine was increased (P = 0.07) in calves with yield grade 1 carcasses versus calves with yield grade 2 and 3 carcasses. Longissimus muscle area was correlated (P < 0.05) with concentrations of prolactin (r = 0.24), triiodothyronine (r = 0.22), and thyroxine (r = 0.35). Other serum metabolites were not related to subsequent carcass characteristics. Heifers had decreased (P < 0.05) concentrations of IGF-I (152 vs 199 ng/mL), and greater (P < 0.01) concentrations of creatinine (2.62 vs 2.08 mg/mL) than steers. However, the percentage of heifers and steers grading Choice did not differ (P > 0.10). These data support previous studies indicating that serum LDH activity could be used as an early physiological marker of carcass composition.

Key Words: Lactate Dehydrogenase, Cattle, Quality Grade, Carcass Quality

Introduction

Ultrasound technology is the most commonly employed method to predict carcass traits in live cattle (Greiner et al., 2003; Crews et al., 2002). However, prediction accuracies are variable and are generally more accurate for carcass fat (Wallace et al., 1977; Brethour, 1992) than for longissimus muscle area (Smith et al., 1992; Waldner et al., 1992). Variation among animals, technicians, and machines interact to influence ultrasound accuracy. Moreover, accuracy may vary within a similar population of cattle scanned by the same technician over a period of successive years (Greiner et al., 2003). Ultrasound may not necessarily be the most cost-effective method to predict carcass traits in cattle due the cost associated with equipment and the need for trained technicians. Therefore, other alternatives to predict carcass traits must be made available to beef cattle producers. The use of physiological markers may provide one such alternative. Blood serum metabolites as potential markers are promising as only a single blood sample may be suffice to run a series of laboratory analysis. Serum metabolites may then be subsequently utilized to predict carcass characteristics. May et al. (2004) recently reported that serum lactate dehydrogenize at weaning was lower in calves whose carcasses ultimately graded USDA Choice vs. USDA Select. Thus, lactate dehydrogenase is one serum enzyme that may be utilized to predict carcass traits in live cattle. Other serum metabolites may similarly serve as potential candidates to predict carcass traits. Therefore, our objective was to quantify several sera metabolites from fall born Brangus-crossbred calves at time of feedlot entry to determine relationships among serum metabolites and carcass characteristics.

Materials and Methods

Eighty-five Brangus-crossbred calves (BW = 324 ± 9 kg) were used in this study. Calves were developed for 144 d on mixed pastures of bermudagrass and tall fescue. Calves were then placed in the feedlot (Flintrock Feeders, Gruver, TX) and fed a high energy ration consisting of corn, alfalfa hay, fat, molasses, and vitamin/mineral premix. Calves were weighed, and blood samples collected 2 d after arrival at the feedlot. After harvest, longissimus muscle and ribfat thickness were measured, and USDA quality and yield grades were assigned to each carcass following a 5 mo finishing phase. All cattle were slaughtered at a commercial packing plant.

Serum samples were collected (jugular venipuncture) to measure serum lactate dehydrogenase (LDH) activities, hemoglobin, protein, creatinine, prolactin, triiodothyronine, thyroxine, cortisol, testosterone, and IGF-I. Serum was harvested by centrifugation at 1500 x g for 25 min and stored at -20°C until analyzed. Serum thyroxine was quantified by solid phase RIA (Richards et al., 1999) utilizing components of a commercially available kit from Diagnostics Products Corp. (DPC, Los Angeles, CA). Serum concentrations of

triiodothyronine and cortisol were also determined with Coat-A-Count Kit (DPC) according to manufacturers instructions. The procedures of Richards et al. (1999) were used to determine concentrations of testosterone. Concentrations of prolactin and IGF-I were determined as described by Spoon and Hallford (1989) and Berrie et al. (1995), respectively. Serum concentrations of LDH activity, hemoglobin, protein, and creatinine were determined with enzymatic reagents and procedures provided by Sigma-Aldrich Co. (St. Louis, MO). The coefficients of variation were less than 10% for all assays.

Lactate dehydrogenase is the enzyme responsible for the inter-conversion of pyruvate and lactate. Forward LDH (fLDH) activity measures the activity associated with LDH converting pyruvate to lactate. Conversely, reverse LDH (rLDH) activity measures the activity associated with LDH converting lactate to pyruvate.

Serum metabolites for each quality grade and yield grade were analyzed with a one-way ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Least squares means were compared using the PDIFF statement of SAS when protected by a significant ($P < 0.05$) treatment effect. Pearson correlation coefficients were calculated utilizing the CORR procedure of SAS to assess the relationship between serum metabolites and quality and yield grades.

Results and Discussion

Sixty-eight percent of carcasses were assigned Choice grade while carcass distributions among yield grade were 18, 52, and 26%, for yield grade 1, 2, and 3, respectively. Carcass quality grade and yield grade was influenced by rLDH activity (Table 1). Serum rLDH activity was lower ($P = 0.07$) in calves with Choice carcasses than calves with Select carcasses. May et al. (2004) reported that serum LDH activity was lower at weaning in calves that ultimately graded Choice compared to calves that graded Select. Lactate dehydrogenase is the primary enzyme involved in the inter-conversion of pyruvate to lactate and has been found to have a relationship with both cattle (Paria, 1997) and pig (Enfalt et al., 1993; Martoccio et al., 1995) carcass traits. Jurie et al. (1995) reported that semitendinous muscle from male Limousin cattle had greater LDH activity than thoracicus muscle. Lactate dehydrogenase activity has been shown to be positively correlated with carcass lean content and negatively correlated with carcass fat content in young Limousin bulls (Renand et al., 1995). In the present study, calves whose carcasses ultimately graded Choice vs. Select had reduced rLDH activity in sera. The exact physiological mechanisms responsible for this observation are not clear. However, LDH plays a critical role in lipogenesis as growing cattle may utilize lactate as a carbon source for the synthesis of fatty acids (Whitehurst et al., 1981). Increased fat deposition in muscle tissue attributed to increased fatty acid synthesis may influence subsequent quality grade.

Thyroxine was increased ($P = 0.07$) in calves with yield grade 1 carcasses versus calves with yield grade 2 and 3 carcasses. Longissimus muscle area was correlated ($P < 0.05$) with concentrations of prolactin ($r = 0.24$), triiodothyronine ($r = 0.22$), and thyroxine ($r = 0.35$). Other serum metabolites were not related to subsequent carcass characteristics. Thyroxine has been reported to be an indicator of protein deposition (Hayden et al., 1993) and a positive relationship has been shown between thyroxine and growth rate in cattle (Graf and Grosser, 1979). Furthermore, triiodothyronine is involved in normal and androgen related growth and protein accretion in beef steers (Kahl et al., 1992). Heifers had decreased ($P < 0.05$) concentrations of IGF-I (152 vs. 199 ng/mL), and greater ($P < 0.01$) concentrations of creatinine (2.62 vs. 2.08 mg/mL) than steers. However, the percentage of heifers and steers grading Choice did not differ ($P > 0.10$).

Implications

The present study provides further evidence and supports previous observations by our laboratory indicating LDH activity could be used as an early physiological marker of carcass composition. Serum rLDH activity at time of feedlot entry was lower in calves with Choice carcasses compared with Select carcasses. Beef cattle producers in different production systems may predict carcass traits in live cattle based on activity of LDH in sera at weaning or feedlot entry.

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Table 1. Least square means from serum metabolites in carcass characteristics from Brangus-crossbred calves (n = 85)[a]

	Quality Grade			Yield Grade			
Variable	Choice	Select	Pooled SE	1	2	3	Pooled SE
Forward lactate dehydrogenase, IU/mg	875.2	889.3	30.8	875.2	929.0	926.1	31.5
Reverse lactate dehydrogenase, IU/mg	361.4[b]	413.0[c]	25.0	373.1	415.1	425.7	23.3
Hemoglobin, µg/mL	16.1	15.5	3.0	17.9	17.8	14.1	3.0
Protein, mg/mL	114.2	118.1	7.3	127.4	116.7	112.7	7.5
Creatinine, mg/mL	2.5	2.2	0.15	2.5	2.4	2.3	0.2
Prolactin, ng/mL	29.8	29.8	7.7	45.3	22.7	18.7	7.8
Triiodothyronine, ng/mL	3.7	3.4	0.21	4.1	3.5	4.0	0.2
Thyroxine, ng/mL	100.3	100.9	4.6	109.0[b]	92.4[c]	95.3[c]	4.7
Cortisol, ng/mL	61.2	60.6	4.0	59.4	64.5	59.1	4.0
Testosterone, ng/mL	0.03	0.04	0.02	0.03	0.04	0.02	0.02
IGF-I, ng/mL	174.1	176.8	20.0	159.3	180.5	198.9	20.3

[a]Blood serum metabolites were measured 2 d following entry into the feedlot. Calves were approximately 12 mo of age at time of feedlot entry. At harvest, longissimus muscle and ribfat thickness were measured, and USDA quality and yield grades were assigned to each carcass following a 5 mo finishing phase.

[bc]Within each quality and yield grade, means without a common superscript letter differ ($P = 0.07$).

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EFFECTS OF FORAGE CULTIVAR AND ANTHELMINTIC ON STEER RESPONSES TO IMMUNE CHALLENGE

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ABSTRACT: Animal toxicoses may be observed in animals grazing endophyte-infected tall fescue. A grazing trial was designed to determine the effects of anthelmintic treatment and forage cultivar on immune and metabolite responses of steers. Steers (n = 6/pasture; BW = 314 ± 19 kg) were allotted to pastures (1.62 ha) of either toxic endophyte-infected (n = 3 pastures; K31+) or non-toxic endophyte-infected tall fescue (n = 2 pastures; HiMag 4) for 92 d. Steers were given daily access to a mineral containing fenbendazole (1 mg/kg BW). Within pasture, one-half of the steers received either ivermectin (0.2 mg/kg BW) or fenbendazole (15 mg/kg BW) every 21 d. On d-84, all calves were given an injection (i.p.) of lipopolysaccharide from *Salmonella typhimurium* (31.8 ug/45 kg BW). Blood samples were collected at 0, 6, 12, and 24 h post-injection. Whole blood was analyzed for blood cell counts, hemoglobin, and hematocrit. Sera were analyzed for concentrations of prolactin, cortisol, NEFA, and insulin. Fecal egg counts were determined and no internal parasite eggs were detected. Steers grazing HiMag 4 had greater ($P < 0.05$) weight gain than steers grazing K31+; however, ivermectin did not consistently improve steer gains. Serum concentrations of prolactin were decreased ($P < 0.05$) at each time point for steers grazing K31+ compared with steers grazing HiMag 4. Concentration of cortisol was decreased ($P < 0.05$) at time 0 for steers consuming K31+. At 6, 12, and 24 h post-injection, NEFA concentrations were lower ($P < 0.05$) in the sera of steers consuming K31+. Blood cell profiles were not consistently altered by forage cultivar or anthelmintic. Endophyte-infected tall fescue decreased steer gain and may alter blood metabolites associated with immune response.

Key Words: Tall fescue, calves, anthelmintic

Introduction

Plants have developed numerous defenses against herbivory including the production of toxins (for review see Cheeke, 1995). Endophyte-infected (*Neotyphodium coenophialum*) tall fescue (*Festuca arundinacea*) has been shown to produce toxins that result in physiological and production changes in animals (for review see, Strickland et al., 1993; Stuedemann and Hoveland, 1988). Those changes are collectively called fescue toxicosis syndrome. That toxicosis includes symptoms of decreased body weight gains and feed consumption, increased core body temperature, rough hair coats, and overall poor appearance and performance. Several agronomic and animal management solutions have been implemented that improve animal productivity, but to date there are no pharmacological products that conclusively improve animal performance while consuming endophyte-infected tall fescue forage.

Ivermectin is an anthelmintic that has reportedly increased weight gains and decreased symptoms of fescue toxicity for grazing calves (Bransby, 1997; Ellis et al., 1989). It has been suggested that ivermectin can reduce core body temperature during heat stress associated with fescue toxicosis (Spiers et al., 2005). However, other experiments found no benefit to using ivermectin as a treatment for or prevention of fescue toxicosis (Goetsch et al., 1988; Rosenkrans et al., 2001). Our objective was to determine the effects of toxic tall fescue and ivermectin on steer response to immune challenge.

Materials and Methods

Animals and pastures. Thirty Angus crossbred steers (BW = 314 ± 19 kg; ~ 9 mo of age) were randomly allotted to one of five pastures (1.62 ha). The steers, and their dams, were developed on mixed grass pastures that included toxic tall fescue, clovers, bermudagrass, and native grasses. Each of the trial pastures consisted of tall fescue (*Festuca arundinacea*). In three pastures, the tall fescue (K31+) was infected with a toxic wild-type endophytic fungus (*Neotyphodium coenophialum*), and in two pastures the tall fescue was infected with a non-toxic endophytic fungus (HiMag 4). Each pasture had been harvested for hay approximately four weeks prior to trial start, and 21 d prior to trial initiation each pasture was fertilized with 56 kg/ha of nitrogen. The trial was initiated on June 17, 2004.

Anthelmintic treatments. On d 0, 21, 42, 63, and 84 of the trial steers were weighed, fecal sampled, and treated with anthelmintic. On each treatment day, within each pasture three steers were given a subcutaneous injection of ivermectin (0.2 mg/kg BW), and three steers were given an oral drench containing fenbendazole (15 mg/kg BW). Every day all steers were offered a mineral containing fenbendazole (1 mg/kg BW).

Immune challenge. On d 84, all steers were gathered, intermingled and administered an intraperitoneal injection of lipopolysaccharide from *Salmonella typhimurium*

(31.8 µg/45 kg BW). Steers were returned to their test pastures approximately 26 h post-injection. Blood samples were collected at 0, 6, 12, and 24 h post-injection. Blood cell counts, hemoglobin, and hematocrit were determined using EDTA-treated whole blood on a Cell Dyne 3500 analyzer. Validated radioimmunoassays were used to determine serum concentrations of prolactin, cortisol, and insulin. Sera concentrations of NEFA were determined using a validated colorimetric assay. All samples for each metabolite were analyzed in one assay. Intrassay CV were 11, 9, 3, and 9%, respectively for NEFA, cortisol, insulin, and prolactin.

Statistical analyses. Data were analyzed as a split-plot design with pasture as the whole-plot and anthelmintic treatment as the sub-plot. Analysis of variance was conducted using the Mixed procedure (SAS Inst. Inc., Cary, NC). Means separation were performed using the least-squares option with multiple t-tests.

Results and Discussion

Evaluation of the fecal samples found no internal parasite eggs during the trial; therefore, our protocol was effective in eliminating the confounding effects of internal parasites on steer gain and physiological responses. During the 84 d pre-challenge period steers grazing K31+ had lower ($P < 0.05$) ADG, and serum concentrations of prolactin and cortisol than steers grazing HiMag 4 (0.55 vs. 1.08 kg; 2.9 vs. 47.3 ng/mL; 30.0 vs. 55.1 ng/mL; respectively). Anthelmintic treatment did not affect ($P > 0.1$) most pre-challenge steer characteristics. Treating steers with fenbendazole resulted in a larger ($P < 0.05$) concentration (1 x 10^3/µL of blood) of monocytes, at time 0, when compared with steers treated with ivermectin (0.91 vs. 0.59; respectively). Interactions between forage cultivar and anthelmintic treatment were not ($P > 0.1$) a significant source of variation for most items pre-challenge. However, the concentration (1 x 10^3/µL of blood) of neutrophils was affected ($P < 0.05$) by an interaction between forage cultivar and anthelmintic treatment. Steers grazing HiMag 4 and treated with ivermectin had lower ($P < 0.05$) concentrations of neutrophils when compared to steers grazing K31+ and treated with ivermectin (1.21, 2.1, 2.24, 1.68; respectively for HiMag 4 ivermectin, HiMag 4 fenbendazole, K31+ ivermectin, and K31+ fenbendazole).

Steer rectal body temperature at 6 h post-challenge was elevated in all animals, and was higher ($P < 0.05$) in steers consuming K31+ when compared to steers consuming HiMag 4 (41.2ºC vs. 40.3ºC; respectively). Steer rectal temperature had returned to normal by 12 h after injection with lipopolysaccharide. Post-challenge blood cell profiles, ADG, and serum concentrations of cortisol and insulin were not affected ($P > 0.1$) by forage cultivar, anthelmintic treatment, or their interaction. Steers consuming K31+ had lower ($P < 0.05$) serum prolactin and NEFA concentrations at 6, 12, and 24 h post-challenge when compared with steers grazing HiMag 4.

Discussion

Our results indicate that ivermectin does not consistently improve steer gains when consuming toxic tall fescue. In addition, ivermectin did not appreciably alter the immune response of steers. Spiers et al. (2005) indicated that ivermectin reduced the core body temperature of cattle challenged with ergot alkaloids under heat stress conditions. Several conditions may explain our contradictory results. Spiers et al. administered 12 mg/d of ivermectin compared with our label rate of ~ 2.5 mg/d. In addition, our trial was a grazing trial; therefore, the steers were exposed to ambient day and night temperatures, not the constant temperature of an environmental chamber.

Steers used in this study had been developed on mixed grass pastures that included tall fescue. We did not attempt to assess the susceptibility of these steers to ergot alkaloid poisoning prior to the trial; however, tolerance to toxins has been the subject of much discussion. That tolerance of fescue toxins has been observed for cow/calf efficiencies, heterosis, and post-weaning calf productivity (Brown et al., 1997, 1999). Symptoms of fescue toxicosis are varied and related to environmental stress, specifically hyperthermia and increased respiratory rates (Aldrich et al., 1993). While the steers used in this study were exposed to temperatures that exceeded 35ºC during this trial they did have access to shade that could have reduced their environmental stress.

Previously we observed that ivermectin improved oxygen carrying capacity of steers consuming toxic tall fescue hay (Rosenkrans et al., 2001). Oliver et al. (2000) found differences in blood cell distributions and chemistry between steers grazing toxic vs. non-toxic tall fescue. However, those profiles and activities were considered to be within normal biological ranges. In this study, we did not detect differences in blood cell numbers or properties. In addition, we did not detect ivermectin or cultivar effects on immune response. That observation is not consistent with previous reports of ivermectin effects on lamb and calf immune challenges (Stankiewicz et al., 1995; Yang et al., 1993).

The precise mechanism by which ivermectin influences animal physiology is not known; however, ivermectin is known to be a modest gamma-amino butyric acid (GABA) agonist (Turner and Schaeffer, 1989). Receptors for GABA are widely distributed throughout the central nervous system and peripheral organs (Erdo, 1990). Therefore, it is assumed that ivermectin can alter the physiology of steers in the absence of parasites and presumably via GABA receptors distributed throughout the body.

Implications

Ivermectin injections did not consistently improve animal gain or response to immune challenge. Toxic tall fescue depressed steers gains, but did not alter the blood cell response to immune challenge. These data suggest that other management strategies are needed to maximize animal performance while grazing toxic tall fescue.

Acknowledgements

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INCIDENCE AND CHARACTERISTICS OF IRIDESCENT BOVINE MEAT

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ABSTRACT. To determine the incidence of iridescence and their associated factors in bovine fresh meat, two muscles were used (semitendinosus and rectus femoris) from 629 animals. The variables measured were pH and temperature at 45 min and 24 hr postmortem, drip loss and color (L* a* b*) at 24 hr postmortem, the iridescence was visually identified in a color box. !9.4% of semitendinosus muscle samples showed abundant iridescence, 25.8% moderate, 27.7% slight and 27.2% did not had iridescence; the rectus femoris muscle only showed moderate and slight iridescence (0.3% and 6.4%, respectively). The pH for the semitendinosus muscle at 45 min and 24 hr was lower (P< .05) as the iridescence was more intense; The values for brightness (L*) and yellow intensity (b*) were higher (P< .05) in fresh meat showing iridescence; The red color intensity (a*) did not vary significantly. Most meat samples that showed iridescence were from young animals (less than 36 months old) slaughtered principally in winter. In conclusion, bovine fresh meat that shows iridescence is more acid, bright and yellowish and had a drip loss below the normal range for bovine meat; The semitendinosus muscle is more susceptible to show iridescence and this phenomen is more frequently observed in young animals.

Key words: Iridescence, bovine, fresh meat.

Introduction

Color is the principal trait used by the consumers to evaluate meta quality at the counter, for fresh meat cherry bright red is the preferred color, any deviation from this color is associated to poor meat quality. Iridescence is an abnormal color appearance observed in meat products and some muscles from beef cattle carcasses; it resembles a bright rainbow and is frequently observed in cured or precooked meats(Swatland, 1984).Most common iridescence has strong green color, followed by red/ orange combination (Lawrence et al., 2002).

Some researchers (Swatland, 1984) mention that iridescence is caused by microstructural diffraction of the muscular fibers. It must be also noted that in connective tissue frequently are observed iridescent colors, but they really do not contribute to muscular iridescence. For other authors (Deitzel, 2002) this phenomenon is a prismatic effect caused by surface diffraction. Water and phosphates addition to cured or precooked meat products notably increases iridescence (Wang, 1991) by protein extraction that fill empty spaces among muscle fibers increasing refractance and intense iridescence (Water, 2003). Carcass muscles frequently showing iridescence are *semitendinosus* and *semimembranosus,* with 90.6 and 34.4 % respectibly (Kukowsky et al., 2004). Because its great incidence and scarce information about this phenomenon in fresh meat, it was decided to investigate the characteristics of this meat and maturity and animal type influence over the presence of this phenomenon in beef cattle produced in tropical conditions.

Materials and Methods

629 animals were randomly sampled in a Federally Inspected Slaughterhouse at Villahermosa, Tabasco Mexico, during three periods, winter 2003/04, spring and summer 2004, factors considered were maturity and animal type. Two muscles *rectus femoris semitendinosus* were used to evaluate incidence of iridescence, but only in *semitendinosus* were evaluated drip loss, pH, Temperature, CIELAB color.

The animals sampled were randomly chosen immediately before slaughter and their maturity and animal type was recorded. Iridescence was evaluated in four categories abundant, moderate, slight and absent 24 h after slaughter using a Judge color box (Macbeth Inc. U.S.A.). For drip loss, four samples (4 g) of each muscle were taken at 30 min postmortem, sample was then string suspended inside a recipient for 24 h at 3 ºC before drip loss was calculated (Honnikel and Kim, 1986). Muscle pH and temperature were recorded at 45 min and 24 h postmortem with a Delta - Trak ISFET pH 101(Delta - Trak, Inc., Pleasanton, CA., EUA). Color was measured with a CM- 2002 Minolta Spectrophotometer (Minolta Inc., New York U.S.A.) at 24 h *postmortem*, in a 200 g sample of each muscle cleaned of surface fat and connective tissue, the values of L* (luminosity) a* (red intensity d) and b* (yellow intensity) of the CIELAB (Commission Internationale pour I´Eclairage) reference were recorded. ,

Statistical Análisis A frequency test was conducted to determine incidence of iridescence of both muscles. Categoric data analysis (Proc catmod, SAS)was used for maturity and animal type effects and the physicochemical variables were evaluated by linear models

Results and Discussion

19.4% of *semitendinosus* (ST) muscle showed abundant iridescence, 25.8% moderate, 27.7 % slight and 27.2 % were of normal color. For *rectus femoris* (RF) only 0.3 and 6.4% showed moderate and slight iridescence, respectively (fig. 1 y 2).

78.8 % of ST samples had iridescence but only 6.7% of RF muscle had it, this values low compared

with those reported by Kukowski et al. (2004), whose found 90.6% of incidente for ST and 12.5% for RF, the diminution in incidence of iridescence observed in this work could be due to type and of the bovines sampled .

Maturity of animals and sampling period affected the proportion of samples having iridescence (P<0.05), were cattle under 36 months old in winter sampling showed higher positive cases (97.30%), however lowest incidence cases were observed in animals older than 36 months, in every sampling period (50.88 % for winter, 62.22 % for spring and 53.66 % for summer; table 1). Sampling period results variation could be attributed to changes in environmental temperature, that in the tropics, it is a determinant factor of stress before slaughter and this directly affect color appearance of meat (Forrest, 1979), thus it is harder to detect iridescence.

The proportion of positive simples showing iridescence was also affected by the interaction between animal type and sampling period. The *semiten*dinosus Muscle sampled from steers had higher percentages of iridiscence in all three periods winter, spring and summer, 83.03, 70.65 and 76.11 % respectively, while cull cows during summer sampling had the lowest iridescence percentage (40.0%; Table2). It is evident that ST muscle have characteristics that influence directly the presence of this phenomenon, that were the reasons to measure the association of them with some physicochemical variables. Each one of the dependent variables measured were affected (P<0.05) by sampling period and iridescence category.

Highest pH values at 45 min *postmortem* were observed in *semitendinosus* without or slight iridescence in spring sampling (6.61 y 6.57 respectively). While the lowest values for pH were recorded in winter and spring for meat with abundant (6.43) and moderate iridescence (6.45), 24 h postmortem Ph showed a similar tendency, however, ST without iridescence had the maximum value(5.98) in summer sampling. But . ST lower pH values were present for abundant and moderate iridescence during spring 5.57 and winter 5.60 sampling periods.

Semitendinosus muscle average temperatures at 45 min were higher in spring for muscles showing slight (31.63°C) and moderate (31.45 °C) iridescence and in winter the average ST muscle temperatures showed lower values independently of iridescence category. The ST muscle 24 h *postmortem* showed similar tendency , it could be observed that, during the periods with higher environmental temperature, the internal ST muscle was also higher. In tropic humid climate during spring and summer the average temperature at shadow is of 40 °C; It could be argued that exterior temperature does not affect chillers inside temperature, but during loading of carcasses it affects because the doors are countinuosly open and the carcasses themselves maintain a high temperature average, This produce an abnormal St muscle temperature reduction that affects the muscle quality, thats why sampling periods somehow masked the results, thus it could be stated that winter sampling had less effect in the ST muscle characteristics and it was easier to detect iridescence.

In respect to color luminosity (L*) of ST muscle increased as iridescence was more intense, this could be better observed in the values of winter sampling were ST muscle with abundant iridescence had an average value of 42.34, followed by same iridescence category muscle average value of 42.09 during summer sampling, while the lowest values for luminosity were observed for winter ST samples without (34.12) or slight (38.23) iridescence

Red intensity (a*) and yellow intensity (b*) showed a small but significant (P<0.05) tendency to diminish as the iridescence was more intense, however, the values for spring and summer are similar, thus is clear that the values of (a*) and (b*) were affected by the internal ST muscle temperature at *rigor mortis.*

Results for drip loss also indicate that ST muscle winter samples showing abundant iridescence had less drip loss (0.94%), followed by those with moderate, slight and absent iridescence (1.37, 1.36 y 1.60 %, respectively), taken in the same period. Spring and summer ST samples for drip loss had similar percentages for all iridescence categories ,but were statistically different to tose observed in winter.

I**mplications**,

Semitendinosus muscle is more susceptible to show iridescence and this phenomenon is more frequent in muscles of finished steers less than 36 months old. Also, Iridescent meat is associated to less drip loss, more luminosity and less red intensity.

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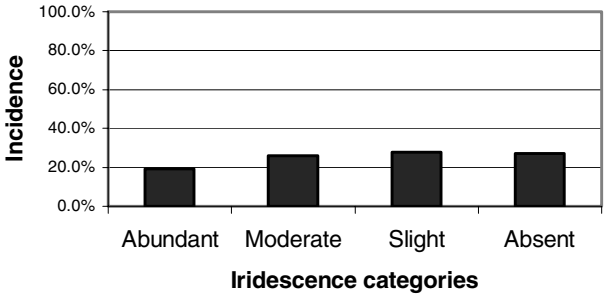

Figure 1. Incidence of iridescence in *semitendinosus* bovine muscle

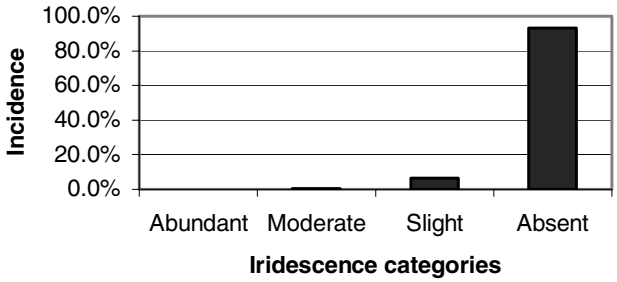

Figure 2. Incidence of iridescence in the *rectus femoris* muscle of bovine

Table 1. Percentage of iridescence by maturity and period in *semitendinosus* bovine muscle

Maturity[1]	Irid[2]	Periods[3]					
		Winter		Spring		Summer	
		N°	%	N°	%	N°	%
a	1	72	97.30[a]	48	71.64[cd]	70	74.47[bc]
	0	2	2.70	19	28.36	24	25.53
b	1	60	85.71[b]	72	67.92[cd]	57	76.00[bc]
	0	10	14.29	34	32.08	18	24.00
c	1	29	50.88[e]	28	62.22[cde]	22	53.66[de]
	0	28	49.12	17	37.78	19	46.34

[a,b,c,d,e] Different letters indicate stastistical diference ($P < 0.05$).
[1]Maturity a= less than 24 months old, b= between 24 and 36 months old, c= more than 36 months old
[2]Iridescence 1= abundant, moderate y slight; 0= absent.
[3]Winter 2003-04; spring and summer, 2004.

Table 2. Percentage of iridescence by animal type and period in *semitendinosus* bovine muscle

Animal type	Irid[1]	Periods[2]					
		Winter		Spring		Summer	
		N°	%	N°	%	N°	%
Steers	1	137	83.03[a]	130	70.65[b]	137	76.11[ab]
	0	28	16.97	54	39.35	43	23.89
Cows	1	24	66.67[bc]	18	52.94[cd]	12	40.00[d]
	0	12	33.33	16	47.06	18	60.00

[a,b,c,d,] Different letters indicate stastistical diference ($P < 0.05$).
[1]Iridescence 1= abundant, moderate y slight; 0= absent .
[2]Winter 2003-04; spring and summer, 2004.

Table 3. Physicochemical characteristics of *semitendinosus* bovine muscle by iridescence category and period

Variable	Periods	Iridescence categories: Abundant N°	Abundant Mean±SE	moderate N°	moderate mean±SE	Slight N°	Slight mean±SE	Absent N°	Absent mean±SE
pH $_{45}$[1]	Inv[9]	45	6.43±.02^{f}	55	6.45±.02^{ef}	61	6.53±.01^{c}	40	6.54±.02^{c}
	Pri[10]	32	6.45±.01^{ef}	55	6.49±.01de	61	6.57±.01^{b}	70	6.61±.01^{a}
	Ver[11]	45	6.51±.01^{cd}	52	6.52±.01^{c}	52	6.52±.01^{c}	61	6.52±.01^{c}
pH $_{24}$[2]	Inv	45	5.60±.01^{ef}	55	5.68±.02^{d}	61	5.73±.02^{cd}	40	5.79±.03^{bc}
	Pri	33	5.57±.02^{f}	55	5.60±.02^{f}	61	5.68±.03de	70	5.80±.03^{b}
	Ver	45	5.74±.03^{cd}	52	5.79±.02^{bc}	52	5.83±.02^{b}	61	5.98±.03^{a}
T_{45}[3]	Inv	45	28.16±.19^{d}	55	28.39±.21^{d}	61	28.30±.18^{d}	40	28.21±.27^{d}
	Pri	32	31.22±.29^{ab}	55	31.45±.20^{a}	61	31.63±.21^{a}	70	31.58±.17^{a}
	Ver	45	30.67±.20^{bc}	52	30.36±.23^{c}	52	30.46±.18^{c}	61	30.09±.20^{c}
T_{24}[4]	Inv	45	5.89±.12^{cd}	55	6.06±.10^{c}	61	5.96±.11^{c}	40	5.53±.13^{d}
		32	10.57±.13^{ab}	55	10.24 ±.13^{b}	61	10.53±.13^{b}	70	10.94±.10^{a}
	Pri								
	Ver	45	10.95±.13^{a}	52	10.85±.14^{ab}	52	10.82±.13^{ab}	61	10.62±.10^{ab}
L*[5]	Inv	45	42.34±.58^{a}	55	39.33±.56^{cde}	61	38.23±.71^{e}	40	34.12±.81^{f}
	Pri	32	41.19±.73^{abc}	55	42.03±.48^{a}	61	41.40±.56^{ab}	70	38.98±.63de
	Ver	45	42.09±.49^{a}	52	41.30±.47^{ab}	52	40.88±.65^{abc}	61	40.26±.71^{bcd}
a*[6]	Inv	45	9.63±.33^{cd}	55	9.24±.36^{d}	61	9.91±.39^{cd}	40	12.04±.57^{a}
	Pri	32	11.19±.52^{ab}	55	11.05±.32^{ab}	61	10.57±.39^{bc}	70	10.42±.42^{bc}
	Ver	45	9.74±.31^{cd}	52	10.50±.27^{bc}	52	10.32±.40^{bc}	61	9.61±.38^{cd}
b*[7]	Inv	45	11.59±.38^{cd}	55	12.23±.53^{bc}	61	13.44±.56^{a}	40	13.23±.74^{ab}
	Pri	32	11.11±.52^{cde}	55	11.42±.33^{cd}	61	11.67±.41^{cd}	70	10.95±.47de
	Ver	45	11.26±.27^{cd}	52	11.51±.34^{cd}	52	10.97±.30de	61	9.80±.41^{e}
	Inv	45	.94±.09^{d}	55	1.36±.07^{b}	61	1.60±.07^{a}	40	1.37±.08^{b}
PAG*[8] *%									
	Pri	32	1.19±.13^{bc}	55	1.09±.08^{cd}	61	1.08±.06^{cd}	70	1.07±.06^{cd}
	Ver	45	1.16±.09^{bcd}	52	1.16±.08^{bcd}	52	1.10±.09^{cd}	61	1.15±.07^{cd}

[a,b,c,d,e] Different letters indicate stastistical diference ($P < 0.05$).

[1] pH $_{45}$= pH at 45 min *postmortem*.

[2] pH $_{24}$= pH at 24 h *postmortem*.

[3] T_{45}= Temperature (°C) at 45 min *postmortem*.

[4] T_2 = Temperature (°C) at 24 h *postmortem*.

[5] L*: 0= black, 100= white.

[6] a* : lower numbers= more green, higher numbers = more red.

[7] b* : lower numbers = more blue, higher numbers = more yellow.

[8] Dri

[11] Ver= Summer 2004.p loss.

[9] Inv= Winter 2003-04.

[10] Pri= Spring 2004.

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EFFECT OF GnRH-ANALOGOUS AND CHROMIUM METHIONINE SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF SOWS

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ABSTRACT: To determine the effect of GnRH-analogous and chromium methionine supplementation on reproductive performance of sows, two hundred thirty nine multiparous hybrid sows were used, in a complete randomized experiment design with a 2 x 2 factorial arrangement. Sows were designed to receive one of four treatments: Diet supplemented or not with 0.4 ppm of chromium (from Cr-methionine; Microplex® Zinpro Co.), during three continues reproductive cycles and application or not of 50 µg of GnRH-analogous (Fertagyl®; Intervet Lab) four days before weaned. During first cycle chromium supplementation had not effect ($P > 0.22$) on total pigs born, live pigs born, litter weight born, and first service farrowing rate. During the second and third cycle Cr supplementation and GnRH-analogous application not affected ($P > 0.56$) the total pigs born, live pigs born, litter weight born, and first service farrowing rate. Across complete experiment Cr supplementation diminished ($P < 0.01$) the interval estrus post weaned (5.36 vs. 4.53 days), while GnRH-analogous application had not effect ($P = 0.32$) this variable; an interaction Cr x GnRH ($P = 0.04$) was found. This result indicates that chromium methionine supplementation is effective to diminishing interval weaning-first estrous in multiparous sows.

Key Words: Chromium, GnRH-analogous, Reproduction, Sows.

Introduction

The time required to the sow restarting its cyclic activity after weaned is an important component of its reproductive efficiency. LH secretion is a key factor on the onset of estrous activity after weaning (King and Martin, 1989). Response of ovaries to gonadotropins can be improved by increments of glucose and insulin in plasma (Boot et al., 1996). Chromium as glucose tolerance factor component (GTF) potentates the action of insulin (Mertz, 1992; Chang and Mowat, 1992), facilitating the union of insulin with its specific cell receptors (Anderson and Mertz, 1997), that becomes essential to carbohydrates metabolism (Amoikon et al., 1995). Campbell (1996) found an increment in the proportion of sows that reinitiate estrous within seven days after weaned as a consequence of chromium picolinate supplementation. Lindemann et al. (2004) supplementing chromium picolinate found an increment on total pigs born and live pigs per litter. GnRH-analogous has been used to contrarrest negative effects of the lactation catabolism (Brüssow et al., 1996), reducing the interval weaning-first estrous (Gooneratne et al., 1989; Szabó et al., 1992). This experiment was carrying out with the objective of determine the effect of GnRH-analogous and Cr-methionine supplementation on reproductive performance of sows.

Material and Methods

The experiment was conducted from February 2003 to October 2004, in the facilities of "El Saucito" Pork Commercial Farm, in Navolato County, Sinaloa in the Northwest of Mexico. The annual mean temperature is 24.6 °C with 41.7 °C and 1.7 °C maximum and minimums, respectively (INEGI,1993). Two hundred thirty nine multiparous hybrid sows were used, in a complete randomized experiment design with a 2 x 2 factorial arrangement. Sows were randomly assigned to receive one of four treatments: 1) Feeding regular diet of the farm during three continues reproductive cycles (Control; n = 71); 2) Feed diet similar to control supplemented with 0.4 ppm of Cr (from Cr-methionine; Microplex® Zinpro Co.), (Cr-Met treatment; n = 63); 3) Feed control diet and were injected with 50 µg of GnRH-analogous (Fertagyl®; Intervet Lab) four days before weaned (GnRH; n = 52); and 4) Feed the Cr-Met supplemented diet and were injected with GnRH-A (Cr-GnRH treatment; n = 53). Pregnant sows during the first 4 week post service received 1.8 kg of a diet for pregnant sows (Table 1), during 5 to 13 weeks received 2 kg of the same diet, and the remainder time until parturition they fed 2.5 kg of lactation diet (Table 1). During lactation phase, they received a lactation diet in amount accord to appetite offered several times by day. The total pigs born, live pigs born, litter weight born, first service farrowing rate, and the interval weaning-first estrous were recorded during three consecutive reproductive cycles.

Statistical analysis. Data total pigs born, live pigs born, litter weight born, and the interval weaning-first estrous were analyzed as a completely randomized experimental design with a 2 x 2 factorial (Steel and Torrie, 1985) arrangement using ANOVA/COV of GLM procedures of Satistix® 8 (Analytical Software; Tallahassee, FL). The data first service farrowing rate was analyzed by Chi-Square test using two by two table procedure of Satistix ® 8.

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Table 1. Composition of the diets used in the experiment.

Ingredients	Diets			
	Pregnant and weaned		Lactating	
	Ctrl.	Cr	Ctrl	Cr
Ground corn	624	623.6	597	596.6
Soybean meal (46% CP)	141	141	215	215
Wheat bran	150	150	68	68
Soybean oil	5	5	40	40
SPX Criacerdina[1]	80	80	0	0
SPX Lactocerdina[2]	0	0	80	80
Microplex[3]	0.0	0.4	0.0	0.4
Total, kg	1000	1000	1000	1000
	Calculated Analyses [4]			
Crude protein , %	15.00	15.00	17.00	17.00
Calcium, %	0.90	0.90	0.88	0.88
Phosphorous, %	0.72	0.72	0.69	0.69
Lysine, %	0.77	0.77	0.94	0.94
Supplementary Chromium, ppm	0.0	0.4	0.0	0.4
ME, kcal/kg	3154	3154	3465	3465

[1] SPX Criacerdina MR. (Purina Mills, Co.), [2] SPX Lactocerdina MR. (Purina Mills, Co.), [3] Microplex MR (Zinpro, Co.) chromium methionine premix containing 1 g of Cr per kg, [4] Calculated from published values (NRC, 1988)

Results and Discussion

The effects of chromium supplementation and GnRH-analogous application on the interval estrous post weaned in multiparous sows, during three continuous reproductive cycles, are shown in table 2.

Table 2. Effect of chromium supplementation and GnRH-analogous application on the interval weaning-first estrous in multiparous sows.

Items	Treatments				SEM[1]
	Ctrl.	Cr	GnRH	Cr + GnRH	
Sows, n	147	134	109	114	
IWFS, days [2]	5.86	4.36	4.87	4.70	0.16

Main effects	*P*-value
Cr	0.01
GnRH	0.32
Cr x GnRH	0.04

[1] Standard error of the mean.
[2] Interval weaning-first estrous.

The chromium supplementation diminished ($P < 0.01$) from 5.36 to 4.53 days the length of interval estrous post weaned. These results are in concordance with observations of Campbell (1996), and Hagen et al. (1998), that found an increment in the proportion of sows that shown estrous within seven days next to weaned, supplementing diets with 0.2 ppm of Cr from chromium picolinate. This response could be attributable to the known action of chromium in improving the insuline activity to enhance the use of glucose by the cells (Garcia et al., 1997). An increment in glucose availability *via* a better insulin activity, improves the activity of cell of the hypothalamus-hypophysis-ovaries axis to stimulate the pulse generator of GnRH, and increase the frequency of LH pulses (Cox et al., 1989). LH pulses (Shaw and Foxcroft, 1985; King and Martin, 1989) and loss of insulin activity (Tokach et al., 1992) are inversely correlated with length of interval estrus post weaned.

The effects of chromium supplementation on total pigs born, live pigs per litter, and litter weight at born time are shown in tables 3 and 4, and the effects on first service farrowing rate are presented in tables 5 and 6.

Table 3. Effects of chromium supplementation on total pigs born, live pigs born and litter weight born during the first experimental cycle.

Items	Treatments		SEM[1]	*P*-value
	Ctrl	Cr		
Sows, n	120	116		
Total pigs born, n	11.61	11.17	0.22	0.31
Live pigs born, n	10.11	9.85	0.19	0.50
Litter weight born, kg	14.02	14.47	0.26	0.39

[1] Standard error of the mean

Table 4. Effect of chromium supplementation and GnRH-analogous application, on total pigs born, live pigs born and litter weight born, during the second, and third experimental cycles.

Items	Treatments				SEM[1]
	Ctrl.	Cr	GnRH	Cr + GnRH	
Sows, n	94	87	73	72	
TPB[2], n	11.41	11.23	11.48	11.32	0.19
LPB[3], n	10.38	10.16	10.30	10.36	0.17
LWB[4], kg	13.69	14.07	13.96	14.21	0.21

Main effects, *P*-value			
	TPB[2]	LPB[3]	LWB[4]
Cr	0.64	0.81	0.48
GnRH	0.84	0.86	0.64
Cr x GnRH	0.97	0.68	0.88

[1] Standard error of the mean.
[2] total pigs born.
[3] live pigs born.
[4] litter weight born.

Chromium supplementation has not effect ($P > 0.20$) on total pigs born, live pigs per litter, litter weight at born time and first service farrowing rate. These results are consistent with those observed by Campbell (1996) in multiparous sows; however Lindemann et al. (2004) found an quadratic effect on number of live pigs per litter in sows fed with diets containing 0.2, 0.6 and 1 ppm of Cr from chromium tripicolinate. In young sows several authors (Lindemann et al., 1995a; Lindemann et al.,

1995b; Trottier and Wilson, 1998), has been observed an increment on total piglet born and live born as consequence of chromium supplementation.

The GnRH-analogous administration not affected (P = 0.32) the interval estrus post weaning (table 2), however a Cr x GnRH interaction (P = 0.04) was observed, which indicates that when Cr and GnRH-analogous are administered together, the efficiency of Cr on this variable becomes minimized.

Table 5. Effects of chromium supplementation on first service farrowing rate during the first experimental cycle.

Items	Treatments	
	Control	Cr
Sows, n	123	116
Farrowed sows, n	108	108
Non farrowed sows, n	15	8
Farrowing rate, % [1]	88	93

[1] There are not statistical difference, P = 0.23

GnRH-analogous application has not effect (P > 0.50) on total pigs born, live pigs born, litter weight born and first service farrowing rate. Similar results has been obtained by Romo et al. (2001) when GnRH-A was injected at estrous time both multiparous sows and gilts.

Table 6. Effect of chromium supplementation, and GnRH analogous application on first service farrowing rate during the second and third experimental cycles.

Items	Treatments			
	Ctrl.	Cr	GnRH	Cr + GnRH
Sows, n	107	102	82	82
Farrowed sows, n	88	88	68	68
Non farrowed sows, n	19	14	14	14
Farrowing rate, % [1]	82	86	83	83

[1] There are not statistical difference, P > 0.56

The absence of effect GnRH-analogous on reproductive performance of sows, could be due, that if is true that a single 50 µg GnRH-analogous injection trigger GnRH plasma concentration, not necessarily support a consistent presence of high GnRH levels during enough time to reactivate the cyclic activity of the sows. The results of this experiment indicates that chromium methionine supplementation, is an available tool to improve reproductive performance of the sow, whereas GnRH injection has not effect and its administration together with chromium, can minimizes the efficiency of these micro-mineral.

Implications

The supplementation with Cr from chromium methionine during the reproductive cycle reduces the interval weaning-first estrous in multiparous sows.

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EFFECT OF GnRH-ANALOGOUS AND CHROMIUM METHIONINE SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF YOUNG SOWS.

J.A. Romo [1], R. Barajas [1,2] , J.J. Valencia [3], J. Castañeda [4], E. Silva [5], and A. Orihuela [6]

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ABSTRACT: To determine the effect of GnRH-analogous (GnRH-A) and chromium methionine supplementation on reproductive performance of young sows, one hundred twenty eight hybrid young sows were used, in a completely randomized experiment design with a 2 x 2 factorial arrangement. Sows were designed to receive one of four treatments: Diet supplemented or not with 0.4 ppm of chromium (from Cr-methionine; Microplex® Zinpro Co.), during two continues reproductive cycles and application or not of 50 µg of GnRH-A (Fertagyl®; Intervet Lab) four days before weaning. During first cycle chromium supplementation has not effect ($P > 0.16$) on total pigs born, live pigs born, litter weight born, and first service farrowing rate. During second cycle Cr supplementation and GnRH-A application has not affect ($P > 0.07$) on total pigs born, live pigs born, litter weight born; however, the first service farrowing rate was improved ($P < 0.01$) both chromium supplementation and GnRH-A application (69 *vs.* 84 %). Across complete experiment Cr supplementation diminished ($P < 0.01$) on 37 % the interval weaning-first estrous (8.12 *vs.* 5.14 days), while GnRH-A application not affected ($P = 0.19$) this variable; interaction Cr x GnRH was not observed ($P = 0.07$). This result indicates that chromium methionine supplementation is effective to reduce interval weaning-first estrous in young sows, and both chromium methionine supplementation and GnRH-A improves the first service farrowing rate in primiparous sows.

Key Words: Chromium, GnRH, Young sows.

Introduction

The young sows integrate proximately the 50 % of herd in commercial farm. Its low reproductive efficiency during their first and second parturition affects strongly the reproductive indicators of the farm (English et al., 1981).

LH secretion is a key factor to restarting estrous after weaning (King and Martin, 1989). The response of ovaries to gonadotropins can be improves by increments of glucose and insulin plasma levels (Boot et al., 1996). Chromium as component of the glucose tolerance factor (GTF) potentiality the action of insulin (Mertz, 1992; Chang and Mowat, 1992), favoring the union of insulin with its specific cell receptors (Anderson and Mertz, 1997), that becomes essential to carbohydrates metabolism (Amoikon et al., 1995). Feeding diets supplemented with 0.2 ppm of chromium from chromium tripicolinate increase farrowing rate (Campbell, 1996), and live pigs born in young sows (Lindemann et al., 1995a). Injection of GnRH-A has been used to contrarrest negative effects of the lactation catabolism (Brüssow et al., 1996), reducing interval weaning-first estrous (Gooneratne et al., 1989; Szabó et al., 1992). The objective of this experiment was determine the effect of GnRH-A and chromium methionine supplementation on reproductive performance of young sows.

Material and Methods

The experiment was conducted from march 2003 to march 2004, in the facilities of "El Saucito" Pork Commercial Farm, in the Sinaloa State in the Northwest of Mexico. The annual mean temperature is 24.6 °C with 41.7 °C and 1.7 °C maximum and minimums, respectively (INEGI, 1993). One hundred twenty eight hybrid gilts were used, in a completely randomized experiment design with a 2 x 2 factorial arrangement. Gilts were randomly assigned to receive one of four treatments: 1) Feeding regular diet of the farm during two continues reproductive cycles (Control; n = 34); 2) Feed diet similar to control supplemented with 0.4 ppm of Cr (Cr-Met; n = 32) from Cr-methionine (Microplex® Zinpro Co.); 3) Feed control diet and injected with 50 µg of GnRH-A (Fertagyl®; Intervet Lab) four days before weaned (GnRH; n = 33); and 4) Feed the Cr-Met supplemented diet and GnRH-A injection (Cr-GnRH; n = 29). Upon completed finishing period (159 ± 4 days of age), the gilts were selected and transferred to service area, where fed *ad libitum* with a gestation diet until receiving natural serve (age average = 204 ±10 days). Pregnant sows during the first 4 week after service were feed with 1.8 kg of a diet for pregnant sows (Table 1), during 5 to 13 weeks received 2 kg of the same diet, and the remainder time until partous they fed 2.5 kg of lactation diet (Table 1). During lactation phase, they were feed a lactation diet in amount accord to appetite offered several times by day. Total pigs born, live pigs born, litter weight born, first service farrowing rate, and the interval weaning-first estrous were recorded during two consecutive reproductive cycles.

Statistical Analysis. Data of total pigs born, live pigs born, litter weight born, and the interval weaning-first estrous were analyzed as a completely randomized experimental design with a 2 x 2 factorial (Steel and Torrie, 1985) arrangement using ANOVA/COV of GLM procedures of Satistix ® 8 (Analytical Software;

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Tallahassee, FL). First service farrowing rate data were analyzed by Chi-Square test using two by two table procedure of Satistix ® 8.

Table 1. Composition of the diets used in the experiment.

Ingredients	Diets			
	Pregnant and weaned		Lactating	
	Ctrl.	Cr	Ctrl	Cr
Ground corn	624	623.6	597	596.6
Soybean meal (46% CP)	141	141	215	215
Wheat bran	150	150	68	68
Soybean oil	5	5	40	40
SPX Criacerdina[1]	80	80	0	0
SPX Lactocerdina[2]	0	0	80	80
Microplex[3]	0.0	0.4	0.0	0.4
Total, kg	1000	1000	1000	1000
	Calculated Analyses [4]			
Crude protein , %	15.00	15.00	17.00	17.00
Calcium, %	0.90	0.90	0.88	0.88
Phosphorous, %	0.72	0.72	0.69	0.69
Lysine, %	0.77	0.77	0.94	0.94
Supplementary Chromium, ppm	0.0	0.4	0.0	0.4
Metabolizable Energy, kcal/kg	3154	3154	3465	3465

[1] SPX Criacerdina MR (Purina Mills, Co.), [2] SPX Lactocerdina MR (Purina Mills, Co.), [3] Microplex MR (Zinpro, Co.) chromium methionine premix containing 1 g of Cr per kg, [4]Calculated from published values (NRC, 1988).

Results and Discussion

The effects of chromium supplementation and injection of GnRH-A on the interval weaning-first estrous in young sows, during two continuous reproductive cycles are shown in table 2. The chromium supplementation diminished ($P < 0.01$) in 37 % (8.12 *vs.* 5.14 days) the length of interval weaning-first estrous. These results are in concordance with observed by Campbell (1996), Hagen et al. (1998), and Trottier and Wilson (1998), whose found increment in percentage of sows that shown estrous within seven days next to weaning, and diminished the length of interval weaning-first estrous supplementing diets with 0.2 ppm of Cr from chromium picolinate. This response could be attributable to the known fact, that chromium improving the insuline activity and enhance the use of glucose by the cells (Anderson and Mertz, 1997; Garcia et al., 1997). An increment in glucose availability way a better insulin activity, improves the activity of cell implicates in the hypothalamus-hypophysis-ovaries axis to stimulate pulse generator of GnRH, that stimulate increments on frequency of LH pulses (Cox et al., 1989). LH pulses (Shaw and Foxcroft, 1985; King and Martin, 1989) and loss of insulin activity (Tokach et al., 1992) are inversely correlated with length of interval weaning-first estrous.

Table 2. Effect of chromium supplementation and GnRH-A application on the interval weaning-first estrous during the first and second reproductive cycles of the sows.

Items	Treatments				SEM[1]
	Ctrl.	Cr	GnRH	Cr + GnRH	
Sows, n	52	39	55	48	
IWFE, days [2]	9.69	4.89	6.56	5.39	0.91
Main effects	*P*-value				
Cr	0.003				
GnRH	0.19				
Cr x GnRH	0.07				

[1] Standard error of the mean.
[2] Interval weaning-first estrous.

The effects on first service farrowing rate are presented in tables 3 and 4. Chromium supplementation had not effect ($P = 0.16$) on first service farrowing rate in the first parturition. The first service farrowing rate in the second parturition was improved ($P < 0.01$) both by chromium supplementation as GnRH-A injection**.** These results are consistent with observed by Campbell (1996) in primiparous sows, who reported improvement in the first service farrowing rate during first and second parturition of the sows, when were feed with a 0.2 ppm chromium tripicolinate supplemented diet during gestation.

Table 3. Effects of chromium supplementation on first service farrowing rate during the first reproductive cycle of young sows.

Items	Treatments	
	Control	Cr
Sows, n	67	61
Farrowed sows, n	60	50
Non farrowed sows, n	7	11
Farrowing rate, % [1]	89	82

[1] There are not statistical difference, $P = 0.16$

The effects of chromium supplementation on total pigs born, live pigs born, and litter weight born are shown in tables 5 and 6. These results are similar to found by Lindemann et al. (1995a), Lindemann et al. (1995b), and Trottier and Wilson, 1998).

The GnRH-A administration has not effect ($P = 0.19$) on the interval weaning-first estrous (table 2), interaction Cr x GnRH was not observed ($P = 0.07$). GnRH application has not effect ($P > 0.38$) on total pigs born, live pigs born, and litter weight born. These results are in concordance with observed by Romo et al. (2001) when injected GnRH-A at estrous time both multiparous sows and gilts. The results of this experiment indicates that chromium methionine supplementation, is an available tool to improve reproductive performance of the young sows, while that a

single 50 µg GnRH-A injection joint with chromium methionine supplementation improves the first service farrowing rate of the young sows in their second parturition.

Table 5. Effects of chromium supplementation on total pigs born, live pigs born and litter weight born, during the first parturition of young sows.

Items	Treatments		SEM[1]	*P*-value
	Ctrl	Cr		
Sows, n	58	53		
Total pigs born, n	10.53	10.17	0.25	0.47
Live pigs born, n	9.57	9.20	0.23	0.43
Litter weight born, kg	12.65	12.18	0.30	0.44

[1] Standard error of the mean.

Table 6. Effect of chromium supplementation and injection of GnRH-A on total pigs born, live pigs born and litter weight born, during the second parturition.

Items	Treatments				SEM[1]
	Ctrl.	Cr	GnRH	Cr + GnRH	
Sows, n	24	18	27	22	
TPB[2], n	10.88	8.50	10.11	10.04	0.34
LPB[3], n	9.79	8.00	9.59	9.27	0.31
LWB[4], kg	13.96	12.03	13.26	12.77	0.38

	Main effects, *P*-value		
	TPB[2]	LPL[3]	LWBT[4]
Cr	0.07	0.10	0.38
GnRH	0.56	0.39	0.98
Cr x GnRH	0.09	0.24	0.35

[1] Standard error of the mean.
[2] Total pigs born.
[3] Live pigs born.
[4] Litter weight born.

Implications

The chromium methionine supplementation during the reproductive cycle reduces the interval weaning-first estrous and first service farrowing rate of the young sows. GnRH-A application improves the first service farrowing rate in the second parturition of young sow.

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THE EFFECTS OF EARLY WEANING ON COW PERFORMANCE AND GRAZING BEHAVIOR IN THE INTERMOUNTAIN WEST

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ABSTRACT: Our objective was to determine the influence of early weaning (130 ± 2 d; EW) and traditional weaning (209 ± 2 d; TW) on cow performance and grazing behavior within three 810-ha pastures. In addition, cow winter feed costs were compared. One hundred fifty-six cow/calf pairs (130 ± 2 d lactation; 78 steer calves and 78 heifer calves) were used in a Randomized Complete Block design in this first year of a two-year study. Cows were stratified by calf sex, BCS, and age and assigned randomly to one of two treatments (TRT) and one of three pastures. Two cows from each TRT and pasture were fitted with global positioning system collars to evaluate grazing behavior. EW calves were allotted to one of three pens (17 x 21 m), in a manner consistent with their dams blocking allocation, and provided meadow hay daily at approximately 2.5% of BW (DM basis) from EW to TW (79 d). The TW calves grazed with their dams during this time. In addition, EW calves were provided 1.0 kg•hd^{-1}•d^{-1} (DM basis) of a supplement formulated to contain 26% CP. All cows were removed from pastures following TW and placed in six separate 25 ha pastures. The same cow groups (blocks) remained intact; however, EW and TW cows were separated and randomly allotted to pastures. Cows were fed 110 d to attain a similar BCS (minimum of 5) by approximately 1 mo prior to calving. The TW cows lost 0.5 BCS and 44 kg while the EW cows gained 0.4 BCS and 12 kg from EW to TW ($P < 0.01$). After 110 d of feeding, there was no difference between EW and TW cow BCS ($P = 0.59$). However, winter feed costs were $28 greater ($P = 0.07$) for TW compared with EW cows. Grazing time, distance traveled, number of visits to water, and cow distribution in rangeland pastures were unaffected ($P > 0.10$) by TRT. Results suggest that EW can improve cow BCS entering the winter feeding period, thereby, decreasing winter feed costs. Cow grazing behavior was not affected by weaning treatment.

Key words: Behavior, Cow, Economics, Management, Weaning

Introduction

Early weaning spring born calves can economically yield heavier calves compared with calves left alongside their dams on sagebrush-bunchgrass range until mid-October (Wallace and Raleigh, 1961). Also, early weaning has additional potential benefits. These include: 1) the cow does not have the additional nutrient requirement of lactation and shouldn't lose as much body condition; 2) the total number of animal units on the range is decreased, thereby extending the number of days cows can remain on range without hay feeding; and 3) dry-gestating cows may cover more range and be better distributed over the grazing area.

Annual winter feed costs in the Intermountain West often total $100 to $200 per cow, representing a significant economic hardship for cow/calf producers. Winter feed costs normally include the cost of harvested forage and supplement necessary to sustain, or increase, cow BCS prior to calving. This is necessary to optimize conception rate and to maintain a 365-d calving interval (Herd and Sprott, 1986). The objective of this study was to compare the effects of early weaning and traditional weaning on cow performance, grazing behavior, and subsequent winter feed costs.

Materials and Methods

Experimental Sites

Grazing research was conducted in three 810-ha pastures at the Northern Great Basin Experimental Range, 72 km west-southwest of Burns, OR. Vegetation has been described previously (Ganskopp, 2001).

Post-weaning management of calves and winter feeding of cows was conducted at the Eastern Oregon Agricultural Research Center, 6 km south of Burns, OR. Early weaned (**EW**) calves were managed in a feedlot and bunk-fed. Winter feeding of cows took place in six 25-ha native flood meadow pastures that were previously harvested for hay.

Available standing crop in each pasture at the Northern Great Basin Experimental Range was measured at the beginning and conclusion of the grazing period by clipping 20 randomly (randomized from pasture UTM coordinates) placed 1-m^2 quadrats in each pasture. Clipped herbage was dried at 55°C for 48 h and weighed for determination of standing crop.

Experimental Design

One hundred fifty-six spring-calving Angus x Hereford cows (78 with steer calves and 78 with heifer calves) were used in the first year of a planned two year early-weaning study. The experimental design was a Randomized Complete Block and was approved by the Institutional Animal Care and Use Committee at Oregon State University. The first year of the experiment began August 2, 2004 (EW date) and concluded February 15, 2005 (approximately 1 month prior to calving). One wk prior to EW, cows were stratified by calf sex, BCS, and age and assigned randomly to one of two weaning treatments

and one of three pastures. All animals were then managed in a common pasture as a single group until the date of EW. Early weaned calves (39 steers; 39 heifers) were 130 ± 2 d of age (on August 2) and traditional-weaned calves (**TW**; 39 steers; 39 heifers) were 209 ± 2 d of age (on October 20). All cows were weighed and evaluated for BCS following an overnight shrink (16 h) at EW and TW. Also, all calves were weighed at EW and TW following a 16-h shrink (overnight).

Early-weaned calves were allotted to one of three pens (17 x 21 m), in a manner consistent with their dams blocking allocation, and provided meadow hay daily at approximately 2.5% of BW from EW to TW (79 d). The TW calves grazed along side their dams during this time. In addition, EW calves were provided 1.0 kg•hd^{-1}•d^{-1} (DM basis) of a supplement consisting of 63.5% ground beet pulp, 33% soybean meal, and 3.5% mineral/salt mix (7.3% Ca, 7.2% P, 27.8% Na, 23.1% Cl, 1.5% K, 1.7 % Mg, .5% S, 2307 ppm Mn, 3034 ppm Fe, 1340 ppm Cu, 3202 ppm Zn, 32 ppm Co, 78 ppm I, 85 ppm Se, 79 IU/kg vitamin E, and 397 kIU/kg vitamin A). The supplement was formulated to contain 26% CP (DM basis). The quantity of meadow hay provided to each pen was noted daily.

Early weaned cows and TW cows and calves were returned to their respective pastures at the Northern Great Basin Experimental Range approximately 1 week after EW. Each pasture had 26 EW cows and 26 TW cow/calf pairs. Water and mineral/salt placement within each pasture were maintained in the same location throughout the experiment. The mineral/salt mix described above was available free choice.

Six cows from each treatment (2 cows•pasture^{-1}•treatment^{-1}) were fitted with global positioning system (**GPS**) collars (Lotek GPS_2000 Collars; Lotek, 115 Pony Drive, Newmarket, Ontario, Canada, L3Y7B5) to obtain data related to grazing behavior. Collars are equipped with head forward/backward and left/right movement sensors, a temperature sensor, and a GPS unit. The collars were programmed to take position readings at 5-min intervals for three 7-d periods evenly distributed between EW and TW dates to estimate grazing time (h/d), distance traveled (m/d), frequency of visits to water (visits/wk), maximum distance from water (m/d), and cow distribution (percentage of ha occupied•pasture^{-1}•wk^{-1}). Collar data were retrieved after each 7-d period, downloaded to a computer, and converted from latitude/longitude to Universal Transverse Mercator as described by Ganskopp (2001). Grazing time was estimated through generation of a prediction model for each cow. Each collared cow was visually observed for 8-12 h. Activities monitored included: grazing, resting (standing or lying down), and walking. Prediction models for estimating grazing time were developed via forward stepwise regression analysis for each cow (S-Plus 2000, Mathsoft Inc., Seattle, WA). The dependent variable was grazing time (min/5 min interval) and the independent variables from GPS collar data included: head forward/backward and left/right movement sensor counts, sum of forward/backward and left/right movement counts, ambient temperature, and the distance traveled (m) by the cow within each 5-min interval. Distance traveled (used for predicting grazing time and distance traveled/d) is underestimated because straight-line pathways were assumed between successive coordinates. Cow distribution within pastures was estimated with Geographic Information System software (Idrisi32 For Windows, Clark Univ., Worcester, MA) using 1-ha grids.

All cows were removed from the three Northern Great Basin Experimental Range pastures following weaning of the TW calves, palpated for determination of pregnancy, and pregnant cows placed in the six separate pastures at the Eastern Oregon Agricultural Research Center. The same cow groups (blocks) were maintained from the Northern Great Basin Experimental Range pastures to the Eastern Oregon Agricultural Research Center pastures; however, EW and TW cows were separated and randomly allotted (by previous blocking structure) to pastures. The amount of hay, alfalfa, and inputs specifically associated with each cow group were recorded daily. The EW and TW cows were fed to attain a similar BCS by February 15, approximately 1 mo prior to calving.

The production costs associated with each weaning treatment were compared for economic analysis. The economic components of the study included winter feed costs and the overall net return for EW and TW cows. Actual feed costs were used whenever possible.

Before study initiation, calves were vaccinated with Vira Shield® 5 and Clostri Shield® 7 (Novartis Animal Health US, Inc.) at approximately 30 d of age. Four weeks later calves were revaccinated with Clostri Shield® 7. Two weeks prior to weaning calves were vaccinated with Vira Shield® 5 + Somnus and a Clostri Shield® 7 booster. At weaning, calves received a booster of Vira Shield® 5 + Somnus.

Approximately 1 month prior to calving, all cows were vaccinated with Vira Shield® 5 and Clostri Shield® 7. Also, all cows were vaccinated with Vira Shield® 5 + VL5 (Novartis Animal Health US, Inc.) at TW.

Statistics

Available standing crop, cow and calf performance data, and cow and calf economical data were analyzed as a Randomized Complete Block using the GLM procedure of SAS. The model included treatments (EW and TW) and pasture (n = 3). A Fisher's protected LSD ($P \leq 0.05$) was used for mean separations (Fisher, 1966).

The experimental design for cow behavioral data (grazing time, distance traveled, frequency of visits to water, maximum distance from water, and cow distribution) was a randomized complete block with three replications (pastures) and two factors: treatments (EW and TW) and sampling periods (n = 3). Data were analyzed as a split-plot with treatments as whole plots and periods as subplots (Petersen, 1985). A Fisher's protected LSD was used as previously to separate treatment means.

Results and Discussion

Standing Forage

Initial and final standing forage at the Northern Great Basin Experimental Range was not affected by pasture ($P > 0.46$) and averaged 297 and 181 kg/ha,

respectively. Initial standing forage was greater than final standing forage ($P = 0.04$). Precipitation for the crop year was 82% (229 mm) of the 67-year average (279 mm; WRCC, 2005); however, it is of interest to note that precipitation during the grazing period of the study (August through October) was 144% of average.

Behavior

Weaning treatment did not influence time spent grazing, resting, or walking ($P > 0.34$; Table 1). In addition, distance traveled (m/d) and average distance to water (m/d) were similar for EW and TW cows ($P > 0.16$). The number of visits to water each week and the percentage of ha occupied per pasture each week by EW and TW cows were not different ($P > 0.15$). We are aware of no other research evaluating the effects of weaning on grazing behavior of beef cows.

Cow Performance

During the 79 d between EW and TW, BCS of EW cows increased 0.4 while TW cows lost 0.5 ($P < 0.01$; Table 2). Similarly, weight change during the same period was 12 and -44 kg for EW and TW cows, respectively ($P < 0.01$). These results agree with other research that has demonstrated increased cow weight and/or BCS with EW compared with TW (Short et al., 1996; Story et al., 2000). During the winter feeding period, TW cows gained 0.8 more BCS and 32 kg compared with EW cows ($P < 0.03$). Consequently, overall total cow BCS change was not affected by weaning treatment ($P = 0.38$). Nevertheless, overall weight change for EW cows was 24 kg greater than what was observed for TW cows ($P = 0.01$). Total feed costs for EW cows during the winter feed period were \$149.49 compared with \$177.42 for TW cows ($P = 0.07$; data not shown). The greater cost associated with TW cows is because of the alfalfa (and related costs) required to obtain a similar BCS to EW cows by 1 mo prior to calving.

Calf Performance

Calves of EW and TW cows weighed 175 ± 3 kg at EW (8/02/2004) and 229 ± 4 kg at TW (10/20/2004) with no difference because of weaning treatment ($P > 0.91$; data not presented). As a result, calf ADG from EW to TW was approximately 0.69 kg for both groups of calves ($P = 0.68$). This was expected of the EW calves because of the meadow hay and supplement provided; however, we did not anticipate TW calves to perform at this level. This erroneous assumption was based on long-term calf performance data from the Northern Great Basin Experimental Range where calf ADG from August to October is approximately 0.23 kg (Wallace and Raleigh, 1961; Turner and DelCurto, 1991). This may partially be explained by a combination of the low total precipitation in the 2003-2004 crop year and timely precipitation during the grazing portion of the study. Ganskopp and Bohnert (2001) demonstrated that forage nutritional quality is greater in those years with below average compared with above average precipitation. Also, the above average precipitation from August through October allowed for fall green up which should have improved the nutritional quality of the diet selected by calves, thereby improving ADG. The quantity of meadow hay offered to EW calves averaged 4.3 $kg \bullet hd^{-1} \bullet d^{-1}$ (DM basis). The total cost of feeding the EW calves from EW to TW was \$47.95/hd, resulting in a cost of gain of \$0.88/kg. No calf morbidity (temperature > 40.5°C) was observed in EW or TW calves.

The unexpected gain of TW calves from EW to TW, a calf market that didn't have much of a spread between 175 and 230 kg calves, and the current practice that a spring-calved cow/calf pair is 1.0 animal unit (NRCS, 2003), resulted in TW returning more income to the cow/calf producer than EW given the conditions of the current study (\$32/cow if EW and TW calves were each sold at weaning; \$18/cow if TW calves sold at TW and EW calves sold at TW after 79 d in feedlot; data not shown).

Implications

Early weaning calves of spring calving cows at approximately 130 days of age will improve cow body condition score entering the winter feeding period and decrease winter feed costs compared with cows traditionally weaned at approximately 205 days of age in the Intermountain West. However, the overall economic effect of early weaning is dependent on a number of factors including timing and amount of precipitation, calf performance during the late summer and early fall, calf prices, and costs associated with winter feeding (feedstuffs, labor, and fuel).

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Table 1. Influence of weaning treatment on grazing behavior of cows pastured on sagebrush-bunchgrass range in southeastern Oregon[a]

Item	Early Weaned	Traditional Weaned	SEM	P-value
Grazing Time, h/d	9.7	9.6	0.34	0.77
Resting Time, h/d	13.4	13.7	0.33	0.66
Walking Time, h/d	0.81	0.74	0.049	0.35
Distance Traveled, m/d	5916	5405	233.9	0.17
Avg. Distance to water, m/d	1221	1131	62.1	0.35
Weekly visits to water	5.8	5.7	0.26	0.77
Distribution, %[b]	21	20	0.6	0.16

[a] Early and traditional weaned cow's calves were weaned at 130 ± 2 d and 209 ± 2 d of age, respectively. Grazing behavior was measured from early weaning to traditional weaning; therefore, only traditional weaned cows had calves at their side.
[b] Percentage of ha occupied per pasture each week

Table 2. Influence of weaning treatment on cow performance[a]

Item	Early Weaned	Traditional Weaned	SEM	P-value
Grazing Period[b]				
8/2/04 BCS	4.9	4.9	0.01	0.10
10/20/04 BCS	5.3	4.5	0.04	0.01
BCS Change	0.4	-0.5	0.04	0.004
8/2/04 Wt., Kg	500	500	2.9	0.94
10/20/04 Wt., Kg	511	456	4.4	0.01
Wt. Change, Kg	12	-44	1.5	0.001
Hay Feeding Period[c]				
11/23/04 BCS	5.5	4.9	0.04	0.01
12/21/04 BCS	5.7	5.2	0.47	0.02
1/18/05 BCS	5.4	5.2	0.05	0.06
2/15/05 BCS	5.6	5.6	0.56	0.59
BCS Change	0.3	1.1	0.08	0.02
11/23/04 Wt., Kg	554	514	0.5	0.0003
12/21/04 Wt., Kg	561	527	0.4	0.0003
1/18/05 Wt., Kg	574	542	6.7	0.08
2/15/05 Wt., Kg	591	567	4.9	0.07
Wt. Change, Kg	79	111	1.0	0.002
Total BCS Change	0.7	0.6	0.05	0.38
Total Wt. Change, Kg	91	67	0.02	0.01

[a] Early and traditional weaned cow's calves were weaned at 130 ± 2 d and 209 ± 2 d of age, respectively. Grazing behavior was measured from early weaning to traditional weaning; therefore, only traditional weaned cows had calves at their side.
[b] Early weaning occurred on 8/2/04 and traditional weaning occurred on 10/20/04.
[c] Hay feeding began on 10/20/04 and concluded on 2/15/05. The early weaned cows received only meadow hay (15.1 kg/hd daily; DM basis) while the traditional weaned cows received meadow hay (14.7 kg/hd daily; DM basis) plus alfalfa (3.34 kg/hd three days a week; DM basis).

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USE OF GREEN FODDER PRODUCED IN HYDROPONICS SYSTEMS AS SUPPLEMENT FOR SALERS LACTATING COWS DURING THE DRY SEASON

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ABSTRACT: In order to analyze the productive and reproductive behavior of lactating Salers cows with calf there was used hydroponics green fodder (HGF) as supplement from may 1st to July 2 of 2002. There were 35 cows used with 42±4.55 open days. They were paired according to the calving date and randomly assigned to one of two treatments: rangeland forage plus HGF as supplement plus green fodder (GF) and rangeland forage plus irrigated prairie forage (PF). The consumption of forage was 1.07 and 1.32 kg/DM/d of GF and 2.66 and 0.88 kg/DM/d of PF in may and june, respectively. Both groups were bred with fertility tested registered Salers bulls. The cows on PF lost weight (P<0.05) from day 28 (496.88±13.34 kg) to day 56 (459.99±13.34 kg) of the experiment, the GF cows maintained the live weight on the same period (506.13±13.73 and 497.60±13.73 kg, respectively). The cows showed daily live weight lost (P<0.05) on PF and daily weight gain on GF (-0.684±0.09 *vs*. 0.196±0.09 kg/d respectively). The calves showed differences (P<0.05) between treatments in live weight at day 56 (106.68±2.97 *vs*. 118.51±3.07 kg on PF and GF respectively). The daily average weight gain of calves from day 0 to 56 was 0.535±0.04 *vs*. 0.759±0.05 kg/d on PF and GF, respectively. There was an effect of treatment on cow body condition BC (P<0.10). The treatment means were 4.63±0.07 *vs*. 4.81±0.07 units of BC for PF and GF, respectively. The cycling cows were 17.65, 23.53 and 76.47% for GF and 11.11, 22.22 and 38.89% for PF on the first, second and third samplings, respectively. The estrous cycle was determined on the animal by the presence of more than 1 ng/ml of progesterone on blood serum. The treatments show difference (P<0.05) on the last sampling period being higher for GF. There were no differences (P>0.05) for pregnancy rates on November 2 of 2002 (88.24 vs. 70.59% for PF and GF, respectively). The HGF show being a viable supplement for the lactating cows to sustain weight on rangelands with an acceptable weight gain of calves.

Key Words: Green fodder, Supplement, Dry season, Hydroponics.

Introduction

One problem on the north of Mexico's beef cattle production is the long drought period, it's necessary to supplement the animals according with their physiology status to sustain them in good body condition during that period (Gutierrez, 1969). The supplementation on grasslands is made to complement the protein (protein supplementation) and mineral (mineral supplementation) requirements, trying to maintain the animal required levels and complementing the energy requirement. The reproductive behavior on beef cows is close related with the body condition score and the weight change before the breeding period, the supplementary protein produce an positive effect and enhance the exit possibility on the breeding when the cows are in good condition, an unadecuated availability of protein before and after the calve depress the reproductive behavior (Charmley *et al*., 1999) although the body condition score (BCS) is an important weighting on cattle to estimate the energy reserves (Burke *et al*., 1998). The intensive production of aliments by hydroponics systems is an alternative on lands not adequate to the agriculture, with extreme ambient conditions and scarce water availability; the developed systems to obtain "hydroponics green fodder" are an viable alternative to the cattle feeding as cause for its nutritive value, digestibility and good consume by the animals on grasslands (Rodriguez y Morales, 2002), considering the arid and semiarid conditions on the regional extensive production systems, its supposed on this work that the supplementation with "hydroponics green fodder" for lactating cows on grasslands can have advantages. The objective of this work was to evaluate the productive behavior, the body condition score, the ciclicity percentage and pregnant percentage of lactating Salers cows supplemented with "hydroponics green fodder" during the drought period on grassland.

Materials and Methods

The job was carried out on "Nopales" ranch, Chihuahua, latitude of 28º 17', west longitude 106º 15', with an altitude of 1630 msnm. The ranch counts with 950 ha should rangeland, the rangeland coefficient is 15 ha per animal unit (COTECOCA, 1978).

Animals description. 35 registered Salers breed lactating cows with calve were used (42±4.55 d open), assigned to two treatments in the following way, they were grouped by two in two for calve date and divided randomly after.

Treatments. Control (PF): grassland plus 1 h daily of meadow (n=18), this was a mixture of oat, rye grass, centeno and triticalle; sown at end of October of 2001; it was sown again with sudan sorgum during april and may of 2002 to prolong its use; it was divided into

five plots, and grassed 5 d each one. The production was estimated by plot in the meadow by means of direct cut at the level of the soil (three samples; plots of 1 m^2) before each plot be grassed (method of cut before and after; Cook et al., 1948 cited by Gutierrez, 1991). The experimental processing (GF) the animals (n=17) remained in grassland and supplemented daily with HGF at noon. The HGF production was obtained on 15 metallic structures (modules) with 12 sheets (varnishes), a system of irrigation with a deposit for water of marks commercial of 400 L, a submergible bomb of water of 1/3 hp; the distribution of the water and fertilizing soluble in water (1.0 g / L; 19 - 19 - 19, N, P, K more minerals outline) was carried out with PVC tubes of 1" of diameter with drillings each 10 cm, placed in the upper part of the modules. There was utilized 2.5 kg of oat "Babicora" by varnishes, the procedure of pregermination and germination was done according to the methodology described by Rodriguez (2002) that consists of disinfection of the seed with chlorine (0.5% of chlorine in water), rinse (1 to 2 times), pregermination (humid seed in containers during 24 hours), sows (distribution of the seed in the varnishes), development and irrigation (12:00 to 19:00), crop of the HGF, and disinfection of the varnish. They were utilized daily 12 varnish from may 1st to may 30 and 15 varnish from may 31 to July 8, 2002, the HGF offered was weighted daily.

Samples of meadow and HGF. The samples taken for the estimation of production in meadow and 22 samples of HGF taken in different days, were dried partially for their conservation until carrying out the analyses of laboratory and to determine content of DM, PC (A.O.A.C., 1990), FDN and FDA (Van Soest, 1982). Management of the animals. Both groups had mounts natural utilizing a bull with test of fertility during the experiment, they had free access to water and mineral salt, one of the mineral mixtures (NUTRILAG®) has 60% of non protein nitrogen (NPN), the consumption of minerals was estimated by means of the direct weight of the refusal. It takes of data.

On may 1st of may, may 29 and june 26 the weight of the cows was determined (PV), weight of the calves (Pc), they were taken blood samples to determine the percentage of cycling cows (VC) with a repetition to the 6th d (repetitions may 7, june 4 and july 2) by caudal punction with vacuum tubes (Vacutainer), body condition scores (BCS) of cows (1=ematiated to 9 = extremely fat), there was estimated the loss or daily profit of weight in cows (GDPV) and the daily weight gain on calves (GDPC) from may 1st to june 26.

The pregnant percentage of cows was obtained (VP) by rectal palpation (November 2, 2002). The determination of cycling cows. The blood samples where used to the quantification of progesterone (P_4), they were centrifuged at 3000 rpm during 20 min to obtain the serum, which was stored to temperature of congealment, the concentration was determined in the laboratory of investigation of the State University again Mexico, United States, by means of the technique of radioimmunoassay described by Hallford *et al*. (1982). It was considered that a cow was cycling when presented levels of P_4>1 ng/ml (Vizcarra et al., 1998 cited by González, 2000) in blood serum in any one of the two repetitions of each sample.

Statistical analysis. The data of PV and Pc were analyzed with the procedure MIXED of the statistical package you ARE (1994), considering like fixed effects the treatment, sample moment (time effect) and their interaction, the animal was considered random effect. The differences of weight inside processing were analyzed among the weights for PV and Pc. They were used like co variables the Julian days for PV and the age in days to the login of the experiment and birth weight for Pc. The data of GDPV and GDPC from the begin to the end of the experiment, were analyzed with the effect of the treatment only by means of the GLM procedure of the SAS (1994) package using like co variables, Julian days at partum and initial weight for GDPV and age in days at the experiment beginning and initial weight for GDPC, to analyze the data of BCS was utilized the Statistical package SAS (1994), including processing, takes of data and their interaction as fixed effects and as random effect the animal, the days were utilized Julian's upon leaving and the initial weight of the cows as co variables of BCS. An exact test was carried out of Fisher for comparison of frequencies in the percentage of cycling cows of the first sample period and the percentage of pregnant cows and an analysis of X 2 for the percentage of cycling cows of the second and third sample period. The analyses were carried out with the procedure FREQ of the statistical package you SAS (1994).

Results and Discussion

Nutrients content of meadow and HGF. The meadow fodder consumption was estimated in 2.66±1.27 kg/DM and 0.88±0.22 kg/DM by animal^{-1} d^{-1} in may and june respectively, the meadow had 26.31 and 37.70% of DM kg-1, 17.36 and 14.64% of PC, 54.79 and 58.41% of FDN, 37.40 and 35.37% of FDA by kg DM during may and june respectively. The consumption of HGF was estimated in 1.09 ± 0.22 kg/DM and 1.32 ± 0.22 kg/DM by animal-1 d-1 in may and june respectively, the HGF had 20.45 and 17.78% of DM kg-1, 17.40 and 21.40% of PC, 47.53 and 43.75% of NDF, 24.69 and 24.11% of ADF by kg DM during may and june respectively.

Cows performance. The interaction between treatment and sample time was significant (P<0.05), on GF was observed an increment of weight on the cows from the first (486.60±13.73 kg) to the intermediate weight (506.13±13.73 kg; P<0.05) indicating a probably compensatory weight gain, there was not difference (P>0.05) between the second and the third weight (497.60±13.73 kg) for GF. For PF, the behavior indicate that the cows sustain the weight from the first (498.32±13.34 kg) to the intermediate (496.88±13.34 kg) weighting (P>0.05), due to the high consumption of fodder of meadow, the cows lost weight significantly (P<0.05) from the second to the third (459.99±13.34 kg) weighting, by the lower production of the meadow and the effect of the conditions of low nutrition.

The change of weight inside GF shows that is feasible the sustainment of weight of cows on grasslands with HGF as supplement. For the loss of weight of the cows in PF (38.33 kg) it should be considered the condition from the meadow which by the date presented each time less production (daily weight change - 0.684±0.09 *vs*. 0.196[b]±0.09 kg for PF and GF respectively; P<0.05).

There has been mentioned that the fountain of protein as supplement for animals in grassland is not so important, satisfying 70 g of protein not degradable in rumen and avoiding the deficiency with a degradable fountain (Sletmoen-Olson et al., 2000).

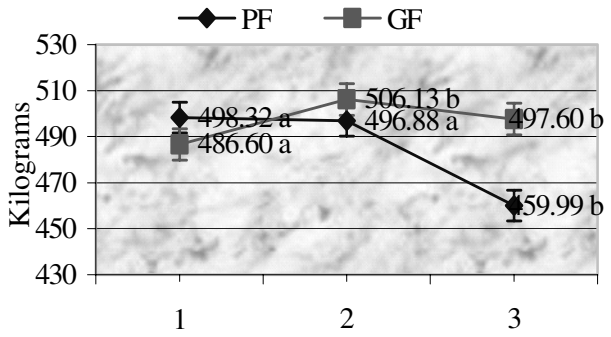

Figure 1. Live weight of the cows during the treatment period (minimum square means ± standar error).

Calves weight. There were no difference on the weight at the first weighting and the intermediate weighting (P>0.05) among treatments (76.73±2.97 *vs*. 76.01±3.07 and 91.90±2.97 *vs*. 88.80±3.07 kg for calves on PF and GF respectively).

There was difference between treatments on the third weighting in favor of the treatment on GF (P<0.05). The daily profit of weight during the experiment was greater on claves of the GF (106.68±2.97 *vs*. 118.51±3.07 kg; P<0.05). In both treatments difference is shown among weightings (P<0.05) due to the constant increase of weight of the calves. The fact of simply providing additional protein to the diet can enlarge the productive behavior of the animals when the quantity of fodder is limited (Rusche et al., 1993), the milk production affects directly the weight of the calves (Drewry et al., 1959 cited by Lopez and Garcia, 1996) and this is increased when the cow is supplemented in grassing condition (Berzaghi et al., 1996), the quantity of supplement offered to the animals could maintain a good milk production in the cows, maintaining a constant profit of weight in the calves (P<0.05) based on the differences among weighting from the beginning to the last weight; the weight of the GF calves was greater (P<0.05) in the last weight; the decrease of weight in the cows of PF affected the weight gain of the calves (0.535±0.04 *vs*. 0.759±0.05 kg for PF and GF respectively; P<0.05) to the last weighting of the experiment, probably they utilized the majority of the food consumed to maintain an acceptable milk production.

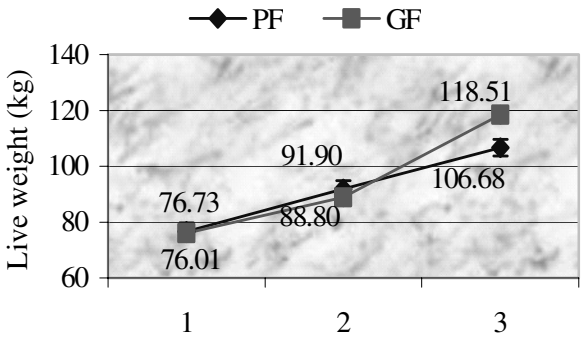

Figure 2. Live weight of the calves during the treatment period (minimum square means ± standar error).

Body condition score. Difference among treatments was not found, there is change (P<0.05) on the body condition score (BCS) of all the cows by effect of the sampling time (change through the time). For cows in good condition, the reserves of energy can be removed to compensate the limiting availability of energy contributed by the diet (Houghton et al., 1990 and Marston and Lusby, 1995 cited by Charmley et al., 1999). The BCS decreased (P<0.05) on cows with PF from the intermediate (4.79±0.08) to the last (4.49±0.08) sampling due probable to a lack on forage consumption, whereas it was not different (P>0.05) to the initial BCS. The BCS of the GF cows shows the highest value (P<0.05) at the intermediate sampling (4.89±0.09) inside of the treatment, the final BCS (4.81±0.09) was similar (P>0.05) to the intermediate and initial (4.72±0.09) BCS. The change explains an sustain of the BCS. The average of all the animals showed that the intermediate data of BCS (4.84±0.06, n = 35) was the highest one finishing with a similar BCS than that of the beginning.

Percentage of cycling cows. The reproductive behavior of the cows is related to the BCS and its changes, with better condition there will be a greater success on the breeding, the restriction of energy on the diet results in corporal losses of weight and BCS, finishing with a cease of the estrus cycle (Richards et al., 1989 and Surround et al., 1996 cited by Bossis et al., 1999). The percentage of cycling cows were of 11.11, 22.22 and 38.89% in PF and of 17.65, 23.53 and 76.47% in GF for the first, second and third samplings; the cycling percentage cows was greater (P<0.05) for cows in GF in the third sampling (38.89 in PF *vs*. 76.47 in GF), indicating influence of the quantity consumed of supplements, the sustain of the BCS maintains a constant increase of the cyclicity of the cows; in PF together with the decrease of BCS is observed a smaller percentage of cyclicity of the second to the third takes of data, there was effect of the meadow fodder consumption decrease in PF showing a greater ciclicity on cows supplemented with HGF. There are fails on the ovulation by restriction of the food on heifers, besides a corporal loss of weight and BCS (Bossis et al., 1999). Cows stimulated with presence of bull showed concentrations of P_4>1.0 ng/ml as of the week six pospartum, the period of the anestrum becomes of until 13 weeks for cows with BCS of 4.5 (Madrigal et

al. ,2001), this explains in part the time elapsed among the begin and the biggest incidence of cows cycling at the end on this work. A cow has more opportunities of remaining pregnant during the breeding upon log on its cycling activity in the first 80 days postpartum (Madrigal et al., 2001).

Percentage of pregnant cows. To the moment of the palpation there were 88.24% of pregnant cows (15/17) in PF and in GF 70.59% (12/17), without difference (P>0.05) among treatments. The supplemented protein on diets for cows has influence upon the reproductive efficiency, an inadequate addition can depress the reproductive behavior, showing that the excess of degradable protein on rumen can affect negatively the percentage of conception in cows (Marston et al., 1995 cited by Charmley et al., 1999), the cows of GF had greater cyclicity during the experiment, suggesting a loss of embryos or presence of cycles with smaller fertility. The BCS was adequate to obtain acceptable values on percentage of pregnant cows.

Implications

The hydroponics green fodder is a viable supplement for the sustain of the weight of cows with raises on lactation on grasslands with a good answer in the increase of weight of the calves. It is recommended to do an evaluation of the effect of the supplementation with HGF upon the consumption, utilization of fodder and its implications on the rumen, as well as a financial evaluation of its utilization besides seeking to compare with the use of the meadow and or other supplements with a longer sampling period. The quantity of supplemented protein presented acceptable an acceptable sustain of the BCS of cows of GF, showing a good percentage of cyclicity cows, however upon seeming the cows presented embryonic losses or well the estrus on the cows had less fertility, is necessary a greater number observations to evaluate the fertility on similar works.

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YEARLY FORAGE PRODUCTION OF IRRIGATED PASTURES GRAZED BY COW-CALF PAIRS AS AFFECTED BY THE TIMING OF SPRINKLER IRRIGATION APPLICATION

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ABSTRACT: In desert states such as Utah where human populations are growing rapidly, efficient water use in agricultural production systems is increasingly imperative. The effectiveness of any irrigation system is a matter of coordinating plant water needs with water application. Information is largely lacking regarding the effect of irrigation procedures on forage plant production when pastures are under management intensive grazing practices using cow-calf pairs. The objective of this study was to measure the yearly production of forage from cool-season grass pastures as affected by frequency of sprinkler irrigation while being intensively grazed by cow-calf pairs. A well established 1.64 ha plot of 60% tall fescue (Festuca arundinacea), 30% orchard grass (Dactylis glomerata) and 10% Kentucky bluegrass (Poa pratensis) was grazed for approximately 170 days per year for each of two consecutive years by six cow (557 kg)-calf (194 kg) pairs. Management intensive grazing was used with the cattle receiving a fresh pasture allotment each 24 hours, the boundaries of which were controlled by portable electric polywire fencing. There were four grazing circuits across the plot each year. On alternate circuits sprinkler irrigation was applied either: 1) starting 7 days postgrazing at a rate of 10.16 cm or 2) starting 14 days postgrazing at the same rate. Nitrogen fertilizer was applied at a rate of 44.9 kg/ha using ammonium nitrate prior to irrigation. Forage harvested was measured using a corrected visual appraisal method just before and just after grazing. Corrections were made using clip-plots each seven days. Although the same resources were applied, postponing the beginning of irrigation to 14 days postgrazing reduced total yearly forage DM production by 19.8% ($P < 0.05$). Beginning irrigation seven days postgrazing resulted in a yearly DM production of 9905 kg/ha, while only 8269 kg/ha was produced if irrigation was postponed to 14 days.

Key Words: Irrigated Pastures, Beef Cattle, Irrigation Frequency

Introduction

In many desert states in the Intermountain West cow-calf operators face two major problems: 1) issues associated with curtailment of public land use and 2) growing human populations. Public land issues are difficult to predict and are often controlled by changing public sentiment and political policy. An alternative that may be considered by some operators currently heavily dependant on the grazing of public lands is intensive cow-calf production on privately owned irrigated pastures. A major problem associated with irrigated pasture production in desert states is competition for water with a growing human population. Research at this station has reported that high carrying capacity on irrigated pastures is imperative for financial success (Meek et al., 2004). Carrying capacity is highly dependant on irrigation practices.

The objective of this study was to determine the importance of the timing of sprinkler irrigation application on the productivity of pastures after being intensively grazed by cow-calf pairs.

Materials and Methods

A well-established (12 yr) 1.64 ha (102m x 163m) cool-season grass pasture was used for the study. The forage composition of the pasture was approximately: 60% tall fescue (*Festuca arundinacea, Alta*), 30% orchardgrass (*Dactylis glomerata, Patomac*), and 10% Kentucky bluegrass (*Poa pratensis*). The longer side of the pasture was oriented east-west. Six cow-calf pairs grazed the pasture for approximately 170 d (May-October) for two consecutive years. The average BW of the cows through the grazing season was 557 kg and that of the calves was 194 kg. The cattle grazed across the pasture from east to west with management intensive grazing and received a new allotment of pasture forage each 24 h. The boundaries of daily pasture allotments were controlled by portable electric polywire fencing. The size of each allotment was adjusted daily to allow ad libitum forage intake, while leaving a 10 cm stubble height. This was accomplished by a visual appraisal of standing forage yield (Stockdale, 1984) that was corrected weekly by determining the forage DM yield of a 0.1 m^2 clip plot. There were four grazing circuits across the pasture each of the two years of the study. The number of days associated with each of these circuits depended on the forage yield during that period. The objective was to have the east end of the pasture prepared for grazing by the time the cattle grazed the last daily allotment on the west end, and thus fairly continual grazing. Forage DM harvested by the cattle was recorded each day.

Grazing started the first week of May each year. Irrigation water was available the first or second week of June each year. So the first grazing circuit each year was fed by snow melt and rain. Commercial fertilizer application on the pasture did not commence until June to help control rapid forage growth during late spring, which was greatly in excess of that which cows with young calves could

consume if fertilizer was applied in April or May. Starting in June N was applied to the pastures at a rate of 44.9 kg/ha before each grazing circuit in the form of ammonium nitrate. The pastures were harrowed shortly after the cattle grazed during each grazing circuit.

The pasture was irrigated with a single hand-changed sprinkler line running in a north-south orientation across the 102 m side of the pasture. Each irrigation set covered about 0.14 ha and delivered 10.16 cm of water. This arrangement allowed irrigation to easily follow the cattle through each grazing circuit. The irrigation management treatments applied to the pastures are described in Table 1. Two irrigation treatments were applied that resulted in the same amount of water being applied after each circuit, but the commencement of irrigation application was delayed either 7 d or 14 d.

Data were analyzed using the Proc MIXED procedure in SAS (SAS Institute, Cary, NC) with yearly forage production as the dependant variable and irrigation treatment as the independent variable. Year was used as a repeated measure. Multiple comparisons were made with P-values adjusted using Tukeys procedure. A $P < 0.05$ was considered significant.

Results and Discussion

The effect of irrigation management on the yearly forage DM production of pastures grazed by cow-calf pairs is summarized in Table 2. Although the same resources were expended to the pasture, when irrigation water was applied at either a 7 d or 14 d post-grazing delay, the 7 d delay resulted in a 19.8% increase in yearly forage DM production compared to that of the 14 d delay. The average forage DM consumption of the cow-calf pairs on this study through the grazing period was 18.3 kg DM/pair/d. The grazing season averaged 170 d during each of the two years of the study. Hence, the carrying capacity of the pasture with a 7 d postgrazing delay was 3.83 pairs/ha, while that of the 14 d delay was only 3.20 pairs/ha. Thus, carrying capacity of the pasture was substantially increased without adding resources simply by applying irrigation water as soon as possible after the cow-calf pair intensively grazed. Meek et al. (2004) demonstrated the importance of high carrying capacity to the profitability of cow-calf production on improved irrigated pastures. Pastures with carrying capacity of 3.59 pair/ha or less were deemed unprofitable. In this study the 7 d post-grazing irrigation delay would therefore be profitable while 14 d post-grazing irrigation delay would not.

Implications

This study demonstrated that if pastures are irrigated within 7 d after being intensively grazed by cow-calf pairs the yearly forage DM production can be increased by nearly 20% compared to delaying irrigation another seven days to 14 d. This simple irrigation management practice will affect the overall profitability of cow-calf production on irrigated pastures.

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Table 1. Post-grazing irrigation management treatments applied to pasture intensively grazed by cow-calf pairs.

	Year	
Grazing Circuit	1	2
	---days post-grazing when irrigation commenced---	
1. (May-June)	natural[a]	natural
2. (June-July)	7[b]	14[c]
3. (August-September)	14	7
4. (September-October)	7	14

[a] Moisture from natural snow melt and rain
[b] Irrigation began 7 d after pasture was grazed by cow-calf pairs
[c] Irrigation began 14 d after pasture was grazed by cow-calf pairs

Table 2. Yearly forage dry matter production of pastures grazed by cow-calf pairs when post-grazed irrigation was delayed either 7 or 14 days.

	Days post-grazing when irrigation commenced			
Item	7	14	SEM[a]	P[b]
Yearly forage harvested[c], kg DM/ha^{-1}	9905[d]	8269[e]	36.4	0.0001

[a] Standard error of mean
[b] Probability greater than F score
[c] Includes forage DM production during the first non-irrigated grazing circuit
[d,e] Means in the same row with different superscripts differ, $P < 0.05$

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LONG-TERM USE OF LOW-QUALITY FORAGES IN BEEF COW NUTRITIONAL MANAGEMENT: EFFECTS ON PRODUCTIVITY AND ECONOMICS

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ABSTRACT: Low-quality forages (LQF) such as cereal straws are often used as an economical feed source in the wintering of beef cow herds, especially during emergency feed shortages. Most studies reporting the effects of LQF use on beef cow productivity seldom involve more than two consecutive years of comparison. The objective of this study was to evaluate the use of LQF in the wintering diets of beef cows on a long-term basis regarding days of use per year and consecutive years of use. Twenty-four crossbred beef cows (545 kg) were stratified into two groups of 12 cows each based on age, body weight (BW), breed type and productivity of calves. Each group was randomly assigned to one of two dietary wintering programs: 1) average-quality meadow grass hay (GH) or 2) ammoniated wheat straw supplemented with alfalfa hay (AWS/AH). The duration of the wintering period was from December-April. Cows remained on these dietary wintering programs for five consecutive years. Both groups were bred to the same bulls and grazed in common on native meadow pastures May-October. During the entire wintering period BW and BCS change of the cows were similar: -4.9 kg versus -3.8 kg (P = 0.47) and -0.17 versus -0.12 (P = 0.46) for GH and AWS/AH diets, respectively. The only difference in BW change detected was that cows wintered on AWS/AH consistently gained 3.9 kg more than cows wintered on GH during the latter half of the grazing season (P = 0.05). No difference in milk production (P = 0.819) or calf weaning weight (P = 0.639) could be detected. No differences in postpartum interval to rebreeding (P = 0.61), pregnancy rate (P = 0.329), or keep/cull rate (P = 0.709) could be detected. Economic analysis showed a 17.7% increase in profitability when the cows were wintered on AWS/AH. This study demonstrated that long-term use of LQF in the nutritional management of beef cow had no negative effects on biological productivity and improved profitability.

Key Words: Low-Quality Forage, Beef Cow, Productivity & Economics

Introduction

In the Intermountain West winter feed costs usually account for 50-60% of variable costs (Field and Taylor, 2002). Mechanically harvested feeds, such as grass hay, is most commonly used for this purpose. When on-ranch winter feed supplies are curtailed due to drought or other emergencies, producers must purchase off-ranch feeds if cow numbers are to be maintained. Purchase of low-quality forages (LQF) are often considered during such situations due to the relatively low cost per unit of NE_m. The relatively low DDM, DMI and vitamin-mineral deficiencies associated with LQF can be rectified with proper supplementation programs and/or chemical treatments of the LQF (Wiedmeier et al. 2003). There have been many studies reporting the successful wintering of beef cows on LQF diets with various supplementation systems and/or chemical treatments of LQF (Paterson et al. 1994). However, these studies usually involve only one wintering period. One could surmised that continual feeding of LQF could eventually have a negative impact on the productivity of beef cows due to factors such as increased abrasion of gastro-intestinal epithelia by undigested fiber, increased chewing/rumination time associated with high-fiber diets, or a high ruminal acetate: propionate ratio. The objective of this study was to compare the biological and economic productivity of beef cows wintered on LQF diets for a prolonged number of days per years and for several consecutive years, compared to that of cows wintered on a traditional grass hay diet.

Materials and Methods

Cows – Twenty-four mature beef cows (568 kg, BCS 5) were used for the study. Due to the long duration of the study (5 consecutive years) cows of 5 to 7 years of age were selected. Twelve of the cows were straight-bred Hereford and the other 12 were crossbreds with Hereford crossed with various other breeds (Angus, Tarentaise, Simmental). Cows were stratified into 6 groups of 4 cows each so that each group was composed of two straight-bred Hereford and two crossbred cows, and were as similar as possible with regard to age, BW, BCS and average 205-d weight indices of calves produced.

These cows remained in these groups for winter feeding for the 5-year duration of the study. When replacement of a cow was necessary due to reproductive failure or unsoundness, a cow with similar characteristics was selected as a replacement. Cows were spring-calving and bred to calve March-April of each year. Breeding was by a battery of 50% Hereford and 50% Angus bulls of moderate weaning and yearling weight indices. Bulls remained with the cows from the first week of June each year until August 15 when they were removed.

Cow Management During the Wintering Period – Cows were wintered in six 6.2m x 21.5m pens constructed of steel livestock panels. The long side of the pens was oriented north/south. The south end of the pen was bedded with a mound of wood shavings, which was replenished as needed to keep cows clean and

comfortable. The north end of each pen was equipped with a 1.0 m x 2.2m steel feeder constructed to accommodate large amounts of bulky feed with minimal waste. Each pen was also equipped with a frost-resistant waterer surrounded by a concrete apron. One group of cows was randomly assigned to each of these pens. Pens were then randomly assigned to one of two wintering diets, three pens per diet. The two wintering diets were: 1) ammoniated wheat straw (AWS) supplemented with alfalfa hay (AH) and 2) medium-quality grass hay (GH). Cows received these winter diets from December-April for each of the five years of the study.

Winter Forages – Wheat straw that was ammoniated for the study was harvested from one of two adjacent fields that were being rotated between wheat and alfalfa hay production. The wheat was of the same variety of hard red spring (Stevens) each year. Straw was baled in the small square bale package size as soon after wheat harvest as possible. Baling was from 0500 to 0900 so as to incorporate as much dew as possible to enhance the ammoniation process. Bales were removed from the field as soon as possible with a mechanical stack wagon and stacked at the feeding site in stacks of 2.2m wide x 3.7m high x 21.5m long. Stacks were then ammoniated according to the stack method described by Sundstøl and Coxworth (1984).

Alfalfa hay was used as a supplement for cattle wintered on AWS. During the months of December and January AH was offered at a rate of 2.1 and 2.8kg DM/cow/d, respectively. During the months of February-April AH was offered at a rate of 3.7kg DM/cow/d. The objective was to use hay of consistent quality each year: 17-18% CP and 46-48% NDF. When hay of this quality could not be obtained from experiment station stocks, it was purchased privately.

The GH used for the study was harvested in small square bales from one of two adjacent fields for each of the 5-years of the study. This helped insure the consistence of GH quality throughout the study. The hay was a mixture of three cool-season grasses: 70% tall fescue (*Festuca arundinacea*), 20% quackgrass (*Agropyron repens*) and 10% Kentucky bluegrass (*Poa pratensis*).

Vitamin-Mineral Supplements – Two supplements were formulated to rectify vitamin and mineral deficiencies predicted for the AWS/AH and GH diets. Ground barley was used as a palatable carrier for the vitamin and mineral premixes used in the formulations. Both supplements were formulated to be fed at a rate of 0.45kg DM/cow/d. Formulas of the two supplements are presented in Table 1.

Feeding Management of Cows During Wintering Period – Cows were fed assigned diets daily at 1600. The first item offered each d was the vitamin-mineral supplement. Appropriate amounts of assigned supplement were evenly spread in the feeder of each pen allowing all cows in the pen equal access. Next, appropriate amounts of AWS and GH were carefully weighed and delivered to the feeders in assigned pens. The amount of these forages offered each d was determined by the amount remaining in the feeder from the previous day's offering. The goal was to allow ad libitum access but insure the cows were not sorting the most nutritious or palatable portions of the forages. Lastly, the appropriate amount of AH was carefully weighed and delivered to feeders in pens where AWS was assigned. The AH was evenly spread over the top of the AWS in the feeder to allow all cows in the pen equal access.

Animal Measurements Taken During Wintering Period – Intake of each feed and forage was recorded for each pen daily. During the last 6-d of December, January and February in vivo DMD was estimated by sampling of feeds offered and AM-PM fecal sampling. Acid insoluble ash was used as an internal marker to determine apparent DMD (Van Keulen and Young, 1977)

During the first week of each month BW and BCS of the cows was recorded. Since shrunk BW was not practical the cows were weighed AM and PM on weigh dates. The average of these two weights was recorded. The BCS system used was that described by Wagner et al. (1988). During the first week of May, marking the end of winter feeding, the milk production of the cows was determined using the weigh-suckle-weigh method (Dawson et al., 1960).

Pasture Grazing Season – The grazing season usually last from May-October each year depending on weather and forage conditions. The pastures were considered feral with evidence of some improvements in the past but had since reverted to a native state. The pasture was separated into three, 9.7 ha parcels through which the cow-calf pairs rotated each 15-20 d. Pastures were watered with a combination of flood and sub-irrigation. No commercial fertilizer was used. The annual forage production of the pasture was 6225kg DM/ha.

The first week of August each year cows and calves were weighed and cows received a BCS. This marked the end of the first half of the grazing season. Milk production of the cows was also measured. Similar measurements were made the third or fourth week of October marking the end of the second half of the grazing season. Calves were weaned at this time. Dry, pregnant cows grazed grass hay crop aftermath during the month of November. Cows were then returned to the winter feeding pens December-April.

Statistical Analysis – Data from this study were analyzed as a completely randomized design, using the Proc MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Randomization was based on individual pen and a 2 x 5 (2 treatments and 5 years) factorial treatment structure was used with year being a repeated measure. Compound symmetry was used as the covariance structure because it provided the best goodness of fit based on the Schwarz-Bayesian test. Initial winter weight and BCS were included as a covariate for weight and BCS responses, respectively. Least square means were calculated and are reported. Mean separation was done using assigned superscripts based on the least significant differences. In all analyses, significance was declared at $P < 0.05$.

Results and Discussion

Yearly Body Weight and Body Condition Score Changes of Cows – Body weight and BCS changes of the cows wintered on either AWS/AH or GH diets is presented in Tables 2 and 3, respectively. Although there were differences detected in BW and BCS change due to year, the type of diet consumed by the cows during the winter had no effect on these important production characteristics. Averaged through the 5-y of the study, BW and BCS of the two groups of cows was fairly constant and stable under the environmental and nutritional variable imposed. It was surprising however to detect that cows wintered on AWS/AH gained 4.2kg more BW during the grazing season than those wintered on GH (P = 0.08). Even though these cows began the grazing period in similar BW and BCS, those wintered on the AWS/AH diet were behaviorally or physiologically elicited in some way to gain more BW during this period. This is apparently a real effect because it persisted over the duration of the 5-y study. This small amount of BW increase of course was not detectable by the visual BCS method used (Table 3).

The BW and BCS responses of the cows are corroborated by the DMI, DMD and DDMI exhibited by the cows during the wintering period (Table 4). Although cows wintered on the AWS/AH diet consumed about 5.3% more DM than those wintered on the GH diet, total energy intake as described by DDMI did not differ. Thus, it would be expected that the performance of the cows as measured by BW and BCS change would not differ.

The average NE_m and CP requirements of the cows during late gestation was estimated to be 11.48 Mcal/d and 0.892 kg/d, respectively (NRC, 1996). Since the cows performed adequately during this period it can be assumed that both diets were supplying these amounts of nutrients. The CP concentration in the GH averaged 9.85% DM, so the cows were receiving 1.162 kg CP/cow/d or about 119% of their estimated requirement. Since the cows assigned to the AWS/AH diet performed as expected, it can be assumed they were also receiving 11.48 Mcal NE_m/cow/d and adequate CP from the diet.

It was difficult to estimate the NE_m and CP requirements of the cow during March and April because varying proportions of the cows were in late gestation and early lactation. We estimated the average NE_m and CP requirements of the cows to be 12.49 Mcal/cow/d and 1.056 kg/cow/d, respectively. Since the cows performed adequately during this period we assume they were receiving these amounts of NE_m and CP.

During the spring/summer/fall grazing season cow milk production averaged 4.92 and 4.98 kg/cow/d for those wintered on AWS/AH and GH diets, respectively. Thus, there was no difference in milk production between the two groups (P = 0.8167). No difference in calf weaning weight (225.1 versus 226.8 kg, P = 0.6392), respectively, could be detected due to winter diet.

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Economic Analysis – The economic analysis of the AWS/AH and GH wintering programs are presented in Table 5. Based on 10-y averages of the value of winter feed used for the study as well as grazing costs and calf market values, it was shown that through the 5-y of the study the cow-calf operation based on wintering the cows on AWS/AH was about 17.7% more profitable than that in which cows where wintered on a more traditional diet of GH.

Implications

This study demonstrated that beef cows wintered on a diet based on LQF such as AWS if properly supplemented will exhibit production similar to that of cows wintered on a traditional grass hay diet. There appears to be no negative impacts on the productivity of beef cows continually wintered on a diet based on AWS if properly supplemented, but the use of such a diet resulted in a 17.7% improvement profitability.

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Table 1. Ingredient composition of vitamin-mineral supplements used to fortify ammoniated wheat straw/alfalfa hay and grass hay winter diets

	Winter diet	
Ingredient, % of DM	AWS/ AH[a]	Grass hay
Ground barley	61.59	68.61
Dicalcium phosphate	12.72	-
Ground limestone	-	6.59
Vitamin premix[b]	8.20	8.20
Mineral premix[c]	8.89	8.01
Salt	8.60	8.60

[a] Ammoniated wheat straw supplemented with alfalfa hay

[b] Vitamin A, 1100 KIU/kg; vitamin d, 110 KIU/kg; vitamin E, 6600 IU/kg

[c] Zinc, 6000 mg/kg; manganese, 5000 mg/kg; copper, 2000 mg/kg; iodine, 200 mg/kg; selenium, 50 mg/kg; cobalt, 50 mg/kg

Table 2. Body weight changes of beef cows as affected by winter diets of either ammoniated wheat straw supplemented with alfalfa hay or a diet of grass hay through five consecutive years

	Winter diet			
Live BW change, kg	AWS/ AH[a]	Grass hay	SEM[b]	P > F[c]
Initial BW, kg	551.3	551.1	14.20	0.95
Late gestation period	36.8	35.2	0.74	0.15
Entire wintering period	-3.8	-4.9	1.00	0.47
Grazing period I	2.1	1.9	1.20	0.93
Grazing period II	4.7	0.8	1.30	0.05
Entire grazing period	6.9	2.7	1.60	0.08
Entire year	0.3	-0.4	3.8	0.87

[a] Ammoniated wheat straw supplemented with alfalfa hay

[b] Standard error of mean

[c] Probability greater than F score

Table 3. Body condition score changes of beef cows as affected by winter diets of either ammoniated wheat straw supplemented with alfalfa hay or a diet of grass hay through five consecutive years

	Winter diet			
BCS change	AWS/AH[a]	GH	SEM[b]	P > F[c]
Initial BCS	5.42	5.17	0.09	0.06
Late gestation	-0.11	-0.10	0.03	0.90
Entire winter	-0.12	-0.17	0.04	0.46
Grazing period I	0.02	-0.02	0.03	0.32
Grazing period II	0.08	0.09	0.05	0.81
Entire grazing period	0.08	0.09	0.05	0.83
Entire year	-0.05	-0.04	0.09	0.93

[a] Ammoniated wheat straw supplemented with alfalfa hay

[b] Standard error of mean

[c] Probability greater than F score

Table 4. Diet DMI and DMD of beef cows wintered on either ammoniated wheat straw supplemented with alfalfa hay or on a diet of grass hay through five consecutive years

	Winter diet			
Month	AWS/ AH[a]	Grass hay	SEM[b]	P > F[c]
December				
DMI, kg	11.2[h]	10.7[i]	0.15	0.0363
DMD, %	49.3	50.3	0.40	0.0950
DDMI, kg	5.5	5.4	0.08	0.4395
January				
DMI, kg	12.2[h]	11.7[i]	0.15	0.0363
DMD, %	50.3	50.7	0.37	0.4067
DDMI, kg	6.1	6.0	0.11	0.5801
February				
DMI, kg	14.0[h]	13.1[i]	0.12	0.0001
DMD, %	51.2	50.9	0.17	0.4188
DDMI, kg	7.2	6.7	0.08	0.4924
March				
DMI, kg	13.9[h]	12.8[i]	0.19	0.0006
April				
DMI, kg	13.5	13.0	0.17	0.0701

[a] Ammoniated wheat straw supplemented with alfalfa hay at a rate of: 2.05, 2.82 and 3.68 kg DM/cow/d through December, January and February-March-April, respectively

[b] Standard error of mean

[c] Probability greater than F score

Table 5. Economic analysis of the profitability associated with a cow-calf operation in which cows are wintered on either ammoniated wheat straw supplemented with alfalfa hay or a grass hay diet for five consecutive years

	Winter diet		
Item	AWS[a]	AH	Grass hay
Winter feed costs[b], $/cow/yr	76.11	46.75	135.46
Grazing costs, $/cow/yr	71.02		71.02
Yearly non-feed costs, $/cow/yr	92.81		92.81
Weaning percentage	85.00		85.00
Calf weaning weight, kg	225.00		227.00
Breakeven price for calves[c], $/kg	1.50		1.56
Ranch value of calves, $/calf	337.50		354.12
Market value of calves, $/calf	423.00		426.76
Profit/loss, $/cow/yr	85.50		72.64

[a] Ammoniated wheat straw

[b] Based on 10-year average values of AWS, alfalfa hay and grass hay December through April

[c] (Winter feed cost + grazing costs + non-feed costs) / (weaning weight of calves x weaning percentage)

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PREDICTING NUTRITIVE VALUE OF IRRIGATED PASTURES USING NEAR INFRARED REFLECTANCE SPECTROPHOTOMETER

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ABSTRACT: NIRS is a fast and accurate method to evaluate chemical composition of cereals, forages and other types of organic compounds. The objective of the present work was to evaluate the use of the NIRS for the analysis of mixed forages of irrigated pastures predicting their nutritive value. Two hundred and fifty four sample mixes of five forages (oat, var. Cocker; barley, var. wintermore; wheat, var. Tam; triticale, var. AN31 and ryegrass, var. Oregon) which have a wide range of chemical and physical characteristics were used and their spectra read with a monocromator NIRS (5000) within a wave length of 1108 and 2500 nm. Calibration coefficients (R^2) and the standard error of the cross validation (SECV) for crude protein (CP) were 0.99 and 0.703 respectively; ashes (A) 0.97 and 0.563respectively; phosphorus (P) 0.96 and 0.028 respectively; neutral detergent fiber (NDF) 0.95 and 2.088 respectively; acid detergent fiber (ADF) 0.93 and 1.506 respectively; dry matter (DM) 0.93 and 0.559 respectively; in vitro organic matter digestibility (IVOMD) 0.86 and 2.953 respectively; in vitro dry matter digestibility (IVDMD) 0.78 and 4.23; These results show the high potential of NIRS for routine analysis of mixed forages, observing acceptable R^2 higher than and SECV values within the average for this types of measurements, indicating both the accuracy of the calibration equations.

Key Words: Near infrared, chemical composition, irrigated pastures, calibrations.

Introduction

Routine laboratory analysis show several limitations mainly due to the response velocity and the high costs related to the conventional chemical methods, which determine the imposibility to analyze rapidly, economically and accuratively all the samples received. Near infrared reflectance spectophotometry as a automatized method of instantaneous response, has showed its capacity to produce simultaneous information of different attributes of the feedstuffs such as chemical composition, digestibility, energy values, etc. It is a non-destructive technique, no chemical reagents involved and it does not require previous preparation of the sample, which can satisfy most of the requirements of a good quality standards control (Norris et al., 1976; Shenk et al., 1979; Shenk et al., 1981). However, several limitations have been found to the present method, since depends on a accurate and precise laboratory analysis of the samples to be scanned in the equipment for calibration and validation and concurrently it is a secundary technique. Also this procedure requires time and the error of the analysis by NIRS is higher than the primary method.

Materials and Methods

Forage samples were obtained from four different locations in Chihuahua State and they were: Avena (Avena sativa), var Cocker; Barley (Secale secale) var wintermore; Red Wheat (Triticum aestivum) var. Tam; Triticale (X. Triticosecale wittman) var AN31; Ryegrass (Lolium multiflorum) var. Oregon. Samples were dried at 55º C and ground in a Wiley mill with a 1mm mesh and chemically analyzed for dry crude protein (CP), ashes (A), phosphorus (P), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter (DM), in vitro organic matter digestibility (IVOMD) and in vitro dry matter digestibility (IVDMD). All samples were run by duplicate and according to the AOAC (1990). Ruminal liquor for in vitro analysis was provided by an adult male pelibuey ovine.

Table 1. Mixes of irrigated pastures

Treatments	Mixes
1	Oat + Barley + Ryegrass
2	Oat + Wheat + Ryegrass
3	Oat + Triticale + Barley
4	Wheat + Barley + Ryegrass
5	Wheat + Barley
6	Barley

A NIRS monocromator (NIRSystem 5000; Perstop Analytical Inc. Silver Spring, M.D.)with a wavelength between 1108 and 2500 were used for spectra recollection of the two hundred and fifty four samples, which were dried and ground previously to the scanning. Once the calibration equation was established, the observations not included were utilized for the process of the validation equation (Shenk, et al., 1981). NIRS equation precision were evaluated according to the standard deviation of the chemical analysis and standard error of the cross validation. If this relation is more than 0.33, the equations have a low prediction capacity (Murray, 1993, citado por Cozzolino 2002). Cross validation was utilized to avoid the over adjustment of the model and a SNV Detrend correction was applied (Barnes et al., 1989, cited by Cozzolino, 2002). The mathematical treatment applied to

the spectra was 1,4,4,1 and the statistics of the calibrations calculated, included the standard error of the calibration (SEC), the determination coefficient of the calibration (R^2) and the standard error of the cross validation (R_{val}) (Shenk and Westerhaus, 1993).

Results and Discussion

Table 2 shows the chemical composition of the samples of the mixes of the forages with the values used for calibration and validation.

Table 2. Chemical composition of samples of mixes of irrigated pastures.

	Calibration					Validation				
Chemical fraction	n	Mean	SD	High value	Low value	n	Mean	SD	High value	Low value
CP	105	17.4	2.7	28.0	10.2	10	21.3	3.3	34.0	10.2
NDF	88	55.8	1.7	69.3	41.9	10	56.9	1.6	68.9	43.4
ADF	62	27.1	1.9	38.4	20.3	10	24.6	1.8	34.2	19.5
IVDMD	74	80.9	1.4	92.9	64.5	10	83.5	1.4	95.3	70.0
IVOMD	66	76.4	1.4	84.6	60.8	10	78.7	1.4	92.5	65.0
Ashes	100	11.0	2.3	15.8	7.0	10	13.9	1.9	20.0	10.3
DM	95	90.7	1.1	93.1	86.9	10	9.03	1.1	91.8	85.5
P	88	0.5	2.6	0.80	0.31	10	0.45	2.3	0.7	0.3

In Table 3 are shown the values for the NIRS calibration for each of the chemical fractions. For protein, values of 0.99 and 0.16 for R^2 and SECV/SD respectively were obtained by the model, which are in agreement to the results obtained by Murray (1993, cited by Cozzolino, 2002) working with forages under grazing. NDF showed acceptable values for both parameters, similar to those found by Norris et al., (1976). However, were higher than the obtained by Marten et al., (1985). For ADF the R^2 and the SECV/SD were quite similar compared to NDF and also with the values reported by Cozzolino (2002) of .0.93 and 0.29 for R^2 and SECV/SD respectively. Cozzolino (2002) mentioned that in the infrared region is difficult to determine complex chemical entities like NDF or ADF, since the method is based in the reading of the chemical bounds which are associated with chemical structures or arbitrary entities such as the fiber fraction.

Table 3. NIRS calibration for chemical fractions of samples of mixes of irrigated pastures

Chemical fraction	n	Mean	SD	R^2	SECB	SECV/SD	T
Ashes	100	11.0	2.3	0.97	0.56	0.25	0.94
P	88	0.5	0.08	0.96	0.03	0.33	0.89
NDF	88	55.8	6.4	0.95	2.1	0.33	0.89
CP	105	17.4	4.5	0.99	0.70	0.16	0.97
ADF	62	27.1	3.7	0.93	1.51	0.41	0.83
IVDMD	74	80.9	6.1	0.78	4.23	0.70	0.60
IVOMD	66	73.4	5.3	0.86	2.95	0.56	0.74
DM	95	90.7	1.4	0.93	0.56	0.41	0.86

Values for IVDMD resulted lower than expected (R^2 = 0.78 and SECV/SD = 4.23) in comparison to the results reported by Coelho et al., (1988) of 0.90 and 0.6 for R^2 and SEC respectively, giving an inadequate prediction value for this parameter. IVOMD showed a better response (0.86 and 2.95) in comparison to IVDMD perhaps due to the elimination of ashes which are a source of variation and error for the determination. For ashes, dry matter and phosphorus, values of prediction are adequate with high R^2 and acceptable SECV/SD values, but no much information are available in the literature to compare them.

Implications

According to the results obtained, use of the NIRS is a very accurate method from the chemical point of view, added to the fact that is a reduce-cost analysis and the time of response is fast giving a very good confidence and moreover, a good reproducibility. It is recommended to add a large number of samples, in order to expand the calibration and give a better prediction equation.

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FORAGE QUALITY AND AGRONOMIC CHARACTERISTICS OF SPRING BARLEY CORE COLLECTION LINES COMPARED TO COMMERCIAL BARLEY CULTIVARS

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Abstract: Forage barley breeding programs commonly select new barley lines based on yield and awnless characteristics despite the importance of forage quality. Our objectives were: (1) to evaluate a subset of the World Barley Core Collection (WC) and commercially available barley cultivars for forage quality and agronomic characteristics and (2) assess suitability of WC lines for use in a forage barley breeding program. The subset, containing 10 WC barley lines and 10 commercial cultivars, was grown in a replicated field trial (r=4) under irrigated conditions in 2004 near Bozeman, MT. Agronomic data collected included plant height, % lodging, maturity score, and yield (t/ha). Forage clip samples were collected at three stages of maturity; boot, anthesis, and at harvest for hay. A 15-cm clip sample was cut at stubble height and dried at 60°C for 48 h. Forage samples were ground to pass a 1-mm screen for boot and anthesis. Samples were ground to 5-mm for harvest and then sub-sampled and ground through a 1-mm screen. All samples were analyzed for DM, NO_3-N, ADF and NDF. Samples at harvest were evaluated for 48 h ISDMD. Nitrate-N did not differ ($P > 0.10$) between WC lines and commercial cultivars at boot, anthesis, or harvest stages of maturity (average 0.12, 0.15, and 0.28%, respectively). Acid detergent fiber content did not differ ($P > 0.10$) between WC lines and commercial cultivars at boot or anthesis stages of maturity (29.9 and 36.0%, respectively). Commercial cultivars had 4% greater ($P < 0.01$) ISDMD when compared to WC lines (68.31 vs. 65.84 %, respectively). World collection lines were shorter ($P = 0.07$) and had narrower ($P = 0.01$) flag leaves when compared to commercial cultivars (86.1 vs. 82.2 cm and 11.5 vs. 9.7 mm; respectively). Yield tended to greater ($P = 0.11$) for WC lines when compared to commercial cultivars (7.77 vs. 7.27 t/ha). Based on yield and NO_3-N results, selected world barley core collection lines were comparable to commercially available barley cultivars and could be utilized in a forage barley breeding program for improved forage quality.

KEYWORDS: Barley, Forage Quality, Selection

Introduction

In Western Canada and the Northern Great Plains, several hundred thousand acres of hay barley are produced annually. In Montana, hay barley varieties account for approximately 13.4% of the total barley acres seeded (Montana Agricultural Statistics Service, 2004). From 1995 to 2002 there was almost a four-fold increase in production of forage barley. One explanation for this increase may be the current drought conditions and the use of hay barley as a temporary hay crop. Additionally, small grains such as barley are used in crop rotations to rejuvenate alfalfa stands, especially under irrigation. This methodology is an effective way to reduce costs associated with weed and disease control. Much of the annual hay crops (including cereal hay) are fed on-site, therefore, an improvement in forage quality of hay barley varieties would be beneficial to the livestock producer and well as the hay producer.

The spring barley core subcollection for the USDA-ARS National Small Grains Collection has been evaluated for forage yield and forage ADF (Surber et al., 2002). These researchers assessed 1531 lines. They determined that there was a wide range in variation in the spring barley core collection for yield and ADF content. With the availability of larger variation greater improvement of barley feed quality can be expected through selection. The authors suggested that this variation could be explored to develop new barley cultivars with improved forage quality.

Forage barley breeding programs commonly select new barley lines based on yield and awnless characteristics despite the importance of forage quality. Our objectives were: (1) to evaluate a subset of the World Barley Core Collection (WC) and commercially available barley cultivars for forage quality and agronomic characteristics and (2) assess suitability of WC lines for use in a forage barley breeding program.

Materials and Methods

The USDA-ARS National Small Grains Collection contains approximately 25,000 barley accessions. The barley core collection has 1,600 lines with the spring growth habit (USDA-ARS, NGRP, 2000) and 10 were selected from previous research of Surber et al. (2002) to represent the genetic diversity found in the larger collection.

The subset of barley lines grown in this experiment contained 10 2-rowed WC lines and 10 commercial cultivars. The subset was grown in a replicated field trial (r=4) under irrigated conditions in 2004 near Bozeman, MT. Agronomic data collected at hay harvest included plant height, flag leaf height and width, % lodging, maturity score, and yield (t/ha). Hay harvest samples were collected on the same day for all genotypes, and plant maturity was scored (maturity score; 4 = late anthesis, 5 = milk, 6 = soft dough). Forage clip samples were collected at three stages of maturity; boot, anthesis, and at harvest for hay. A 15-cm clip sample was cut at stubble height and dried at 60°C for 48 h. Forage samples were ground to pass

a 1-mm screen in a Wiley mill for boot and anthesis sampling periods. Hay harvest samples were ground to pass a 5-mm screen in a Wiley mill for harvest samples and then sub-sampled and ground through a 1-mm screen. All samples were analyzed for DM, NO_3-N, (AOAC, 2000) ADF and NDF (Van Soest et al., 1991). Samples at harvest were evaluated for 48 h ISDMD.

Plot served as the experimental unit. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to test the effects of WC versus commercial cultivars on forage and agronomic characteristics. When a significant F-value was found ($P < 0.05$), means were separated using LSD.

Results and Discussion

Statistical analyses comparing forage quality characteristics of world collection and commercial cultivars are presented in Table 1. Nitrate-N did not differ ($P > 0.10$) between WC lines and commercial cultivars at boot, anthesis, or harvest stages of maturity (average. 0.12, 0.15, and 0.28%, respectively). However, an unusual trend occurred when NO_3-N concentrations were examined over time (Figure 1). Higher levels of nitrates are generally present in immature plants and decrease as plants mature (Wright and Davison, 1964). It is unclear why nitrate concentrations increased with advancing maturity in this study, however, a number of factors could have contributed to higher NO_3-N levels such as plant stress (i.e. drought), and shading from lodging. Forage barley is particularly prone to nitrate accumulation, and nitrate toxicity in animals can result from feeding hay barley with elevated nitrate levels. The Montana State University Extension Service estimated between 2000 and 2002 greater than 38 % of hay barley samples from Montana counties were considered "hazardous" for nitrate levels (Cash et al., 2003). This resulted in the loss of approximately 165,000 tons of hay during this time. Purchasing hay at $75/ton to replace unsafe hay could cost livestock producers approximately $12 million. Selecting new barley varieties based on low nitrate potential has obvious social and economic benefits.

Neutral detergent fiber content did not differ ($P > 0.10$) between WC lines and commercial cultivars at boot, anthesis, and harvest stages of maturity (average 54.4, 61.0 and 56.5%, respectively). Similarly, ADF content did not differ ($P > 0.10$) between WC lines and commercial cultivars at boot, anthesis, and harvest stages of maturity (average 29.9, 36.0 and 31.8%, respectively). It is interesting to note that ADF content declined at the harvest stage of maturity. These results agree with Khorasani et al. (1997) who found that as plants reached maximum maturity, NDF and ADF began to decline for barley, oat and triticale forage species. They suggested that as the plant matures, leaves and stem are more fibrous but in later stages of maturity the higher fiber is offset by the increased starch development as the head fills.

Commercial cultivars had 4% greater ($P = 0.002$) ISDMD when compared to WC lines (68.31 vs. 65.84%, respectively). Nylon bag digestibility gives an approximation of digestible dry matter. Digestible dry matter is often used as a proxy of digestible energy (Coleman and Moore, 2003). This is an unexpected result as WC lines used in this study were selected for low ADF and high in yield. Acid detergent fiber (ADF) is negatively related to digestibility potential for the animal and can be used to estimate the digestibility and energy value (Reid et al., 1988). We expected these selected WC lines to have greater digestibility when compared to commercial cultivars.

Agronomic characteristics of WC lines and commercial cultivars are presented in Table 2. World collection lines were shorter ($P = 0.07$) and had narrower ($P = 0.008$) flag leaves when compared to commercial cultivars lines (86.1 vs. 82.2 cm; and 11.5 vs. 9.7 mm; respectively). There were two commercial cultivars that were noted to have especially wide leaves and may have skewed the results for that dataset. Lodging percent was lower ($P = 0.001$) for commercial cultivars lines when compared to WC lines (24.4 vs. 63.2%, respectively). Maturity scores did not differ ($P = 0.46$) between barley types (average 5.0; milk stage of maturity).Yield tended to greater ($P = 0.11$) for WC lines when compared to commercial cultivars (7.77 vs. 7.27 t/ha). This result was expected as WC lines used were selected specifically to have high yields.

Implications

Based on yield, NO_3-N content, and forage quality results, selected world barley core collection lines were comparable to commercially available barley cultivars and could be utilized in a forage barley breeding program for improved forage quality.

Acknowledgments

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Table 1. Forage quality characteristics at different stages of maturity as affected by barley type.

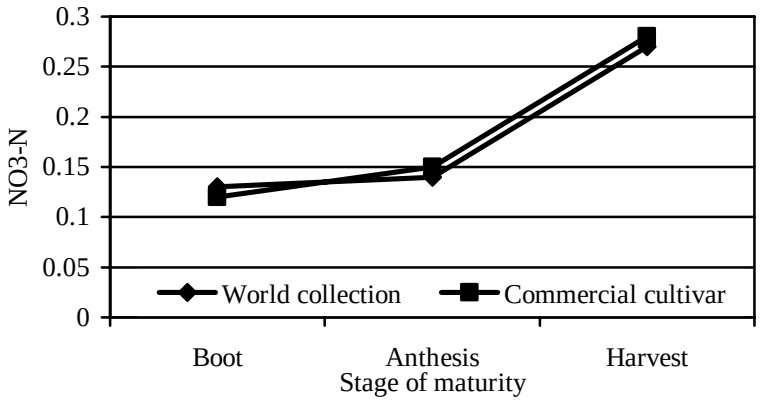
	Barley Type			
Item	World Collection	Commercial Cultivar	SE	*P*-value
Boot				
NDF, % of DM	55.0	53.7	0.53	0.10
ADF, % of DM	30.3	29.4	0.42	0.15
NO_3-N, % of DM	0.13	0.12	0.009	0.38
Anthesis				
NDF, % of DM	61.2	60.7	0.35	0.36
ADF, % of DM	36.4	35.6	0.79	0.45
NO_3-N, % of DM	0.14	0.15	0.009	0.28
Harvest				
NDF, % of DM	56.2	56.8	0.55	0.38
ADF, % of DM	31.4	32.1	0.45	0.26
NO_3-N, % of DM	0.27	0.28	0.026	0.85
ISDMD, % at 48 h	65.8	68.3	0.55	0.002

Table 2. Forage agronomic characteristics as affected by barley type.

	Barley Type			
Item	World Collection	Commercial Cultivar	SE	*P*
Plant height, cm	82.2	86.1	1.50	0.07
Flag leaf height, cm	71.6	74.2	1.25	0.14
Flag leaf width, mm	9.7	11.5	0.48	0.008
Maturity score[a]	5.0	4.9	0.13	0.46
Lodging, %	63.2	26.4	5.44	<0.0001
DM yield, t/ha	3.4	3.2	0.10	0.11

[a] Maturity score; 4 = late anthesis, 5 = milk, 6 = soft dough.

Figure 1. Nitrate concentration trends as stage of maturity advance.



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EFFECTS OF SAMPLING DATE ON THE FORAGE QUALITY AND QUANTITY OF STOCKPILED NATIVE RANGE IN SOUTHWESTERN NORTH DAKOTA

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ABSTRACT: The objective of this study was to characterize changes in biomass and nutrient concentration of stockpiled native range from early November to late January. In each of four years, dry beef cows were grazed in a single pasture. For sampling purposes, the pasture was separated into two halves with five permanent sampling sites established in each half. Forage samples were collected from each sampling site on 14-day intervals. In the last three years, forage samples were pooled within each site, sampling date and pasture half for nutrient analysis. Forage samples were analyzed for concentrations of crude protein (CP), acid (ADF) and neutral (NDF) detergent fibers, calcium (Ca), phosphorus (P), magnesium (Mg), and potassium (K). Total digestible nutrient (TDN) concentration was calculated using a standard procedure. The concentration of all reported nutrients, with the exception of P (P>.35), was affected by advancing season. Concentrations of CP (P<.1) and Ca (P<.05) increased and then decreased with advancing season. Concentrations of TDN (P<.02), Mg (P<.1) and K (P<.1) decreased, and ADF (P=.01) increased, linearly with advancing season. NDF concentration (P<.1) increased in one year and remained constant in the other two years. Average total forage biomass available for grazing varied with year (P=.03). The lowest level was in year 1 and the highest in year 2 (900, 1584, 1370 and 1151 kg/ha for years 1, 2, 3, and 4, respectively). Biomass disappearance per animal unit (AU) was estimated to be 35.0, 30.9 and 4.1 kg/d for total, grass and forbs, respectively. Stockpiled native range is a readily available source of grazing for use in extending the grazing season of dry beef cows into the late fall and early winter. Nutritional supplementation that offsets declining forage quality, coupled with appropriate stocking rates, will be essential for optimizing the use of this grazing resource.

Key Words: Stockpiled Forage Quality, Biomass Disappearance

Introduction

Extending the grazing period can potentially reduce the dependence on harvested forage for the winter. The forage availability and nutrient content in native range pastures in southwestern North Dakota may limit it's usefulness as a grazing resource for beef cows in late fall and early winter. Forage stockpiling is a method considered to be somewhat successful at extending the grazing period past the normal growing season. This method allows forage to grow during the times of active growth and saved for use at some other time of the year. Stockpiling native range has been shown to be a viable mechanism for extending the grazing season. However, stockpiled forage may not always meet the cattle's nutrition requirements and additional dietary supplementation may be required. An understanding of the forage quality and quantity of stockpiled native range throughout an extended grazing period is essential for designing effective supplementation strategies. The objective of this study was to characterize changes in forage quality and quantity of stockpiled native range from early November to late January.

Materials and Methods

In each of four years (Table 1), dry pregnant beef cows were grazed on a single stockpiled native range pasture (approximately 116.6 ha) during a period from early November to late January. Forage available for grazing was sampled at regular intervals throughout the grazing period to detect changes in forage dry matter (DM) available for grazing. For sampling purposes, the pasture was virtually divided into an east and a west section and five permanent sampling sites were established in each section. On each sampling date, total forage was removed from two randomly selected 0.25-m^2 areas at ground level at each site and separated into forbs and grasses. Forage samples were dried (55° C until constant weight) and dry matter yields calculated. In three years, forage samples were then pooled within section and sampling day for nutrient analysis. Forage samples were analyzed for crude protein (CP), acid (ADF) and neutral (NDF) detergent fiber, calcium (Ca), phosphorus (P), magnesium (Mg), and potassium (K) in a commercial laboratory. Total digestible nutrients (TDN) concentration was calculated using a standard procedure.

Forage composition and DM available for grazing were analyzed for the effects of sampling year and date using a split-plot in time analysis within a randomized complete block design. Pasture section represented the blocking factor. A commercial statistical software program (SAS; Cary, NC) was used to perform all analytical procedures.

Results and Discussion

Average nutrient concentrations of ADF (P=.32), Ca (P=.20) and P (P=.79) did not differ among years (Table 2). Concentrations of CP (P = 0.10), TDN (P = 0.11), NDF (P<.05), Mg (P<.05) and K (P<.05) varied significantly in at least one sampling year.

Changes in forage quality with advancing season are shown in Figures 1 and 2. Concentrations of CP (P = .09) and Ca (P = 0.05) changed quadratically with both concentrations increasing and then decreasing as grazing season advanced. Concentrations of TDN (P = 0.02), Mg (P = 0.08) and K (P = .09) decreased, and ADF (P = 0.01) increased, linearly with advancing season. The change in NDF concentration with advancing season was not consistent across years (P = 0.09). NDF increased in one year, while remaining constant in the other two years, as season advanced.

Total grazing days per season ranged from 63 to 85 (Table 1). Annual animal unit grazing days per ha ranged from 16.1 to 19.4 and annual stocking rate ranged from 1.56 to 1.90 ha per animal unit month.

Changes in forage biomass available for grazing (kg DM/ha) among years and with advancing season are shown in Table 2 and Figure 2, respectively. Average annual total, grass and forbs biomass available for grazing varied across years (P<.05) and declined linearly with advancing season (P<.01). The average rate of DM disappearance was 34.9, 30.7 and 4.2 kg per animal unit grazing day for total, grass and forbs, respectively.

Forage quality of stockpiled native range in the late fall and early winter period varies both within and across years. However, with the exception of NDF concentration, forage quality changes with advancing season seem to be consistent across years. Since the opportunity exists for grazing animals to select a diet higher in quality than the average of what is offered, further work in the forage quality of stockpiled native range needs to focus on quantifying potential animal selectivity.

Forage quantity of stockpiled native range also changes with year and advancing season. The response to advancing seasons seems to be linear and is related to accumulated animal unit grazing days. Assuming a 50% utilization of total DM, the total DM removal rate suggests that this pasture would support approximately 11 animal unit grazing days per acre or require approximately 2.8 acres per animal unit month of grazing at this time of the year.

Implications

Stockpiled native range is a readily available source of grazing for use in extending the grazing season of dry beef cows into the late fall and early winter. Nutritional supplementation to offset declining forage quality and appropriate stocking rates will be essential for optimizing the use of this grazing resource

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Table 1. Animal and grazing information.

	Year			
	1	2	3	4
Number of cows	23	21	24	24
Initial:				
Body weight, lb	604 ± 41	629 ± 64	509 ± 39	572 ± 59
Body condition score[a]	5.8 ± .7	6.8 ± .6	4.5 ± 1.1	4.8 ± 1.0
Grazing Dates:				
Beginning	November 22	November 14	November 6	December 4
End	January 31	January 23	January 29	February 5[c]
Total grazing days	70	70	85	63
AU[b] grazing days/ha	16.2	16.1	19.1	19.4
Hectares/(AUM[b])	1.86	1.90	1.60	1.56

[a] Estimate of body fatness (1 to 9 scale; Encinias and Lardy, 2000).

[b] Animal unit (AU) and animal unit month (AUM).

[c] Biomass sampling stopped in mid January of Year 4..

Table 2. Annual averages for nutrient concentrations (% DM) and forage available for grazing (kg DM/ha).

	Year			
	2000-2001	2001-2002	2002-2003	2003-2004
Nutrient				
Crude protein	--	4.8[a]	5.3[b]	5.4[b]
Acid detergent fiber	--	47.5	48.0	48.3
Neutral detergent fiber	--	70.8[x]	73.5[z]	60.9[x]
Total digestible nutrients	--	52.6[b]	51.5[a]	51.3[a]
Calcium	--	.65	.67	.47
Phosphorus	--	.073	.076	.076
Magnesium	--	.097[y]	.095[y]	.069[x]
Potassium	--	.34[y]	.16[x]	.15[x]
Forage available for grazing				
Total	900.7[x]	1584.5[z]	1369.1[yz]	1150.6[xy]
Grass	793.5[x]	1402.8[z]	1240.8[yz]	1106.3[y]
Forb	107.2[y]	181.7[z]	128.3[y]	44.5[x]

[a,b] Means within a row with differing superscripts differ ($P < 0.15$).
[x,y,z] Means within a row with differing superscripts differ ($P < 0.05$)

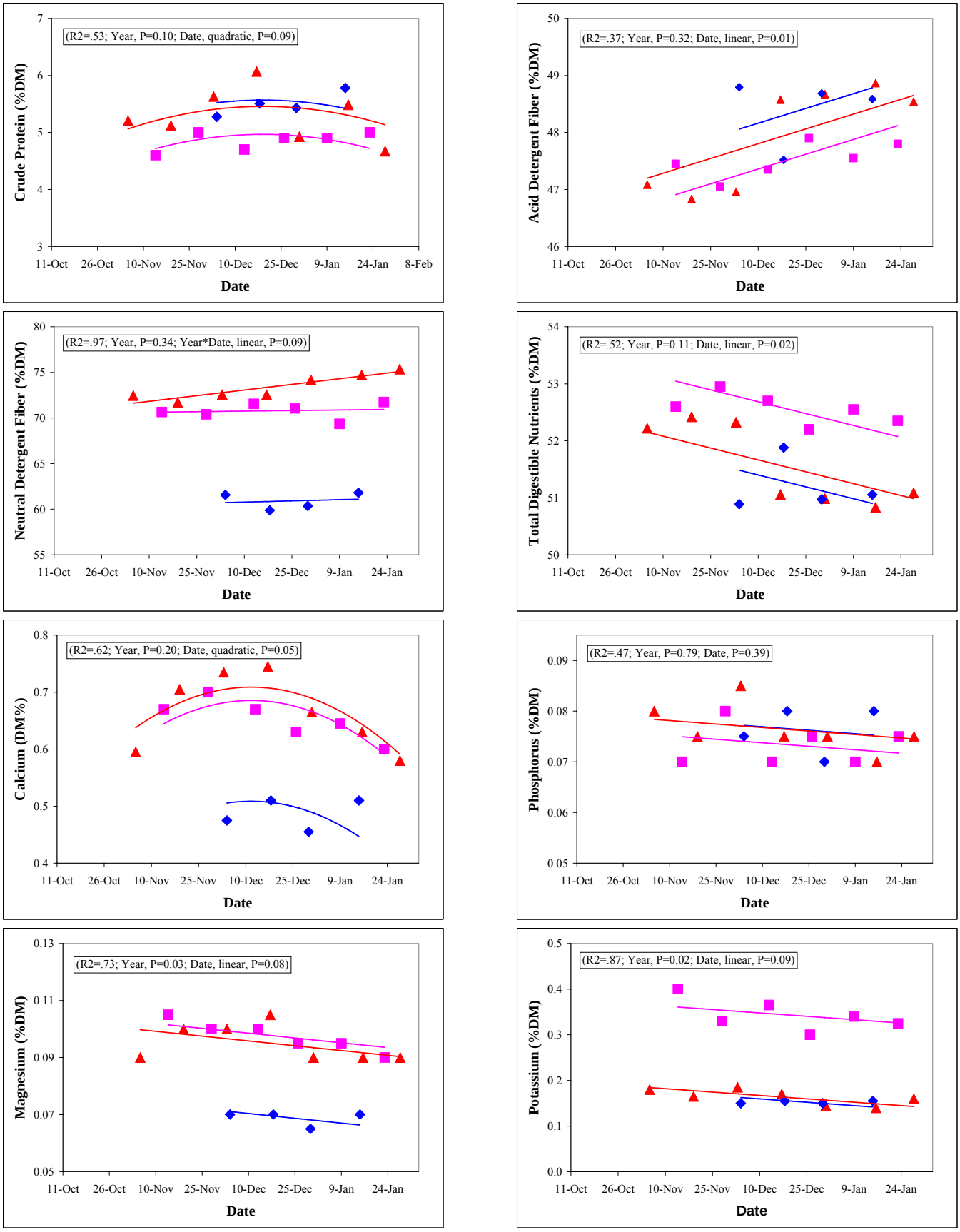
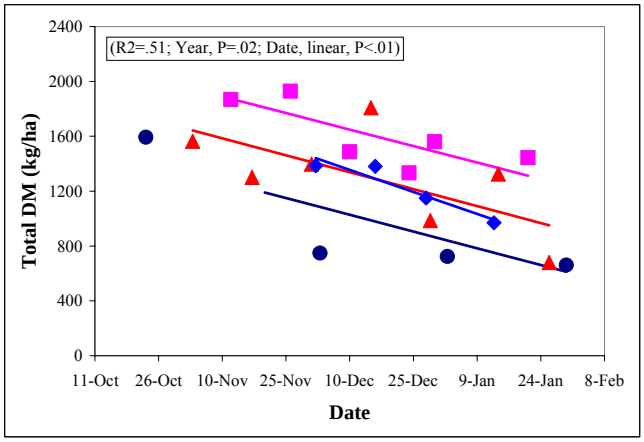
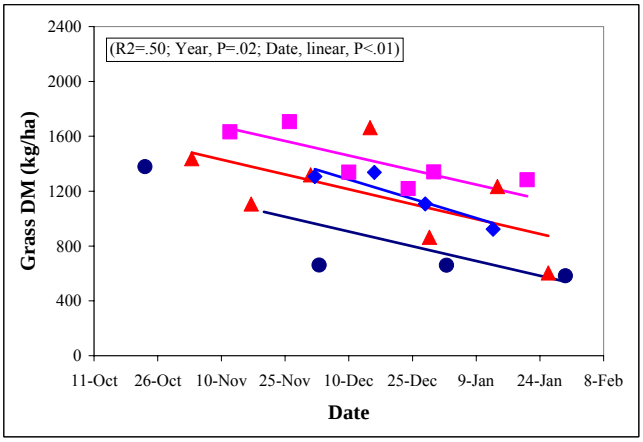


Figure 1. Effect of year and advancing season on nutrient concentrations (%DM) of stockpiled native range. Solid lines indicate annual change with advancing season (■=year 2, ▲=year 3, and ♦=year 4).





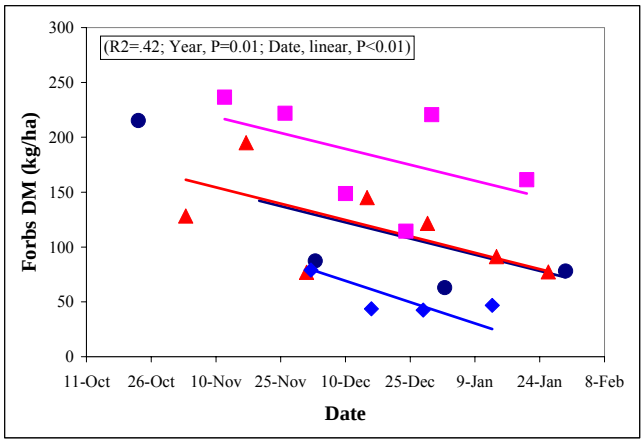


Figure 2. Effect of year and advancing season on total, grass and forbs DM available for grazing (kg/ha). Solid lines indicate DM disappearance for each biomass type per accumulated animal unit grazing days assuming a year dependent intercept and a common rate of DM disappearance (●=year 1, ■=year 2, ▲= year 3 and ♦=year 4).

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EFFECTS OF WEANING DATE AND RETAINED OWNERSHIP ON CATTLE PERFORMANCE AND FORAGE DISAPPEARANCE IN SPRING CALVING BEEF SYSTEMS

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ABSTRACT: Weaning calves early from spring calving cows can have multiple impacts on beef production systems. The objective of this three-state study was to evaluate the effects of mid-August (AW) versus early-November weaning (NW) on cow and calf production traits and forage utilization. Three hundred-seventeen cow-calf pairs from the NDSU-Dickinson Research Extension Center (DREC; n=88), SDSU-Antelope Research Station (ANT; n=136) and the University of Wyoming Beef Unit (UW; n=93) were stratified by BW and body condition score (BCS) and assigned to either AW (calves weaned at approx. 140 d of age) or NW (calves weaned at approx. 215 d of age). Cows grazed native range between the two weaning dates. At AW date, a subset of cows from each treatment at DREC were randomly assigned to six 20-ha. pastures (three pastures/treatment) to measure biomass disappearance between AW and NW dates. Steer calves at ANT and DREC were weaned and backgrounded 7.3 wk and finished in a commercial feed yard. Steers at UW were backgrounded 42 d and finished on site. Treatment by location interactions were detected for cow BW change, BCS change, calf ADG, and gain:feed. At each location, AW cows lost less weight (P<0.01) between weaning dates than NW cows. Similarly, cow BCS change was improved (P<0.01) for AW vs. NW at DREC (0.39 and -1.20), ANT (0.34 and -0.02), and UW (-0.05 and -0.78). Forage biomass disappearance, between weaning dates, was reduced by 27.7% (P=0.15) when calves were AW. AW steers at DREC had higher (P<0.01) ADG during backgrounding than NW, and AW steers at DREC and ANT were more efficient (P<.01) during backgrounding. During finishing, AW steers grew slower (P<.01), were less efficient (P<.01) at ANT, and required 61 more days on feed to reach harvest endpoint. Weaning spring-born calves at 140 d compared to 215 d reduced forage utilization, improved cow BCS change, and resulted in similar calf performance.

Key Words: Early Weaning, Cow Performance, Forage Disappearance

Introduction

Profit margins in cow/calf production are slim due to high production costs (Taylor and Field, 1995) and lost opportunity to capture value from marketable ranch products (NASS, 1999). Development of systems that lower production costs while adding value to calves would be beneficial to sustaining and improving rural communities in the drier regions of the Western United States. The majority of costs in cow/calf businesses are for harvested feed (Taylor and Field, 1995). Systems that rely more on grazing and less on harvested and purchased feedstuffs have a higher potential to be profitable (Adams et al., 1994).

Body condition of cows at time of calving has been shown to influence subsequent pregnancy rates (Richards et al., 1986), and the body condition score of spring calving cows grazing winter range is influenced by body condition score in the fall (Adams et al., 1987). Lamb et al. (1997) showed spring calving cows grazing native range lost 0.4 of a body condition score if nursing a calf from September to November, whereas cows that had their calves weaned in September maintained condition from September to November. Management of body condition score by weaning early can improve subsequent reproduction and/or reduce the requirements for non-grazed feed inputs that would be required for thin cows.

The Beef Cattle NRC (1996) predicts a spring calving cow lactating in August will have a 9% greater daily intake of range forage than a dry cow. Weaning calves early may allow standing forage to be spared, reducing late season supplemental feed requirements.

Performance of early-weaned calves during the backgrounding and finishing phase is important. Research has shown calves weaned at 100 to 150 days of age were heavier and younger at slaughter than normal weaned (weaned at 225-250 days) calves (Peterson et al., 1987). Meyers et al. (1999) reported that 40% more early weaned steers graded average choice or higher than their normal weaned counterparts. Carcass quality improvement in early weaned calves managed for maximum economic yield parallels value-based marketing trends (Cattle-Fax, 2003).

The objective of this multi-state investigation is to evaluate the impact of early weaning and retained ownership decisions on the relationship between weaning date and herbage availability, cattle performance, and economic returns.

This report encompasses only the first year of a two-year investigation.

Materials and Methods

Cow herds from the SDSU Antelope Range and Livestock Research Station (136 cows), the NDSU Dickinson Research Extension Center (88 cows) and the UW Beef Unit (93 cows) were used in the study. At each location, spring-born calves were weaned from cows at approximately 140 days (mid-August, **AW**) or 215 days of age (early-November, **NW**). Cow body weight and body condition score changes were monitored between the August and November weaning dates to determine the impacts of weaning on cow performance.

Calf weaning weights were recorded at each location. The steer calves from Antelope Station and Dickinson REC were transported immediately after weaning to the NDSU Hettinger Research Extension Center for backgrounding. The steers were backgrounded either 49 (AW) or 54 (NW) days, using a diet consisting of locally grown forage and a commercial pellet consisting of regionally available co-product feedstuffs (soyhulls, wheat middlings, barley malt sprouts). Two-to-four weeks prior to each weaning date, calves were immunized against calfhood diseases and were administered a booster vaccination at weaning.

Following the seven- to eight-week backgrounding phase, Antelope and Dickinson REC calves were transported to Decatur County Feed Yard, Oberlin, Kansas, for finishing. An ACCU-TRAC® electronic cattle management system was employed at Decatur to determine final end point, based on an external fat depth of 10 mm in the computer model. Steers were slaughtered at a commercial plant and carcass data were collected.

Both steers and heifers from UW Beef Unit were managed in a similar protocol as described for Antelope and Dickinson REC steers. Cattle were backgrounded at the UW Beef Unit, Laramie, Wyoming, for either 43 (AW) or 40 (NW) days. Following the backgrounding period, cattle remained at the UW Beef Unit for the experiment's finishing phase. Cattle were marketed in three groups, March 29, May 10 and May 25, 2004 based on ultrasound backfat depth and percent intramuscular fat measured between the 12th and 13th ribs. Cattle were slaughtered at a commercial plant and carcass data were collected

Grazing, backgrounding, and finishing performance were analyzed by ANOVA using a PROC GLM of SAS (SAS Inst. Inc., Cary, NC). Since treatment by location interactions were identified, treatment means were compared within location.

Vegetation samples were collected at the Dickinson REC to determine the magnitude of biomass disappearance among cows suckling calves from August to November (NW; n=3 pasture groups) versus dry cows grazing from August to November (AW; n=3 pasture groups). A 240 ha pasture was subdivided into 12 20-ha pastures in a wagon-wheel configuration with central watering. A subset of cattle from each treatment at the Dickinson REC, were rotated into six previously ungrazed pastures at the August weaning date (3 pastures/treatment; 8 cows/pasture).

Clipped forage samples were obtained in the six pastures just prior to the AW date and again at the end of grazing when all cows were removed from the pastures in November. Samples (0.25 m^2) were cut to ground level, using battery-powered electric shears. Samples were oven dried. Forage disappearance was calculated as the difference between pre- and post-grazing estimates.

Analysis of variance was used to evaluate weaning treatment effect on biomass disappearance.

Results and Discussion

In this multi-state weaning date study, early weaning impacted cows positively by maintaining or improving body weight (P<.01) and body condition score (P<.01) at each location (Table 1).

Early weaned cows at SDSU Antelope Station gained 15 kg from mid-August to the first week of November and improved 0.34 condition score, while normally weaned cows lost 21 kg. and 0.02 body condition score. Cows with calves weaned early in August at the UW Beef Unit gained 10 kg., and maintained body condition from August to November, whereas normally weaned cows lost 29 kg and 0.8 body condition score during the August-to-November period.

At the NDSU Dickinson Research Extension Center, early weaned cows lost 5 kg, but overall, improved 0.39 body condition score, whereas normally weaned cows lost 89 kg and 1.20 body condition score during the same August–to-November period. The 89-kg loss was larger than expected and may have been attributed to a snow storm (approximately 254 cm) one week before the NW date that partially covered forage until weaning.

The AW system utilized 72% of the available biomass when compared to the NW system. Forage disappearance for cows that had calves weaned early was estimated to be 803 kg per ha, whereas forage disappearance among cows that continued to nurse their calves for an additional 75 days was estimated to be 1109 kg per ha (P = 0.15). The difference in forage utilization was attributed to calf removal and less trampling.

Postweaning backgrounding performance for steers from Antelope, Dickinson and UW is shown in Table 2. Normally weaned steers were heavier at the end of the backgrounding phase (P<.01) at each location. Dickinson calves that were early weaned had a higher (P<.01) average daily gain during backgrounding than normally weaned calves, whereas calves from Antelope had similar gains across weaning dates. In contrast, calves from UW that were early weaned gained less than those that were weaned in November (P< 0.01). Dry matter intake (P<.05) and F:G (P<.01) of AW calves were improved compared to NW at Antelope and Dickinson.

Finishing performance for the two management systems is shown in Table 3. Normally weaned steers were an average 77 kg heavier on arrival (P<.01); however, final harvest weight did not differ. On average, NW steers required 61 fewer days on feed (P<.01), and SDSU's NW steers were less efficient during finishing (P<.01).

Final harvest end point for steers at Antelope and Dickinson were determined using the ACCU-TRAC® electronic cattle management system (ECM) whereas the

UW used ultrasound fat depth and percent intramuscular fat to make the determination. Fat depth at the UW was 2.75 mm greater (P<.05) for the early weaned steers. Yield and quality grades did not differ at Antelope and Dickinson. Carcass measurements for the three sites did not differ for hot carcass weight or rib-eye area. However, early weaned steers had greater yield (P<.05) and quality grades (P <.10) at UW that resulted when the early weaned group was fed to a higher degree of finish. The number of Choice grading steers was low for NDSU and SDSU cattle, suggesting that final end point determination using the ECM system underestimated days on feed needed to attain higher quality grade.

Implications

These data suggest that weaning spring-born calves 75 days early (140 versus 215 days) can reduce late summer native forage utilization and improve cow body condition without negatively impacting postweaning calf performance.

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Table 1. Body weight and condition score change among early and normally weaned cows located at the NDSU-Dickinson REC, SDSU-Antelope Station and UW-Beef Unit (2003).

	NDSU Dickinson REC		SDSU Antelope Station		UW Beef Unit	
Item	Weaning Period		Weaning Period		Weaning Period	
	Early	Normal	Early	Normal	Early	Normal
August Cow Wt., kg	583	605	609	603	548	564
November Cow Wt., kg[a]	578	515	624	582	557	535
Cow Wt. Change, kg[a]	-5	-89	15	-21	10.0	-29
August BCS	5.52	5.52	5.63	5.65	5.43	5.59
November BCS[a]	5.91	4.32	5.97	5.63	5.38	4.82
BCS Change[a]	0.39	-1.20	0.34	-0.02	-.05	-.78
August Calf Wt., kg[b]	175	184	185	183	201	198
November Calf Wt., kg	-	247	-	264.	-	276

[a]Treatments at each location differ (P<.01)
[b]Treatments at Dickinson location differ (P<.10)

Table 2. Summary of backgrounding performance for early and normally weaned steers at the NDSU-Dickinson REC, SDSU-Antelope Station and UW-Beef Unit (2003)

	NDSU Dickinson REC		SDSU Antelope Station		UW Beef Unit	
Item	Early	Normal	Early	Normal	Early	Normal
No. Steers	40	38	36	35	26	23
Days on Feed	49	54	49	54	43	40
Start Wt., kg[a]	185	251	188	272	202	282
End Wt., kg[a]	262	325	258	347	243	326
ADG, kg[b]	1.56	1.36	1.43	1.39	0.97	1.16
DM Intake, kg[c]	5.44	5.67	5.31	5.99	5.28	7.44
F:G[d]	3.43	4.17	3.71	4.31	5.47	6.45
G:F, kg/100kg[d]	29.15	23.98	26.95	23.20	18.28	15.50

[a]Treatments at each location differ (P<.01)
[b]Treatments at Dickinson and UW locations differ (P<.01)
[c]Treatments at Dickinson and Antelope locations differ (P<.05)
[d]Treatments at Dickinson and Antelope locations differ (P<.01)

Table 3. Feedlot finishing performance and carcass measurements. (Decatur County Feed Yard, Oberlin, Kansas, and UW Livestock Center, Laramie, Wyoming)

	NDSU Dickinson REC		SDSU Antelope Station		UW Beef Unit	
Item	Early[a]	Normal	Early	Normal	Early	Normal
Receiving Wt., kg[b]	254	318	255	338	243	326
Harvest Wt., kg	516	533	504	533	553	560
Days at Feed Yard, da[b]	188	129	183	133	224	150
ADG, kg	1.39	1.67	1.36	1.47	1.40	1.55
F:G[c]	5.20	5.18	5.18	5.86	6.07	6.17
G:F, kg/100kg[c]	19.23	19.31	19.31	17.06	16.47	16.21
Hot Carcass Wt., kg	326	327	319	329	334	333
Rib-eye Area, sq. cm.	78.6	82.8	78.4	80.1	74.6	78.5
Fat Depth, mm.[d]					13.75	11
Yield Grade,[d]	2.61	2.54	2.68	2.7	2.76	2.45
Quality Grade[e]	2.95	2.78	3.00	2.8	4.95	4.38
Percent Choice, %	26.4	25.7	13.9	23.5	85.7	59.1

[a]Two steers died of bloat during finishing.
[b]Treatments at each location differ (P<.01)
[c]Treatments at the Antelope location differ (P<.01)
[d]Treatments at the UW Beef Unit differ (P<.05)
[e]Treatments at the UW Beef Unit differ (P<.10)

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UTILIZATION OF FIELD PEA AND SUNFLOWER MEAL AS DIETARY SUPPLEMENTS FOR BEEF COWS

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ABSTRACT: The objectives of this study were to determine if field pea and sunflower meal can be used effectively as dietary supplements and whether energy or protein is a first-limiting nutrient for beef cows grazing stockpiled native forage in the late fall and early winter. Beef cows grazed a pasture of stockpiled predominately native range in western North Dakota from November through January in each of two years. At the end of the grazing portion of the experiment each year, all cows were combined into one group and managed similarly. Grazing treatments included a control (CON) and three supplemented groups. Supplemental treatments were chosen to supply additional energy and gradient levels of protein. Supplemental treatments were a barley-, field pea- and sunflower meal-based pellet. Dietary treatment did not affect BW change on day 14 of grazing (P>.7). Supplementation improved BW change compared to CON on days 42 (P≤.1) and 70 (P≤.05) in both years and on day 84 (P≤.01) in year 2. Overall, supplementation improved weight change during grazing by 29.6 and 27.2 kg in years 1 and 2, respectively. Body condition score (BCS) change was improved by supplementation on day 42 in year 1 (P=.08) and on day 84 in year 2 (P=.02). Under common management for 28 days post-grazing, overall BW change (P>.5) did not differ among treatments in year 1. However, in year 2 after 42 days post-grazing, supplemented cows were still 25 kg heavier than CON cows. Overall change in BCS with common post-grazing management (P=.8 and .18 in years 1 and 2, respectively) was not affected by dietary treatment. Supplemental treatment did not affect BW (P>.19) or BCS (P>.13) change in either year. Weight change in beef cows grazing stockpiled native forage from mid November to late January was improved by supplementation. Energy appeared to be a first limiting nutrient and source of supplemental energy (barley, field pea or sunflower meal) did not affect BW change.

Key Words: Stockpiled Native Forage, Supplementation, Winter Range

Introduction

Narrow profit margins in the cow/calf sector of the beef industry require careful attention to production costs and associated levels of output. Extended grazing periods have been shown to decrease winter feed costs (a major component of overall cow/calf expenses; Adams et al., 1994). Management of precalving cow weight and condition change can enhance overall reproductive efficiency (Dunn and Moss, 1992). Nutritional supplementation regimes may be necessary to manage cow weight and condition during extended fall/winter grazing periods. Dietary protein has been suggested to be the first-limiting nutrient in cattle grazing winter range. There are alternative crops and processing co-products that are higher in crude protein than typical feed grains that might be used effectively in protein supplements formulated for cattle grazing stockpiled perennial forage. Stockpiling refers to the practice of allowing forage to accumulate in the absence of grazing for use at a later time.

Objectives on this study were to (1) determine whether field pea (*Pisum sativum* L)and sunflower (*Helianthus annuus* L.) meal can be used effectively as dietary supplements for beef cows grazing stockpiled perennial forage in the late fall/early winter and (2) determine whether either energy or protein is the first-limiting nutrient for beef cows grazing stockpiled perennial forage in late fall/early winter.

Materials and Methods

Dry, pregnant beef cows grazed a pasture (116.6 ha) of stockpiled predominately native range in southwestern North Dakota from November through January in each of two years (Table 1). In each year (2001-2002 and 2002-2003), cows were randomly allotted into four groups and groups were then assigned one of four dietary treatments. Treatments included an unsupplemented control (CON) and three supplemented groups. Supplemental treatments were a barley (BAR)-, field pea (PEA)- and sunflower meal (SFM)-based pellet. Supplemental treatments were chosen to supply additional energy and gradient levels of rumen-degradable protein (Table 2). Supplements were provided to individual cows in the supplemental treatments three times a week. Supplemental intake was limited to 3.0 lb/hd per day or 7.0 lb/hd per feeding. To monitor carry-over effects, at the end of grazing in each year all cows were combined into one group and managed similarly. Cows were moved to a corn field that had been previously grazed by beef heifers and fed grass hay ad libitum. Cows remained at this facility until grazing commenced the following spring.

Cows were weighed (BW) and condition scored (BCS; Encinias and Lardy, 2000) at 14-day intervals throughout the course of the grazing period. Weight and BCS was also recorded either 28 or 42 days post-grazing in year 1 and 2, respectively.

Animal data were analyzed by year utilizing a completely random design with four treatments replicated across cows. Treatment represented a fixed effect and animal within treatment served as the experimental unit. Means were separated using a set of orthogonal contrasts.

Specific contrasts included 1) CON vs supplemental treatments, 2) BAR vs PEA and SFM and 3) PEA vs SFM.

Results and Discussion

In general, cows were heavier and in better body condition in year 1 compared to year 2 (Table 1). The seasonal stocking rate (ha per animal unit month) was greater in year 2. This resulted from lighter cows and a longer grazing period in year 2. Initial forage available for grazing was not different between years (Poland et al., 2005).

Year 1. Dietary treatment (P > .3; Table 3) did not affect BW change on day 14. On this day, cows had lost an average of 64.5 kg. Supplementation reduced BW loss compared to CON on days 42 (P = .10) and 70 (P < .01). Overall, supplementation reduced BW loss during grazing by 28.6 kg. Loss of BCS (P < .10; Table 3) was reduced by supplementation on day 42. Supplemental treatments did not affect BW (P>.4) or BCS (P>.1) changes.

Under common management for 28 days post-grazing, overall BW and BCS change did not differ among dietary treatments (P > .2; Table 4). In general during late fall and early winter, BW increased 34.0 kg and BCS decreased .3 units with 70 days of grazing and 28 days of recovery.

Year 2. Dietary treatment (P>.7; Table 4) did not affect BW change on day 14 (average gain was 34.9 kg). Supplemental treatments improved BW change on days 42 (P<.05), 70 (P<.01) and 84 (<.01). Overall, supplementation increased BW gain during grazing by 27.2 kg. Supplementation improved BCS change (P<.05) on day 84. Supplemental treatments did not affect BW (P>.15) or BCS (P>.1) changes.

Under common management for 42 day post-grazing, overall BCS change (P>.1; Table 4) was not affected by dietary treatment. However, previous supplementation improved BW change (P<.01) 25.0 kg. There were no difference among supplemental treatments in overall BW (P>.5) and BCS (P>.1) change. In general, BW increased 70.4 kg and BCS increased .9 units with 85 days of grazing and 42 days of recovery.

Despite cows starting from very different BW and BCS between the two years, BW change in beef cows grazing stockpiled perennial forages in southwestern North Dakota from mid November to late January was improved with dietary supplementation. Energy appeared to be the first limiting nutrient and source of supplemental energy (barley, field pea or sunflower meal) did not affect body weight change. Field pea and sunflower meal appear to be suitable feed ingredients in the formulation of supplements for beef cows grazing stockpiled perennial forage.

Implications

Beef cows can be managed in the late fall and early winter on stockpiled perennial forages in southwestern North Dakota and weight change during grazing can be improved with supplementation. Supplemental energy appears to be the first limiting nutrient for beef cows grazing this type of forage. Field pea and sunflower meal appear to be suitable feed ingredients in the formulation of supplements for beef cows grazing stockpiled perennial forage.

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Table 1. Initial animal and grazing information.

	Year 1	Year 2
Total number of cows[a]	21	24
Initial		
Body weight, lb	629.1 ± 64.5	509.3 ± 39.0
Body condition score[b]	6.8 ± .64	4.5 ± 1.1
Grazing dates		
Beginning	November 14	November 6
End	January 23	January 29
Total grazing days	70	85
Cow grazing days/ac[c]	5.1	7.1
Acres/cow/month[d]	6.0	4.3

[a] In year 1, there were 6 cows in the control treatment and 5 cows in each of the supplemental treatments. In year 2, all treatments had 6 cows.
[b] Estimate of body fatness (1 to 9 scale; Encinias and Lardy, 2000).
[c] Total pasture area was 288 acres
[d] One month equals 30 days.

Table 2. Composition of total digestible nutrients (TDN), crude protein (CP) and ruminally degraded crude protein (DIP) in stockpiled perennial forage, barley, field pea and sunflower meal[a].

	Forage	Barley	Field Pea	Sunflower Meal
TDN (%DM)	53	84	87	74
CP (%DM)	4.9	13	25	45
DIP (%DM)	-	10.3	19.5	34.2
DIP (%CP)	-	79	78	76

[a] Sources: NRC, 1984, 1985, 1996; Hickling, 1994; and Transtrom, et al., 2003.

Table 3. Effect of supplementation on body weight and body condition score changes in year 1.

Day of	Treatment[a]					Probability[b]		
Trial	CON	BAR	PEA	SFM	SE	1	2	3
Body weight change, lb								
14	-58.1	-59.9	-63.5	-46.3	13.34	.90	.76	.37
42	-82.6	-46.3	-61.2	-48.1	16.28	.10	.68	.57
70	-74.4	-49.9	-44.0	-43.1	10.89	.03	.63	.96
Hay28	29.5	29.0	43.1	34.5	10.02	.59	.44	.55
Body condition score[c] change								
14	-.3	-.4	-.6	-.4	.24	.62	.74	.56
42	-1.2	-.6	-1.0	-.4	.25	.08	.74	.11
70	-1.0	-.4	-1.0	-.8	.27	.37	.14	.60
Hay28	-.3	0.0	-.4	-.4	.26	.81	.23	1.00

[a] Treatments include an unsupplemented control (CON) and three supplements. Supplemental treatments were a barley (BAR)-, field pea (PEA)- and sunflower meal (SFM)-based pellet.
[b] Probability of a significant orthogonal contrast. Specific contrasts were (1) CON vs supplemental treatments, (2) BAR vs PEA and SFM, and (3) PEA vs SFM.
[c] Estimate of body fatness (1 to 9 scale; Encinias and Lardy, 2000).

Table 4. Effect of supplementation on body weight and body condition score changes in year 2.

Day of	Treatment[a]					Probability[b]		
Trial	CON	BAR	PEA	SFM	SE	1	2	3
Body weight change, lb								
14	33.6	38.1	29.9	37.2	5.03	.77	.47	.32
42	12.2	23.1	27.2	24.5	4.76	.03	.67	.69
70	20.4	41.3	42.6	43.5	4.45	.003	.72	.89
84	-14.5	11.3	18.1	8.6	5.03	.001	.73	.19
Hay42	51.7	78.9	78.5	73.0	6.94	.005	.70	.58
Body condition score[c] change								
14	.2	.3	.2	.2	.18	.79	.46	1.00
42	.5	.8	.7	.8	.30	.43	.82	.69
70	.5	1.2	1.2	.7	.32	.19	.53	.29
84	-.3	.5	.8	.2	.30	.02	1.00	.13
Hay42	.5	1.5	1.0	.7	.35	.18	.13	.50

[a] Treatments include an unsupplemented control (CON) and three supplements. Supplemental treatments were a barley (BAR)-, field pea (PEA)- and sunflower meal (SFM)-based pellet.
[b] Probability of a significant orthogonal contrast. Specific contrasts were (1) CON vs supplemental treatments, (2) BAR vs PEA and SFM, and (3) PEA vs SFM.
[c] Estimate of body fatness (1 to 9 scale; Encinias and Lardy, 2000).

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EFFICACY OF A SELF-FED SMALL SUPPLEMENT FOR PREPARTUM COWS GRAZING DORMANT PINON-JUNIPER RANGELAND

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ABSTRACT: A 3-yr study was conducted at the Corona Range and Livestock Research Center, NM to evaluate efficacy of small amounts of a self-fed protein-mineral supplement for BW and body condition score (**BCS**) maintenance in prepartum Angus and Angus-cross cows grazing dormant pinon-juniper/blue grama range. Cows were supplemented with 1) a 36% CP supplement (**CON**) composed mostly of oilseed meals fed 3 times/wk; 2) a supplement comprised of 25% feather meal, 25% blood meal, 27% minerals, 19% salt and 4% distillers dried grains (40% CP, **SMP**), or 3) manager fed cows CON at discretion (**VAR**) based on perceived environmental stress. This treatment (VAR) functioned as a negative control. Supplementation periods were 27 d (yr 1), 62 d (yr 2) or 93 d (yr 3). Across years, mean supplement consumption was 0.63, 0.23, and 0.04 kg/d for CON, SMP, and VAR. Supplementation occurred in November, December, and/or January and terminated two weeks prior to initiation of calving. Year impacted BW and BCS measures ($P < 0.01$), but no treatment X year interactions were noted ($P > 0.33$). Feeding CON or SMP had minimal impact on BW change (0.5 kg and 2.1 kg respectively), while cows fed VAR lost (-12.3kg) BW (SE ± 3.9, $P = .06$). Initial BCS of cows was similar among treatments (5.0, 4.9, and 5.0 ± 0.1 for CON, SMP, and VAR). Feeding CON or SMP resulted in minimal changes in BCS (-0.1 score) while VAR treated cows lost 0.4 BCS score ($P = 0.10$). Mean feed costs ($/cow) were 10.08, 4.70, and 0.60 for CON, SMP and VAR. In this study, cows required supplemental nutrients to maintain BW and BCS, and SMP was utilized most efficiently for this purpose. Relative to VAR, SMP efficiency was 1.1 (weight difference:consumption) while CON efficiency was 0.3. Improved efficiency resulted in substantial reductions in the cost of cow maintenance.

Key Words: beef cows, prepartum, supplementation

Introduction

Previous studies have demonstrated that low amounts of supplemental protein, particularly sources high in undegraded intake protein (**UIP**), may enhance the efficiency of nitrogen utilization (Sawyer et al., 1998; Coomer et al., 1993). Additionally, nutrient restriction increases the efficiency of nitrogen utilization in cows (Freetly and Nienaber, 1998). The use of a supplement based on small quantities of high UIP ingredients combined with salt and minerals was demonstrated to maintain ruminal function with low quality forage diets (Sawyer et al., 2000) and this same supplement exhibited controlled and consistent consumption patterns by cows grazing desert range (Stalker et al., 2002). The objective of this study was to field validate these previous findings by evaluating the efficacy of a small package size, self-fed protein supplement for maintaining body weight and body condition score (**BCS**) of gestating cows grazing dormant rangeland forage.

Materials and Methods

This study was conducted over a three-year period at New Mexico State University's Corona Range and Livestock Research Center, 12 km east of Corona, NM. Elevation at the study site is 1900 m. Annual precipitation averages 400 mm, with approximately 70% of annual precipitation occurring from May to October. Rangeland at his site is characterized as a pinon-juniper woodland, with a moderate to dense overstory of one-seed juniper (*Juniperus monosperma*) and pinon pine (*Pinus edulus*). Herbaceous vegetation is predominately blue grama (*Bouteloua gracilis*) with minor components of wolftail (*Lycurus phleoides*), sideoats grama (*Bouteloua curtipendula*), threeawn (*Aristida spp.*), sand dropseed (*Sporobulus cryptandrus*), and black grama (*Bouteloua eriopoda*) as described by Knox (1998).

Each year, gestating Angus and Angus-cross cows ranging from 2.5 to 8.5 years old were utilized in this study. Cows were stratified by breed and weight at weaning and randomly assigned to one of six replications or sub-herds, such that sub-herds contained the same proportion of Angus and crossbred cows. Each sub-herd was then randomly assigned to one of six pastures containing at least 260 ha. Treatments were then randomly assigned to each pasture, resulting in 2 sub-herd replications/treatment within each of the three years.

Treatments were supplementation strategies designed to be reflective of commonly applied practices, rather than as fixed protocols. Reflecting this approach, and due to variation in annual forage conditions and grazing constraints, the duration of the supplementation period varied by yr. In yr 1, supplements were fed for 27 d; in yr 2, 62 d, and in yr 3, 93 d. In all yrs, strategies were designed so that supplementation ended 2 wks prior to the expected initiation of parturition in the herd based on breeding season dates. Under management conditions in this study, the prepartum supplementation period ended the first wk in February each year.

A positive control strategy (**CON**) was developed based on a hand-fed, 36% CP pellet. Under this strategy, supplement was delivered to cows 3 times weekly. This strategy reflects common practice when prepartum supplementation is applied in this region. The CON supplement was composed of 57 % cottonseed meal, 10% soybean meal, 1.2 % urea , 21% wheat middlings, 9% molasses and fortified with trace minerals and Vitamin A. The CON supplement was priced at $26.46/100 kg ($240/ton). Consistent with the annual variation in forage conditions, the feeding rate for CON varied by year. When prorated to a per day feeding rate, CON was supplied at 953 g/d (Year 1), 757 g/d (Year 2), or 454 g/d (Year 3). Cows receiving CON had ad libitum access to a salt-mineral supplement.

A negative control strategy was also developed. This strategy allowed for brief and intermittent supplementation due to periods of environmental stress, such as snow cover, and is best described as variable supplementation (**VAR**). This strategy relied on managerial discretion to supply feed when required, but with the directive to minimize usage of supplemental feed. The VAR strategy utilized the same supplement formulation at the same cost as CON, and when supplied, was always fed twice weekly, prorated to 454 g/d. Cows receiving VAR were fed for the equivalent of 9.5 d in yr 1, 8 d in yr 2, and 0 d in yr 3. The very low amount of supplement input with this strategy allows it to be considered a negative control. Cows receiving VAR had ad libitum access to a salt-mineral supplement.

To meet experimental objectives, a strategy utilizing a small package size, self-fed supplement (**SMP**) was developed based on previous findings (Sawyer et al., 2000; Stalker et al., 2002). This supplement was formulated to contain 40% CP and was composed of 25% feather meal, 25% blood meal, 27% minerals, 19% salt and 4% distillers dried grains. The mineral portion of the SMP supplement was designed to provide the same level of mineral intake as the ad libitum supplement supplied to cows receiving CON and VAR treatments. The SMP supplement was priced at $35.69/100 kg ($323.75/ton). Target intake rate of this supplement was 200 g/d. Cows actually consumed 281 g/d of supplement in yr 1, 172 g/d in yr 2, and 249 g/d in yr 3. The mean intake across years for SMP (weighted by duration of supplementation period) was 230 g/d. For clarity, feeding rate, duration of supplemental feeding periods, and total consumption are shown for each supplementation strategy by year in Table 1.

Cows were weighed and body condition scores (BCS) were assigned on a 1-9 scale (1 = emaciated, 9 = obese) at weaning (October) of each year, at the initiation of the supplementation period (January, December, and November for years 1, 2 and 3, respectively), and at termination of the supplementation period (February). Feed deliveries were recorded and feed remaining (SMP) was recorded for each strategy to validate consumption rates.

Response data were analyzed as a completely randomized design with a 3 X 3 factorial treatment arrangement using General Linear Models procedures of SAS v.9 (SAS Institute, Cary, NC, USA). Year and supplementation strategy served as factors in the model. Treatments were applied per pasture, and therefore pasture was used as the experimental unit for all responses. Responses are expressed on a per cow basis for clarity. When model effects were significant ($P < 0.10$), means were separated using Fisher's Least Significant Difference.

Table 1. Feeding rate, duration of the supplementation period, and total amount of supplement fed to cows receiving different supplemental feeds during three years.

Item	CON	SMP	VAR
Year 1			
Rate, g/d	953	281	454
Duration, d	27	27	9.5
Total fed, kg	25.7	7.6	4.3
Year 2			
Rate, g/d	757	172	454
Duration, d	62	62	8
Total fed, kg	46.9	10.7	3.6
Year 3			
Rate, g/d	454	249	0
Duration, d	93	93	0
Total fed, kg	42.2	23.2	0

Results and Discussion

No significant year by supplementation strategy interactions were observed. The lack of interactions indicates that despite variation in duration and rate of supplementation among years, cows responded to these strategies consistently across years. Additionally, the lack of interaction indicates that any differential responses due to annual variation in feeding rate or supplementation duration would be wholly explained by main effect terms.

Year had significant impacts on measured responses (Table 2). Yearly differences in BW recovery from weaning to initiation of supplementation justify the variation in annual strategies. In years 1 and 2, cows gained weight from weaning until the initiation of supplementation, indicating that supplemental nutrients were not required during that time. In year 3, cows apparently lost weight from weaning to the initiation of supplementation, justifying the earlier intervention and longer duration of supplemental treatments during that year. Additionally, when pooled across supplementation strategies, cows either maintained (years 1 and 3) or lost BW (year 2) during the supplementation period. This indicates that cow nutrient requirements were not being met by forage alone, and again, that the timing of intervention was appropriate within each year. Differences in BW at the beginning of supplementation (initial BW) among years are reflected in cow body condition score at the beginning of the supplementation period.

While not a primary objective of this experiment, the observations derived from year effects regarding the suitability of supplementation strategies is potentially important. Whitson et al. (1982) clearly demonstrated that the use of protein supplements increases the financial stability of range cow-calf operations. However, purchased feed inputs are among the highest variable costs incurred by such operations, and are related to ranch profitability

(McGrann et al., 2004). The results of this study suggests that strategic implementation of supplemental feeding can successfully resolve these apparently conflicting effects. Strategic application of supplements in accordance with forage conditions and availability, rather than by calendar date, successfully mitigated BW loss. Mitigation of BW loss should reduce production variability and thus increase operational stability, and achieving this goal with minimum inputs optimizes the variable cost function.

Table 2. Year effects on measures of cow body weight and condition score.

Item	Yr 1	Yr 2	Yr 3	SE[a]	P
Body Weight Responses					
Wean BW, kg[b]	442[e]	486[f]	542[g]	17	<0.01
Initial BW, kg[c]	474[h]	573[i]	530[j]	14	<0.01
Final BW, kg	472[h]	558[i]	536[i]	14	<0.01
Change[d], kg	-2.2[h]	-15.1[i]	6.3[h]	3.9	0.01
Change, %	-0.4[e]	-2.5[f]	1.4[e]	0.8	0.02
Body Condition Responses					
Initial BCS	4.4[h]	5.4[i]	5.0[j]	0.1	<0.01
Final BCS	4.3[h]	5.4[i]	4.7[j]	0.1	<0.01
BCS change	-0.2[e]	0.0[f,h]	-0.4[g,i]	0.1	0.01

[a]n = 6
[b]Cow weight at weaning in October
[c]Cow weight at initiation of supplementation period
[d]Cow weight change (Final – Initial)
[e,f,g] Means differ, P < 0.10
[h,i,j] Means differ, P < 0.05

Table 3 depicts the effects of different supplementation strategies on cow responses across years. Cows assigned to different strategies had similar BW at weaning (P = 0.98), and gained weight from weaning until the beginning of supplementation, so that BW at the initiation of supplementation was also similar among treatments (P = 0.78). Supplementation strategy influenced weight change during the supplementation period whether expressed as absolute weight change (P = 0.06) or as a percentage of initial BW (P = 0.09). Cows receiving CON or SMP exhibited similar BW changes, neither of which were different from zero, essentially reflecting BW maintenance. Cows receiving VAR lost BW during the supplementation period. These results indicate that nutrient limitations existed during this period, and that these deficiencies were corrected by provision of either CON or SMP. Weight changes are reflected in BCS changes. Supplements affected BCS change (P = 0.10), with cows receiving CON or SMP exhibiting minimal BCS reduction during the supplementation period, while cows receiving VAR lost condition.

It is well established that BCS at parturition is related to the duration of the postpartum interval and to conception rate in beef cows (Houghton et al., 1990). In this study, cows entered the supplementation period in moderate body condition, a level that has been suggested to maintain adequate reproductive performance.. Additionally, it has been demonstrated that cows that are on a negative plane of nutrition as they approach parturition have reduced reproductive success compared to those at maintenance or on increasing planes of nutrition, even when initial BCS is similar (Selk et al., 1988; Wiltbank et al., 1962). If mitigation of BW and condition loss prepartum reduces the risk of production failures, then strategic supplements would be effective at minimizing this form of production risk.

Table 3. Body weight and body condition responses of gestating cows to different supplementation strategies.

Item	CON	SMP	VAR	SE[a]	P
Body Weight Responses					
Wean BW, kg[b]	488	492	490	17	0.98
Initial BW, kg[c]	522	521	534	14	0.78
Final BW, kg	522	523	521	14	0.99
Change[d], kg	-0.2[e]	1.8[e]	-12.6[f]	3.9	0.06
Change, %	0.1[e]	0.5[e]	-2.2[f]	0.8	0.09
Body Condition Responses					
Initial BCS	5.0	4.9	5.0	0.1	0.49
Final BCS	4.9[e]	4.9[e]	4.6[f]	0.1	0.12
BCS change	-0.1[e]	-0.1[e]	-0.4[f]	0.1	0.10
Total Feed Consumed, kg	38.2	13.7	2.6	--	--

[a]n = 6
[b]Cow weight at weaning in October
[c]Cow weight at initiation of supplementation period
[d]Cow weight change (Final – Initial)
[e,f,g] Means differ, P < 0.10

In this study, both CON and SMP were effective at maintaining cow BW and condition during late gestation. However, the supplements were used with different efficiencies. Efficiency of supplement utilization can be expressed as the difference in BW change between supplemented and unsupplemented groups (i.e., relative to VAR) per unit of supplement fed. Using this calculation, CON was used with an efficiency of 0.32 kg BW spared/kg supplement fed. SMP supplement was used with an efficiency of 1.05 kg BW spared/kg supplement consumed, a 228% increase in apparent utilization efficiency. Efficiency of these supplements can also be evaluated on the basis of quantity of CP consumed, rather than quantity of total supplement, to accommodate differences in supplement composition. On a CP basis, CON spared 0.90 kg BW/kg CP supplied; SMP spared 2.63 kg BW/kg CP supplied, a 192% difference.

These enhancements in utilization efficiency are consistent with previous reports. Sawyer et al. (1998) demonstrated that 40 g of CP/d from a blood meal:feather meal combination was as effective at promoting nitrogen retention as 160 g CP from urea or cottonseed meal. When blood meal and feather meal were combined with a salt-mineral mix (Sawyer et al., 2000), ruminal function and microbial CP production were maintained relative to 160 g CP from cottonseed meal alone, resulting in higher apparent utilization efficiency with the small package supplement. These efficiency enhancements may be a result of improved N recycling (Coomer et al., 1993), increased ruminal N scavenging (Russell and Strobel, 1987), or increased efficiency of whole body N metabolism due to nutrient restriction (Freetly and Nienaber, 1998). Regardless of the

mechanism, the results of this study demonstrate a greater utilization efficiency for the small-package supplementation strategy.

Increased utilization efficiency resulted in decreased feed costs of maintenance for cows fed SMP relative to CON despite higher per unit feed costs for SMP. Applying the unit feed costs for CON, SMP and VAR to the total consumption pooled across years results in per cow costs of $10.08, $4.70, or $0.60/cow, respectively. Because cows receiving VAR failed to maintain BW, SMP was the most economical strategy for BW maintenance in this study. This cost comparison does not include additional charges for labor and equipment that might be associated with any of the feeding strategies employed. Conceivably, application of these charges would further separate CON and SMP.

Implications

Strategic supplementation was effective for maintenance of BW and BCS in prepartum gestating cows. Use of a self-fed, small package supplement was equally effective as use of a traditional hand-fed, oilseed-based supplement. The small package supplement was used with higher efficiency and was more cost effective. Although either supplement might serve to mitigate production risk through reduced weight and condition losses, the small-package supplement was more efficacious at optimizing the cost of risk reduction.

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SEED BANDWIDTH, PLANT POPULATION AND MATURITY STAGE EFFECTS ON BARLEY FORAGE WHEN USING AIR DRILL TECHNOLOGY

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Abstract: Barley management options were evaluated by varying seed band width, population densities, and harvest endpoint under dryland conditions using commercial air drill technology. The trial was established in the spring under dryland conditions as a randomized complete block (r=6) with a factorial arrangement of treatments and was conducted for three years (2001, 2002 and 2003). Plots were 15.24 m by 3.04 m consisting of ten rows on 30.5 cm centers. Treatments consisted of cultivar ('Harrington' and 'Haybet'), bandwidth (narrow, 12.5 cm and wide, 19.3 cm), and population rate (140, 184 and 226 seeds/m^{-2}). The initial forage cut (EARLY) was taken at soft dough, while the late (LATE) cut was seven days later. Data were analyzed using the GLM procedure in SAS. Altering the bandwidth in which the seed is placed (NARROW versus WIDE) did not affect yield, plant height or forage quality (P>0.05). Nitrate-N for the EARLY harvest was reduced 15% (P = 0.0019; NARR = 0.105% and WIDE = 0.090%). LATE harvest nitrate-N was reduced 22% (NARR = 0.092% and WIDE = 0.071%). At the highest population (226 seeds/m^{-2}) DM yield was increased both for the EARLY and LATE harvest (P < 0.05). WIDE seed configuration for increasing population rates for both the EARLY and LATE harvest have a significant negative linear relationship for nitrate-N concentration (P = 0.0611 and P = 0.0378, respectively). Narrow seed configuration for increasing population rates for both the EARLY and LATE harvest have a significant quadratic relationship (P = 0.0243 and P = 0.0330, respectively). This quadratic relationship indicates that the potentially worst scenario to harvest barley forage is at the moderate seed rate in a narrow seed configuration. It appears forage quality can be altered by manipulating management options associated with seed placement and populations using air drill technology. Nitrate-N was reduced 15 and 22% by planting the seed in a wide versus a narrow row configuration. Increasing populations employing a wide bandwidth configuration decreased nitrate-N significantly as population increased, however nitrate-N levels were highest at the moderate seeding rate in the narrow row configuration.

Key words: Barley Forage, Air Drill, Population and Bandwidth

Introduction

Producers in the semi-arid Northern Great Plains are asking researchers to develop strategies that improve options for dryland barley production where rainfall levels vary drastically from year to year. Barley is currently second only to wheat in number of acres of small grain crops grown at 1.0 million acres, and constitutes approximately $94 million in gross revenue to the economy of Montana (Montana Agricultural Statistics, 2003). Barley planted solely for forage production has been increasing rapidly in recent years (Montana Agricultural Statistics, 2001, 2002, and 2003). The use of cereal annual forages (**AF**) is becoming widespread in the feedlot areas of Canada, from Medicine Hat west to Lethbridge and North to Calgary, Alberta to Saskatchewan. Cereal annual forages generate vast quantities of silage used in beef cattle rations during the finishing phase in beef production and to increase winter feed supplies and grazing opportunities in the Northern Great Plains (Entz et al,. 2002). Barley and oats are commonly the choice in these short-season areas (Jedel and Salmon, 1995).

As forages approach maturity, fiber components (ADF) increase and the digestibility of the forage decreases (Merchen, 1988). Barley had the lowest ADF, highest IVDDM and highest CP when compared to the other species (Cherney et al., 1983 and Helsel and Thomas, 1987). Different plant species differed markedly on the amounts of nitrate accumulated; oats > barley > triticale in a trial conducted near Edmonton, Alberta under similar management and harvest maturity levels (Khorasani et al., 1997). Cultivars differ in whole plant forage quality and nitrate-N accumulation. 'Haybet' had lower better NDF (55.69 and 61.04%), ADF (29.00 and 34.49%), and nitrate-N (0.18 and 0.46 nitrate-N%) levels than 'Westford' barley (Surber et al., 2001).

Investigating altering barley management inputs and evaluating those affects on barley forage are limited in the arid dryland regions of the west. Air drills are becoming more common in West and are a rapid and effective way to plant cereals. However, barley's response to seed band width and seeding rate has not been extensively explored for either grain or forage production.

Utilizing air drill technology, the objective was to evaluate barley cultivar and management options by varying seed band width, population densities, and harvest endpoint under dryland production conditions with barley harvested as forage.

Materials and Methods

The trial was conducted for three years (2001, 2002 and 2003) and was established in the spring of the year. The study was conducted at Northern Agricultural Research Center, Montana State University, Hill County south of

Havre, Montana. The plots were established in a randomized complete block with 6 replications using a research scale replica of a commercial air drill. Weed control, fertilizer rates and other agronomic management decisions were performed as normal for barley grain production in the Northern Great Plains. The treatments consisted of cultivar seed, bandwidth and population density. Cultivars tested were 'Harrington' and 'Haybet'. The bandwidths evaluated were narrow (**NARROW**, simulating traditional seed placement with a 10.2 cm sweep and a reverse seed boot spreader) and wide (**WIDE**, achieved with a 25.4 cm sweep and a wide seed boot spreader). Population densities represent recommended populations for barley harvested to be utilized as grain in the malt (**MALT**) industry, as grain in animal diets (**FEED**) industry and as a forage (**FORAGE**) fed to ruminants (140, 184 and 226 seeds/m^{-2}, respectively; Hensleigh et al., 2001). Plots were hand cut (91 cm) at ground level at the soft dough stage and then again with in the same row 7 d after the initial cutting. Samples were dried in a forced air oven at 60°C for 48 hours. Forage samples were ground to pass a 1 mm screen in a Wiley mill and evaluated for ADF, NDF (Van Soest et al., 1991) DM, CP and nitrate-N (AOAC, 2002).

Data were analyzed using the GLM procedure in SAS. Fixed effects were cultivar, bandwidth and population, with replication within year as the random term. The experiments were analyzed across years since variances were homogeneous. Least squares means were obtained for cultivar, bandwidth and population. When the F-tests indicated significant differences ($P < 0.05$) for the main effect by year interaction, the 'slice' command was used to determine simple effects. Least squares means are reported with associated standard errors. The appropriate error term was used when the main effect by year interaction was significant.

Results and Discussion

There were no significant three- or four-way interactions ($P > 0.05$). Year was a significant source of variation in most analyses ($P < 0.05$). Two way interactions with year that were significant ($P < 0.05$) were separated into simple effects with the 'slice' command of SAS.

Forage yield and quality measures for cultivar are presented in Table 1. EARLY harvest yield and plant heights were greatest for Haybet ($P < 0.05$). The percent by weight of the heads and stems were very dependent upon year. Harrington had the greatest head weight ($P = 0.0001$) in 2002, a year that was characterized as approaching normal for rainfall. In 2003, a year of severe drought, Haybet appeared to have greater weight in the head, however the yields of grain in the same trial (data not reported) averaged only 484 kg ha^{-1}. Traditionally, Harrington, an awned 'grain type', yields more than Haybet, a hooded 'forage type', when harvested for seed (Hensleigh et al., 2004). Nitrate-N was not different for the EARLY harvest. Harrington for the LATE forage harvests in 2001 and 2002 contained lower ADF and NDF. Nitrate-N was greatest in 2003 for the LATE harvested Haybet and was opposite in rank for years 2001 and 2002. Surber et al., (2001) reported cultivar differences in nitrate-N concentration in barley forages.

Altering the bandwidth in which the seed is placed (NARROW vs. WIDE) did not affect yield, plant height or forage quality ($P > 0.05$). Nitrate-N for the EARLY harvest was reduced 15% ($P = 0.0019$; NARROW = 0.105% and WIDE = 0.090%). LATE harvest nitrate-N was reduced 22% (NARROW = 0.092 and WIDE = 0.071%).

Population effects for forage yield and quality are presented in Table 2. At the highest population (226 seeds/m^{-2}) DM yield was increased both for the EARLY and LATE harvest ($P < 0.05$). In Lacombe, Alberta, three seeding rates (250, 375, and 500 seeds/m^{-2}) were evaluated by quantifying yield and quality on cereal forages of barley, oat and triticale. Little effect on forage or biomass on a land basis was observed (Juskiw, 2000). We documented a nitrate-N reduction ($P < 0.05$) for the 226 seeds/m^{-2} treatment over the lower seeding rates.

When the banding pattern x population rate interaction was investigated, it illustrated no beneficial increases in DM yield ($P > 0.05$). Two way interactions for bandwidth x population rate on forage nitrate-N concentration are illustrated in Fig. 1. WIDE seed configuration for increasing population rates for both the EARLY and LATE harvest have a significant negative linear relationship ($P = 0.0611$ and $P = 0.0378$, respectively).

Two way interaction for bandwidth x population rate on forage nitrate-N concentration is illustrated in Fig. 2. Narrow seed configuration for increasing population rates for both the EARLY and LATE harvest have a significant quadratic relationship ($P = 0.0243$ and $P = 0.0330$, respectively). This quadratic relationship indicates that the potentially worst scenario to harvest barley forage is with a moderate seed rate in a narrow bandwidth configuration; a scenario commonly employed by producers using conventional drills that have a narrow bandwidth planting style.

Implications

It appears forage quality can be altered by manipulating management options associated with seed placement and populations using air drill technology. Nitrate-N was reduced 15 and 22% by planting the seed in a wide configuration versus a narrow bandwidth configuration. Increasing populations while employing a wide row configuration decreased Nitrate-N significantly as population increased, however nitrate-N levels were highest at the moderate seeding rate in the narrow bandwidth configuration.

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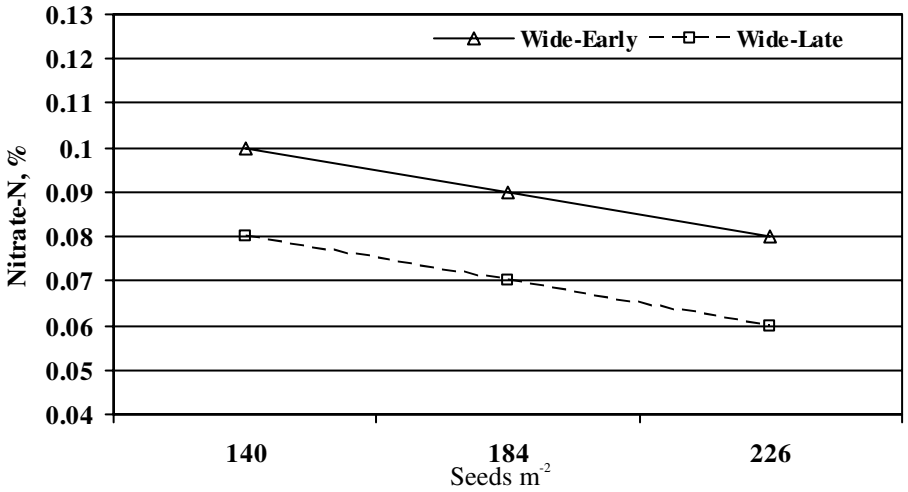

Figure 1. Two way interaction for bandwidth x population rate on forage nitrate-N concentration. Wide seed configuration for both the EARLY and LATE harvest have a significant negative linear relationship (P=0.0611 and P=0.0378, respectively).

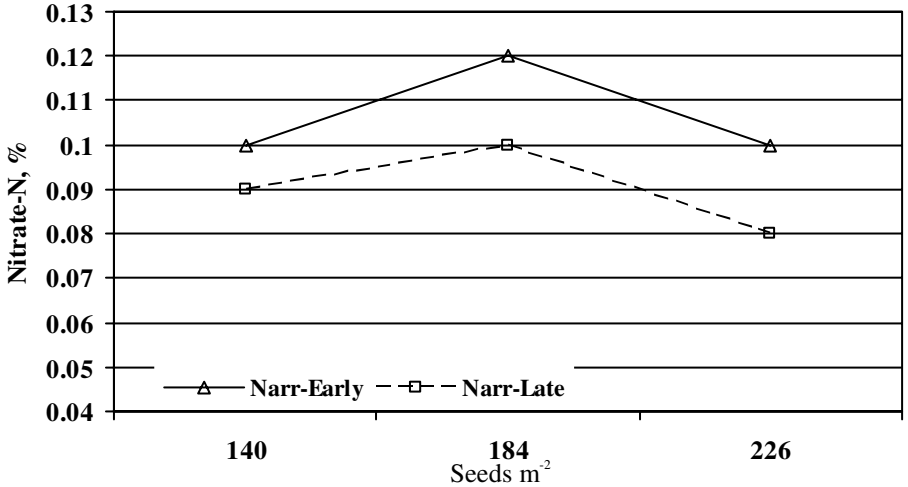

Figure 2. Two way interaction for bandwidth x population rate on forage nitrate-N concentration. Narrow seed configuration for both the EARLY and LATE harvest have a significant quadratic relationship (P=0.0243 and P=0.0330, respectively).

Table 1. Forage yield and quality when harvested at the soft dough stage and 7 d after the initial harvest by year for Harrington (HARR) and Haybet (HAY) barley cultivars. Both forage cuts were taken within row nine. Row nine was selected for harvest so all opener and packer relationships were the same for all treatments. When the F-tests indicated significant differences (P<0.05) for the main effect by year interaction, the 'slice' command was used to determine simple effects. Least squares means are reported with associated standard errors. Nutrient values are on DM basis.

Trait	2001		2002		2003		Main Effects		Year*Cultivar		Main Effects	
Cultivar	HARR	HAY	HARR	HAY	HARR	HAY	HARR	HAY	SE	P =	SE	P =
					Early cut, soft dough							
Plant Height, cm	37.8[a]	45.2[b]	58.4[a]	65.0[b]	43.9[a]	47.9[b]	46.7	52.7	0.61	0.0086	0.35	0.0001
DM Yield, kg	2804	2980	6206	6301	3394	3848	4135[a]	4376[b]	150.0	0.4464	87.0	0.0513
Percent Head, %	29.7	31.1	46.0[b]	41.3[a]	34.4[a]	36.2[b]	36.7	36.2	0.66	0.0001	0.38	0.3630
Percent Stem, %	70.3	68.9	54.0[a]	58.7[b]	65.6[b]	63.8[a]	63.3	63.8	0.66	0.0001	0.38	0.3630
DM, %	93.7[a]	94.3[b]	93.2[a]	93.7[b]	93.4	93.5	93.4	93.8	0.08	0.0004	0.04	0.0001
CP, %	16.6	16.7	10.7	10.5	14.5	14.2	13.9	13.9	0.15	0.6952	0.19	0.6276
ADF, %	25.1	27.2	26.5[a]	27.0[b]	28.8	29.2	26.8	27.8	0.26	0.0012	0.16	0.0001
NDF, %	47.7	48.3	48.2	48.7	54.2	54.3	50.0	50.4	0.35	0.6901	0.20	0.1864
Nitrate-N, %	0.08	0.10	0.05	0.04	0.16	0.15	0.09	0.10	0.006	0.1842	0.02	0.2936
					Late cut, 7 d later							
Plant Height, cm	37.8	54.0	58.2	64.7	46.2	48.6	47.4[a]	55.8[b]	5.08	0.3403	2.95	0.0467
DM Yield, kg	3400	3127	5935	6273	3410[a]	3877[b]	4488	4426	165.8	0.0194	96.2	0.6607
Percent Head, %	44.5	43.6	52.9[a]	44.1[b]	40.4	41.3	46.0	43.0	0.76	0.0001	0.44	0.0001
Percent Stem, %	55.5	56.4	47.1[a]	55.9[b]	59.5	58.7	54.0	57.0	0.76	0.0001	0.44	0.0001
DM, %	94.2[a]	94.5[b]	93.6[a]	94.0[b]	94.4	94.5	94.1	94.3	0.46	0.0009	0.02	0.0001
CP, %	12.1	12.0	10.4	9.9	13.7	14.0	12.1	12.0	0.17	0.0791	0.10	0.4101
ADF, %	26.1[a]	29.0[b]	24.8[a]	27.0[b]	26.8	27.0	25.8	27.7	0.30	0.0001	0.17	0.0001
NDF, %	49.1[a]	51.6[b]	46.9[a]	46.8[b]	52.5	52.1	49.5	51.2	0.40	0.0001	0.23	0.0001
Nitrate-N, %	0.06	0.05	0.04	0.03	0.14[a]	0.16[b]	0.08	0.08	0.006	0.0409	0.02	0.2920

[a, b] Within year and main effects cultivars lacking a common superscript differ (P<0.05).

Table 2. Forage yield and quality when harvested at the soft dough stage and 7 d after the initial harvest by year for three populations (140, 184 and 226 seeds m^{-2}). Both forage cuts were taken within row nine. Row nine was selected for harvest so all opener and packer relationships were the same for all treatments. When the F-tests indicated significant differences (P<0.05) for the main effect by year interaction, the 'slice' command was used to determine simple effects. Least squares means are reported with associated standard errors. Nutrient values are on DM basis.

Trait	2001			2002			2003			Main Effects			Year*Population		Main Effects	
Population, seeds m^{-2}	140	184	226	140	184	226	140	184	226	140	184	226	SE	P =	SE	P =
							Early cut, soft dough									
Plant height, cm	42.7	41.4	40.4	62.2	61.9	60.8	46.0	46.0	45.7	50.3	49.8	48.9	0.75	0.6924	0.43	0.0944
DM yield, kg	2598	3137	2940	6163	615	6460	3436	3525	3902	4066[a]	4266[ab]	4434[b]	183.7	0.3863	106.5	0.0530
Percent Head, %	28.4	31.6	31.1	42.9	43.1	45.0	36.3	34.5	35.2	35.9	36.3	37.1	0.81	0.0084	0.47	0.1753
Percent Stem, %	71.6	68.4	68.9	57.1	56.9	55.0	63.7	65.8	64.8	64.1	63.6	62.9	0.81	0.0084	0.47	0.1753
DM, %	94.1	94.0	94.0	93.3	93.4	93.5	93.5	93.3	93.5	93.7	93.6	93.6	0.09	0.3687	0.05	0.6375
CP, %	17.0	16.2	16.7	10.6	10.9	10.4	14.2	14.9	13.9	14.0	14.0	13.6	0.40	0.2839	0.23	0.4897
ADF, %	25.8	26.1	26.5	27.0	27.0	26.2	28.4	28.4	29.3	27.4	27.2	27.3	0.33	0.0371	0.19	0.7153
NDF, %	48.1	47.8	48.0	49.1	48.8	47.4	54.5	53.4	54.9	50.6	50.0	50.1	0.43	0.0203	0.25	0.2247
Nitrate-N, %	0.10	0.09	0.08	0.04	0.05	0.03	0.16	0.17	0.14	0.10[b]	0.10[b]	0.09[a]	0.008	0.3750	0.005	0.0154
							Late cut, 7 d later									
Plant height, cm	41.9	55.8	40.0	62.3	61.6	60.3	48.0	47.9	46.2	50.8	55.1	48.8	6.22	0.6282	3.61	0.4534
DM yield, kg	3335	3186	3269	6291	6037	7052	3517	3554	3862	4381[a]	4258[a]	4727[b]	203.2	0.1836	117.8	0.0161
Percent Head, %	46.3	44.2	41.7	49.0	46.9	49.5	42.5	39.8	40.3	45.9	43.6	43.8	0.93	0.0553	0.54	0.0048
Percent Stem, %	53.7	55.8	58.3	51.0	53.1	50.5	57.5	60.2	59.7	54.1	56.3	56.2	0.93	0.0553	0.54	0.0048
DM, %	94.4	94.4	94.	93.7	93.9	93.9	94.4	94.4	94.4	94.2	94.2	94.2	0.06	0.0319	003	0.1671
CP, %	12.4	11.9	11.8	9.8	10.6	10.1	13.8	14.0	13.7	12.0	12.2	11.9	0.21	0.0615	0.12	0.1605
ADF, %	27.4	27.6	27.7	26.1	26.0	25.6	26.6	27.0	27.0	26.7	26.9	26.8	0.37	0.8330	0.21	0.8636
NDF, %	49.6	50.0	51.4	48.7	48.5	47.9	51.7	52.5	52.7	50.0	50.3	50.7	0.49	0.0912	0.28	0.2717
Nitrate-N, %	0.07	0.05	0.05	0.03	0.05	0.03	0.16	0.16	0.14	0.09[b]	0.08[b]	0.07[a]	0.032	0.0553	0.004	0.0102

[a, b, c] Within year and main effects population rates lacking a common superscript differ (P<0.05).

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EFFECTS OF FLUNIXIN MEGLUMINE ON PREGNANCY ESTABLISHMENT IN BEEF CATTLE[1]

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ABSTRACT: Our objective was to determine effects of a single injection of the prostaglandin inhibitor Flunixin Meglumine (FM; 1.1 mg/kg BW, i.m.) approximately 13 d after AI on pregnancy establishment. Three experiments were conducted using estrus-synchronized heifers and cows. Technicians and AI sires were equally represented across treatments within locations and experiments. Bulls were introduced following FM treatment (approximately 13 d after AI). Pregnancy to AI was diagnosed 28 to 50 d after AI using ultrasonography. In Exp 1, beef heifers (n = 1,221) were divided within five locations to receive FM or no further treatment (Control). At insemination, heifers were divided into two similar pastures or pens and approximately 13 d later, one group of heifers within each location was worked through an animal handling facility to administer FM treatment. Location had no effect (P > 0.10) on AI pregnancy rates, so data were pooled. Pregnancy rates to AI were reduced (P < 0.025) among heifers receiving FM (65%) compared to control heifers (71%). In Exp 2, cows (n = 719) were assigned within two locations to receive FM or no further treatment (Control) 13 d after AI. At insemination, Control and FM cows were divided into separate pastures and only FM cows were handled after AI. Pregnancy rates differed by location (P < 0.01), but there was no location by treatment interaction (P > 0.10) so data were pooled. Pregnancy rates to AI did not differ (P > 0.10) between FM (57%) and Control cows (59%). In Exp 3, heifers (n = 247) and cows (n = 335) from one location were assigned at AI to receive FM or Control treatment approximately 13 d later. In Exp. 3, all cows and heifers were handled through a working facility but only half of each age group received FM treatment. Pregnancy rates to AI between FM (45%) and Control cows (42%) or FM (56%) and Control (55%) heifers were not different (P > 0.10). We conclude FM administration at the current dosage of 1.1 mg/kg BW approximately 13 d after AI did not improve pregnancy establishment in beef cows and heifers and that the additional stress of handling heifers at this time may decrease pregnancy establishment.

[1] Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana AES, 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.

Key Words: Pregnancy, Cattle, Flunixin Meglumine

Introduction

It is estimated that fertilization is successful in 90 to 95% of cows that are bred regardless of whether this mating is by natural service or AI. However, conception rates to a single service are generally less than 65 to 75%, suggesting that embryonic loss occurs in 15 to 30% of females. This loss has been characterized (Hanly, 1961), but is difficult to measure because the most reliable means of pregnancy diagnosis (ultrasound visualization of a fetal heartbeat) is not discernable until approximately d 27 after breeding. It is hypothesized that the majority of embryonic loss occurs because the developing embryo produces insufficient interferon tau to signal its presence around day 14 following breeding, resulting in failure of maternal recognition of pregnancy (Bazer et al., 1991). Without sufficient interferon tau, the uterus produces prostaglandin $F_{2\alpha}$ (PGF), which causes corpus luteum regression and loss of progesterone secretion, allowing the female to return to estrus (Binelli et al., 2001; Thatcher et al., 2001).

Flunixin Meglumine (FM) is a non-steroidal anti-inflammatory compound that inhibits PGF synthesis. Administration of FM at the time of transportation stress 12 to 15 days after AI increased pregnancy rates by 15% over transported cows not receiving FM and by 8% over non-transported controls (Merrill et al., 2003). Merrill et al. (2003) reported no differences in concentration of the metabolite of PGF (PGFM) in serum collected from cows before or after a 4 hr trucking stress (± FM) or control treatment. In a subsequent study (Merrill et al., 2004), heifers and cows that received FM 14 d after AI (± trucking stress) had higher AI pregnancy rates than heifers and cows receiving no FM (± trucking stress). The objective of this study was to determine effects of a single injection of the PGF inhibitor Flunixin Meglumine approximately 13 d after AI on pregnancy establishment in the absence of transportation stress.

Materials and Methods

In Exp. 1, 1,221 predominantly Angus heifers were divided within five locations to receive FM or no further treatment (Control). At insemination, heifers were divided into two similar pastures or pens and

approximately 13 d later, one group of heifers within each location was worked through an animal handling facility to administer FM (1.1 mg/kg BW, i.m.) treatment. Immediately after FM, these heifers were placed into the same pasture as control heifers and bulls were introduced for the remainder of the breeding season. Pregnancy to AI was diagnosed among heifers approximately 35 d after AI using transrectal ultrasonography with a 5MHz probe.

In Exp. 2, Angus cross cows (n = 719) within two locations received FM or Control treatment as described in Exp. 1. At insemination, Control and FM cows were divided into separate pastures and only FM cows were handled after AI. Immediately after FM, these cows were placed into the same pasture as control cows and bulls were introduced for the remainder of the breeding season. Pregnancy to AI was diagnosed approximately 47 d after AI using transrectal ultrasonography with a 5MHz probe.

In Exp. 3, heifers (n = 247) and cows (n = 335) from one location were assigned at AI to receive FM or Control treatment approximately 13 d later. The genetic composition of cows and about one-half of the heifers in this experiment was CGC Composite (½ Red Angus, ¼ Charolais, ¼ Tarentaise), while the remaining heifers were predominantly Angus. All cows and heifers were handled through a working facility but only half of each age group received FM treatment. Bulls were not introduced into either age group of cows until after d 13. Pregnancy was diagnosed approximately 29 d after AI using transrectal ultrasonography with a 5MHz probe.

Statistical Analysis. Factors affecting pregnancy rates in Exp. 1 were evaluated using logistic regression in SAS (SAS Inst. Inc., Cary, NC). The initial model included fixed effects of treatment and location, interval from AI to day of FM (one half day increments) as a continuous variable, and the interactions of treatment by time interval and treatment by location. Factors not affecting pregnancy rate (P > 0.2) were sequentially removed from the model using a step-down approach. Inseminators and AI sires were not consistent across locations, but each was approximately equal across treatments within location. Chi Square analyses were used to compare pregnancy rates between FM and control groups from Experiments 2 and 3.

Results and Discussion

In Exp. 1, pregnancy rates to AI were reduced (P < 0.025) among heifers that were worked through a chute and given FM (65%) compared to Control heifers (71%; Table 1). Pregnancy rates were numerically higher for Control heifers compared to FM heifers across all locations (Table 1). Because the interval from AI to FM treatment varied based on interval to estrus following synchronization, we evaluated the effect of interval from AI to FM on pregnancy rate. Interval from AI to FM had no effect (P > 0.10) on pregnancy rate to AI (data not shown). Others have reported increased pregnancy rates when FM was administered to cattle after AI (Merrill et al., 2003; 2004) or at the time of embryo transfer (Schrick, et al., 2001; Purcell et al., 2005). However, controls in those studies were also worked throough a chute, in contrast to Exp. 1 where all heifers were not handled and exposed to stress after AI.

Table 1. Pregnancy rate to AI for heifers in Exp. 1 that received Flunixin Meglumine (FM) or no further treatment (Control) approximately 13 d after AI

	AI pregnancy rate, no. pregnant/total no. (%)		
Location	FM	Control	Combined
B	51/74 (69)	57/74 (77)	108/148 (73)
P	42/62 (68)	51/68 (75)	93/130 (72)
R	119/193 (62)	132/198 (67)	251/391 (64)
S	51/74 (69)	58/77 (75)	109/151 (73)
W	130/202 (64)	143/199 (72)	273/401 (68)
Total	393/604 (65)[a]	441/617 (71)[b]	834/1221 (68)

[a,b] Numbers within a row with different superscripts differ ($P < 0.025$).

In Exp. 2, pregnancy rates to AI did not differ (P > 0.10) between FM (57%) and Control cows (58%). We anticipated an increase in pregnancy rate among cows based on earlier studies (Schrick, et al., 2001; Merrill et al., 2004; Purcell et al., 2005). In contrast to Exp 1, where pregnancy rates were reduced in heifers that were processed through a chute and given FM when compared to non-disturbed controls, this treatment did not appear to adversely affect pregnancy in cows. Thus, it is possible that heifers may have been more sensitive than cows to either the stress of handling and (or) negative effects of FM injection, or that FM was effective at alleviating the stress of handling in cows but not heifers.

Table 2. Pregnancy rate to AI for cows in Exp. 2 that received Flunixin Meglumine (FM) or no further treatment (Control) approximately 13 d after AI

	AI pregnancy rate, no. pregnant/total no. (%)		
Location	FM	Control	Combined
P	65/143 (45)	57/126 (45)	122/269 (45)[a]
W	152/236 (64)	141/213 (66)	293/449 (65)[b]
Total	217/379 (57)	198/339 (58)	415/718 (58)

[a,b] Numbers within a column with different superscripts differ ($P < 0.01$).

In Exp. 3, pregnancy rates to AI did not differ (P > 0.10) between FM (45%) and Control cows (42%) or FM (56%) and Control (55%) heifers. The design of Exp. 3 differed from Exp. 1 and 2 in that all heifers and cows were gathered and processed through a working facility in Exp. 3, but only the FM females were processed in Exp. 1 and 2.

When the results are considered from the 3 experiments, a logical conclusion is that processing of heifers through a chute appears to cause sufficient stress to reduce pregnancy rate (as observed in FM treated heifers in Exp 1). However, similar responses were not observed in cows (Exp. 2), and treatment of heifers with a single i.m. injection of 1.1 mg FM/kg BW was not affective at alleviating the negative affects of processing in heifers because no benefit of FM was observed in Exp 3, where both Control and FM were processed. Other researchers have reported that stress from transportation at critical time periods following breeding can affect

pregnancy establishment (Harrington et al., 1995). Merrill et al. (2004) reported that cortisol concentration in serum collected before transportation from heifers was higher than that of cows. Transportation for 2.5 to 3 h resulted in an approximate two-fold increase in cortisol concentration among cows, but very little increase in cortisol concentration among heifers. Thus, it would appear gathering and handling may be perceived as a greater stressor to heifers than to cows, which may have become more accustomed to handling stress. Merrill et al. (2004) also reported that FM treated heifers and cows had higher pregnancy rates to AI than heifers and cows that did not receive FM regardless of whether they received transportation stress. Serum concentration of PGFM decreased after FM treatment of these cows and heifers (data unpublished). Serum collected from heifers before transportation or FM treatment had higher concentrations of PGFM than cows, and FM resulted in a sharper decrease in PGFM concentrations among transportation stressed heifers. Associations between PGFM and cortisol in heifers and cows may indicate one mechanism by which stress occurring around the time of maternal recognition of pregnancy may decrease pregnancy rates. Based on differences in response to FM after AI in the present study, a likely interpretation would be that FM administration may decrease embryonic loss among cows and heifers experiencing unavoidable stress by suppressing PGF concentrations in the bloodstream. However at the dosage evaluated in the present studies, a single administration of FM cannot improve pregnancy establishment above that of non-stressed females.

Implications

During maternal recognition of pregnancy, the embryo must prevent PGF release by the uterus in order to survive. Subjecting cattle to stressors, such as handling stress, may be sufficient in some females to interfere with embryonic inhibition of PGF release that signals maternal recognition of pregnancy. Experiments reported here provide evidence that handling stress may interfere with this process more in heifers than cows. A single injection of Flunixin Meglumine (1.1 mg/kg BW, i.m.) was inadequate to overcome the impacts of stress.

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Effects of Protein and Energy Feeding on Ovine Oocyte Production and Developmental Capacity

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SUMMARY

A study was conducted to determine the effects of protein and energy on oocyte production in ewes. Eighty-one whiteface ewes were randomly divided into three feeding treatments, and penned in groups of three, which served as the experimental unit. Ewes were placed on one of three feeding treatments, wheat hay (maintenance), mixed grain with added protein, or mixed grain with added energy. Ewes were fed for 35 d on their respective feed treatments then harvested and ovaries removed, trimmed, weighed, lacerated and rinsed with TL Hepes. Recovered oocytes were graded and matured in the incubator for 24 h in 5% CO_2 at 38.5°C. Following incubation, oocytes were removed, rinsed and activated to begin development. Oocytes were incubated in a blood gas mixture for 7 d and evaluated at d 4 and d 7 for cleavage rates and morula formation. Results yielded no difference ($P > 0.05$) among feeding treatments with respect to ovarian weights, oocyte numbers, quality scores, or developmental rates.
Key Words: Sheep, Oocytes, Ovaries, Protein, Energy

INTRODUCTION

Reproductive capabilities of livestock have long been associated with their nutritional state. The methods in which nutrition, genetics, health, and reproduction correlate together are not well understood (Ayalon, 1978). Animals are placed into a negative energy balance when they do not receive enough energy or protein in their diet to meet the demands of energy expenditure for locomotion, milk production, reproduction, and maintenance (Dunn and Moss, 1992). Once an animal cannot meet its maintenance needs it begins to falter in other areas such as reproduction in order to maintain its body functions to survive.

Research has shown increased amounts of protein in the diet of dairy cows can have a detrimental effect on their reproductive success (Jordan and Swanson, 1979). Elrod and Butler (1993) found a decrease in the rate of pregnancy from 82% in the normal feeding group to 61% in the group of heifers fed high levels of protein. Protein excesses have continually shown a deleterious effect towards reproductive performance. Energy feeding has also been shown to affect reproductive traits in cattle. Butler and Smith (1989) state that energy can be a limiting factor on reproductive performance in postpartum cows. Wiltbank et al. (1965) stated energy limitations could adversely affect reproductive success of beef cattle more than protein. However, an increase in dietary energy seems to enhance reproduction while protein inhibits reproductive ability.

Therefore, this study was designed to evaluate the effects of protein and energy on oocyte production, quality and developmental capacity in white-face ewes.

MATERIALS AND METHODS

Animals and Dietary Treatments

Eighty-one whiteface ewes were randomly divided into three groups of 27. Each of the groups were then subdivided into nine groups of three. Each subgroup of three formed an experimental unit that was assigned to either a mixed grain diet containing more available energy (Treatment 1), a mixed grain diet containing more protein (Treatment 2), or a maintenance diet of wheat hay (Treatment 3) intended to meet only maintenance needs (Table 1). Ewes were fed at a rate of 2.5% of body weight to meet the NRC recommended amount of total digestible nutrients for maintenance. One ewe died and the feeding ratio was adjusted accordingly for that pen. Treatment 1 consisted of a higher amount of corn (19.6% vs. 9.7%) to add energy to the diet, than the protein diet, which had a higher level of cottonseed meal (11.8% vs. 3.1%), to ad protein to the diet.

Before the ewes were assigned to their dietary trial, they were fed a diet of strictly wheat hay that meets the NRC requirements for maintenance to allow them to adjust to the environment (adjustment). For purposes of time management, the three groups of ewes were staggered one week apart when placed on their respective diets. Three subgroups, consisting of three ewes each from each feeding treatment, were placed on their dietary rations. Due to the amount of time involved in the harvesting of the ewes and the collection and processing of their ovaries, it was necessary to divide the overall feeding, collection, and processing into thirds. Therefore, the first subgroup was on the adjustment diet for two weeks, the second group for three weeks, and the third group for four weeks. Once assigned to their feed trial period, all ewes stayed on their designated feed for five weeks.

Ovary Retrieval

Upon the conclusion of the five-week feeding trial, each set of ewes was taken to Ranchers' Lamb of Texas Inc. (San Angelo, Texas) for harvest. Ovaries from each ewe were removed by manually pinching them off the reproductive tract. They were immediately placed in phosphate buffered saline for transportation to the

laboratory for processing. The ovaries were trimmed of excess tissue and a weight was taken on a per pen basis.

Cumulus Oocyte Complexes Retrieval

Protocols used for cumulus oocyte complexes (COC) retrieval, oocyte maturation, storage and culture, were provided by Ovagenix Laboratories (East 37th Street San Angelo, Texas 76903). Ovaries were sliced with a scalpel blade and rinsed with TL Hepes, modified Tyrode's medium; with 0.03% bovine serum albumin fraction V (BSA), commonly used for most embryo and oocyte holding and culture media. The ovaries were then chopped and placed in a 50 ml conical tube (Fisher Scientific, Houston, TX) with 10 mls of TL Hepes, shaken, then drained and rinsed into another 100 ml petri dish. The COC's were removed from the original dishes and placed in a clean dish of TL Hepes for rinsing and sorting. Cumulus oocyte complexes were counted into a third dish for a third wash and grouped according to quality.

Oocyte Grading

Quality scores were based on those of Deloose et al. (1989) and Tripp et al. (1999). Scores ranged from A (excellent) to D (poor) based on cumulus orientation and ooplasm regularity. Category A consisted of oocytes that are surrounded by at least 3 layers of compact cumulus cells and contained a normal cytoplasm. Cumulus oocyte complexes scoring a B were surrounded by 2 layers of compact cumulus cells and held a normal cytoplasm. Category C contained oocytes surrounded by 1 layer of compact cumulus cells and contained a normal cytoplasm. Category D included COC's with less than 1 layer of cumulus cells or an unstable layer of cells with an abnormal cytoplasm. Normal cytoplasms appeared smooth and complete in structure without defects. Abnormal cytoplasms had color blotches and voided areas. The COC's scoring A and B were grouped together for maturation, culture, and overall assessment. The COC's scoring C were kept alone and the D scoring groups were disposed of as it was highly unlikely that the D groups would be capable of maturation (Deloose et al. 1989).

Oocyte Maturation

The COC's were placed in a maturation medium containing 88% M199 with Earle's salts (Gibco), 0.5% luteinizing hormone (LH), 0.5% recombinant bovine follicle stimulating hormone (bFSH), 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin and placed in incubation with 5% CO_2 at 38.5˚C for 24 h. The M199 is a complex cell culture medium used as the base medium for oocyte maturation and embryo culture and Earle's salts is bicarbonate buffered and must be used in an atmosphere of 5% CO_2 to maintain proper pH (Ovagenix Laboratories). Luteinizing hormone, bFSH, and FBS were used in oocyte maturation. This procedure was done to mature the COC's relatively close to the same developmental stages and equilibrate them into the new environment outside of the follicle (Ovagenix Laboratories).

Oocyte Rinsing and Storage

After 24 h of incubation, the matured oocytes were removed and placed in 1 ml of TL Hepes in a 15 ml conical tube. The oocytes were vortexed at high speed for 2 min 15 s to remove cumulus cells and reveal the actual oocyte. The oocytes were rinsed from the conical tube into a 60 mm petri dish with TL Hepes. The oocytes were rinsed using a multiple step activation protocol. First, they were rinsed in a combination of 5% ionomycin in TL Hepes for four min to disinfect them from any outside contaminants. The lights were turned down to a minimum and the ionomycin was kept covered at all possible times since it is light sensitive. Second, the oocytes were rinsed in a wash of TL Hepes for four min to remove ionomycin debris. Third, they were rinsed in a wash of M199 with 10% FBS and 10% 200 mM dimethlyaminopurine (DMAP; Genetic Savings and Clone, Bryan, TX) to remove all other liquid associates. Finally, they were stored in a fresh mixture of M199 with 10% FBS and 10% 200 mM DMAP for 5 h at 38.5°C with a 5% CO_2 atmosphere. DMAP was added to the medium to maintain oocytes in a state of meiotic arrest once they have been removed from the follicle. The addition of DMAP allowed the oocytes to be incubated at a fixed point in development without advancement beyond the stage of initiation. This allowed time for the oocytes to re-equilibrate with the holding environment before they were activated for culture.

Oocyte Culture

At the completion of 5 h, the oocytes were removed from the incubator, then rinsed and stored in a solution called Barc's (Genetic Savings and Clone, Bryan, TX) designed to cause the oocytes to undergo cleavage and divide as if they had been fertilized and become embryos. The oocytes were cultured for 7 d at 38.5°C in a medical grade, blood gas mixture containing 90% nitrogen, 5% carbon dioxide, and 5% oxygen (Airgas, San Angelo, TX) to enhance tissue development and growth. After 4 d of culture, the oocytes were removed and evaluated for cleavage rates. After the oocytes were evaluated and the findings recorded, they were placed back into the gas mixture and cultured for the remaining 3 d. Once the oocyte completed the full 7 d culture period, they were removed and assessed for morula formations, and blastocyst development. Oocytes evolving to blastocyst were considered fully developed because that is as far as an oocyte can transform without the assistance of DNA from sperm.

Data Collection and Procedures

Collection and handling procedures of ovaries and oocytes were similar to the methods of Rho et al. (2001) and Khurana and Niemann (2000). Body weights were taken prior to the initiation of each groups' feeding trial and after the completion of each trial before harvest. Means were calculated and recorded according to experimental units and feed groups. Totals were recorded for ovarian mass, oocyte production, oocyte grades, and oocyte development for each of the treatment groups.

Statistical Analysis

Pen of three ewes was considered the experimental unit and day of harvest was included in the model. Body

weight, ovarian weight, oocyte numbers (total and per classification), percent cleavage rates and development rates (morula and blastocyst) were analyzed using the General Linear Models of SAS (SAS Inst., Inc., Cary, NC.). Duncan's Least Significant Difference was used to separate means. Treatments were considered different at $P < 0$ .05.

RESULTS AND DISCUSSION

Animal Performance

No difference ($P > 0.05$) in weight gain or loss among the different treatment groups in relation to oocyte production. Neither ovarian weights, oocyte production, oocyte grades, nor oocyte development were affected ($P > 0.05$) by weight gain or loss of the ewes.

Dietary Treatments

Feed analysis were performed by Dairy One DHI Forage Testing Laboratory and the results were: treatment 1 contained 19.6% corn grain on a DM basis, where treatment 2 contained only 9.7% corn grain on a DM basis. Treatment 3's energy availability was derived from the nutritional value of the wheat hay with no supplementation (Table 1). After analysis, the quality of the hay was higher than anticipated. Thus, the maintenance diet was not present, but two diets had protein levels above those reported for maintenance. The differences were then forage versus mixed grain.

Ovarian Weights, Oocyte Totals, Grade Percentages, and Development

No differences ($P > 0.05$) in ovarian weights, oocyte totals, oocyte grades (Table 2), or blastocyst development were seen among treatment groups (Table 3). No differences were found that would indicate nutritional levels: 1) affected the way the ovary grows due to follicular or tissue development, 2) caused the oocyte to produce more primary, secondary, or tertiary follicles, 3) resulted in a higher or lower abundance of retrievable oocytes, 4) produced varying amounts of A, B, C, or D quality oocytes, 5) influenced the developmental capacity of the oocytes to reach the blastocyst stage.

One area of interest that was not approached in this study was the feeding of different quantities and the effect that it has on ovary production. Papadopoulos et al. (2000) conducted a study where they fed grass meal to sheep at either 2.0 times maintenance energy requirements (MER) or 0.5 times MER. They found that ewes on the 0.5 MER diet produced less overall follicles than the ewes on the 2.0 MER diet. Despite these findings no difference in the rate of oocyte retrieval due to feeding amounts was observed. Animals on the 2.0 MER did have a lower cleavage rate than those on the 0.5 MER diet however, no differences in blastocyst rate, blastocyst-hatching rate, or blastocysts cell number were seen when expressed as percentage of cleaved oocytes. The development of more follicles in their study could affect the weights of the ovaries whereas in our study, the weights were unaffected by the three feeding groups. The altering of the amount of the grain diets and forage diet could have shown a difference in the weights of ovaries retrieved due to follicle production. Similar to these results, our study did not show a difference in the total number of oocytes derived from the three treatment groups. This evidence suggests that neither feeding amounts, nor the type of feed affects the total oocyte production of the ovary. Papadopolous et al. (2000) concurs with the current study that neither different feeding amounts, nor nutritional amounts or types affect the development of oocytes after cleavage occurs. Callaghan et al. (2000) also supports these findings. When crossbred ewes were assigned to one of three diets and fed levels of 0.5, 1.0, or 2.0 times maintenance energy requirements, no difference in oocyte morphology was found associated with the feeding trials. Another study corresponding to these, was conducted by Nolan et al. (1998). Heifers were fed an *ad libitum* or restricted diet of grass silage/concentrate at a 10:1 ratio. These feeding treatments had no apparent effect on oocyte grades or oocyte development in an *in vitro* setting. Results appear to be conclusive that feeding amounts do not effect ($P > 0.05$) the quality and development of oocytes, but do affect the ovulation rates as expressed by Henniawati and Fletcher (1986) in an experiment with Indonesian sheep and goats. Sheep and goats were placed on either a maintenance level or a supramaintenence level of nutrition containing elephant grass and a supplemental commercial concentrate. The supramaintenece diet showed a strong improvement of ovulation over the maintenance diet. Research has shown that ovulation rates and follicle formation can be affected by feeding amounts to allow for an area of improvement in reproduction.

Other research supports the evidence that differences in ewes offered half maintenance energy requirement diets vs. ewes offered double the maintenance energy requirements is insignificant in regards to oocyte morphology (Boland et al. 2001). However, other evidence suggests that a relation between the *in vitro* development of oocytes from harvested heifers that were restricted from energy intake prior to slaughter (McEvoy et al., 1997). McEvoy et al. (1997) also found that the blastocyst yields from heifers on low energy intake rather than high-energy intake were enhanced by the nutritional diets. Nolan et al. (1998) also supports these results in their study with enhancement of oocytes collected trans-vaginally to blastocyst development *in vitro* from heifers restricted of dietary intake. These research studies further emphasize the role that nutrition plays on reproduction is very important. Enough evidence exists to suggest a relationship exists between nutrition and reproduction at the level of the oocyte.

Feeding different types of diets, forages or concentrates, may be capable of producing different outcomes in regards to oocyte production. An experiment was conducted on heifers that were fed either a diet of barley concentrate or a diet of a citrus/beet pulp mixture. Both feeds were designed to contain 14% crude protein. Heifers on the citrus/beet pulp diet produced more freezable and transferable embryos than heifers on the barley diet. However, the numbers of retrieved embryos with lower grades, and unfertilized ova were not affected by concentrate type (Yaakub et al., 1998). This study did not take into account different amounts of nutritional

protein in the diet, or energy levels; nor did it evaluate the results of the diets used on the production and development of oocytes. The information provided does give an implication that a different effect was found on the production of embryos according to the feed types. This insinuates that a possible impact on the ovaries or the oocytes before the time of conception. It is possible a greater number of quality oocytes were produced that led to a larger amount of quality grade embryos from the feeding of citrus/beet pulp as compared to the feeding of barley concentrate. This would give hope to the assumption that the production and development of oocytes can be affected by the influence of the type of feed and the nutritional components of the feed.

An abundance of data exists revealing connections with reproduction and nutrition at the level of the ovary and the oocyte. While some of the evidence is contradictory, it all shows a connection in one way or another. Whether it is the effect of nutrient deficiencies, feeding abundances, or different types of feeds involved, many of these studies show an effect on the production of the ovary and the oocyte. Some of the studies show increases in follicular production and enhanced oocyte developmental capacities, and others provide information contradicting these findings showing decreases in follicular production and oocyte developmental capacities. Several of the studies indicated decreases in the nutritional feeding of animals causes a positive outcome with ovarian production and some insist that enhanced feeding is required to reach the same effects. A few of the studies even offer the explanation that energy is beneficial to reproductive success and protein is deleterious to reproductive success. It is apparent that the dynamics of nutritional effects on reproduction are still not fully understood. So many of the studies offer contradictory findings that a definite need for further investigation in the search for answers regarding nutritional effects on reproductive success exists.

CONCLUSIONS

The primary goal of this study was to determine if added protein or energy to a maintenance diet would increase oocyte production rates, oocyte quality or developmental capacity in mature ewes, therefore enhancing the reproductive rate and success of sheep. This study did not take into account physiological characteristics or digestibility factors of different types of feeds. It also did not address the issues of nutritional stress from limit feeding or the effects of over feeding. These are areas that should be further explored as a large amount of research exists that indicating these areas have an effect on reproduction in one way or another.

Based on the literature reviewed, we hypothesized an increase in protein would have detrimental affects on oocyte production through decreased numbers, quality, and survivability. Also, that energy increases should have shown beneficial results in the respective fields of oocyte production. This hypothesis was not proven in this study. We did not see differences in any of the respective areas in association to the diets used.

Results of this study indicate that increased amounts of protein in a ewe's diet will not increase oocyte production or developmental capacity thus not having an effect on conception rate. Therefore, it may prove more economically efficient for producers to feed energy instead of protein to ewes during the breeding season since energy is more cost efficient than protein. Also, researchers in the field of oocytes may not need to account for differences in nutritional values of feeds when evaluating the developmental capacities of oocytes from ewes when maintenance requirements are met by the diet.

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Table 1. Ingredients and nutritional analysis, DM basis, of dietary treatments fed to ewes at 2.5% of body weight per day[a]

	Treatment[b]		
Ingredients, %	1	2	3
Corn Grain	19.6	9.7	0.0
Soybean Hulls	30.4	30.2	0.0
Sheep Premix	2.7	2.7	0.0
Cane Molasses	4.2	4.2	0.0
Cotton Hulls	40.0	41.3	0.0
Cotton Seed Meal	3.1	11.8	0.0
Wheat Hay	0.0	0.0	100.0
Nutrients, %			
Crude Protein	12.1	15.9	19.6
Total Digestible Nutrients	71.0	72.0	59.0
Acid Detergent Fiber	42.4	43.1	29.7
Neutral Detergent Fiber	51.8	48.6	51.6

[a] Randomized complete block design with pen of three ewes serving as the experimental unit
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

Table 2: Ovarian weights, oocyte totals, and oocyte grade percentages from ewes offered three dietary treatments.[a]

	Treatments[b]			
	1	2	3	SE
Ovarian weight, g	10.76	9.72	10.42	0.476
Oocyte totals, g	39.78	47.11	55.00	6.968
Percent A/B	41.56	46.89	38.43	3.68
Percent C	19.27	20.15	21.51	1.73
Percent D	39.56	32.95	36.86	3.15

[a] Randomized complete block design with pen of three ewes serving as the experimental unit.
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

Table 3. Percentages of oocyte cleavages, morulas, and blastocyst formations from ewes offered three dietary treatments.[a]

	Treatments[b]			
Item, %	1	2	3	SE
A/B Cleavage	92.75	91.42	81.88	4.84
A/B Blastocysts	60.60	57.29	51.82	6.60
A/B Morulas	8.35	11.62	9.71	2.54
C Cleavage	72.09	80.69	73.21	7.58
C Blastocysts	41.54	37.00	21.62	8.05
C Morulas	10.41	19.85	10.27	6.26

[a] Randomized complete block design with pen of three ewes serving as the experimental unit
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

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UTILIZATION OF WHOLE SOYBEANS OR CORN MILLING CO-PRODUCTS IN BEEF HEIFER DEVELOPMENT DIETS

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ABSTRACT: Three experiments were conducted utilizing soybeans (SB) and either dried distillers grains (DDG) or wet corn gluten feed (WCGF) in heifer development diets. In Exp. 1 and 2, spring-born heifers (n=104 and n=100, respectively) were fed diets containing 1.25 kg (DM) SB or 2.0 kg WCGF (Exp. 1) and 1.25 kg SB or 1.25 kg DDG (Exp. 2). Treatment diets were initiated at 10 and 6 months of age for Exp. 1 and 2, respectively. Heifers were synchronized with MGA/$PGF_{2\alpha}$, artificially inseminated, and exposed to bulls for approximately 60 d beginning 10 d after the last AI. Pregnancy to AI was determined by ultrasound 45 d after the last AI. Experiment 3 was conducted over two years with 100 summer-born heifers (n=50 each year). Heifers were supplemented with either 1.23 kg SB and 0.40 kg corn or 1.65 kg DDG during the development period, and synchronized with $PGF_{2\alpha}$ at the end of the feeding period. Dominant follicles (DF) were measured and aspirated using an ultrasound-guided probe. Heifers were exposed to bulls 14 d after aspiration for 45 d. Heifers fed SB in Exp. 1 had a lower ($P < 0.05$) synchronization rate (81 vs. 96%) and delayed ($P = 0.05$) estrous response to synchronization (3.2 vs. 2.9 d after $PGF_{2\alpha}$). There were no differences ($P > 0.10$) in AI conception (76.5 and 68.5%), AI pregnancy (67 and 59%), or final pregnancy rates (92 and 90%) in Exp. 1 or 2, respectively. There was no difference ($P > 0.05$) in follicle characteristics or pregnancy rates (88%) in Exp 3. The reason for lower synchronization rate and delayed estrus response in Exp. 1, but not Exp. 2, is unknown. However, HPLC analysis of the extracted soybeans detected presence of three phytoestrogens. The interaction of phytoestrogens and age of heifers at the time of feeding may have induced the altered reproductive response in soybean-fed heifers in Exp. 1. In summary, SB, DDG and WCGF all appear to be feasible protein and energy supplements in heifer development diets at the inclusion rates used in these studies.

Keywords: Protein Supplements, Heifer Development, Fertility

Introduction

Effective replacement heifer development is a critical segment of the integrated management program in an efficient beef cow production system (Corah and Hixon, 1999). Economical heifer development diets of sufficient nutritional value to promote adequate growth and development for successful reproductive function can be achieved with high forage diets supplemented with high-energy sources of protein. Fat supplementation has been shown to have positive reproductive effects independent of its energy contribution. In pregnant and postpartum beef females, supplemental fat has improved follicle growth and steroidogenic potential, enhanced luteal characteristics and progesterone levels, increased first-service conception rates, and shortened the anestrous period (Williams and Stanko, 1999). Research on supplemental fat in peripubertal heifers is limited and results have been inconclusive.

Soybeans are grown throughout the Midwest, and are high in fat, protein, and energy. Soybeans are also high in linoleic acid which has been associated with improved reproductive function in beef heifers (Lammoglia et al., 2000) and mature cows (Wehrman et al., 1991; Ryan et al., 1992). In years of adequate precipitation, soybeans can be an extremely cost-effective way to deliver a nutrient-dense feedstuff to beef cattle. Use of corn milling co-products, such as corn gluten feed and distillers grains, is becoming common practice in the feedlot industry because of their high nutrient value and ability to increase feed efficiency in finishing cattle. These co-products have the potential to be useful to cow-calf producers, however, currently they are not as widely used. While both co-products are higher in energy than corn, DDG provides moderate levels of fat and high levels of undegradable intake protein (UIP) to the diet. Both of these nutrients can be utilized as energy in certain situations (Coppock and Wilks, 1991; Stalker et al., 2004), and potentially benefit reproductive function independent of an energy contribution (Wiley et al., 1991; Williams & Stanko, 1999). Additional research on co-product usage in development and gestation diets of beef cattle is needed to further establish these ingredients as viable, or even preferred, alternatives to traditional energy and protein supplements. Therefore, the objectives of this study were 1) determine the effects of supplemental fat (soybeans) or corn milling co-products on reproductive characteristics in developing heifers and 2) determine the effect of reproductive status (prepubertal vs. postpubertal) on response to fat supplementation.

Materials and Methods

Three experiments were conducted utilizing whole soybeans (**SB**) and either dried distillers grains (**DDG**) or wet corn gluten feed (**WCGF**) in heifer

development diets to determine diet effects on reproductive response and follicle characteristics in virgin beef heifers. Crossbred spring-born heifers (n=104 and n=100, Exp. 1 and 2, respectively) were allotted by weight and randomly assigned to receive diets (Table 1) containing 1.25 kg (DM) SB or 2.0 kg WCGF (Exp. 1) and 1.25 kg SB or 1.25 kg DDG (Exp. 2) as part of a total mixed diet. In Exp. 2, heifers within treatment groups were allotted by weight to one of four pens. Heifers in Exp. 1 weighed 300 kg and were approximately 10 mo old when treatment diets were initiated, and heifers were fed once daily for 110 d. In Exp. 2, heifers weighed 229 kg and were approximately 6 mo of age at diet initiation, and were fed once daily for 226 d. Diets containing SB had an increased level of dietary fat compared to WCGF and DDG diets. Experiment 1 contained added fat levels of 3.2 and 0.5%, respectively, and Exp. 2 contained 3.3 and 1.9% added fat, respectively. Diets within study were formulated to be approximately isocaloric and isonitrogenous (Table 1).

Two blood samples were taken 7 (Exp. 1) or 10 (Exp. 2) d apart before and during the feeding period to determine cycling status. Concentrations of progesterone (**P_4**) in serum were determined using a solid-phase RIA kit (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA) without extraction as previously described (Stewart et al., 1996). Serum progesterone concentrations ≥ 1 ng/mL were considered indicative of cyclicity. Body weights were determined at the time of blood collection and at the end of the feeding period. Heifers in Exp. 1 and 2 were synchronized using 14-d melengestrol acetate (MGA, 0.5 mg/d, Pfizer Animal Health, New York, NY) treatment 19 d before $PGF_{2\alpha}$ injection (25 mg i.m. ProstaMate, Phoenix Scientific, Inc., St. Joseph, MO) given on d 110 (Exp. 1) or 226 (Exp. 2) of trial. Heifers were artificially inseminated 12 h after visual detection of estrus. Ten days after the last AI, heifers were placed on native pasture with bulls for approximately 60 d. Pregnancy to AI was detected by ultrasound approximately 45 d after the last AI. Final pregnancy rates were determined at approximately 90 d of gestation. Body weights were determined at the time of each ultrasound.

Table 1. Soybean (SB) and wet corn gluten feed (WCGF) diets, Exp. 1; SB and dried distillers grains (DDG) diets, Exp. 2

Experiment 1			Experiment 2		
Ingredient (%DM)	**SB**	**WCGF**	**Ingredient (%DM)**	**SB**	**DDG**
Silage	**32.7**	**30.1**	Silage	**28.0**	**24.4**
Straw	**47.8**	**21.3**	Straw	**24.2**	**21.8**
Soybeans	**15.9**	**0**	Soybeans	**16.7**	**0**
WCGF	**0**	**13.3**	DDG	**0**	**16.9**
Brome Hay	**0**	**31.9**	Brome Hay	**27.0**	**32.9**
Supplement	**3.5**	**3.5**	Supplement	**4.1**	**4.1**
Nutrient Composition[1]			**Nutrient Composition**[1]		
CP	**11.2**	**10.8**	CP	**12.2**	**11.3**
TDN	**65.3**	**64.2**	TDN	**64.4**	**65.5**
Fat	**5.0**	**2.3**	Fat	**5.1**	**3.6**

[1]Percent (%) of DM.

Experiment 3 was conducted over two years with 100 summer-born heifers (n=50 each year) to determine diet effects on follicle characteristics and pregnancy rates. Crossbred heifers averaging 198.8 kg and 8 mo of age were allotted by weight and randomly assigned to one of two groups, and to one of two pens per group. Heifers were fed meadow hay (CP = 9%) ad libitum, and supplemented daily with either 1.23 kg SB ground with 0.40 kg corn or 1.65 kg DDG (CP = 31% for both supplements). Blood samples were taken for serum progesterone analysis as described in Exp. 1 and 2. Body weight was determined at the time of blood collection. At the end of the feeding period heifers were synchronized with two injections of $PGF_{2\alpha}$ 14 d apart. Sixty hours after the second injection, dominant follicles (DF) were measured and aspirated using an ultrasound-guided transvaginal probe (Aloka 500V ultrasound with a 7.5 MHz probe; Aloka, Wallingford, CT). Blood samples were taken 48 and 60 h after the second injection of $PGF_{2\alpha}$ for serum 17β-estradiol (E_2) analysis. Granulosa cells were harvested from aspirates, and follicular fluid (FF) samples were stored at -80º until subsequent steroid analysis could be performed. Heifers were placed with bulls for 45 d beginning 14 d after aspiration.

Concentrations of P_4 in non-extracted FF were determined as described in Exp. 1 and 2. Concentrations of E_2 in extracted serum samples and non-extracted FF samples were determined by RIA as described by Kojima et al. (1992). Estrogen-activity of the DF was determined by the E_2:P_4 ratio and E_2 concentrations (ratio ≥ 1.0 and > 100 ng E_2/mL of FF indicates DF is E_2-active; Roberts and Echternkamp, 2003).

Statistical Analysis. Differences in body weight, ADG, timing of estrus, DF diameter, hormone levels in serum and FF, and calving date were analyzed by ANOVA using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). Means, least squares means and SEM were generated by PROC GLM. Differences in synchronization, conception and pregnancy rates, cyclicity, and number of E_2-active follicles were analyzed using Chi-square analysis by PROC GENMOD of SAS. Means for binomial data were determined by SAS Frequency Table. Non-significant ($P > 0.10$) covariates (i.e. pen, sire, year) were removed from the statistical model, and data were reanalyzed with only treatment and significant covariates remaining in model.

Results and Discussion

Exp. 1. Heifers weighed 380 kg at the time of $PGF_{2\alpha}$ injection, and ADG during the feeding period was not different between treatment groups (0.69 kg/d; Table 2). At the 45-d ultrasound, SB heifers were heavier ($P = 0.02$) and gained more ($P < 0.01$) weight from the time of synchronization to first ultrasound. The difference in weight also existed at time of the 90-d ultrasound, but with less magnitude ($P = 0.07$). Average daily gain for the period between AI ultrasound and final ultrasound was not different between groups, but the difference in ADG

Table 2. Performance data: Wt (kg) and ADG (kg/d), Exp. 1 and 2

Study	Treatment	Wt.[1]	Wt.[2]	Wt.[3]	ADG[4]	Wt.[5]	ADG[6]	Wt.[7]	ADG[8]	ADG[9]
Exp. 1	SB	299.40	349.74	381.40	0.70	382.56[a]	0.03[a]	414.48[a]	0.66	0.35[a]
	WCGF	299.75	342.66	379.90	0.68	367.91[c]	-0.27[d]	402.08[b]	0.72	0.24[c]
	P-value	0.95	0.27	0.83	0.50	0.02	0.0001	0.07	0.30	0.0001
	SEM	9.18	10.0	10.7	0.04	9.80	0.085	10.35	0.078	0.039
Exp. 2	SB	229.53	368.64[a]	375.70[a]	0.65[a]	383.02[a]	0.15	441.87	1.51	0.56[a]
	DDG	229.04	380.04[c]	389.87[c]	0.71[d]	395.44[c]	0.11	450.98	1.42	0.51[b]
	P-value		0.04	0.01	0.0001	0.03	0.50	0.14	0.22	0.10
	SEM	6.61	8.69	8.74	0.02	8.82	0.08	9.61	0.11	0.04

[1]Average of two weights taken at the beginning of the experimental period.
[2]Weight taken at 4th blood collection.
[3]Weight taken at the time of $PGF_{2\alpha}$ injection.
[4]ADG during feeding period.
[5]Weight taken at the time of 1st ultrasound, 45 d after the last AI.
[6]ADG from $PGF_{2\alpha}$ injection to 45-d ultrasound.
[7]Weight taken at the time of 2nd ultrasound, ~90 d after AI.
[8]ADG from 45-d ultrasound to 90-d ultrasound.
[9]ADG from $PGF_{2\alpha}$ injection to 90-d ultrasound.
[a,b] Numbers within a column within experiment with different superscripts have a tendency to differ ($P < 0.10$).
[a,c] Numbers within a column within experiment with different superscripts differ ($P < 0.05$).
[a,d] Numbers within a column within experiment with different superscripts differ ($P < 0.01$).

from synchronization to 45-d ultrasound resulted in a higher ($P < 0.01$) overall rate of gain in the SB group for the period between synchronization and 90-d ultrasound (Table 2). Heifers previously developed on SB diet appeared to acclimate more favorably while grazing native pasture.

Treatment did not affect cycling status at any time measured. At treatment initiation, 82% of the heifers were cycling, and 98% had reached puberty at mid-point of the feeding period (d 55-62 of treatment). More ($P < 0.05$) heifers on the WCGF diet (96%) exhibited estrus during the 5-d breeding period compared to heifers fed SB (81%). Among the SB heifers exhibiting estrus during the synchronization period, there was a delay in the average time of estrus compared to WCGF heifers (3.2 d vs. 2.9 d; $P = 0.05$). Diet did not affect the percent of heifers detected in estrus becoming pregnant to AI (AI conception rate), the percent of all heifers in each group becoming pregnant to AI (AI pregnancy rate), or the percent of heifers in each group becoming pregnant to AI or natural service (final pregnancy rate; Figure 1).

The reason for the reduced synchronization rate and delay in estrus is not known. However, upon analysis of soybeans by HPLC, three phytoestrogens were detected: genistein = 1095 ppm; daidzein = 940 ppm; and glycitein = 100 ppm. The interaction of these phytoestrogens and the physiological maturity of heifers at the time of feeding may have altered the reproductive response in heifers fed SB. Heifers in this trial were predominantly pubertal when treatment began. Structural similarities between E_2 and phytoestrogens allow phytoestrogens to bind to estrogen receptors (**ER**), and elicit a response that varies from being similar to that of E_2 to acting antagonistically to E_2 (Adams, 1995). In pubertal females, E_2 acts positively on the hypothalamus and pituitary to stimulate GnRH and gonadotropin secretion, respectively. In prepubertal females, however, E_2 inhibits GnRH secretion at the level of the hypothalamus and subsequently suppresses LH and FSH release (Day et al., 1987; Kinder et al., 1995). We hypothesize response to exogenous estrogens in SB may be affected by physiological status (prepubertal vs. postpubertal) of heifers at the time of feeding; therefore, heifers in Exp. 2 were fed SB at a younger age and lighter weight assumping that more would be prepubertal.

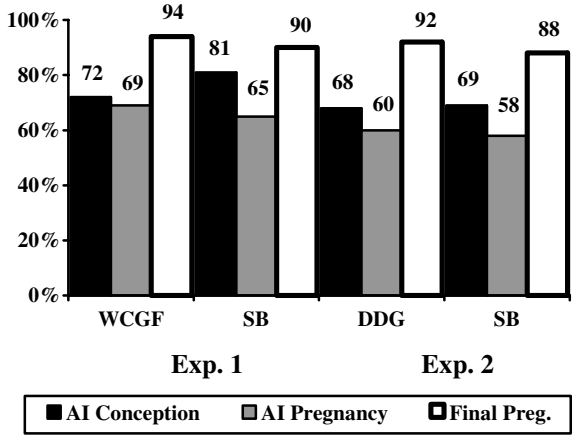

Figure 1. AI Conception, AI Pregnancy, and Final Pregnancy Rates, Exp. 1 and 2 ($P > 0.10$).

Exp. 2. Heifers fed DDG were heavier ($P = 0.04$) at the fourth blood collection, and heavier ($P = 0.01$) at the time of $PGF_{2\alpha}$ injection (Table 2). Average daily gain for the feeding period was higher ($P < 0.01$) in DDG heifers. Heifers did not differ in ADG from $PGF_{2\alpha}$ injection to 45-d ultrasound, however, heifers previously fed DDG continued to be heavier ($P = 0.03$). There was no weight difference between groups at the time of the 90-d ultrasound, and no difference in ADG for the period between AI ultrasound and final ultrasound. There was a trend ($P = 0.09$) for SB heifers to have higher ADG for the grazing period (Table 2). Diets were formulated to be similar in energy and protein; however, energy values have not been clearly established for DDG in high roughage diets, so it is possible the energy level of DDG was underestimated. Treatment did not affect cycling status at any time measured. Eighteen percent of heifers had reached puberty at treatment initiation, and 99% had cycled at least once by the fourth blood collection. There was no difference in synchronization rate (86%) or average time of estrus (2.9 d after $PGF_{2\alpha}$) between groups. Diet had no affect on AI conception, AI pregnancy, or final pregnancy rates (Figure 1). Decreased AI conception and AI pregnancy rates in Exp. 2 compared to Exp. 1 could be partially explained by sire and technician effects in Exp. 2 (Table 3).

As observed in Exp. 1, heifers previously developed on SB diets tended to have a greater ADG during the grazing period following AI. It is not clear why heifers previously fed whole SB adapt more favorably to grazing conditions. In contrast to Exp. 1, reproductive response was not altered when predominantly prepubertal heifers were fed SB.

Table 3. Chi-Square analysis of AI Conception Rates, Exp. 2

Source	DF	Chi-Square	Pr > ChiSq
TRT	**1**	**0.15**	**0.6997**
Pen(TRT)	**6**	**2.87**	**0.8250**
Sire	**2**	**4.66**	**0.0971**
Technician	**3**	**5.62**	**0.1315**

Exp. 3. Body weight did not differ between treatment groups at any time measured, however, ADG was higher ($P = 0.02$) in DDG heifers than SB heifers (0.87 vs. 0.82 kg/d). Year of experiment (yr 1 vs. yr 2) had a significant ($P < 0.01$) effect on beginning weights (210.63 vs. 186.96 kg, respectively), and ADG (0.89 vs. 0.80 kg/d, respectively). Due to pen availability, treatment diets were initiated earlier in yr 2 than yr 1, making yr 1 average weights lighter. At the end of the feeding period, however, weights were not different (344.0 vs. 352.06; weights taken at the time of the second $PGF_{2\alpha}$ injection) between yrs. Treatment had no effect on cyclicity at any time measured, and there was no treatment × yr interaction. Progesterone analysis of serum indicated 28% of heifers were cycling at treatment initiation, and 94% had cycled by the end of the feeding period. In yr 1, more ($P = 0.02$) heifers were cycling (38 vs. 18%) at the beginning of the feeding period, which is probably due to beginning the trial later in yr 1, allowing heifers to be older and heavier at diet initiation than heifers in yr 2. At the end of the feeding period, however, there was a tendency ($P = 0.07$) for more heifers the second yr to be cycling than the first yr (98 vs. 90%).

There was a tendency ($P = 0.06$) for yr of experiment to have an affect on follicle diameter, yr 2 having a larger diameter (12.6 vs. 11.9 mm) than yr 1. There was also a tendency ($P = 0.09$) for a treatment × year interaction, with SB heifers having larger (12.2 vs. 11.6 mm) follicles than DDG heifers yr 1, but not yr 2 (Table 4). Follicular fluid concentrations of E_2 and P_4 were not affected by treatment. There was no difference in number of E_2-active follicles between groups, and no difference in FF E_2 or P_4 concentrations when only E_2-active follicle data was analyzed. Circulating concentrations of E_2 in serum samples collected 48 and 60 h after the second injection of $PGF_{2\alpha}$ were not affected by treatment, and were not different between years. Pregnancy rates were not affected by development diet (88%).

Table 4. Follicle Data, Exp. 3

Study	Treatment	DF diameter[4]	FF E_2[5]	FF P_4[6]	E_2 – activity[7]
Exp. 3 (yr 1)	**SB**	**12.21**	**2055.8**	**139.55**	**84.2**
	DDG	**11.55**	**1891.2**	**146.67**	**65.0**
Exp. 3 (yr 2)	**SB**	**12.51**	**1493.7**	**86.84**	**68.2**
	DDG	**12.75**	**1959.4**	**99.55**	**70.0**
Exp. 3 overall	**SB**	**12.37**	**1763.3**	**119.91**	**75.6**
	DDG	**12.18**	**1925.3**	**116.76**	**67.5**
	***P*-value**	**0.64**	**0.72**	**0.92**	**0.42**
SEM[1]	**SB**	**0.28**	**292.45**	**20.09**	
	DDG	**0.29**	**296.08**	**20.34**	
Year[2]	***P*-value**	**0.07**	**0.56**	**0.08**	**0.15**
Trt×yr[3]	***P*-value**	**0.09**	**0.45**	**0.73**	**0.67**

[1] SEM of LS Means for treatment group.
[2] Main effect of yr 1 vs. yr 2.
[3]Treatment × Yr interaction.
[4]Dominant follicle (DF) diameter (mm).
[5]Follicular fluid (FF) estrogen (E_2) concentrations (ng/mL).
[6]FF progesterone (P_4) concentrations (ng/mL).
[7]Percent of follicles with E_2: P_4 ratio > 1.0 and ng E_2/mL FF > 100.

In summary, spring-born heifers fed diets containing SB after a high percent of heifers had attained physiological maturity exhibited a decreased response to synchronization, and among those that did respond, timing of estrus was delayed. When spring-born heifers were fed diets containing SB while the majority of heifers were prepubertal, no difference was seen in reproductive response. Conception and pregnancy to AI, and final pregnancy rates were similar across diets regardless of when SB diets were initiated. Dominant follicle characteristics of summer-born heifers supplemented with

SB or DDG were similar, and pregnancy rates were similar throughout the two-year experiment.

Implications

Soybeans, DDG, and WCGF appear to be feasible protein and energy supplements in heifer development diets at the inclusion rates used in these studies. Physiological status of heifers at the time of feeding may affect reproductive response to diets containing estrogenic feedstuffs such as SB.

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INTRALUTEAL TREATMENT OF BOVINE CORPUS LUTEUM WITH INDOMETHACIN

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ABSTRACT: The objective of the study was to determine if inhibition of cyclooxygenase with intraluteal indomethacin prevented or delayed luteolysis in beef cows. Corpora lutea (d 7 to 9; ovulation = d 0; n = 5 per treatment) were randomly assigned to one of four treatments in a 2 x 2 factorial design. Treatments included control (C; Atrigel only) or Atrigel with indomethacin (I; 10 mg), with or without PGF_{2a} (P and NP, respectively; C+P, I+P, C+NP, and I+NP respectively). Atrigel (300 µL) was infused (C or I) into the CL via transvaginal ultrasound guided needle (0 h). Prostaglandin F_{2a} (25 mg, i.m., Lutalyse) was given 72 h post-infusion. Blood samples collected at 12 h intervals from 0 to 72 h and 6 h intervals from 72 to 108 h were analyzed for serum progesterone (P_4). Areas of CL were determined at 0 h, 72 h, and 108 h via ultrasonography. At 108 h, ovariectomy was performed and CL weights recorded. Data were analyzed using Mixed procedure of SAS. To control for pretreatment variability, P_4 concentrations from 12 to 72 h were analyzed as a percent of 0 h. Serum P_4 concentrations from 12 to 72 h in I-infused animals did not differ (123%; $P = 0.40$) from those treated with C (110 ± 11%). Progesterone concentrations from 78 to 108 h, analyzed as a percent of 72 h, declined ($P < 0.001$) in P-treated cows (31%) compared to NP (84 ± 6 %). Areas of CL were similar ($P = 0.40$) among treatments (5.3, 5.2, 5.3, and 4.2 ± 0.6 cm^2 for C+NP, I+NP, C+P, and I+P, respectively). Weights of CL receiving NP were heavier (5.3 g; $P = 0.004$) than those receiving P (3.0 ± 0.5 g) with no differences observed ($P = 0.82$) between C and I. Loss of luteal function in I+P indicates Atrigel was ineffective in releasing indomethacin or intraluteal indomethacin was ineffective in preventing or delaying luteolysis.
Keywords: Corpus luteum, Luteolysis

Introduction

The corpus luteum (CL) is a transient endocrine gland that develops on the ovary after ovulation from granulosa and theca cells. It has long been accepted that prostaglandin (PG) F_{2a} of uterine origin is the luteolysin in domestic livestock (McCracken, 1971). The bovine CL, however, also produces $PGF_{2\alpha}$ in response to uterine $PGF_{2\alpha}$ stimulation of the cyclooxygenase (Cox)-2 enzyme (Shemesh et al, 1975; Milvae and Hansel, 1983). The CL expresses mRNA for Cox-2 (Tsai and Wiltbank, 1998) and PGF synthase (Tsai and Wiltbank, 1998).

The exact role of luteal PGF_{2a} in luteolysis has yet to be determined. Because the luteolytic mechanism is not functional in the early luteal phase, luteal $PGF_{2\alpha}$ has been suggested to have a permissive effect through a paracrine positive feedback on oxytocin (Griffeth et al., 2002). This suggests that inhibition of luteal $PGF_{2\alpha}$ could impair luteolysis, resulting in an extended CL lifespan and prolonged progesterone secretion. In support of this theory, the positive feedback pathway, through which PGF_{2a} is produced in the CL, is not present until late in the luteal phase after luteolytic capacity has been acquired (Diaz et al., 2002).

Wu and Wiltbank (2001) elucidated a positive feedback pathway in the ovine large luteal cell in which uterine or exogenous PGF_{2a} stimulates luteal PGF_{2a} synthesis. Additionally, PGF_{2a} administered in luteolytic amounts during the mid-luteal stage resulted in a rapid increase in luteal PGF_{2a} secretion during the first 4 h and again at 24 h, coinciding with increased Cox-2 mRNA expression (Hayashi et al., 2003). These findings suggest that luteal PGF_{2a} is amplificatory rather than permissive in luteolysis. The objective of this study was to determine if blocking luteal PGF_{2a} synthesis with a Cox inhibitor administered directly into the mid-luteal bovine CL prevents or delays PGF_{2a}-induced luteolysis.

Materials and Methods

Corpora lutea (d 7 to 9; ovulation = d 0) from normally cycling, mature beef cows were randomly assigned to four treatments in a 2 x 2 factorial design. Treatments included control (C; Atrigel only), or Atrigel with indomethacin (I: 10 mg; Sigma Aldrich Inc.; St Louis, MO), with or without PGF_{2a} (P and NP, respectively; 25 mg; Lutalyse, Upjohn; Kalamazoo, MI) resulting in C+P, I+P, C+NP, and I+NP, respectively (n = 5 per treatment).

Atrigel (Atrix Laboratories; Fort Collins, CO), a liquid drug delivery system, consists of biodegradable polymers dissolved in biocompatible carriers. When injected into tissue, fluid in the tissue causes the polymer to solidify, resulting in an in situ implant. The biodegradable polymer of the gel matrix allows treatments to be released in a controlled manner over time (Dunn et al., 1990). In this study, Atrigel was used to deliver indomethacin, a Cox inhibitor, directly to the CL for the purpose of blocking or preventing the synthesis and secretion of luteal PGF_{2a}.

Cows were estrus synchronized via injections of GnRH (100 µg, i.m.; Cystorelin, Abbot Laboratories; North Chicago, IL) followed by PGF_{2a} seven d later (25 mg, i.m;). Cows that failed to ovulate 48 h after PGF_{2a} received an additional injection of GnRH (100 µg, i.m). Daily ultrasonography from d of PGF_{2a} administration through ovulation was performed to determine d of ovulation (d 0). An Aloka 500v ultrasound console (Corometrics Medical Products; North Wallingford, CT) equipped with a 7.5 mHz rectal transducer was used to record disappearance of the

largest follicle, which defined d of ovulation. Intraluteal infusions were administered on d 7 to 9 post-ovulation.

Treatments were prepared prior to infusion by aliquoting 300 µL Atrigel. Indomethacin (10 mg) was added to the Atrigel for the I treatment and mixed with a syringe until a uniform distribution was achieved. Atrigel and Atrigel containing indomethacin were transferred into individual 18 gauge, 53 cm infusion needles (Cook, Queensland, Australia) via tuberculin syringe.

Immediately prior to infusion, cows were sedated with 7.5 mg Acepromazine (i.v., Vedco, Inc., St. Joseph, MO). A caudal epidural anesthetic was administered by inserting an 18 gauge, 4.0 cm needle between the last sacral and first coccygeal vertebrae until contact was made with the floor of the spinal canal, followed by injection of 5 ml of 2% lidocaine. To prevent the luteolytic effects of uterine PGF_{2a} stimulated by manipulation of the reproductive tract during the infusion, Banamine® (Flunixin Meglamine; Schering-Plough Animal Health, Kenilworth, NJ) was administered (1.1 mg per kg body weight).

Following aseptic preparation of the perineum, a 50 cm transvaginal, needle-guided probe housing a 7.5 MHz transducer was inserted into the vagina. The CL-containing ovary was palpated per rectum and placed on the transducer to visualize the CL on the ultrasound monitor. An infusion needle, containing C or I, was inserted into the needle guide of the probe. The vaginal wall was punctured and penetration of the CL was observed on the ultrasound monitor. Upon CL penetration, a stylet was used to slowly expel treatment from the needle (0 h). Infusion success was visually determined by the appearance of a white mass within the CL on the ultrasound monitor. Corpora lutea were held on the transducer for approximately 60 sec to ensure Atrigel solidification within the CL before the infusion needle was removed.

Prostaglandin F_{2a} (25 mg, i.m) or no PGF_{2a} (P or NP) was administered 72 h post infusion to cows receiving C or I.

Blood samples were collected at 12 h intervals from 0 to 72 h and 6 h intervals from 72 to 108 h via caudal venipuncture. Serum was analyzed for progesterone concentration by solid phase RIA (Diagnostic Products Corporation, Los Angeles, CA), with modifications as described by Schneider and Hallford (1996). Intra- and inter-assay CV's were 13.7% and 12.2%, respectively.

Areas of CL, measured as width by height in cm^2, were determined at 0 h, 72 h, and 108 h via ultrasonography.

At 108 h, unilateral ovariectomy was performed via colpotomy. Cows received Acepromazine and caudal epidural as described above. As described by Williams and Forrest (1989), with modifications, a small puncture in the vaginal wall made by a trocar provided access to the ovary. The ovarian stalk was clamped and the ovary was removed within the vaginal cavity. Corpora lutea were dissected from the ovaries and implants were recovered and removed. Following ovariectomy, CL and implant weights were recorded.

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

Statistical Analysis: Progesterone, CL area and weight, and implant weight data were analyzed using Mixed procedure of SAS (SAS, Inst. Inc., Cary, NC). Progesterone and CL area were analyzed for repeated measures using compound symmetry and autoregressive as the respective covariate structures. Corpus luteum was the experimental unit. To control for pretreatment variability, progesterone concentrations from 12 to 72 h were analyzed as a percent of 0 h (pre-infusion). Progesterone concentrations from 78 to 108 h were analyzed as a percent of 72 h (pre-PGF_{2a} administration). Indomethacin, time, and indomethacin by time were included in the model statement in the analysis of progesterone concentrations from 12 to 72 h. Indomethacin, PGF_{2a}, time, and their interactions were included in the model statements in the analyses of progesterone concentrations from 78 to 108 h and CL area. Indomethacin, PGF_{2a}, and indomethacin by PGF_{2a} were included in the model statement in the analysis of CL and implant weight. Because an interaction existed ($P = 0.03$) between PGF_{2a} and time for progesterone from 78 to 108 h, data were analyzed by time. No other interactions were observed in analyses of progesterone, CL area and weight, and implant weight ($P > 0.05$).

Results

Progesterone concentrations from 12 to 72 h did not differ ($P = 0.40$; Figure 1) between C- and I-treated CL, confirming that Atrigel or indomethacin did not alter the CL's ability to synthesize or secrete progesterone.

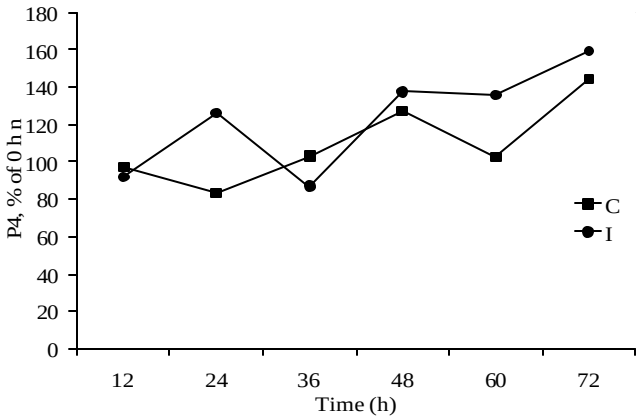

Figure 1. Effect of infusion of the mid-luteal bovine CL with 300 µL Atrigel or 300 µL Atrigel containing 10 mg indomethacin on serum progesterone concentrations from 0 through 72 h. Serum progesterone concentrations of C-treated CL were similar ($P = 0.40$) to I-treated CL (110 and 123 ± 11% for C and I, respectively).

Progesterone concentrations of C- and I-treated CL were similar (50.6 and 64.9 ± 6.0% $P = 0.11$) from 78 to 108 h. Because an interaction was observed ($P = 0.03$) between PGF_{2a} and time, data were analyzed by time. Progesterone concentrations of NP-treated CL were greater at all times ($P < 0.01$) than P-treated CL (Figure 2).

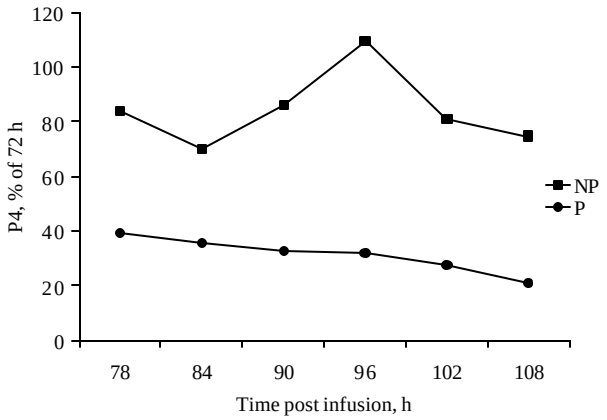

Figure 2. Effects of PGF_{2a} administration 72 h after infusion of the mid-luteal bovine CL with 300 µL Atrigel or 300 µL Atrigel containing 10 mg indomethacin on serum progesterone concentrations from 78 through 108 h. Serum progesterone concentrations of NP-treated CL were higher than P-treated CL at all collection times ($P < 0.01$).

Areas of CL were similar between C- and I-treated CL (5.3 and 4.7 ± 0.4 cm^2; $P = 0.3$), and P- and NP-treated CL (4.8 and 5.2 ± 0.4 cm^2; $P = 0.5$). Weights of CL receiving NP were heavier (5.3 g; $P = 0.004$) than those receiving P (3.0 ± 0.5 g) with no differences observed ($P = 0.82$) between C and I (4.2 and 4.0 ± 0.5 g, respectively).

Implants recovered from C- and I-treated CL were similar in weight (0.18 and 0.10 ± 0.03 g; $P = 0.07$). Implants recovered from P-treated CL were heavier (0.2 g; $P = 0.008$) than implants recovered from NP-treated CL (0.08 ± 0.03 g).

Discussion

This research investigated the role of luteal PGF_{2a} in luteolysis. We hypothesized that by blocking luteal PGF_{2a} synthesis with locally placed indomethacin, PGF_{2a} induced luteolysis would be inhibited or delayed.

Results of this study were not consistent with our hypothesis. Because progesterone concentrations were similar between I- and C-treated CL and NP-treated CL had greater progesterone concentrations than P-treated CL at all collection times from 78 to 108 h, we conclude that indomethacin did not effectively inhibit PGF_{2a}-induced luteolysis possible due to insolubility in Atrigel. In a similar study conducted by Griffeth et al. (2002), serum progesterone concentrations of ewes implanted with Atrigel containing 0, 1, or 10 mg of indomethacin all were less than 200 pg/ml by d 17 post estrus. These authors concluded that intraluteal PGF_{2a} was not necessary for functional luteolysis.

The trends expected for serum progesterone were also expected for luteal weights. We hypothesized that C+NP, I+NP, and I+P groups would have similar luteal weights, and be heavier than C+P-treated CL. However, treatment with PGF_{2a} decreased weights of CL treated with either C or I. Thus, infusion of indomethacin in an Atrigel matrix did not effectively prevent or delay PGF_{2a} induced luteolysis.

Implications

Loss of luteal function in I+P indicates Atrigel was ineffective in releasing indomethacin or intraluteal indomethacin was ineffective in preventing or delaying luteolysis.

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ARTIFICIAL INSEMINATION PREGNANCY RATES USING A MODIFIED CO-SYNCH PROTOCOL FOR PRIMIPAROUS SUCKLED COWS EXPOSED TO THE BIOSTIMULATORY EFFECT OF BULLS[1]

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ABSTRACT: The objective was to evaluate whether exposing primiparous suckled beef cows to the biostimulatory effect of bulls alters breeding performance associated with an estrous synchronization (ES) protocol that included GnRH, $PGF_{2\alpha}$ (PG), and fixed-time AI. The hypotheses tested were that: 1) proportions of cows that exhibit estrus after PG; and 2) AI pregnancy rates do not differ between primiparous cows exposed or not exposed to the biostimulatory effect of bulls. This is a composite analysis of 3 experiments that evaluated: 1) effects of bull exposure at different d after calving (Yr 1); 2) biostimulatory effects of bull excretory products (Yr 2); and, 3) biostimulatory effects of familiar and unfamiliar bulls (Yr 3) on resumption of cycling activity. In all studies cows were exposed (BE; n = 84) or not exposed (NE; n = 70) to bulls or excretory products of bulls for at least 55 d before the start of the ES protocol. Average calving date did not differ among yr and was 52 ± 5 d (± SE). Start of the ES protocol was the same date each yr. Year did not affect the proportions of BE and NE cows that were cycling at the start of the ES protocol. However, a greater ($P < 0.01$) proportion of BE than NE cows were cycling at this time. Each cow was given GnRH, followed by PG 7 d later. Cows were observed for estrus twice daily (am:pm) after PG. Cows that showed estrus before 54, 60, and 64 h after PG were inseminated by AI 12 h later in each yr, respectively. Cows that failed to show estrus were given GnRH and TAI at 62, 72, and 72 h after PG in each yr, respectively. Pregnancy rates (PR) were determined ultrasonically 35 d after TAI. Proportions of BE and NE cows that showed estrus after PG and before TAI did not differ. Artificial insemination PR did not differ between BE and NE cows; however, TAI PR tended to be greater ($P = 0.07$) for BE cows than for NE cows. We conclude that TAI but not overall AI pregnancy rates in an ES protocol that includes GnRH, PGF2a, and TAI may be improved by the biostimulatory effect of bulls.

Key Words: Biostimulation, Estrous Synchronization, Postpartum Anestrus, Fixed Timed AI

Introduction

Extended postpartum anestrus is the major cause of primiparous suckled cows failing to rebreed or breeding late in their next breeding season (Short et al., 1994). This problem can create a challenge to successfully synchronizing estrus or ovulation and using artificial insemination (AI). The presence of bulls accelerates resumption of postpartum ovarian activity in primiparous suckled beef cows (Custer et al.. 1990; Fernandez et al., 1993). Berardinelli (1987) reported that 33% more primiparous cows exposed to bulls before the start of the breeding season were bred by artificial insemination (AI) during the first 21 d of a 55-d breeding season than cows not exposed to bulls. Subsequently, Fernandez et al. (1993) found that AI pregnancy rate, over a 21-d breeding season, for cows exposed to bulls before the breeding season was 40% greater than that for cows not exposed to bulls.

Using estrous synchronization (ES) protocols that incorporate AI allows for a majority of cows to be inseminated artificially in a very short period of time. Jordan et al. (2002) indicated that gonadotropin releasing hormone (GnRH)-based ES protocols are more successful if postpartum cows have resumed cycling activity. Thus, the biostimulatory effect of bulls may be used as a management strategy to improve the breeding performance of primiparous cows using this type of ES protocol.

The objective of this study was to evaluate whether exposing primiparous suckled beef cows to the biostimulatory effect of bulls alters breeding performance associated with an ES protocol that included GnRH, $PGF_{2\alpha}$, and fixed-time AI. The hypotheses tested were that: 1) proportions of cows that exhibit estrus after PG; and 2) AI pregnancy rates do not differ between primiparous cows exposed or not exposed to the biostimulatory effect of bulls before the breeding season.

Materials and Methods

Animals, Years, and Treatments

One-hundred sixty-one, spring-calving, 2-yr-old Angus x Hereford crossbred, primiparous beef cows were used in this longitudinal study conducted over a 3-yr-period at the Montana State University Livestock Teaching and Research Center, Bozeman, Montana.

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Animal care, handling, and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

In each experiment (Year), cows were stratified by calving date, calf birth weight, sex of calf, and body condition score before they were assigned to treatments. Average calving date among years was 52 ± 5 d (± SE). For the purposes of this study, cows that were exposed to the physical presence of bulls or exposed to excretory products of bulls are designated "BE"; cows not exposed to bulls or excretory products of bulls are designated "NE".

Year 1. Fifty-six cows were assigned randomly to one of six treatments in a completely randomized design using a 2 x 3 factorial arrangement. Factors were exposure type (BE or NE) and day exposed postpartum (15, 35, or 55). Day 15, 35, or 55 after calving represented d 0 for each treatment, respectively. Four 2-yr-old, epididymectomized Angus bulls were used in this experiment. Bull to cow ratio was approximately 1:9. Cows were in their respective treatments for 62 ± 2 d at the start of the ES protocol.

Year 2. Sixty-one cows were assigned randomly to one of four treatments: exposed continuously to presence of a bull (BE) or excretory products of bulls (BE), and not exposed to a bull (NE) or exposed to excretory products of cows (NE) beginning on d 35 ± 2 d after calving. Five, 3-yr-old, epididymectomized Angus bulls were used in this experiment. Bull to cow ratio for each was approximately 1:15. Cows exposed to excretory products of bulls or cows were placed into an enclosure that was approximately one-third (~245 sq. m) of the area of the pen and was used to alternately house bulls and cows. Bulls (n = 4) were place into this enclosure at approximately 0800 h and removed at 1830. Cows were then moved into the enclosure overnight between 1830 and 0800 h. Cows had been in their respective treatments for 63 ± 2 d at the start of the ES protocol.

Year 3. Fifty cows were assigned randomly to one of two treatments; exposure to bulls (BEF) or exposure to mature OVX cows (NEF) from 5 to 35 d after calving. On d 35 after calving, 12 BEF cows were assigned randomly to be exposed to a different bull (BEU); likewise, 12 NEF cows were assigned to be exposed to unfamiliar OVX cows (NEU). The cow to bull and cow to OVX cow ratio was approximately 1:12, respectively, during the first 35 d after calving, and then 1:6, respectively, for the remainder of the experiment. Cows in the BEF and BEU treatments are designated BE cows, while cows in the CEF and CEU treatments are designated NE cows for this evaluation. Cows were in their treatments for either 95 d (BEF and NEF) or 60 d (BEU and CEU) before the start of the ES protocol.

Lots Used for Exposure Type

Year 1, 2, and 3. Two lots were used in each experiment, designated north and south by their geographic location. Each lot contained four 41 m x 18 m (L x W) pens that were identical in east-west configuration, bunk space, aspect, slope, and connection to open-shed shelters. Lots were approximately 0.35 km apart. Animals housed in one lot were not able to see or smell animals housed in the other lot; however, there was a possibility that sounds made by animals in one area could be heard by animals in the other area. Pens within each lot were isolated from each other by draping and securing tarpaulins over the 3 m fences that separated pens. Cows exposed to bulls had had no contact with bulls throughout pregnancy and after calving until they were placed into pens with bulls (Years 1, 2, and 3) or excretory products of bulls (Year 2). Cows not exposed to bulls had no contact with bulls throughout pregnancy and the experiments.

Nutrition

Year 1, 2, and 3. Cows and calves had free access to good quality mixed-grass alfalfa hay and any pasture grasses that were available before they were moved into their respective pens. Once cows were moved into pens they were given free access to the same hay (chopped), 0.25 kg cracked barley per animal daily, water, and a mineralized-salt supplement until the end of the experiment in each year. The TDN of these diets exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996). Bulls were fed the same diet as cows within each year.

Estrous Synchronization and AI

Year 1, 2, and 3. On May 18th of each year, each cow was injected i.m. with GnRH (100 μg/hd) on d –7, followed by an i.m. injection of $PGF_{2\alpha}$ (25mg/hd; d 0) 7 d later. Cows were then observed for estrus twice daily (0700 and 1900 h) beginning on d -7 until fixed time AI (TAI). Cows that exhibited estrus before 54 (Year 1), 60 (Year 2), and 64 (Year 3) h after $PGF_{2\alpha}$ were inseminated artificially 12 h later by one of two experienced AI technicians; each assigned randomly to breed a cow. Within each year, cows were inseminated with frozen semen from a single sire of proven fertility. Cows that failed to show estrus were given a second injection of GnRH (100 μg/hd) and TAI at 62, 72, and 72 h after $PGF_{2\alpha}$ for Years 1, 2, and 3, respectively. In Year 1, bulls were removed from cows at TAI; in Year 2, cows remained in their treatments for 5 d after TAI; and in Year 3, cows remained in their treatments for 7 d after TAI. Pregnancy rates were determined by transrectally ultrasonography 35 d after TAI.

Statistical Analyses

Calving date, calf BW, calf sex ratio, cow BW and BCS, and change in cow BW and BCS over the experimental period were analyzed by separate by analyses of variance for a completely random design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment (BE and NE), Year, and

their interaction. Means were evaluated with the PDIFF option of SAS (SAS Inst. Inc., Cary, NC).

Proportions of cows among treatments that exhibited resumption of ovarian cycling activity at the start of the ES protocol, estrous response after $PGF_{2\alpha}$, pregnancy rates for AI at 12 h after estrus and TAI, and overall AI pregnancy rates among years were analyzed separately with the CATMOD PROC of SAS. The model for each analysis included year, treatment, and the year by treatment interaction. If a factor was not statistically important ($P > 0.10$) then data for the variables were pooled and re-analyzed. Data for cows that showed estrus before injection of $PGF_{2\alpha}$ were excluded from the analyses.

Results

Calving date, calf BW, calf sex ratio, cow BW and BCS, and change in cow BW and BCS did not differ between treatments or among years. Two, 1, and 3 cows (5 of 161; 3.1%) exhibited estrus before injection of $PGF_{2\alpha}$.

Table 1. Number of cows per treatment, percentages of cows cycling at the start of the modified Co-Synch protocol, percentages that showed estrus after $PGF_{2\alpha}$, and pregnancy rates for cows bred by AI 12 h after estrus, bred at fixed time AI (TAI), and overall AI for primiparous cows exposed to bulls or their excretory products (BE) or not exposed to bulls or their excretory products (NE)

	Treatment			
Variable	BE	NE	X^2	*P* value
n	84	70		
% cycling before the start of ES protocol	86.3 [b]	31.3 [c]	42.0	< 0.05
% showing estrus after $PGF_{2\alpha}$	50.0 [b]	50.0 [b]	0.00	= 0.96
AI pregnancy rate (%) [a]	72.6 [b]	62.9 [b]	2.70	= 0.11
Pregnancy rate for AI 12 h after estrus	84.6 [b]	81.8 [b]	0.07	= 0.80
Pregnancy rate for TAI	59.5 [b]	37.1 [c]	3.20	= 0.07

[a]Pregnancy rates determined ultrasonographically 35 d after TAI

[b,c]Percentages within rows that lack a common superscript differ.

There was no interaction between treatment (BE and NE) and Year (1, 2, and 3) for percentages of cows cycling at the start of the ES protocol, percentages of cows exhibiting estrus after $PGF_{2\alpha}$, pregnancy rates for AI at 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates. Therefore, data these variables were pooled over Years and re-analyzed.

A greater ($P < 0.05$) proportion of BE than NE cows were cycling at the start of the ES protocol. Proportions of BE and NE cows that showed estrus after $PGF_{2\alpha}$ and before TAI did not differ (Table 1). Artificial insemination pregnancy rates did not differ between BE and NE cows; however, TAI pregnancy rate tended to be greater ($P = 0.07$) for BE cows than for NE cows (Table 1).

Discussion

Berardinelli (1987) reported that 33% more primiparous cows exposed to bulls before the start of the breeding season were bred by artificial insemination (AI) during the first 21 d of a 55-d breeding season than cows not exposed to bulls. Subsequently, Fernandez et al. (1993) found that AI pregnancy rate, over a 21-d breeding season, for cows exposed to bulls before the breeding season was 40% greater than that for cows not exposed to bulls. Estrous synchronization was not incorporated into the breeding season of these two experiments. Subsequently, Jordan et al. (2002) suggested that gonadotropin releasing hormone (GnRH)-based ES protocols are more successful if postpartum cows have resumed cycling activity. We hypothesized that the biostimulatory effect of bulls would improve breeding performance of primiparous beef cows using an estrous synchronization (ES) protocol that included GnRH, $PGF_{2\alpha}$, and fixed-time AI.

This present study represents a compilation of breeding performance data collected over a 3-yr period from individual experiments that evaluated factors related to the biostimulatory effect of bulls. Cows in these experiments were exposed to the physical presence of bulls or the excretory products of bulls for longer than 60 d by the start of the ES protocol. The ES protocol began on the same calendar date for each year of these experiments, and the same ES protocol was employed in each experiment, except for minor differences in the timing of the second GnRH and TAI injection after $PGF_{2\alpha}$. The AI technicians used for these experiments were the same individuals for all 3 yr, and semen was from the same bull within years. Thus, it would be reasonable to evaluate the biostimulatory effect of bulls on breeding performance of the primiparous cows over these experiments. This idea is supported by the fact that there was no interaction between treatment and year for any of the variables evaluated in this study.

We found that the percentage of cows that exhibited estrus after injection of $PGF_{2\alpha}$ did not differ between BE and NE cows, even though there were more BE cows cycling before the start of the ES protocol than NE cows. This indicated that the first injection of GnRH may have induced cycling activity in more anestrous NE cows than in anestrous BE cows. Furthermore, AI pregnancy rates for cows bred 12 h after estrus did not differ between BE

and NE cows. This result indicates that the biostimulatory effect of bulls does not improve AI pregnancy rates of when cows are bred 12 h after a synchronized estrus.

Overall AI pregnancy rate was numerical greater for BE cows than for NE cows. This result appears to be consistent with those obtained by Anderson et al. (2002) and Berardinelli et al. (2004) in individual experiments with these cows. This advantage is reflected in the difference in TAI pregnancy rates between BE and NE cows. Exposing cows to bulls before the start of and during the ES protocol appeared to increase TAI pregnancy relative to cows not exposed to bulls. Again, this is similar to the result reported by Anderson et al. (2002) and indicates that exposure to bulls may affect TAI pregnancy rates.

In conclusion, results of the analyses of this 3-yr longitudinal study indicated that timed AI pregnancy rates may be improved by exposing primiparous beef to bulls before and during an estrous synchronization protocol that included GnRH, $PGF_{2\alpha}$, and fixed time AI.

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EFFECTS OF SUPRANUTRITIONAL LEVELS OF SELENIUM (SE) ON VASCULAR DENSITY AND CELL PROLIFERATION IN THE SHEEP PLACENTA.

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ABSTRACT: To examine effects of dietary Se on sheep placenta, 16 pregnant Targhee ewe lambs were randomly allotted to one of two treatments: control (CON; 0.1 ppm Se) and Se-wheat (SW; 3.0 ppm Se). SW diet was formulated using 32% high selenium wheat. Diets were similar in CP (15.5%) and energy (2.68 Mcal of ME) and fed to meet or exceed requirements. Treatments were initiated at 50 ± 5 d of pregnancy. Placentomes were collected at 135 ± 3 d of gestation. One hour before slaughter, ewes were administrated bromodeoxyuridine (BrdU; 5 mg/kg BW) to determine cell proliferation rate. For each ewe, separate placentomes were fixed with Carnoy's solution by vascular perfusion of the caruncular (CAR; maternal portion of the placentome) or cotyledonary (COT; fetal portion of the placentome) tissues. After paraffin embedding tissues were sectioned and stained with hematoxylin and periodic acid Shiffs's for vascularity and anti BrdU for cell proliferation. The CAR and COT tissues were evaluated for: capillary area density (CAD), capillary no. density (CND), capillary surface density (CSD), area per capillary (APC). Fetal and placental weights were not different, for CON vs. SW. Total numbers of placentomes were less ($P < 0.07$) in SW vs. CON (66.5 vs. 82.85 ± 5). For CAR (maternal placental) CAD, CND, CSD, and APC were not affected by diet, for COT (fetal placental) the CND was greater ($P < 0.06$) but APC was smaller ($P < 0.08$) in SW vs. CON. For COT there was no effect of diet on total cellular density (TCD, no. of cells/unit tissue area; $P = 0.57$) or cell proliferation rate (PR; dividing cells vs. non dividing; $P = 0.49$). In contrast, for CAR the TCD was greater ($P < 0.001$) and PR was less ($P < 0.03$) in SW vs. CON. Data indicate that supernutritional levels of Se have effects on CAR cell proliferation and COT vascularity.

Key Words: Lambs, Placenta, Selenium, Vascularity

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Introduction

The trace element Selenium (Se) has an inhibitory effect on neo-angiogenesis of cancerous tissues (Jiang et al., 1999b). Also, recent work (Clark et al., 1996; Combs and Lu, 2001) indicates that supranutritional levels of Se can decrease combined incidence of lung, colorectal and prostate cancer by as much as 50% in humans. During pregnancy all of the respiratory gases, nutrients, and wastes exchanged between the maternal and fetal systems are transported via the placenta (Faber and Thornburg, 1983; Ramsey, 1982; Reynolds and Redmer, 2001a; Reynolds and Redmer, 1995). The importance of placental function is probably best exemplified by the close relationship between fetal weight, placental size, and uterine and umbilical blood flows in many mammalian species (Magness, 1998; Reynolds and Redmer, 2001b; Reynolds and Redmer, 1995). Establishment of functional fetal and placental circulations is one of the earliest events during embryonic/placental development (Ramsey, 1982). It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on the dramatic growth of the placental vascular beds and the resultant large increases in uterine and umbilical blood flows (Reynolds and Redmer, 2001c; Reynolds and Redmer, 1995). We are aware of no research evaluating the influence of supranutritional levels of Se on placental growth, placental vascular development or fetal development in sheep. Therefore our hypothesis was that supranutritional levels of Se would alter placenta mass, cellular proliferation, and vascularity in pregnant ewe lambs.

Materials and Methods

Animals and Diets

Sixteen pregnant Targhee ewe lambs (45.6 ± 10.5 kg; 330 ± 30 d of age) were randomly allotted to one of two treatments in a randomized design. The North Dakota State University Institutional Animal Care and Use Committee reviewed and approved animal care and use protocols used during this study. Treatments were control (CON; 1 ppm Se) and Se-wheat (SW; 3.0 ppm Se). Both diets contained 32% wheat; however, the SW diet was formulated using a high SE (8 to 10 ppm) wheat source. Diets were similar in CP (15.5%) and energy (2.86 Mcal ME), and fed to meet or exceed NRC requirements (NRC, 1985). Diets were initiated at day 50 ± 5 of gestation. The SW diet provided 75 µg/kg BW daily of SE. Diets were delivered in a pelleted form, and ewes were fed twice daily.

Slaughter Procedures

At day 135 ± 3 ewes were infused i.v. with 5-bromo-2-deoxy-uridine (BrdU; 5 mg/kg BW). Ewes were slaughtered 1 h after BrdU infusion and gravid uteri were obtained. The BrdU is a thymidine analog that is incorporated into DNA during the S-phase of cell cycle and allows estimating the cell proliferation rate in live animals (Jablonka-Shariff et al., 1993; Jin et al., 1994). To provide a quantitative description of vascular growth, we developed a perfusion fixation procedure. For each ewe, separate placentomes were fixed with Carnoy's solution by perfusion of the main arterial vessel supplying the caruncular (CAR; maternal portion of the placentome) or cotyledonary (COT; fetal portion of the placentome) tissue. These perfusion procedures are similar to those previously described (Jablonka-Shariff et al., 1996). Fetal weights, placental weights and number of placentomes also were recorded for each ewe.

Placentome Vascularity and Cell Proliferation

After perfusion and fixation, several cross-sections (approximately 0.5 cm thick and taken full-thickness from the fetal to maternal side) of each placentome were post-fixed by immersion in Carnoy's solution. Subsequently, each placentomal cross-section was embedded in paraffin, sectioned at 5 µm, mounted onto glass slides using standard histological techniques, and stained with hematoxylin and periodic acid-Schiff's for vascularity measurements using procedures we have previously reported (Reynolds and Redmer, 1992; Reynolds and Redmer, 1998). Pictures were taken at 20x magnification using a Nikon DXM 1200F digital camera. Vascularity was then determined by image analysis (Image-Pro Plus®, Media Cybernetics). We analyzed 10 COT and 10 CAR tissue areas per placentome (340032 µm/tissue area) with the following parameters determined for each section: CAR (maternal) tissue area, COT (fetal) tissue area, shrinkage area (effect of fixation that was subtracted from total tissue area), capillary area density (CAD; capillary area/unit tissue area), capillary number density (CND; capillary no./unit tissue area), capillary surface density (CSD; capillary surface/unit tissue area), and the average cross-sectional area per capillary (APC). We also determined the total capillary volume (TCV) for CAR and COT (CAD x total CAR or COT tissue mass). For relative rate of cell proliferation, a separate set of slides was prepared. The presence of BrdU in specific nuclei was localized using immunohistochemistry with anti-BrdU primary antibody (Mouse anti-BrdU monoclonal, Boehringer, Mannheim, Indianapolis, IN) and a biotinylated secondary antibody (Horse anti-mouse IgG; Vector Lab., Burlingame, CA) in combination avidin-biotin-horseradish peroxidase complex (ABC) reagents, and 3,3` diaminobenzidine (DAB; Vectastain, Vector Lab.) substrate (Jablonka-Shariff et al., 1993). Sections were counterstained briefly with Harris' hematoxylin to visualize unlabeled nuclei. Photomicrographs of BrdU incorporation were analyzed by image analysis (Image-Pro Plus®, Media Cybernetics) to quantify cell proliferation rate (PR; percentage of cells proliferating).

Statistical Analyses

General linear models (GLM) procedures (SAS Inst., Inc., Cary, NC) were used for statistical analysis. Data are presented as means with respective standard errors.

Results and discussion

Supernutritional levels of selenium in the SW diet had no effect on fetal or placental weights ($P = 0.13$; $P = 0.16$; Table 1). Total numbers of placentomes were less ($P < 0.06$) in SW vs.CON ewes (Table 1). For CAR (maternal placenta), CAD, CND, CSD, APC and TCV were not affected by dietary treatment. For COT (fetal placenta), CAD, CSD and TCV were not affected by diet; however, CND was greater ($P < 0.06$) but APC was smaller ($P < 0.08$) for SW vs. CON (Table 2). For COT there was no effect of diet on total cellular density (TCD, no. of cells/unit tissue area; $P = 0.57$) or cell proliferation rate (PR; dividing cells vs. non dividing; $P = 0.49$). In contrast, for CAR the TCD was greater ($P < 0.001$) and PR was less ($P < 0.03$) in SW vs. CON (Table 3).

These data indicate that dietary selenium can influence angiogenesis and cell proliferation in sheep placenta. This agrees with recent data that show anti-angiogenic activity of selenium in vivo (Jiang et al., 1999a). Although high dietary selenium did not affect fetal or placental size, placentome number was reduced by high dietary selenium. As the role of dietary selenium supplementation in cancer prevention increases (Combs and Lu, 2001) there is a need to further investigate the influence of Se on rapidly developing tissues of the placenta and growing fetus.

Implications

Thus, a high-Se wheat diet fed to healthy ewe lambs during gestation decreased average size of blood vessels on the fetal side of the sheep placenta and reduced number of placentomes. Despite these effects, the total mass and total capillary volume of CAR and COT was similar between dietary treatments. Selenium treatment also affected maternal placental cell turnover, but had no effect on fetal placental cell proliferation. The reduction in cell proliferation rate of the maternal placenta appeared not to affect fetal or placental weight.

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Table 1. Effect of dietary Se on fetal and placentomal weight and number of placentomes on day 130 of gestation in pregnant ewe lambs.

	Treatment			
Item	Control	Se-wheat	SE	*P*-value
No. of observations	8	8		
Fetus, g	4137	3380	326.5	0.13
Placenta, g	305	242	30	0.16
Placentomes, no./ewe	83	67	5.3	**0.06**

Table 2. Effect of dietary Se on vascular development in maternal placental (CAR) and fetal placental (COT) tissues on day 130 of gestation in pregnant ewe lambs.

	Treatment			
Item	Control	Se-wheat	SE	*P*-value
No. of observations	8	8		
CAR				
CAD, %	41.6	40.2	1.6	0.58
CND, no./mm^2	1275.1	1473.3	81.8	0.23
CSD, µm/area[b]	7497.7	7737.8	374.5	0.66
APC, µm^2	361.6	308.9	23.0	0.15
TCV, mL	28.1	23.4	3.3	0.37
COT				
CAD, %	15.2	15.4	0.8	0.82
CND, no./mm^2	917.4	1100.8	64.5	**0.06**
CSD, µm/area[b]	29485.0	14392.5	8433.2	0.36
APC, µm^2	170.0	146.4	8.9	**0.08**
TCV, mL	35.8	26.8	6.1	0.12

[a] capillary area density – CAD, capillary no. density – CND, capillary surface density – CSD, area per capillary – APC, total capillary volume – TCV.
[b] Area analyzed = 340032 µm^2 per picture.

Table 3. Effect of dietary Se on cellular proliferation in maternal placental (CAR) and fetal placental (COT) tissues on day 130 of gestation in pregnant ewe lambs.

Item	Treatment		SE	*P*-value
	Control	Se-wheat		
No. of observations	8	8		
CAR				
Total cellular density, no. of cells/unit tissue area	187	238	7.6	**0.001**
Cell proliferation, %	0.66	0.44	0.06	**0.03**
COT				
Total cellular density, no. of cells/unit tissue area	105	107	3.025	0.57
Cell proliferation, %	4.04	4.26	0.295	0.61

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ASSOCIATIONS OF GROWTH AND CARCASS TRAITS WITH ATTAINMENT OF PUBERTY IN HEIFERS FED AT TWO LEVELS [1]

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ABSTRACT: Associations of growth and ultrasound carcass measurements with attainment of puberty by 13.5 mo of age were evaluated in heifers developed under two levels of feeding during a 160-d postweaning study. Heifers from the CGC composite (½ Red Angus, ¼ Charolais, ¼ Tarentaise) were randomly assigned to either control (fed to appetite; n=63) or restricted (fed at 80% of that consumed by controls adjusted to a common BW basis; n=62) feeding. Heifers were individually fed a diet of 67% corn silage, 18% alfalfa and protein-mineral supplement (DM basis) in pens equipped with Calan gates. Ultrasound measurements of LM area, intramuscular fat (IMF), and subcutaneous fat depth (FT) were made at d 140 (about 1 yr of age). Restricted feeding decreased ($P < 0.01$) DMI (4.2 vs. 5.7 kg/d), BW (297 vs. 322 kg on d 140), ADG (0.61 vs. 0.78 kg/d), LM area (53 vs. 59 cm^2), IMF (3.4 vs. 3.7 %) and FT (4.1 vs. 4.7 mm) when compared to control. Concentrations of progesterone in blood samples collected at 9 to 11 d intervals were used to ascertain pubertal status, which did not differ between restricted (29%) and control heifers (41%) by 13.5 mo of age (d 180). Logistical regression was used to analyze effects of treatment and individual traits on pubertal status at d 180. Proportion of heifers that were pubertal increased ($P < 0.05$) with increases in fat (IMF or FT) or ADG, irrespective of treatment. Increases in hip height, BW and LM area at 140 d were each associated ($P < 0.05$) with increases in puberty in control, but not restricted heifers. Pubertal status did not vary in association with gain to feed ratio or residual feed intake. Increased propensity for fat deposition and ADG positively influenced attainment of puberty whereas variations in efficiency of gain or residual feed intake did not.

Key Words: Heifer, Puberty, Efficiency

Introduction

Profitability of producing beef is influenced by factors that affect level and cost of production. Reproductive performance is one of the most important factors influencing level of production and expenses associated with winter-feed are a major factor influencing annual cost of production. Thus, producers are faced with a challenge of optimizing reproductive efficiency while minimizing feed costs. Technical limitations in measuring feed intake by individual animals have limited true measures of efficiency as units of output per unit input. Much of the research in this area has focused on efficiency of converting feed to body weight of growing animals (Herd et al., 2003); and some limited research on level of nutrient intake on milk production in beef cattle (Jenkins et al., 2000). Associations between feed efficiency and reproductive traits have not been explored.

During the past several years, interest and emphasis on selection for carcass traits has been increasing. Obvious benefits to producers using these selection approaches arise from premiums received, either directly or indirectly, for carcass traits. Because of potential genetic correlations between carcass and reproductive traits, it is important to establish how selection to improve carcass attributes may affect production characteristics of females retained in the cowherd. Puberty is the first expressed trait associated with reproduction and much of the research on feed efficiency to date has focused on the postweaning developmental period, therefore objectives of this study were to evaluate associations of feed intake, growth, feed efficiency and ultrasound carcass measurements with attainment of puberty in heifers developed under two levels of feeding during a 160-d postweaning period.

Materials and Methods

Heifers used in this study were from the CGC composite (½ Red Angus, ¼ Charolais, ¼ Tarentaise). Heifers represent a randomly selected population produced by mating GCG dams and sires (n=14) with consideration given to minimize inbreeding. Heifers were born between March 28 and May 30, 2003 (average date of birth 4/13/03 $\pm$ 13 days) and weaned on October 8 (177 $\pm$ 13 d of age; 196 $\pm$ 29 kg). Subsequent to weaning, heifers were divided into groups of six based on weaning weight ranking in order to minimize size variation within groups. Groups were randomly assigned to one of 22 pens. Each pen was approximately 5.8 m by 11 m, and fit with six individual feed bunks equipped with electronic Calan gates to allow for individual feeding. Heifers were allowed approximately one month for adaptation to the pens and to become trained to the head gates. During this time, heifers were allowed ad libitum access to the test diet fed once daily. Four heifers failed to learn to use the Calan gates, and were removed from the pens, leaving 4 pens with five rather than six heifers. Heifers were randomly assigned within pens

[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.

to either a control (n=64) or restricted (n=63) level of feeding. Feed restriction was initiated on December 2, 2003 and feeding treatment ended on May 10, 2004. Controls heifers were fed to appetite and restricted heifers were fed at 80% of that consumed by controls adjusted to a common BW basis. The diet consisted of 67% corn silage, 18% alfalfa, and 15 % protein-mineral supplement (DM basis). The diet was 36.1 % DM, 15.1 % CP, 24.6 % ADF and provided 1.57 Mcal/kg NEm and 0.97 Mcal/kg NEg. Weight of feed offered was recorded daily. Weigh back of uneaten feed was recorded as necessary to insure fresh feed was provided for each heifer on a daily basis. At the conclusion of the 160 d feeding period, one heifer was omitted from each feeding level due to chronic illness.

An average BW and hip height recorded on 2 separate days at the onset of the study (December 1 and 3) and at approximately 1-yr of age (April 19 and 21) were used as on test and 140 d measurements. Ultrasound measurements of LM area and ratio (width to height), intramuscular fat (IMF), and subcutaneous fat thickness (FT) were also collected at d 140, using an Aloka SSD-500 ultrasound machine with a 17.2 cm transducer (Aloka Co., Ltd, Wallingford, CT). Ultrasound images were collected and interpreted with Beef Image Analysis software (Designer Genes Technologies, L.L.C, Gustine, TX). Single measures of BW were recorded at approximately 28 d intervals throughout the study, and on d160. The serial BW measures were used to adjust feed level of restricted heifers using the following formula: (0.80 * (mean BW of restricted/mean BW control) * mean daily feed intake of controls. At completion of the feed restriction (d160), heifers were transferred out of individual feeding pens to larger pens for subsequent synchronization of estrus and artificial insemination.

Gain to feed ratio was calculated for each heifer by dividing BW change during the 140 d period by amount of feed consumed (DM basis). An average of the on test BW and 140 d BW was calculated for each heifer, and raised to the 0.75 power to provide a midpoint metabolic BW. Average daily gain was calculated for the 140 d period for each heifer. Residual feed intakes were obtained for heifers within feeding treatment for each heifer using the regression of daily DMI on average midpoint metabolic BW and ADG for the 140d period.

Blood samples were collected from the tail vein at 9 to 11 d intervals beginning at approximately 11.5 mo of age and ending on June1 (d 180, approximately 13.5 mo of age), the date when synchronization of estrus was initiated. Serum concentrations of progesterone in these blood samples were determined directly without extraction by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA) as reported previously (Bellows et al., 1991). Week in which puberty occurred was determined as the serum sample for which progesterone concentration exceeded 1 ng/mL. Because the majority of heifers failed to reach puberty by the end of the sampling period, data on pubertal status were treated as discontinuous (i.e., either yes or no by d180).

Following synchronization of estrus, heifers were subjected to AI and subsequently exposed to bulls for 6 wk. Bulls were removed from heifers on July 26. One month later, heifers were evaluated for pregnancy by transrectal ultrasonography with a 5 MHz transducer.

Effects of feeding level on feed intake, growth and ultrasound measurements of carcass were evaluated with the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included weaning weight as a covariate and feeding level as a class variable. Chi square analysis was used to compare proportions of heifers that reached puberty and pregnancy rates between the two feeding levels. The logistical regression procedure of SAS was used to analyze effects of feed level and performance/carcass traits on pubertal status. Separate regressions were run for each trait, fitting feeding level as a class variable, the individual trait as a continuous variable, and the interaction. The interaction and feeding level effects were removed from the model if not significant ($P > 0.14$). Because residual feed intakes were calculated within level of feeding, logistical regressions were run separately for each feed level. Predicted values for proportion of pubertal heifers were obtained from the logistic regressions to facilitate interpretation of the results.

Results and Discussion

Restricted feeding resulted in an average of 1.5 kg less ($P < 0.01$) DMI/d compared to control fed heifers (Table 1). When compared at d 140 of the study, feed restriction decreased BW, ADG, LM area, IMF and FT (Table 1). Feed restriction improved G:F and also influenced shape of the LM (i.e., decreased the width to height ratio, Table 1) but did not influence LM area/unit BW (data not shown). Hip height at d 140 (118 $\pm$ 4 cm) did not differ ($P = 0.95$) between feeding levels. The differences observed between the two levels of feeding are consistent with slower accretion of lean and fat in the restricted group, but no detectable difference in skeletal size. The improved G:F ratio is consistent with lower maintenance requirements of smaller animals, and may also be associated with earlier physiological stages of growth. However, differences in efficiency may not persist if compared at a common BW endpoint (Freetly, et al., 2001).

Table 1. Effects of feed restriction on feed intake, growth, gain to feed ratio (G:F) and ultrasonic measurements of LM, intramuscular fat (IMF) and fat thickness (FT) at d 140[1]

Trait	Restricted	Control	SE	$P <$
DMI, kg/d	4.17	5.67	0.04	0.001
BW, kg	294	323	2.4	0.001
ADG, kg/d	0.61	0.78	0.01	0.001
G:F, kg/kg	0.147	0.138	0.003	0.045
LM area, cm^2	52.7	59.6	0.9	0.001
LM ratio	0.59	0.62	0.005	0.002
IMF, %	3.4	3.7	0.06	0.006
FT, mm	4.1	4.7	0.2	0.03

[1] Values are least squares means and the larger SE.

Proportion of heifers that were pubertal by d 180 of the study, 13.5 mo average age, did not differ between restricted (29%) and control heifers (41%). Results from the logistic regression analyses of pubertal status on the various traits indicated that three traits (IMF, FT and ADG) influenced

pubertal status irrespective of feed level ($P > 0.14$ for feed level and interaction of feed level by trait). Probability of heifers being pubertal increased ($P < 0.01$) with increases in 140 d measures of fat (IMF or FT) or ADG. Estimated odds ratios for these traits are shown in Table 2. Interpretation of this statistic is that for every unit increase in the independent trait, there is an odds ratio fold increase in chance of a heifer being pubertal. Thus, for every one percent increase in IMF, chance of a heifer being pubertal increases by 1.66 fold. Magnitudes of the estimated odds ratios are influenced by the size of units used for expressing each trait, and thus direct comparisons of odds ratios across traits are not appropriate. Positive associations among fat deposition and rate of gain with pubertal status are consistent with genetic correlations among these traits reported previously (MacNeil et al., 1984). Collectively, these findings support the concept that traits indicative of early maturation of growth are paralleled by early maturation of the reproductive axis.

Increases in hip height, BW and LM area at 140 d were each associated with increased chance of control fed heifers being pubertal ($P < 0.02$), but not restricted heifers ($P \leq 0.09$ for interaction of trait and feed level). Odds ratio estimates for these traits are also shown in Table 2. The reason that these phenotypes were indicative of pubertal status in control heifers, but not the restricted heifers may be due to the fact that control fed heifers had greater opportunity to express differences associated with their genetic potential for these traits. Pubertal status did not vary in association with G:F ratio or residual feed intake. Because neither of these traits were associated with pubertal status, improved efficiency for maintenance and growth may not be associated with greater partitioning of nutrients for physiological process associated with attainment of puberty.

Table 2. Odds ratio estimates and upper and lower 95% confidence limits (CI) from logistic regression of effects of various traits on pubertal status

Trait	Odd ratio[2]	Lower CI	Upper CI	*P* <
IMF, %	3.24	1.42	7.43	0.006
FT, mm	1.66	1.27	2.17	0.001
ADG, kg/d	55.1	3.0	>999	0.008
[1]Hip Ht, cm	1.24	1.04	1.49	0.02
[1]BW, kg	1.04	1.02	1.06	0.001
[1]LM area, cm^2	1.12	1.04	1.21	0.003

[1]These traits were only significant in control fed heifers.
[2]Note that magnitudes of estimates are influenced by magnitude of unit used for each trait.

To provide additional interpretation of results from the logistic regression, predicted values for pubertal status were obtained for the range of each trait analyzed. Values for each trait that resulted in predictions of 25 and 45 percent of heifers being pubertal are shown in Table 3. This Table illustrates the relative change for each trait that would be associated with a 20 % increase in pubertal status. Hip height values shown for 25 and 45 % pubertal columns in Table 3 reflect 85 and 88 % of the average hip height in mature (> 4 yr of age; 134.6 cm) CGC cows. Likewise, BW values shown for these same two columns represent 55.5 and 60.1 % of BW (546 kg, BCS 5) taken on mature cows prior to breeding in June.

Table 3. Values of various traits that result in predicted proportions of pubertal heifers of 25 and 45 percent

Trait	25 % pubertal	45 % pubertal
IMF, %	3.23	3.99
FT, mm	3.56	5.33
ADG, kg/d	0.59	0.81
[1]Hip Ht, cm	114.6	118.7
[1]BW, kg	303	328
[1]LM area, cm^2	53.5	61.3

[1]These traits were only significant in control fed heifers.

Pregnancy rates to AI did not differ between the two levels of feeding (60 and 61 % for control and restricted heifers, respectively). Likewise, overall pregnancy rates were not different between control (95 %) and restricted (90 heifers

Implications

Factors that influence age of puberty can have large affects on reproductive success of replacement heifers. Increased propensity for fat deposition and average daily gain positively influenced attainment of puberty. Thus potential negative impacts on reproductive performance must be considered if selecting against fat accretion. Variations in efficiency of gain or residual feed intake did not appear to influence attainment of puberty.

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HEPARIN LEVEL EFFECT ON SPERM CAPACITATION OF FRESH AND FROZEN-THAWED BOVINE SEMEN

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ABSTRACT: To evaluate the effect of heparin level (0, 6, 8 and 10 μg/mL) on *in vitro* sperm capacitation as assessed by the chlortetracycline fluorescence assay on fresh and frozen-thawed semen, 3 Charolais (CH), 4 Hereford (HE) and 6 Limousin (LI) bulls were used. Semen was obtained by electroejaculation. From each ejaculate, 1 mL of diluted fresh semen was used to evaluate sperm capacitation, and the rest was frozen in straws. Either two or four frozen straws were thawed to evaluate sperm capacitation of frozen semen. Semen was purified through Percoll gradients and each sample divided into four subsamples that were randomly assigned to the four heparin treatments. Subsamples were incubated with heparin for 4 h at 38.5°C, under 5 % of CO_2 in air and 95 % humidity. A microscope with blue-violet illumination was used to evaluate rates of capacitated spermatozoa, both acrosome-intact (B pattern) and acrosome-reacted (AR). A model with fixed effects of heparin level, breed and their interaction, and random effect of bull within breed, was adjusted for analyses capacitation rates. Analyses were run separately for fresh and frozen semen There were no effects ($P > 0.05$) for the B pattern of capacitated frozen semen. The breed by heparin interaction effect was important ($P < 0.08$) for the B pattern of fresh semen. Capacitated, acrosome inctact spermatozoa increased with heparin level in semen from CH and HE, but no for LI bulls. The interaction was not important ($P > 0.10$) for AR spermatozoa. Proportions of AR spermatozoa increased ($P < 0.05$) as heparin level increased, up to 8 μg/mL, and was greater ($P < 0.05$) for fresh semen of LI bulls than for semen of HE and CH bulls (36.5 ± 2.2, 14.4 ± 2.7 and 8.7 ± 3.1 %, respectively). In conclusion, percentage of capacitated, acrosome-intact (pattern B) spermatozoa increased with heparin level in fresh semen, but not in frozen semen; however, heparin effect on sperm capacitation of fresh semen might vary among breeds.

Key Words: Sperm Capacitation, Bovine Semen, Heparin

Introduction

A step of *in vitro* fertilization protocols is sperm capacitation, for which an *in vitro* capacitating factor is normally used. Results reported by Pérez *et al.* (1996) suggest that probably this factor is not required with frozen-thawed semen, given that freezing causes a capacitation process similar to that obtained with addition of heparin to semen (Bailey *et al.*, 2002). However, McHugh and Rutledge (1998) found some variation on the effect of the freezing process on sperm capacitation of semen among species and among bulls within the same breed.

In our laboratory, Carrera (2003, personal communication) compared the binding patterns obtained with the chlortetracycline epifluorescence assay (CTC; Ward and Storey, 1984) for spermatozoa of fresh and frozen-thawed semen, with and without the addition of heparin, and observed that heparin was required for *in vitro* sperm capacitation of fresh semen but not for frozen-thawed semen; however, results were not clear for proportions of acrosome reacted spermatozoa. Adding heparin to semen resulted in less acrosome reacted spermatozoa for frozen-thawed semen than for fresh semen, which was not expected. Lu and Gordon (1988) reported that heparin level is important on sperm capacitation. In the study mentioned before, only one heparin level (10 μg/mL) was used for bulls of different breeds, which could have affected the observed results.

The objective of this study was to evaluate the effect of heparin level on in vitro sperm capacitation as assessed by the CTC assay on fresh and frozen-thawed semen.

Materials and Methods

Semen samples were obtained by electoejaculation of 3 Charolais (CH), 4 Hereford (HE) and 6 Limousin (LI) bulls from different breeders in the state of Chihuahua, México. Just after semen was collected, volume, appearance and color were checked. Then, the semen sample was put into a water bath at 36°C before a microscopic evaluation was conducted to decide wether the sample was adecuate for freezing or was not, using a citrate-yolk based extender.

From each ejaculate, 1 mL of diluted fresh semen was used to evaluate sperm capacitation and the rest was frozen in 0.5 mL straws using liquid nitrogen. Either two or four frozen straws were thawed by plunging them into a water bath (37°C) for 1 min to evaluate sperm capacitation of frozen-thawed semen. After thawed, semen was evaluated in the microscope for viability.

Semen was purified through Percoll gradients (45/90 %), centrifuged at 2000 rpm for 30 min, and each sample divided into four subsamples that were randomly assigned

to four heparin treatments (0, 6, 8 and 10 µg/mL). Subsamples were incubated with heparin for 4 h at 38.5°C, under 5 % of CO_2 in air and 95 % humidity.

Rates of sperm capacitation were evaluated by the chlortetracycline (CTC) method modified by Chamberland *et al.* (2001), using an Olympus BX41 microscope with blue-violet illumination and 40x epifluorescence lens. For each sample, 100 spermatozoa were counted and classified according to the CTC patterns: F, showing uniform fluorescence on the head, indicating uncapacitated, acrosome intact spermatozoa; B, represented by a fluorescence-free band on the post acrosomal region, indicating capacitated, acrosome intact spermatozoa; and AR, represented by a uniformly fluorescence-free head and with a fluorescence band in the equatorial region, indicating acrosome reaction. Percentages of the B and AR patterns were statistically analyzed separately for fresh and frozen-thawed semen using PROC MIXED of SAS (1999). The model included fixed effects of heparin level, breed of bull and their interaction, and random effect of bull within breed. When heparin effect was significant, a trend analysis was run by regression analyses, accross breeds when the interaction was not significant and by breed when the interaction was significant.

Results and Discussion

For frozen-thawed semen, there were no effects ($P > 0.05$) of heparin level, bull breed or their interaction, on the percentages of the B pattern spermatozoa. The freezing-thawing process causes some damage on the sperm membrane which is similar to the sperm capacitation process (Bailey *et al.*, 2002). Poor calcium control in cryopreserved sperm inhibits normal cell physiology, and the ability of the frozen-thawed sperm to regulate their levels of intracellular calcium has been found to be associated with *in vivo* fertility outcome (Bailey and Buhr, 1994).

In the case of fresh semen, the interaction of bull breed by heparin level was important ($P < 0.08$) for the percentages of the B pattern spermatozoa. For the CH and HE bull breeds, there was an increase (linear regression coeficients = 5.18 and 3.15, respectively) in the proportion of B pattern spermatozoa as the heparin level increased, but this was not ($P > 0.05$) the case for the LI bull breed (Figure 1).

For the percentages of AR pattern spermatozoa, there was not an interaction effect ($P > 0.05$) of heparin level by bull breed effect, for either fresh or forzen-thawed semen. The proportion of AR pattern spermatozoa was increased ($P < 0.05$) as the heparin level was increased (Figures 2 and 3), and there was a larger ($P < 0.05$) proportion of AR pattern spermatozoa in fresh semen of LI bulls than for HE and CH bulls (36.5 ± 2.2, 14.4 ± 2.7 and 8.7 ± 3.1 %, respectively; Figure 4). McHugh and Rutledge (1998) have found that each breed has a different response to heparin level.

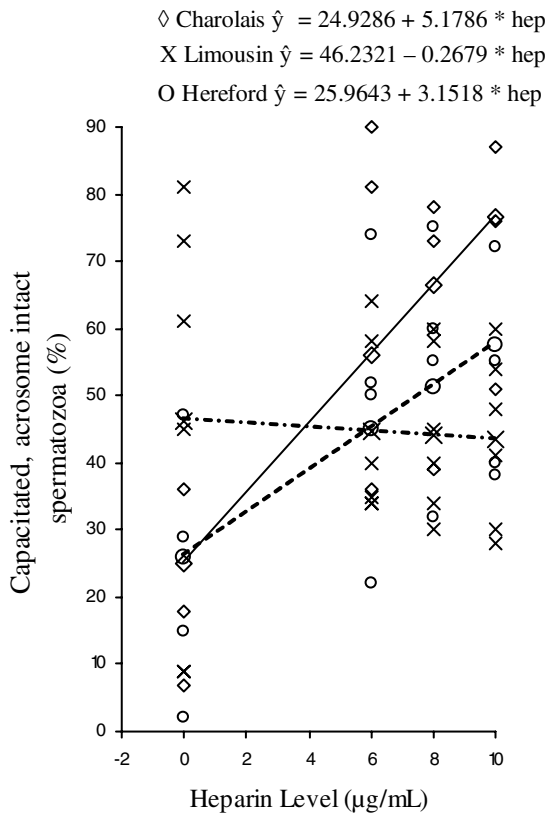

Figure 1. Percentages of capacitated, acrosome intact spermatozoa (B) in fresh semen of Charolais, Hereford and Limousin bulls, with different levels of heparin.

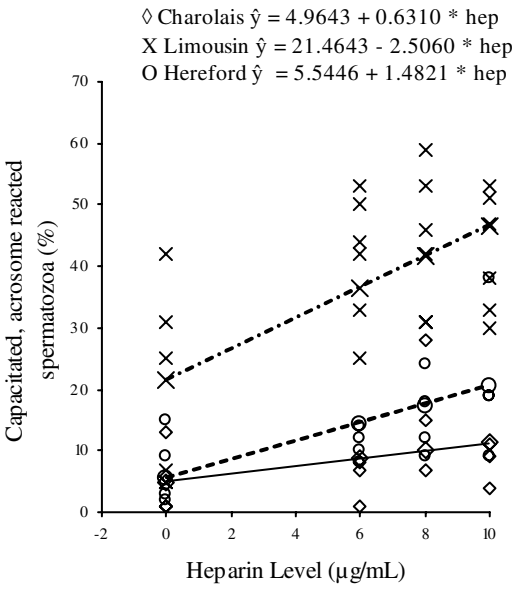

Figure 2. Percentages of capacitated, acrosome reacted spermatozoa (AR) in fresh semen of Charolais, Hereford and Limousin bulls, with different levels of heparin.

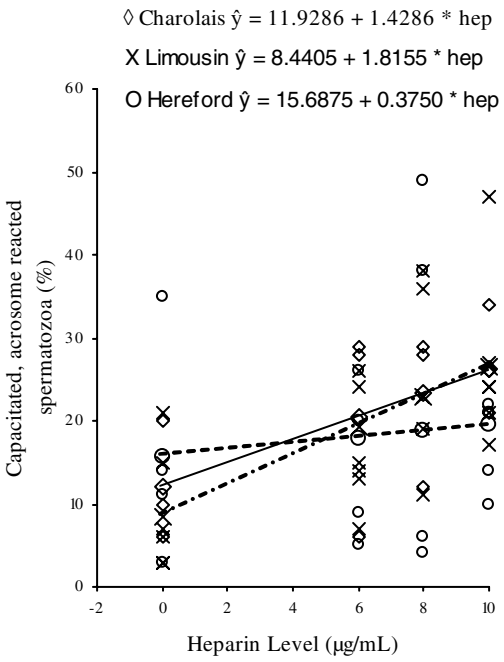

Figure 3. Percentages of capacitated, acrosome reacted spermatozoa (AR) in frosen-thawed semen of Charolais, Hereford and Limousin bulls, with different levels of heparin.

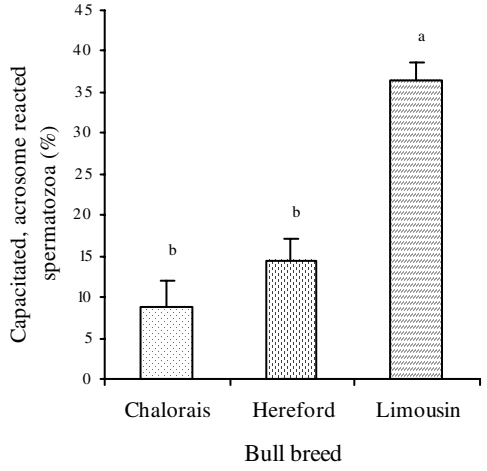

Figure 4. Lsmeans (± E.E.) of percentages of capacitated, acrosome reacted spermatozoa (AR) in fresh semen of Charolais, Hereford and Limousin bulls.

Implications

Percentage of capacitated, acrosome-intact (B pattern) spermatozoa increased with heparin level in fresh semen, but not in frozen-thawed semen; however, heparin effect on sperm capacitation of fresh semen might vary among breeds. Given that for frozen-thawed semen, addition of heparin did not increase the percentage of capacitated spermatozoa any further compared to the 0 µg/mL, heparin may not be required for the *in vitro* capacitation process of frozen-thawed bovine semen used for *in vitro* fertilization protocols. For *in vitro* sperm capacitation in fresh semen, the optimal heparin level to be used must be defined according to the bull breed and, probably, according to the particular bull to be used.

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QUALITY STANDARDS FOR SOMATIC CELL COUNTS AND ACCURACY OF POOLED SAMPLES TO DETECT SUBCLINICAL MASTITIS IN EWE'S MILK

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ABSTRACT: Forty-eight multiparous and primiparous lactating Rambouillet ewes were used to 1) establish a standard somatic cell count (SCC) value for classifying normal and abnormal ewe milk, and 2) examine whether pooling milk from the right and left udder halves would indicate an accurate SCC, comparable to taking the average of individual sides. At weaning, 50 mL milk samples were collected from each side, after which 15 mL from each side was pooled. Samples collected from the left and right side and the pooled sample were classified as normal (<500,000 cells/mL^{-1}) or having subclinical mastitis ($\geq$ 500,000 cells/mL^{-1}); values were transformed into log10. To establish the 99% confidence interval (CI) and individual upper limit range for SCC in milk considered to be normal, two parameters were examined: SCC difference between right and left udder halves and the SCC for pooled samples. The 99% CI for the mean SCC ranged between 1.9 log10/µl and 3.2 log10/µl for the difference between sides, with an individual animal's upper prediction limit not exceeding 6.7 log10/µl. The 99% CI for pooled SCC ranged between 3.8 log10/µl and 4.5 log10/µl, with an individual animal's upper prediction limit not exceeding 6.4 log10/µl. Once the ranges for normal milk were established, mastitis samples were added to the data set and evaluated. All samples classified as mastitis exceeded the difference and pooled upper limits and were outside the range associated with normal milk. Milk SCC averaged from the right and left side did not differ (P = .70) from the pooled SCC values (6.2 and 6.3 $\pm$ .14 log10/µl; mean $\pm$ SE, respectively). The SCC were increased (P < .0001) in ewes with mastitis compared to normal ewes (4.18 and 8.30 $\pm$ .22 SE log10/µl, respectively). This data helps establish an SCC value range that can be used to classify milk in ewes with and without mastitis. Additionally, a pooled sample can be used to accurately determine SCC for the left and right side in normal and mastitic ewes.

Keywords: Ewe, Mastitis, Milk

Introduction

Mastitis is defined as an inflammation of the mammary gland caused by infection or excessive stress to the mammary tissue (Keisler et al., 1992; Forde et al., 2003). Mastitis causes a decrease in milk production, which in turn causes poor growth performance in lambs, reduction in milk quality and quantity, premature culling, and in severe cases, death (Maisi et al., 1987), thereby decreasing profits for the producer. However, treatment of this disease many times is based on cost versus benefit and longevity of the infected animal.

Somatic cell counts are widely used to determine udder health and incidence of non-symptomatic subclinical mastitis in dairy cattle (Haenlein, 2002). Specific standards for SCC have been set by the dairy industry, which are used to indicate if an animal has subclinical mastitis. A SCC value above 5.3 log10/µl is the standard used by the Dairy Herd Improvement Association. Haenlein (2000, 2001) reported similar standard values for small ruminants are not available and suggested the standard for dairy cattle may be inappropriate for sheep and goats. Fthenakis et al. (1991) reported that values exceeding 6.9 log10/µl were indicative of subclinical mastitis if samples were collected from what appeared to be healthy mammary glands. Other studies conducted in sheep have reported values as low as 6.4 log10/µl in two French populations to over 7.6 log10/µl in a population in Cyprus, giving a large variation as an indicator of subclinical mastitis (Cremoux, 1998; Mavrogenis et al. 1995). It would be much more relevant to the sheep industry if a minimal SCC threshold standard could be established that could be used to predict udder infection before the animal showed clinical signs of mastitis. A producer could decide whether to treat and keep or cull the infected animal.

In the dairy industry, a pooled sample from the four quarters can be used to determine SCC and detect potential mastitis problems. The test is very inexpensive and costs about $.50/sample. The same procedure can also be used to determine SCC in sheep milk, however, because a ewe has two udder halves two samples are required for each animal. How accurate a pooled sample is in determining incidence of subclinical mastitis, in sheep particularly, if only one side is infected, is unknown, but would be financially beneficial to the producer, decreasing the cost by half.

The objectives of this study are to 1) establish a standard SCC value for classifying normal and abnormal ewe milk, and 2) examine whether pooling milk from the right and left udder halves would indicate an accurate SCC, comparable to taking the average of the individual sides.

Materials and Methods

Animals. Forty-eight multiparous and primiparous lactating Rambouillet ewes (3 to 7 yr old) were selected for this study. Ewes lambed within a 4-wk period starting 3/24/04. The ewes were group-fed alfalfa

pellets (2.72 $kg^{-1} \cdot head^{-1} \cdot d^{-1}$) and 0.23 kg $corn^{-1} \cdot head^{-1} \cdot d^{-1}$ for the first 30 d of lactation. Following the 30-d period, ewes and their offspring were moved to pasture and maintained there until lambs were weaned.

Sampling Procedures. Lambs were weaned at 98 ± 7 days of age and put on pasture. Following weaning, ewes were taken off feed and maintained in the dry lot overnight. Ewes had free access to water. Milk was allowed to accumulate in their udders' during the 24-h period. The next morning, milk samples were collected from each side of the ewe's udder. Prior to sampling, the teats were disinfected using nolvasan spray and then wiped with Pampers baby fresh alcohol-free wipes. The first ~3 mL of milk from each side was discarded, after which 50 mL were collected from each side. The samples were kept cool until brought to the lab. In the lab, 15 mL from each side sample was then pooled and gently mixed. The right, left, and pooled samples (all kept cool) were sent to the Dairy Herd Improvement Association (DHIA) laboratory located in Fresno, CA the same day of collection. Samples were analyzed for SCC percentage in right, left, and pooled samples.

Statistical Procedure. SAS software version 9.1 (SAS Corporation, Cary, NC) was used to perform all statistical analysis. Excel was used to chart scatterplot graphs. Ewes with SCC's above 500,000 cells/mL^{-1} (6.2 log10/µl) were classified as having mastitis and ewes below that value to be mastitis-free. This value was selected based on previous studies conducted by McFarland (2000) and Surian (2001). Seven ewes were classified as having mastitis and 41 ewes were classified as mastitis-free. All SCC values were transformed to the logarithm to the base of 10 to decrease variation before statistical analysis. The SAS MACRO UNIVAR (Fernandez, 2002) was used to determine the 99% confidence interval (CI) mean and upper limit SCC for individual ewes for the average SCC between the right and left udder halves and the pooled sample in ewes considered to be mastitis-free. The SAS MACRO FIXONEQL (Fernandez, 2004) was used to perform a one-way ANOVA to determine the effect of mammary status (mastitis or mastitis-free) on SCC difference between right and left sides and the pooled samples. A two (mastitis status) x two (sample type) factorial arrangement ANOVA using SAS MACRO FXQLQL (Fernandez, 2005), was used to examine a pooled sample versus average sampling between the right and left udder halves. The average of two sides was used because it represented ewe's SCC status as compared to separate sides.

Results and Discussion

Objective 1. The 99% CI for the mean SCC ranged between 1.9 log10/µl and 3.2 log10/µl for the difference between sides, with an individual animal's upper prediction limit not exceeding 6.7 log10/µl. The 99% CI for pooled SCC ranged between 3.8 log10/µl and 4.5 log10/µl, with an individual animal's upper prediction limit not exceeding 6.4 log10/µl. This indicated that ewes could be classified as having normal SCC with counts not exceeding a 99% CI of 4.5 and an upper limit of 6.4. Ewe data with mastitis (those with at least one udder half or pooled sample above 6.2 log10/µl) were then added to the normal ewe data and graphed on a scatterplot (Figure 1). The mastitic ewes that exceeded difference and pooled upper limits were considered outliers.

Because of the extensive research conducted in dairy cattle, there are set quality standards for SCC in milk. The current maximum levels are less than 5.3 log10/µl for individual animals (National Mastitis Council, 2004). Sheep data is highly varied and every study seems to have a different acceptable level, plus factors such as stage of lactation and time of sampling must be taken into account when examining milk SCC. Fthenakis (1996) found that SCC increased with advanced stage of lactation. He concluded that 6.9 log10/µl could be considered the individual upper limit for SCC in milk from healthy ewes and that non-pathological factors can also affect SCC. Cremoux (1998) reported that two French sheep populations had an SCC range between 6.4 log10/µl and 6.7 log10/µl, in both normal and mastitic ewes, depending on stage of lactation. Mavrogenis et al. (1995) found the average SCC for non-infected Chios ewes was 7.4 log10/µl while infected Chios ewes had 7.6 log10/µl. It was the suggested that a threshold level for subclinical mastitis in sheep should be about 7.3 log10/µl. In contrast, Saratsis et al. (1999) found that sheep induced with *S. epidermidis* showed SCC above 6.9 log10/µl while control ewes were below 6.9 log10/µl. All of these data indicate the wide range of SCC that is acceptable in ewe's milk and that there is no set standard. Our results suggest that the upper limit should be considerably lower with an upper acceptable limit of 6.4 log10/µl for normal ewes.

Objective 2. Milk SCC averaged from the right and left side did not differ (P=.70) from the pooled SCC values (6.2 and 6.3 ± .14 log10/µl; mean ± SE, respectively). The SCC were increased (P<.0001) in ewes with mastitis compared to normal ewes (4.18 and 8.30 ± .22 SE log10/µl, respectively). This data indicates that there is no difference in SCC between an average and a pooled sample of both sides. Therefore, a pooled sample gives an accurate measure of SCC for the right and left side and can be used to indicate normal or abnormal ewe milk.

While it is common practice to pool the four quarters of the udder milk in dairy cattle for one sample for SCC and microbiology testing, collecting individual samples from both the right and left udder half is the common practice in ewes. However, individual testing of sides is not as economical as pooling the right and left sample in that it would decrease producer cost in half. Testing is essential despite cost, as Haenlein (2001) reported that the greatest economic importance to the producer is the SCC, which indicates the ewe's udder health status and is the widely applied health quality standard for accepting consumable milk. Allowing a pooled sample to be tested can allow the producer to save money and know the health status of his flock, therefore producing a higher quality product.

Implications

This study indicates a much lower SCC value should be used when classifying ewe milk for determining subclinical mastitis then previously reported. Further research needs to be conducted to validate these values, however, these data should help toward establishing a quality standard for the sheep industry. This data also suggests that a pooled sample can be used to accurately determine SCC for the left and right side in non-mastitic ewes and detect mastitis in a ewe with at least one side that is infected.

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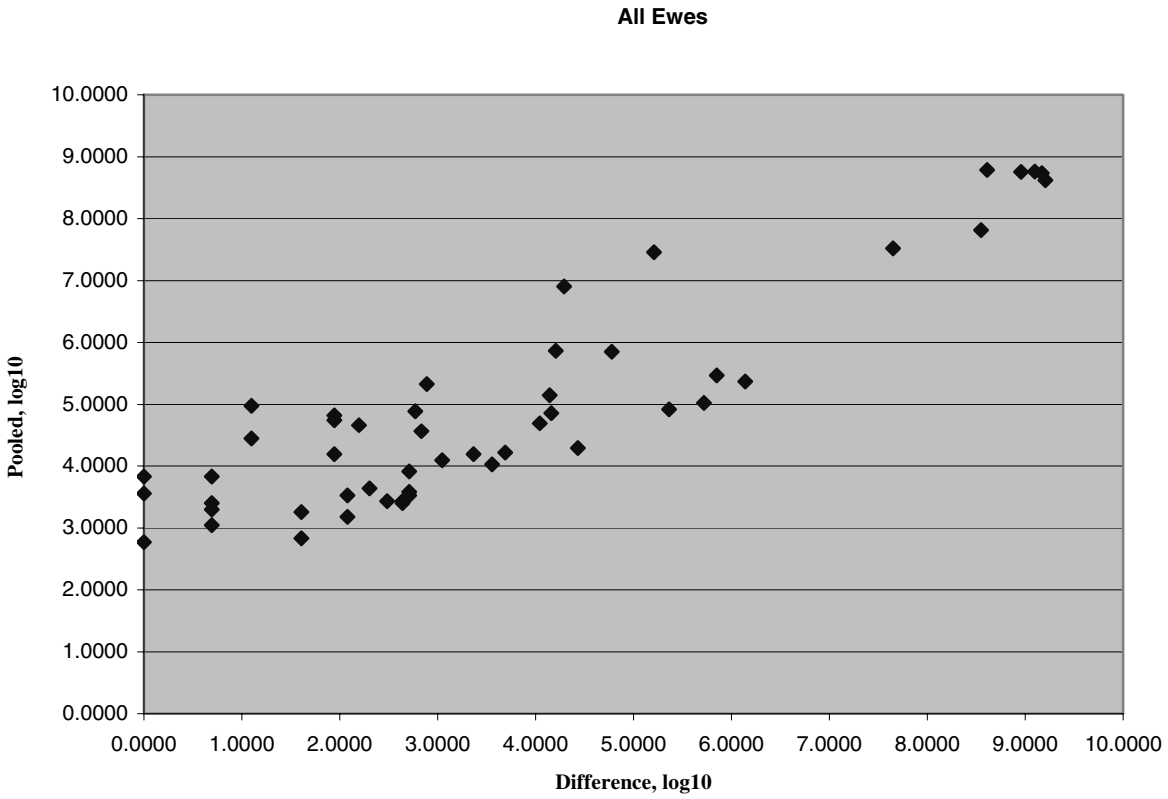

Figure 1. Scatterplot of ewes classified as having mastitis or being mastitis-free as determined by difference (difference between right and left udder halves) and pooled (pooled sample of right and left udder halves). Ewes with a pooled SCC above 4.5 log10/µl and a difference above 6.7 log10/µl were classified as having subclinical mastitis.

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CHANGES IN PITUITARY GENE EXPRESSION BETWEEN SURGICALLY CASTRATED AND LHRH IMMUNOCASTRATED MALE RATS

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ABSTRACT: The objective of the study was to characterize changes in regulation of pituitary gene expression in both surgically castrated and LHRH immunocastrated male rats using microarray analysis. This model provides a basis to study the molecular aspects of immunocastration as a means to suppress reproduction in livestock. Sprague Dawley male rats (8 wk of age) were randomly divided into four treatment groups: intact (n = 12); castrated (n = 12); LHRH immunized (n = 15); and LHRH immunized and castrated (n = 12). Rats immunized against LHRH received a primary injection and two booster injections of an LHRH fusion protein, while intact and castrated animals received vehicle injections on d 0, 35, and 96. Blood samples were collected monthly for 208 d to quantify LHRH antibody binding and testosterone concentrations. Body weight among treatment groups was similar on d 0 (P = 0.8; mean = 323 ± 8.4 g). By d 63, testosterone concentrations decreased for castrated and immunocastrated treatment groups compared to intact males (P < 0.01). Similarly, paired testes, and epididymal weights at sacrifice were greater (P < 0.01) for intact (3.6 ± 0.2 and 1.6 ± 0.02 g, respectively) than LHRH immunized (0.84 ± 0.2 and 0.4 ± 0.01 g, respectively) males. Seminiferous tubule atrophy associated with LHRH immunization decreased tubule diameter for immunized rats to half that of intact controls (100 ± 0.8 µm vs 260 ± 0.7 µm; P < 0.01). Histological examination of pituitaries demonstrated the formation of hypertrophic castrate cells in surgically castrated but not LHRH immunized rats. Microarray analysis identified 67 pituitary genes that changed 2-fold or greater in the LHRH immunized group compared to the castrate group. Higher expression of several genes in the castrate group was confirmed in independent samples by real-time PCR. This study has begun to identify pituitary genes that are uniquely regulated by LHRH immunocastration. These data can be used to understand cellular and molecular changes that occur in animals following LHRH immunocastration.

Key Words: Immunocastration, Microarray, Male, Pituitary, Rat

Introduction

The physiological aspect of active immunization against LHRH as a mechanism by which to modulate reproduction has been investigated extensively in livestock (Falvo et al., 1986; Brown et al., 1994; Aïssat et al., 2000) and rodents (Fraser et al., 1974; Awoniyi et al., 1993). Interest in the cellular basis of LHRH active immunization arises from the knowledge that although immunocastration and surgical castration are both effective methods used to suppress reproduction, the manner by which each is achieved is unique. Following surgical castration and complete removal of testosterone negative feedback on the pituitary and hypothalamus, concentrations of LH and FSH increase dramatically (Schanbacher, 1982). Conversely, active immunization against LHRH results in immunoneutralization of LHRH by binding the decapeptide before it recognizes its membrane receptors on gonadotropes. This effectively suppresses the synthesis and secretion of LH and to a lesser extent FSH, which results in a subsequent decline in gonadal steroids (Brown et al., 1994; Aïssat et al., 2000). The downstream molecular signals altered by LHRH immunocastration have yet to be elucidated. Therefore, the objective of the present study is to characterize genes in the pituitary that respond differentially to surgical or immunological castration and evaluate histological changes in the anterior pituitary associated with each type of castration.

Materials and Methods

Preparation of Antigen

Ovalbumin-LHRH-7 fusion protein was prepared using recombinant DNA technology as previously described (Zhang et al., 1999). The *E. coli* strain BL21(DE3) (Novagen Inc., Madison, WI) was used to express the fusion protein. The recombinant fusion protein expresses a 6-histidine sequence (His-tag, Novagen, 1994) at the carboxyl terminus to facilitate purification of the LHRH fusion protein via nickel affinity chromatography. Fifty µg of purified recombinant ovalbumin or recombinant ovalbumin LHRH-7 was emulsified in a water-oil adjuvant to yield a total injectable volume of 100 µl/rat. *Mycobacterium butyricum* (CalBiochem, San Diego, CA),

was used as an immunostimulant for the primary immunization and the bacterial proteins were excluded in subsequent booster injections.

Animals and Treatments

All procedures were approved by the Washington State University Institutional Animal Care and Use Committee. Sprague Dawley male rats (8 wk of age = d 0) had ad libitum access to food and water and were exposed to a 14L:10D photoperiod. Following a one-month acclimation period, rats were randomly assigned to one of four treatment groups: intact; castrated; LHRH immunized; and LHRH immunized and castrated. The intact control (n = 12) and castrate groups (n = 12) received 50 µg/rat of recombinant ovalbumin protein, while LHRH immunized (n = 15) and LHRH immunized and castrate (n = 12) rats received 50 µg/rat of recombinant ovalbumin LHRH-7 as a single subcutaneous injection at the base of the tail on d 0, 35 and 96. Surgical castration occurred on d 40 of the study. All rats were sacrificed on d 208; testes, epididymal, prostate, and seminal vesicles were collected and weighed. Testicular tissue samples obtained from each animal were placed in Bouin's fixative for histological examination (Sigma-Aldrich, St. Louis, MO). Three representative rat pituitaries from each treatment group were collected and preserved in 10% buffered formalin for histological examination.

Blood samples and body weights were collected monthly for the duration of the study. Blood was collected via the saphinous vein and allowed to clot at 4° C overnight; serum was separated by centrifugation at 1,500 x g for 20 min at 4° C, decanted into plastic vials and stored at - 20° C until assayed.

Hormone Analysis

Serum testosterone and LHRH antibody binding were evaluated as an indication of vaccine efficacy. Testosterone was quantified by solid-phase RIA utilizing components of a commercial kit from Diagnostic Systems Laboratory (DSL, Webster, TX). LHRH antibody titers were evaluated by percentage of ^{125}I –LHRH bound after serum was diluted 1:1000 as described by Johnson et al. (1988).

Affymetrix GeneChip Analysis

Anterior pituitaries from nine rats per group were collected, snap frozen in liquid nitrogen, and stored at –80 °C. To obtain necessary amounts of RNA, three anterior pituitaries per group were pooled. RNA was purified using TRIzol, according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Total cellular RNA (10 µg) from two independent pituitary pools was used to synthesize the microarray target for each treatment group. Affymetrix rat 230AGeneChip containing 16,000 genes, was used in all hybridizations. Results were analyzed using Affymetrix Microarray Suite 5.0 (MAS) software.

Statistical analysis

Percent LHRH antibody binding, testosterone, and body weight were analyzed using Proc Mixed procedures of SAS (SAS Inst. Inc, Cary, NC). The model included treatment and was tested using animal with treatment as the error term. Day and treatment x day interaction was tested using the residual mean square. When a significant treatment x day interaction was detected, treatment effects were examined within day. Orthogonal contrasts were used to compare treatment groups with the expected similar and different biological response for a specific trait. Reproductive weights were subjected to a one-way completely random design and least square means are reported.

Results

Body Weight

A treatment x day interaction was detected for body weight ($P > 0.01$). At the initiation of the study (d 0) no difference was detected in body weight ($P = 0.47$; average BW = 325 ± 8.3 g). Likewise, body weights among treatment groups were similar on d 35 and 63 ($P > 0.10$). By d 129, body weight was greater ($P > 0.01$) for intact controls (586 ± 14.9 g) than castrate, LHRH immunized and LHRH immunized and castrated groups (521, 520, 532 ± 14.9 g, respectively). Similarly, greater body weight for intact controls was demonstrated on d 161 and at the end of the study (d 208; $P < 0.01$), with no detectable difference among castrated, LHRH immunized or LHRH immunized and castrated groups.

LHRH Antibody Binding and Serum Testosterone

Repeated measures analysis revealed a treatment by day interaction ($P = 0.01$); therefore, effect of treatment on LHRH antibody bound was examined within day. LHRH antibodies were detected by d 35 in LHRH immunized rats (5.7 ± 1.4 %) and LHRH immunized and castrated rats (6.8 ± 1.5). Percent LHRH binding increased following each injection and was higher at the time of sacrifice (d 208) compared to rats receiving the vehicle alone.

A treatment x day interaction ($P < 0.01$) for testosterone necessitated examination of testosterone concentration within day. Testosterone concentrations at initiation of the study varied such that castrated and intact rats had higher circulating testosterone (5.8 ± 0.8 ng/mL and 6.7 ± 0.9 ng/mL, respectively) than LHRH immunized or LHRH immunized and castrated (3.3 ± 0.8 ng/mL and 3.0 ± 0.9 ng/mL, respectively) rats ($P < 0.01$). Serum testosterone concentrations decreased dramatically after surgical castration. A decline in testosterone concentrations were observed in LHRH immunized rats 35 d after primary immunization. All immunized and castrated groups had similar concentrations of testosterone by d 63 ($P = 0.88$). Intact rats continued to display a greater testosterone concentration compared to all LHRH immunized rats for the duration of the study, but a gradual decline in testosterone was noted over the course of the study.

Testes and Pituitary Measurements

At sacrifice testis samples were collected from each rat. Seminiferous tubule atrophy in LHRH immunized rats resulted in a marked decrease in tubule

diameter compared to intact controls (100 ± 0.8 μm vs 260 ± 0.7 μm; $P < 0.01$). Testes of immunized rats were completely devoid of spermatozoa whereas normal spermatogenesis was observed in controls. Additionally, vacuolization of basophilic cells in the pituitary was noted in surgically castrated male rats (Figure 1). Intact rats or those receiving immunocastration alone or in combination with surgical castration did not develop the hypertrophic pituitary cells.

Paired testis and epididymal weight at the time of sacrifice was greater ($P < 0.01$) for intact rats (3.6 ± 0.2 and 1.6 ± 0.02 g, respectively) than immunized rats (0.84 ± 0.2 and 0.4 ± 0.01 g, respectively).

Genes involved in surgical and immunological castration

Differential gene expression was determined using absolute and comparison analysis with the use of MAS 5.0. All possible combinations of microarray comparison analysis were made among the different treatment groups. After examination of the various analyses, the most biologically relevant treatment group comparisons were determined to be LHRH immunized and castrate vs. castrate, and LHRH immunized vs. castrate. In both cases immunized groups were compared using the castrate group as baseline expression data. Pituitaries from LHRH immunized and castrated rats showed a total of 99 genes that were different from the castrate baseline by at least two-fold. Similarly, LHRH immunized rat pituitaries showed 67 genes that differed from the castrate baseline by at least two-fold. When these comparisons were examined for genes that were similar between both groups, sixteen were identified.

Genes that changed two-fold or greater were clustered based on gene ontology. Several genes in this group related to signaling and immune function. Expression of FSHβ, LHβ, Rgs4, and Kcnmb4 were decreased in LHRH immunized rats. The down regulations of these genes in relation to the castrate group were verified by real-time PCR.

Discussion

This study indicates that immunization against LHRH acts to suppress reproduction in a manner that uniquely modulates pituitary gene expression from that of surgical castration. However, the effect of immunization against LHRH on body weight is consistent with weight gains for intact bulls compared to steers (Huxsoll et al., 1998; de A. Ribeiro et al., 2004). In this study no difference in growth weight was observed in intact controls until 129 d after the primary injection. However, for each weight collected after this point the intact controls were heavier compared to castrated, LHRH immunized or rats which received both LHRH immunization and surgical castration.

LHRH antibody titers were effective in down regulating the expression of LHβ and FSHβ genes and consequently suppressing serum concentrations of testosterone. Long-term suppression of LH and FSH, caused by immunization against LHRH, has been shown to cause testicular atrophy that subsequently leads to decreased testosterone concentrations and eventual disruption of spermatogenesis (Awoniyi et al., 1989; Schanbacher, 1982; Cook et al., 2000). The effect of immunocastration on pituitary LH and FSH secretion are vastly different than those seen in surgically castrated animals. Gonadectomy leads to LH concentrations of 5 to 10 times greater than intact males (Parlow, 1964). Furthermore, this increase in gonadotrophins results in vacuolization of the multihormonal basophilic pituitary cells, known as castrate cells. Interestingly there are no castrate cells in rats that were immunized against LHRH alone or in concert with surgical castration. In fact, LHRH immunized and LHRH immunized and castrated rats had pituitary morphology comparable with that of intact controls. This absence of castrate cells in the pituitary following immunocastration is consistent with findings previously reported in LHRH immunized pigs (Molenaar et al., 1993).

Following immunocastration, the expression of genes in the pituitary is suppressed when compared to expression in the pituitary of castrated rats. Genes related to hormonal regulation of reproduction were repressed following immunization against recombinant ovalbumin LHRH-7. The expression of FSHβ and LHβ were significantly repressed following immunocastration. This was an expected effect since immunoneutralization of LHRH has been shown to suppress circulating concentrations of the pituitary gonadotropins, LH and FSH (Brown et al., 1994; Aïssat et al., 2002). Similarly, a decrease was detected for regulator of G-protein signal 4 (Rgs4). Though this gene had not previously been identified to respond to active immunization against LHRH, its expression may be expected as Rgs4 has GTPase activity (De Vries and Farquhar, 1999; Wieland and Mittmann et al., 2003). Additionally, expression of Cd74, Ifitm3I, and H2-M3, genes related to immune function, were affected by immunocastration when compared to surgical castration. Again this might be an expected result due to the immunomodulatory effects of active immunization against LHRH. These results are likely attributed to the effects of immunocastration and not a result of either the adjuvant or the vehicle since castrated rats received the same amount of adjuvant and vehicle as the immunocastrated groups.

Implications

Oligonucleotide microarrays were utilized to provide information on gene expression of the anterior pituitary gland after animals are exposed to either surgical or immunological castration. Information from these analyses can be used to further understanding of the molecular and cellular mechanisms by which immunological castration is effective.

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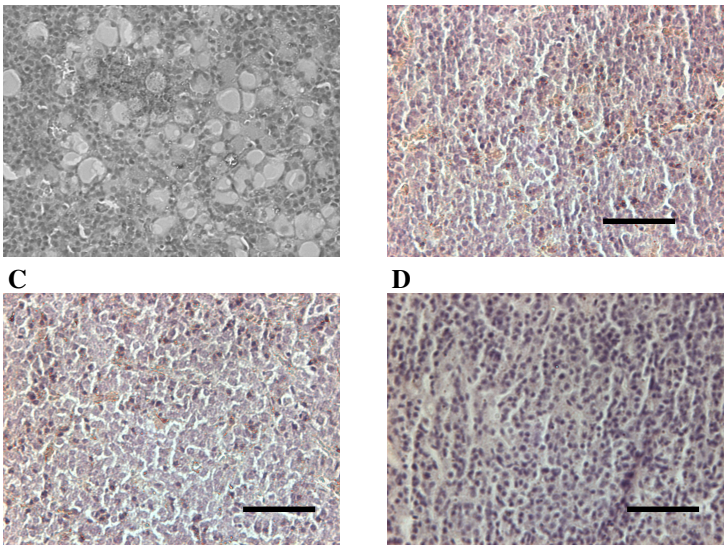

Figure 1. Photomicrograph of pituitary cross sections from A) castrated, B) LHRH immunized, C) LHRH immunized and castrated, and D) intact male Sprague Dawley rats. 100 x magnification. Bar = 100 µm. Note that the castrate cells in A are not found in LHRH immunized, LHRH immunized and castrate or intact rat pituitaries.

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CALCIUM ABSORPTION AND CALCIUM KINETICS IN SHEEP FED NON CONVENTIONAL PHOSPHATES

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ABSTRACT: To determine calcium absorption and calcium kinetics in sheep fed non conventional phosphates, 20 crossbred West African lambs were supplemented with two Venezuelan sedimentary phosphates, Riecito (RIO) and Montefresco (MONTE), and a fertilizer, triple superphosphate (TSP), using a dicalcium phosphate as reference control, with (DICAL+F) and without (DICAL) F addition, as NaF (500 ppm). The experimental diets contained 14% CP, 2.2 Mcal ME/kg, 0.36 total P and 0.60% Ca. Calcium apparent absorption (AA) and F apparent retention were measured using a total collection method, with four animals/treatment. Sheep were kept in metabolic crates, 14 days for adaptation and 7 days to record feed intake and faecal excretions. The animals were previously fed the same diets during a 12 month period. Calcium kinetics was determined by orally dosing the sheep with 200 µCi ^{45}Ca. Blood samples were taken periodically after dosing and 168 h later the animals were slaughtered to remove the femur for chemical analyses. In addition, bone mineralization data were recorded. Calcium AA (%) was greater (P<0.05) in animals fed DICAL (71.43), DICAL+F (70.87) and RIO (69.74) and lower (P<0.05) for MONTE (62.67) and TSP (59.06). Fluorine apparent retention (%) was higher (P<0.05) for DICAL+F (78.52) followed by MONTE (56.65), TSP (52.41) and RIO (54.23). Bone ash content (%) was higher (P<0.05) for DICAL (61.8) and TSP (63.6), followed by DICAL+F (60.3) and RIO (60.7) and (P<0.05) MONTE the lowest (57.8) .The entry of ^{45}Ca from DICAL treatment to the central pool (t½; h) was faster (1.8; P<0.05) than TSP (8.48) and DICAL+F (18.29), and these more than RIO (38.72) and MONTE (33.16). Blood specific activity showed similar trend. Radioactive Ca leaving the central pool showed a bi-exponential pattern with all phosphorus sources. Bone ^{45}Ca was higher in the less mineralized bone tissue. It is concluded that Ca utilization could be impaired in sheep fed high fluorine phosphates.
Key Words: Absorption, Kinetics, Phosphates, Calcium, Ruminant

Introduction

In Venezuela, cattle, sheep and goat production is a pastoral activity, subject to climatic and soil fertility conditions that determine the quality and quantity of forage available to animals. Under these conditions, phosphorus deficiency is one of the most important limiting factors for grazing animals (McDowell *et al.*, 1993). Calcium deficiency is also generally associated to phosphorus deficiency. In most cases, feed grade calcium-phosphate supplements are expensive, so that non conventional calcium-phosphorus sources, such as raw rock phosphates and fertilizers, are often used.

In preceding papers (Godoy *et al*., 2002; Godoy y Chicco, 2004) productive and apparent digestibility data of Venezuelan sedimentary phosphates and of a high fluorine phosphate fertilizer were reported. However, very little information is available on calcium absorption of these phosphates in ruminants.

Therefore, the purpose of this paper is to present data on calcium absorption and calcium kinetics of raw rock phosphates most frequently used in Venezuela, including a phosphorus fertilizer (triple superphosphate)

Materials and Methods

To measure Ca absorption and Ca kinetics in sheep fed non conventional phosphates, 20 West African yearlings, averaging 48.5 kg body weight, were supplemented with two Venezuelan sedimentary phosphates, Riecito (RIO) and Montefresco (MONTE) and a fertilizer, triple superphosphate (TSP), using a dicalcium phosphate as a reference control, with (DICAL+F) and without (DICAL) F addition, as NaF (500ppm).

The experimental diets contained 14% CP, 2.2 Mcal ME/kg, 0.36 total P and 0.60% Ca. Calcium apparent absorption (AA) and F net retention were measured using a total collection method, with four animals/treatment. Sheep were kept in metabolic crates, 14 days for adaptation and 7 days to record feed intake and fecal excretions. Prior digestibility trial, the animals were fed the same diets during a 12 month period.

Calcium kinetics was determined by orally dosing the sheep with 200 µCi ^{45}Ca (CaHPO4). Blood samples were taken periodically after dosing and 168 h later the animals were slaughtered to remove the femur for chemical analysis by conventional methods (AOAC, 1990). Radioactive Ca half life time (t½, h) entering to the central pool (plasma) and bi-exponential equations to measure ^{45}Ca leaving the blood were used to describe Ca kinetics (Underwood, 1977, 1981). Bone ^{45}Ca specific activity was also measured.

Data were treated by ANOVA and Tukey's test was used for mean comparison. In addition, regression and correlation analyses between response variables were carried out (Steel and Torrie, 1988).

Results and Discussion

Calcium AA (%) was greater (P<0.05) in animals fed DICAL (71.43), DICAL+F (70.87) and RIO (69.74) and lower (P<0.05) for MONTE (62.67) and TSP (59.06) (Table 1). In general terms, the presence of F in the phosphates with fluorine tended to diminish Ca absorption, in some cases significantly. This is supported by Ramberg *et a*l. (1982), who suggest a possible negative effect of F on the primary absorption of Ca trough mechanisms related to calcium transport.

Table 1. Calcium apparent absorption in sheep fed non conventional phosphates

Item	DICAL	D+F	RIO	MONTE	TSP
Ingested	6.05	4.39	5.70	6.73	6.02
g/day	±1.45	±0.58	±1.21	±1.45	±1.82
Excreted	1.66	1.28	1.75	2.53	2.42
g/day	±0.32	±0.28	±0.82	±1.05	±0.74
AA, %	71.43[a]	70.87[a]	69.74[a]	62.67[b]	59.06[b]
	±6.54	±4.49	±4.45	±6.08	±7.03

[1]Four sheep/treatment
[2]Apparent absorption (AA)=Ingested-Excreted/ingested x 100
[a, b, c] Means with different superscripts are different (P<0.05)

Fluorine apparent retention (%) was higher (P<0.05) for DICAL+F (78.52) followed by MONTE (56.65), TSP (52.41) and RIO (54.23) (Table 2). The proportion of F excreted trough the faeces were higher than the urinary excretion. The latter represented 33.03, 21.51, 11.61 y 8.33 % of total fluorine excretion for DICAL+F, TSP, RIO and MONTE, respectively. Similar results were previously reported by the authors (Godoy *et al*., 2000) in cattle fed sedimentary phosphates.

Table 2. Fluorine retention in sheep fed non conventional phosphorus sources

Item	D+F	TSP	RIO	MONTE
Ingested	264.85	276.11	215.85	444.78
mg/day	±34.9	±83.6	±83.6.	±162.1
Excreted	56.64[a]	132.25[b]	100.21[b]	186.78[c]
mg/day	±13.11	±39.4	±38.1	±74.2
ANR[2],%	78.52[a]	52.41[b]	54.23[b]	56.65[b]
	±4.59	±7.84	±9.34	±12.85

[1]Four sheep/treatment
[2] ANR: Apparent net retention
[a, b, c] Means with different superscripts are different (P<0.05)

After dosing, entry of ^{45}Ca (Table 3) from DICAL treatment to the central pool (t½; h) was faster (1.86; P<0.05) than TSP (8.48) and DICAL+F (18.29), and these more (P<0.05) than RIO (38.72) and MONTE (33.16). Blood specific activity showed similar trend. These findings suggest a lower P absorption from the gut of RIO and MONTE phosphates, probably due to a more complex molecular structure and the presence of high levels of F in these sedimentary sources. DICAL+F and TSP treatments, with $t_{1/2}$ lower than RIO and MONTE, but higher than DICAL, also indicate that F may interfere Ca absorption (Ramberg *et al.*, 1982). These results corroborate previous data that show a lower absorption of Ca from sedimentary phosphates (Godoy and Chicco, 2004).

Radioactive Ca leaving the central pool (Table 4) had a bi-exponential pattern with all phosphorus sources. In the faster rate, blood clearance and $t_{1/2}$ (h) of ^{45}Ca in DICAL treatment were higher (P<0.05) than TSP and DICAL+F and the sedimentary phosphates, RIO and MONTE. No significant differences were found in the slow rate blood clearance of ^{45}Ca among different phosphates, while $t_{1/2}$ (h) movement out of the blood was faster (P<0.05) for DICAL than the other phosphates. These results indicate that radioactive Ca sowed a similar kinetic pattern than the stable element (Georgievskii, 1982) and that blood clearance of ^{45}Ca suggests that F may have impaired primary and secondary absorption of Ca from the gut (Ramberg *et al.*, 1982)

Table 3. Entry of ^{45}Ca into the plasma of sheep fed different non conventional phosphorus sources

Source	A_1	k_1	R^2	$T_{1/2}$
DICAL	0.3881	0.3731	0.79	1.86
D+F	0.2333	0.0379	0.63	18.29
RIO	0.2176	0.0179	0.65	38.72
MONTE	0.2765	0.0204	0.35	33.16
TSP	0.3418	0.0817	0.37	8.48

[1] Four sheep/treatment
[2] $y= A_1e^{k1t}$
[3] T ½ half life time (hours) = ln 2/k

Table 4. Equations to describe kinetics of ^{45}Ca moving out from the plasma in sheep fed different non conventional phosphorus sources

Source	Compart-ment[2]	A	k	R^2	$T_{1/2}$[3], hour
DICAL	A_1	0.4334	-0.1187	0.79	5.84
	A_2	0.2000	-0.0053	0.87	130.8
D+F	A_1	2.4154	-0.0463	0.63	14.97
	A_2	0.3500	-0.0022	0.91	315.1
TSP	A_1	0.7220	-0.0407	0.50	13.15
	A_2	0.2600	-0.0024	0.84	315.1
RIO	A_1	0.7368	-0.0527	0.66	16.12
	A_2	0.2500	-0.0022	0.86	223.6
MONTE	A_1	0.4117	-0.0430	0.35	17.03
	A_2	0.3650	-0.0031	0.92	288.8

[1] Four sheep/treatment
[2] A_1: fast; A_2: slow; $y= A_1e^{k1t} + A_2e^{k2t}$
[3] $T_{1/2}$:= ln2/k Half life time (hours)

Capture of ^{45}Ca ($x10^{-7}$) from bone tissue (Table 5) tended to be higher in the less mineralized femur, with

greater values (P<0.05) for MONTE (2.15), RIO (1.52) and TSP (1.54) than DICAL+F (0.85) and DICAL (0.72). The higher ^{45}Ca specific activity in the bone tissue corroborates previous findings with ^{32}P (Chicco *et al*., 1967) which indicate that capture of the element is greater in less mineralized bone.

Table 5. Bone composition of sheep fed non conventional phosphorus sources

Item	DICAL	D+F	RIO	MONTE	TSP
Ash, %	61.8 [a]	60.3 [bc]	60.8 [b]	57.9[c]	63.6 [ab]
	±0.88	±1.17	±1.49	±2.38	±0.32
SA	0.72[c]	0.85[c]	1.52[b]	2.15[a]	1.54[b]
$^{45}Cax10^{-7}$	±0.12	±0.05	±0.35	±0.38	±0.32

[1] Four sheep/treatment
[2] SA: Specific activity
[a, b, c] Means with different superscripts are different (P<0.05)

Implications

Absorption and kinetic data indicate that Ca utilization of sedimentary phosphates and of a triple superphosphate was lower than a dicalcium phosphate, and that calcium utilization may be impaired by the presence of fluorine.

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EFFECT OF LEVEL AND SOURCE OF SELENIUM ON GROWTH AND CELLULAR PROLIFERATION OF MATERNAL JEJUNAL TISSUE IN GROWING PREGNANT EWE LAMBS[1]

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ABSTRACT: To examine effects of source (organic vs. inorganic) and level (0.1, 3, and 15 ppm) of dietary Se on maternal jejunal cellular proliferation, 32 pregnant Targhee ewe lambs (45.6 ± 2.3 kg; 330 ± 30 d) were randomly allotted to one of four treatments in a completely randomized design. Treatments consisted of control (CON; 0.1 ppm), Se-wheat (SW; 3 ppm), selenate (S3; 3 ppm), and selenate (S15; 15 ppm). The SW diet was formulated using 32% high Se wheat (with a concentration of 8 ppm Se). All diets were similar in CP (15.5%) and energy (2.68 Mcal ME), and fed to meet or exceed nutritional requirements. Diets were initiated at 50 ± 5 d of gestation. Formulation of SW and S3 (supranutritional levels) provided 75 µg/kg BW of Se, while the S15 treatment provided 375 µg/kg BW of Se daily. At day 135 of gestation, ewes were slaughtered and tissues harvested. Jejunal (total and mucosal) mass was unaffected by treatment. Jejunal mucosal DNA concentrations were greater ($P < 0.05$) in SW compared with CON (6.39 vs. 5.19 ± 0.33 mg/g). Total mucosal cells were greatest ($P = 0.08$) in SW compared with other treatments (188.6 vs. 141.4 17.1 x 10^9). Percentage of proliferating cells within the mucosa increased ($P = 0.10$) in Se compared with CON fed ewes (15.3 vs. 10.4 ± 2.1%). Total proliferating cells within the jejunal mucosa were greater ($P = 0.09$) in Se fed compared with control ewes (24.0 vs. 13.6 ± 3.8 x 10^9). These data agree with our previous observations in steers and indicate that supranutritional levels of both organic and inorganic forms of Se increase jejunal cellular proliferation within the intestinal mucosa of growing pregnant ewe lambs. The mechanisms by which Se stimulates cell proliferation (increased by 76.5%), and implications for nutrient transfer and energy metabolism needs to be determined.

Key Words: Intestinal Growth, Selenium, Sheep

Introduction

Selenium is an essential trace nutrient for normal growth and development in both livestock and humans (McDowell, 2003; Sunde, 1997). Both Se deficiency and excess has resulted in economic liabilities for livestock producers (McDowell, 2003: Underwood and Suttle, 2001). Recent work (Clark et al., 1996; Combs and Lu, 2001) indicates that supranutritional levels of Se from yeast (2- to 4-fold above normal requirements) can reduce the combined incidence of lung, colorectal, and prostate cancers in humans by as much as 50%. Additionally, research using rodent cancer models has demonstrated that the positive response to supranutritional (2 to 3 ppm) levels of Se may be dependent upon the molecular form of Se (Finley et al., 2000; Wagner et al., 2000; Finley and Davis, 2001).

These studies and others have spawned a large effort regarding high Se foods and human health benefits. This is of particular interest to the beef (and other livestock) industries because beef and wheat are the single largest sources of Se in typical diets consumed by North Americans (Schubert et al., 1987; Pennington and Young, 1991). Lawler et al. (2004) recently reported that steers fed 60 to 70 Φg Se/d provided as high-Se wheat had similar intakes, gains, and efficiencies compared with controls that were fed 7 to 12 Φg Se/d. They also reported that the Se content of harvested product was high enough to consider beef as a possible option for providing supranutritional levels of Se in human diets. In a companion study, Soto-Navarro et al., (2004) evaluated effects of supranutritional Se (3 ppm) from high-Se wheat on intestinal mass, crypt cell proliferation, and vascular density in beef steers fed finishing diets. They found that the percentage of jejunal cellular proliferation was unaffected by high Se; however, jejunal mass was increased in steers fed high Se treatments. Consequently, when cellular proliferation estimates were coupled with jejunal mass the total number of jejunal proliferating cells was almost double in steers fed high-Se wheat. Based upon this previous data, we hypothesized that both source and level of selenium would increase intestinal growth and cellular proliferation in pregnant ewe lambs.

Materials and Methods

Thirty-two pregnant Targhee ewe lambs (45.6 ± 2.3 kg, 330 ± 30 d of age) were randomly allotted to one of four treatments in a completely randomized design. Treatments consisted of control (CON; 0.1 ppm), Se-wheat (SW; 3 ppm), selenate (S3; 3 ppm), and selenate (S15; 15 ppm). The SW diet was formulated using 32% high Se wheat (with a concentration of 8 ppm Se). All diets were similar in CP (15.5%) and energy (2.68 Mcal of ME), and

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fed to meet or exceed nutritional requirements. Diets were initiated at 50 ± 5 d of gestation. Formulation of SW and S3 (supranutritional levels) provided 75 µg/kg BW of Se, while the S15 treatment provided 375 µg/kg BW of Se daily. Ewe lambs were housed individually at the Animal Nutrition and Physiology Center on the North Dakota State University Campus. The North Dakota State University Institutional Animal Care and Use Committee approved animal handling and experimental protocols.

Ewes were assigned randomly to a slaughter schedule over a 5 d period. One hour prior to slaughter, ewes were injected with 5-bromo-2-deoxy-uridine (BrdU; 5 mg/kg BW), which is a thymidine analog that is incorporated into cellular DNA during the S-phase of the cell cycle (Jablonka-Shariff et al., 1993; Jin et al., 1994). Ewes were stunned via captive bolt and exsanguinated. Immediately following evisceration, visceral organs were obtained. Intestinal segments were located, and demarcations made (Schaeffer et al., 2004b).

Jejunal and jejunal mucosal samples were taken for protein, DNA, and RNA analysis as previously described (Scheaffer et al., 2004a,b). Jejunal samples consisted of a 2-cm wide cross section of tissue, and a 10 to 15 cm portion of the jejunum was used to obtain the mucosal scrape sample. Samples were frozen in liquid nitrogen and stored at -80˚C until analyzed for DNA, RNA, and protein concentrations (Reynolds et al., 1990; Reynolds and Redmer, 1992). Tissue homogenates were analyzed for concentrations of DNA and RNA by using the diphenylamine (Johnson et al., 1997) and orcinol (Reynolds et al., 1990) procedures. Protein in tissue homogenates was determined with Coomassie brilliant blue G (Bradford, 1976), with bovine serum albumin (Fraction V; Sigma Chemical) as the standard (Johnson et al., 1997). The 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). Intestinal DNA, RNA, and protein contents were calculated by multiplying DNA, RNA, and protein concentrations by fresh tissue weights (Swanson et al., 2000; Scheaffer et al., 2003).

The presence of BrdU in specific nuclei was localized in Carnoy's-fixed intestinal sections using immunohistochemistry previously described in order to estimate the rate of cell proliferation (Jablonka-Shariff et al., 1993; Jin et al., 1994; Swanson et al., 1999). Tissues were placed into paraffin blocks, sectioned at 4 µm, and mounted on glass slides for analysis of cellular proliferation (Fricke et al., 1997; Scheaffer et al., 2004b). A computerized image analysis system (Image Pro Plus 4.5; Media Cybernetics) was used to evaluate BrdU staining.

Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). When the overall F-test for treatment was significant ($P < 0.10$), means were separated using contrasts.

Results and Discussions

Concentrations of jejunal DNA (mg/g) were lower ($P = 0.01$; Table 1) in Se treated compared with control fed ewes. Jejunal DNA concentations were also lower ($P = 0.06$) in ewes fed 3 ppm selenate compared with those fed 15 ppm. Jejunal RNA:DNA ratios were higher ($P = 0.10$) in Se treated compared with control fed ewes and in ewes fed 3 ppm selenate compared with ewes fed 15 ppm ($P = 0.06$). Selenium treatment did not alter concentrations of RNA or protein within jejunal tissue. Likewise jejunal protein:DNA ratios were unaffected by Se.

Within the jejunal mucosa, DNA concentrations were higher ($P < 0.02$) in ewe lambs fed high Se wheat treatments when compared with controls (6.39 vs. 5.19 ± 0.33 mg/g). These data agree with Soto-Navarro et al., (2004) and indicate that supranutritional levels of selenium from wheat will increase hyperplasia within the jejunal mucoal tissue. Our responses within the jejunal mucoal scrapes differ from those observed within whole jejunal tissue samples. This is likely due to differing proportions and activity within jejunal non-mucosal support tissues. Mucosal DNA concentrations were higher ($P = 0.02$; Table 1) in high-Se wheat compared with Se-salt fed ewes, indicating that source of Se may partially dictate responses in rapidly proliferating intestinal mucosal tissues. Protein:DNA ratios were lower ($P = 0.01$) in the mucosa of high-Se wheat vs. salt fed ewes and also were lower ($P = 0.03$) in the mucosa of ewes treated with selenate at 3 ppm compared with 15 ppm. In the mucosa, the RNA:DNA ratio was lower ($P = 0.07$) in high-Se wheat fed ewes compared those fed Se-salt treatments. Above indices of hyperplasia and hypertrophy suggest that mucosal tissues in high Se-wheat treated pregnant ewe lambs were composed of smaller more numerous cells. Concentrations of RNA and protein in the mucosa were unaltered ($P = 0.21$, and $P = 0.26$) by Se treatments.

Within jejunal tissue, DNA, RNA, and protein contents (tissue mass x concentration) were unaffected ($P = 0.46$ to $P = 0.90$; Table 2) by Se. In contrast, mucosal DNA contents were higher ($P < 0.02$) in Se-wheat compared with control fed ewe lambs (1.25 vs. 0.86 ± 0.08 g), which agrees with Soto-Navarro et al., (2004). In addition, mucosa DNA contents were higher ($P = 0.05$) in Se-wheat compared with salt fed ewes. Total mucocal RNA and protein contents were unaltered by Se.

Percent proliferating nuclei within jejunal mucosal crypts were higher ($P = 0.05$; Table 3) in ewe lambs fed Se compared with controls. In the jejunum, tissue mass (g), total cells (x 10^9), and total cells proliferating (x 10^9) were not altered ($P = 0.48$ to $P = 0.52$) by Se treatments. In the mucosa, total cells (x 10^9) were higher ($P < 0.02$) in Se-wheat compared with control fed ewes (188.6 vs. 130.1 ± 17.1). In addition, total cells within the mucosa were higher ($P = 0.05$) in high-Se wheat treated ewes compared with those fed Se-salt treatments. Total proliferating cells in the mucosal were lower ($P = 0.02$) in control versus selenium fed ewes. Mucosal mass (g) was unaltered ($P = 0.55$) by Se treatments.

Implications

These data imply that supranutritional levels of selenium alter gut mucosal growth and cell proliferation. In addition, these data imply that the form of selenium provided within the diet may partially dictate responses

observed. Impact of observed responses on nutrient transfer, energy metabolism, and whole animal responses are unknown and warrant further investigation.

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Table 1. Effects of source and level Se on DNA, RNA, and protein (mg/g) in tissue from pregnant ewe lambs (fresh basis)

	Treatments[a]						Contrast[b]		
Item	Control	SW	S3	S15	SE	P	C vs. Se	SW vs. Salt	S3 vs. S15
Jejunum									
DNA, mg/g	5.49	4.85	4.35	5.03	0.25	0.03	0.01	0.60	0.06
RNA, mg/g	4.13	4.40	4.64	4.04	0.46	0.79	0.67	0.92	0.37
Protein, mg/g	43.11	35.00	38.42	42.67	3.35	0.25	0.26	0.16	0.35
Protein:DNA	7.94	7.36	9.22	8.60	0.87	0.48	0.48	0.45	1.62
RNA:DNA	0.75	0.90	1.07	0.82	0.09	0.09	0.10	0.66	0.06
Mucosa									
DNA, mg/g	5.19	6.39	5.53	5.16	0.33	0.05	0.21	0.02	0.45
RNA, mg/g	4.43	4.42	4.64	3.84	0.27	0.21	0.66	0.60	0.05
Protein, mg/g	39.80	37.56	34.09	41.98	2.83	0.26	0.57	0.89	0.06
Protein:DNA	7.75	5.85	6.43	8.15	0.55	0.02	0.16	0.01	0.03
RNA:DNA	0.86	0.70	0.86	0.76	0.05	0.06	0.13	0.07	0.15

[a]Treatments were: Control = 2.5 µg Se·kg BW^{-1}·d^{-1}; SW and Selenate (3 ppm) = 75 µg Se·kg BW^{-1}·d^{-1}; Selenate (15 ppm) = 375 µg Se·kg BW^{-1}·d^{-1}. [b]Contrasts were: C vs. Se (Control vs. all selenium treatments); SW vs. Salt (high-Se wheat vs. Salt treatments); S3 vs. S15 (3 ppm Salt vs. 15 ppm Salt).

Table 2. Effects of source and level Se on DNA, RNA, and protein content in tissue from pregnant ewe lambs (fresh basis)

	Treatments[a]						Contrast		
Item, g	Control	SW	S3	S15	SE	P	C vs. Se	SW vs. Salt	S3 vs. S15
Jejunum									
DNA	1.30	1.38	1.14	1.27	0.11	0.49	0.77	0.20	0.41
RNA	0.96	1.26	1.22	1.05	0.15	0.46	0.23	0.50	0.42
Protein	10.2	10.0	9.77	11.0	1.26	0.90	0.99	0.81	0.47
Jejunal mucosa									
DNA	0.86	1.25	1.02	0.93	0.11	0.08	0.11	0.05	0.57
RNA	0.73	0.88	0.87	0.71	0.10	0.45	0.42	0.43	0.25
Protein	6.62	7.36	6.45	7.72	0.99	0.75	0.60	0.81	0.36

[a]Treatments were: Control = 2.5 µg Se·kg BW^{-1}·d^{-1}; SW and Selenate (3 ppm) = 75 µg Se·kg BW^{-1}·d^{-1}; Selenate (15 ppm) = 375 µg Se·kg BW^{-1}·d^{-1}. [b]Contrasts were: C vs. Se (Control vs. all selenium treatments); SW vs. Salt (high-Se wheat vs. Salt treatments); S3 vs. S15 (3 ppm Salt vs. 15 ppm Salt).

Table 3. Effects of source and level of dietary Se on jejunal tissue mass and cellular proliferation.

	Treatments[a]						Contrast[b]		
Item	Control	SW	S3	S15	SE	P	C vs. Se	SW vs. Salt	S3 vs. S15
Proliferating Nuclei. %	10.4	12.6	17.4	15.8	2.1	0.10	0.05	0.13	0.58
Jejunum									
Mass, g	250.5	285.8	262.2	256.5	18.4	0.52	0.41	0.22	0.82
Total Cells, x 10^9	196.8	208.7	172.5	192.0	16.5	0.49	0.77	0.20	0.41
Total Cell Prolif., x 10^9	21.3	25.8	29.6	31.1	4.7	0.48	0.18	0.44	0.83
Mucosa									
Mass, g	169.7	195.3	200.5	175.0	19.8	0.55	0.36	0.71	0.30
Total Cells, x 10^9	130.1	188.6	153.9	140.3	17.1	0.08	0.11	0.05	0.57
Total Cell Prolif., x 10^9	13.6	23.0	26.9	22.1	3.8	0.09	0.02	0.73	0.36

[a]Treatments were: Control = 2.5 µg Se·kg BW^{-1}·d^{-1}; SW and Selenate (3 ppm) = 75 µg Se·kg BW^{-1}·d^{-1}; Selenate (15 ppm) = 375 µg Se·kg BW^{-1}·d^{-1}. [b]Contrasts were: C vs. Se (Control vs. all selenium treatments); SW vs. Salt (high-Se wheat vs. Salt treatments); S3 vs. S15 (3 ppm Salt vs. 15 ppm Salt).

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THE INFLUENCE OF LENGTH OF SUPRA-SELENIUM SUPPLEMENTATION ON SELENIUM STATUS, FEEDLOT PERFORMANCE, AND CARCASS CHARACTERISTICS OF FINISHING LAMBS

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ABSTRACT: Dietary selenium (Se) affects the Se concentration of skeletal muscle in ruminants. However, limited data are available describing the effects of length of supra-selenium supplementation to lambs on selenium status, feedlot performance, or carcass characteristics. Therefore, our objectives were to evaluate the influence of length of supra-selenium supplementation on muscle and plasma Se status, feedlot performance, and carcass characteristics of finishing lambs. One-hundred forty four wethers (BW = 34 ± 0.2 kg) were blocked by weight and stratified by breed and assigned randomly to one of four dietary treatments (n = 4): supra-selenium supplementation with selenoyeast for the final 56 d, 28 d, 14 d, or 0 d (CON) of finishing. Diets were similar in ingredient composition (84% corn and 15% wheat middlings based supplement; DM basis), isonitrogenous and isocaloric (17% CP, 3.85 Mcal DE/kg DM), and offered once daily (0800) to provide ad libitum intake for 84 d. Daily selenium intake for CON and supra-selenium supplemented wethers was 19 and 75 µg/kg BW, respectively. Two-day weights were recorded on d 0 and 84 and plasma samples were collected on d 0 and 84 for determination of circulating Se status. At the end of the trial, wethers were slaughtered; carcass data recorded; and skeletal muscle samples collected for Se analysis. Dry matter intake decreased linearly ($P = 0.02$) as length of supra-selenium supplementation increased. Total gain, gain to feed, and ADG did not differ ($P > 0.32$). Hot carcass weight exhibited a quadratic decrease ($P = 0.04$). No differences ($P > 0.10$) were observed for backfat thickness, longissimus muscle area, quality grade, and yield grade. Final plasma Se concentration increased linearly ($P = 0.005$) and muscle Se concentration increased quadratically ($P = 0.04$) because of treatment. Supra-supplementation of Se to finishing lambs did decrease intake and hot carcass weight. Plasma and muscle Se concentration increased and did not appear to plateau as length of supra-selenium supplementation increased.

Key Words: Lamb, Muscle, Selenium

Introduction

Selenium (**Se**) deficiency in humans is not considered to be an issue in the United States, however, recent research suggests that humans who consume in excess (2 to 4 fold) of the recommended dietary allowance (RDA = 55 μg Se/d) of Se may reduce their chance for developing lung, colorectal, and prostate cancer by 30, 50, and 70%, respectively (Clark et al., 1996). Although tablets of Se as selenite or high Se yeast are available, the American Dietetic Association encourages people to consume nutrients through food whenever possible, including meats and grains (ADA, 2005).

Taylor et al. (2002) documented that circulating plasma Se concentration of finishing beef steers was elevated within 21 d by feeding high Se (60 to 70 µg • kg^{-1} BW • d^{-1}) wheat, hay, or sodium selenate supplement. Additionally, Lawler et al. (2004) reported that beef steers fed feedstuffs naturally high in Se (65 µg • kg^{-1} BW • d^{-1}) had Se concentration in semitendinosus muscle greater than those fed control or selenate. Results by Lawler et al. (2004) were similar to those of Hintze et al. (2001), who reported that a moderate sized portion of high Se beef would supply the Se necessary to achieve the cancer protection benefits described by Clark et al. (1996). However, the research by Lawler et al. (2004) was conducted over a 126 d finishing period and the minimum length of time to achieve elevated Se concentration was not determined.

We are aware of no research evaluating the influence of length of supra-supplementation of selenium to finishing lambs on muscle selenium status, feedlot performance, or carcass characteristics. Therefore, our objectives were to evaluate the influence of length of supra-selenium supplementation on muscle and plasma Se status, feedlot performance, and carcass characteristics of finishing lambs.

Materials and Methods

One-hundred forty four wethers (BW = 34 ± 0.2 kg) were used in a randomized complete block design to evaluate the influence of duration of supra-selenium supplementation in finishing rations on a) lamb skeletal muscle and plasma Se concentration; b) finishing period body weight gain and feed efficiency; and c) carcass

characteristics. The North Dakota State University Institutional Animal Care and Use Committee reviewed and approved animal care and use protocols used during this study. Wethers were blocked by weight (heavy BW = 36 ± 0.2 kg and light BW = 31 ± 0.5 kg) and stratified by breed (Rambouillet and Rambouillet X Suffolk) and assigned randomly to one of four dietary treatments (n = 4): supra-selenium supplementation with selenoyeast for the final 56 d (**56 d**), 28 d (**28 d**), 14 d (**14 d**), or 0 d (**CON**) of finishing. Wethers were dewormed with Ivomec® on day 0. Diets were similar in ingredient composition (84% corn and 15% wheat middlings based supplement; DM basis), isonitrogenous and isocaloric (17% CP, 3.85 Mcal DE/kg DM), and offered once daily (0800) to provide ad libitum intake (105% of previous d intake) for 84 d (NRC, 1985; Table 1). Feed offered was recorded daily and feed refusals collected and recorded once per week. Daily selenium intake for CON and supra-selenium supplemented wethers was 19 and 75 µg/kg BW, respectively (Table 1). Initial and final weights were two-day un-shrunk weights, and single day interim weights were taken once every 14 d to aid in monitoring health and potential Se toxicity. Blood (jugular venipuncture into EDTA) was collected on day 0 and 84 to determine the change in circulating Se status. Blood samples were centrifuged, plasma collected, and frozen. Plasma samples were frozen at -30°C until analysis for Se. Wethers were harvested at Iowa Lamb Corp. in Hawarden, IA. Skeletal muscle samples (approximately 5 g of front foreleg) were removed for the determination of Se concentration. Skeletal muscle samples were frozen at -30°C until analysis for Se. Skeletal muscle samples (0.3 to 0.5 g) and plasma samples were analyzed for Se by inductively coupled plasma-mass spectrometry after acid digestion (minimum detection limit = 10 ng/mL, inter assay CV = < 7%, intra assay CV = < 4%; Utah Veterinary Diagnostic Laboratory, Logan, UT). Additionally, carcass characteristics were evaluated following harvest. Hot carcass weight, backfat thickness, and ribeye area were measured. Yield and quality grades were determined subjectively by a USDA grader. Gain to feed (**G:F**) ratios and ADG were calculated. Dry matter intake (**DMI**), body weight (**BW**), G:F, ADG, blood and skeletal muscle Se concentration, and carcass characteristic data were analyzed as a randomized complete block design with the GLM procedure of SAS (SAS Inst. Inc., Cary, NY) using pen as the experimental unit. The model included block and treatment using the residual error term. The two-way interaction of treatment X block was tested prior to testing of main affects, and when not significant ($P > 0.05$) was retained in the error term. Orthagonal contrast statements included 1) Control vs. supra-selenium supplementation; 2) linear effect of supra-selenium supplementation; and 3) quadratic effect of supra-selenium supplementation.

Results and Discussion

There was no block by treatment interaction for response variables tested ($P \geq 0.15$). We observed no signs of potential Se toxicity. Length of supra-selenium supplementation did not effect performance measures of final weight, total gain, G:F, and ADG ($P \geq 0.15$; Table 2), carcass characteristics (fat depth and REA; $P \geq 0.13$; Table 2), or carcass quality ($P \geq 0.11$; Table 2). However, DMI decreased linearly ($P = 0.02$) as length of supplementation increased. Additionally, hot carcass weight exhibited a quadratic decrease ($P = 0.04$; Table 2). Initial plasma Se concentration was similar for all lambs on day 0 ($P \geq 0.69$; Table 3). Final plasma Se concentration increased linearly ($P = 0.005$) and muscle Se concentration increased quadratically ($P = 0.04$) because of treatment (Table 3).

Body weight gain, ADG, G:F, carcass characteristics, and carcass quality traits of the lambs in this study were not affected by the length of supra-selenium supplementation. These results are similar to results in steers (Hintze et al., 2002; Lawler et al., 2004). However, we observed a linear decrease in DMI and a quadratic decrease in hot carcass weight as length of supra-selenium supplementation increased. The apparent cause of the decrease in intake is not readily available, as this response is not consistent with other research (Hintze et al., 2002; Lawler et al., 2004). One possibility is a decrease in palatability for the selenoyeast. However, the 14 d treatment did not exhibit a decrease in intake.

Muscle Se concentration in our trial increased quadratically as length of supra-selenium supplementation increased. These results are similar to results of other researchers. Van Ryssen et al. (1989) reports that mature ewes fed high-Se wheat at 1 mg of Se/kg diet had increased liver, wool, and muscle Se concentrations compared with sheep supplemented with a similar quantity of sodium selenite. Additionally, Ehlig et al. (1967) reported that lambs supplemented with 0.4 mg Se/day had higher muscle Se concentrations for supplementation with selenomethionine compared to selenite. Lawler et al. (2004) reported similar muscle Se concentrations (4.41 ppm DM) in beef cattle finished on high selenium diets, specifically, with high Se wheat.

North Americans acquire their daily Se requirement primarily from wheat grain and beef (Schubert et al., 1987; Holden et al., 1991; Hintze et al., 2001). A ¼ lb portion of lamb from lambs fed a supra-selenium supplemented diet would provide approximately 155, 112, 95, and 76 μg Se/day (wet basis; 56 d, 28 d, 14 d, and 0 d supra-selenium supplementation, respectively). While the non-selenium supplemented treatment in this trial provided adequate selenium to meet the recommended dietary allowance for selenium in humans (RDA = 55 μg Se/d for females and 70 μg Se/d for males), the selenium concentration in lamb skeletal muscle tissue for the 56 d selenium supplemented treatment would provide approximately 281% of the RDA for selenium. This level falls within the range indicated by Clark et al. (1996; 2 to 4 fold the RDA) for humans to reduce their chance of developing lung, colorectal, and prostate cancer. The four state region of ND, SD, WY, and MT has soils high in Se (Lawler et al., 2002), however, the variability of Se concentration in forages (and subsequently grazing livestock) indicate that a feeding program for finishing livestock is necessary to ensure animals of both low and high Se status can achieve the desired Se

concentration in skeletal muscle. Our results provide one possible alternative for finishing programs to increase the muscle Se status of finishing lambs.

Implications

Lambs supra-supplemented with selenium for 56 days have skeletal muscle selenium concentrations at levels that may prevent lung, colorectal, and prostate cancer in humans. These results indicate that future efforts are needed to ascertain the targeted selenium concentration in lamb muscle, the likely level of demand for a high-selenium lamb product, and the marketing techniques required to deliver that product to the consumer. Development of a feeding protocol for achieving high selenium lamb will aid current lamb cooperatives and individual producers in developing a niche market for the sale of lamb as an organic selenium supplement, as well as adding value to locally grown forages through the finishing of lambs.

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Table 1. Dietary ingredient and nutrient composition of lamb finishing diets

Ingredient	Feedstuff Se concentration, ppm	Diets[a]	
		CON	Selenium Diet
		%, DM basis	
Corn	0.42	84.25	84.04
Supplement[b]	0.92	15.00	15.00
Selenoyeast	666	---	0.21
Vitamin E	---	0.25	0.25
Ammonium Chloride	---	0.50	0.50
Nutrient Composition of Diet			
CP, %		16.9	16.9
DE, Mcal/kg		3.85	3.85
ADF, %		4.56	4.56
Calcium, %		0.79	0.79
Phosphorus, %		0.31	0.31
Vitamin E, IU/kg diet		27.83	27.83
Selenium, ppm		0.49	1.89
Selenium intake, $\mu g \cdot kg^{-1}$ BW$\cdot d^{-1}$		19.47	74.83

[a]CON = no supra-selenium supplementation with selenoyeast; Selenium diet = supplementation with selenoyeast for the final 14, 28, or 56 days of finishing.
[b]Supplement: Decoquinate = 0.03 g/kg; CP = 43%; Ca = 4.8%; P = 0.65%; Na = 1.45%; S = 0.7%; K = 1.5%; Mg = 0.35%; Fe = 258 ppm; Mn = 671 ppm; Cu = 20 ppm; Zn = 701 ppm; Vitamin A; 16,364 IU/kg; Vitamin D = 1,636 IU/kg; Vitamin E = 34 IU/kg.

Table 2. The influence of supra-selenium supplementation on feedlot lamb performance and carcass characteristics

	Treatment[a]				*P*-value[c]		
Item	CON	14 d	28 d	56 d	SEM[b]	CON vs. Supp.	Linear	Quadratic
Dry Matter Intake, kg/d	1.87	1.95	1.80	1.79	0.03	0.47	0.02	0.24
Final BW, kg	60	62	60	59	1.0	0.75	0.26	0.15
Gain, kg	27	29	26	25	1.1	0.98	0.27	0.34
Average Daily Gain, kg/d	0.32	0.34	0.31	0.31	0.01	0.92	0.33	0.38
G:F	0.17	0.17	0.17	0.17	0.01	0.73	0.99	0.60
Hot Carcass Weight, kg	33	34	33	32	0.4	0.70	0.09	0.04
Backfat thickness, cm	0.71	0.71	0.69	0.64	0.05	0.44	0.13	0.38
Ribeye Area, cm^2	17.74	17.35	16.52	17.23	0.39	0.14	0.20	0.18
Quality Grade[d]	3.1	3.1	3.1	3.0	0.04	1.0	0.75	0.48
Yield Grade	3.1	3.2	3.1	2.9	0.08	0.72	0.11	0.13

[a]CON = no supra-selenium supplementation with selenoyeast; 14 d = supplementation with selenoyeast for the final 14 days of finishing; 28 d = supplementation with selenoyeast for the final 28 days of finishing; 56 d = supplementation with selenoyeast for the final 56 days of supplementation.
[b]Standard Error of Mean; n = 4 .
[c]*P*-value for CON vs. supra-selenium supplemented treatments and linear and quadratic affect of supra-selenium supplementation.
[d]1 = utility; 2 = good; 3 = choice; 4 = prime.

Table 3. The influence of supra-selenium supplementation on feedlot lamb muscle and plasma selenium concentration

	Treatment[a]					*P*-value[c]		
Item	CON	14 d	28 d	56 d	SEM[b]	CON vs. Supp.	Linear	Quadratic
Initial plasma Se concentration (ppm)	0.18	0.18	0.18	0.18	0.004	0.84	0.69	0.95
Final plasma Se concentration (ppm)	0.20	0.31	0.30	0.35	0.03	0.003	0.005	0.20
Muscle Se concentration (ppm; DM)	2.87	3.64	4.33	6.10	0.20	< 0.001	< 0.001	0.04
Muscle Se concentration (ppm; wet)	0.76	0.95	1.12	1.55	0.05	< 0.001	< 0.001	0.02

[a]CON = no supra-selenium supplementation with selenoyeast; 14 d = supplementation with selenoyeast for the final 14 days of finishing; 28 d = supplementation with selenoyeast for the final 28 days of finishing; 56 d = supplementation with selenoyeast for the final 56 days of supplementation.
[b]Standard Error of Mean; n = 4 .
[c]*P*-value for CON vs. supra-selenium supplemented treatments and linear and quadratic affect of supra-selenium supplementation.

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BODY CONDITION SCORE AT PARTURITION AND POSTPARTUM SUPPLEMENTAL FAT EFFECTS ON METABOLITE AND HORMONE CONCENTRATIONS OF BEEF COWS[1]

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Abstract: Three-year-old Angus × Gelbvieh beef cows nutritionally managed to achieve a BCS of 4 ± 0.07 (BW = 479.3 ± 36.3 kg) or 6 ± 0.07 (BW 579.6 ± 53.1 kg) at parturition were used in a 2-yr experiment (n = 36/yr) to determine the effects of body condition score at parturition and postpartum lipid supplementation on blood metabolite and hormone concentrations. Beginning 3 d postpartum, cows within each BCS were randomly assigned to be fed hay and a low-fat control supplement or supplements with either cracked high-linoleate safflower seeds or cracked high-oleate safflower seeds until d-61 of lactation. Diets were formulated to be isonitrogenous and isocaloric, and safflower seed supplements provided 5% DMI as fat. Preprandial and hourly postprandial (0 h, 1 h, 2h, 3 h, and 4 h) blood samples were collected on d 31 and 61 of lactation. Serum insulin (P = 0.27) and glucose (P = 0.64) were not affected by BCS at parturition. Overall concentrations of plasma NEFA (P = 0.08) and β-hydroxybutyrate (P = 0.08) tended to be greater and serum IGF-1 was greater (P < 0.001) in BCS 6 cows. Conversely, serum GH was greater (P = 0.003) for BCS 4 cows indicating that the IGF/GH axis may have been uncoupled in BCS 4 cows. Postpartum diet did not affect NEFA (P = 0.94), glucose (P = 0.15), IGF-1 (P = 0.33), or GH (P = 0.62). Oleate supplemented cows had greater (P = 0.03) overall serum insulin, whereas control cows had greater (P = 0.01) plasma β-hydroxybutyrate. NEFA (P = 0.05) and glucose (P < 0.001) were greater and β-hydroxybutyrate tended (P = 0.08) to be greater at d 30, whereas IGF-1 was greater (P = 0.01) at d 60 of lactation. Similar levels of IGF-1 and NEFA indicate that nutritional status of beef cows during early lactation was not influenced by lipid supplementation.
Key Words: Beef Cattle, Lipid Supplementation, Metabolites, Hormones

Introduction

Dietary lipids have been hypothesized to be nutraceuticals (Williams and Stanko, 1999), acting as nutrient partitioning agents to shift energy utilization from one metabolic process to another. For example, Bottger et al. (2002) attributed maintenance of greater BCS during lactation in beef cattle to supplementation with linoleic acid, whereas dietary oleic acid increased milk fat synthesis. Increasing the BCS of thin lactating beef cows by repartitioning nutrients toward adipose tissue reserves rather than milk fat synthesis could lead to improved reproduction (Houghton et al., 1990) and decreased maintenance requirements (Wagner et al., 1988). Metabolic hormones play a major role in coordinating nutrient partitioning between milk synthesis, adipose tissue storage and mobilization, as well as oxidation in different tissues (Brockman and Laarveld, 1986). Lipid supplementation in lactating dairy cows has been associated with increased plasma glucose and decreased ketone body concentrations (Kronfeld et al., 1980), effectively increasing substrate availability for milk fat synthesis and concurrently reducing the demand for mobilized substrate from energy reserves.

We hypothesized that the aforementioned nutrient partitioning effects attributed to BCS and dietary lipids may be associated with changes in concentrations of metabolites and metabolic hormones. Therefore, our objective was to evaluate the interaction of BCS at parturition and supplementation of cracked high-oleic acid or high-linoleic acid safflower seeds on circulating metabolite and hormone concentrations associated with nutrient utilization.

Materials and Methods

General

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for the following study. Three-year-old Angus × Gelbvieh beef cows nutritionally managed to achieve a BCS (1 = emaciated, 9 = obese, Wagner et al., 1988) of 4 ± 0.07 (BW = 479.3 ± 36.3 kg) or 6 ± 0.07 (BW 579.6 ± 53.1 kg) at parturition were used in a 2-yr experiment (n = 36/yr).

Cows were randomly assigned within BCS group to postpartum dietary treatment as they calved. Beginning 3 d postpartum, cows were placed into one of six pens (six animals per pen) with individual feeding stanchions and were randomly assigned to be fed hay and a low-fat control supplement or supplements with either cracked high-linoleate safflower seeds or cracked high-oleate safflower seeds until d 61 of lactation. Cows were fed twice daily in individual feeding stanchions. Previous research at the

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University of Wyoming reported that cows of similar genetics produced 9 kg of milk during peak lactation (Bottger et al., 2002). Therefore, diets (Table 1) were formulated to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk at peak lactation. Diets were also formulated to provide equal quantities of N and TDN within each year. Dietary CP was higher in yr 2 due to differences in hay used during yr 1 (bromegrass hay; CP = 8.5 %) vs. yr 2 (foxtail millet hay; CP = 10.6 %). Dietary TDN was similar between years, and lipid supplemented diets were formulated to be isolipidic, providing 5% DMI as fat.

Beginning at 0500 on d 31 and 61, cow blood samples were collected through indwelling jugular catheters. Preprandial blood samples (10 mL) were collected immediately prior to feeding. Cows were allowed 1 h 45 min to consume feed and water. Postprandial blood samples (10 mL) were taken beginning at 2 h from the onset of feeding (0 h) and continued every 15 min for 4 h. Plasma was harvested from blood samples collected in heparanized tubes. Blood samples were immediately refrigerated for 12 h and centrifuged at 13000 × *g* for 30 min. Serum and plasma aliquots (4 mL) were stored at -4°C for metabolite and hormone assays.

Laboratory Analyses

Preprandial serum samples were analyzed for IGF-1 concentration (Echternkamp et al., 1990). Preprandial, 0 h, and 4 h postprandial plasma samples were analyzed for NEFA and β-hydroxybutyrate using commercially available kits (NEFA-C and Autokit 3-HB, respectively; Wako Chemicals, Richmond, VA). Intra- and inter assay CV were 12 and 16%, 7.6 and 8.3%, and 4.3 and 11.9 % for IGF-1, NEFA, and β-hydroxybutyrate, respectively. Preprandial and hourly postprandial serum samples were analyzed for insulin (Reimers et al., 1982) and glucose (Infinity Glucose Hexokinase kit; Thermo Trace, Louisville, CO). Preprandial and postprandial serum samples taken every 15 min were analyzed for GH concentration (Hoefler et al., 1987). Intra- and inter-assay CV was 8 and 5%, 6 and 7.5 %, and 7 and 7 % for insulin, glucose, and GH, respectively.

Statistical Analyses

All data were analyzed as a repeated measure with a 2 × 3 arrangement of treatments in a randomized complete block design using the MIXED procedures of SAS (SAS Institute, Cary, NC). Year was used as a block and the model included the effects of BCS at parturition, dietary treatment, day of sampling and all possible interactions. Cow within BCS at parturition × dietary treatment was used as the RANDOM statement.

Results and Discussion

Effects of Body Condition Score at Parturition on Cow Serum Metabolite and Hormone Concentrations. A BCS × time of sample collection interaction ($P = 0.04$) was noted for serum NEFA concentrations (data not shown). Preprandial ($P = 0.23$) and 0 h postprandial ($P = 0.60$) serum NEFA concentrations did not differ between cows in BCS 4 or 6; however, 4 h postprandial NEFA concentrations were greater ($P = 0.02$) in BCS 6 cows compared with BCS 4 cows. Concentrations of NEFA ($P = 0.08$) and β-hydroxybutyrate ($P = 0.08$) tended to be higher in cows nutritionally managed to achieve a BCS of 6 at parturition (Table 2). In agreement with the current study, greater levels of circulating NEFA in beef (Vizcarra et al., 1998) and dairy cows (Busato et al., 2002) has been reported for cows in better condition during early lactation. This observation was most likely reflective of greater fatty acid mobilization from adipocytes of the fatter cows. Serum concentrations of insulin ($P = 0.27$) and glucose ($P = 0.64$) were not different due to BCS at parturition. Changes in insulin usually correspond with changes in circulating glucose concentrations (Busato et al., 2002). Therefore, the lack of change in insulin concentration due to BCS at parturition may have been due to the lack of effect of BCS on glucose concentration. Serum concentrations of IGF-1 ($P < 0.001$) were higher for BCS 6 cows at parturition. Conversely, GH concentrations were greater ($P = 0.003$) for BCS 4 cows at parturition. Serum IGF-1 concentrations may be indicative of nutritional status (Zulu et al., 2002), with cows in better condition having greater circulating concentrations of IGF-1 (Lalman et al., 2000). Under conditions of adequate nutrient intake GH stimulates hepatic secretion of IGF-1 (Thissen et al., 1994; Roberts et al., 1997). However, increased GH secretion in cows in negative energy balance was reported (Breier et al., 1988; Zulu et al., 2002) with a concomitant decrease in circulating IGF-1 (Thissen et al., 1994; Busato et al., 2002; Meikle et al., 2004). Dietary energy restriction results in greater concentrations of GH, decreased concentrations of IGF-1, and decreased IGF-1 responsiveness to circulating GH (McGuire et al., 1992). Reduced hepatic binding of GH may explain the loss of sensitivity of IGF-1 to GH (Breier et al., 1988). Increased synthesis and secretion of GH during energy restriction is a result of decreased levels of circulating IGF-1, reducing the negative feedback on the hypothalamic-pituitary regulation of GH synthesis (McGuire et al., 1992). Results from the current study support the contention of an uncoupling of the somatropic axis. A potential decrease in hepatic binding of GH may account for the greater circulating GH and lower circulating IGF-1 concentrations.

Lalman et al. (2000) concluded that increased circulating IGF-1 and decreased concentrations of GH were indicative of animals maintained in superior nutritional status. Meikle et al. (2004) reported improved reproductive performance in dairy cows with greater circulating IGF-1 concentrations. Likewise, Roberts et al. (1997) indicated that circulating IGF-1 concentrations were associated with the capacity of beef cows to resume cyclicity after parturition. Results from the current study agree with these conclusions because cattle in a BCS of 6 at parturition had greater circulating IGF-1 concentrations coupled with greater overall pregnancy rates (Lake et al., 2004).

Although circulating GH was greater in BCS 4 cows, the potential uncoupling of the GH/IGF-1 axis resulted in circulating IGF-1 concentrations insufficient to achieve desirable reproductive performance.

Effects of Dietary Treatment on Cow Circulating Metabolite and Hormone Concentrations. The main effects of dietary treatment are presented in Table 2. Dietary supplementation had no effect on concentrations of plasma NEFA ($P = 0.94$), glucose ($P = 0.15$), IGF-1 ($P = 0.33$), or GH ($P = 0.62$). Circulating concentrations of NEFA are a reliable index of adipose tissue mobilization (Bauman et al., 1988). Dietary lipid supplementation had little or no effect on basal lipolysis (Baumgard et al., 2002; Selberg et al., 2004). A primary response to lipid supplementation in lactating cows may be increased availability of glucose for lactose synthesis, or for purposes other than milk fat synthesis. The premise behind increased lactose synthesis, or glucose sparing, is that fatty acid oxidation would increase relative to glucose oxidation. Additionally, utilization of glucose for NADPH synthesis to support mammary fatty acid synthesis would be decreased. The lack of difference in circulating glucose concentration among postpartum dietary treatments in the current study was consistent with the lack of differences in milk lactose or total milk yield (Lake et al., 2004). Oleate supplemented cows had greater ($P = 0.03$) serum concentrations of insulin, whereas control cows had greater ($P = 0.01$) plasma concentrations of β-hydroxybutyrate. Concentrations of β-hydroxybutyrate in plasma can be affected by rumen epithelial metabolism of butyrate, or ketone body formation in animals experiencing negative energy balance. Due to the lack of differences detected for most measured indices of nutritional status in the current study, decreased plasma β-hydroxybutyrate concentrations in lipid supplemented cows was most likely due to alterations in ruminal fermentation.

Circulating IGF-1 concentrations have been implicated as a mediator of nutritional status (Roberts et al., 1997). Therefore, concentrations of IGF-1 would be dependent on energy balance. Consistent effects of lipid supplementation on circulating IGF-1 concentrations were not evident in the current study because nutritional status was similar among cows fed different postpartum diets.

Effects of Day of Lactation on Cow Circulating Metabolite and Hormone Concentrations. A day of lactation × time of sampling interaction ($P = 0.001$; data not shown) was noted for plasma β-hydroxybutyrate concentration. No difference between preprandial ($P = 0.54$) and 4 h ($P = 0.26$) postprandial plasma β-hydroxybutyrate concentrations were noted between d 30 and 60. However, 0 h plasma β-hydroxybutyrate concentrations were greater ($P < 0.001$) at d 30 than 60. The main effects of day of lactation are presented in Table 2. No effects for day of lactation were detected for serum concentrations of insulin ($P = 0.62$) and GH ($P = 0.40$). Plasma concentrations of β-hydroxybutyrate tended ($P = 0.08$) to be greater at d 30 compared with d 60 of lactation. Likewise, concentrations of NEFA ($P = 0.05$) and glucose ($P < 0.001$) were greater at d 30 than d 60 of lactation; however, serum IGF-1 concentrations were greater ($P = 0.01$) at d 60 of lactation. Serum glucose concentrations in forage fed ruminants is derived almost exclusively from gluconeogenesis, and low glucose levels can also be the consequence of nutritional restriction or higher glucose needs. Glucose requirements are highest in lactating ruminants during peak milk production (Busato et al., 2002); therefore, the decrease in serum glucose concentration as peak lactation approached was expected. Plasma concentrations of NEFA are indicative of adipose tissue mobilization as a consequence of negative energy balance associated with increased nutrient demand, inadequate nutrition, or a combination of both. Greater concentrations of β-hydroxybutyrate with a concomitant increase in NEFA suggests enhanced ketogenesis as a consequence of negative energy balance. Although higher NEFA concentrations are generally associated with peak milk production, increased NEFA at d 30 in the current study was supported by greater loss of body condition at d 30 of lactation compared with d 60 (Lake et al., 2004). Levels of circulating IGF-1 concentrations increase as lactation progresses (Busato et al., 2002). Because circulating IGF-1 concentrations reflect energy balance (Zulu et al., 2002), the increase in circulating IGF-1 from d 30 to 60 was consistent with the decrease in NEFA and increase in BCS at d 60 (Lake et al., 2004).

In conclusion, postpartum dietary lipid supplementation did not appear to impact metabolic signals associated with nutrient partitioning in beef cows during early lactation. Cows in BCS of 4 at parturition had lower circulating NEFA and greater serum GH; however, an uncoupling of the IGF/GH axis in cows managed to achieve a BCS of 4 at parturition may have detrimental impacts on postpartum cow performance.

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Table 1. Ingredient and chemical composition of diets consumed by lactating beef cows[a]

Item	Diet year 1			Diet year 2		
	Control	High-linoleate	High-oleate	Control	High-linoleate	High-oleate
	---------- Ingredients, % of DM----------					
Hay[b]	79.3	85.4	85.3	87.2	89.7	89.6
High-linoleate safflower seed	-	11.8	-	-	8.1	-
High-oleate safflower seed	-	-	9.6	-	-	7.6
Soybean meal	2.8	-	2.1	0.7	-	0.7
Molasses	0.8	0.8	0.8	0.6	0.6	0.6
Beet pulp	15.0	-	-	10.0	-	-
Minerals	2.8	2.1	2.1	1.6	1.6	1.6
	----------Chemical composition, % of DM----------					
CP	10.4	10.2	10.4	11.2	11.4	11.4
TDN[c]	70.6	71.1	72.0	69.7	70.1	70.1
Crude fat	1.2	5.0	5.0	2.2	5.0	5.0
	----------Dietary fatty 0acid profile, % of DM----------					
16:0	28.7	9.9	7.8	19.8	10.0	8.0
18:0	5.6	3.2	0.3	2.7	3.2	0.2
18:1	10.1	10.2	73.2	10.4	10.3	71.3
18:2	20.3	69.7	10.4	22.4	68.1	10.9
18:3	6.6	0.6	0.1	1.7	0.4	0.6

[a]Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk during peak lactation. Lipid supplemented diets were formulated to provide 5% DMI as fat.
[b]Bromegrass hay (CP = 8.5%) was fed in yr 1, foxtail millet hay (CP = 10.8%) was fed in yr 2.
[c]TDN for hay samples was estimated from ADF values (Linn and Martin, 1989), whereas tabular values (NRC, 1982) were used to calculate TDN of supplemental ingredients.

Table 2. Main effects of BCS at parturition, postpartum dietary treatment, and day of lactation on circulating metabolite and hormone concentrations in beef cows during early lactation[a]

	BCS		Treatment			Day			*P* Value		
Item	4	6	C	L	O	30	60	SEM[b]	BCS	TRT	DAY
n	36	35	24	23	24	71	71	-	-	-	-
NEFA, mEq/L	0.37	0.42	0.39	0.40	0.39	0.42	0.37	0.02	0.08	0.94	0.05
BHB, µmol/L	0.35	0.38	0.39	0.35	0.35	0.38	0.35	0.01	0.08	0.01	0.07
Glucose, mg/dL	70.7	71.7	70.8	73.9	68.9	76.6	65.8	1.85	0.64	0.15	<0.001
Insulin, ng/mL	0.89	0.94	0.85	0.89	1.01	0.93	0.90	0.04	0.27	0.03	0.62
IGF-1, ng/mL	52.4	84.9	68.0	63.1	74.8	53.5	75.0	5.99	<0.001	0.33	0.01
GH, ng/mL	7.91	6.75	7.07	7.42	7.5	7.15	7.51	0.41	0.003	0.62	0.34

[a]Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk during peak lactation. Lipid supplemented diets were formulated to provide 5% DMI as fat. Refer to Table 1 for diet description.
[b]Greatest SEM are presented.

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EFFECT OF THE UREA CONCENTRATION IN PROTEIN SUPPLEMENT ADDED TO DRY GRASS ON THE *IN VITRO* PRODUCTION OF GAS, VOLATILE FATTY ACIDS AND AMMONIA

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ABSTRACT: The effects of urea concentration (UL) in protein supplements, and supplementation level (LS) were determined on the in vitro gas production (GP) volatile fatty acids (VFA) and ammonia (NH3) for dry grass diet. Kinetics of GP were evaluated using asymptotic gas production (GP-A), time of half gas formation after incubation (GP-B), constant determining of the switching profile (GP-C), point of inflection (GP-t1), fractional rate of substrate digestion (GP-R) and time of maximum fractional rate (GP-tRm). The protein supplements were 0, 2.5, 5 and 7.5% of urea and supplementation levels were 6, 12 and 18% of the samples incubated. The dry grass used was from northwest of Chihuahua, Mexico. An interaction (P<0.03) was observed between UL and LS for GP, from 6 h until 12 h (P<0.01) when changes in treatments were registered and from this time it kept similar until 96 h (P<0.001). The lowest GP occurred with LS 18% with UL 0% (31.50 ml / 200 mg DM) and the highest value was LS 6% with UL 5% (34.33 ml / 200 mg DM). These changes indicate differences in the fermentation profiles with the different combination of substrates. There was an interaction (P<0.07) of UL and LS for GP-B, GP-t1 and GP-tRm and GP-R. It was assumed that the increasing LS accelerated fermentation and UL increased the substrate availability. Supplementation level had linear effect (P<0.02) on GP-A and GP-C. GP-A and GP-C diminished when LS increased, this means that fermentation increased velocity. Level of supplementation linearly increased (P<0.0001) NH3 concentration, while UL showed a tendency for cubic effect (P<0.13). The highest NH3 concentrations were obtained with UL 2.5 and 7.5% and the lowest with 0 and 5% of UL, probably due to more efficient protein synthesis. There was no effect (P>0.05) on VFA. The best yield in the ruminal parameters evaluated resulted from 5% urea with the level of supplementation at 12%.

Key Words: Urea level, Gas Production and Protein Cubes

Introduction

The urea is the composed one of Nitrogen Not Protein (NNP) but extensively utilized in diets for ruminants everywhere. The urea has been utilized to diminish the costs of the commercial food, without considering its effects in the production. The microbial protein formed from the NNP has a high nutritious value, any incorporation of nitrogen originating from the urea to the corporal weaves, will depend on the microbial unease (Church and Pond, 1992). The concentration of ammoniac ruminal has been used like an indicator of the degradation microbial of protein *in vivo* and *in vitro*, and also of the utilization of NNP (Broderick and Kang, 1980). The replace excessive of protein true with NNP can carry to a deficiency of protein in highly productive animals (NRC, 1976). The urea is better taken advantage of when the diets are of high energy, and lowers in protein. The high diets in concentrated are going to provide more energy for the synthesis of bacterial protein as of ammoniac and to increase the quantity of ammoniac retained in the ruminal liquid due to a low pH ruminal (NRC, 1984). The ammoniac is the main protein metabolite in the rumen; it is the main final product of the microbial degradation of the protein and is the form of nitrogen required by many types of ruminal bacteria (Broderick and Kang, 1980). Various models have been utilized and compared to describe the production of gas of diverse types of food (Cone et to the., 1998; Groot et to the., 1996; Kitessa et to the., 1999). The escape of gas, the effect of the pressure of the gas accumulated in the solution of the gas and the size of the container of incubation relating to the space upon the substrate, are some factors that can affect the Kinetics of fermentation of the food. However, the advantages of this method, compared to other enzymatic methods, are that takes less time and reduces the proper errors upon washing, filtration and passage (Kitessa et to the., 1999). In the rumen, the supply of urea or some sources of NNP, provokes that these nitrogen compound are attacked for the flora ruminal in the presence of VFA, which they provide skeletons of carbon and they are transformed to amino acids, that is the way that the AGV themselves produce ammoniac (NRC, 1976).

Materials and Methods

The dry grass was obtained of Teseachi Experimental Ranch, property of the University of Chihuahua, which is located to the northwest of the state of Chihuahua, Mexico. Four levels of urea combined with three levels of protein cubes with dry grass. The levels of urea in the protein cubes were 0, 2.5, 5 and 7.5%. The levels of protein cubes in the samples were 6, 12 and 18% (As fed basis) and the remainder of the diet was of dry grass. The variables measured were: production of gas

(ml / 200 mg M.S.) which was evaluated through the time to different hours of incubation (2, 4, 6, 8, 12, 24, 48, 72 and 96 h), thus same of the model one monobasic not lineal for production of gas proposed by Groot et. al. (1996), they were evaluated with six parameters which were (1) asymptotic of production of gas (parameter A, (2) medium time of formation of gas after incubation (parameter B), (3) indicator constant of the change of the profile (parameter C), (4) point of inflection (parameter t1), (5) fractional rate of fermentation (parameter R) and (6) time of maximum fractional rate of fermentation (parameter tRm); Also it was evaluated the concentration of VFA (mM / L) and the concentration of ammoniac nitrogen (N-NH3; mg / 100 ml). The samples of dry grass were recollected to the chance of four transects representative of the area of a barn of the ranch. Subsequently the samples of dry grass utilized for the elaboration of the processing as well as the of protein cubes, they were triturates in a mill marks Wiley® model 3375-E50 with a sieve of 1 mm, and they elaborated you mix homogeneous with the adequate proportions of the processing previously described.

They were incubated in vitro in rumen fluid buffer and the production of gas was measured in different intervals of time as it describes Menke and Steingass (1988). For each processing weighed 200 ± 4 mg of each one of them you show priory dried to the air, subsequently the heavy samples (200 mg) were placed in syringes of plastic (60 ml). The syringes remained during 24 h to a temperature of 39 ºC inside an incubator (Incubator shaker model I2400); to each syringe they were added it 30 ml of mixture buffer with fluid ruminal. The readings of the displacement were taken of the pistons at 2 o'clock, 4, 6, 8, 12, 24, 48, 72 and 96 h. The concentration of N-NH3 went determined in the liquid one ruminal subsequently of the digestion carried out in the measurement of the production of gas. The refrigerated samples were passed for paper filter, and they were analyzed the VFA following the technique described by Galyean and May (1995), with an injection of 1 µl. The VFA analyzed they were the acetic acid, propionic acid and butyric acid. The design of the experiment utilized was an arrangement factorial 4 X 3, with four levels of urea and three levels of block; and five repetitions by processing were utilized. The variables measured were production of gas, ammoniac and volatile fatty acids.

Results and Discussion

The gas average production was increased in agreement advanced the time of incubation in all the processing. In the first hours of incubation ferments the protein fraction of the fodders due to that is highly soluble (Cone et to the., 1999a). Given that the dry grass utilized in this study has a low content of protein (4.74%) is possible that not they have been observed differentiates significant among processing in the first hours of incubation, due to the absence of carbohydrates highly soluble. Cone et. al. (1999b) indicated that the degradation of diets of dry grass is more drop compared with another type of fodders. They began to observe differentiates significant (P<.03) among processing as of the 6 h, where the processing with extreme value minimum and most maximum of block and of urea they resulted to have the value average but under and the but high respectively, that is to say, the T4 had the one that some substrates with a high gas production volume they have comparatively more protein and fermentable carbohydrates, which explains it occurred with the processing T5 and T7. In the processing evaluated the quantity of protein and fermentable carbohydrates does not increase the quantity of gas produced in lineal form, but is the combination of these the one that increases the availability of the substrates. A compensatory production of gas was observed in the measurements of the last hours for the processing that they obtained productions of gas you lower in the first hours, may be to it lowers availability of the substrates and its resistance to be fermented by the microorganisms of the rumen during the first hours of incubation, and on the other hand, also to the lack of products quickly fermentable in the processing.

Asymptotic Production of gas (parameter A). The value of the parameter A is related to the quantity of fermentation of a component in individual of a substrate. The interaction of the effects level of urea and level of block was not significant neither important (P>.05) upon the values average of the parameter A of the not lineal model. An effect was observed (P<.02) of the level of block upon the values average of the parameter A. The effect found of the level of block is of lineal type (P<.01) where to the extent that the level of block is increased in the diet the values average of the parameter A diminish. This owes to that to the extent that the quantity of block is increased, the quantity of components is being increased soluble in the diet, this does that be reached but quick the rate fractional of fermentation and by consequence is reached quicker the asymptotic of the gas production curve (parameter A). Groot et al. (1996) they mention that the rate fractional maximum of fermentation (Rm) can be reached quickly in the incubation of the components soluble, this coincides with the results obtained in the present medium study Time of formation of gas after the incubation (parameter B). An interaction among the effects of the level was found of urea and of the level of protein cubes, which turned out to be highly significant and important (P<.0005) upon the values average of the Parameter B. The values of the parameter B tend to diminish when a fraction exists soluble present in the food, while for the fractions not soluble happens the contrary thing (Cone et to the., 1998). The interaction shows that the high levels of block but low in level of urea they had high values of the parameter B and these values diminish in agreement enlarged the level of urea.

In agreement diminish the values of the parameter B can be understood like acceleration in the fermentation ruminal, what suggests that the addition of urea produces an increase in the availability of substrates for the microorganisms of the rumen before the components soluble contained in the diets.

Constant indicator of the change of the profile (parameter C). This parameter measures the change of

the profile of the gas production curve. The interaction of the effects level of urea and level of block was not significant neither important (P>.05). Point of inflection (parameter t1) was found that an interaction among the effects of the level exists of urea and the of the level of block, which turned out to be significant and important (P<.01) upon the values average of the parameter t1.

Fermentation fraccional rate (parameter R). The parameter R is increased in the initial phases of the incubation, for which the population microbial tends to be multiplied and to colonize the substrate until reaching a maximum (Rm), that is to say, when the size of the population microbial does not limit more the fermentation, and the digestion is not seen impeded by the chemical or structural components of the food, then Rm can be reached quickly in the incubation of the soluble components, which does not require of the microbial colonization under high densities of microorganisms (Groot et to the., 1996). The interaction among the effects of the level of urea and level of block turned out to be significant and important (P<.07) for the values average of the parameter R. The diets that contain a high proportion of soluble carbohydrates and protein, which they are found highly available and quickly they are fermented, they have low values of tRm and very high values of Rm (Groot et al. 1996). The levels higher of protein cube with the lowest of urea had the low values of the parameter R, that is to say, they present a smaller quantity of soluble components. These values of the parameter R tend to enlarge in agreement they enlarge the levels of urea in the block. In base to what they mention us Groot et al. (1996). Parameter R was reached more quickly in the processing with a greater quantity and availability of the soluble components, and is observed that the level of urea in the block enlarged the availability of the substrates to the ruminal microorganisms. The values of Rm are low for fodders, but once Rm has been reached, the rate fractional of fermentation can decline quick or gradually. A quick fall of the values of the parameter R can occur after that they have been consumed the components of the substrate; It is common in the case that the diet have a high content of soluble components.

Maximum Time of fermentation fractional rate (parameter tRm). When the population microbial multiplies and colonizes the substrate to digest the insoluble components of the food, then the maximum tiem of fractional fermentation rate is reached (Groot et al. 1996), and the time of incubation in which this process occurs is the tRm. An interaction among the effects of the level was observed of urea and of the level of protein cubes, which turned out to be significant and important (P<.005) upon the values average of the parameter tRm. The values of the parameter tRm in the different levels of block have a tendency to diminish in agreement enlarges the level of urea in the block. This indicates that the time of maximum rate frictional of fermentation diminishes with the addition of urea in the protein cube. The insoluble components of the substrate are colonized more quickly with higher levels of urea. It was observed that the lowest levels of block had higher values of the parameter tRm, which suggests that upon enlarging the level of block is reached but quickly the time of maximum rate frictional of fermentation, that is to say, the fermentation accelerates. When the proteins are fermented in vitro the process carries to the formation of bicarbonate of ammonia (NH_4HCO_3) as of the CO_2 and the NH_3, by consequence the contribution of CO_2 to the production of gas is reduced (Menke and Steingass, 1988), for which a possible one should keep in mind itself sub-estimation of the gas production values by the methods in vitro.

Ammonia Nitrogen. The interaction among the effect of the level of urea and the level of block was not significant neither important (P>.05) upon the contained average of ammonia nitrogen. A significant effect was observed (P<.0001) of the level of block upon the contained average of ammonia nitrogen. The effect found is of lineal type (P<.001) where to the extent that the level of block is increased in the diet, the quantity average of ammoniac is also increased in the liquid one ruminal. This is of being supposed because enlarges the quantity of nitrogen total in the diet as is observed in the Picture 1, where the processing with greater content of block have greater content of protein. The concentration of ammonia nitrogen in the rumen depends directly of the rumen degradability of the nitrogen fractions that they compose the diet, so that in agreement enlarges the proportion of protein not degradable in rumen the concentration of ammonia nitrogen in the rumen diminishes (Cecava et al., 1991).

Implications

The concentration of ammonia nitrogen in the rumen liquid after the digestion, has a tendency to enlarge in agreement they enlarge the block consumption levels of protein and the levels of urea in the block; however this increment in the concentration of ammonia nitrogen greater is affected for the level of block in the diet, to comparison of the level of urea in the block, which indicates that is important to monitor that the consumption of food by the animals be the adequate one to improve the productive performance. The level of 12% of block in the diet presented the better parameter's of fermentation ruminal for which is recommended that the consumption of block in the grass land go among 8 and 14% (B.S.) of the total of the diet, being a recommendable standard consumption for bovine of adult meat in grass land from 0.7 to 1.2 kg of block of protein by animal for day, this can vary depending. The nutritional content of the product and can also be seen affected by the age and the type of the animals. So that the commercial products comply with the objective of optimizing the animal production, is important that they have these ranks of consumption. Lower levels of consumption would not have the effect expected in the total digestibility of the diet, and therefore in the animal answer; and the highest levels of consumption would modify the rumen conditions and there would be a tendency to sub-utilize dry grass. For the elaboration of blocks of protein the level of 5% of urea in the block

showed the best fulfillment upon the parameters of fermentation ruminal evaluated, given that levels but high of urea they conduct to an increase in the concentration of ammonia nitrogen in the rumen liquid after the digestion, and these high concentrations of ammoniac in the rumen tend to diminish the production of volatile fatty acids therefore diminish the animal answer.

It is recommendable for subsequent studies the use of automatic equipment for a better measurement of production of gas. The measurement of true protein would be a parameter useful to estimate the synthesis of microbial protein.

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In vitro and in vivo rumen fermentation and gas production: influence of corn and mineral oils and their bloat potential

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ABSTRACT: The influence of mineral (MO) and corn oil (CO) on in vitro ruminal gas production, bloat potential and weight gain of steers was investigated in a series of in vitro and in vivo grazing studies. Overall objectives were to: 1) quantify in vitro the effect of source of rumen fluid, type of oils and different level of oils on rate of gas production, foam height and foam strength and 2) quantify the influence of CO on rumen fluid (RF) bio-film production, bloat potential and weight gain of steers grazing wheat pasture. In Exp. 1, duplicated analysis of in vitro gas production was measured from 0 to 6 h rumen incubation periods. In vitro rumen foam production and foam strength were measured. In Exp. 2, eighteen ruminally cannulated steers (330 ± 22 kg) were randomly allocated to one of three CO treatments (0, 1, and 2 % of DMI). Steers grazed on wheat from December 28 2004 to January 28 2005. Rumen contents were collected from wheat forage diet on d 0, 20, and 30 for ethanol perceptible bio-film complexes. Bloat was visually scored (from 0 to 3) 5 d a wk. In Exp. 1, in vitro rate of gas production was greater ($P < 0.01$) for MO than for CO addition to Bermuda grass RF. Wheat forage RF exhibited no response to oil addition. Changing the sources of RF from Bermuda grass hay to wheat forage increased ($P < 0.01$) the rate of gas and potential gas production. Addition of either MO or CO decreased ($P < 0.01$) foam height and foam strength in a dose dependant manner. Feeding CO to steers grazing wheat forage had no effect on animal weight gain. Mean bloat score was lower ($P < 0.01$) for CO addition than for control, but bio-film complexes in clarified RF increased ($P < 0.01$) at 1.5 % CO over control. It is concluded that addition of CO reduced bloat severity principally through reducing foam production and foam strength in the rumen.

Key Words: Oil addition, Ruminal gas production, Steer bloat

Introduction

There are conflicting reports on the effects of added fat and vegetable oil on rumen fermentation and animal production. Some workers have reported that added fat had no adverse effects on ruminal digestibility (Palmquist and Conrad, 1978), while others reported reductions in digestibility and food intake (Eastridge and Firkins, 2000). Several authors have pointed out that dietary fat and oil alter the ruminal microbial ecosystem and, in particular, prevention of legume bloat in cattle, but had no effect on animal performance (Reid and Johns, 1957; Lovette et al., 2003). The studies reported here were designed to the influence of mineral (**MO**) and corn oil (**CO**) on ruminal gas production and bloat potential were investigated in a series of in vitro and in vivo grazing studies. Overall objectives were to: 1) quantify in vitro the effect of source of rumen fluid, type of oils and different level of oils on rate of gas production, foam height and foam strength and 2) quantify the influence of CO on rumen fluid (**RF**) bio-film production, bloat potential and weight gain of steers grazing wheat pasture.

Materials and methods

In Exp. 1, duplicated analysis of in vitro gas production was measured as plunger displacement (ml) at 0, 1, 2, 3, 4, 5, and 6 h incubation periods (Min et al., 2005). The RF was collected from three cannulated steers fed either Bermuda grass hay or winter wheat forage diets, mixed and strained through four layers of cheesecloth and flushed with CO_2 gas for in vitro rumen incubation. Bermuda grass hay was used because it is a bloat safe forage, and therefore was used as a control diet.

The effect of oil addition on rumen foam production and strength was measured at 0 and 6 h in vitro rumen incubation (pH 6.8) with minced wheat forage, according to Okine et al. (1989). Wheat forage samples were collected randomly selected and cut to ground level twice a wk and stored at –20 ^{O}C for in vitro ruminal

gas and foam production analyses. Forage was minced prior to all in vitro experiments (Min et al., 2005). Total CP from forage samples was determined by the Kjeldahl digestion procedure (AOAC, 1990).

In Exp. 2, eighteen ruminally cannulated steers (330 ± 22 kg) were randomly allocated to one of three CO treatments (0, 1, and 2 % of DMI). Steers grazed on wheat from December 28 2004 to January 28 2005. Rumen contents were collected from wheat forage diet on d 30, 40 and 50 for bio-film production and RF microbial protein characteristics (data not shown) measurements. The RF protein fractions were assayed using the method described by Min et al. (2005). Ethanol-precipitable bio-film complexes in clarified RF (16,000 x *g* for 30 min) were assayed using the method described by Gutierrez et al. (1963). Bloat was visually scored (from 0 to 3) 5 d a wk at approximately 0800 h. The scoring system of Min et al. (2005) was employed to characterize the incidence and severity of bloat.

Statistical analysis. Data are presented as mean values, together with the standard error of the mean (SEM). Response variables included frothy bloat, forage protein, in vitro ruminal gas production, ruminal foam production and strength, and associated interactions was analyzed using the MIXED procedure of SAS (SAS, Inst., Inc., Cary, NC). In vitro gas production rate was calculated using the exponential Eq. of Ørskov and McDonald (1979). The constants parameters for each treatment were calculated with the method described by Min et al. (2000) using the Non-Linear Regression (NLIN) procedure from SAS.

Results and Discussion

As expected, total CP content was lower ($P < 0.01$) for Bermuda grass hay (10.7 g CP/kg DM) than for wheat forage (25.6 g CP/kg DM). In Exp. 1, in vitro rate of gas production was greater ($P < 0.01$) for MO than for CO addition to Bermuda grass RF (Table 1). However, wheat forage RF exhibited no response to oil addition. Changing the sources of RF from Bermuda grass hay to wheat forage resulted in increased ($P < 0.01$) rate of gas and potential gas production. Rate of gas production exhibited a source of RF x dose levels of oil interaction ($P < 0.01$), that resulted from rate of gas production increasing with oil addition to wheat forage RF, while decreasing or remaining relatively unchanged in Bermuda grass hay fed RF. This observation is similar trends with the findings of Getachew et al. (2001), who observed no effect on in vitro gas production by supplementation of tallow, but increased in vitro gas production when addition of yellow grease. However, CO decreased in vitro gas production and had no effect of digestibility and total VFA production (Getachew et al., 2001).

Addition of either MO or CO decreased ($P < 0.01$) foam height and foam strength in a dose dependant manner (Table 2). The source of RF x dose level and type of oil x dose level interactions were significant ($P < 0.01$) for foam height, suggesting that foam height decreased with level of oil addition, and it decreased more with corn oil. Negligible foam height and foam strength were detected when wheat forage was incubated with different source of RF after 6 h ruminal incubation

Feeding CO to steers grazing wheat forage had no effect on animal weight gain (Table 3) and RF microbial protein characteristics (data not shown). Mean bloat score was lower ($P < 0.01$) for CO addition than for control. This is consistent with Colvin et al. (1959), who reported that addition of vegetable oil (Wesson oil) decreased the rate of eructation, the volume of gas expelled, intra-rumen pressure, and reduced bloat frequency in dairy cattle receiving freshly harvested alfalfa forage. In addition, bio-film complexes in clarified rumen fluid increased ($P < 0.01$) at 1.5 % CO over control, suggesting that addition of CO might be modified by rumen microbial populations. The rate and extent of hydrolysis of fats and oils by rumen microorganisms both in vitro and in vivo need to be study.

Implications

Our results show that addition of CO reduced bloat severity principally through reducing foam production and foam strength in the rumen. The absence of negative effects on rumen microbial protein fractions as well as in vitro gas production by inclusion of vegetable oil in the form of unsaturated fat and mineral oil, suggested that types of oil have much less effect on rumen fermentation than source of RF and their corresponding diets. Further research is required to define bloat and animal growth responses to increasing lengths of feeding time on oil addition.

Acknowledgements

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Table 1. The influence of source of rumen fluid (RF) and types of oil addition on the in vitro ruminal gas production from Bermuda grass hay and fresh wheat forage[1]

	Ruminal gas production	
	Rate of gas	Potential gas
Item[a]	c (ml/h)	a + b (ml/12 h)
Source RF		
Bermuda grass hay diet		
Corn oil	14.1[b]	96.3
Mineral oil	17.9[a]	106.2
Mean	16.0[B]	101.3[B]
Wheat forage diet		
Corn oil	22.7	121.7
Mineral oil	21.0	117.8
Mean	21.9[A]	119.7[A]
SEM	0.70	2.86
Types and levels of oil addition		
Corn oil (% DM)		
0	18.6	109.9
1	16.7	103.5
2	19.9	113.6
Mean	18.4	109.0
Mineral oil (% DM)		
0	17.7	104.2 [0.13]
1	20.5	115.2
2	20.1	116.5
Mean	19.4	112.0
SEM	1.21	4.96

[1] Least squares means for each collection period in vitro rumen incubated with oil additives treatment. [A vs. B, a vs. b] Numbers within a column superscript letters are significantly different ($P < 0.05$).

Table 2. The influence of source of rumen fluid (RF) and types of oil addition on the in vitro ruminal foam production from Bermuda grass hay and fresh wheat forage[1]

	Foam height (FH) and strength (FS)			
	FH		FS	
Item[a]	0 h	6 h	0 h	6 h
Source RF				
Bermuda grass hay diet				
Corn oil	130.3[b]	49.2	2.8	1.2
Mineral oil	163.3[a]	50.0	2.4	0.9
Mean	146.8[B]	49.6	2.6	1.1
Wheat forage diet				
Corn oil	111.7[b]	47.5	2.6	1.0
Mineral oil	143.3[a]	48.3	2.8	0.9
Mean	127.5[A]	47.9	2.7	0.9
SEM	5.95	1.44	1.72	0.21
Types and levels of oil addition				
Corn oil (% DM)				
0	230.0[a]	45.0[b]	6.9[a]	2.6[a]
1	73.0[b]	50.0[a]	0.4[b]	0.3[b]
2	60.0[c]	50.0[a]	0.5[b]	0.1[b]
Mean	121.0[B]	48.3	2.7	1.1
Mineral oil (% DM)				
0	217.5[a]	51.3[a]	7.1[a]	2.6[a]
1	122.5[b]	50.0[ab]	0.6[b]	0.1[b]
2	120.0[b]	46.3[b]	0.4[b]	0.1[b]
Mean	153.3[A]	49.2	2.6	0.9
SEM	7.29	1.77	2.10	0.26

[1] Least squares means for each collection period in vitro rumen incubated with oil additives treatment. [A vs. B, a vs. b] Numbers within a column superscript letters are significantly different ($P < 0.05$).

Table 3. Effects of level of corn oil addition on the average daily gain (ADG), bloat potential and bio-film complexes (mg DM/ml) of rumen cannulated steers grazing winter wheat forage (n = 6).

Item	Levels of corn oil (% of DMI)				
	0	0.75	1.5	SEM	*P*-value
Initial BW (kg/hd)	336	321	332	21.5	0.88
Final BW (kg/hd)	376	353	376	19.4	0.66
ADG (43 days; kg/d)	0.9[ab]	0.8[b]	1.0[a]	0.1	0.05
Mean bloat score	0.5[a]	0.2[b]	0.02[b]	0.1	0.01
Steers that bloated	6[a]	3[b]	1[b]	1.05	0.01
Bio-film complexes	3.7[b]	3.5[b]	4.4[a]	0.21	0.01

[a vs.b] Numbers within a row with different superscripts are different ($P < 0.05$). SEM = Standard error of the mean.

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KINETICS OF RUMINAL DEGRADATION OF FOUR CORN SILAGE HYBRIDS USING IN VITRO GAS PRODUCTION

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ABSTRACT: The kinetics of ruminal fermentation of four corn silage hybrids was evaluated using in vitro gas production (IVGP). The chemical composition of the hybrids 31G98, DK641, H9403 and 238W was determined. Protein was determined to be most important nutrient influencing fermentation (P<0.05) among hybrids. The CP was the highest for H9403 (9.5%) and the lowest for the 238W hybrid (8.1%). The accumulative IVGP was different (P<0.05) among hybrids at 2, 4 and 24 h. At 2 h the H9403 hybrid had the highest gas volume (P<0.05) when compared to others but at 24 h was the lowest IVGP (P<0.05). The superiority of IVGP of the H9403 hybrid at 2 h was attributed to the relationship between IVGP and the degraded substrate. Hybrid H9403 was converted to quickly fermentable products that do not need a complete colonization to be utilized. The IVGP data were adjusted with the model described by Groot et al., (1996). In monophasic model, the asymptote (A) did not differ among hybrids, but the maximum fractional rate of substrate digestion (R_M) was different, the DK641 had a higher R_M (P<0.05) than the 31G98 and H9403 but it was similar to the 238W (P = 0.07, 0.05, 0.05 and 0.06; respectively). In relation to the time of occurrence of R_M (tR_M) there were not differences among hybrids. In the multiphasic model no differences were found among asymptotes (Ai) of hybrids. In the Phase 1, the R_{M1} was reached faster by the H9403 hybrid (P<0.05) compare to others hydrids due to degradation of soluble cell components. In the Phase 2, the value of tR_{M2} of H9403 was higher (P<0.05) than the 31G98, DK641 and 238W hybrids, which indicates that in the H9403 hybrid the only fraction remaining in this phase was insoluble.
KEYWORDS**:** *in vitro* gas production, corn hybrids

Introduction

The digestibility of forages can be estimated by biological methods, trying to mimic the digestive processes. Until now there are three main techniques to asses the nutritive value of feedstuffs: (1) Tilley and Terry method (1963) or the gas production method (Menke *et al,* 1979), that use ruminal microorganisms for digestion; (2) The cellulase technique (Jones and Hayward, 1975) that uses fungi enzymes to carry the feed degradation, and (3) ruminal incubations *in situ* (Mehrez and Ørskov, 1977) of samples contained in nylon or Dacron bags placed inside the rumen. Prediction of intake and digestibility using regression equations to predict digestibility from feedstuffs chemical composition of is not satisfactory for many forages (Van Soest, 1994).

The *in sacco, in situ (IS)* or known commonly as nylon bag, has been used during some time to provide estimations of the rate of disappearance and potential degradability of the chemical constituents of forages. Some disadvantages of this method is that a small number of forages simples can be placed in each cannulated animal, at least three animals have to be used to avoid too much variation among animals, and special care has to be taken care: bag characteristics (fabrics, size of bag, size of pores), incubation time, withdrawal of bag, amount of sample, sample preparation (drying method, temperature, size of particle), host animals and diets (Huntington and Givens, 1995). The objective of this study was to evaluate the fermentation kinetics of four corn hybrids silages using the IVGP technique.

Materials and Methods

This trial was carried on in the Sheep Production Unit of the Universidad Autonoma de Chihuahua. Four corn varieties were used: Dekalb DK 641, Eagle 238W, Golden Harvest H9403 and Pioneer 31G98. Microsilos were used to make the silage, and samples were obtained to approximately the same degree of maturity, corresponding it to 1/3 of milk line, and then frozen until chemical analysis. Four castrated males (40 ± 2.5 kg), fitted with permanent ruminal cannulas, consuming a maintenance ration, were used as source of inoculums. A 96 h gas production essay was carried on following the Menke and Steingass procedure (1988) with some modifications. Data was fitted to follow the model proposed by Groot *et al.* (1996), using the equation of the multiphasic model used of Koops.

$$G = \sum_{i=1}^{n} \frac{A_i}{1 + \frac{B_i^{C_i}}{t^{C_i}}}$$

Where G (ml g^{-1} DM) is the amount of produced gas per g of incubated DM, t is time after incubation, A_i (ml g^{-1} DM) represents the asymptote of GP. B_i (h) is time of incubation on which half of total gas has been formed, and C_i is a constant that determines the curve. The term i indicates the number of phases of the profile of GP. The parameters B y C, can provide the fractional rate of degradation of substrate (R) if we assume a linear relationship between the fermentation of substrate and GP. The ratio of potential digestible substrate is given by $P_i(t) = [A_i - G(t)]/A_i$, so R can be computed by

$$R = 1/P\ dP/dt = Ct^{c-1}/B^c + t^c$$

The time to achieve the maximum rate (t_{RM}) and R_M can be computed with the following equations:

$$t_{RM} = B(C-1)^{1/c}$$

$$R_M = Ct_{RM}^{c-1} / B^c + t_{RM}^{c-1}$$

Results and Discussion

In table 1 is presented the chemical composition of four corn silages. DM was the same for all silages, with the exception of 238W that had a higher DM content, 22.6% (P<0.05). Important differences were found for CP (P<0.05). H9403 had the highest content (9.5%), and 238W the lowest (8.1%). 31G98 and DK641 hybrids were statistically equal, but differed with respect to the rest. In relation to NDF, DK641 was different (P<0.05) to 31G98 and 238W (64.4, 67.6 y 68%, respectively), but equal to H9403 (65.3%). 31G98 (67.6%) was equal to H9403 and 238W (68%). In respect to ADF, made up mainly by cellulose and lignin, DK641 was different (P<0.05) to 31G98, H9403, y 238W (38.6, 43.4 41.5 y 42.2; respectively).

Table 1. Composition of four corn silages

Hybrid	DM	OM	CP	NDF	ADF	CE	HEM	LA	pH
	(% DM)								
31G98	19.4[b]	94.2[a]	9.0[b]	67.6[a]	43.4[a]	26.7[b]	24.2[a]	11.2[a]	3.5[a]
DK641	20.2[b]	94.1[a]	9.1[b]	64.4[b]	38.6[b]	24.5[b]	25.7[a]	9.6[a]	3.6[a]
H9403	19.8[b]	92.4[a]	9.5[a]	65.3[a][b]	41.5[a]	26.5[b]	23.8[b]	10.7[a]	3.5[a]
238W	22.6[a]	93.7[a]	8.1[c]	68.0[a]	42.2[a]	29.8[a]	25.7[a]	9.4[a]	3.5[a]
S. E.	0.38	0.42	0.05	0.63	0.57	0.53	0.39	0.44	0.03

S.E. = Standard error
[abc] = Numbers within a column with different superscripts are different ($P < 0.05$).
DM = Dry matter, OM = Oarganic matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber CE = Cellulose; HEM = Hemicellulose; LA = Lactic acid
pH = Potetial of hydrogen, % DM = Dry matter basis

In table 2 the volume of gas produced by corn silages are shown. GP increased as incubation time advanced, but only in h 2 differences were noticeable. Hybrid H9403 had a larger GP volume (8.42 ml), as compared with the other three hybrids (P<0.05), but in h 24 this hybrid had the lowest GP (P<0.05). Similar results have been reported by Kamalak *et al.* (2002) when comparing four corn silages and found significant differences among them at h 24. Blümmel *et al.* (1997b) found that some substrates with higher GP have more carbohydrates and protein to be fermented. The superiority of IVGP of hybrid H9403 at h 2 can be attributed to the relationship between the gas produced and the substrate, which can be due to the utilization of readily fermentable products by microorganisms at the onset of incubation.

Correlation between IVGP and chemical components of forages are shown in table 3. MO was strongly correlated with IVPG at 24, 48, 72 y 96 h, whereas CP show a high correlation only at 4 h. Getachew *et al.* (2004) found a high correlation between chemical composition, degradation of DM and IVPG of several feedstuffs, finding a negative correlation (P<0.001) between CP and IVGP. These results concur with this study, with the exception of 4 h.

Table 2. Gas production of four corn silages

Hybrid	Incubation time (h)								
	2	4	6	8	12	24	48	72	96
31G98	5.70[b]	10.16	14.54	17.32	24.63	39.66[a]	47.60	48.76	48.76
DK641	6.29[b]	9.89	13.07	16.19	22.90	37.92[ab]	47.34	48.93	49.21
H9403	8.42[a]	11.05	13.19	15.68	20.79	33.43[b]	43.66	46.22	46.37
238W	5.29[b]	9.01	11.97	15.69	22.64	37.09[ab]	45.90	48.38	48.38
E. E.	0.50	0.60	0.80	0.89	1.03	1.20	1.12	1.16	1.15

S.E.= Mean standard error.
[abc] = Numbers within a column with different superscripts are different ($P < 0.05$).
DM = Dry matter.

Table 3. Pearson correlation coefficients between IVGP at different time with some nutritive entities of four corn silages

	Incubation time (h)								
	2	4	6	8	12	24	48	72	96
OM	-.87	-.63	0.24	0.65	0.91	0.97[a]	.99[b]	.99[b]	.98[a]
CP	0.81	0.95[a]	0.58	0.15	-.31	-.38	-.28	-.50	-.44
NDF	-.63	-.53	-.02	0.28	0.48	0.39	0.18	0.25	0.14
ADF	-.21	-.01	0.32	0.38	0.32	0.15	-0.08	-0.13	-0.23
CEL	-.40	-.55	-.51	-.29	-.03	-.06	-0.24	-0.07	-0.16
HEM	-.65	-.85	-.64	-.27	0.19	0.37	0.46	0.66	0.69

[a] Significant at<0.05).
[b] Significant at (P<0.01).
OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, CEL = Cellulose; HEM = Hemicellulose.

The observed correlation between IVGP and h 4 can be explained by the assumption that IVGP at the onset of fermentation was strongly influenced by the soluble components of silage, some of them of protein origin. There was no correlation between IVGP and fiber, as Getachew *et al.* (2004) reported. Because the proteic fraction in forages is usually very soluble, it is likely that this fraction is fermented during the early phase of incubation. (Cone *et al.,* 1999).

In table 4 it is reported the asymptote of gas production (A_1) and other kinetic parameters for the corn silages using the monophasic model. A_1 went from 50.73 to 54.02 ml / 200mg substrate DM. These findings does not concur with the work reported by Kamalak *et al.* (2002), who did not find differences between the asymptotes of four corn silage asymptpates. Such discrepancy can be due to the VFA profiles observed among both studies, suggesting that the genotype of the corn varieties of both studies were different.

The parameters obtained using the multiphasic model are shown in table 5. IVGP of phases 1 and 2 represent the soluble and insoluble fraction of corn

silage, respectively. No differences were found in asymptotes of silages. Values of A_1 were among 11.81 to 17.81 ml / 200mg MS, whereas A_2 had values from 31.83 to 38.39. In opposition to the monophasic model, R_M did not register significant differences between corn hybrids between phases.

Table 4. Parameters of fermentation kinetics of four corn silages using the monophasic model

Hybrid	A (ml/200 mg MS)	t_M (h)	R_M (h)	t_{MAX} (h)	dg/dt (ml/200 mg MS / h)
31G98	52.84	6.71	0.05 [b]	3.62	2.74
DK641	54.02	8.31	0.07 [a]	4.66	2.89
H9403	50.73	5.68	0.05 [b]	3.03	2.50
238W	52.79	8.11	0.06 [ab]	4.55	2.64
S. E.	1.32	0.76	0.004	0.44	0.14

S. E. Standard error of the least square mean
[abc] = Numbers within a column with different superscripts are different ($P < 0.05$).
A = Gas asymptote
R_M (h) = Maximum fractional of digestión of substrate.
t_M (h) = Time of occurrence of R_M.
t_{MAX} (h) = Time of occurrence of the maximum rate of gas production.
dg/dt = Maximum rateo f gas production.
DM = Dry matter.

Table 6. Parameters of fermentation kinetics of four corn silages using the multiphasic model

Hybrid	A_1 (ml/200 mg MS)	A_2 [d] (ml/200 mg MS)	t_{M1} (h)	R_{M1} (h)	t_{M2} (h)	R_{M2} (h)	t_{MAX1} (h)	t_{MAX2} (h)	dg/dt_1 (ml/200 mg MS/h)	dg/dt_2 (ml/200 mg MS/h)
31G98	17.81	31.83	2.40 [a]	0.32	19.83 [b]	0.22	1.34 [a]	12.22 [b]	3.63	1.49 [ab]
DK641	11.81	38.39	1.76 [ab]	0.44	19.69 [b]	0.17	1.00 [ab]	11.99 [b]	3.87	1.74 [a]
H9403	14.85	32.63	0.73 [b]	0.38	27.68 [a]	0.23	0.39 [b]	17.36 [a]	5.66	1.34 [b]
238W	12.18	37.35	1.88 [ab]	0.49	20.33 [b]	0.18	1.05 [ab]	12.38 [b]	5.99	1.55 [ab]
S. E.	1.66	1.82	0.34	0.04	1.84	0.04	0.19	1.24	2.38	0.10

S. E. Standard error of the least square means
[abc] = Numbers within a column with different superscripts are different ($P < 0.05$).
A_1 = Asymptote in the first phase.
A_2 = Asymptote in the second phase.
R_{M1} (h) = Maximum fractional rate of digestion of substrate estimated in the first phase.
R_{M2} (h) = Maximum fractional rate of digestion of substrate estimated in the second phase
t_{M1} (h) = Time of occurrence of R_{M1}.
t_{M2} (h) = Time of occurrence of R_{M2}.
t_{MAX1} (h) = Time of occurrence of the maximum rate of gas production in the first phase.
t_{MAX2} (h) = Time of occurrence of the maximum rate of gas production in the second phase.
dg/dt_1 = Maximum rate of gas production in the first phase.
dg/dt_2 = Maximum rate of gas production in the first phase.
DM = Dry matter.

Implications

The comparison of fermentation kinetics of IVGP of mono and multiphasic models permit the insight of the mechanistics relationships among the way that the ruminal microorganisms act upon the different substrates that comprises forages. When possible, the multiphasic approach should be taken.

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VALINE LIMITS NITROGEN RETENTION OF GROWING LAMBS

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ABSTRACT: Six ruminally cannulated Rambouillet wether lambs (46 ± 1.3 kg BW) were used in a 6 × 6 Latin square to determine if branched-chain amino acids (**AA**) limit N retention. Lambs were limit-fed (0.80 kg DM/d) twice daily a diet (80% soybean hulls, 15% alfalfa hay, 3.5% molasses, 0.35% urea, 1.5% minerals/vitamins) low in rumen undegradable protein. All lambs received continuous infusions of acetate and propionate into the rumen to supply additional energy. Treatments were composed of abomasal infusions of a solution (500 mL/d) containing 1) a mixture of 10 essential AA, 2 nonessential AA, and glucose (**12AA**), 2) 12AA with Leu removed, 3) 12AA with Ile removed, 4) 12AA with Val removed, 5) 12AA with Leu, Ile, and Val removed, and 6) 12AA with all AA removed. Periods consisted of 7 d with 3 d for adaptation to treatments, and 4 d for collections. Retained N decreased ($P < 0.10$) in response to removal of all AA, demonstrating that at least one AA was limiting. Retained N also decreased ($P < 0.10$) in response to removal of all branched-chain AA, and to the removal of only Val. However, removal of Leu and Ile did not affect N retention. These results demonstrate that Val limits N retention and may limit lean tissue deposition of lambs when fed a diet low in ruminally undegradable protein.

Key Words: Amino acids, N retention, Lambs

Introduction

When dietary CP consists predominantly of ruminally degradable protein, microbial protein is the major source of amino acids (**AA**) available for absorption by ruminants (Merchen and Titgemeyer, 1992). Storm and Ørskov (1984) reported that microbial protein of sheep maintained by intragastric nutrition was limiting in Met, Lys, His and Arg. Recent research by Nolte et al. (2004) demonstrated that Met and at least one of the branched-chain AA were limiting when growing lambs were fed a diet containing predominantly ruminally degradable protein. Our objective was to determine which branched-chain AA limit N retention of lambs when fed a diet low in ruminally undegradable protein.

Materials and Methods

Experimental procedures were approved by New Mexico State University's Institutional Animal Care and Use Committee. Six ruminally cannulated Rambouillet wether lambs (46 ± 1.3 kg BW) in a 6 × 6 Latin square experiment were housed individually in metabolism crates in a room with continuous lighting. The lambs had free access to fresh water and were limit-fed (0.80 kg/d, DM basis) a soybean hull-based diet (Table 1) in equal portions twice daily. The diet was formulated to contain low concentrations of ruminally undegradable protein so that microbial protein would be the predominant source of AA supplied to the small intestine. To supply additional energy to the animal without greatly altering rumen microbial growth, all lambs were continuously infused with VFA (41 g/d acetate and 14 g/d propionate) into the rumen and glucose (75 g/d) into the abomasum. Infusions into the rumen were made by placing flexible tubing through the rumen cannula, and abomasal infusions were made by extending a similar tube through the reticulo-omasal orifice of lambs. The tubes were secured in the abomasum with a rubber flange (3 cm in diameter).

Treatments were continuous abomasal infusions of a solution (500 mL/d) containing 1) a mixture of 10 essential AA, 2 nonessential AA, and glucose (**12AA**), 2) 12AA with Leu removed, 3) 12AA with Ile removed, 4) 12AA with Val removed, 5) 12AA with Leu, Ile, and Val removed, and 6) 12AA with all AA removed. The AA supplied by the 12AA treatments were (g/d): DL-Met (2.0), L-Lys (7.0), L-His (3.4), L-Thr (3.3), L-Arg (6.6), L-Phe (3.7), L-Trp (0.5), L-Leu (8.2), L-Ile (2.0), L-Val (4.7), L-Glu (11.0), and Gly (6.0).

Experimental periods were 7 d, which allowed 3 d for adaptation to treatments, and 4 d for collection of feces and urine. Total feces and a representative sample of urine (5%) were saved, composited by period for each lamb, and frozen for later analysis. Urine was collected into bottles containing 50 mL 6 *N* HCl to prevent NH_3 loss. Feed and fecal samples were dried at 55°C in a forced-air oven and ground to pass a 1-mm screen. Dietary and fecal samples were analyzed for DM (105°C for 24 h) and OM (500°C for 8 h), and dietary, fecal, and urinary samples were analyzed for N (LECO FP-528, LECO Corporation, St. Joseph, MI) to calculate N retention.

Data were analyzed statistically using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included effects of period and treatment, with lamb as a random effect. Data are presented as least squares means, and differences were considered significant when $P < 0.10$.

Table 1. Diet composition

Item	% of DM
Ingredient	
Soybean hulls	79.6
Alfalfa hay	15.0
Cane molasses	3.5
Mineral/Vitamin premix[a]	0.80
Sodium bicarbonate	0.50
Urea	0.35
Salt	0.20
Elemental sulfur	0.05
Nutrient	
OM	91.6
CP	14.3
RDP[b]	12.1

[a] Composition: Ca (14.0 to 16.8%), P (≥ 11.0%), NaCl (11.0 to 13.2%), Mg (≥ 0.50%), K (≥ 0.10%), Cu (5.0 to 7.0 ppm), Se (≥ 15 ppm), Zn (≥ 1980 ppm), Vit A (660 KIU/kg), Vit D (165 KIU/kg), Vit E (1.32 KIU/kg).
[b] Ruminally degraded protein, calculation based on table values (NRC, 1996).

Results and Discussion

To identify limiting AA, infusions of VFA into the rumen and glucose into the abomasum ensured that the basal supply of energy would not limit responses to changes in postruminal AA supply. Also, abomasal infusions of all essential AA (12AA) in a profile that matched the lamb's requirement as closely as possible, ensured that a single limiting AA could be evaluated without several essential AA being co-limiting. Then, a response in N retention to the removal of a single AA from the 12AA mixture would demonstrate that the basal supply of that essential AA was limiting, whereas no response would demonstrate that the basal supply of that AA was not limiting.

Nitrogen retention of lambs decreased ($P < 0.10$) by 58% when all AA were removed from the 12AA mixture (Table 2). This demonstrated that the basal supply of AA to the small intestine (dietary plus microbial) was limiting in protein or in at least one AA. These results are consistent with those of Nolte et al. (2004), who also demonstrated large decreases (67 to 73%) in N retention when all AA were removed from a 12AA mixture. A 15% decrease ($P < 0.10$) in N retention in response to the removal of all branched-chain AA (Leu, Ile, plus Val) is consistent with reports by Nolte et al. (2004), who concluded that Met and at least one of the branched-chain AA were limiting for growing lambs. In the current study, N retention of lambs decreased ($P < 0.10$) by 15% in response to the removal of Val, but was not affected by the removal of Leu and Ile from the 12AA mixture. These results indicate that the basal supply of postruminal essential AA was limiting in Val, and not Leu and Ile. Our finding that Val was limiting is in agreement with a report for cattle (Löest et al., 2001).

Implications

The results of this experiment demonstrate that the branched-chain amino acid, valine, may limit growth of lambs when fed a diet containing protein that is predominantly degraded in the rumen.

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Table 2. Effects of removing AA from postruminal infusions on N balance of growing lambs.

	Treatments[a]						
Item	12AA	-LEU	-ILE	-VAL	-BCAA	NC	SEM
	---------- g/d ----------						
Feed N	15.8	17.8	16.7	16.9	17.9	17.2	0.96
Feed + Infused N	24.7	25.8	25.4	25.2	25.1	17.2[c]	0.96
Fecal N	6.9	7.4	6.8	6.9	8.4	6.7	0.60
Urinary N	9.9	10.9[c]	11.3[c]	11.6[c]	9.9	7.0[c]	0.46
Retained N	8.0	7.4	7.3	6.8[c]	6.8[c]	3.4[c]	0.55
	---------- % ----------						
Retained N Efficiency[b]	32.2	28.7	27.9	27.0[c]	26.8[c]	19.2[c]	2.07

[a] 12AA = mixture of 10 essential AA, 2 nonessential AA, and glucose; -LEU = Leu removed from 12AA; -ILE = Ile removed from 12AA; -VAL = Val removed from 12AA; -BCAA = Leu, Ile, and Val removed from 12AA; NC = negative control with all AA removed from 12AA.
[b] Retained N Efficiency = (N retained / N from feed plus infusion) × 100.
[c] Different from 12AA ($P < 0.10$).

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EFFECTS OF VARIOUS EXPOSURE ROUTES OF SWAINSONINE ON SERUM CONSTITUENTS, RUMEN CHARACTERISTICS, AND NUTRIENT METABOLISM OF SHEEP

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ABSTRACT: Nineteen mixed breed wethers (42.6 ± 1.5 kg initial BW) were used in a two period (14-d treatment period plus 8-d post-treatment period) experiment to determine the effects of swainsonine via subacute ruminal, abomasal, or intravenous exposure on ruminal characteristics, serum constituents, diet digestibility, and N retention. The animals were fed once daily at 1.8% of BW (DM basis) a diet of 95% fescue hay and 5% alfalfa hay (DM basis). Treatments were: 1) no (CON; n = 5), 2) ruminal (RUM; n = 5), abomasal (ABO; n = 4), and 4) intravenous (IV; n = 5) infusions of a locoweed extract to deliver 0.6 mg of swainsonine/kg BW/d on d 1 to 14 of the experiment (subacute exposure). Serum concentrations of aspartate aminotransferase were greater ($P < 0.05$) for RUM, ABO, and IV versus CON on d 8, 12, 16, 18, and 20 of the experiment. Serum alkaline phosphatase was greater ($P < 0.05$) for RUM when compared to CON on d 2, 4, 8, 12, 16, and 18, but were not different ($P > 0.11$) among ABO, IV, and CON on d 4 to 20. No differences ($P > 0.25$) were observed for serum concentrations of glucose, non-esterified fatty acids, and serum urea N. Ruminal concentrations of VFA, pH, and ammonia collected on d 14 and d 23 of the study were not different ($P > 0.15$) among treatments. Nutrient intake and digestibility, and N retention were not affected ($P > 0.11$) by treatments. Results demonstrated that subclinical intoxication occurs when sheep are exposed to swainsonine, but appears to have little effect on ruminal fermentation, nutrient digestibility, and N retention.

Key Words: Swainsonine, Nutrient metabolism, Sheep

Introduction

Swainsonine, an indolizidine alkaloid, is the primary toxicant to cause locoism in sheep and beef cattle that have consumed locoweed. Most of the published research has defined symptoms associated with locoweed intoxication and the impact on livestock productivity. No research has been conducted describing the effects of various exposure routes of swainsonine on nutrient metabolism and blood metabolistes. Goss et al. (1994) reported that when swainsonine was administrated intravenously in human at 0.15 mg swainsonine/kg BW/d, serum swainsonine levels were approximately 4 µg/mL. Stegelmeier et al. (1995), on the other hand, dosed sheep orally with 1.5 mg swainsonine and reported serum swainsonine levels of only 0.4 µg/mL. Although differences in serum swainsonine levels between humans and ruminants may differ due to different clearance rates from the body, the lower serum swainsonine concentrations for sheep could also be due to degradation or alteration of swainsonine in the rumen. Therefore, the objective of this study was to determine effects of swainsonine during subacute ruminal, abomasal, and intravenous exposures on ruminal characteristics, serum constituents, diet digestibility, and N retention of sheep fed a forage-based diet.

Materials and Methods

Animals and Diet. Experimental protocols were approved by the Institutional Animal Care and Use Committee of New Mexico State University. Nineteen ruminally cannulated mixed breed wethers (42.6 ± 1.5 kg initial BW) were housed in individual metabolism crates (130 cm × 46 cm), had free access to water, and were fed once daily at 1.8% of BW (DM basis) a diet of chopped fescue and alfalfa hay (Table 1). Wethers were allowed 14 d to adapt to the diet and metabolism crates before initiation of the experiment.

Experimental Design and Treatments. The experiment consisted of a 14-d treatment period, followed by an 8-d post-treatment period. Treatments were: 1) no (**CON**; n = 5), 2) ruminal (**RUM**; n = 5), abomasal (**ABO**; n = 4), and 4) intravenous (**IV**; n = 5) infusions of a locoweed extract to deliver 0.6 mg of swainsonine/kg BW/d at 1 h after daily feeding during the 14-d treatment period. The locoweed extract was prepared as described by Taylor and Strickland (2002) and the final water-soluble fraction of this extract contained 4.51 mg/mL swainsonine (α-mannosidase inhibition assay; Taylor and Strickland, 2002). Sterile physiological saline was infused (0.133 mL/kg BW) into those sites (i.e., rumen, abomasum, and jugular vein) of CON, RUM, ABO, and IV animals where locoweed extract was not infused. Polyethylene tubing (Tygon; 1/16 mm i.d.) was used for infusions into the rumen and abomasum. Abomasal infusion lines were passed through the rumen and reticulo-omasal orifice, and were secured with a rubber flange (4-cm diameter) in the abomasum (Löest et al., 2001). Intravenous treatments were delivered using 21 gauge needles (HENKE SASS WOLF GMBH, Tuttlingen,

Germany) that were 25 mm in length. To insure sterility, locoweed extract was autoclaved (STERILMATIC, Market Forge Industries INC, Everett, MA 02149) for 15 min at 121°C.

Table 1. Diet composition

Item	% of DM
Ingredient	
Fescue hay	95.0
Alfalfa hay	5.0
Nutrient	
NDF	51.6
ADF	28.7
CP	13.4
Ca	0.88
P	0.23

Sample Collections and Analysis. Blood samples (10-mL) were collected via jugular venupuncture (Corvac serum separator, Sherwood, St. Louis, MO) from each animal 4 h after exposure to treatments (equivalent to 5 h after feeding) on d 0, 1, 2, 4, 8, and 12 of the treatment period and on d 16, 18, and 20 (post-treatment period). Blood samples were allowed to clot (60 min at room temperature), and then centrifuged at 1,500 × g for 30 min at 4°C. Serum was stored at -20°C until subsequent analyses for alkaline phosphatase (ALK-P) and aspartate aminotransferase (AST). Also, serum was analyzed for urea N (Thermo DMA, Louisville, CO, 80027, Cat No. TR-12321), non-esterified fatty acids (NEFA C, Wako Chemicals USA, INC., Richmond, VA 23237, Cat No. 994-75409E), and glucose (Thermo DMA, Louisville, CO, 80027, Cat No. 1530-500) using modified endpoint protocols for a 96-well microtiter plate reader (MRX HD, Dynex laboratories, Chantilly, VA).

Total feces and urine were collected, weighed, and recorded on d 10, 11, 12, and 13 (treatment period), and d 20, 21, 22, and 23 (post-treatment period). Representative samples (10% for feces; 5% for urine) were composited for each animal by period (treatment period vs post-treatment period) and frozen at -20°C for later analyses. Urine was collected into bottles containing 6 N HCl (50 mL) to prevent loss of ammonia. Feed samples, feed refusals, and fecal samples were dried at 55ºC in a forced-air oven, air-equilibrated, ground (2-mm screen), and analyzed for DM (100ºC for 24 h), OM (500ºC for 8 h), NDF and ADF (Ankom 200 fiber analyzer, Ankom Technology Cooperation, Fairport, NY), and N (LECO N analyzer FP-528, LECO Corporation, St. Joseph, MI). Urinary samples were also analyzed for N.

On d 14 (treatment period) and on d 23 (post-treatment period), ruminal contents was collected at 30 min before treatment administration and at 3, 6, 9, and 12 h after treatment administration, strained through four layers of cheesecloth, and the pH was measured. A 10-mL aliquot was acidified with 6 N HCl (0.5 mL) and frozen for later analysis of ammonia by the procedure of Broderick and Kang (1980) modified for micro analysis using 96 well microtiter plate reader. An additional 10 mL was acidified with 2 mL of 25% metaphosphoric acid and frozen for VFA analysis (May and Galyean, 1996) using gas chromatography (Star 3400, Varian, Walnut Creek, CA).

Statistical Analysis. Data were analyzed using the MIXED procedure of SAS (v 8.1, SAS Inst. Inc., Cary, NC). For serum data, fixed effects included treatment, day, and treatment × day, with repeated measures utilizing compound symmetric covariance structure. For ruminal VFA, ammonia, and pH, fixed effects were treatment, hour, and treatment × hour. Data for each day (d 14 vs. d 23) were analyzed separately, and analyses include repeated measures utilizing compound symmetric covariance structure. For nutrient intake and digestibility, and N retention, fixed effects included treatment only. Data for each period were analyzed separately. For all data, least square means were separated using appropriate pair-wise t-tests if the fixed effects were significant ($P < 0.05$).

Results and Discussion

For this experiment, a dose of swainsonine to the rumen was used to mimic oral intake of swainsonine similar to that for an animal consuming locoweed. Dosing swainsonine into the abomasum would evaluate effects on the post-ruminal gastrointestinal tract, thus separating the effects in the rumen from the rest of the tract, whereas infusing swainsonine intravenously would evaluate its direct effects on post-absorptive metabolism.

Serum concentrations of AST (Figure 1) exhibited a treatment × day interaction ($P < 0.05$). No differences ($P > 0.13$) were detected in serum AST among treatments before the treatments were initiated (d 0) and on d 1 and 2 of the treatment period, but serum AST increased ($P < 0.05$) on d 4, 8, and 12 for RUM, and on d 8 and 12 ($P < 0.05$) for ABO and IV when compared to CON. Serum levels of AST continued to be greater ($P < 0.05$) for RUM, ABO, and IV vs. CON on d 16, 18, and 20 (post-treatment period), indicating that effects of intoxication did not subside within 6 d after the exposure to swainsonine was terminated**.** Also, serum AST concentrations were greater ($P < 0.01$) for RUM than for ABO and IV on d 18, 4 d after swainsonine exposure was terminated.

A treatment × day interaction ($P < 0.05$) was noted for serum ALK-P (Figure 1). There were no differences ($P > 0.26$) among treatments on d 0 and d 1, but serum ALK-P levels increased ($P < 0.05$) at d 2 for RUM, ABO, and IV when compared to CON. Serum levels of ALK-P continued to be greater ($P < 0.05$) throughout the remainder of the treatment period (d 4 to 14) for RUM vs. CON, whereas serum ALK-P levels for ABO and IV returned to baseline levels and were not different ($P > 0.11$) to CON for the remainder of the treatment period and the post-treatment period (d 16 to 20). However, serum levels of ALK-P for RUM continued to be greater ($P < 0.05$) than CON on d 16 and 18 (post-treatment period), but returned to baseline levels on d 20 of the experiment. These results agree with Bachman et al. (1992) and Pulsipher et al. (1994) who demonstrated decreases in serum ALK-P levels 7 to 14 d after swainsonine exposure was terminated. A brief elevation in serum ALK-P for ABO and IV on d 2 only, was surprising, because all previous studies reported that

ALK-P remains high during the entire period of swainsonine exposure (Bachman et al., 1992; Pulsipher et al., 1994). However, elevated serum ALK-P levels for RUM are consistent with other reports in the literature. It is likely that elevated serum ALK-P levels in ruminants consuming locoweed are due to ruminal metabolism of swainsonine to a compound that is more toxic to the animal, or due to the production of other toxic compounds in the rumen in response to swainsonine.

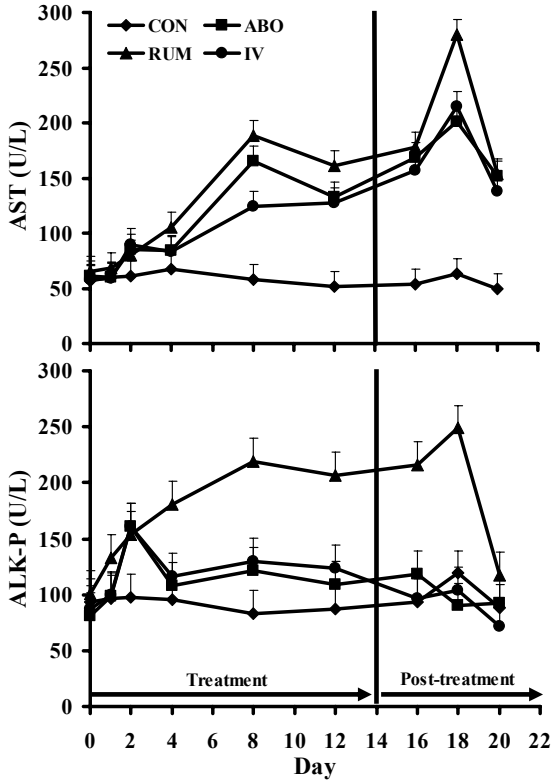

Figure 1. Serum concentrations of aspartate amino-transferase (AST) and alkaline phosphatase (ALK-P) collected from sheep 5 h after feeding during a treatment (d 1 to 14) and post-treatment (d 15 to 23) period. Treatments were: 1) no (CON; n = 5), 2) ruminal (RUM; n = 5), abomasal (ABO; n = 4), and 4) intravenous (IV; n = 5) infusions of 0.6 mg of swainsonine/kg BW/d delivered by locoweed extract (4.51 mg swainsonine/mL).

No treatment × day interactions ($P > 0.40$) and no treatment effects ($P > 0.25$) were noted for serum concentrations of glucose, NEFA, and urea N (Figure 2). These results are consistent with reports by Pulsipher et al. (1994) and Taylor et al. (2000) who found no differences in serum concentrations of glucose and urea N when sheep were fed locoweed for 28 to 35 d. A day effect ($P < 0.05$) was noted for serum glucose and NEFA, and a tendency for a day effect ($P = 0.09$) was observed for urea N.

No treatment × time interaction ($P > 0.15$) and no treatment effects ($P > 0.16$) were detected for molar concentrations of VFA, pH, and ammonia among treatments during the treatment and post-treatment periods (Table 2). It was hypothesized that swainsonine may alter ruminal fermentation profiles due to potential toxic effects on rumen microorganisms. However, rumen fermentation profiles of this study indicated that rumen microbial activity was not affected by exposure to swainsonine in the rumen. These results are consistent with Reed (2004) who found no effects of swainsonine on ruminal VFA concentrations of sheep fed locoweed at 0, 0.2, and 1.6 swainsonine/kg BW/d for 23 d.

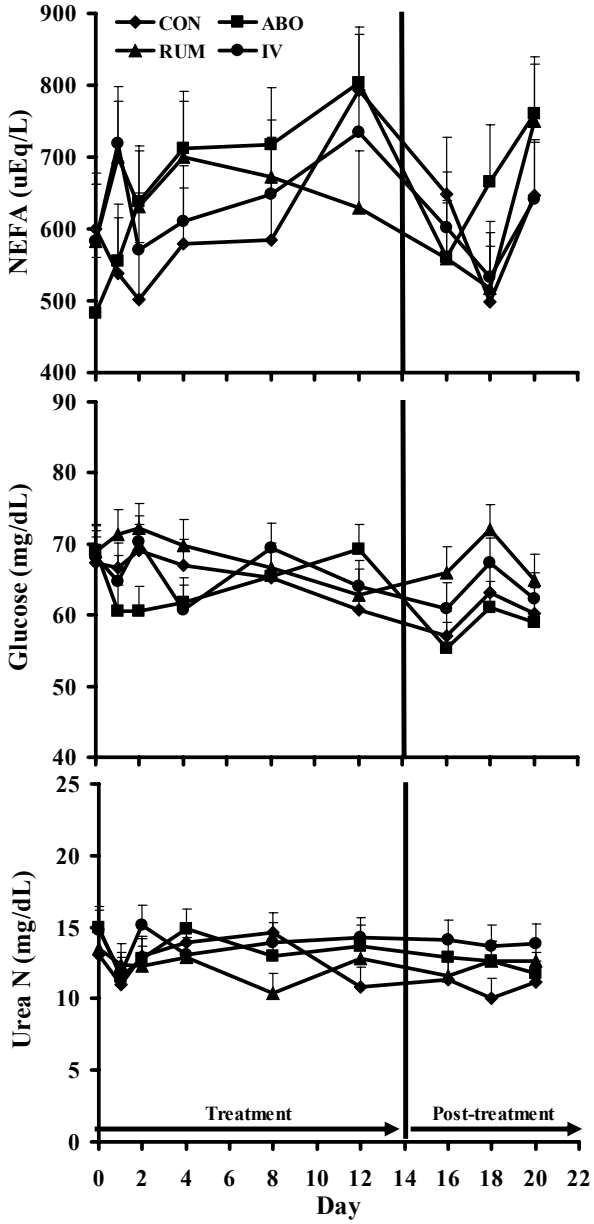

Figure 2. Serum concentrations of non-esterified fatty acids (NEFA), glucose, and urea N collected from sheep 5 h after feeding during a treatment (d 1 to 14) and post-treatment (d 15 to 23) period. Treatments were: 1) no (CON; n = 5), 2) ruminal (RUM; n = 5), abomasal (ABO; n = 4), and 4) intravenous (IV; n = 5) infusions of 0.6 mg of swainsonine/kg BW/d delivered by locoweed extract (4.51 mg swainsonine/mL).

Intake, fecal excretion, and digestibility of DM, OM, CP, NDF, and ADF are presented in Table 3. No differences ($P > 0.11$) in apparent total tract digestibility for DM, OM, CP, NDF, and ADF were observed among

treatments during the study except for NDF during the post-treatment period. Digestibility of NDF tended to be greater (P = 0.08) for RUM when compared to CON, ABO, or IV treatments. However, Reed (2004) observed no differences in digestibility of DM, CP, NDF, and ADF when sheep were fed levels of locoweed that supplied 0, 0.2, and 1.6 mg swainsonine/kg BW/d.

Implications

Results of this study indicate that administering swainsonine into the rumen, abomasum, and intravenously at 0.6 mg of swainsonine/kg BW/d caused intoxication of sheep based on serum concentrations of aspartate aminotransferase. However, serum concentrations of alkaline phosphatase responded only in those animals exposed to swainsonine via their rumen. Subacute ruminal, abomasal, and intravenous exposure to swainsonine had little impact on ruminal fermentation characteristics, total tract digestibility of nutrients, and nitrogen retention. Therefore, consumption of locoweed (i.e., swainsonine) by ruminants should have little, if any, negative effects on utilization of nutrients by sheep.

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Table 2. Ruminal pH, ammonia, and VFA concentrations from sheep on d 14 and 23.

	Treatments[a]				
Item	CON	ABO	RUM	IV	**SEM[b]**
Treatment period[c]					
pH	6.65	6.64	6.47	6.68	0.97
Ammonia, mM	4.60	4.12	3.92	3.73	0.91
Total VFA, mM	74.95	78.83	88.62	83.50	7.33
Acetate, mM	51.01	53.72	59.49	56.41	4.72
Propionate, mM	15.04	15.45	17.85	17.31	2.24
Butyrate, mM	6.65	7.56	8.13	7.44	0.92
Post-treatment period[c]					
pH	6.83	6.98	6.84	7.06	0.16
Ammonia, mM	5.52	4.07	5.67	4.13	0.98
Total VFA, mM	84.65	78.58	68.57	72.74	13.79
Acetate, mM	57.66	55.56	48.15	50.65	9.23
Propionate, mM	16.25	13.17	12.29	13.74	3.03
Butyrate, mM	8.01	7.19	5.88	6.12	1.62

[a]Treatments were: 1) no (CON; n = 5), 2) ruminal (RUM; n = 5), abomasal (ABO; n = 4), and 4) intravenous (IV; n = 5) infusions of 0.6 mg of swainsonine/kg BW/d delivered by locoweed extract (4.51 mg swainsonine/mL).
[b]n = 5.
[c]Collections were made on d 14 (treatment period) and on d 23 (post-treatment period) at 30 min before treatment administration and at 3, 6, 9, and 12 h after treatment administration.

Table 3. Intake, excretion, and digestibility of OM, NDF, and N retention of sheep.

Item	Treatments[a]				SEM[b]
	CON	ABO	RUM	IV	
Treatment period					
OM					
Intake, g/d	733.7	671.9	781.4	800.7	76.9
Feces, g/d	229.8	189.9	237.0	241.9	30.1
Digested, g/d	503.9	482.1	544.5	558.7	51.5
Digestibility, % of intake	68.5	72.8	70.0	69.8	1.8
NDF					
Intake, g/d	401.7	371.0	427.7	437.1	39.7
Feces, g/d	145.8	121.7	140.9	152.8	19.1
Digested, g/d	255.9	249.3	286.8	284.3	23.5
Digestibility, % of intake	63.8	68.7	67.3	65.1	2.2
N					
Intake, g/d	16.5	15.0	17.7	17.9	1.63
Feces, g/d	5.7	4.6	6.0	5.9	0.74
Urine, g/d	6.3	6.6	8.3	8.4	1.17
Digested, g/d	10.8	10.5	11.7	12.1	0.99
Retained, g/d	4.5	3.8	3.4	3.6	1.02
Retention, % of intake	26.1	28.3	20.1	19.7	5.79
Post-treatment period					
OM					
Intake, g/d	670.0	639.1	560.4	656.1	110.8
Feces, g/d	205.6	193.2	159.8	198.7	39.0
Digested, g/d	464.4	445.9	400.6	457.4	73.7
Digestibility, % of intake	69.7	69.8	72.8	70.5	1.9
NDF					
Intake, g/d	366.0	350.1	335.5	360.6	55.1
Feces, g/d	129.2	121.0	96.9	127.4	25.6
Digested, g/d	236.8	229.2	238.6	233.2	31.1
Digestibility, % of intake	65.9	65.8	73.3	65.7	2.9
N					
Intake, g/d	14.8	13.8	12.3	14.6	2.50
Feces, g/d	5.2	4.8	4.2	5.3	0.93
Urine, g/d	6.5	7.5	5.8	7.1	1.32
Digested, g/d	9.6	8.9	8.1	9.3	1.65
Retained, g/d	4.5	1.4	2.3	2.3	1.20
Retention, % of intake	26.8	9.7	17.6	13.7	7.42

[a]Treatments were: 1) no (CON; n = 5), 2) ruminal (RUM; n = 5), abomasal (ABO; n = 4), and 4) intravenous (IV; n = 5) infusions of 0.6 mg of swainsonine/kg BW/d delivered by locoweed extract (4.51 mg swainsonine/mL).

[b]n = 5.

[c]Collected were made on d 10, 11, 12, and 13 (treatment period), and d 20, 21, 22, and 23 (post-treatment period) composited for each animal by period.

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FATTY ACID COMPOSITION OF PLASMA, MEDIAL BASAL HYPOTHALAMUS, AND UTERINE TISSUE IN PRIMIPAROUS BEEF COWS FED HIGH-LINOLEATE SAFFLOWER SEEDS

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ABSTRACT:[1] Experimental objectives were to evaluate the influence of supplemental high-linoleate safflower seeds on fatty acid concentrations in plasma, brain, and reproductive tissues and 13,14-dihydro-15-keto $PGF_{2\alpha}$ metabolite (PGFM) in serum of primiparous beef cows during early lactation. Beginning 1 d postpartum, 18 primiparous, crossbred beef cows (BW = 410 ± 24.2 kg) were fed Foxtail millet hay at 2.1% of BW and either a low-fat control supplement (61.2% corn, 32.1% safflower seed meal, 3.7% liquid molasses; **Control**) at 0.37% BW (n = 9) or a supplement containing 94% cracked high-linoleate (77% 18:2) safflower seeds and 6.0% liquid molasses (**Linoleate**) at 0.24% of BW (n = 9). Diets were formulated to provide similar quantities of N and TDN and the Linoleate diet was formulated to contain 5% fat. Starting 1 d postpartum, cattle were bled every 3 d for collection of sera and plasma. Cattle were slaughtered after 37 ± 3 d for collection of the medial basal hypothalamus, myometrium, endometrium, caruncular tissue, intercaruncular tissue, and oviduct. Feeding Linoleate increased (P = 0.001) plasma concentrations (mg/g) of 18:2, 18:2$^{cis\text{-}9\ trans\text{-}11}$, and total unsaturated fatty acids, however, 18:1$^{trans\text{-}11}$ did not differ (P = 0.19) between treatments. Concentrations of individual fatty acids in the medial basal hypothalamus did not differ (P = 0.17 to 0.95) or reflect the fatty acid composition of the plasma. The oviduct had the greatest (P < 0.05) fatty acid concentrations compared with other uterine tissues. Cows fed Linoleate had higher (P = 0.05) endometrial 18:3 and lower (P = 0.06) myometrial 18:2$^{cis\text{-}9\ trans\text{-}11}$ concentrations than Control. The provision of supplemental linoleic acid increased (treatment × d postpartum, P = 0.01) PGFM compared with Control between d 1 and 9 postpartum. Supplemental high-linoleate safflower seeds increased plasma and oviduct fatty acid concentrations, as well as serum PGFM whereas medial basal hypothalamus and uterine tissue fatty acid concentrations were virtually unaffected by lipid supplementation during the early postpartum period.

Key Words: Beef Cattle, Brain, Fatty Acids, Linoleic Acid, Uterine Tissue, Prostaglandin Metabolite

Introduction

Provision of supplemental lipids to beef cows has stimulated the interest of many researchers because of the potential to improve reproduction (Williams and Stanko, 2000; Funston, 2004; Hess et al., 2005). Staples et al., (1998) and Williams and Stanko (2000) suggested that the improvement in reproduction for cattle consuming supplemental lipids may be related to an increase in the pool of linoleic acid available for metabolism. However, as an early precursor to PG (Funston, 2004), a modest increase in metabolizable linoleic acid may be counterproductive during critical reproductive events (Hess et al., 2005). Increasing intestinal supply of linoleic acid nearly 3-fold (Scholljegerdes et al., 2004) led to an increase in $PGF_{2\alpha}$ metabolite (**PGFM**) in cows fed high-linoleate safflower seeds during the postpartum period (Grant et al., 2005). Filley et al. (1999) reported an increase in plasma linoleic acid and concentrations of PGFM in postpartum beef heifers receiving an i.v. infusion of soybean oil (~53% linoleic acid).

Burns et al. (2003) demonstrated that plasma and endometrial fatty acids were altered in non-lactating beef cows consuming a diet supplemented with PUFA. Our hypothesis was that supplemental safflower seeds high in linoleic acid will alter fatty acid composition of plasma and tissues involved in the reproductive process as well as serum PGFM. Therefore, the objectives of this trial were to evaluate the fatty acid composition of plasma, medial basal hypothalamus, and uterine tissues and serum PGFM concentrations in primiparous beef cows fed a forage-based diet and supplemental high-linoleate safflower seeds.

Materials and Methods

Animals and diets

Starting on d 1 postpartum, 18 primiparous crossbred beef cows (avg BW = 410 ± 24.2 kg; BCS = 5.25 ± 0.20) were fed Foxtail millet hay (*Setaria italica cv. White Wonder*) at 2.1% of BW and either a low-fat control (64.2% cracked corn, 32.1% safflower seed meal and 3.7% liquid molasses; **Control**) or a cracked high-linoleate safflower seed supplement (94.0% cracked high-linoleate (77% 18:2) safflower seeds and 6.0% liquid molasses; **Linoleate**). Supplements were formulated to provide equal quantities of N and TDN, with Linoleate containing 5% DM as fat. All experimental procedures were conducted in accordance to an approved University of Wyoming Animal Care and Use Committee protocol.

Sampling

Plasma and serum were collected every 3 d from d 1 through d 33 postpartum for analysis of fatty acids and

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PGFM, respectively. Blood was immediately refrigerated for 12 h after which serum was harvested by centrifugation at 2000 x *g* for 30 min. On d 37 ± 3 d cattle were transported to a commercial slaughter facility. The medial basal hypothalamus (Moss et al., 1980) and uterine tissues were obtained approximately 15 min postmortem. Upon removal from the carcass, both horns of the uteri were incised. Approximately 1 cm × 1 cm section of intercaruncular and caruncular tissues was sampled (both intercaruncular and caruncular tissues included the endometrium, myometrium, and perimetrium) and as much of the endometrium and myometrium as possible were dissected from the perimetrium and both oviducts (from the ampula to the uterotubual junction) were trimmed of connective tissue and ommental fat. Samples of the medial basal hypothalamus, oviduct and uterine tissues were immediately placed into liquid N and subsequently stored at -80°C.

Sample Analysis

Plasma, medial basal hypothalami, uterine, and oviductal tissues were lyophilized (Genesis SQ 25 Super ES Freeze Dryer, The VirTis Co., Gardiner, NY) and ground with a mortar and pestle. These samples were analyzed for fatty acids as described by Alexander et al. (2002). Serum samples were analyzed for concentrations of the PGFM by RIA as described by Silvia and Niswender (1984) with the following modifications. A volume of 0.5 mL of serum was used for extraction. Extracts were stored at -20°C in the 12 × 75 mm borosilicate glass tubes used for the extraction. One day before analysis, the extracts were thawed and centrifuged at 1500 × *g* for a minimum of 20 min, and the supernatant was transferred to 1.5-mL microfuge tubes. The tubes were stored at 4°C overnight after which 50 µL was dispensed for the assay. The samples were analyzed in a single assay with an intra-assay CV of 7.5%.

Statistical Analysis

All data were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized designed experiment. Dietary treatment effects were tested with the dietary treatment as the error term (error a), whereas the tissue type and dietary treatment × tissue type interactions were tested with residual error (error b). Only two treatment × tissue interactions were detected for 18:2 ($P = 0.01$) and 18:3 ($P = 0.03$). Additionally, MIXED procedures of SAS were used for analysis of plasma fatty acid concentrations and serum PGFM over the postpartum period, which included the effects of animal, treatment, time, and treatment × time. Animal was used to specify variation between animals using the RANDOM statement and d postpartum was used as the repeated effect and animal (treatment) was the nested effect using the SUBJECT statement. Autoregression order one was determined to be the most desirable covariance structure according to the Akaike's Information Criterion.

Results and Discussion

Fatty Acid Intake and Plasma Fatty Acids

Intake of individual and total fatty acids was greater ($P = 0.001$) for Linoleate compared with Control (Table 1). A treatment × day postpartum interaction ($P = 0.001$ to 0.04) was detected for plasma 18:0, 18:1$^{trans\text{-}11}$, 18:2, 18:3, others, total SFA, MUFA, PUFA, total unsaturated fatty acids, and total fatty acids. All of these interactions were related to changes in magnitude of difference in plasma fatty acid concentration at the onset of the trial; Figure 1 illustrates the treatment × d postpartum interaction for 18:2 which was similar to that of the other fatty acids. Plasma 18:2 was equal on d 1 and 3 and then increased for Linoleate thereafter. The treatment × d postpartum noted for total SFA, MUFA, PUFA, total unsaturated fatty acids, and total fatty acids were an artifact of an interaction noted for 18:0, 18:1$^{trans\text{-}11}$, 18:2, 18:3 because the values for these variables are a sum of the appropriate fatty acids in the particular categories.

Table 1. Influence of supplemental high-linoleate safflower seeds on fatty acid intake (g/d) in primiparous beef cows consuming a forage-based diet

	Treatments[a]			
Item	Control	Linoleate	SEM[b]	*P*
16:0	12.3	45.7	0.60	0.001
18:0	2.2	17.1	0.22	0.001
18:1$^{cis\text{-}9}$	21.4	55.9	1.0	0.001
18:2	16.5	470.7	6.2	0.001
18:3	2.9	3.5	0.06	0.001
Other	55.6	63.4	1.2	0.001
Total SFA[c]	14.5	62.8	0.8	0.001
MUFA[d]	21.4	55.9	1.0	0.001
PUFA[e]	20.1	476.8	6.3	0.001
Total unsaturated[f]	41.6	532.8	7.1	0.001
Total[g]	111.5	658.9	8.7	0.001

[a]Treatments = All cows were fed Foxtail millet hay at 2.13% of BW and either Control (61.2% corn, 32.1% safflower seed meal, and 3.7% molasses) at 0.37% of BW or Linoleate (94.0% cracked high-linoleate safflower seeds, and 6.0% molasses) at 0.24% of BW.
[b]n=9/treatment.
[c]Total SFA = 16:0 and 18:0.
[d]MUFA = 18:1$^{cis\text{-}9}$.
[e]PUFA = 18:2 and 18:3.
[f]Total unsaturated fatty acids = MUFA and PUFA.
[g]Total fatty acids = Total SFA, MUFA, PUFA, and other fatty acids.

Plasma 18:1$^{cis\text{-}9}$, 20:4, 22:6, and MUFA were not affected ($P = 0.19$ to 0.71) by dietary treatment (Table 2). However, plasma 18:2 increased ($P = 0.001$) from 11.9 to 19.2 mg/g of freeze dried plasma with supplemental high-linoleate safflower seeds. Other researchers (Filley et al., 1999; Whitney et al., 2000) demonstrated that provision of supplemental lipids increased plasma linoleic acid. Due to ruminal biohydrogenation of 18C fatty acids 18:1$^{trans\text{-}11}$, 18:2$^{cis\text{-}9\ trans\text{-}11}$, and 18:2$^{cis\text{-}10\ trans\text{-}12}$ were present in the blood but not in the feed. Plasma 18:1$^{trans\text{-}11}$ did not differ ($P = 0.19$) across treatment even though our laboratory (Scholljegerdes et al., 2004) reported increased duodenal supply of 18:1$^{trans\text{-}11}$ when cattle are fed high-linoleate safflower seeds. This apparent lack of concurrence would indicate that tissues were removing the 18:1$^{trans\text{-}11}$ from circulation. Mammary tissue and milk from these cows (data not shown) did have higher amounts of 18:1$^{trans\text{-}11}$ in the Linoleate treatment. Supplemental high-linoleate safflower seeds increased ($P = 0.001$) plasma 18:2$^{cis\text{-}9\ trans\text{-}11}$.

Medial Basal Hypothalamus Fatty Acids

The medial basal hypothalamus is a site of GnRH synthesis and storage. Scholljegerdes (2005) postulated that hormone production within the hypothalamus may be influenced by dietary fatty acids and subsequent alterations in brain fatty acid composition.

Table 2. Influence of supplemental high-linoleate safflower seeds on plasma fatty acids (mg/g of freeze dried plasma) in primiparous beef cows consuming a forage-based diet

	Treatments[a]			
Item	Control	Linoleate	SEM[b]	P
16:0	6.2	7.7	0.25	0.001
18:0	7.1	11.0	0.51	0.001
18:1$^{trans\text{-}11}$	6.2	6.8	0.33	0.19
18:1$^{cis\text{-}9}$	0.43	0.38	0.09	0.71
18:2	11.9	19.2	0.80	0.001
18:2$^{cis\text{-}9\ trans\text{-}11}$	0.87	1.2	0.07	0.001
18:2$^{cis\text{-}10\ trans\text{-}12}$	0.01	0.03	0.01	0.47
18:3	1.2	1.6	0.14	0.08
20:4	0.11	0.07	0.02	0.23
20:5	0.02	0.07	0.01	0.001
22:6	0.24	0.38	0.09	0.27
Other	18.2	18.9	1.0	0.62
Total SFA[c]	13.6	19.1	0.70	0.001
MUFA[d]	6.6	7.2	0.40	0.28
PUFA[e]	14.4	22.6	0.92	0.001
Total unsaturated[f]	21.0	29.7	1.2	0.001
Total[g]	52.4	66.6	2.4	0.001

[a]Treatments = All cows were fed Foxtail millet hay at 2.13% of BW and either Control (61.2% corn, 32.1% safflower seed meal, and 3.7% molasses) at 0.37% of BW or Linoleate (94.0% cracked high-linoleate safflower seeds, and 6.0% molasses) at 0.24% of BW.
[b]n=9/treatment.
[c]Total SFA = 16:0 and 18:0.
[d]MUFA = 18:1$^{cis\text{-}9,}$ and 18:1$^{trans\text{-}11}$.
[e]PUFA = 18:2, 18:2$^{cis\text{-}9,\ trans\text{-}11}$,18:2$^{trans\text{-}10,\ cis\text{-}12}$, 18:3, 20:4, and 20:5.
[f]Total unsaturated fatty acids = MUFA and PUFA.
[g]Total fatty acids = Total SFA, MUFA, PUFA, and other fatty acids.

However, with the exception of 20:5, which tended (P = 0.10) to be greater in Linoleate cows, fatty acid composition of the medial basal hypothalamus did not differ (P = 0.17 to 1.0) between treatments (Data not shown).

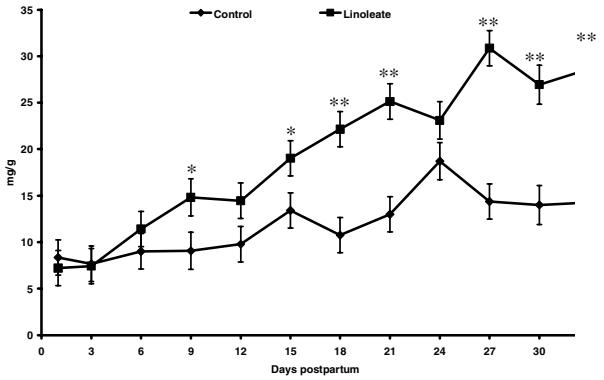

Figure 1. Influence of supplemental high-linoleate safflower seeds on plasma 18:2 (mg/g of freeze dried plasma) from d 1 to 33 postpartum in primiparous beef cows consuming a forage-based diet (SEM = 2.0). *P < 0.05; ** P < 0.01 for differences between means.

In contrast, MacDonald et al. (1996) noted that rats fed corn oil had higher 18:2 levels in both the plasma and cerebra compared to rats fed beef tallow, but other fatty acids did not differ across dietary treatments. To our knowledge no published reports on fatty acids profile or content in the medial basal hypothalamus of beef cows exists. The overall lack of differences between fatty acids could be due to the brain's ability to strictly regulate fatty acid production and fatty acid transport out of the blood (Sperry et al., 1940; Edmond, 2001). The brain can readily synthesize non-essential fatty acids (Sperry et al., 1940) and transport *n*-6 (Edmond, 2001) and *n*-3 (Pawlosky et al., 1996) fatty acids via fatty acid transporters that have specificity toward location and occurrence of double bonds.

Uterine and Oviduct Fatty Acids

No differences were noted for uterine and oviductal 15:0 (P = 0.30) and 20:5 (P = 0.89), respectively (Table 3). All other fatty acids differed (P = 0.001 to 0.05) across uterine tissue types. Although caruncular and intercaruncular tissue included both the endometrium and myometrium, fatty acid concentrations were numerically lower than either the endometrium or myometrium alone. This was probably due to a dilution effect associated with the *tunica serosa* (perimetrium) covering of the uterus. The oviduct had the highest concentration of 16:0, 18:1$^{trans\text{-}11}$, 18:2, 18:3, total, MUFA, PUFA and total unsaturated fatty acids of all tissues.

Table 3. Influence of supplemental high-linoleate safflower seeds on fatty acid composition (mg/g of freeze dried tissue) of pooled uterine tissues and oviducts of primiparous beef cows consuming a forage-based diet

Fatty	Uterine tissues[a]					
acid	Caruncle	Intercaruncle	Endometrium	Myometrium	Oviduct	SEM[b]
16:0	2.4^{c}	3.2^{c}	4.3^{c}	6.1^{c}	48.7^{d}	5.6
18:0	3.5^{c}	0.03^{c}	5.3^{c}	15.0^{d}	8.8^{c,d}	4.0
18:1$^{trans\text{-}11}$	2.9^{c}	3.5^{c}	5.0^{c}	7.1^{c}	41.9^{d}	6.2
18:1$^{cis\text{-}9}$	1.6^{c}	1.1^{c}	0.22^{c}	3.6^{c}	14.5^{d}	4.0
18:2	2.9^{c}	5.2^{c}	4.1^{c}	7.0^{c}	28.3^{d}	5.1
18:2$^{cis\text{-}9\ trans\text{-}11}$	0.06^{c}	0.03^{c}	0.26^{d}	0.26^{d}	0.26^{d}	0.08
18:2$^{cis\text{-}10\ trans\text{-}12}$	0.16^{c}	0.01^{c}	0.92^{d}	0.14^{c}	0.20^{c}	0.24
18:3	0.07^{c}	0.15^{c}	0.85^{c}	0.21^{c}	1.5^{d}	0.38
20:4	2.8^{d}	0.33^{c}	2.0^{c}	3.2^{d}	1.0^{c}	1.2
20:5	2.6	3.1	2.9	4.1	2.2	1.5
22:6	0.33^{d}	0.07^{c}	0.33^{d}	0.29	0.19^{cd}	0.11
Other	44.2^{c,d}	19.4^{c}	69.4^{d}	53.3^{c,d}	59.2^{d}	18.7
Total SFA[f]	5.9^{c}	3.2^{c}	9.6^{c,d}	21.1^{d}	57.5^{d,e}	7.7
MUFA[g]	4.5^{c}	4.6^{c}	5.3^{c}	10.7^{c}	56.4^{d}	6.3
PUFA[h]	8.9^{c}	8.9^{c}	11.4^{c}	15.3^{c}	33.8^{d}	5.9
Total unsaturated[i]	13.4^{c}	13.4^{c}	15.9^{c}	24.2^{c}	90.2^{d}	10.9
Total[j]	65.1^{c,d}	36.4^{c}	98.3^{d}	103.6^{d}	213.7^{e}	28.3

[a]Treatments = All cows were fed Foxtail millet hay at 2.13% of BW and either Control (61.2% corn, 32.1% safflower seed meal, and 3.7% molasses) at 0.37% of BW or Linoleate (94.0% cracked high-linoleate safflower seeds, and 6.0% molasses) at 0.24% of BW.
[b]n=18/tissue.
[c,d,e]Means on the same row lacking a common superscript differ (P < 0.05).
[f]Total SFA = 16:0 and 18:0.
[g]MUFA = 18:1$^{cis\text{-}9}$ and18:1$^{trans\text{-}11}$.
[h]PUFA = 18:2, 18:2$^{cis\text{-}9,\ trans\text{-}11}$,18:2$^{trans\text{-}10,\ cis\text{-}12}$, 18:3, 20:4, and 20:5.
[i]Total unsaturated fatty acids = MUFA and PUFA.
[j]Total fatty acids = Total SFA, MUFA, PUFA, and other fatty acids

Supplemental high-linoleate safflower seeds did not lead to large increases (P = 0.13 to 1.0) in many of the fatty acids within the uterine tissues (data not shown). Of the fatty acids influenced by dietary treatment, endometrial 18:3 increased (P = 0.05) from 0.16 to 1.54 mg/g of freeze

dried tissue with safflower seed supplementation, whereas, myometrial 18:2$^{cis\text{-}9,\ trans\text{-}11}$ tended to be higher ($P = 0.06$) in cows fed Control supplement. Oviductal 18:2, PUFA, and total unsaturated fatty acids were greater ($P < 0.05$) for Linoleate, and oviductal 18:3 was greater ($P = 0.01$) for Control cows (Table 4).

Table 4. Influence of supplemental high-linoleate safflower seeds on oviductal tissue fatty acids (mg/g of freeze dried tissue) of primiparous beef cows consuming a forage-based diet

	Treatments[a]			
Item	Control	Linoleate	SEM[b]	*P*
16:0	46.5	51.0	8.5	0.69
18:0	14.4	3.1	6.1	0.17
18:1$^{trans\text{-}11}$	37.4	46.3	9.4	0.48
18:1$^{cis\text{-}9}$	15.0	14.1	6.1	0.91
18:2	8.7	48.0	7.7	0.001
18:2$^{cis\text{-}9\ trans\text{-}11}$	0.27	0.25	0.12	0.91
18:2$^{cis\text{-}10\ trans\text{-}12}$	0.20	0.20	0.38	0.99
18:3	2.6	0.47	0.58	0.01
20:4	1.8	0.31	1.9	0.55
20:5	2.3	2.1	2.2	0.94
22:6	0.25	0.13	0.18	0.58
Other	68.4	50.0	24.4	0.62
Total SFA[c]	60.9	54.2	10.1	0.66
MUFA[d]	52.4	60.4	8.2	0.53
PUFA[e]	16.1	51.5	7.7	0.004
Total unsaturated[f]	68.5	11.9	14.3	0.05
Total[g]	204.2	223.4	37.1	0.73

[a]Treatments = All cows were fed Foxtail millet hay at 2.13% of BW and either Control (61.2% corn, 32.1% safflower seed meal, and 3.7% molasses) at 0.37% of BW or Linoleate (94.0% cracked high-linoleate safflower seeds, and 6.0% molasses) at 0.24% of BW.
[b]n=9/treatment.
[c]Total SFA = 16:0 and 18:0.
[d]MUFA = 18:1$^{cis\text{-}9}$ and 18:1$^{trans\text{-}11}$.
[e]PUFA = 18:2, 18:2$^{cis\text{-}9,\ trans\text{-}11}$,18:2$^{trans\text{-}10,\ cis\text{-}12}$,18:3, 20:4, and 20:5.
[f]Total unsaturated fatty acids = MUFA and PUFA.
[g]Total fatty acids = Total SFA, MUFA, PUFA, and unidentified fatty acids.

The oviduct was the only tissue that had higher ($P = 0.001$) 18:2 concentrations when cows consumed high-linoleate safflower seeds. In an effort to examine the effects of fish meal supplementation on plasma and endometrial fatty acid composition, Burns et al. (2003) fed nonlactating cycling cows either corn gluten meal or fish meal for 64 d. Fish meal is a source of both 20:5 (3.5% of total fatty acid content) and 22:6 fatty acids (4.9% of total fatty acid content). In that study, fish meal increased plasma 20:5 and 22:6 along with endometrial 20:5, but did not increase 22:6. Similar to our results plasma fatty acid composition does not necessarily reflect reproductive tissue fatty acid composition.

Plasma PGFM

There are varying reports on the influence of dietary 18:2 on PG synthesis (Williams and Stanko, 1999, Funston, 2004, Hess et al., 2005). Greater concentrations of 18:2 in plasma has increased (Burke et al., 1997; Filley et al., 1999, Grant et al., 2005), reduced (Cheng et al., 2001) or did not affect (Webb et al., 2001) serum PGFM. A treatment × d postpartum interaction ($P = 0.01$) was observed for serum PGFM (Figure 2). Serum PGFM was greater for the Linoleate treatment from d 3 to 9 postpartum with the two treatments converging later in the postpartum period. When pooled across d postpartum, Linoleate tended to have increased ($P = 0.07$) serum PGFM (data not shown). This is in agreement with Lammoglia et al. (1997) who reported that beef cows fed a high-fat diet tended to have greater PGFM peak concentrations from d 1 of the first estrous cycle until the first dominant follicle of the second estrous cycle was detected (usually d 5 of the second estrous cycle).

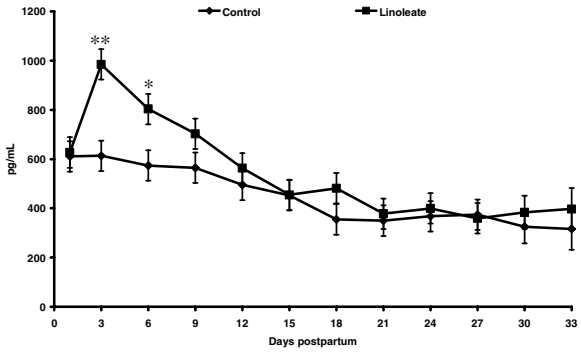

Figure 2. Influence of supplemental high-linoleate safflower seeds on serum the $PGF_{2\alpha}$ metabolite 13,14-dihydro-15-keto $PGF_{2\alpha}$ metabolite (PGFM) from d 1 to d 33 postpartum in primiparous beef cows consuming a forage-based diet (SEM = 85.0). *$P < 0.05$; ** $P < 0.01$ for differences between means.

Grant et al. (2005) demonstrated that supplemental high-linoleate safflower seeds increased PGFM from 25 to 80 d postpartum in multiparous cows. In our experiment and that of Mattos et al. (2004), cattle provided a diet high in linoleic acid had a greater PGFM after parturition than did cattle given a low fat control within the first 5 d postpartum.

It is often difficult to evaluate data concerning the effects of supplemental fat on reproduction. This difficulty arises because of the differences in stage of production when measurements are taken as well as dietary differences, especially when comparing research between dairy and beef cattle (Funston, 2004). Additionally, the length of time that fat is supplemented and the age of the cattle may produce varied results further complicating comparisons (Hess, et al., 2002; Funston, 2004). Therefore, from this experiment it is clear that more work needs to be done that further investigates if indeed certain reproductive tissues differentially sequester fatty acids from the circulation. In addition, work needs to be done to evaluate any differences in the expression of lipoprotein lipase or fatty acid binding proteins amongst reproductive tissues during the various phases of reproduction.

Implications

The provision of supplemental high-linoleate safflower seeds increased circulating levels of fatty acids; however, plasma fatty acid concentrations were not always indicative of reproductive tract tissue fatty acid composition. The subtle differences in tissue fatty acid concentrations may evoke physiological responses that could improve or hinder reproductive success, but more research is needed to evaluate changes in reproductive tract tissue fatty acid composition as beef cows come into estrus.

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FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND HEALTH RESPONSES OF STEERS FED INORGANIC AND ORGANIC SOURCES OF COPPER, MANGANESE, AND ZINC

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ABSTRACT: Mixed breed beef steers (n = 210; initial BW = 285 ± 7.6 kg) were stratified by BW and randomly assigned to one of nine weight blocks (two pens/BW block) to examine effects of organic forms of Cu, Mn, and Zn on performance, health, and carcass characteristics. One pen (15.2 x 30.4 m, 11 to 12 steers/pen) from each block was assigned to one of two treatment groups (nine pens/treatment). Treatments were inorganic (control) or organic (LiquiTrace®, LT; Global Animal Products, Inc., Amarillo, TX) forms of Cu, Mn, and Zn fed during the receiving and finishing periods. Diets were formulated to deliver 21, 56, and 82 ppm Cu, Mn, and Zn, respectively. Animals were observed twice daily during the first 28 d for visual signs of respiratory disease. If rectal temperature was 39.8°C or greater, steers were considered morbid and treatment was initiated. Body weights were recorded at 28-d intervals. During the 28-d receiving period, approximately 59 % of animals in each treatment group were considered morbid. Of morbid steers, 38.5 % of controls and 30.0 (± 5.2) % of steers fed LT received a second treatment (P = 0.20). Body weights (536 and 541 ± 12 kg on d 140), DMI (8.3 and 8.4 ± 0.2 kg/d), ADG (1.72 and 1.76 ± 0.05 kg), and G:F (0.21 and 0.21 ± 0.002) were similar (P > 0.30) in control and LT-fed steers, respectively, over the feeding period. Neither HCW, ribeye area, fat thickness, KHP, nor yield grade differed (P > 0.50) between groups. Percentages of carcasses grading choice were 30.5 for controls and 36.0 (± 4.0) for those receiving LT (P = 0.16). Results suggest that effects of organic forms of Cu, Mn, and Zn on growth, health, and carcass merit warrant further investigation.

Introduction

The beef cattle feeding industry continues to be faced with economic losses resulting from the morbidity, mortality, and poor performance of cattle suffering from bovine respiratory disease (BRD) (Galyean et al., 1999). These losses result from the cost of treatment and lost production (Perino, 1992). Respiratory disease has its largest effects on newly-received lightweight calves due to the added stresses of weaning and shipping resulting in a weakened immune system (Blecha et al., 1984), exposure to infectious agents via common practices of marketing, transport, and feedlot management procedures, and subsequent infection (Galyean et al., 1999). As a result, these cattle tend to experience a decrease in feed intake upon arrival at the feedlot. A diet formulated to meet nutrient requirements based on expected intake calculated from BW may fail to provide adequate nutrients to highly stressed calves unless this intake depression is also considered (Cole, 1993). If a calf becomes deficient in any nutrient, recovery from these stressors would likely be delayed, performance impaired, and changes in carcass quality are likely. Improved performance has been reported with increased concentrate and (or) protein levels in starting diets (Galyean et al., 1999). Furthermore, supplemental trace elements such as Cu, Cr, Fe, Mn, Se, and Zn can positively influence immune function (Chandra and Dayton, 1982; Cole, 1993). Additionally, disease resistance may take priority for limited micronutrients, leaving other processes vulnerable (Suttle and Jones, 1989). Contrasting results have been reported pertaining to the advantage of supplementing organic versus inorganic sources of minerals. Salyer et al. (2004) found no differences between source (polysaccharide vs. sulfate) of Cu or Zn on ADG, DMI, G:F, or BRD morbidity in lightweight, newly-received heifers. Galyean et al. (1992) reported that neither Zn source level nor Cu-lysine supplementation affected steer performance or morbidity during a 28-d receiving period. Also, DMI was improved by Zn supplementation regardless of source (sulfate vs. methionine) although ADG, feed efficiency, and carcass measurements were not affected by source or level of Zn. Similar results were reported by Spears and Kegley (2002) when comparing Zn-oxide with Zn-proteinate. Although Zn source (oxide vs. methionine) did not affect steer average daily intake, ADG, or feed efficiency, Greene et al. (1988) reported higher marbling scores, increased external fat and KPH, and higher USDA quality grades in steers fed Zn-methionine rather than Zn-oxide or no supplemental Zn. In a summary of 22 feedlot studies evaluating effects of Zn-methionine, an improvement of 3.3 % and 4.0 % in ADG and G:F, respectively (Anonymous, 1994). Neither Cu source nor level altered performance of growing steers but ADG, DMI, and G:F were reduced by supplementing Cu during the finishing period (Engle and Spears, 2000). The objective of the study reported herein was to examine effects of organically chelated Cu, Mn, and Zn on growth, carcass, and morbidity characteristics of feedlot steers.

Materials and Methods

Animals. All procedures were approved by the New Mexico State University Animal Care and Use Committee. Three loads of 78, 70, and 74 calves (steers and bulls) arrived at the Clayton Livestock Research Center (CLRC), Clayton,

NM, on three consecutive days after being in transit for 15 to 19 h. All cattle were processed immediately upon arrival. Processing included: vaccination against clostridia infectious bovine rhinotracheitis, bovine viral diarrhea (type 1 and type 2), parainfluenza 3, and bovine respiratory syncytial virus. Animals were also individually identified, treated for internal and external parasites, dehorned and weighed. Cattle were held in pens for 1 to 3 d and were fed wheat hay and a 70% concentrate diet before beginning the experiment. On the first day of the experiment (before feed delivery), all cattle were implanted (Ralgro; Schering-Plough Animal Health, Union NJ) and weighed individually and then allowed access to water. The average of individual incoming and current (d 0) BW was obtained and used for sorting cattle into a randomized complete block experiment of nine blocks (22 to 24 steers per block), two pens per block (10 to 12 steers per pen) with each treatment being represented in each block.

Experimental Diets. Cattle were incrementally stepped up from a 60% concentrate diet to the experimental diet containing 90% concentrate. The control finishing diet was typical of those fed at CLRC and contained only inorganic sources of mineral supplementation. Dietary ingredients included: steam-flaked corn (76.7%), alfalfa hay (10%), molasses (3.5%), tallow (3%), cottonseed meal (3%), supplement (2.5%), urea (1.04%), and dicalcium phosphate (0.25%). Diets were formulated to contain 13% CP, 2.18 Mcal/kg NEm, 1.5 Mcal/kg NEg, 0.88% Ca, and 0.35% P. The treatment diet was similar to the control diet and differed only in terms of the mineral supplement in which a portion of the Cu, Mn, and Zn was supplied by an organic liquid supplement (LiquiTrace®, **LT**, Global Animal Products, Inc., Amarillo, TX). Specifically, addition of 28 g of the liquid supplement/steer/d delivered 100 mg of Cu (Cu-betaine), 200 mg of Mn (Mn-methionine), and 400 mg of Zn (as Zn-methionine). The two complete diets were formulated to be iso-trace mineral delivering a total of 21, 56, and 82 ppm Cu, Mn, and Zn, respectively.

Results and Discussion

At the beginning of the experiment, all animals weighed 285 ± 7.6 kg. On d 84, control steers weighed 373 kg compared with 378 (± 9.5) kg for those receiving LT (P = 0.47). Weights remained similar between treatments for the remainder of the experiment with controls and LT-fed steers weighing 536 and 541 (±11.5) kg, respectively, on d 140 (P = 0.65). Similar results were reported in a North Carolina State University study using LiquiTrace® in feedlot steers (J. W. Spears, personal communication).

Gain, intake, and efficiency data are presented in Table 2. Neither ADG, DMI, nor G:F differed (P > 0.18) in controls and LT-fed steers during the first 84 d of the experiment. Similarly, Salyer et al. (2004) reported no difference in ADG, DMI, or G:F in lightweight, newly-received heifers. Galyean et al. (1992) showed improved (P < 0.10) DMI during a 161-d finishing period with Zn supplementation regardless of source although there were no differences in

Animal Responses. Steers were weighed on d 0, 28, 56, 84, 112, 140, 168, and 182 and ADG, DMI, and G:F were calculated for each period. If necessary, orts were measured (DM basis, DMB) on each 28-d weigh day to be subtracted from feed delivery records for calculating actual DMI for that period. Steers were evaluated daily for clinical signs of BRD and treated if rectal temperature was 39.8°C or greater. An individual steer was considered morbid after the initial treatment for BRD. If subsequent treatments were required, the steer was recorded as having been retreated. Retreat values were considered an indication of the ability to recover from disease after a single treatment for BRD. For analysis, morbidity and retreat data were expressed as percentages of the total number of animals per pen and as a percentage of morbid steers in each pen, respectively.

Slaughter dates were assigned based on pen average BW on d 112 with an attempt to slaughter groups with similar weights without dividing a weight block. Blocks 7, 8, and 9 were slaughtered on d 140, blocks 4, 5, and 6, on d 168, and blocks 1, 2, and 3 on d 182. Carcass data were collected from all steers in the experiment by a professional carcass data collection service (Cattlemen's Carcass Data Service, West Texas A&M University, Canyon, TX) and certified USDA graders at the commercial facility (Tyson/IBP, Amarillo, TX). Pen average values for HCW, marbling score, fat thickness, REA, KPH fat, yield grade, color score, and liver abscesses were calculated for analysis and quality grades were analyzed as percentages by pen.

Statistical Analysis. Performance (BW, DMI, ADG, and G:F), morbidity (% morbid and % retreat), carcass (HCW, marbling score, % choice, % select, fat thickness, ribeye area, KPH, yield grade, color scores, and liver scores) data were subjected to analysis of variance appropriate for a randomized complete block design using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pen (n = 18) was used as the experimental unit and block (n = 9) as a random variable.

ADG, feed efficiency, and carcass characteristics. In this experiment, between d 85 and 112, steers that received the control diet had greater ADG (P = 0.06) and DMI (P = 0.05) than the LT-fed steers but G:F was not different (P = 0.58) between groups. Conversely, LT-fed steers tended to have improved ADG (P = 0.16) and G:F (P = 0.17) between d 113 and 140. Steers that received the LT diet also tended (P = 0.15) to have increased DMI between d 113 and 140 and between d 169 and 182. Overall (d 0 through d 182), no differences were observed between treatment groups for ADG, DMI, or G:F (P > 0.30).

During the 28-d receiving period, approximately 59 % of animals in each treatment group were considered morbid. Of morbid steers, 38.5 % of controls and 30.3 (± 5.2) % of steers fed LT received a second treatment (P = 0.20) with no treatment differences for morbidity values. These results are supported by previous research by Galyean et al. (1992) who found no treatment effects on morbidity during a 28-d receiving period when Cu-Lys was supplemented along with either Zn-sulfate or Zn- methionine.

Steers fed the control and LT diets, respectively, produced carcasses that weighed 339 and 340 (± 4.9) kg (P = 0.82), had ribeye area of 86.9 and 86.4 (± 1.2) cm^2 (P = 0.78), fat thickness of 11.9 and 11.4 (± 0.5) mm (P = 0.52) and KHP values of 3.34 and 3.27 (± 0.10) % (P = 0.11). Carcasses from both groups had yield grades of 2.6 ± 0.1. Although Greene et al. (1988) reported improved marbling scores, external fat and KPH values with supplemented Zn-methionine, these results more closely agree with those of Galyean et al. (1992) and Spears and Kegley (2002). Interestingly, 30.5 ± 4.0 % of control carcasses graded choice compared with 36.0 ± 4.0 % of carcasses from LT-fed steers (P = 0.13). These data agree with results reported by Greene et al. (1988) where Zn-methionine supplemented steers yielded carcasses that graded higher than carcasses from steers receiving Zn-sulfate or no added Zn supplement.

Implications

Although major benefits of feeding organic sources of copper, manganese and zinc were not observed in this study, effects on retreating morbid steers and carcass grade warrant further evaluation.

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Table 1. Average daily gain (kg), dry matter intake (kg/d), and efficiency of gain of steers fed inorganic or combined inorganic/organic sources of Cu, Mn, and Zn [a].

Period, day	Variable	Mineral supplement		SE [d]	OSL [e]
		Control [b]	Treatment [c]		
0 to 28	ADG	1.50	1.55	0.12	0.71
	DMI	5.50	5.59	0.28	0.77
	G:F	0.27	0.28	0.011	0.58
29 to 56	ADG	1.62	1.75	0.06	0.18
	DMI	7.95	8.13	0.26	0.55
	G:F	0.20	0.21	0.007	0.25
57 to 84	ADG	2.26	2.16	0.09	0.22
	DMI	8.83	8.68	0.25	0.63
	G:F	0.26	0.25	0.009	0.32
85 to 112	ADG	2.01	1.93	0.04	0.06
	DMI	9.36	9.01	0.19	0.05
	G:F	0.21	0.21	0.007	0.58
113 to 140	ADG	1.56	1.74	0.09	0.16
	DMI	9.27	9.72	0.27	0.15
	G:F	0.17	0.18	0.009	0.17
141 to 168	ADG	1.35	1.38	0.05	0.73
	DMI	9.32	9.34	0.19	0.95
	G:F	0.14	0.15	0.007	0.69
169 to 182	ADG	0.88	0.82	0.19	0.81
	DMI	8.33	8.54	0.37	0.15
	G:F	0.11	0.10	0.020	0.73
Overall, 0 to 182	ADG	1.72	1.76	0.05	0.54
	DMI	8.34	8.40	0.18	0.77
	G:F	0.21	0.21	0.002	0.35

[a] Iso-trace mineral diets were formulated to deliver a total of 21, 56, and 82 mg/kg Cu, Mn, and Zn, respectively
[b] Inorganic sources of Cu, Mn, and Zn
[c] 100 mg Cu, 200 mg Mn, and 400 mg Zn from organic source with additional inorganic sources to meet iso-trace mineral formulations
[d] Based on 9 pens per treatment
[e] OSL = Observed significance level

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EFFECT OF CREEP FEED SUPPLEMENTATION AND SEASON ON INTAKE, MICROBIAL PROTEIN SYNTHESIS AND EFFICIENCY, SITE OF DIGESTION, AND RUMINAL FERMENTATION IN NURSING CALVES GRAZING NATIVE RANGE IN SOUTHEASTERN NORTH DAKOTA

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ABSTRACT: Nine ruminally and duodenally cannulated crossbred steer calves (172 ± 23 kg initial BW) were used to evaluate effects of supplementation and advancing season on intake, microbial protein synthesis and efficiency, digestion, and ruminal fermentation. Treatments were no supplement (Control) and supplement fed at 0.45% BW (DM basis) daily. The supplement consisted of 55% wheat middlings, 38.67% soyhulls, 5% molasses, and 1.33% limestone. There were three collection periods which were 15 d in length, occurring in June, July, and August. On d 1 of each collection period, rumens were evacuated and masticate samples were collected. Duodenal and fecal samples were taken on d 7 to 11 at 0, 4, 8, and 12 h after supplementation. Ruminal fluid was drawn on d 9 and used as inoculant for in vitro digestibility. Ruminal fluid was collected and pH recorded at –1, 0, 2, 4, 8, and 12 h post-feeding on d 11. Milk intake was estimated using weigh-suckle-weigh on d 15. Forage organic matter intake (OMI) tended ($P = 0.13$) to decrease in supplemented calves, while there was no difference ($P = 0.84$) in milk OMI. Total OMI (kg/d and % BW) increased (P # 0.02) in supplemented calves compared with controls. Supplementation had no affect on grazed diet or milk composition. Apparent total tract OM disappearance increased ($P = 0.03$) and apparent total tract N disappearance tended ($P = 0.11$) to increase in supplemented calves. Microbial efficiency was not affected ($P = 0.50$) by supplementation. There were no differences in ruminal pH ($P = 0.40$) or total VFA ($P = 0.21$) between treatments, while ruminal ammonia-N increased ($P = 0.03$) in supplemented compared with control calves. These data indicate that supplementation with a wheat middlings and soyhulls-based creep feed increases total OMI and total tract OM and N digestion and has no effects on ruminal fermentation.

Key Words: Calves, Creep Feed, Forage, Intake, Supplementation, Digestibility

Introduction

Creep feeding has traditionally been used to increase weaning weights, reduce grazing pressure, and improve feed intake at weaning. Creep feeding studies have consistently shown increases in weaning weight in creep-fed calves (Faulkner et al., 1994; Lardy et al., 2001; Loy et al., 2002). Faulkner et al. (1994) and Myers et al. (1999) reported that creep-fed calves had greater feed intake upon feedlot entry. Many researchers have shown that calves consume less forage when supplemented with creep feed (Cremin et al. 1991; Faulkner et al. 1994; Lardy et al., 2001). However, research in the Northern plains is somewhat inconclusive: Maddock and Lardy (2002), Soto-Navarro et al. (2004), and Loy et al. (2002) reported no differences in forage intake between creep-fed and non creep fed calves, while Gelvin et al. (2004) reported increased forage intake with creep feed supplementation.

Measurement of microbial crude protein synthesis via ruminal and duodenal fistulas has not been reported in nursing calves grazing native range. A better understanding of microbial efficiency and protein supply should lead to a greater understanding of supplementation needs, more efficient creep feeding programs, and optimal weight gains. Therefore, we hypothesized that a wheat middlings, soyhulls-based supplement would improve total intake, not alter ruminal fermentation or microbial efficiency, and improve performance in nursing calves grazing native range.

Materials and Methods

Study Site and Climatic Conditions. The study was conducted at the Albert Ekre Grasslands Preserve southwest of Walcott, ND. Precipitation and temperature data were recorded using an average of two weather stations located at Leonard, ND (approximately 25 km north of study site) and Wyndmere, ND (approximately 25 km south of study site).

Animals and Diets. Nine ruminally and duodenally cannulated nursing beef steers (172 ± 23 kg initial BW) were used in a split-plot design. Calves were allotted to one of two treatments 1) control (no supplement; n = 4) and 2) supplemented calves (n = 5). Supplemented calves were fed at 0.45% BW (DM basis) once daily at 0800. Amount of supplement fed was adjusted at the beginning of each period based on beginning BW for that period. The supplement was pelleted and consisted of 55% wheat middlings, 38.67% soy hulls, 5% molasses, and 1.33% limestone (DM basis). Cattle had ad libitum access to water and a commercial trace mineralized salt mix (Trouw Nutrition, Willmar, MN).

Sample Collection. Calf responses to treatment and seasonal effects were measured during three experimental periods. Collection periods were June 24 to July 8 (June), July 15 to July 29 (July), and August 5 to 19 (August). Chromic oxide (5 g) was ruminally dosed twice daily at 0800 and 2000 on d 2 to 11 via gelatin capsules (Torpac, Inc., Fairfield, NJ) to determine DM flow rate and total fecal output.

Duodenal fluid samples (200 mL) were collected on d 7 to 11. Samples were collected at 0, 4, 8, and 12 h after

supplementation and were composited within steer for each period.

On d 9, ruminal fluid was drawn from each steer to serve as inoculum for in vitro analysis. Ruminal fluid from each steer was used as inoculum for their corresponding masticate sample.

Ruminal fluid samples were collected on d 11 of each period. Ruminal fluid samples were collected at -1, 1, 2, 4, 8, 12, and 24 h after supplementation. Ruminal fluid was collected with a suction strainer and pH was recorded using a pH meter and combination electrode (Model 2000, Beckman Instruments, Inc., Fullerton, CA).

Milk intake was measured on d 16 using a 12 h weigh-suckle-weigh technique according to the methods described by Boggs et al. (1980). On d 15 of each period, a milk sample (approximately 150 mL) was extracted by hand from the dams of the cannulated calves for milk composition analysis.

Statistical Analysis. Data were analyzed as a split-plot design using the Proc Mixed procedure of SAS (SAS Inst., Cary, NC). The model contained effects for treatment, period, and treatment x period. Ruminal data over time were analyzed as a repeated measures design using the Mixed procedures of SAS (SAS Inst., Cary, NC). The model included effects for period, treatment, sampling time, treatment x period, treatment x sampling time, treatment x period x sampling time. The three-way interaction was used in the error term to test for treatment effects. Two-way and three-way interactions involving sampling time were usually significant but were largely due to magnitude; therefore, *P* values were not presented and means were averaged across sampling time. Period means were separated using linear and quadratic contrasts.

Results and Discussion

Climatic Conditions. Rainfall was 12.33, 2.63, and 1.63 cm for June, July, and August, respectively. Overall average rainfall is 8.62, 8.25, and 3.86 cm for June, July, and August, respectively. Average temperature over the course of the study was 20.6^{o} C. Overall average temperature for June, July, and August is 20.0^{o} C (NDAWN, 2004).

There were no treatment x period interactions detected for any components of the grazed forage. Grazed diet composition was not affected (P = 0.49 to 0.86) by treatment. Grazed diets averaged 10.8% CP and 61.6% in vitro OM disappearance (IVOMD). There was a quadratic seasonal affect (P < 0.01) for CP, OM, IVOMD, NDF, and Ca with advancing season. Crude protein, OM, IVOMD, and Ca were highest in July, while NDF was lowest in July. There was heavy rainfall in June particularly during the final nine days of the month. Heavy rainfall likely promoted new forage growth which was higher in CP, OM, IVOMD, and Ca and lower in NDF, causing the quadratic affect. Acid detergent fiber (P = 0.45) and P (P = 0.18) were not affected by season.

There were no treatment x period interactions detected for milk composition. Milk composition was not affected by treatment or period.

There was a treatment x period interaction for supplement OMI (kg/d; P = 0.07). The interaction was due to magnitude of change and not a change in treatment ranking; therefore, only main effect means are presented. There were no other treatment x period interactions for OMI. By design, supplemented calves had increased (P < 0.001) supplement OM intake (OMI, kg/d) compared with controls (Table 1). There were no treatment differences (P = 0.84) for milk OMI, while forage OMI tended (P = 0.13) to be lower in supplemented calves. Similarly, Loy et al. (2002) reported no differences in milk or forage intake between supplemented and control calves grazing the same pasture as used in our study. Total organic matter intake (kg/d, P = 0.001 and % BW, P = 0.02) was greater in supplemented compared with control calves. Greater total OMI in supplemented calves was due to increased supplement OMI. Across season, total OMI (kg/d) increased (P < 0.001) linearly. This result is due to increasing calf size and thus nutrient requirements. Total OMI (% BW) also increased linearly (P = 0.03) across season.

There were no treatment x period interactions for ruminal OM disappearance. Apparent ruminal OM disappearance (% intake) was greater (P = 0.03; Table 1) in supplemented calves compared with controls. In contrast, Soto-Navarro et al. (2004) reported no differences in apparent ruminal OM disappearance in supplemented versus control nursing calves consuming a soybean hulls, wheat middlings-based supplement and brome hay. True ruminal OM disappearance (% intake) was not affected (P = 0.15) by treatment. This result concurs with the findings of Soto-Navarro et al. (2004).

Apparent ruminal OM disappearance (% intake) increased (P = 0.02; Table 1) as season progressed, which is likely due to increased ruminal function. True ruminal OM disappearance (% intake) across season was not different (P = 0.50).

Intestinal apparent OM disappearance (% intake) was lower (P = 0.09; Table 1) in supplemented compared with control calves. The decrease in intestinal apparent OM disappearance (% intake) likely reflects increased ruminal apparent OM disappearance.

There was not a treatment x period interaction for apparent total tract OM disappearance (P = 0.24). Apparent total tract OM disappearance was greater (P = 0.03) for supplemented compared with control calves and was not (P = 0.40) affected by season (Table 1). The supplemented calves consumed a diet that was higher in IVOMD, this likely caused the increase in apparent total tract OM disappearance. Also, the supplement was based on digestible fiber sources and was relatively high in CP (17.39% CP; 69.89% DIP, % of CP) which may have positively influenced fiber digestion.

There was not a treatment x period interaction for nitrogen intake (P = 0.16). Total nitrogen (N) intake (g/d) was not affected (P = 0.19) by treatment; however, N intake was affected (P = 0.02) by season (Table 1). There was a

quadratic affect (P = 0.008) for N intake across season with N intake being greatest in July and lowest in August. This was caused by the CP content of grazed forage (11.90, 12.81, and 7.68% CP for June, July, and August, respectively).

There was not a treatment x period interaction for microbial efficiency (P = 0.52). Microbial efficiency (grams of N per Kg of organic matter truly digested) was not affected (P = 0.50; Table 1) by treatment. Microbial efficiency values were greater than those reported by Soto-Navarro et al. (2004; 11.8 and 12.0 g N/kg OMDT for control and supplemented, respectively) in nursing calves. Microbial efficiency decreased linearly (P = 0.007) as season progressed.

There was a treatment x period interaction (P = 0.03) for ruminal N disappearance (% intake). Ruminal N disappearance for control calves decreased from June to July and July to August, while ruminal N disappearance for supplemented calves increased from June to July and decreased from July to August. Ruminal N disappearance (apparent, P = 0.06 and true, P = 0.01; % of intake) was greater in supplemented compared with control calves. Similarly, Reed et al. (2004) reported increases in apparent and true ruminal N disappearance (% intake) with increasing field pea supplementation for moderate quality forage diets. Ruminal N disappearance (apparent and true; % of intake) decreased quadratically (P = 0.02) as season progressed being highest in July and lowest in August. This follows the same pattern as forage quality. Forage IVOMD and CP were highest in July and lowest in August.

There was a treatment x period interaction for intestinal apparent N disappearance (% intake; P = 0.03). Intestinal apparent N disappearance (% intake) in control calves was similar between June and July and increased in August, while intestinal apparent N disappearance (% intake) in supplemented calves decreased from June to July and increased from July to August. Intestinal apparent N disappearance (% intake) was lower (P = 0.07; Table 1) in supplemented compared with control calves. This likely resulted from greater ruminal N disappearance in supplemented calves. Soto-Navarro et al. (2004) also observed decreased intestinal N disappearance (% intake) in supplemented calves and suggested that lower intestinal N digestibility in supplemented calves resulted from a tendency toward greater N intake in supplemented compared with control calves because N (g/d) disappearing intestinally was similar between treatments. Intestinal apparent N disappearance (% intake) responded quadratically (P = 0.01) across season being lowest in July and highest in August. This pattern coincides with ruminal N disappearance (% intake) which was highest in July and lowest in August.

There was a treatment x period interaction for apparent total tract N disappearance (%; P = 0.06). Apparent total tract N disappearance (%) in control calves decreased from June to July and July to August, while apparent total tract N disappearance (%) in supplemented calves increased from June to July and decreased from July to August. Overall, apparent total tract N disappearance (%) tended (P = 0.11) to increase in supplemented calves and responded quadratically (P = 0.04) across season (Table 1). The percentage of forage OMI to total OMI continually increased throughout season while the percentage of milk and creep OMI to total OMI decreased, this likely explains a decrease in apparent total tract N digestibility in August.

There was not a treatment x period interaction for ruminal pH (P = 0.72). Ruminal pH was not different (P = 0.40; Table 1) between treatments. Soto-Navarro et al. (2004) reported similar results. Ruminal pH was likely not affected by supplementation because the supplement in our study and that of Soto-Navarro et al. (2004) was based on wheat middlings and soyhulls which are relatively low in starch and high in potentially fermented fiber components (Soto-Navarro et al., 2004). Others have reported reductions in ruminal pH when supplementing nursing calves with supplements containing greater levels of rapidly fermented carbohydrates (Cremin et al., 1991; Faulkner et al., 1994; Gelvin et al., 2004).

There was a treatment x period interaction for ruminal ammonia-N concentration (P = 0.01). Treatment ranking did not change across period, thus, we believe the interaction was caused by magnitude of change. Therefore, only main effect means are reported. Ruminal ammonia-N concentration was greater (P = 0.03; Table 1) in supplemented compared with control calves. Similarly, Gelvin et al. (2004) and Soto-Navarro et al. (2004) reported increased ruminal ammonia-N concentrations in supplemented calves. Gelvin et al. (2004) suggested the increase was due to increased CP intake from a field-pea based supplement, and Soto-Navarro et al. (2004) suggested increased ruminal ammonia-N concentrations were due to numerically greater N intakes in supplemented calves. Ruminal ammonia-N concentration responded quadratically (P = 0.001) to advancing season being highest in July and lowest in August. This result follows the same pattern as grazed forage CP.

There was not a treatment x period interaction for total VFA (P = 0.32). Total VFA concentrations were not different (P = 0.21; Table 1) between treatments. In contrast, Gelvin et al. (2004), Faulkner et al. (1994), and Soto-Navarro et al. (2004) reported increased total VFA concentrations in supplemented compared with control calves suggesting that supplements were more rapidly fermented than forage. Gelvin et al. (2004) and Faulkner et al. (1994) fed supplements that were greater in starch than our study, which likely explains differences in total VFA. Soto-Navarro et al. (2004) fed a similar supplement to the supplement in our study, but they (Soto-Navarro et al., 2004) fed a lower quality forage (7.43% CP) compared with our study (10.8% CP).

There were treatment x period interactions for acetate:propionate ratio (P = 0.006), acetate (P = 0.001) and butyrate (P = 0.003) molar proportions. Treatment ranking never changed across period, thus, we believe the interaction was caused by magnitude of change.

Molar proportions of acetate decreased (P < 0.001) and molar proportions of propionate increased (P = 0.002) in

supplemented compared with control calves (Table 1). Therefore, the acetate:propionate ratio decreased (P = 0.001) in supplemented compared with control calves. Lower acetate:propionate ratio in supplemented calves has been reported by numerous researchers (Faulkner et al., 1994; Gelvin et al., 2004; Tarr et al., 1994). Decreased molar proportions of acetate likely reflect the trend for decreased forage intake in supplemented calves, and increased proportions of propionate in supplemented calves are likely the result of consumption of a more energy dense diet.

Molar proportions of butyrate (P = 0.002) increased with supplementation (Table 1). Soto-Navarro et al. (2004) suggested that increases in butyrate may be explained by fermentation of AA originating from the supplements.

In summary, supplementing nursing calves with wheat middlings and soyhulls-based creep feed increased OMI, total tract OM disappearance, ruminal N disappearance, and total tract N disappearance; and had no affects on ruminal pH, total VFA concentration, or microbial efficiency.

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Table 1. Effect of creep feeding and grazing season on OM and N intake and digestion in nursing steer calves

	Treatment			Period					P values[a]		Contrasts[b]	
Item	Control	Creep	SEM	June	July	August	SEM	Trt[c]	Period	Trt x Period	L	Q
OM intake, kg/d												
Supplement	0.00	0.86	0.02	0.38	0.44	0.48	0.03	0.001	0.07	0.07	0.02	0.76
Milk	0.59	0.56	0.10	0.55	0.73	0.44	0.11	0.840	0.21	0.19	0.51	0.10
Forage	1.65	1.54	0.05	1.09	1.57	2.14	0.06	0.13	0.001	0.34	0.001	0.55
Total OM intake, kg/d	2.24	2.96	0.13	2.01	2.73	3.05	0.15	0.001	0.001	0.34	0.001	0.27
Total OM intake, % BW	1.17	1.41	0.07	1.12	1.35	1.39	0.08	0.02	0.07	0.74	0.03	0.36
Digestion[d]												
Apparent ruminal	21.7	39.2	4.8	22.3	30.9	38.2	4.3	0.03	0.02	0.95	0.007	0.87
True ruminal	61.3	68.8	3.4	64.8	63.5	66.8	2.8	0.15	0.50	0.85	0.48	0.35
Intestinal	40.0	28.8	4.3	445.4	323.7	264.0	3.4	0.09	0.001	0.44	0.001	0.29
Total tract	61.8	68.0	1.7	66.8	63.3	64.6	1.8	0.03	0.40	0.24	0.40	0.29
N intake, g/d	98.7	115.4	8.6	99.6	135.5	86.1	10.9	0.19	0.02	0.16	0.41	0.008
Microbial efficiency[e]	18.6	17.1	1.6	20.0	18.6	14.9	1.4	0.50	0.02	0.52	0.007	0.43
Digestion[d]												
Apparent ruminal	3.4	21.8	6.3	22.4	32.2	-16.9	8.3	0.06	0.004	0.03	0.007	0.02
True ruminal	33.0	52.1	4.8	49.7	57.1	21.1	6.1	0.02	0.003	0.06	0.007	0.02
Intestinal	70.3	56.2	4.9	58.5	48.3	83.0	6.3	0.07	0.01	0.03	0.02	0.01
Total tract	73.7	78.0	1.7	80.9	80.5	66.1	2.4	0.11	0.001	0.06	0.001	0.04
pH	6.69	6.61	0.07	6.76	6.47	6.72	0.05	0.40	0.001	0.72	0.23	0.001
NH_3-N, mM	3.26	4.73	0.41	3.91	4.51	3.56	0.30	0.03	0.002	0.01	0.13	0.001
Total VFA, mM	62.56	68.04	2.91	48.36	77.78	69.75	2.43	0.21	0.001	0.32	0.001	0.001
Acetate:propionate	4.96	4.45	0.07	4.62	4.94	4.57	0.05	0.001	0.001	0.006	0.35	0.001
Acetate, mol/100 mol	72.06	69.63	0.15	70.15	71.78	70.61	0.17	0.001	0.001	0.001	0.07	0.001
Propionate, mol/100 mol	14.59	15.74	0.18	15.31	14.64	15.55	0.14	0.002	0.001	0.11	0.06	0.001
Butyrate, mol/100 mol	8.65	9.74	0.16	9.56	9.07	8.94	0.13	0.002	0.001	0.003	0.001	0.08

[a]Two-way and three-way interactions involving time were usually significant and mostly due to magnitude; therefore, *P*-values are not presented and means are averaged across time. [b]Contrasts were L = linear amd Q = quadratic. [c]Probability value for the F-test for an overall treatment (trt) effect. [d]Digestion as a percentage of intake. [e]Grams of nitrogen per kg of OM digested, true (corrected for OM of bacterial origin).

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INFLUENCE OF DIETARY FAT ON DIGESTIVE FUNCTION OF BEEF STEERS FED A STEAM-FLAKED CORN-BASED FINISHING DIET CONTAINING WET CORN GLUTEN FEED

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ABSTRACT: Nine ruminally cannulated mixed breed beef steers (436 ± 16 kg) were used in a completely random metabolism experiment to evaluate effects of added dietary fat on total tract digestion, digesta kinetics, and ruminal fermentation profiles in steers fed a finishing diet (90% concentrate) containing wet corn gluten feed (WCGF, Sweet Bran®, Cargill). Steers were assigned randomly to one of three treatments: 1) standard finishing diet with 3% added fat (STD3); 2) steam-flaked based diet containing 25% (DM basis) WCGF with no added fat (WCGF0); or 3) steam-flaked based diet containing 25% (DM basis) WCGF with 3% added fat (WCGF3). Diets contained (DM basis) 9% alfalfa hay, 2.5% supplement, 0 or 3% added fat (yellow grease), and 0 or 25% WCGF with the remainder consisting of steam-flaked corn. Cobalt-EDTA and Yb-labeled steam-flaked corn were intraruminally infused at 0600 on d 3 of the 6-d collection period. Ruminal samples were collected at 0, 2, 4, 6, 8, 10, 12, 14, 18, 26, 34, and 50 h after the 0600 feeding. Steers fed WCGF3 consumed more (P = 0.01) DM than did those fed STD3 or WCGF0 (23.5 > 18.4 = 18.8 ± 0.9 g/kg BW daily, respectively). A similar response was observed for OM, NDF, and ADF intake. Fluid dilution rate (7.5 > 5.7 = 5.2 ± 0.4 %/h) and particulate passage rate (5.9 > 4.2 = 3.6 ± 0.4 %/h) differed (P < 0.03) in steers fed WCGF3, WCGF0, and STD3, respectively. Ruminal pH also differed (P < 0.09) among dietary treatments (6.0 > 5.5 = 5.3 ± 0.16; WCGF0, WCGF3, STD3, respectively). Total tract digestibility of DM, OM, NDF, and CP were similar (P > 0.15) among treatments while ether extract digestibility differed (P < 0.01) as expected with dietary fat (93.5 > 90.4 > 88.6 ± 0.3%; WCGF3, STD3, WCGF0, respectively). Fat added at 3% to diets containing 25% WCGF resulted in increased intake and fluid and particulate passage rates.

Key Words: Digesta Kinetics, Cattle, Dietary Fat

Introduction

Feed intake is generally considered the single most important factor affecting production by feedlot cattle. Ingredients and (or) practices that improve intake can be very important performance enhancers. Recently, many feedlots have begun using wet corn gluten feed (**WCGF**), a by-product of the wet corn milling industry, as an energy and protein source, replacing a portion of the steam-flaked corn and supplemental protein in growing and finishing diets. Previous research has shown inclusion of WCGF in finishing diets to increase DMI (Ham et al., 1995; Parsons et al., 2001; Scott et al., 2003) as well as gains (Ham et al., 1995; Scott et al., 2003). The effect of WCGF on feed efficiency is not consistent; however many researchers have reported improvements (Ham et al., 1995; Hussein and Berger, 1995; Parsons et al., 2001; Sindt et al., 2002). Montgomery et al. (2004) reported improved digestibility of OM, NDF, and starch in steers fed steam-flaked corn-based diets containing WCGF (40% DM basis). The same researchers also reported increased fluid and particulate passage rates. It is speculated that increased passage rates are due to decreased negative associative effects of starch on fiber digestion (Firkins, 1997).

Because the energy source in WCGF is highly fermentable fiber instead of starch, as in corn, feeding WCGF may help decrease the occurrence of subacute acidosis, a common cause of variable feed intake during growing and finishing phases. Krehbiel et al. (1995) and Montgomery et al. (2004) reported increased pH in cattle fed finishing diets containing WCGF.

Adding supplemental fat to ruminant diets is a common practice aimed at increasing caloric density. However, reports show fat to have detrimental effects on fiber digestion (Palmquist, 1988, as cited by Lodge et al., 1997). A previous study conducted by Defoor et al. (2003) at the Clayton Livestock Research Center, Clayton, NM, examined effects of WCGF and added fat level on performance and carcass quality of finishing beef steers. This research demonstrated an increase in DMI resulting in greater gain, carcass weight, and overall profitability of steers fed wet corn gluten feed. The objective of the study reported herein was to evaluate effects of added dietary fat on total tract digestibility and ruminal disappearance of nutrients by steers fed finishing diets containing 25% WCGF.

Materials and Methods

General. A completely random metabolism experiment was conducted at the Clayton Livestock Research Center, Clayton, New Mexico. Nine ruminally cannulated mixed-breed steers (436 ± 16 kg) were assigned randomly to one of three treatments: 1) standard finishing diet with 3% added fat (**STD3**); 2) 25% (DM basis) WCGF with no added fat (**WCGF0**); or 3) 25% (DM basis) WCGF with 3% added fat (**WCGF3**). Dietary ingredients and composition are shown in Table 1. Steers were individually penned (1.5 x 4 m) in a barn and had free access to fresh water. All surgical procedures, post surgical care, and the experimental protocol were approved by the New Mexico

State University Institutional Animal Care and Use Committee. The experiment consisted of an 18-d adaptation period and a 6-d collection period. Before the adaptation period, cattle were incrementally stepped up from a 60% concentrate diet to the experimental diet containing 90% concentrate. The adaptation period began (d 0) with feeding the treatment diet and continued through d 17. Steers where adapted to the barn, stalls, and fecal bags during the final 3 d of the adaptation period (d 15 to 17).

Collections. Steers were fed once daily at 0600. To prevent spoilage, diets were mixed for 2 d of feedings. Samples from each mixing were collected and composited. Total feces were collected once daily on d 1 through 5 of the collection period. Daily feces and feed refusals (orts) were weighed and recorded and a 10% subsample was composited. Feed, fecal, and ort samples were refrigerated (4°C) until later analysis. Cobalt-EDTA and Yb-labeled steam-flaked corn were utilized as markers of fluid and particulate passage rates, respectively. Both mineral markers were prepared as described by Varga and Prigge (1982). On d 3 of the collection period, steers were intraruminally dosed with 200 mL Co-EDTA and 1 kg Yb-labeled steam-flaked corn at 0600, before feeding. Ruminal fluid and particulate samples were collected before dosing (h 0) and at 2, 4, 6, 8, 10, 12, 14, 18, 26, 34, and 50 h after dosing. Ruminal fluid was collected via the ruminal cannula using a suction strainer, which was rinsed with warm water between each sampling. At the time of sampling, pH of filtered ruminal fluid was measured (combination electrode), and a 200-mL sample retained after adding 3 mL of concentrated hydrochloric acid. Ruminal fluid and ruminal particulate samples were frozen (-20°C) for later analysis.

Analyses. Refrigerated feed, fecal, and ort samples were dried at 60° C in a forced-air oven for 48, 24, and 48 h, respectively, then allowed to equilibrate at room temperature. Samples were then ground to pass a 2-mm screen in a Wiley mill, and a 1 g subsample was then dried at 100° C in a forced-air oven for 24 h then at 525° C for 8 h to determine DM and OM, respectively (AOAC, 1990). Neutral detergent fiber and ADF were determined using Ankom 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Samples were also analyzed for CP using LECO FP-528 (LECO Corporation, St. Joseph, MO). Total starch and ether extract content were sent to a commercial laboratory (DairyOne, Ithaca, NY) for analysis**.**

After thawing ruminal fluid samples were centrifuged at 4000 x g and the supernatant was retained for subsequent analysis of VFA and Co-EDTA concentrations. Cobalt concentration was determined with an air-plus-acetylene flame using atomic absorption spectroscopy. Volatile fatty acid concentrations were analyzed according to Goetsch and Galyean (1983).

Ruminal particulate samples were dried in a forced-air oven at 60° C for 24 h, allowed to equilibrate at room temperature, then ground to pass a 2-mm screen in a Wiley mill. Ytterbium was extracted from these samples as outlined by Hart and Polan (1984) and marker concentration was determined by atomic absorption spectroscopy using a nitrous oxide-plus-acetylene flame.

Calculations. Fluid and particle passage rates were calculated as the absolute slope of the regression equation of time after dosing and the natural log of Co and Yb concentration, respectively. Rumen volume was calculated as concentration (mg) of Co in dose divided by ruminal concentration extrapolated to 0 h. Outflow from the rumen or fluid flow rate (h) was calculated as rumen volume (L) multiplied by the dilution rate. Turnover time was calculated as one divided by the absolute value of the fractional dilution rate.

Intake values (g/d) were calculated as the concentration of the nutrient in feed less the concentration of the nutrient in orts. Intake values (g/kg BW) were calculated as nutrient intake (g) divided by BW. Dry matter fecal output values were calculated as the average fecal output (wet basis) multiplied by its DM concentration. Fecal nutrient output was calculated by multiplying fecal output (DM basis) by the nutrient concentration. Total tract digestibility values were calculated by subtracting fecal nutrient output from nutrient intake and dividing that value by nutrient intake.

Statistical Analysis. Body weight, intake, fecal output, digestibility data, and ruminal kinetics were subjected to analysis of variance appropriate for a completely random design, with steer as the experimental unit. Analyses were computed using GLM of SAS (SAS Inst. Inc., Cary, NC). Ruminal pH and VFA were subjected to analysis of variance for repeated measures using the mixed procedure of SAS. The model included treatment as a fixed effect, time as a repeated measure, and steer (treatment) as the error term. The covariance structure that most appropriately fit the data was compound symmetry. No treatment by sampling interactions were detected ($P > 0.20$); therefore, effects of treatment on pH and VFA were examined across time. For all variables, when significant treatment effects were detected, treatment means were separated using the predicted difference test of SAS.

Results and Discussion

Nutrient intake, fecal content, and digestibilities are shown in Table 2. Steers fed WCGF3 consumed more ($P = 0.01$) DM than did those fed STD3 or WCGF0 (23.5 > 18.4 = 18.8 ± 0.9 g/kg BW daily, respectively). A similar response was reported by Ham et al. (1995), Parsons et al. (2001), Defoor et al. (2003), and Scott et al. (2003). Organic matter (g/kg BW), NDF, and ADF intakes were greater ($P < 0.02$) for WCGF3-fed steers than steers fed STD3 and WCGF0. Intake of ether extractable nutrients also differed ($P < 0.001$) among the three diets (0.7 > 0.4 > 0.3 ± 0.025 g/d for WCGF3, STD3, and WDGF0, respectively). Fecal output of OM, NDF, ADF, CP, starch, and ether extractable nutrients were similar ($P > 0.11$) among treatments. Likewise, total tract digestibilities of DM, OM, NDF, ADF, and CP were not different ($P > 0.15$) in the three groups. However, ether extract digestibility differed ($P = 0.002$) among diets (93.5 > 90.4 > 88.6 ± 0.3% for WCGF3, STD3, and WCGF0, respectively).

Ruminal volume (43.4, 38.8, 32.0 ± 3.3 L) and fluid flow rate (2.2, 2.3, 2.4 ± 0.2 L/h) were similar ($P > 0.12$) in steers fed STD3, WCGF0, and WCGF3, respectively. Ruminal fluid dilution rate (7.5 > 5.7 = 5.2 ±

0.4 %/h) and particulate passage rate (5.9 > 4.2 = 3.6 ± 0.4 %/h) were increased (P < 0.03) in steers fed WCGF3 compared with those fed WCGF0 and STD3, respectively. As would be expected from the increase in passage rates, turnover time in the WCGF3-fed steers was decreased (P = 0.03) compared with those of WCGF0 and STD3 (13.3 < 17.6 = 19.5 ± 1.2 h, respectively).

Ruminal pH tended (P = 0.09) to be greater in steers fed WCGF0 than in those fed WCGF3 and STD3 (6.0 > 5.5 = 5.3 ± 0.16, respectively). The tendency for WCGF0 to have a higher pH than commonly observed with high concentrate feedlot diets agrees with reports by Sindt et al. (2003) and Montgomery et al. (2004). The lower pH resulting from adding 3% fat to WCGF may have resulted from effects of dietary fat on fibrolytic bacteria in the rumen (Palmquist, 1988, as cited by Lodge et al., 1997) or possibly the increased intake resulting from addition of fat to the WCGF diet.

Implications

Addition of 3% fat to diets containing 25% wet corn gluten feed does not appear to detrimentally affect fiber digestibility. Ruminal pH is decreased when 3% fat is added to a 25% wet corn gluten feed diet which may affect acidosis. Increased intakes resulting from adding wet corn gluten feed to feedlot diets results from increased fluid and particulate passage rates.

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Table. Ingredients and nutrient composition of concentrate diets fed to beef steers

	Treatment[a]		
Item	STD3	WCGF0	WCGF3
Ingredient, % (DM basis)			
Steam-flaked corn	74.9	63.3	59.5
Alfalfa hay, mid bloom	9.0	9.0	9.0
Wet corn gluten feed		25.0	25.0
Molasses	4.0		
Cottonseed meal	5.7		0.8
Urea	0.9	0.2	0.2
Finish supplement[b]	2.5	2.5	2.5
Tallow (yellow grease)	3.0		3.0
Composition, % (DM basis)			
Ash	2.0	1.3	2.4
Crude Protein	13.7	13.9	14.1
Neutral detergent fiber	11.2	15.2	15.8
Acid detergent fiber	3.0	3.3	3.9
Ether extract	5.2	4.0	7.2
Starch	53.7	47.0	44.8

[a] STD3 = standard finishing diet with 3% added fat; WCGF0 = 25% (DM basis) wet corn gluten feed with no added fat; WCGF3 = 25% (DM basis) wet corn gluten feed with 3% added fat.
[b] Supplement supplied the following (DM basis): Ca = 0.60%, P = 0%, K = 0.10%, Mg = 0.08%, S = 0.03%, NaCl = 0.30%, Ammonium sulfate = 0.10%, Co = 0.20 ppm, Fe = 66.47 ppm, I = 0.50 ppm, Mn = 40.09 ppm, Se = 0.05 ppm, Zn = 75.0 ppm, Cu = 10.00 ppm, vitamin A = 2,200 IU/kg, vitamin E = 17.5 IU/kg, monensin = 30 g/ton, Tylosin = 10 g/ton, crude protein = 0.30%

Table 2. Characteristics of digestion in steers fed wet corn gluten feed and (or) supplemental fat

	Treatment[a]				
Item[b]	STD3	WCGF0	WCGF3	SE	*P*
Animals, no.	3	3	3		
BW, kg	473.6	435.8	403.9	16.5	
DM intake, kg	8.7	7.3	9.5	0.6	0.36
DM intake, g/kg BW	18.4[b]	18.8[b]	23.5[c]	0.9	0.01
OM intake, g/day	8.4	7.8	8.9	0.5	0.39
OM intake, g/kg BW	17.7[b]	17.8[b]	22.1[c]	0.8	0.02
NDF intake, g/day	0.98[b]	1.2[b, c]	1.5[c]	0.09	0.02
ADF intake, g/day	0.2[b]	0.2[b]	0.3[c]	0.02	0.02
CP intake, g/day	1.2	1.1	1.4	0.08	0.21
Starch intake, g/day	4.7	3.7	4.1	0.2	0.08
EE intake, g/day	0.4[b]	0.3[c]	0.7[d]	0.02	0.001
Fecal OM output, g/day	1.2	1.1	1.4	0.09	0.22
Fecal NDF output, g/day	0.6	0.7	0.8	0.07	0.11
Fecal ADF output, g/day	0.3	0.3	0.4	0.04	0.50
Fecal CP output, g/day	0.3	0.2	0.3	0.02	0.21
Fecal starch output, g/day	0.03	0.03	0.03	0.005	0.72
Fecal EE output, g/day	0.04	0.03	0.04	0.003	0.22
Total tract nutrient digestion, % of intake					
DM	81.9	81.6	81.1	0.8	0.73
OM	85.4	85.4	84.4	0.6	0.47
NDF	39.8	45.8	43.3	3.9	0.58
CP	77.9	80.6	80.4	0.9	0.15
Starch	99.4	99	99.2	0.1	0.28
EE	90.4[b]	88.6[c]	93.5[d]	0.3	0.002

[a] STD3 = standard finishing diet with 3% supplemental fat; WCGF0 = 25% (DM basis) wet corn gluten feed with no supplemental fat; WCGF3 = 25% (DM basis) wet corn gluten feed with 3% supplemental fat.
[b, c, d] Row values with different superscripts differ.

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EFFECT OF DISTILLER'S GRAIN BASED CREEP FEED AND SEASON ON INTAKE, MICROBIAL PROTEIN SYNTHESIS AND EFFICIENCY, DIGESTION, AND RUMINAL FERMENTATION IN NURSING CALVES GRAZING NATIVE RANGE IN SOUTHEASTERN NORTH DAKOTA

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ABSTRACT: Nine ruminally and duodenally cannulated commercial Angus steer calves (145 ± 21 kg initial BW) were used to evaluate the effects of dry distiller's grain with solubles (DDGS) in creep feed and advancing season on intake, microbial protein synthesis and efficiency, digestion, and ruminal fermentation, while grazing native range. Calves were assigned to one of two treatments: a creep feed containing 41% soybean meal, 26.25% wheat midds, 26.25% soyhulls, 5% molasses, and 1.5% limestone (Control) or a creep feed containing 50% DDGS, 14.25% wheat midds, 14.25% soyhulls, 14% soybean meal, 5% molasses, and 1.5% limestone. Calves were offered creep feed individually (0.45% of BW) once daily. There were three collection periods, occurring in June, July, and August. Total OMI was not different (kg/d and % BW) between treatments. Ruminal true OM disappearance (% intake), intestinal apparent OM disappearance (% intake), and total tract apparent OM disappearance were not different between treatments. In addition, microbial efficiency was not affected by treatment. Apparent total tract N disappearance decreased (P = 0.04) in calves fed DDGS. Total ruminal VFA and ruminal pH were not different between treatments. Propionate proportion tended (*P* = 0.11) to increase in calves fed DDGS and the acetate:propionate ratio was lower (*P* = 0.08) in calves fed DDGS compared with controls. Inclusion of 50% DDGS in creep feed resulted in no differences in OMI, OM disappearance, microbial efficiency, total VFA concentration, or ruminal pH. Calves supplemented with DDGS had decreased apparent total tract N disappearance and a lower acetate:propionate ratio compared to controls. Dry distiller's grain with solubles appears to be a suitable ingredient in creep feed and has no negative effects on ruminal fermentation when offered at 50% of the creep feed.

Key Words: Distiller's Grain, Creep Feed, Forage

Introduction

Creep feeding can provide many benefits to cow-calf producers including increased weaning weights, reduced grazing pressure, and improved feed intake at weaning. Creep feeding studies have consistently shown increases in weaning weight in creep-fed calves (Faulkner et al., 1994; Lardy et al., 2001; Loy et al., 2002).

Many researchers have shown that calves consume less forage when supplemented with creep feed (Cremin et al. 1991; Faulkner et al. 1994; Lardy et al., 2000). However, research at NDSU is somewhat inconclusive: Soto-Navarro et al. (2004), and Loy et al. (2002) reported no differences in forage intake between creep-fed and non creep-fed calves. However, Gelvin et al. (2004) reported increased forage intake and Benz et al. (2004) reported decreased forage intake with creep feed supplementation.

Evaluation of dry distiller's grain with solubles (DDGS) in creep feed on forage intake is warranted because previous data are inconclusive and because changes in processing technology have resulted in differing products. Therefore, our objectives were to determine the effects of DDGS in creep feed on intake, microbial protein synthesis and efficiency, digestion, and ruminal fermentation.

Materials and Methods

Animals and Diets. Nine ruminally and duodenally cannulated nursing beef steers (319 ± 46 lbs initial BW) were used in a split-plot design. Calves were assigned to one of two treatments: a pelleted creep feed containing 41% soybean meal, 26.25% wheat midds, 26.25% soyhulls, 5% molasses, and 1.5% limestone (Control) or a pelleted creep feed containing 50% DDGS, 13.9% soybean meal, 14.55% wheat midds, 14.55% soyhulls, 5% molasses, and 2.0% limestone (DDGS; DM basis). Calves were fed individually (0.45% of BW) once daily at 0800. Amount of supplement fed was adjusted at the beginning of each period based on beginning BW for that period. Cattle had ad libitum access to water and a commercial trace mineralized salt mix (Trouw Nutrition, Willmar, MN).

Sample Collection. Calf responses to treatment and seasonal effects were measured during three experimental periods. Collection periods were June 24 to July 8 (June), July 15 to July 29 (July), and August 5 to 19 (August). Two-day weights were taken at the beginning and end of the experiment and one-day weights were taken at the end of each period. Supplement samples were collected twice weekly and composited within period for laboratory analysis. Chromic oxide (5 g) was ruminally dosed twice daily at 0800 and 2000 on d 2 to 11 via gelatin capsules (Torpac, Inc., Fairfield, NJ) to determine DM flow rate and total fecal output. On d 1, ruminal evacuation techniques were used to collect masticate samples. Masticates were frozen (!20 C) in preparation for lyophilization. Duodenal fluid samples (200 mL) were collected on d 7 to 11. Samples were collected at

0, 4, 8, and 12 h after supplementation and were composited within steer for each period. On d 9, ruminal fluid was drawn from each steer to serve as inoculum for in vitro analysis. Ruminal fluid samples were collected on d 11 of each period. Ruminal fluid samples were collected at -1, 1, 2, 4, 8, 12, and 24 h after supplementation. Milk intake was measured on d 16 using a 12 h weigh-suckle-weigh technique according to the methods described by Boggs et al. (1980).

Statistical Analysis. Data were analyzed as a split-plot design using the Proc Mixed procedure of SAS (SAS Inst., Cary, NC). The model contained effects for treatment, period, and treatment x period. Ruminal data over time were analyzed as a repeated measures design using the Mixed procedures of SAS (SAS Inst., Cary, NC). The model included effects for period, treatment, sampling time, treatment x period, treatment x sampling time, treatment x period x sampling time. The three-way interaction was used in the error term to test for treatment effects. Two-way and three-way interactions involving sampling time were usually significant but were largely due to magnitude; therefore, *P* values were not presented and means were averaged across sampling time. Period means were separated using linear and quadratic contrasts.

Results and Discussion

Crude protein, OM, in vitro organic matter disappearance (IVOMD), Ca, and P levels of the grazed diet were not different between treatments (data not shown). Calves consuming DDGS selected forage that was higher (P # 0.07) in NDF (P = 0.07) and ADF (P = 0.06). Loy et al. (2002) reported no differences in grazed diet quality between treatments on the same pasture as our study. Season did not affect level of CP, OM, IVOMD, ADF, Ca, or P of the grazed diet. Similarly, Loy et al. (2002) reported no differences in grazed diet quality across season. Neutral detergent fiber of the grazed diet responded quadratically (P = 0.01) to season (data not shown). Neutral detergent fiber of the grazed diet decreased from June to July and increased from July to August.

There were no treatment x period interactions for OM intake or digestion. Supplement OM intake (OMI; kg/d) was not different (P = 0.59; Table 1) between treatments. Supplement was fed at 0.45% of BW (DM basis) and level of supplement was adjusted according to BW at the beginning of each period; therefore, supplement OMI was not different between treatments and increased (P = 0.01) as season progressed.

Forage OMI (kg/d) was not different (P = 0.76; Table 1) between treatments. Forage intake was likely not affected because the forage was of moderate quality (10.8% CP) and nutrient profiles of the creep feeds' were similar. Forage OMI increased (P = 0.001) as season advanced. This effect is likely due to increasing rumen function and increasing calf size.

Milk OMI (kg/d) was not different (P = 0.82; Table 1) between the treatments. Others have reported similar milk OMI when comparing intakes of calves consuming different types of creep feed (Loy et al., 2002; Faulkner et al., 1994). There was a quadratic affect (P = 0.002) for milk OMI across season. Milk OMI was highest in July and lowest in August.

There were no differences in ruminal, intestinal, or total tract OM digestion between the treatments (Table 1). This was likely an effect of having similar total OMI and similar IVOMD for the supplement and grazed diets. Intestinal OM digestion decreased (P = 0.07) linearly as season progressed. There were no other seasonal differences for digestion.

There was a treatment x period interaction for N intake (P = 0.08). During June and August N intake was less in DDGS calves, but in July N intake was greater in DDGS calves than in controls. Nitrogen intake was not affected (P = 0.17; Table 1) by treatment. Crude protein content of the supplements was similar (26.54 and 26.50%, DM basis, for control and DDGS, respectively) and CP of the grazed diet and milk were not affected by treatment; therefore, N intake was not affected by treatment.

True ruminal and intestinal N disappearance were not affected by treatment (Table 1). However, total tract N disappearance was lower (P = 0.04) in calves fed DDGS. True ruminal and intestinal N disappearance declined linearly (P = 0.001) as season progressed. This affect may be due to increasing proportions of forage in the diet which was less digestible than supplement and milk.

There was not a treatment x period interaction for microbial efficiency (P = 0.51). Microbial efficiency was not affected by treatment (Table 1). This is likely an effect of similar N intakes and similar diet IVOMD between treatments. Also, microbial efficiency was not affected (P = 0.95) by season.

There was a treatment x period interaction for ruminal pH (P = 0.001). In June and July ruminal pH was lower for DDGS calves, but in August ruminal pH was greater for DDGS calves than for controls. There was not a treatment x period interaction for total VFA (P = 0.27). Ruminal pH and total VFA were not different between treatments, suggesting that ruminal fermentation was similar between treatments (Table 1). Ruminal pH declined linearly (P = 0.001) as season progressed, which is likely an effect of increased ruminal function and fermentation. Decreased ruminal pH coincided with total VFA, which increased quadratically (P = 0.05) as season progressed. Total VFA decreased from June to July and increased from July to August.

There was not a treatment x period interaction for acetate (P = 0.61); but, there was a treatment x period interaction for propionate (P = 0.02). Propionate was lower for DDGS calves in June and higher in July and August compared with controls. Acetate was not affected by treatment; however, propionate tended (P = 0.11) to increase in calves fed DDGS and the acetate:propionate ratio was lower (P = 0.08) in calves fed DDGS compared with controls (Table 1). Reduced acetate:propionate ratio has been reported by others when supplementing increased levels of starch to nursing calves (Cremin et al., 1991; Faulkner et al., 1994; Tarr et al., 1994).

Inclusion of 50% DDGS in creep feed resulted in no differences in OMI, OM disappearance, microbial efficiency,

total VFA concentration, or ruminal pH. Calves supplemented with DDGS had decreased apparent total tract N disappearance and a lower acetate:propionate ratio compared to controls. Dry distiller's grain with solubles appears to be a suitable ingredient in creep feed and has no negative effects on ruminal fermentation when offered at 50% of the supplement.

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Table 1. Effect of DDGS in creep feed and grazing season on intake, microbial efficiency, digestion and ruminal fermentation in nursing steer calves

	Treatment			Period				P values[a]			Contrasts[b]	
Item	Control	DDGS	SEM	June	July	August	SEM	Trt[c]	Period	Trt x Period	L	Q
OM intake, kg/d												
Supplement	0.69	0.66	0.03	0.60	0.66	0.76	0.03	0.59	0.01	0.58	0.004	0.59
Milk	0.35	0.36	0.04	0.37	0.48	0.21	0.044	0.82	0.001	0.28	0.02	0.002
Forage	1.77	1.72	1.08	1.05	1.78	2.41	0.12	0.76	0.001	0.57	0.001	0.78
Total OM intake, kg/d	3.10	2.95	0.26	3.11	2.62	3.35	0.39	0.68	0.48	0.70	0.69	0.26
Total OM intake, % BW	1.61	1.62	0.06	1.52	1.59	1.74	0.12	0.98	0.50	0.81	0.26	0.79
Digestion[d]												
Apparent ruminal	248.1	227.4	37.9	193.2	246.0	273.9	81.6	0.70	0.83	0.68	0.560	0.92
True ruminal	557.3	563.2	23.6	558.7	585.9	536.2	48.9	0.86	0.83	0.71	0.78	0.59
Intestinal	420.8	443.8	38.4	540.0	399.1	357.8	50.6	0.67	0.07	0.68	0.03	0.46
Total Tract	66.9	67.1	1.4	73.3	64.5	63.2	4.0	0.91	0.30	0.52	0.16	0.53
N intake, g/d	75.3	68.5	3.3	64.5	69.5	81.7	3.4	0.17	0.006	0.08	0.002	0.37
Microbial efficiency[e]	17.1	19.5	2.6	19.7	17.8	17.3	4.8	0.50	0.95	0.51	0.76	0.92
Digestion[d]												
Apparent ruminal	12.2	11.3	5.1	-0.6	27.0	9.0	4.9	0.90	0.002	0.23	0.14	0.001
True ruminal	35.9	36.1	5.3	19.9	50.3	37.9	5.1	0.99	0.001	0.19	0.01	0.002
Intestinal	62.6	62.0	4.6	77.3	49.2	60.5	4.5	0.92	0.001	0.19	0.009	0.001
Total Tract	74.9	73.3	1.2	76.7	76.1	69.5	1.4	0.34	0.006	0.74	0.003	0.11
pH	6.53	6.43	0.06	6.55	6.47	6.42	0.04	0.22	0.001	0.001	0.001	0.61
Total VFA, mM	65.2	68.4	2.0	65.7	63.1	71.6	2.2	0.27	0.04	0.26	0.07	0.05
Acetate:propionate	4.29	4.03	0.09	3.89	3.73	4.86	0.07	0.08	0.001	0.02	0.001	0.001
Acetate, mol/100 mol	67.2	66.1	0.6	65.8	64.0	70.2	0.4	0.21	0.001	0.61	0.001	0.001
Propionate, mol/100 mol	16.0	16.7	0.3	17.3	17.3	14.6	0.2	0.108	0.001	0.02	0.001	0.001
Butyrate, mol/100 mol	10.1	11.4	0.3	10.2	12.1	10.0	0.2	0.01	0.001	0.001	0.27	0.001

[a]Two-way and three-way interactions involving time were usually significant and mostly due to magnitude; therefore, *P*-values are not presented and means are averaged across time. [b]Contrasts were L = linear and Q = quadratic. [c]Probability value for the F-test for an overall treatment (trt) effect.
[d]Digestion as a percentage of intake. [e]Grams of nitrogen per kg of OM digested, true (corrected for OM of bacterial origin).

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EFFECTS OF CREEP FEEDING AND SEASON ON INTAKE, PERFORMANCE AND GRAZE DIET QUALITY IN NURSING STEER CALVES GRAZING NATIVE RANGE IN SOUTHEASTERN NORTH DAKOTA

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ABSTRACT: Sixteen intact and nine ruminally cannulated commercial Angus steer calves (181 ± 36 kg initial BW) were used to evaluate the effect of dry distiller's grain with solubles (DDGS) in creep feed on intake, performance, and grazed diet quality in nursing calves grazing native range at the Albert Ekre Grasslands Preserve in southeastern North Dakota. Calves were assigned to one of two treatments: a creep feed containing 41% soybean meal, 26.25% wheat midds, 26.25% soyhulls, 5% molasses, and 1.5% limestone (Control) or a creep feed containing 50% DDGS, 14.25% wheat midds, 14.25% soyhulls, 14% soybean meal, 5% molasses, and 1.5% limestone. Calves were offered creep feed individually (0.45% of BW) once daily. There where three collection periods, occurring in June, July, and August. Total fecal collections were performed for 5 d. Masticate samples were collected for diet quality analysis. Ruminal fluid was drawn and used as inoculate for in vitro organic matter disappearance (IVOMD) analysis, which was used to estimate forage intake based on fecal output. Creep feed OM intake (OMI; % BW) tended (P = 0.09; 0.380 and 0.366% for Control and DDGS, respectively) to decrease in calves fed DDGS. Forage OMI (% BW) was not different (P = 0.52; 1.16 and 1.20% for Control and DDGS, respectively) and increased (P = 0.001; 0.79, 1.31, and 1.45% for June, July, and August, respectively) as season advanced. Milk OMI (% BW) was not different between treatments. Final weight, ADG, and gain efficiency (G:F) were not affected by treatment. Crude protein, OM, IVOMD, Ca, and P levels of the grazed diet were not affected by treatment, however calves receiving DDGS tended to select forage that was higher (P # 0.07) in NDF and ADF. Inclusion of 50% DDGS in creep feed resulted in few differences in OMI, performance, and grazed diet quality. Dry distiller's grain with solubles appears to be a suitable ingredient in creep feed when offered at 50% of the creep feed.

Key Words: Calves, Creep Feed, Forage, Intake, Supplementation

Introduction

Creep feeding can provide many benefits to cow-calf producers including increased weaning weights, reduced grazing pressure, and improved feed intake at weaning. Weaning weight is an important factor affecting profitability of producers who sell their calves at weaning. Creep feeding studies have consistently shown increases in weaning weight in creep-fed calves (Faulkner et al., 1994; Lardy et al., 2001; Loy et al., 2002). Faulkner et al. (1994) and Myers et al. (1999) reported that creep-fed calves had higher feed intake when newly received into the feedlot. In addition, Myers et al. (1999) reported that creep-fed calves had decreased respiratory morbidity compared to non-creep fed calves.

Many researchers have shown that calves consume less forage when supplemented with creep feed (Cremin et al. 1991; Faulkner et al. 1994; Lardy et al., 2001). However, research at NDSU is somewhat inconclusive: Maddock and Lardy et al. (2002), Soto-Navarro (2004) and Loy et al. (2002) reported no differences in forage intake between creep-fed and non creep-fed calves. Gelvin et al. (2004) reported increased forage intake and Benz et al. (2003) reported decreased forage intake with creep feed supplementation. Evaluation of dry distiller's grain with solubles (DDGS) in creep feed on forage intake is warranted because previous data are inconclusive and changes in processing technology has resulted in differing products.

Therefore, our objectives were to determine effects of dry distiller's grain with solubles in creep feed on intake, performance, milk intake, and grazed diet quality in nursing calves grazing native range North Dakota.

Materials and Methods

Twenty-five commercial steer calves (181 ± 36 kg initial BW) were used to evaluate the effect of DDGS in creep feed on intake, performance, and grazed diet quality in nursing calves grazing native range at the Albert Ekre Grasslands Preserve in southeastern North Dakota.

Calves were stratified by pair weight and assigned randomly to one of two treatments (Table 1): a pelleted supplement containing SBM (41%), wheat midds (26.25%), soyhulls (26.25%), molasses (5%), and limestone (1.5%) with no DDGS (Control) or a pelleted supplement containing DDGS (50%), soybean meal (14%), soyhulls (14.25%), wheat midds (14.25%), molasses (5%) and limestone (2.5%). Calves were fed individually (0.45% of BW) once daily and treatments were formulated to contain similar levels of crude protein. There were three research periods of approximately one month in length in June, July, and August.

Intact calves (n = 16, 202 ± 20 kg initial BW) were fitted with fecal collection bags during each collection week. Total fecal collections were performed for 5 d in order to estimate forage intake. Fecal contents were weighed, sub-sampled, and frozen for later analysis of DM,

organic matter (OM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Forage intake was estimated by measuring daily fecal output and indigestibility of the forage, correcting for creep feed intake and indigestibility.

Two-day weights were taken at the beginning and end of the study and one-day weights were taken at the end of each period to determine average daily gain (ADG) and gain efficiency (G:F). Milk intake was measured on d 16 using a 12 h weigh-suckle-weigh technique according to the methods described by Boggs et al. (1980). On d 15 the cows from the cannulated calves were hand milked in order to determine milk composition.

Cannulated calves (n = 9, 145 ± 21 kg initial BW) were used to monitor seasonal changes in forage quality of grazed native range by collecting masticate samples. Masticate samples were collected following ruminal evacuations on d 1 each collection period. Ruminal contents were evacuated and calves were allowed to graze for approximately 1 h. Masticate samples were then removed from the rumen, frozen, and lyophilized prior to laboratory analysis. Masticate samples were analyzed for DM, ash, N (AOAC, 1990), ADF, NDF (ANKOM, Fairport, NY) and IVOMD (Tilley and Terry 1963).

Statistical Analysis

Data were analyzed as a completely randomized design using the PROC Mixed procedure of SAS (SAS Inst., Cary, NC). The model contained effects for period, animal, and treatment. Period means were separated using linear and quadratic contrasts.

Results and Discussion

Crude protein, OM, IVOMD, Ca, and P levels of the grazed diet were not different between the treatments (Table 2). Calves consuming DDGS selected forage that was higher in NDF ($P = 0.07$) and ADF ($P = 0.06$). Loy et al. (2002) reported no differences in grazed diet quality between treatments on the same pasture as our study. When comparing a field pea-based creep feed to no creep feed, Gelvin et al. (2004) reported that the nutrient composition of grazed diet samples was not different between treatments, except for CP, which was greater for calves receiving the field pea-based creep feed. Season did not affect level of CP, OM, IVOMD, ADF, Ca, or P of the grazed diet. Similarly, Loy et al. (2002) reported no differences in grazed diet quality across season. Neutral detergent fiber of the grazed diet responded quadratically ($P = 0.01$) to season. Neutral detergent fiber of the grazed diet decreased from June to July and increased from July to August.

Creep feed OM intake (OMI) as a percentage of body weight (% BW) tended ($P = 0.09$) to decrease in calves fed DDGS (Table 2). Creep feed intake is often variable and in this study there were two calves on the DDGS treatment that regularly did not completely consume all of their creep feed. This likely explains the tendency for reduced creep intake observed in this study. In our study, creep feed was fed at 0.45% of BW (DM basis) and level of creep was adjusted according to BW at the beginning of each period; therefore, as season progressed, creep feed OMI (kg/d) increased ($P < 0.001$).

Forage OMI (% BW) was not different between treatments ($P = 0.52$; Table 2). Forage intake was likely not affected because the forage was of moderate quality (10.8% CP) and the nutrient profiles of the creep feeds' were similar (Table 1). Forage OMI increased ($P = 0.001$) as season advanced. Increased forage OMI intake was likely due to increased rumen function and calf size.

Milk OMI (% BW) was not different ($P = 0.46$) between the treatments (Table 2). Numerous researchers have reported no differences in milk intake when comparing creep-fed to non creep-fed calves (Gelvin et al., 2004; Soto-navarro et al., 2004; and Loy et al., 2002). Additionally, Loy et al. (2002) and Faulkner et al. (1994) reported no differences in milk intake when comparing different types of creep feed. There was a quadratic affect ($P = 0.02$) for milk OMI across season. Milk OMI (% BW) decreased from June to July and increased from July to August. This result was unexpected as milk production typically declines, as the cow gets further into lactation (NRC, 1996). Final weight ($P = 0.70$), ADG ($P = 0.82$), and G:F ($P = 0.83$) were not affected by treatment (Table 2). Other researchers have reported no differences in calf performance when comparing different types of creep feeds. Loy et al. (2002) reported no differences in calf performance when comparing an energy supplement, a degradable intake protein supplement, and a degradable with undegradable intake protein supplement. Faulkner et al. (1994) reported no differences in calf performance when comparing corn to soyhulls.

There were no differences in calf performance between treatments. Calves supplemented with DDGS tended to have reduced supplement OMI and selected a diet higher in NDF and ADF. Dried distiller's grain with solubles produced using modern processing technology seems to be a suitable feedstuff for inclusion in creep feed.

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Table 1. Composition of creep feed

	Treatment	
Ingredient, %	Control	DDGS
Dry Distiller's grain with solubles	0.00	50.00
Soybean meal	41.00	14.00
Soyhulls	26.25	14.25
Wheat midds	26.25	14.25
Molasses	5.00	5.00
Limestone	1.50	2.50
----------Laboratory analysis, % DM----------		
CP	30.01	29.61
OM	91.87	92.08
NDF	33.33	36.43
ADF	17.94	18.36
Ca	0.67	0.64
P	0.39	0.35
Starch	7.95	8.79
IVOMD[a]	85.67	79.33

[a]In vitro organic matter disappearance

Table 2. Effect of inclusion of dry distiller's grain with solubles in creep feed and grazing season on grazed diet composition, OM intake, and performance of nursing steer calves in southeastern North Dakota

Item	Treatment			Period				P values			Contrasts[a]	
	Control	DDGS	SEM	June	July	August	SEM	Trt[b]	Period	Trt H Period	L	Q
Grazed diet composition, %												
Crude Protein	10.8	10.8	0.5	11.8	11.0	9.5	0.7	0.99	0.11	0.08	0.04	0.74
Organic Matter	84.6	78.5	3.3	85.1	82.6	77.0	3.8	0.21	0.34	0.31	0.16	0.76
IVOMD	47.7	44.7	1.4	48.6	45.4	44.6	1.8	0.15	0.27	0.99	0.13	0.61
NDF	63.0	66.6	1.3	67.4	61.9	65.2	1.3	0.07	0.02	0.21	0.26	0.01
ADF	37.0	41.7	1.4	39.7	37.7	40.7	1.9	0.06	0.58	0.68	0.72	0.33
Ca	1.05	1.43	0.31	0.88	1.30	1.54	0.38	0.37	0.41	0.22	0.19	0.80
P	0.51	0.45	0.03	0.47	0.54	0.43	0.04	0.20	0.25	0.31	0.50	0.15
Organic matter intake, % BW												
Creep	0.38	0.37	0.01	0.38	0.37	0.37	0.01	0.09	0.35	0.58	0.20	0.51
Forage	1.16	1.20	0.04	0.79	1.31	1.45	0.06	0.52	0.001	0.76	0.001	0.007
Milk	0.17	0.18	0.02	0.20	0.14	0.19	0.02	0.46	0.08	0.96	0.88	0.02
Total	1.71	1.75	0.05	1.37	1.82	2.01	0.06	0.55	0.001	0.81	0.001	0.09
Calf Performance												
Initial weight, kg	224.6	221.3	5.5	201.9	219.3	247.7	6.8	0.68	0.001	0.98	0.001	0.51
Final weight, kg	291.2	289.2	10.5	219.3	247.7	290.2	7.4	0.70	0.001	0.99	0.001	0.44
ADG, kg	1.13	1.11	0.04	0.83	1.02	1.52	0.05	0.82	0.001	0.27	0.001	0.02
DMI, kg	4.84	4.71	0.11	3.47	5.18	5.68	0.13	0.39	0.001	0.002	0.001	0.00
Gain:feed	0.23	0.24	0.01	0.24	0.20	0.27	0.01	0.83	0.001	0.55	0.11	0.001

[a]Contrasts were L = linear amd Q = quadratic

[b]Probability value for the F-test for an overall treatment (Trt) effect

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EVALUATION OF PERFORMANCE AND COSTS OF TWO HEIFER DEVELOPMENT SYSTEMS

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ABSTRACT: Early weaned (EW) heifers must be developed for a longer period of time and can have elevated development costs. Developing EW heifers on native range may reduce costs. Dried distillers grains plus solubles (DDGS) offers protein and energy that compliment native forages for developing heifers. The objective of this study was to evaluate the performance and costs of two heifer development systems in northwest South Dakota. Sixty-five nulliparous crossbred beef heifers were randomly allotted to one of two systems: 1) heifers (n=33) weaned at 132 d of age (209 kg) and developed on range with a DDGS supplement (0.8 to 2.9 kg/hd/d) from Sept. 25 to May 18 (Range); 2) heifers (n=32) weaned at 218 days of age (274 kg) and developed in a drylot with grass hay and a conventional supplement (1.2 to 1.6 kg/hd/d) from Dec. 2 to May 18 (Normal). Supplement levels were established to result in both groups of heifers reaching 65% of mature weight at breeding (392 kg). All heifers were managed similarly after May 18. Heifers were synchronized with a shot of $PGF_{2\alpha}$ and bred natural service beginning June 14. As necessary for target weights to be reached, ADG through the feeding period was greater ($P<0.05$) for Range (0.76 kg/d) than Normal (0.61 kg/d). Range heifers tended ($P=0.12$) to be heavier on May 18 (390 and 376 kg, respectively) and were heavier ($P<0.05$) at breeding (415 and 378 kg, respectively). Weight differences in May were a result of higher than expected gains by the Range heifers in the spring. From May 18 to June 14, Range heifers gained more ($P<0.05$) than Normal (0.94 and 0.15 kg/d, respectively). Synchronized conception and overall pregnancy rates were similar ($P>0.25$) between the Range and Normal heifers (58% vs. 50% and 91% vs. 88%, respectively). Supplement and forage costs for the Range system was similar ($122/hd) to the Normal ($117/hd). Range development provides an alternative method for developing early-weaned heifers that reduces daily costs.

Key Words: early weaning, heifer development, distillers grains

Introduction

Cow-calf production systems that rely heavily on harvested and purchased feeds have less potential to be profitable (Adams et al., 1994). At the Antelope Range and Livestock Research Station near Buffalo, South Dakota, ongoing research is evaluating the effectiveness of early weaning in managing forage supplies and cow body condition in order to reduce the requirement for harvested feeds. An important part of any early weaning system is the reproductive performance and costs associated with developing heifers. Indeed, early-weaning heifers from dams results in more days that heifers must be managed and fed, potentially increasing the costs of the heifer development program. If available, forage spared by early weaning may be used in developing the early-weaned heifers.

Developing heifers on range is not a common practice in northern South Dakota due to the perception that adequate reproduction cannot be maintained in such a system. Recent reports showed that bred heifers could be managed on range with no hay during late gestation by feeding dried corn-gluten feed (Loy et al., 2004), a source of protein and fiber based energy. It is hypothesized that a similar management system could be used to develop replacement heifer calves.

Dried distillers grains plus solubles (**DDGS**) has a unique combination of fat, fiber, and protein that makes the product valuable to young beef female management programs. Both fat (Bellows, 1997) and undegradable intake protein (Patterson et al., 2003) supplemented to bred heifers during late gestation has been shown to increase reproductive rates. The effect of DDGS supplementation on reproduction in replacement heifers has not been well documented. Due to the low cost of both protein and energy in DDGS, the product may also be valuable in replacing expensive hay inputs in heifer development programs. Since DDGS compliments native winter range, it has promise as a supplement to heifers being developed on grass.

Materials and Methods

Sixty-five nulliparous crossbred beef heifers at the Antelope Range and Livestock Research Station, located near Buffalo, SD, were randomly allotted into one of two heifer development systems. In the first system (**Range**), heifers (n = 33) were weaned on August 12, 2003, averaging 132 days of age (209 kg). Heifers were fed a weaning ration in the drylot consisting of grass hay and 1.6 kg (DM) of weaning pellet (pellet contained adequate protein, vitamins, and minerals and 66 mg/kg Decoquinate). On September 25, 2003, the heifers were turned out to native range and supplemented with DDGS (loose meal; Table 1). The DDGS was fed daily in feed bunks at rate of 0.8 to 2.9 kg/hd/d (DM basis). The feeding rate was established to result in heifers weighing approximately 65% of mature weight at breeding in June (392 kg), for an average daily gain of 0.68 kg/day during the trial (assuming 0.91 kg/day following treatments in early summer). The feeding rate changed over the winter to account for heifer size, weather conditions, expected forage quality and

observed interim performance. The level of DDGS supplementation (DM basis: per hd/d) was 0.8 kg in September and increased to 1.6 kg on November 24, 2.0 kg on December 2 and 2.9 kg on February 12. The supplementation level was then decreased to 2.0 kg on April 20 and 1.0 kg on May 4. Hay was fed on two days when snow cover prevented grazing (4.7 kg/hd/d).

The second system (**Normal**), heifers (n = 32) were weaned on November 6, 2003, averaging 218 days of age (274 kg). Heifers were fed the same weaning ration as the early-weaned heifers for 37 days. On December 13, immediately following the weaning period, heifers remained in the drylot and were placed on a diet consisting of ad-libitum access to grass hay (8.1% CP, 66% NDF; DM basis) and a conventional supplement fed at a rate of 1.2 to 1.6 kg/hd/d (DM basis; Table 1). The supplement was fed at a rate to achieve approximately 65% of mature weight at breeding in June (392 kg), for an average daily gain of approximately 0.59 kg/day during the trial (assuming heifers would gain 0.91 kg/day following treatments in early summer). Although hay was fed ad-libitum, each hay bale was weighed to record hay usage.

Both treatments were terminated on May 18, 2004, when all the heifers were turned out to native range as a single group.

Heifers were weighed at weaning, the initiation of winter treatments (September 25 and December 13), at the termination of winter treatments on May 18, and at approximately 30-day intervals throughout the treatment period. Heifers were also weighed at the initiation of breeding on June 14 and at time of pregnancy determination on November 9.

On June 14, all heifers were exposed to bulls as a single group. On June 18, heifers were given an injection of $PGF_{2\alpha}$ (25 mg i.m. Lutalyse, Pfizer Animal Health, New York, NY) to synchronize estrus. Bulls were removed 5 d later, on June 23, for a 14 d period so that synchronized conception rates could be determined. Synchronized conception rates were determined by transrectal-ultrasonography 51 d after synchronization. Overall pregnancy was determined by rectal palpation 99 d after the breeding season. Two blood samples were taken 2-weeks apart prior to synchronization to determine estrous cycling status.

The effects of treatments on heifer weights and body condition scores were analyzed by ANOVA with Proc GLM of SAS (SAS Inst. Inc., Cary, NC). The effects of treatments on estrous cycling status, synchronized conception rates and pregnancy rates were analyzed by Chi-Square.

Results and Discussion

Range heifers weighed less ($P < 0.05$) at the initiation of their treatment protocol (September 25) than did Normal heifers at the initiation of their treatment protocol (December 2; Table 2). Range heifers were able to overcome their lighter initial weights by gaining 0.15 kg/d more than the Normal heifers during the experimental period ($P < 0.05$; Table 2). There was a slight difference in ADG between the Range and Normal heifers (0.61 and 0.54 for Range and Normal, respectively; $P = 0.13$) from December through February. The average daily gains between the winter months were lower for both systems than anticipated. This could be attributed to cold weather in December (avg. min. -11 ºC; avg. max. 20 ºC), January (avg. min. -15 ºC; avg. max. –5 ºC) and February (avg. min. -13 ºC; avg. max. 0 ºC). In addition, from December through February there were 44 days when snow cover was measured (average depth of 10 cm). Range heifers had higher ($P < 0.05$) ADG through March (0.97 and 0.56 for Range and Normal, respectively) and April (1.17 and 0.81 for Range and Normal, respectively).

Due to the greater than expected gain in the spring, the Range heifers tended ($P = 0.12$) to be heavier than the drylot heifers (390 kg and 376 kg, respectively) on May 18, the termination of treatment application. Interestingly, there was a difference ($P < 0.05$) between average daily gain of heifers from the two systems from May 18 to June 14, after treatments were applied (0.94 and 0.15 kg/d for Range and Normal, respectively). Although both groups of heifers were near their target weight of 392 kg at breeding on June 14 (Table 2), Range heifers were heavier at breeding ($P < 0.05$) than Normal heifers. The Normal heifers did not overcome the weight difference by November ($P < 0.05$; Table 2).

There was no difference between treatments in the percentage of heifers that were estrous cycling before the start of the breeding season ($P > 0.25$; 94% and 100% for Range and Normal, respectively). Synchronized conception rates and overall pregnancy rates did not differ ($P > 0.25$) between the Range and Normal heifers (Table 2).

Supplement and forage costs for the Range heifers was similar ($122/hd) to the Normal group ($117/hd). Cost per day for the Range and Normal systems were $0.52 and $0.74, respectively (Tables 3).

Loy et al. (2004) reported that bred heifers could be maintained during the winter without hay feeding. These data show that heifer calves may also perform adequately without significant hay inputs. We observed heifers foraging through snow-cover. It is possible that the increased level of supplementation in February and March was not necessary since the heifer gains were higher than expected in the spring and early summer. It is important to note that more severe winter conditions may result in a requirement for more hay feeding to sustain performance. The improvement in gains for Range heifers compared to Normal during the early summer was higher than expected and also contributed to their weights being higher at breeding. It is not clear if this was due to physiological or behavioral differences in the heifers during the early summer months.

Implications

These results showed that early-weaned heifers developed on range with dried distiller grains supplement weighed more at breeding and achieved similar reproductive performance as normal-weaned/drylot developed heifers, but at a lower cost per day. The range system reduced the high costs of developing early-weaned heifers and resulted in more developed young cows.

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Table 1. Nutrients in DDGS and Conventional Supplement (DM basis)

Item	DDGS	Conventional Supplement
Crude Protein (%)	29.7	31.0
Calcium (%)	0.06	0.37
Phosphorus (%)	0.79	1.11
Potassium (%)	1.09	1.31
Magnesium (%)	0.34	0.45
Copper (mg/kg)	6	61
Zinc (mg/kg)	99	112
Manganese (mg/kg)	18	56

Table 2. Performance of heifers that were weaned in August and developed on range (Range) compared to November-weaned heifers developed in a drylot (Normal)

Treatment	Range ± SEM	Normal ± SEM
No. Head	33	32
Initial BW, kg[e]	209 ± 9.3[a]	274 ± 9.5[b]
Final BW, kg[f]	390 ± 12.9[c]	376 ± 13.1[d]
Overall ADG, kg/d[g]	0.76 ± 0.03[a]	0.61 ± 0.03[b]
BW - 6-14-04, kg	415 ± 12.7[a]	378 ± 12.9[b]
BW - 11-9-04, kg	490 ± 13.7[a]	470 ± 13.7[b]
ADG 5/18 to 6/14, kg/d	0.94 ± 0.12[a]	0.15 ± 0.12[b]
% pubertal before the breeding season[h]	94	100
Synchronized Conception Rate[i]	58	50
Final Pregnancy Rate[j]	91	88

[a,b] Within a row, means with unlike superscripts differ ($P < 0.05$)

[c,d] Within a row, means with unlike superscripts differ ($P = 0.12$)

[e] Weight at the beginning of treatments
Range: 9-25-03; Normal: 12-2-03

[f] Weight at the end of treatments - both groups 5-18-04

[g] Average daily gain from initial to final weight

[h] Percent of heifer estrous cycling before the start of the breeding season

i Percent pregnant during the 10 d synchronization period to natural service

[j] overall pregnancy (34 d breeding season)

Table 3. Supplement and Forage Costs for heifers that were weaned in August and developed on range (Range) compared to November-weaned heifers developed in a drylot (Normal)

	Range[a]		Normal[b]	
	Total Feed (kg)	**Total Cost**	**Total Feed (kg)**	**Total Cost**
Hay	341	$27.07	29,633	$2,352.28
DDGS	16,402	$2,061.58		
Range[c]		$1,947		
Conventional Supplement			7,836	$1,382.40
	Total Cost	$4,035.65	**Total Cost**	$3,734.68
	$/hd/day	**$0.52**	**$/hd/day**	**$0.74**

[a] 33 early weaned heifers developed on range and DDGS for 236 d

[b] 32 normal-weaned heifers developed in drylot and conventional supplement for 158 d

[c] Rate at $7.50/AUM

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EFFECT OF LEVEL AND SOURCE OF DIETARY SE ON SE CONCENTRATIONS IN MATERNAL AND FETAL TISSUES OF PREGNANT YEARLING EWES[1]

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ABSTRACT: Pregnant Targhee ewe lambs (n = 32; BW = 45.6 ± 2.3 kg) were randomly allotted to one of four treatments in a completely randomized design to examine the effects of dietary selenium (Se) source and level on maternal and fetal liver, kidney, and muscle Se concentrations. Diets contained (DM basis) either no added Se (CON) or supranutritional Se added as high Se-wheat at 3.0 ppm (SW) and sodium selenate added at 3 (S3) and 15 (S15) ppm. Diets were similar in CP (15.5%) and energy (2.68 Mcal), and fed to meet or exceed requirements. Treatments were initiated at 50 ± 5 d of gestation. The CON, SW, S3, and S15 treatment diets provided 2.5, 75, 75, and 375 μg/kg BW of Se, respectively. In the ewe, liver and kidney mass on d 135 gestation were not different among treatments; however, Se concentrations (fresh basis) were greater ($P < 0.01$) in the respective tissues for Se treatments compared with CON. Selenium concentrations in the liver were 2.1, 5.9, 3.9, and 15.9 ± 1.7 μg/g; and in the kidney were 2.1, 3.2, 3.4, and 5.5 ± 0.3 μg/g for CON, SW, S3, and S15, respectively. Muscle (longissimus dorsi) Se was greatest in SW compared with all other treatments (0.91, vs. 0.27, 0.40, 0.78 ± 0.05 μg/g; for SW, CON, S3, and S15 respectively. Fetal liver and kidney mass were not different among treatments. Fetal liver and kidney Se concentrations were higher ($P < 0.05$) in SW and S15 compared with CON and S3. Fetal liver Se was 0.6, 2.9, 0.9, 3.5 ± 0.5 μg/g for CON, SW, S3, and S15, respectively. Fetal muscle (longissimus dorsi) Se was greatest ($P < 0.01$) in SW compared with all other treatments (0.82, vs. 0.19, 0.25, 0.59 ± 0.05 μg/g for SW, CON, S3, and S15, respectively). These data indicated organically bound Se crosses the placenta and is retained in fetal tissue more effectively than inorganic sources.

Key Words: Pregnancy, Selenium, Sheep

Introduction

In resent years, selenium has been the focus of much research as a potential cancer preventative. In 1996 it was determined that lung, colorectal, and prostate cancers could be reduced by as much as 50% in men supplemented with supranutritional levels of selenite (200 μg/d; Clark et al., 1996). Jaing et al., (1999) determined that rate of mammary tumor growth was reduced in rats fed 2.0 mg selenite/kg BW per day. As more information is gained in regard to this important trace mineral, consumers will seek demand for additional sources of dietary selenium.

Organic sources include small grains, such as wheat, and most sources of meat from domesticated livestock (Schubert et al., 1987; Holden et al., 1991; and Hintze et al., 2001). Organically bound selenium has been shown to be metabolized differently than inorganic salt forms (Thompson, et al, 1982). In ruminants, Se salts such as selenate are converted to selenocysteine (van Ryssen et al., 1989). Once incorporated into cysteine, the selenocysteine form is utilized to make selenoproteins (Sunde, 1990). Selenocysteine, however, cannot be converted to selenomethionine, and thus is not well incorporated into muscle tissue.

In addition, it has been shown that Se source does influence Se load in pregnant and lactating rats. Taylor et al., (2005) demonstrated a higher accumulation of Se in uterine, placenta, and fetal tissues in pregnant rats fed 2 μg per day of selenomethionine compared with 2 μg per day selenocystine. Selenomethionine can pass through the rumen intact and be incorporated into tissues where methionine is utilized (McDowell, 2003). When steers were fed either organic or inorganic sources of Se (65 μg/kg BW, daily), it was determined that Se accumulated in semitendinosus muscle tissue more efficiently in steers fed a high-Se wheat source versus all other treatments (Lawler et al., 2004). In the same study, it was found that the Se salt treatment (inorganic source) resulted in higher concentrations of Se in the liver compared with the high-Se hay treatment. More research is needed in this area to define the relationship between dietary Se source and level on Se tissue concentrations, and how these sources are transferred across the placenta to the fetus. Objectives of this study were to determine how source and level of selenium affect Se concentrations in maternal and fetal muscle, liver, and kidney tissues in pregnant ewe lambs.

Materials and Methods

Thirty-two pregnant Targhee ewe lambs (45.6 ± 2.3 kg, 330 ± 30 d of age) were randomly allotted to one of four

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treatments in a completely randomized design. Treatments were: control (CON; 0.1 ppm Se), Se-wheat (SW; 3 ppm Se), 3 ppm selenate (S3), and 15 ppm selenate (S15). Both diets contained 32% wheat; however, the SW diet was formulated using a high (8 to 10 ppm) Se wheat source (Table 1). Diets were similar in CP (15.5%) and energy (2.68 Mcal ME) and fed to meet or exceed NRC requirements (NRC, 1985). All diets were delivered in a complete pelleted form and fed twice daily. Diets were initiated at 50 ± 5 d of gestation. The SW and S3 diets (supranutritional Se levels) provided 75 µg/kg BW of Se, while the S15 treatment provided 375 µg/kg BW of Se. On d 135 of gestation ewes were stunned via captive bolt, eviscerated, and tissues harvested. Collected tissues were wrapped in foil, snap frozen in isopentane, and stored at -80° C.

Tissue samples were digested in trace mineral-grade nitric acid under heat. The digests were then diluted with ultra-pure water to a final nitric acid content of 5%, which provided a matrix match for the analytical standards. The prepared samples were analyzed by atomic absorption spectrophotometry (PerkinElmer Instruments, Shelton, CT) and assessed against concentration curves of known standards. Standard curves and quality control samples were analyzed every 5 samples. Total tissue content (mg) of Se was calculated by multiplying their respective concentrations by fresh tissue mass. Maternal and fetal tissue data were analyzed using the PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC). When the overall F-test for treatment was significant ($P < 0.10$), means were separated using the method of least significant difference.

Results and Discussion

Final body weight (BW) and rate of gain in the ewe were not different among treatments; fetal weights were lower ($P < 0.09$) in SW vs. all other treatments (Ward et al., 2004). In the ewe, liver and kidney mass on d 135 gestation were not different among treatments. Blood was collected via venepuncture every 14 d of the study to measure hormone and Se concentrations. Ward et al., (2004), reported data from this study, indicating that plasma Se concentrations were higher ($P < 0.01$) in all Se-treated compared with CON ewes (d 135 = 0.27, 0.55, 0.47, and 1.28 ± 0.06 ppm Se; for CON, SW, S3, and S15, respectively).

Selenium concentrations (fresh basis) were greater ($P < 0.01$) in the maternal liver and kidney tissues for Se treatments compared with CON (Table 1). Ewes provided 375 µg/kg BW daily of Se from selenate had three-fold higher ($P < 0.01$) concentrations of Se in the liver compared with all other treatments. Muscle Se concentration was greatest in SW compared with all other. These data are in agreement with Lawler et al., (2004), who found the greatest accumulation of Se in steers provided a high-Se wheat source. In the pregnant ewe, it appears both organically bound and salt sources of Se increase tissue Se concentrations when fed at supranutritional levels. Though ewes fed high-Se wheat had higher ($P < 0.01$) Se concentrations in muscle tissue, no differences in Se concentrations were observed in maternal liver and kidney tissues between organically bound and inorganic sources fed at 75 µg/kg BW Se. Results differ from Lawler et al., (2004), who reported higher concentrations of Se in kidney and liver tissue of beef steers fed high-Se wheat and high-Se hay vs. selenate when provided at similar dietary levels (65 µg/kg BW Se). These differences in Se accumulation may be due to days on treatment or physiological state. Our pregnant ewe lambs were fed Se treatments for 85 d while the steers used by Lawler et al., (2004) were fed Se treatments for 126 d.

Fetal liver and kidney mass were not different among treatments. Fetal liver and kidney Se concentrations were higher ($P < 0.05$) in SW and S15 compared with CON and S3 (Table 2). This response is different than that observed in the dam, suggesting the additional dietary supply of selenomethionine from high-Se wheat was directly incorporated into the rapidly growing tissues of the fetus. Ward et al., (2004) reported higher ($P < 0.01$) plasma Se concentrations in the SW and S15 fetuses compared with S3 and CON (0.11, 0.33, 0.18, 0.37 ± 0.01 ppm Se for CON, SW, S3, and S15, respectively).

Fetal muscle Se was greatest ($P < 0.01$) in SW compared with all other treatments. These findings indicate that when Se is bound to an amino acid (methionine), it is more efficiently transferred across the placenta and is incorporated into tissues similar to the response in the dam. This is in agreement with Taylor et al., (2005), who reported higher concentrations of Se in the uterine, placental, and fetal tissue in female rats provided 2.0 µg Se/g per d of selenomethionine, compared with female rats provided similar doses of selenocystine. Selenium in the salt form must be provided as much as 50 times (15 ppm) daily recommendations (0.3 ppm) before it will concentrate in fetal tissues at concentrations similar to those of organic sources fed at 10 times (3 ppm) the recommendations.

Implications

Both source and level of dietary selenium fed at supranutritional levels result in higher concentrations of Se in the liver, kidney, and the longissimus dorsi muscle in pregnant ewes and their fetuses. When provided from high-Se wheat, Se is more efficiently incorporated into muscle tissue of both the pregnant ewe and fetus. Further research is needed to better understand to the impacts of maternal dietary Se on fetal, neonatal, and postnatal growth and development.

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Table 1. Effect of source and level of Se on maternal organ mass and Se concentrations (fresh basis) of liver, kidney and the longissimus dorsi muscle in pregnant adolescent ewes.

	Treatment [a]					
Item	CON	SW	S3	S15	SE	*P* value
Liver Mass g	958.60	1044.80	1038.20	1068.10	49.9	0.448
Liver Se, µg/g	2.08[c]	5.90[c]	3.87[c]	15.90[d]	1.69	0.001
Total Se [b], mg	1.90[c]	6.13[c]	4.07[c]	17.47[d]	2.04	0.001
Kidney Mass g	137.90	144.50	139.10	138.50	6.20	0.867
Kidney Se, µg/g	2.07[c]	3.24[d]	3.37[de]	5.52[f]	0.30	0.001
Total Se, mg	0.28[c]	0.47[d]	0.47[d]	0.75[e]	0.04	0.001
Muscle						
Muscle Se, µg/g	0.27[c]	0.91[d]	0.40[e]	0.75[f]	0.05	0.001

[a] Control (CON), selenium wheat (SW; 3 ppm), selenate (S3; 3ppm), and (S15; 15 ppm).
[b] Total Se = tissue (g) X tissue Se µg/g
[c,d,e,f] Means within a row with different superscripts differ ($P < 0.05$)

Table 2. Effect of source and level of Se on fetal organ mass and Se concentrations (fresh basis) of liver, kidney and the longissimus dorsi muscle in pregnant adolescent ewes.

	Treatment [a]					
Item	CON	SW	S3	S15	SE	*P* value
Liver Mass g	118.50	111.50	118.00	113.20	12.10	0.964
Liver Se, µg/g	0.56[c]	2.90[d]	0.89[c]	3.50[d]	0.50	0.001
Total Se [b], mg	0.07[c]	0.34[d]	0.10[c]	0.36[d]	0.50	0.001
Kidney Mass g	23.20	22.10	21.20	19.40	1.50	0.263
Kidney Se, µg/g	0.81[c]	1.34[d]	0.91[c]	1.48[d]	0.11	0.001
Total Se, mg	0.02[c]	0.03[d]	0.02[c]	0.03[d]	0.04	0.001
Muscle						
Muscle Se, µg/g	0.19[c]	0.82[d]	0.25[c]	0.59[e]	0.05	0.001

[a] Control (CON), selenium wheat (SW; 3 ppm), selenate (S3; 3ppm), and (S15; 15 ppm).
[b] Total Se = tissue (g) X tissue, Se µg/g
[c,d,e] Means within a row with different superscripts differ ($P < 0.05$)

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EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SE ON GROWTH AND ESTIMATES OF CELLULARITY IN JEJUNAL, PLACENTAL, AND MAMMARY TISSUES INN PREGNANT EWE LAMBS [1]

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ABSTRACT: Pregnant Targhee-cross ewe lambs (n = 36; 53.8 ± 1.3 kg) were randomly allotted to one of four treatments in a 2 x 2 factorial arrangement to examine effects of nutrient restriction and dietary Se on mass, RNA, DNA, and protein concentrations in maternal jejunal, mammary, and placental tissues. Treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 µg/kg BW [NSe]; no added Se vs. 81.5 µg/kg BW [HSe]; Se enriched yeast). Selenium treatments were initiated 21 d before breeding and restriction treatments on d 64 of gestation. All diets were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. On day 135 of gestation ewes were slaughtered and tissues harvested. Total jejunal, jejunal mucosal, and mammary mass (g) were lower ($P < 0.02$) for R fed ewes compared with M. There were no treatment effects on DNA (estimate of hyperplasia) or RNA concentrations in the jejunum, mammary, and caruncle tissues. Nutrient restriction reduced ($P < 0.02$) total DNA in the jejunal mucosa; 1.97 vs. 1.48 ± 0.13 (g) for M vs. R, respectively. There was a nutrition x Se interaction ($P < 0.03$) in the RNA:DNA ratio in the jejunum; interactive means were 0.70, 0.85, 0.86, and 0.79, for M-NSe, M-HSe, R-NSe, and R-HSe, respectively. Nutrient restriction also reduced ($P < 0.09$) total protein in all tissues analyzed, excluding the caruncle. In the mammary tissue, nutrient restriction reduced ($P < 0.02$) total protein; 52.7 vs. 37.0 ± 4.1 (g) for M vs. R, respectively. Selenium increased ($P < 0.06$) total (g) DNA and RNA, while reducing protein:DNA ratios (estimates of hypertrophy) in the cotyledon. These data indicate that maternal nutrient restriction reduces mass and alters some estimates of cellularity in jejunal, mammary, and placental tissues in pregnant ewe lambs. Within the cotyledon high maternal dietary Se increased estimates of hyperplasia and decreased hypertrophy. Further research is needed to assess impacts of maternal nutrition on growth and production of offspring.

Key Words: Nutrient Restriction, Pregnancy, Selenium

Introduction

Selenium is an essential trace nutrient for normal growth and development in both livestock and humans (McDowell, 2003; Sunde, 1997). Both Se deficiency and excess has resulted in economic liabilities for livestock producers (McDowell, 2003: Underwood and Suttle, 2001). Recent work (Clark et al., 1996; Combs and Lu, 2001) indicates that supranutritional levels of Se from yeast (2- to 4-fold above normal requirements) can reduce the combined incidence of lung, colorectal, and prostate cancers in humans by as much as 50%. Additionally, research using rodent cancer models has demonstrated that the positive response to supranutritional (2 to 3 ppm) levels of Se may be dependent upon the molecular form of Se (Finley et al., 2000; Wagner et al., 2000; Finley and Davis, 2001). Unfortunately, little research has evaluated effects of supranutritonal levels of Se on growth and cellulartiy of normal rapidly proliferating tissues.

Soto-Navarro et al., (2004) evaluated effects of supranutritional Se (3 ppm) from high-Se wheat on intestinal mass, crypt cell proliferation, and vascular density in beef steers fed finishing diets. They found that the percentage of jejunal cellular proliferation was unaffected by high Se; however, jejunal mass was increased in steers fed high Se treatments. Consequently, when cellular proliferation estimates were coupled with jejunal mass, the total number of jejunal proliferating cells was almost double in steers fed high-Se wheat. We have also shown that nutrient restriction during pregnancy reduces maternal jejunal and total small intestinal mass by 17 and 20% respectively (Scheaffer et al., 2004a). In addition, Ward et al., (2004) recently reported that high dietary Se reduced placentome weight and number in gestating ewe lambs. Little data exist in the literature evaluating the combined effects of nutrient restriction and high selenium on growth and cellularity of key nutrient transferring tissue. Therefore our objectives were to investigate the influence of nutrient restriction and supranutritional Se on estimates of hypertrophy and hyperplasia in intestinal, mammary, and placentome tissues in pregnant ewe lambs.

Materials and Methods

Thirty-six pregnant Targhee-cross ewe lambs (53.8 ± 1.3 kg) were randomly assigned to individual pens (.91 x 1.2 m), and allotted to one of four treatments in a 2 x

[1]This project was partially supported by National Research Initiative Competitive Grant no. 2005-35206-15281 from the USDA Cooperative State Research, Education, and Extension Service and by NIH Grant HL 64141, and partially by USDA-IFAFS Grant No. 00-52102-9636.

2 factorial arrangement. Care and management of animals were approved by the North Dakota State University Institutional Animal Care and Use Committee. Dietary treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 µg/kg BW [NSe]; no added Se vs. 81.5 µg/kg BW [HSe]; Se enriched yeast; Alltech Inc., Nicolasville, KY). All diets consisting of alfalfa hay and the respective supplements were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. Diets (Table 1) were fed once daily, with free access to water and salt. Supplements provided to HSe ewes contained Se enriched yeast to meet targeted Se intakes, while NSe ewes were fed supplements without added Se.

Dietary Se supplementation was initiated 21 d prior to breeding, and continued to slaughter (d 135 gestation). Nutrient restriction ewes were offered 60% of diet supplied to maintenance fed ewes. Dietary restriction treatments were initiated on d 64 of gestation. Maintenance ewes were fed to perform within NRC (1985) guidelines.

Table 1. Chemical composition of alfalfa hay and supplements (DM basis) fed to ewes at requirement or restricted (60% of control ewes).

		Supplement[a]	
Item, %	Alfalfa Hay[b]	Control	Selenium
DM	87.4	85.6	86.1
Ash	9.9	1.95	2.3
CP	16.13	10.0	12.7
ADF	27.3	5.5	5.0
NDF	37.2	26.2	26.5
Ca	.01	0.06	0.03
P	.005	0.35	0.42
Se, ppm	-	0.32	43.2

[a] Supplement contained 0.3 ppm and 43.2 ppm Se for Control and Selenium, respectively, and was fed to provide 7.4 µg/kg BW or 81.5 µg/kg BW daily for control and high-Se fed ewes.
[b] Hay was chopped, averaging 3.8 cm in length.

One hour prior to slaughter, ewes were injected with 5-bromo-2-deoxy-uridine (BrdU; 5 mg/kg BW), which is a thymidine analog that is incorporated into cellular DNA during the S-phase of the cell cycle (Jablonka-Shariff et al., 1993; Jin et al., 1994). Ewes were stunned via captive bold and exsanguinated Immediately following evisceration, visceral organs were obtained. Intestinal segments were located, and demarcations made (Schaeffer et al., 2004b).

Jejunal, jejunal mucosal, mammary, caruncular, and colyledonary tissues were collected and analyzed for protein, DNA, and RNA analysis as previously described (Scheaffer et al., 2004a,b). Jejunal samples consisted of a 2-cm wide cross section of tissue and a 10 to 15 cm portion of the jejunum was used to obtain the mucosal scrape sample. Samples were frozen in isopentane and stored at -80˚C until analyzed for DNA, RNA, and protein concentrations (Reynolds et al., 1990; Reynolds and Redmer, 1992). Tissue homogenates were analyzed for concentrations of DNA and RNA by 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 Chemical) as the standard (Johnson et al., 1997). The 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). Intestinal section DNA, RNA, and protein contents are calculated by multiplying DNA, RNA, and protein concentration by fresh tissue weight (Swanson et al., 2000; Scheaffer et al., 2003; Schaeffer et al 2004b).

Data were analyzed as a 2 x 2 factorial using PROC GLM procedures of SAS (SAS inst. Inc., Cary, NC). Because ewe lambs carried both singles and twins, fetal number was used as a covariate. Model contained effects nutrition, (M vs. R), level of Se (NSe vs. HSe), and nutrition x Se interaction. When interactions were present ($P < 0.10$), means were separated by least significant difference.

Results and Discussion

In the jejunum, there were no treatment effects on DNA or RNA concentrations; however, within jejunal mucoal tissue nutrient restriction resulted in lower ($P < 0.02$) DNA content (g); (Table 2). These data indicate that there were fewer mucosal cells present in restricted compared with M ewe lambs. Others (Scheaffer et al., 2004b) have also reported reduced DNA in jejunal tissues of nutrient restricted ewes. Jejunal tissues (total and mucosal) had lower ($P < 0.03$) total protein in R vs. M ewes. There was a Se x Nutrition interaction ($P < 0.03$) with the RNA:DNA ratio (Table 2). These data indicate that effects of nutrient restriction on jejunal hypertropy may be partially dependent on dietary Se concentrations. However, HSe had no effect on jejunal DNA or measures of hypertrophy within the mucosa. Differences between estimates of jejunal and jejunal mucosal cellularity are likely explained by a difference in activity of non-mucosal support tissues, compared with mucosal tissue. .

Dietary nutrient restriction and supranutritional levels of Se had no effect on estimates of cellularity in mammary or caruncular tissues (Table 2). The one exception was mammary tissue protein content was decreased ($P < 0.01$) by nutrient restriction.

Nutrient restriction had no effects on DNA or RNA in cotyledonary tissues (Table 2). However nutrient restriction decreased ($P < 0.09$) protein concentration (mg/g), contents (g) and protein:DNA ratio in the cotyledon, indicating that cell size was likely reduced. When Se was provided at 81.5 µg/kg BW in the diet, DNA and RNA content (g) was increased ($P < 0.06$) in the cotyledon. In the cotyledon, HSe also reduced ($P < 0.07$) the protein:DNA ratio. These data indicate that within the

cotyledon HSe increased cell number and reduced cell size.

Implications

These data imply that key nutrient transferring tissues (intestine and placenta) are responsive to nutrient restriction and Se level. Observed changes may be related to changes in cellular proliferation and/or vascularity. Further investigation is needed to determine the maternal, fetal, and postnatal responses .

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Table 2. Influence of nutrient restriction and high Se on estimates of cellularity in maternal jejunal tissues in pregnant ewe lambs.

Item	Nutrition [a]		Selenium [b]		SE	*P* Value [c]		
	M	R	NSe	HSe		Nut	Se	Se X Nut
Jejunum								
DNA (mg/g)	5.89	6.34	6.13	6.10	0.34	0.352	0.950	0.361
DNA g	2.06	1.85	1.91	2.00	0.13	0.262	0.610	0.941
RNA (mg/g)	4.49	5.18	4.75	4.91	0.29	0.106	0.696	0.486
RNA g	1.57	1.51	1.47	1.61	0.11	0.675	0.371	0.217
RNA:DNA [c]	0.77	0.83	0.78	0.82	0.03	0.262	0.429	0.021
Protein (mg/g)	51.58	38.64	42.82	47.40	3.85	0.021	0.403	0.199
Protein g	17.88	11.01	13.48	15.41	1.37	0.001	0.324	0.648
Protein:DNA	9.09	6.02	7.04	8.07	0.68	0.002	0.288	0.842
Jejunal mucosa								
DNA (mg/g)	8.50	8.00	8.02	8.48	0.61	0.565	0.604	0.618
DNA g	1.97	1.48	1.66	1.78	0.13	0.011	0.518	0.579
RNA (mg/g)	6.73	7.18	6.70	7.22	0.54	0.559	0.513	0.757
RNA g	1.59	1.31	1.38	1.52	0.12	0.114	0.442	0.236
RNA:DNA	0.81	0.93	0.91	0.84	0.06	0.139	0.420	0.226
Protein (mg/g)	45.63	45.04	45.02	45.65	4.52	0.925	0.922	0.619
Protein g	11.48	8.36	9.35	10.49	0.93	0.022	0.390	0.587
Protein:DNA	5.78	6.16	6.40	6.40	5.54	0.691	0.359	0.808
Mammary								
DNA (mg/g)	2.22	2.52	2.17	2.57	0.23	0.344	0.224	0.766
DNA g	1.32	1.14	1.22	1.24	0.14	0.402	0.921	0.833
RNA (mg/g)	4.01	4.16	4.27	3.89	0.52	0.839	0.612	0.284
RNA g	2.48	1.77	2.38	1.87	0.35	0.164	0.321	0.339
RNA:DNA	1.93	1.85	2.13	1.64	0.23	0.805	0.141	0.669
Protein (mg/g)	84.79	80.37	83.37	81.78	4.53	0.492	0.807	0.885
Protein g	52.71	37.02	47.00	42.73	4.11	0.010	0.473	0.801
Protein:DNA	42.78	37.23	42.12	37.87	3.94	0.328	0.456	0.682
Cotyledon								
DNA (mg/g)	3.61	3.41	3.11	3.91	0.31	0.650	0.076	0.383
DNA g	1.18	1.04	0.94	1.28	0.12	0.297	0.054	0.335
RNA (mg/g)	3.73	4.26	3.73	4.26	0.26	0.149	0.153	0.412
RNA g	1.21	1.28	1.09	1.41	0.11	0.666	0.051	0.270
RNA:DNA	1.18	1.29	1.31	1.16	0.10	0.427	0.288	0.781
Protein (mg/g)	50.96	42.88	48.70	45.13	3.20	0.078	0.438	0.732
Protein g	16.07	12.79	14.43	14.43	1.32	0.083	1.000	0.816
Protein:DNA	15.85	12.41	15.84	12.42	1.28	0.062	0.068	0.445
Caruncle								
DNA (mg/g)	1.62	1.89	1.76	1.74	0.21	0.363	0.941	0.543
DNA g	0.16	0.18	0.17	0.17	0.02	0.499	0.909	0.762
RNA (mg/g)	2.91	3.54	2.95	3.49	0.37	0.223	0.296	0.884
RNA g	0.30	0.34	0.29	0.35	0.04	0.489	0.307	0.966
RNA:DNA	2.80	2.39	2.79	2.40	0.66	0.647	0.673	0.974
Protein (mg/g)	41.62	38.44	36.82	43.24	5.06	0.653	0.377	0.333
Protein g	4.00	3.52	3.42	4.09	0.54	0.517	0.388	0.373
Protein:DNA	35.17	24.30	30.49	28.98	7.06	0.272	0.880	0.857

[a] M=non-restricted ewes fed at requirements, R =ewes fed to 60% of M

[b] 7.4 µg/kg BW (NSe); no added Se vs. 81.5 µg/kg BW (HSe)

[c] Interactive means for RNA:DNA were 0.70, 0.85, 0.86, and 0.79 for MN-NSe, MN-HSe, MR-NSe, and MR-HSe, respectively

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EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON MATERNAL AND FETAL METABOLIC HORMONES IN PREGNANT EWE LAMBS[1]

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ABSTRACT:[1] Pregnant Targhee-cross ewe lambs (n = 36; 53.8 ± 1.3 kg) were randomly allotted to one of four treatments in a 2 x 2 factorial arrangement to examine effects of nutrient restriction and dietary Se on maternal and fetal serum IGF-1, T_3, and T_4 concentrations. Treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 µg/kg BW [NSe]; no added Se vs. 81.5 µg/kg BW [HSe]; Se enriched yeast). Selenium treatments were initiated 21 d before breeding and restriction treatments on d 64 of gestation. All diets were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. Serum was collected via vena puncture on days 62, 76, 90, 104, 118, 132, and 135 of gestation. Fetal serum was collected at slaughter, on d 135. Nutrition x Se interactions were not significant for ewe or fetal hormones. Nutrient restriction reduced ($P < 0.03$) maternal serum IGF-1, T_3, and T_4 concentrations (ng/mL) on d 118, 132, and 135 of gestation. Maternal serum T_3 and T_4 were also reduced ($P < 0.07$) at d 90 and tended ($P < 0.13$) to be lower at d 104. Nutrient restriction tended ($P = 0.12$) to reduce maternal IGF-1 on d 104 of gestation. On d 135, IGF-1 concentrations were 142.8 vs. 77.4 ± 12.5 ng/mL, T_3 concentrations were 0.78 vs. 0.58 ± 0.04 ng/mL, and T_4 concentrations were 44.5 vs. 38.1 ± 2.0 ng/mL for M vs. R fed ewes, respectively. Maternal serum levels of T_4 were lower ($P < 0.03$) on d 132 and 135 (d 135 = 37.6 vs. 45.0 ± 1.9 ng/mL for HSe and NSe, respectively). Fetal IGF-1 concentrations were lower ($P < 0.01$) in R compared with M fed ewes (61.9 vs. 77.4 ± 4.1 ng/mL). There was no effect of dietary Se on fetal IGF-1, T_3, or T_4. These data indicate that maternal nutrition impacts both maternal and fetal IGF-1, T_3, and T_4 serum concentrations.

Key Words: Nutrient Restriction, Pregnancy, Selenium

Introduction

Selenium is an essential trace element that has diverse biological function (Sunde, 1997; McDowell, 2003). In addition, research has indicated that supranutritional levels of Se can reduce cancer risk in humans (Clark et al., 1996), and rodent models (Finely and Davis, 2001; Finely et al., 2000; and Jaing et al., 1999). Selenium plays a role in immunity, reproduction, and thioredoxin reductase and glutathione peroxidase function (Thompson et al., 1982). Recent work in beef cattle indicates that 3 ppm of dietary Se from high-Se wheat results in a reduction in jejunal vascularity and increased jejunal mass (Soto-Navarro et al., 2004). Nutrient restriction can reduce intestinal growth (Schaeffer et al., 2004), oxygen consumption (Schaeffer et al., 2003), and whole animal maintenance requirements (Burrin, et al., 1990).

Selenium is needed for proper thyroid function (Hotz et al., 1997). The selenoprotein Type I deiodinase is a key component in the conversion of thyroxine (T_4) into triiodothyronine (T_3), the more active form of the thyroid hormone (Beckett et al., 1987; Awadeh et al., (1997) reported that increasing levels of Se in the diet result in higher levels of serum T_3. In addition, nutrient restriction has been shown to reduce circulating levels of T_3 and T_4 in geldings (Powell et al., 2000). Recent data from our laboratory suggests there is no effect of additional dietary Se on serum T_3, or T_4 concentrations in pregnant ewes or their fetuses, when dietary nutrient supply is adequate (Ward et al., 2004).

Serum IGF-1 levels were found to be lower in children from Se deficient versus Se adequate regions (Aydin et al., 2002). In addition, elevated levels of selenite provided to rats resulted in lower serum IGF-1 versus control (Gronbaek et al., 1995). Ward et al. (2004) reported that serum IGF-1 increased in fetuses from ewes fed 3 ppm dietary Se compared with controls. In the same study, ewes fed Se at 15 ppm had lower serum IGF-1 compared with all other treatments. To our knowledge, combined effects of nutrient restriction and supranutritonal levels of Se on serum IGF-1, T_3, and T_4 have not been previously reported. In addition, combined effects of these treatments on fetal IGF-1, T_3, and T_4 are not understood and could have impacts on prenatal, postnatal, and adult productivity. Therefore, we hypothesized that nutrient restriction and supranutritional Se should alter both maternal and fetal serum concentrations of IGF-1, T_3, and T_4, in pregnant ewes lambs.

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Materials and Methods

Thirty-six pregnant Targhee-cross ewe lambs (53.8 ± 1.3 kg) were randomly assigned to individual pens (.91 x 1.2 m), and allotted to one of four treatments in a 2 x 2 factorial arrangement. Care and management of animals were approved by the North Dakota State University Institutional Animal Care and Use Committee. Dietary treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 µg/kg BW [NSe]; no added Se vs. 81.5 µg/kg BW [HSe]; Se enriched yeast; Alltech Inc., Nicolasville, KY). All diets, consisting of alfalfa hay and the respective supplements, were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. Diets (Table 1) were fed once daily, with ewes having free access to water and salt. All ewes received a corn-based supplement (Table 1). Supplements provided to HSe ewes contained Se-enriched yeast to meet targeted Se intakes, while NSe ewes were fed supplements without added Se. Dietary Se supplementation was initiated 21 d prior to breeding, and continued to slaughter (d 135 gestation). Nutrient restricted ewes received 60% of the diet supplied to maintenance fed ewes. Dietary restriction treatments were initiated on d 64 of gestation. Maintenance ewes were fed to perform within NRC (1985) guidelines.

Table 1. Chemical composition of alfalfa hay and supplements (DM basis) fed to ewes at requirement or restricted (60% of control ewes).

		Supplement[a]	
Item, %	Alfalfa Hay[b]	Control	Selenium
DM	87.4	85.6	86.1
Ash	9.9	1.95	2.3
CP	16.13	10.0	12.7
ADF	27.3	5.5	5.0
NDF	37.2	26.2	26.5
Ca	.01	0.06	0.03
P	.005	0.35	0.42
Se, ppm	-	0.32	43.2

[a] Supplement contained 0.3 ppm and 43.2 ppm Se for Control and Selenium, respectively, and was fed to provide 7.4 µg/kg BW or 81.5 µg/kg BW daily for control and high-Se fed ewes.
[b] Hay was chopped, averaging 3.8 cm in length.

Ten mL of serum was collected with Corvac serum separator Vacutainer tubes (Tyco Healthcare, Mansfield, MA) via venepuncture on days 62, 76, 90, 104, 118, 132, and 135 of gestation. Ten mL of fetal serum was collected on d 135 (slaughter) via cardiac puncture. All blood samples were centrifuged at 1500 X g for 28 minutes. The supernant was pipetted into 2 mL screw cap vials and stored at –20 °C. Serum IGF-1 was analyzed in a single assay according to procedures of Berrie et al. (1995). Thyroxin samples were quantified utilizing components of DPC kits (Diagnostic Products Corp., Los Angeles, CA) with modifications in procedures described by Richards et al. (1999).

Data were analyzed within day of gestation as a 2 x 2 factorial using PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Because ewe lambs carried both singles and twins, fetal number was used as a covariate. Model contained effects nutrition, (M vs. R), level of Se (NSe vs. HSe), and nutrition x Se interaction. When interactions were present ($P < 0.10$), means were separated by least significant difference.

Results and Discussion

Ewe and fetal BW were reduced ($P < 0.01$) in R compared with M fed ewes (Table 2). High Se supplementation had no effect on ewe BW. Fetal BW, however, was higher ($P < 0.08$) in fetuses from HSe ewe lambs. Ewe average daily gain during gestation was lower ($P < 0.01$) in R ewes compared with M ewes (139 vs. 0.7 g/ewe daily, respectively). These results indicate that at d 132 of gestation, control fed ewes lambs (M) were performing within NRC (1985) guidelines, while those fed at restriction were not. Thus, growth responses observed in these ewes followed our experimental objectives.

Nutrient restriction reduced ($P < 0.03$) maternal serum IGF-1, T_3, and T_4 concentrations at d 118, 132, and 135 of gestation (Tables 3, 4, and 5). Nutrient restriction also tended ($P = 0.12$) to reduce maternal IGF-1 on d 104 of gestation. Maternal serum T_3 (Table 4) and T_4 (Table 5) were also reduced ($P < 0.07$) at d 90 and tended ($P < 0.13$) to be lower at d 104, in R compared with M ewes.

Selenium addition had no effect on IGF-1 or T_3 status in the ewes throughout gestation. Maternal serum concentrations of T_4 were lower ($P < 0.03$) on d 132 and 135 in HSe versus NSe ewes (Table 5). Ward et al. (2004) found a numeric reduction of serum T_4 concentration by d 130 of gestation in ewes fed either 3 ppm or 15 ppm Se, compared with controls. This apparent response in late gestation of T_4 to Se addition to the diet has not been well characterized in the literature and may merit further investigation. Nutrient restriction has been shown to reduce circulating IGF-1, T_3, and T_4 concentrations in many models (Hayden et al., 1993; Powel et al., 2002; Bispham et al., 2003). Data from this study support these findings; however, Bisham et al. (2003) reported increases in T_4 as gestation progressed in both nutrient restricted and non-restricted ewes, whereas we observed a steady decline in serum T_4 with advancing gestation in all treatments. The potential cause of these varied responses to T_4 during pregnancy has yet to be established.

Fetal IGF-1 concentrations were lower ($P < 0.01$) in R compared with M fed ewes (Table 6). Insulin like growth factor-1 plays a key role in normal bone development (Yakar et al., 1999). When IGF-1 gene expression was blocked, there was a 10% decrease in bone mineral density and over a 35% decrease in periosteal circumference and cortical thickness of the tibia bone in growing mice (Yakar et al., 2002). Reduction in fetal IGF-1 due to maternal under-nutrition could impact the growth potential of offspring. In our study, maternal nutrient restriction did not alter fetal

serum T_3 or T_4 concentrations. There was no effect of dietary Se on fetal serum IGF-1, T_3, or T_4 concentrations.

When nutrition is restricted to 60% of maintenance in pregnant adolescent ewes, maternal serum IGF-1, T_3, and T_4 concentrations are reduced during the last trimester of pregnancy. Circulating IGF-1 was reduced in the fetus, when level of nutrition was restricted in the ewe. There is no effect of supranutritional dietary Se on maternal or fetal serum IGF-1 and T_3 concentrations, whereas high dietary Se reduced maternal T_4 in late gestation. Selenium, however, is required for the conversion of T_4 to T_3 via type 1 deidodinase, a selenoprotein (Hotz et al., 1997; Beckett, and Arthur, 2005).

Implications

By d 118 of gestation, nutrient restriction in pregnant ewe lambs resulted in lower circulating IGF-1, T_3, and T_4 in the ewe. Nutrient restriction, however, had no impact on T_3 and T_4 concentrations in the fetus. Insulin like growth factor-1 concentrations was lower in fetal serum from ewes experiencing undernutrition. Reductions in IGF-1 may partially explain some effects (lower birth weights, smaller bone diameter) reported in offspring from nutrient restricted dams. Selenium did not affect circulating IGF-1 or T_3, in the pregnant ewe or fetus. Serum T_4 concentrations were lower during the last 27 d of gestation in ewes fed supranutritional levels of organically bound Se compared with controls. Further investigation is needed to better understand the full impact of restricted nutrition and high dietary Se during pregnancy on prenatal, neonatal, and postnatal responses.

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Table 2. Body weight (kg) of pregnant ewe lambs fed different levels of nutrition and organically bound Se.

Gestation, d	Nutrition [a]		Selenium [b]			P Value[c]		
	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
62	54.3	54.0	53.9	54.5	0.8	0.850	0.615	0.751
76	55.6	52.8	53.9	54.6	0.8	0.015	0.311	0.758
90	56.6	52.6	54.3	54.9	0.8	0.001	0.595	0.599
104	58.7	52.8	55.4	56.1	0.8	0.001	0.590	0.804
118	61.6	53.7	57.1	58.2	0.8	0.001	0.393	0.707
132	63.8	54.1	58.1	59.8	0.9	0.001	0.167	0.518
135	66.5	56.1	60.5	62.1	1.0	0.001	0.253	0.738
Fetal BW	4.0	3.6	3.6	4.0	0.2	0.055	0.077	0.575

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.

Table 3. Serum IGF-1 (ng/mL) concentrations in pregnant ewe lambs fed different levels of nutrition and organically bound Se.

Gestation, d	Nutrition [a]		Selenium [b]			P Value[c]		
	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
62	141.98	143.25	145.83	139.40	9.74	0.925	0.645	0.291
76	169.38	154.24	164.62	159.00	9.80	0.272	0.689	0.6.00
90	159.34	144.50	145.48	158.36	10.37	0.317	0.395	0.959
104	194.69	162.09	180.50	176.27	15.15	0.122	0.842	0.495
118	211.54	134.40	168.81	177.33	17.13	0.003	0.722	0.936
132	194.63	99.05	128.87	164.82	21.12	0.002	0.221	0.646
135	142.79	77.41	109.44	110.76	12.52	0.001	0.940	0.869

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe. (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.

Table 4. Serum T_3 (ng/mL) concentrations in pregnant ewe lambs fed different levels of nutrition and organically bound Se.

Gestation, d	Nutrition [a]		Selenium [b]			P Value[c]		
	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
62	1.17	1.11	1.14	1.14	0.06	0.491	0.975	0.623
76	0.95	0.90	0.84	1.01	0.07	0.642	0.118	0.291
90	0.82	0.69	0.72	0.79	0.03	0.007	0.176	0.724
104	0.73	0.65	0.68	0.70	0.04	0.116	0.586	0.518
118	0.76	0.52	0.66	0.62	0.04	0.001	0.429	0.281
132	0.66	0.50	0.61	0.55	0.03	0.001	0.189	0.832
135	0.74	0.58	0.70	0.62	0.04	0.006	0.160	0.909

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe. (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.

Table 5. Serum T_4 (ng/mL) concentrations in pregnant ewe lambs fed different levels of nutrition and organically bound Se.

Gestation, d	Nutrition [a]		Selenium [b]			P Value[c]		
	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
62	64.91	64.41	63.19	66.14	2.69	0.894	0.445	0.621
76	57.64	53.40	54.52	56.52	2.42	0.213	0.566	0.502
90	55.05	48.32	52.03	51.33	2.48	0.063	0.845	0.405
104	47.51	42.75	46.19	44.06	2.23	0.126	0.497	0.347
118	43.54	34.22	40.60	37.08	2.11	0.003	0.249	0.289
132	41.17	33.29	40.88	33.58	2.26	0.014	0.025	0.272
135	44.48	38.15	45.04	37.59	1.96	0.024	0.011	0.218

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.

Table 6. Metabolic serum hormone levels (ng/mL) of fetuses from ewe lambs fed different levels of nutrition and organically bound selenium

Hormone, ng/mL	Nutrition [a]		Selenium [b]			P Value[c]		
	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
IGF-1	77.39	61.92	65.97	73.31	4.09	0.002	0.211	0.226
T_3	0.34	0.32	0.33	0.33	0.01	0.576	0.981	0.557
T_4	104.46	103.46	103.98	103.94	4.09	0.856	0.994	0.903

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; SeN (7.4 µg/kg BW) and SeH. (81.5 µg/kg BW), respectively.

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PHYTIC ACID LEVELS IN BARLEY FOR BEEF CATTLE

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ABSTRACT: Thirty-two heifers (initial BW 421 kg) were individually fed finishing diets (83% barley) based on one of four barley genotypes for 66 days to evaluate the performance and nutritional value of low phytic acid (PAP) barley in beef cattle. The four barleys contained 2.9 (2.9PAP), 1.5 (1.5PAP), 0.9 (0.9PAP), or 0.2 (0.2PAP) mg/g PAP, which represented 0, 40, 70, or 95% reductions in PAP from "normal" levels, respectively. Barley was cracked prior to being fed and diets were formulated to be isocaloric (2.01 Mcal/kg NEm and 1.35 Mcal/kg NEg) and isonitrogenous (2.9% N) with similar Ca:P (2.5). Heifers were weighed on d 0, 23, 39, and 66. Feed, ort, and fecal samples were collected for 7 d beginning on d 39 and analyzed for DM, N, ADF, starch, AIA, P, and PAP in order to estimate total tract digestibility. AIA was used as an internal marker to estimate fecal output. Liver biopsies were taken on d 23 and d 66 for mineral analysis. ADG by heifers did not differ (P = 0.33) due to barley genotype (average 0.9 kg/d). Dry matter intake was similar (P = 0.81) between barley treatments; however, total P intake was greatest ($P < 0.10$) by heifers fed 1.5PAP, followed by 2.9PAP, 0.2PAP, and lowest for those fed 0.9PAP. There were no differences ($P > 0.10$) in DM, N, ADF, starch, or total P digestibility between barley treatments. Digestibility of PAP was greater ($P < 0.10$) in heifers consuming 0.9PAP and 0.2PAP than in heifers consuming 2.9PAP and 1.5PAP. Fecal PAP output was lower ($P < 0.10$) from heifers consuming 0.9PAP and 0.2PAP than from heifers consuming 2.9PAP and 1.5PAP; however, there was no difference ($P > 0.10$) in fecal total P output between barley treatments. Liver copper concentration was lower ($P < 0.10$) in heifers consuming 2.9PAP and 1.5PAP than in heifers consuming 0.9PAP and 0.2PAP. Low PAP barley mutants had similar feeding value to normal barley in beef cattle.

Key Words: Phytic Acid, Barley, Ruminants, Digestibility, Phosphorus

Introduction

The primary storage form of phosphorus (**P**) in most grains is phytic acid-phosphorus (**PAP**), and phytic acid is known to decrease the bioavailability of certain minerals in non-ruminant animal diets (Pallauf and Rimbach, 1997). In addition, excretion of unutilized PAP has led to pollution problems in areas of intensive livestock production (Jongbloed and Lenis, 1998). The enzyme phytase added to non-ruminant diets has increased the availability of PAP, thereby reducing the need to add inorganic P and decreasing environmental pollution from P in animal wastes (Bedford, 2000). Low phytic acid cultivars of corn, rice, soybeans, wheat, and barley (Larson et al., 1998) have also been developed and may have the potential to improve phosphorus efficiency in animal diets. Increased availability of phosphorus from low phytic acid barley has been reported in poultry (Li et al., 2001), swine (Veum et al., 2002), and fish (Sugiura et al., 1999) and consequently, has decreased fecal phosphorus output by 55% in swine (Veum et al., 2002) and 43% in fish (Suguira et al., 1999).

Previous researchers found little or no PAP in the feces of ruminant animals consuming concentrate diets (Nelson et al., 1976; Morse et al., 1992) and concluded that ruminal microbes were able to completely hydrolyze phytic acid. In addition, there are no known negative effects of phytic acid on mineral absorption in ruminants (Van Soest, 1994). However, newer more sensitive assays are now available which may allow for more accurate evaluations of PAP hydrolysis. A decreased need for phosphorus supplementation would reduce feed costs, and decreased phosphorus excretion in ruminants could be an important management tool for feedlot operations. The use of low phytic acid grains has not been previously evaluated in ruminant finishing diets. The objective of this study was to evaluate the effects of low phytic acid-P barley genotypes in finishing diets on performance, digestion, and mineral metabolism in ruminants.

Materials and Methods

Thirty-two heifers (average weight 421 kg) were assigned by weight to 8 pens and fed finishing rations based on one of the four barley genotypes: Harrington (2.9 mg/g PAP; **2.9PAP**), 422 (1.5 mg/g PAP; **1.5PAP**), 635 (0.9 mg/g PAP; **0.9PAP**) and 955 (0.2 mg/g PAP; **0.2PAP**). The low-phytic acid barley genotypes used in this trial were grown by the National Small Grains Germplasm Research Facility, USDA-ARS, Aberdeen, ID and contained similar levels of total P. 'Harrington' (2.9PAP) was considered to be a "normal" or "wild-type" barley high in phytic acid, while 422 was a near iso-genic progeny of the cultivar 'Harrington' and homozygous for the low-phytic acid *lpa-1* allele which causes a 50% reduction in PAP (Larson et al.,

1998; Raboy et al., 2001). The genotypes 635 and 955 are *lpa1*-like mutants that cause 70 and 95% reduction in kernel PAP, respectively (Raboy et al., 2001). Chemical composition of the barley cultivars is presented in Table 1. Heifers were individually fed using Calan-Broadbent gates (American Calan, Inc., Northwood, NH) and each pen contained each of the four treatments (randomized complete block, blocked by pen). Barley was cracked prior to being fed and diets were formulated to be isocaloric (2.01 Mcal/kg NEm and 1.35 Mcal/kg NEg) and isonitrogenous (2.9% N) with similar Ca:P (2.5). Diets were formulated to contain 83% barley, 6% straw, 3% soybean oil, and 8% vitamin/mineral supplement. Heifers were fed the same basal ration from d 0 to 22 in order to adjust to the Calan gates and offered their assigned treatment rations beginning on d 23. Heifers had free-choice access to water and were fed once daily at 0900. Heifers were weighed on d 0, 23, 39, and 66. Animals were cared for under protocols approved by the Montana State University Animal Care and Use Committee.

The 7-d data collection period began on d 39. Feed samples were collected on a daily basis and orts were weighed and sampled in the morning prior to feeding. Feed and ort samples were ground through a Wiley mill (1-mm screen) and analyzed for DM (AOAC, Method 934.01, 1999) and N (Leco Corporation, St. Joseph, MI), total P (Chen et al., 1956; Raboy et al., 2000), PAP by high performance liquid chromatography (Raboy et al., 2000), NDF and ADF (Van Soest et al., 1991), starch (Megazyme, Sidney, Australia), and AIA (4N method; Van Keulen and Young, 1977). Fecal grab samples were also taken at 0800, 1200, and 1500 on three different days. Fecal samples were dried for 48 h in a 60° C forced-air oven, and ground to pass a 1-mm screen in a Wiley mill. Fecal samples were composited for each heifer on an equal dry weight basis and analyzed for DM, N, ADF, starch, AIA, P, and PAP. Acid insoluble ash was used as an internal marker to estimate fecal output so that total tract apparent digestibility of DM, N, ADF, starch, P, and PAP could be calculated. Liver biopsies were taken on d 23 and d 66 (Corah and Arthington, 1994) and analyzed for Ba, Cd, Ca, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, K, Na, S, V, and Zn using inductively coupled plasma atomic emission spectroscopy (Animal Health Diagnostic Laboratory, Michigan State University, East Lansing).

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with diet and pen in the model and animal as the experimental unit. Initial weight was the weight on d 23 when animals went on the treatments diets. Overall ADG was analyzed using the ADG from d 0 to d 22 as a covariate. Sample date (d 23 and 66) was also included in the model for the mineral data. Least square means were separated using the Least Significant Difference method when $P < 0.10$.

Results & Discussion

Average daily gain by heifers did not differ ($P = 0.33$) between barley-based finishing diets, and averaged 0.9 kg/d (Table 2). The duration of the finishing trial was short (43 d), due to limited availability of the experimental low PAP barley genotypes, which could explain the lack of difference in animal performance between treatments in this trial. A phosphorus deficiency results in decreased appetite and growth deficiency (Church and Pond, 1988), therefore, P tied up in phytic acid has the potential to decrease animal performance. Phosphorus requirements for the heifers in our trial were 15-20 g/d (NRC, 1984). Since these heifers consumed >40 g/d of P, with 26 to 63% P from PAP, it is unlikely that P was a limiting nutrient. Pigs fed semi-purified diets containing low phytate barley had greater weight gains than pigs fed semi-purified diets with normal barley; however, pigs fed low phytate barley in practical diets (supplemented with P) had similar weight gains as those fed diets containing normal barley (Veum et al., 2002).

There was no difference in DM intake ($P = 0.81$) between finishing diets (Table 2). Nitrogen intake was lower ($P < 0.10$) for steers consuming 1.5PAP than all other barley genotypes. Acid detergent fiber intake was lowest ($P < 0.10$) for 0.9PAP, intermediate for 1.5PAP and highest for 0.2PAP, with 2.9PAP being similar to 1.5PAP and 0.2PAP. Starch intake was greater ($P < 0.10$) for 0.9PAP than all other genotypes. These differences may reflect differences in the nutrient content of the diets or sorting of the ration.

Total P intake was lowest ($P < 0.10$) by heifers consuming 0.9PAP, followed by 0.2PAP, 2.9PAP, and 1.5PAP. Dicalcium phosphate was added to treatment 1.5PAP in order to balance for Ca:P which may explain it's higher P intake. Phytic acid-P intake differed ($P = 0.001$) between all treatments and reflected the concentration of PAP in the barleys.

There was no difference ($P > 0.12$) in digestibility of DM, N, ADF, starch, or P between barley treatments (Table 2). Digestibility of PAP was greater ($P < 0.10$) in heifers consuming 0.9PAP and 0.2PAP than in heifers consuming 2.9PAP and 1.5PAP. This agrees with the data of Greiner et al. (1993), who reported inorganic P released from phytate actually inhibited activity of phytase. There could also be differences in inherent phytase activity between barley genotypes; however, Li et al. (2001) reported that endogenous phytase activity was similar between a normal barley and a low phytate mutant. This study is the first to report the feeding value and digestion coefficients of low phytic acid barley genotypes in ruminants. Our results are in agreement with Sugiura et al. (1999) who reported that apparent DM digestibility in fish was similar between normal and low phytic acid genotypes of barley. Energy and N digestibility were also similar in pigs fed low phytate and normal barley (Veum et al., 2002).

There was no difference ($P = 0.32$) in fecal total P output between barley treatments (Table 2). Fecal PAP

output was 86% lower ($P < 0.10$) from heifers consuming 0.9PAP and 0.2PAP than from heifers consuming 2.9PAP and 1.5PAP. However, this decrease in fecal PAP output did not result in reduced fecal output of total P. Our results differ from studies conducted in pigs (Veum et al., 2002) and rainbow trout (Sugiura et al., 1999) where low phytic acid barley decreased fecal total P excretion by 55 and 43%, respectively. Sugiura et al. (1999) noted that low phytic acid grains have the potential to reduce P waste provided that P levels in the diet are adjusted appropriately. Our experimental diets contained P in excess of requirements, which likely explains the lack of differences in total fecal P output between treatments.

Liver copper concentration was lower ($P < 0.10$) in heifers consuming 2.9PAP and 1.5PAP than in heifers consuming 0.9PAP and 0.2PAP (Table 2). Liver sodium concentration was also lower ($P < 0.10$) in heifers consuming 2.9PAP and 0.9PAP than in heifers consuming 0.2PAP, while heifers consuming 1.5PAP had an intermediate concentration. Liver concentrations of Ba, Cd, Ca, Cr, Fe, Mg, Mn, Mo, Ni, P, K, S, V, and Zn were not influenced ($P > 0.10$) by barley treatment (data not shown). Copper is stored in the liver (Church and Pond, 1988) and can be bound by phytic acid (Pallauf and Rimbach, 1997). It appears that level of phytic acid in barley influenced liver copper storage in heifers consuming high grain diets despite nearly complete hydrolysis of PAP. In the report of Sugiura et al. (1999) sodium concentration was lower in normal than low phytate mutant barley; therefore, differences in liver sodium concentrations between barley treatments could be due to levels supplied by the different barleys.

Implications

This is the first study to investigate the feeding value of low phytate grains and their effect on performance of cattle consuming finishing rations. The lack of negative effects on performance indicates that low phytic acid mutants have similar feeding value to normal barley and have the potential to decrease phosphorus excretion in feedlot cattle without compromising animal performance. This research has important implications for reducing P in excretion in feedlots, but further research needs to be conducted using rations that are balanced for available P.

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Table 1. Chemical composition of barley genotypes containing decreasing levels of phytic acid-phosphorus (PAP)

	Barley genotype[a]			
	2.9PAP	1.5PAP	0.9PAP	0.2PAP
Composition of grain				
DM, %	92.1	92.0	93.4	93.7
N, %	2.9	3.1	2.8	2.8
ADF, %	4.4	5.9	4.8	4.1
Starch, %	42.6	40.5	45.9	45.7
Total phosphorus, %	0.49	0.39	0.39	0.40
Phytic acid-P, %	0.29	0.15	0.09	0.02
Particle size, μm	1604	1593	1506	1347
3 h ISDMD, %	18.1	21.5	18.7	22.8

[a] 2.9PAP: Harrington barley (2.9 mg/g PAP); 1.5PAP: barley genotype 422 (1.5 mg/g PAP); 0.9PAP: barley genotype 635 (0.9 mg/g PAP); 0.2PAP: barley genotype 955 (0.2 mg/g PAP).

Table 2. Performance, intake, digestibility, fecal output, and liver mineral concentrations of nutrients by beef heifers fed finishing diets based on one of four barley genotypes containing decreasing levels of phytic acid-phosphorus (PAP)

	Barley genotype[a]					
	2.9PAP	1.5PAP	0.9PAP	0.2PAP	SEM	*P*-value
No. of heifers	8	8	8	8		
Initial weight, kg	465	455	471	464	8.8	0.70
43-d ADG, kg	1.0	0.9	0.9	0.7	0.10	0.33
Intake						
DM, kg	10.1	9.8	9.8	9.9	0.26	0.81
Nitrogen, g	278^{c}	254^{b}	278^{c}	276^{c}	6.9	0.07
ADF, kg	0.94^{cd}	0.90^{c}	0.84^{b}	0.97^{d}	0.024	0.007
Starch, kg	3.56^{b}	3.35^{b}	3.82^{c}	3.58^{b}	0.092	0.01
Phosphorus, g	52.0^{d}	76.9^{e}	40.3^{b}	45.7^{c}	1.49	0.001
Phytic acid-P, g	32.8^{e}	23.9^{d}	15.1^{c}	12.1^{b}	0.46	0.001
Apparent digestibility, %						
DM	70.3	71.1	73.7	69.8	2.88	0.77
N	74.6	75.5	80.3	74.8	2.46	0.33
ADF	23.5	28.1	19.9	25.3	7.02	0.86
Starch	94.0	96.6	96.2	92.9	1.21	0.12
Phosphorus	56.3	65.2	54.4	47.2	5.20	0.14
Phytic acid-P	93.8^{b}	91.0^{b}	98.4^{c}	97.8^{c}	2.02	0.06
Fecal output, g per day						
Phosphorus	22.7	27.5	18.7	24.1	3.27	0.32
Phytic acid-P	2.04^{c}	2.25^{c}	0.25^{b}	0.35^{b}	0.535	0.02
Liver concentrations, mg/kg						
Copper	269^{b}	269^{b}	323^{c}	301^{c}	12.0	0.009
Sodium	3,394^{b}	3,674^{bc}	3,380^{b}	3,876^{c}	152.2	0.08

[a] 2.9PAP: Harrington barley (2.9 mg/g PAP); 1.5PAP: barley genotype 422 (1.5 mg/g PAP); 0.9PAP: barley genotype 635 (0.9 mg/g PAP); 0.2PAP: barley genotype 955 (0.2 mg/g PAP).
[b,c,d,e] Within a row, means without a common superscript letter differ ($P < 0.10$).

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INFLUENCE OF CHROMIUM METHIONINE SUPPLEMENTATION ON GROWTH PERFORMANCE OF MEDIUM STRESSED BULL-CALVES DURING THE RECEIVING PERIOD IN THE FEEDLOT

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ABSTRACT: To determine the effect of chromium methionine supplementation on growth performance of medium stressed bull-calves during the receiving period in the feedlot. Sixty Brahman-crossbreed bull calves (206.9 ± 2.18 kg) were used in a 28 days performance experiment. in groups of five were randomly placed in 12 pens (6 x 12 m), The animals were randomly assigned to one of two treatments: 1) Feed a 36:64 forage concentrate starting diet containing 15.6 % CP and 1.6 Mcal of NEm kg $^{-1}$ (control); or 2) Diet similar to control, and supplementation with 2.8 mg of Cr head^{-1} day^{-1} from chromium methionine (Cr). Chromium methionine supplementation tended (P = 0.06) to increase 2.5 % ending weight (240.99 *vs*. 247.11 kg), and improved (P = 0.02) in 22.7 % the average daily gain (1.191 *vs.* 1.462 kg day $^{-1}$). Cr tended (P = 0.09) to increase dry matter intake (6.297 *vs.* 6.573 kg day $^{-1}$) and enhances in 17 % feed/gain ratio (5.410 *vs.* 4.487 kg kg $^{-1}$). Cr improved in 8 % (P = 0.05) retained NEm from the diet (1.722 *vs.*1.867 Mcal kg $^{-1}$), and increased (P = 0.05) in 11.6 % retained NEg from the diet (1.099 *vs*. 1.227 Mcal kg $^{-1}$). The daily mean intake of chromium in Cr-supplemented animals was close to 48 µg by kg of BW $^{0.75}$ day $^{-1}$, and was equivalent to feed a 0.426 ppm Cr-supplemented diet. These results, suggests that chromium supplementation from chromium methionine in amount close to 50 µg by kg of BW $^{0.75}$ day $^{-1}$ or 0.4 ppm of Cr in the diet, is adequate to improves growth performance of bull calves with starting weight near to 210 kg, during the 28 day receiving period in the feedlot.

Key Words: Chromium, Growth-performance, Bull-calves

Introduction

Feedlot cattle may be subjected to a considerable amount of stress and multiple stressors during the marketing process and upon arrival at the feedlot (Loerch and Fluharty, 1999). The stress reduces performance of cattle (Morrison, 1983). Chromium supplementation has shown be effective to diminish adverse effects of stress, reducing cortisol level and improving immunity (Chang and Mowat, 1992; Kegley and Spears, 1995), frequently these facts becomes in enhanced growth performance (Moonsie-Shageer and Mowat, 1993). However, the amount of chromium needed to be supplemented to improve performance remains unclear; Moonsie-Shageer and Mowat (1993), found response with 0.2 and 1.0 ppm of supplementary Cr, but performance was not modifies with 0.6 ppm of Cr. Kegley et al. (1997), increased ADG supplying diets with 0.4 ppm of Cr. Barajas and Almeida (1999), observed increments in ADG supplementing 1.0 ppm of Cr. In other experiment (Barajas et al., 1999), found maximal performance with 0.4 ppm of Cr, while 1.0 ppm of Cr has not effect. This experiment was conducted with the objective of determine the effect of chromium methionine supplementation on growth performance of low stressed bull-calves during the receiving period in the feedlot.

Material and Methods

The experiment was conducted from October 2 to October 30 of 2004, in the facilities of the Experimental Station for Beef Cattle in the Dry Tropic Weather of the Universidad Autonoma de Sinaloa, inside of feedlot Ganadera Los Migueles, S.A. de C.V. in Culiacan, Sinaloa in the Northwest of Mexico.

Early September 2004, Eight hundred Brahman-crossbreed bull calves were acquired from different farms in the state of Nayarit and were truck transported (450 km) to Ganadera Los Migueles. Just arrivals to the feedlot, the calves were weighed, and received a vaccine to prevent infections by *Pasteurella sp*. (One Shoot®; Pfizer Ltd.), after that, the animals were placed in six ground big size pens, without grouping by weight or any other criteria. They were fed with alfalfa hay and a 40:60 forage:concentrate diet and free access to fresh and clean drinking water.

After be rested by at least 15 day, in October 2 (2004), all the animals were weighed, and then sixty Brahman-crossbreed bull calves (206.9 ± 2.18 kg) were selected to be used in a 28 days performance experiment. The sixty selected animals were identified with numerate ear tag, ear implanted (Component TES with Tylan ®;ELANCO Co.), vaccinated against diseases by *Clostridium* and *Haemophilus somnus* (Ultrabac-Somnobact®; Pfizter), dewormed (Albendaphorte®; Lab. Salud y Bienestar), and injected with vitamins A, D and E (ADEphorte®; Lab. Salud y Bienestar), after that, the calves in groups of five were randomly placed in 12 experimental ground pens (6 x 12 m), fitted with a 2.4 m feed bunk and 0.6 m drinker.

In agreement to a completely randomized experiment design (Hicks, 1973), the animals were randomly assigned to receive one of two treatments: 1) Feed a 36:64 forage concentrate starting diet containing 15.6 % CP and 1.6 Mcal of NEm/kg (control); or 2) Diet similar to control, and supplementation with 2.8 g of a chromium methionine premix head^{-1} day^{-1}, equivalent to

2.8 mg of Cr head^{-1} day^{-1} (proximately 50 µg of Cr by kg of initial BW $^{0.75}$). Chromium was supply dispersing 14 g of chromium methionine premix (Microplex®; Zinpro Co.) in ground corn until complete 1 kg , and then was mixed with the diet directly in the fed bunk of each pen (five calves). Calves in the control treatment receiving 1 kg of ground corn mixed with the diet in the feed bunk. The cattle were fed once a day (1600) the diets that appears in table 1, under free access condition. Feed intake was considerate as feed offered minus weekly refusals, feed samples (4 kg) were taken weekly directly from mixer wagon, and were oven dried (110 °C by 48), and dry matter intake was calculate. The animals were weight on day 1 and 28 of experiment. Average daily gain was considered as total weight gain/28 days.

Table 1. Composition of diets used in the experiment.

Ingredients	Diets, % of Dry Matter	
	Days1 to 14	Days 15 to 28
Corn straw	36.8	30.7
Ground corn	29.2	36.3
Sugar cane molasses	12.8	12.8
Canola meal	16.4	14.3
Pork meat meal	2.0	3.1
Ganamin Total [1]	2.8	2.8
Calculated analyses [2]		
Crude protein %	15.66	15.33
NEm, Mcal/kg	1.602	1.674
NEg, Mcal/kg	1.001	1.064

[1] Ganamin Total® Vitamins and mineral premix (Técnica Mineral Pecuaria, S.A. de C.V.).
[2] Calculated from tabular values (NRC, 1996).

Retained energy (RE, mega calories) was derivate from measurements of body weight (BW, kg) and average daily gain (ADG, kg/day) in agreement with the equation: Bull calves RE = (0.0562 PV$^{.75}$) GDP$^{1.097}$ (NRC, 1984). The net energy content of the diet for maintenance and gain was calculated assuming a constant heat increment production (MQ) of 0.077 PV$^{.75}$ Mcal/day (Lofgreen and Garrett, 1968). From the estimating of RE y MQ, the values of NEm y NEg of the diet were obtained by iterative process (Zinn, 1987) fitting a NEg = (.877 ENm) - .41 (NRC, 1984). The values of NE obtained were divided by expected NE values to estimate the efficiency on the use of diet energy by the cattle.

Statistical Analysis. Performance data of bull-calves was analyzed as a completely randomized design (Hicks, 1973), using each pen (mean of five calves) as the experimental unit. General AOV/AOCV procedure of Statistix® 8 (Analytical Software, Tallahassee, FL) was used to perform the analyses, and P-value for F-test was obtained.

Results and Discussion

The effects of chromium methionine supplementation on growth performance of Brahman-crossbreed bull calves, during their first 28 days in the feedlot, are shown in Table 2.

Table 2. Effects of chromium supplementation on growth performance of Brahman-crossbreed bull calves, during their first 28 days in the feedlot.

Variables	Treatments		SEM [1]	*P*
	Control	Cr		
Bull calves, n	30	30		
Pens, replicates, n	6	6		
Days in trial	28	28		
Initial weight, kg [2]	207.7	206.2	2.18	0.51
End weight, kg [2]	241.0	247.1	2.72	0.06
Period wt gain, kg	33.34	40.95	2.75	0.02
ADG, kg/day	1.191	1.462	0.09	0.02
DMI, kg/day	6.297	6.573	0.15	0.09
Feed/gain, kg/kg	5.410	4.487	0.49	0.09
Diet NE, Mcal/kg				
Maintenance	1.722	1.867	0.06	0.05
Gain	1.051	1.175	0.05	0.05
Observed /expected NE				
Maintenance	1.05	1.14	0.04	0.05
Gain	1.05	1.18	0.05	0.05

[1] Standard error of the means.
[2] 4% was pencil removed as fill of digestive tract (NRC, 1984).

Chromium methionine supplementation tended (*P* = 0.06) to increase 2.5 % ending weight (251.38 *vs*. 257.75 kg), Barajas et al. (1999) observed an improvement of 3.5% on 28 days weight, supplementing 0.4 ppm of Cr in the diet.

Cr increased (*P* = 0.02) in 22.7 % the average daily gain (1.191 *vs.* 1.462 kg day $^{-1}$). Kegley et al. (1997) found an increment of 10.7 % on ADG supplementing 0.4 ppm of Cr from Cr-nicotinate. Barajas et al. (1999) observed an increment of 23 % on ADG, when fed diets containing 0.4 ppm of Cr from Cr-methionine to Brahman-crosses bull calves with 219 kg of starter weight, however they did not found effect when fed diets with 1.0 ppm of Cr, while that several authors has been observed benefice effects on ADG when fed diets containing 1 ppm to lightweight calves (Moonsie-Shageer and Mowat, 1993; Barajas and Almeida, 1999).

Cr tended (P = 0.09) to increase dry matter intake (6.297 *vs.* 6.573 kg day $^{-1}$). Moonsie-Shageer and Mowat (1993), observed 14 % of increment on DMI feeding diets supplemented with 0.2 ppm and 1.0 ppm from high-Cr yeast. This animal response can be interpret, that as chromium diminished the adverse effects of stress (Almeida and Barajas, 2002), facilitate the adaptation of cattle to feedlot environment, and is reflected as an increment in voluntary feed intake. Modification of stress-associated behavior may be a mechanism to improve fed intake of newly received calves (Loerch and Fluharty, 1999). Cr tended (P = 0.09) to enhances in 17 % feed/gain ratio (5.410 *vs.* 4.487 kg kg^{-1}). Improvements between 19% and 23% on feed/gain ratio has been reported by others authors (Chang et al., 1995; Barajas et al., 1999).

Cr supplementation enhanced in 8 % (P = 0.05) the retained NEm from the diet (1.722 *vs.*1.867 Mcal kg $^{-1}$), and consequently increased (P = 0.05) in 11.6 % the NEg retained from the diet (1.099 *vs*. 1.227 Mcal kg $^{-1}$). This fact, more that attributes any possible effect of Cr on increasing energy value of the diet, is explained by a diminishing in the NEm requirements of the calves, as consequence of a stress reduction, induced by chromium supplementation (Chang and Mowat, 1992; Kesgley et al., 1997; Almeida and Barajas, 2002), considering that stress increases the necessities of energy for maintenance of cattle (NRC, 1984).

In this experiment, the daily mean consumption of chromium in Cr-supplemented animals was close to 48 µg by kg of BW $^{0.75}$ day $^{-1}$ [(2.8 mg /(((206.16 kg +247.11 kg)/2)$^{0.75}$))/1000 = 48], and was equivalent to fed a 0.426 ppm Cr-supplemented diet (2.8 mg of Cr/6.573 kg DMI = 0.426 mg kg $^{-1}$). This result is in concordance with data of Barajas et al. (1999), when found that 219 kg-Brahman crosses bull calves fed diet containing 0.4 ppm of Cr (proximately 50 µg by kg of BW $^{0.75}$ day $^{-1}$) , shown a better performance than animals fed not supplemented Cr diets or with higher Cr concentrations (0.7 and 1.0 ppm). This findings, permits suppose that the chromium daily requirement for bull-calves of initial body weight near to 210 kg, will be close to 50 µg by kg of BW $^{0.75}$, this chromium intake could be obtained, supplementing 0.4 ppm of Cr in the diet.

Implications

These results, suggests that chromium supplementation from chromium methionine in amount close to 50 µg by kg of BW $^{0.75}$ day $^{-1}$ or 0.4 ppm of Cr in the diet, is adequate to improves growth performance of bull calves with starting weight near to 210 kg, during the 28 day receiving period in the feedlot.

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INCREASING GLUCOGENIC PRECURSORS IN RANGE SUPPLEMENTS ALTERS NUTRIENT PARTITIONING IN YOUNG POSTPARTUM RANGE COWS

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ABSTRACT: Altering nutrient partitioning in young postpartum beef cows from milk production to body weight gain has potential to improve reproductive performance. A 2-yr study conducted at the Corona Range and Livestock Research Center from February to July in 2003 (n = 51) and 2004 (n = 40) evaluated responses of 2- and 3-yr-old postpartum beef cows grazing dormant native range to three protein supplements with increasing glucogenic potential (**GP**). Supplements were fed at 1135 g·cow^{-1}·d^{-1} twice weekly for approximately 70 d and provided 1) 341 g CP, 142 g UIP, 57 g GP (**UIP0**), 2) 341 g CP, 151 g UIP + 80 g propionate salt (NutroCAL™, Kemin Industries, Inc.), 124 g GP (**UIP80**), or 3) 341 g CP, 159 g UIP + 160 g propionate salt, 192 g GP (**UIP160**). A supplement × yr × age interaction was observed for days to first estrus (P = 0.04). Cows fed UIP0 took longer to return to estrus in 2004 than in 2003, with 2-yr-old cows requiring more days than 3-yr-old cows in 2004. The yr difference may be due to pastures receiving less precipitation during the previous growing season in 2004 than 2003, differences in diet quality and/or diet species composition between the two years, or some combination of these factors. Weight loss was greater from start of supplementation to BW nadir in 2004 (P < 0.01; – 12 vs – 49 ± 3 kg for 2003 and 2004, respectively). Milk production exhibited a quadratic (P < 0.01) response to increasing glucogenic precursor content in the supplement with cows fed UIP80 producing the least amount of milk (8278, 7257, and 8684 ± 358 g/d for UIP0, UIP80, and UIP160, respectively). Implications of this study suggest that cows fed the moderate level of GP partitioned nutrients away from milk production and towards reproduction.

Key words: Propionate, Protein Supplements, Reproduction

Introduction

One of the biggest challenges facing range cow/calf producers is poor breed back of 2- and 3-year-old cows. Lack of dietary glucogenic precursors is one of the many factors influencing return to estrus and subsequent conception in young beef cows. In ruminants, nearly 100% of glucose needs are synthesized from products of digestion, including propionate and amino acids. However, high-acetate ruminal fermentation of dormant range yields small and probably inadequate quantities of these precursors, particularly propionate. To add to the deficit of glucose available to the postpartum cow, the glucose requirement has increased dramatically due to the nutrient demands of lactation. Glucose precursors enhance gluconeogenesis, thereby influencing nutrient partitioning by altering tissue sensitivity to nutrients. Waterman et al. (2002b) found cows fed range protein supplement containing glucogenic precursors in the form of 100 g/d of propionate salt returned to estrus 9 d earlier than cows fed a traditional cottonseed meal-based supplement with no additional glucogenic precursors. The objective of the current research was to investigate if 2- and 3-yr-old postpartum cows would benefit if the amount of glucogenic precursors in the supplements was increased. To accomplish this objective, we evaluated return to estrus, milk production, weight change responses, and insulin sensitivity of postpartum 2- and 3-year-old range beef cows to supplements with increasing glucogenic potential (**GP**) provided as 0, 80, or 160 g/d propionate salt.

Materials and Methods

A 2-yr study was conducted at the Corona Range and Livestock Research Center, Corona, NM during late winter and spring of 2003 and 2004. The Corona Range and Livestock Research Center (average elevation = 1900 m; average annual precipitation = 400 mm) is located 300 km northeast of Las Cruces, NM. Predominant forages in experimental pastures included blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*), as well as other less dominant grasses and forbs (Forbes and Allred, 2001). Each year, three ruminally cannulated cows were used to collect diet samples for analysis of CP (AOAC, 2000) and NDF (Van Soest et al., 1991). In 2003, CP and NDF concentrations (OM basis) averaged 15.4% and 81%, respectively. In 2004, CP and NDF concentrations (OM basis) averaged 11.3% and 80%, respectively.

All animal handling and experimental procedures were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of New Mexico State University. Cows (n = 91 total; n = 51 in 2003; n = 40 in 2004) were two (n = 33) and three (n = 58) yr of age and predominantly Angus with some Hereford and Simmental influence. Cows were assigned to treatment by calving date so that similar d postpartum were reflected in each treatment group. Supplements were individually fed at a rate of 1135 g·cow^{-1}·d^{-1} for avg 69 d postpartum. In 2003, supplementation continued through the first 21 d of breeding season (6 June); in 2004, supplementation ceased when breeding season began (15 May). Supplements provided 1) 341 g CP, 142 g UIP, 57 g GP (**UIP0**), 2) 341 g CP, 151 g UIP + 80 g propionate salt (NutroCAL™, Kemin

Industries, Inc.), 124 g GP (**UIP80**), or 3) 341 g CP, 159 g UIP + 160 g propionate salt, 192 g GP (**UIP160**). Glucogenic potential was calculated by the equation of Preston and Leng (1987), where 40% of the undegraded intake protein is considered glucogenic. NutroCAL™ contains 80% propionate, which was assumed to be 100% glucogenic.

Blood samples were collected twice weekly on supplementation days (Monday and Friday) via coccygeal venipuncture beginning approximately 40 d postpartum for analysis of progesterone to determine d to first estrus (2 or more consecutive progesterone concentrations > 1 ng/mL). Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Inter- and intra-assay coefficients of variation were less than 10%. Cows were diagnosed for pregnancy via rectal palpation at weaning (26 September 2003; 24 September 2004).

A subsample of cows (n = 29 in 2003; n = 20 in 2004) were milked with a portable milking machine approximately 57 d postpartum on a d following supplementation using a modified weigh-suckle-weigh technique (Appeddu et al., 1997). Milk weight was recorded to estimate milk yield. Milk subsamples were collected in preservative-coated vials for analysis of protein, lactose, butterfat, and solids non-fat by an independent laboratory (Pioneer Dairy Labs, DHIA, Artesia, NM).

Cows were weighed weekly until termination of breeding season and at weaning. Days to BW nadir were determined from lowest BW obtained postpartum. Pre-planned intervals of weight change were calculated and included beginning of supplementation to BW nadir, BW nadir to end of supplementation, BW nadir to end of breeding, and end of supplementation to end of breeding. Body condition scores (**BCS**; 1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at beginning and end of supplementation, beginning and end of breeding season, and at weaning. Calf birth weights were obtained in the field within 3 d after birth with a portable platform scale. Calves were weighed at weaning and adjusted 205-d weaning weights were used as a measure of calf growth; no adjustment was made for sex of calf or age of dam in the calculation.

A glucose tolerance test (**GTT**) was conducted approximately 53 d (2003) or 65 d (2004) postpartum on a subsample of cows (n = 26 in 2003; n = 20 in 2004) on a day after supplementation. A 50% dextrose solution was infused at 0.5 mL/kg BW via indwelling jugular catheter inserted the morning of the GTT. Blood samples were collected at –1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min relative to infusion. Glucose was analyzed with a commercial kit ([Trinder] method, Sigma Diagnostics, St. Louis, MO or enzymatic endpoint method, Thermo DMA, Louisville, CO). Insulin was analyzed by solid-phase radioimmunoassay (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as validated by Reimers et al. (1982). Intra- and inter-assay coefficients of variation were less than 10%. Serum glucose and insulin areas under the curve (AUC) were calculated using trapezoidal summation. Glucose half-life was estimated by determining time required for 50% decrease in peak serum glucose concentration.

Data were analyzed as a completely randomized design by analysis of variance using GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as experimental unit. The effects of supplement, year, cow age and their interactions were used in the model. Covariates were used when appropriate and included calf gender, calving date and days on feed. When appropriate, orthogonal polynomial contrasts were used to test for linear and quadratic effects of increasing supplemental glucogenic precursors. Pregnancy data were analyzed using GENMOD procedure of SAS (SAS Inst., Inc., Cary, NC).

Results and Discussion

A supplement × yr × age interaction was observed for d to first estrus ($P = 0.04$, Table 1). Return to estrus occurred later in cows fed UIP0 in 2004 than 2003, with 2-yr-old cows requiring more d than 3-yr-old cows in 2004. The yr difference may be due to pastures receiving less precipitation during the previous growing season in 2004 than 2003 (Figure 1), differences in diet quality and/or diet species composition between the two years, or some combination of these factors. More UIP0- and UIP80-fed cows were pregnant ($P = 0.01$) than UIP160-fed cows (100, 100, and 91% pregnant for UIP0, UIP80, and UIP160, respectively). Weight loss was greater from start of supplementation to BW nadir in 2004 ($P < 0.01$; – 12 vs – 49 ± 3 kg for 2003 and 2004, respectively), possibly reflecting previous growing season precipitation differences or diet quality/species composition differences between the years. However, supplement had no effect ($P = 0.72$) on magnitude of BW nadir (– 28, – 31, and – 32 ± 3 kg for UIP0, UIP80, and UIP160, respectively). Milk production exhibited a quadratic ($P < 0.01$) response to increasing GP in the supplement with cows fed UIP80 producing the least amount of milk (Table 2). The decrease in milk production when 80 g of propionate salt was added to the supplement suggests that the combination of glucogenic precursors (metabolizable protein from UIP plus propionate salt) shifted nutrient partitioning away from milk production. Similar results were found by Waterman et al. (2002b). Further, the increase in milk production of UIP160 cows compared to UIP80 cows suggests that the additional GP in the UIP160 supplement was used for milk production. Yet these milk production differences did not affect ($P = 0.64$) calf 205-d weaning weights (241, 238, and 236 ± 4 kg for UIP0, UIP80, and UIP160, respectively).

Supplement did not affect ($P \geq 0.69$) tissue sensitivity to insulin, as glucose and insulin AUC and glucose half-lives were similar among supplement groups (Table 3). Glucose half-lives for all cows were similar to those in Waterman et al. (2002a) and these authors determined that cows fed supplements with increased GP in the form of additional metabolizable protein (UIP) and/or propionate salt had shorter glucose half-lives and were more sensitive to insulin than cows fed a traditional cottonseed meal-based supplement with no additional glucogenic precursors (avg 63 vs 100 min, respectively).

All cows in the present study were fed supplements with additional GP from metabolizable protein and/or propionate salt and exhibited similar glucose half-lives to those of the previous study. Cows in both studies were considered insulin-resistant, as glucose half-lives were at least two-fold higher than the normal value of 35 min described by Kaneko (1997).

Feed costs for the supplementation period of this study were $21.09, $30.27, and $39.89/cow for UIP0, UIP80, and UIP160, respectively. To compare benefits of supplementation strategies, results of Waterman et al. (2002b) and the present study were combined (2000, 2001, 2003 and 2004 experiments). The UIP80 treatment group and the group fed supplement containing 100 g propionate salt from Waterman et al. (2002b) were grouped for this comparison and will be referred to as UIP80; the UIP0 supplement was fed in both studies. In all 4 yr, the UIP80-fed cows produced less milk (6615 vs 7386 g/d for UIP80 and UIP0, respectively) and cycled earlier (89 vs 98 d postpartum for UIP80 and UIP0, respectively). Both groups of cows had equivalent calf growth (1.0 kg/d age) and estrous cycle fertility. Pregnancy rates across the 4 yr averaged 91.4% for cows fed UIP0 and 93.1% for cows fed UIP80. An economic comparison (Table 4) was calculated to predict hypothetical results of two 100-cow herds fed either UIP0 or UIP80 for 69 d postpartum (supplementation period feed cost of $21.09 and $30.27, respectively). Additional feed inputs for the year included free-choice mineral ($7.96) and prepartum supplement ($3.36). The UIP0 calves were assumed to be 205 d at weaning; all calves were valued at $2.20/kg at weaning. Even though feed costs for the year were higher for cows in the UIP80 group, their calves had potential to be heavier at weaning because cows fed UIP80 bred back sooner than cows fed UIP0. This resulted in an increase in income of $19.50/cow when UIP80 was compared to UIP0.

Implications

Cows fed the moderate level of glucogenic potential partitioned nutrients away from milk production and towards reproduction. A combination of supplemental glucogenic precursors may be best suited to shift nutrient partitioning in young postpartum range cows grazing dormant forage.

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Table 1. Days to first estrus for 2- and 3-year-old postpartum range cows fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt) in 2003 and 2004.

		Year							
		2003				2004			
		Cow Age							
Response	Supplement	2	SE[a]	3	SE	2	SE	3	SE
Days to first estrus	UIP0	56	8	59	5	97	6	72	7
supp × yr × age $P = 0.04$	UIP80	73	8	58	5	66	8	62	7
	UIP160	68	6	53	5	67	8	72	6

[a]Standard error.

Table 2. Milk production and constituents for 2- and 3-year-old postpartum range cows fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt) in 2003 and 2004.

	Supplement							Contrast	
Item	UIP0	SE[a]	UIP80	SE	UIP160	SE	OSL[b]	L	Q
24-h milk production, g/d	8278	337	7257	358	8684	341	0.02	0.40	< 0.01
Butterfat, g/d	289	18	271	20	317	18	0.22	0.26	0.19
Protein, g/d	219	10	199	11	236	10	0.06	0.25	0.04
Lactose, g/d	418	18	365	21	433	18	0.05	0.57	0.02
Solids non-fat g/d	712	30	630	34	747	31	0.05	0.42	0.02

[a]Standard error.
[b]Observed significance level.

Table 3. Glucose tolerance test responses of 2- and 3-year-old postpartum range cows fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt) in 2003 and 2004.

	Supplement							Contrast	
Item	UIP0	SE[a]	UIP80	SE	UIP160	SE	OSL[b]	L	Q
Glucose area under the curve, units	8786	975	8007	1036	9047	1073	0.76	0.86	0.48
Insulin area under the curve, units	190	19	185	20	209	21	0.69	0.51	0.58
Glucose half-life, min	75	11	66	11	75	12	0.81	0.99	0.53

[a]Standard error.
[b]Observed significance level.

Table 4. Hypothetical economic comparison of two 100-cow herds fed either UIP0 or UIP80 supplements. Results of 4 yr of experiments were combined (current study and Waterman et al., 2002b). Assumptions are defined in text.

Supplement	Pregnancy rate (%)	d to first estrus	Predicted kg calf weaned/cow exposed	Yearly feed cost ($/cow)	Predicted income difference, ($)
UIP0	91.4	98	195	32.41	-----
UIP80	93.1	89	208	41.59	19.50

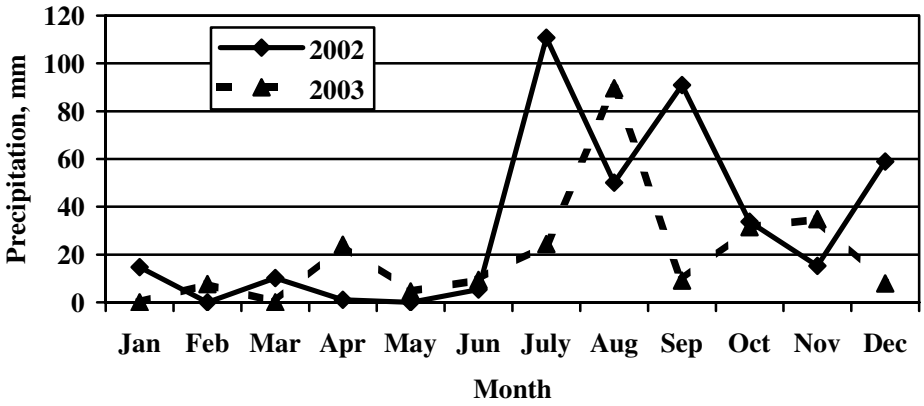

Figure 1. Monthly precipitation of pastures grazed by 2- and 3-yr-old postpartum range cows from February through July for 2002 and 2003. Pastures were grazed in 2003 and 2004 during the present study. Annual precipitation was 391 and 243 mm for 2002 and 2003, respectively.

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EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON SELENIUM CONCENTRATIONS IN MATERNAL AND FETAL TISSUES OF PREGNANT EWE LAMBS[1]

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ABSTRACT: Pregnant Targhee-cross ewe lambs (n = 36; 53.8 ± 1.3 kg) were randomly allotted to one of four treatments in a 2 x 2 factorial arrangement to examine effects of nutrient restriction and dietary Se on maternal and fetal liver, kidney, and muscle (longissimus dorsi) Se tissue concentrations. Treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 μg/kg BW [NSe]; no added Se vs. 81.5 μg/kg BW [HSe]; Se enriched yeast). Selenium treatments were initiated 21 d before breeding and restriction treatments on d 64 of gestation. All diets were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. On day 135 of gestation ewes were slaughtered and tissues harvested. Maternal and fetal liver masses (g) were lower ($P < 0.01$) in R compared with M fed ewes (maternal liver = 367.7 vs. 271.3 ± 13.6 g, and fetal = 102. vs. 81. ± 5.4 g, for R vs. M, respectively). Total Se (mg) was higher ($P < 0.01$) in liver and kidney tissues in HSe compared with NSe fed ewes (0.43 vs. 2.84 ± 0.180; and 0.18 vs. 0.38 ± 0.02 mg, for liver and kidney, respectively). Total Se (mg) was higher ($P < 0.01$) in fetal liver and kidney tissues in HSe compared with NSe (0.03 vs. 0.16 ± 0.01, for liver). Selenium concentrations (μg/g) in maternal and fetal liver were higher ($P < 0.01$) in R-HSe compared with M-HSe. Maternal muscle Se was lower ($P < 0.01$) in R-HSe compared with M- HSe fed ewes (0.83 vs. 1.05 ± 0.07 μg/g). Conversely, fetal muscle Se was higher ($P < 0.01$) in R-HSe compare with M-HSe fed ewes (1.45 vs. 1.02 ± 0.18 μg/g). Dietary Se levels affect Se concentrations in both maternal and fetal liver, kidney, and muscle tissues. When nutrition is limiting, Se may become more concentrated in fetal liver and muscle.

Key Words: Nutrient Restriction, Pregnancy, Selenium

Introduction

In resent years, Se has been the focus of much research as a potential cancer preventative. In 1996 it was reported that lung, colorectal, and prostate cancers could be reduced by as much as 50% in men supplemented with supranutritional levels of selenoyeast (200 μg/d; Clark et al., 1996). Jaing et al., (1999) determined that rate of mammary tumor growth was reduced in rats fed 2.0 mg/kg BW of selenite per day. In addition, Se plays a role in immunity, reproduction, and thioredoxin reductase and glutathione peroxidase function (Thompson et al., 1982). As more information is gained in regard to this important trace mineral, consumers will likely seek additional sources of dietary selenium.

Organic sources include small grains, such as wheat, and most sources of meat from domesticated livestock (Schubert et al., 1987; Holden et al., 1991; and Hintze et al., 2001). Organically bound selenium has been shown to be metabolized differently than inorganic salt forms (Thompson, et al, 1982). In ruminants, Se salts such as selenate, are converted to selenocysteine (van Ryssen et al., 1989). Once incorporated into cysteine, the selenocysteine form is utilized to develop selenoproteins such as glutathione peroxidase and thioredoxin reductase; two strong antioxidant substrates (Sunde, 1990). Selenocysteine, however, cannot be converted to selenomethionine, and thus is not well incorporated into muscle tissue.

In addition, it has been shown that Se source does influence Se load in pregnant and lactating rats. Taylor et al., (2005) demonstrated a higher accumulation in uterine, placental, and fetal tissues in pregnant rats fed 2 μg/g of selenomethionine compared with 2 μg/g selenocystine. Selenomethionine can pass through the rumen intact and be incorporated into tissues where methionine is utilized (McDowell, 2003). When steers were fed either organic or inorganic sources of Se (65 μg/kg/BW), it was determined that Se accumulated in semitendinosus muscle tissue more efficiently in steers fed a high-Se wheat source vs. all other treatments (Lawler et al., 2004). In the same study, it was found that the Se salt treatment (inorganic source) resulted in higher concentrations of Se in the liver compared with the high-Se hay treatment. Dietary intake by livestock consuming potentially high Se diets is variable. Currently, little data are available in the literature investigating the effects of nutrient restriction and high Se on incorporation of Se into livestock tissues. Moreover, in production settings with gestating animals, little research has been published evaluating combined effects of high Se and nutrient restriction. Therefore, objectives were to determine effects of nutrient restriction and high Se on Se concentrations in maternal and fetal muscle, liver, and kidney tissues in pregnant ewe lambs.

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Materials and Methods

Thirty-six pregnant Targhee-cross ewe lambs (53.8 ± 1.3 kg) were randomly assigned to individual pens (.91 x 1.2 m), and allotted to one of four treatments in a 2 x 2 factorial arrangement. Care and management of animals were approved by the North Dakota State University Institutional Animal Care and Use Committee. Dietary treatments were nutrition (maintenance [M] vs. 60% maintenance [R]) and dietary Se (7.4 µg/kg BW [NSe]; no added Se vs. 81.5 µg/kg BW [HSe]; Se enriched yeast; Alltech Inc., Lexington, KY). All diets consisting of alfalfa hay and the respective supplements were similar in CP (16.0%) and energy (2.12 Mcal/kg) density. Diets (Table 1) were fed once daily, with free access to water and salt. All ewes received a corn based supplement (Table 1). Supplements provided to HSe ewes contained Se enriched yeast to meet targeted Se intakes, while NSe ewes were fed supplements without added Se. Dietary Se supplementation was initiated 21 d prior to breeding, and continued to slaughter (d 135 gestation). Nutrient restriction ewes were offered 60% of the diet supplied to maintenance fed ewes. Dietary restriction treatments were initiated on day 64 of gestation. Maintenance ewes were fed to perform within NRC (1985) guidelines.

Table 1. Chemical composition of alfalfa hay and supplement (DM basis) fed to ewes at requirement or restricted (60% of control ewes).

		Supplement[a]	
Item, %	Alfalfa Hay[b]	Control	Selenium
DM	87.4	85.6	86.10
Ash	9.9	1.95	2.30
CP	16.13	10.0	12.70
ADF	27.3	5.5	5.00
NDF	37.2	26.2	26.50
Ca	.01	0.06	0.03
P	.005	0.35	0.42
Se, ppm	-	0.32	43.20

[a] Supplement contained 0.3 ppm and 43.2 ppm Se for Control and Selenium, respectively, and was fed to provide 7.4 µg/kg BW or 81.5 µg/kg BW daily for control and high-Se fed ewes.
[b] Hay was chopped, averaging 3.8 cm in length.

Ten mL of serum was collected with Corvac serum separator Vacutainer tubes (Tyco Healthcare, Mansfield, MA) via venepuncture on days 62, 76, 90, 104, 118, 132, and 135 of gestation. The last collection period was 3 d on average prior to slaughter (d 135). Ten mL of fetal serum was collected on d 135 (slaughter) via cardiopulmonary stick. All blood samples were centrifuged at 1500 X g, for 28 minutes. Supernant was pipetted into 2 mL screw cap vials and stored at –20 °C. On d 135 of gestation ewes were euthanized via captive bolt, eviscerated, and tissues harvested. Approximately 5 grams of tissue from the liver, kidney, and longissimus dorsi was removed from both the ewe and fetus.

The collected tissues were wrapped in foil, snap frozen in isopentane, and stored at -80° C. Tissue samples were digested in trace mineral-grade nitric acid under heat. The digests were then diluted with ultra-pure water to a final nitric acid content of 5%, which provided a matrix match for the analytical standards. The prepared samples were analyzed by atomic absorption spectrophotometry (PerkinElmer Instruments, Shelton, CT) and assessed against concentration curves of known standards. Standard curves and quality control samples were analyzed every 5 samples. Total tissue content (mg) of Se was calculated by multiplying their respective concentrations by fresh tissue mass. Plasma Se was analyzed by the Utah State Veterinary Diagnostic Laboratory, Logan, UT; utilizing ICP-MC techniques.

Data were analyzed within day of gestation as a 2 x 2 factorial using PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Because ewe lambs carried both singles and twins, fetal number was used as a covariate. Model contained effects nutrition (M vs. R), level of Se (NSe vs. HSe), and nutrition x Se interaction. When interactions were present ($P < 0.10$), means were separated by least significant difference.

Results and Discussion

Ward et al., (2004), reported higher plasma Se concentrations in the ewes receiving 75 µg/kg BW and 375 µg/kg BW daily of Se compared with control. In this study, plasma Se concentrations were higher ($P < 0.01$) in HSe on d 62, 90, and 135 of gestation compared with NSe (Table 2). Fetal plasma Se concentrations were also higher ($P < 0.01$) on d 135 in HSe compared with NSe (Table 2).

Final body weight (BW), liver, and kidney masses were lower ($P < 0.01$) in R compared with M fed ewes (Table 3). This is in agreement with Schaeffer et al., (2004), who showed lower BW, liver, and kidney masses in ewes fed at 60% of maintenance controls.

Selenium concentrations (fresh basis) were greater ($P < 0.01$) in maternal liver, kidney, and muscle tissues for Se treatments compared with controls (Table 3). This is in agreement with Lawler et al., (2004), who reported higher concentrations of Se in kidney and liver tissue of beef steers fed supranutritional levels (65 µg/kg BW daily) of Se from high-Se wheat, high-Se hay, and selenate sources, compared with controls. Nutrient restriction did not affect Se (µg/g) concentrations in maternal kidney and muscle tissue, but increased ($P < 0.01$) Se concentrations in maternal liver. In addition, there were nutrient restriction x dietary Se level interactions ($P < 0.05$) observed in Se concentrations of the maternal liver and muscle tissue (Table 5). In the maternal liver of R fed ewes on HSe, Se concentrations were almost two-fold higher ($P < 0.01$) compared with M ewes fed HSe.

The response was different in maternal muscle. Ewes fed M-HSe had higher Se concentrations in muscle tissue compared will all other treatments.

Total Se (mg) in maternal liver and kidney tissues was not affected by nutrient restriction. Selenium concentrations (μg/g) in muscle, kidney, and liver tissue from HSe ewes were higher ($P < 0.01$) in HSe compared with NSe. These data are in agreement with others (Hintze et al., 2002; Lawler et al., 2004; and Ward et al., 2005) who reported increased muscle Se concentrations due to increases in dietary Se from organic sources.

Fetal body weight (BW) and liver masses were lower ($P < 0.01$) in the R compared with M (Table 4). Schaeffer et al. (2004) showed lower fetal BW, liver, and kidney masses from ewes fed at 60% of maintenance vs. control. In addition, fetal BW tended ($P < 0.08$) to be greater in HSe vs. NSe groups. Selenium concentrations (fresh basis) were greater ($P < 0.01$) in fetal liver, kidney, and muscle (longissimus dorsi) tissues for Se treatments compared with NSe (Table 4). Similarly, Total Se was greater ($P < 0.02$) in fetal liver and kidney in HSe vs. NSe. Ward et al., (2004) reported higher Se concentrations in fetuses of ewe lambs fed 75 μg/kg BW Se from a high-Se wheat source, compared with selenate fed at similar dietary levels. These data may indicate that Se is transferred to the fetus more efficiently from ewes fed an organic source versus a salt form. As in the ewe, there were nutrition x Se interactions ($P < 0.09$) observed in liver and muscle Se concentrations in the fetus (Table 5). The interactions observed in fetal liver are similar to those in the ewe. Selenium concentrations in fetal liver tissue from R-HSe fed ewes were higher ($P < 0.01$) compared with M-HSe fetuses. Selenium concentrations in fetal muscle, however, were greatest ($P < 0.01$) in fetuses from R-HSe, compared with all other treatments. When total mg of Se was determined for fetal liver and kidney, nutrient restriction was not significant. Nutrient restriction did not impact Se concentration in fetal muscle tissue.

Implications

Nutrient restriction caused a reduction in body, kidney, and liver mass in ewe lambs. As a consequence of this nutrient restriction, fetal weight, liver, and kidney masses were also reduced. Plasma Se concentrations were increased due to higher dietary levels of Se. Tissue concentrations of Se were also higher in both maternal and fetal tissues when ewes were fed 81.5 μg/kg BW Se from an enriched yeast source, compared with controls. Further investigation is needed to better understand not only the impact nutrient restriction has on the dam and her subsequent offspring, but the role Se may play in animals exposed to a lower plane of nutrition.

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Table 2. Serum selenium levels (ppm) of pregnant ewe lambs fed different levels of nutrition and organically bound selenium

Item	Treatments [a]				SE
	M-NSe	M-HSe	R-NSe	R-HSe	
Gestation, d					
-21	0.24	0.23	0.24	0.24	0.01
0	0.22	0.25	0.21	0.27	0.03
62	0.20[b]	0.43[c]	0.22[b]	0.42[c]	0.02
90	0.19[b]	0.45[c]	0.20[b]	0.53[d]	0.02
135	0.18[b]	0.40[c]	0.21[b]	0.45[d]	0.02
Fetal Se (ppm)	0.12[b]	0.30[c]	0.14[b]	0.32[c]	0.02

[a] M=non-restricted ewes fed at requirements, R =ewes fed to 60% of M, NSe = 7.4 µg/kg BW; no added Se, HSe = 81.5 µg/kg BW.
[b,c,d] Treatment means differ ($P < 0.10$).

Table 3. Effect of level of nutrition and dietary Se on Se concentrations of maternal liver, kidney, and the longissimus dorsi muscle in pregnant ewes lambs.

Item	Nutrition [a]		Selenium [b]		SE	*P* Value[c]		
	M	R	NSe	HSe		Nut	Se	Se X Nut
Body Wt, kg[d]	66.50	56.10	60.50	62.10	1.00	0.001	0.253	0.738
Liver g	687.50	564.1	620.30	631.30	13.58	0.001	0.288	0.876
Liver Se, µg/g [e]	4.01	6.43	1.44	9.01	0.42	0.001	0.001	0.005
Total Se, mg [f]	1.48	1.80	0.43	2.84	0.18	0.224	0.001	0.367
Kidney g	143.49	126.98	129.40	141.08	3.82	0.004	0.038	0.338
Kidney Se, µg/g	1.98	2.13	1.42	2.68	0.11	0.364	0.001	0.555
Total Se, mg	0.29	0.27	0.18	0.38	0.02	0.490	0.001	0.211
Muscle [e]								
Muscle Se, µg/g	0.62	0.53	0.21	0.94	0.05	0.215	0.001	0.050

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.
[d] Final BW measured on d 135 gestation (slaughter weight).
[e] Interactive means for fetal liver and muscle are shown in Table 5.
[f] Total Se = (tissue, g X tissue Se, µg/g)/1000.

Table 4. Effect of level of nutrition and dietary Se on Se concentrations of fetal liver, kidney, and the longissimus dorsi muscle in pregnant ewes lambs.

	Nutrition [a]		Selenium [b]			*P* Value [c]		
Item	M	R	NSe	HSe	SE	Nut	Se	Se X Nut
Body Wt, kg [d]	4.05	3.62	3.64	4.03	0.16	0.055	0.077	0.575
Liver g	102.00	81.33	87.85	95.44	5.43	0.008	0.313	0.939
Liver Se, µg/g [e]	0.91	1.13	0.35	1.69	0.06	0.018	0.001	0.015
Total Se, mg [f]	95.2	94.2	31.0	158.3	8.60	0.937	0.001	0.659
Kidney g	21.62	19.95	19.93	21.64	0.88	0.170	0.162	0.292
Kidney Se, µg/g	0.79	0.86	0.47	1.18	0.11	0.621	0.001	0.849
Total Se, mg	0.02	0.02	0.008	0.025	0.002	0.983	0.001	0.879
Muscle [e]								
Muscle Se, µg/g	0.77	0.92	0.46	1.23	0.11	0.998	0.001	0.084

[a]Nutritional treatments were M (maintenance) and R (60% of maintenance), respectively.
[b]Selenium treatments were daily intake of organically bound Se; NSe (7.4 µg/kg BW) and HSe (81.5 µg/kg BW), respectively.
[c] Probability values for effects of nutritional levels (Nut), selenium (Se), and the interaction.
[d] Final BW measured on d 135 gestations (slaughter weight).
[e] Interactive means for fetal liver and muscle are shown in Table 5.
[f] Total Se = (tissue, g X tissue Se µg/g)/1000.

Table 5. Interactive means Se concentrations based on level of nutrition and dietary Se in maternal and fetal liver and longissimus dorsi muscle tissues in pregnant ewes lambs

	Treatments [a]				
Item	M-NSe	M-HSe	R-NSe	R-HSe	SE
Ewe					
Liver Se, µg/g	1.12 [b]	6.90 [c]	1.75 [b]	11.11 [d]	0.63
Muscle Se, µg/g	0.18 [b]	1.05 [c]	0.23 [b]	0.83 [d]	0.07
Fetal					
Liver Se, µg/g	0.35 [b]	1.47 [c]	0.35 [b]	1.92 [d]	0.10
Muscle Se, µg/g	0.52 [b]	1.02 [c]	0.40 [b]	1.45 [d]	0.18

[a] M=non-restricted ewes fed at requirements, R =ewes fed to 60% of M, NSe = 7.4 µg/kg BW; no added Se, HSe = 81.5 µg/kg BW.
[b,c,d] Treatment means differ ($P < 0.10$).

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DIGESTA KINETICS OF STEERS FED FORAGE KOCHIA (*KOCHIA PROSTRATA*) IN A LOW QUALITY FORAGE DIET[1]

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ABSTRACT: Forage kochia is a shrub that is useful in the Western US for rehabilitating degraded rangelands. Little information is known about its nutritional value for grazing beef cattle. The objective of this study was to evaluate digesta kinetics in diets using different ratios of forage kochia and tall wheatgrass (*Agropyron elongatum*). Four ruminally fistulated beef steers (mean initial BW = 348 kg) were allocated to four treatments in a 4 × 4 Latin square design. Treatments were: 100% wheatgrass (0K); 75% wheatgrass:25% kochia (25K); 50% wheatgrass:50% kochia (50K); and 25% wheatgrass:75% kochia diet (75K). Steers were fed twice daily at 110% of mean intake over the previous 5 d. Steers were allowed an 11 to 13 d adaptation period. Feed intake and fecal output were measured and sampled during the following 7 d. Digesta kinetics was determined using a pulse dose of ytterbium ($YbCl_3$) as an external marker. Each steer was dosed with tall wheatgrass labeled with $YbCl_3$ just before feeding on the morning of the last fecal collection. The Yb-labeled forage was placed in different locations within the rumen. Rectal fecal grab samples were collected at 0 (before Yb was dosed), 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, and 120 h post dosing. Data were analyzed in a Latin square-design in the MIXED procedure of SAS. As the amount of kochia increased in the diets, passage rate increased linearly (P = 0.0006) and mean retention time decreased linearly (P = 0.0009). Animals on the 75K diet had the highest passage rate and the lowest mean retention time. Digestive tract fill on a weight basis (P = 0.06) and on a percentage of body weight basis (P = 0.08) tended to increase linearly as the amount of kochia increased in the diet. We previously reported that intake and rate of DM and NDF digestion increased linearly as kochia increased in the diet. Fill followed the pattern of intake. Kochia affects digestive tract kinetics in a low quality diet by increasing the rate of passage and decreasing retention time as the level of kochia increases in the diet.

Key words: Beef cattle, forage utilization, forage kochia

Introduction

Forage kochia (*Kochia prostrata*) is native to the arid and semiarid regions of Central Eurasia (Keller and Bleak, 1974) and has adapted well to a variety of environmental conditions in the western United States. Forage kochia is a shrub that is useful in the Western US for rehabilitating degraded rangelands. However, little information is known about its nutritional value for grazing beef cattle.

Fall and winter grazing studies have been conducted on forage kochia (ZoBell et al., 2003; Koch and Asay, 2002) but little is known on the effects it will have on digestive kinetics in grazing animals.

The objective of this study was to evaluate digesta kinetics in diets of beef cattle using different dietary ratios of forage kochia and tall wheatgrass (*Elytrigia elongata*) straw.

Materials and Methods

Four ruminally fistulated beef steers (mean initial BW = 348 kg) were allocated to one of four treatments in a 4 × 4 Latin square design. The 4 treatments consisted of varying mixtures of tall wheatgrass straw and forage kochia mixed to provide diets of: 100% wheatgrass (0K); 75% wheatgrass:25% kochia (25K); 50% wheatgrass:50% kochia (50K); and 25% wheatgrass:75% kochia diet (75K) on an as-fed basis. The forage kochia was from an irrigated, pure stand with the intent of the seed being harvested but was instead harvested as hay at full maturity with the seed attached for the purpose of this study. The tall wheatgrass straw was harvested in late fall from an irrigated, pure stand after seed had been harvested. Both forages were intended to mimic stockpiled forage used for winter grazing. The tall wheatgrass and forage kochia were chopped to an average length of 3 cm. Treatment diets were fed as mixed rations.

Steers were housed in individual metabolism crates (2.4 x 1.1 m) located inside a shed that was enclosed on three sides and was open facing the south. Steers were allowed free access to water and a trace-mineralized salt block (Table 1). Steers were fed twice daily at 0700 and 1900. Orts were collected and weighed daily before the morning feeding. Steers were then offered 110% of mean intake over the previous 5 d. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Utah State University.

Experimental periods were 24 to 26 d, with 11 to 13 d of adaptation. Period 1 consisted of a 13-d adaptation period to allow steers to adjust to their respective treatments. Period 2 adaptation was reduced to 12 d and periods 3 and 4 were reduced to 11 d because of a limited supply of forage kochia. The following 7-d period was used to measure feed intake and total fecal output to estimate in vivo digestibility (Stonecipher et al., 2004).

Digesta kinetics were determined using a pulse dose of ytterbium ($YbCl_3$) as an external marker (Krysl et al., 1985;

[1] Research supported by Utah Agricultural Experiment Station.

Teeter et al., 1984). For period 1, 800 g of tall wheatgrass (as is), subsampled from the feed pile, was soaked in a prepared Yb solution that contained 20 g of $YbCl_3$ * 6 H_2O dissolved in deionized water. For periods 2, 3, & 4, 1000 g of wheatgrass (as is) was soaked in a prepared Yb solution that contained 30 g of $YbCl_3$ * 6 H_2O dissolved in deionized water. The solution was then brought to volume with enough deionized water to sufficiently cover the forage. The wheatgrass was soaked for 24 h. Excess water was poured off and the forage was washed with deionized water every hour for a 6-h period. Forage was then dried in a forced-air oven at 60º C for 48 h. On the morning of the last total fecal collection, just before feeding, each steer was pulse-dosed with tall wheatgrass labeled with $YbCl_3$. The labeled forage was administered into various sites in the rumen to achieve uniform dispersion.

For period 1, steers were dosed with 150 g of forage containing 0.311 g Yb g^{-1} forage DM via rumen fistula. For periods 2, 3, & 4, steers were dosed with 180 g of forage containing 0.477 g, 0.442 g, and 0.405 g of Yb g^{-1} forage DM, respectively, via rumen fistula. Rectal fecal grab samples were collected at 0 h, before Yb was dosed, and at 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, and 120 h post dosing. Fecal samples were stored at -20º C until laboratory analysis.

Laboratory Analysis. Feed samples were dried at 60º C and ground in a Wiley mill to pass a 1-mm screen. Subsamples were dried overnight at 105ºC to determine DM (AOAC, 1996). Feed samples were analyzed for NDF and ADF content using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Feed samples were analyzed for N content with the combustion method (AOAC, 1996) using a N analyzer (Leco, St. Joseph, MI) and nitrogen was multiplied by 6.25 to determine CP. Yb-labeled fecal samples were dried at 60º C in a forced-air oven. Fecal samples were then ground through a Wiley mill to pass a 1-mm screen. Duplicate samples were dried at 105ºC to determine DM content (AOAC, 1996) and then ashed overnight at 500º C in a muffle furnace and Yb was extracted following the procedure of Ellis et al. (1980). Yb concentration was then determined by inductively coupled plasma emission spectroscopy using a Thermo Jarrell Ash Iris Advantage (Franklin, MA).

Statistical analysis. Fecal Yb concentration curves were fitted to a one-compartment model (Pond et al., 1987) using the nonlinear regression procedure of SAS (PROC NLIN, SAS Institute, Cary, NC). Passage rate, retention times, and gastrointestinal fill were calculated from the nonlinear regression results. The response of passage rate, retention time, and gastrointestinal fill to wheatgrass:kochia treatment were analyzed using the MIXED procedure of SAS in a Latin square-repeated measure design. The model included treatment, period, and steer. Steer was designated a random effect and period was designated a repeated measure. Linear and quadratic polynomial contrasts were constructed to evaluate the influence of increasing levels of forage kochia in the diet.

Results and Discussion

The low CP and high fiber of the tall wheatgrass typified a low-quality, dormant forage available for winter grazing (Table 2). The higher nutrient concentration, particularly CP, in the forage kochia should induce a positive associative effect, leading to improved fermentation of the fiber in the tall wheatgrass straw.

Passage rate increased linearly (P = 0.0006) and mean retention time decreased linearly (P = 0.0009) as the amount of forage kochia increased in the diet (Table 3). Animals on the 75K diet had the highest passage rate and the lowest mean retention time. Consuming forages with large amounts of stem material could slow passage rate and increase retention times (Hess et al., 1994). The tall wheatgrass fed in this study consisted of a large amount of stem material and passage rate was slowest and mean retention time the highest on diets consisting of only tall wheatgrass (0K).

We previously reported that increasing levels of forage kochia caused a linear increase in the rate of digestion of DM and NDF of the tall wheatgrass (Stonecipher et al., 2004). These concomitant increases in rates of digestion and passage suggest that forage kochia had a positive associative effect on utilization of the tall wheatgrass.

Digestive tract fill on a weight basis (P = 0.06) and on a percentage of body weight basis (P = 0.08) tended to increase linearly as the amount of forage kochia increased in the diet (Table 3). We previously reported that intake increased linearly as kochia increased in the diet (Stonecipher et al., 2004). Fill followed the pattern of intake. Forage kochia has the ability to increase CP supplied to livestock in the winter grazing period (Welch and Davis, 1984; ZoBell et al., 2003). Supplementing diets with protein has been reported to increase particulate passage rates (Hess et al., 1994) and also increase forage intake and digestibility (McCollum and Horn, 1990). Passage rate is believed, to an extent, to be responsible for the regulation of intake (Merchen, 1988). In this study, passage rate and intake increased in direct proportion as forage kochia was added to the diets. McCollum and Galyean (1985) and Caton et al. (1988) reported increases of intake in relation to increases in passage rate and decreases in retention time.

Implications

Incorporating forage kochia into a low-quality grass diet affected digestive tract kinetics by increasing the rate of passage and decreasing retention time as the level of kochia increased in the diet. Forage kochia can be used with poor quality forages to help increase digesta turnover rates, which will contribute to increased nutrient intake by livestock grazing low-quality forages.

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Table 1. Composition of trace-mineralized salt block [a]

Item	Concentration
Salt (NaCl) minimum	95.5 %
Salt (NaCl) maximum	98.5 %
Zinc (Zn) minimum	3,500 ppm
Iron (Fe) minimum	2,000 ppm
Manganese (Mn) minimum	1,800 ppm
Copper (Cu) minimum	280 ppm
Copper (Cu) maximum	420 ppm
Iodine (I) minimum	100 ppm
Cobalt (Co) minimum	60 ppm

[a] Ingredients: salt, zinc oxide, ferrous carbonate, manganous oxide, magnesium oxide, copper oxide, calcium iodate, cobalt carbonate, red iron oxide for color.

Table 2. Chemical composition of tall wheatgrass and forage kochia used in diets

Item	Wheatgrass	Kochia
DM, %	94.1	93.6
	------ % of DM ------	
CP	3.6	9.6
NDF	77.7	53.8
ADF	50.6	32.2

Table 3. Influence of forage kochia on digesta kinetics for cattle consuming tall wheatgrass straw. All results reported on a DM basis

	Treatment						Contrast	
Item	0K	25K	50K	75K	SE	T[a]	L[b]	Q[c]
Particulate passage rate, % h^{-1}	1.81	2.00	2.34	2.47	0.181	0.004	<0.001	0.75
Mean particulate retention time, h	97.6	84.1	73.7	68.5	6.07	0.005	<0.001	0.30
Gastrointestinal fill, kg	2.97	3.34	3.16	3.66	0.186	0.15	0.06	0.73
Gastrointestinal fill, %BW	0.84	0.91	0.86	1.00	0.05	0.17	0.08	0.45

[a] T = ANOVA treatment effect.
[b] L = Linear effect.
[c] Q = Quadratic effect

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Effect of feeding range protein supplement on ruminal methylglyoxal production

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ABSTRACT: When beef cows are grazing dormant winter range nitrogen is typically the first limiting nutrient creating an imbalance with fermentable carbohydrate. Under these conditions nitrogen is often supplemented to remedy this deficiency. Methylglyoxal is a highly reactive electrophile produced by bacteria in response to an imbalance of carbohydrate (excess) to nitrogen (limitation) in growth medium. Therefore, presence of methylglyoxal in ruminal contents could be used as a tool to assess the effectiveness of protein supplementation strategies. An experiment was conducted at the Corona Range and Livestock Research Center to determine the influence of protein supplementation on ruminal production of methylglyoxal, ammonia, pH and blood ketones by cows grazing dormant winter range. Treatments consisted of no supplementation (n=4) or 36% CP cottonseed meal based supplement (n=3) fed at 0.45 $kg \cdot cow^{-1} \cdot d^{-1}$ three times weekly. Supplementation regimen had no effect on ruminal ammonia ($P = 0.13$), pH ($P = 0.99$), methylglyoxal ($P = 0.63$) or blood ketone ($P = 0.42$). Blood ketone concentrations were different depending on day, ($P < 0.01$) with detectable ketones increasing as the trial progressed. Ruminal pH decreased ($P < 0.01$) over day during the length of the experiment. Ruminal ammonia tended to decrease by day ($P = 0.06$) with lower values at the completion of the experiment compared to the start. Methylglyoxal increased ($P = 0.05$) during the duration of the experiment being highest on d 5 and undetectable on d 1. This experiment is the first attempt to quantify ruminal methylglyoxal in cows under winter range conditions. Ruminal methyglyoxal concentration may be a more sensitive to ruminal nitrogen and carbohydrate imbalances than the measurement of ruminal ammonia.

Key Words: methylglyoxal, protein, ruminal bacteria

Introduction

The use of the compound methylglyoxal as a marker to asses the nitrogen status of ruminants is a new concept that deserves deeper exploration. In mammals, methylglyoxal is synthesized by the host animal or microorganisms in the digestive tract. Methylglyoxal-producing bacteria use a variety of pathways such as glycolytic bypass, glycerol degradation, and amino acid catabolism to produce methylglyoxal.

The microorganisms that predominate in the rumen are saccharolytic. Carbohydrates, such as cellulose and other polysaccharides, make up most of the ruminant diet and constitute the main substrate available for fermentation. *Prevotella ruminicola* $B_1$4 accounts for as much as 19% of the cultivable bacteria from the rumen (Russel, 1993). When there is a loss of balance between nitrogen and carbon metabolism in the rumen bacterium *Prevotella ruminicola* $B_1$4 produces methylglyoxal *in vitro* (Russell, 1993). Therefore the hypothesis for this experiment is that methylglyoxal production by ruminal bacteria would indicate a loss of balance between carbon and nitrogen metabolism in the rumen.

When ruminants are consuming dormant range, the ruminal microbes may experience growth conditions where carbohydrate is supplied in excess of microbial needs and nitrogen is limiting to fermentation. Under these conditions ruminal bacteria have developed strategies involving futile cycles to spill energy until the carbon to nitrogen ratio favors the synthesis of microbial crude protein (Russell and Cook, 1994).

To overcome dietary inadequacies, cattle are supplemented with protein supplements formulated to meet their nutrient requirements (NRC, 1996). These protein supplements may contain ruminally degradable protein, undegradable protein, or combinations of both. However, prediction of the effectiveness of the supplementation program, in regards to the degradable protein requirement of the microbial population, is based partially on ammonia (NH_3) concentration in ruminal contents.

Ammonia in the rumen is a pool of several inputs and outputs. Ammonia is derived from degradation of dietary protein and dietary NPN, from the hydrolysis of urea recycled to the rumen, and from the degradation of microbial crude protein (NRC, 1996). Ammonia disappears from the rumen pool due to uptake by the microbes, absorption by the microbes, absorption through the rumen wall, and flushing to the omasum. Changes in any of these factors will alter NH_3 concentration in the rumen (Ørskov, 1982). Thus, NH_3 concentration is too dynamic to be a good indicator of the nitrogen status of the ruminal environment.

An *in vivo* method has been developed to test the feasibility of detecting methyglyoxal under extreme nutrient limitations (Lodge-Ivey et al. 2002). However, the idea of using methylglyoxal as a marker for effectiveness of protein supplementation under a typical range supplementation protocol has not been investigated. Therefore, an experiment was conducted at the Corona Range and Livestock Research Center to determine the influence of protein supplementation on ruminal production of methylglyoxal, ammonia, pH and blood ketones by cows grazing dormant winter range.

Materials and Methods

Seven ruminally fistulated English crossbred cows (BW = 590 ± 15 kg) were used in a completely randomized design. Surgical procedures and post-surgical care had been were reviewed and accepted by the New Mexico State University Institutional Animal Care and Use Committee. Treatments consisted of two feeding regimes a positive control (CON) representative of production practices in this region and a strategic supplementation designed to vary with cow demands (VAR). The CON treatment (n=3) consisted of a 36% CP cottonseed meal based pelleted supplement delivered at 0.45 $kg \cdot cow^{-1} \cdot d^{-1}$, prorated to 3 times per week delivery. The VAR treatment (n=4) consisted of provision of the 36% supplement used in CON on an as needed basis. Need was determined by visual assessment of cow body condition change and forage conditions. Due to favorable weather and range conditions during the course of this experiment, animals on the VAR treatment did not receive supplement. Cows on both treatments were assigned to separate pastures and were allowed *ad libitum* access to range forage and water. Additionally, free choice salt and mineral mix was available in the pastures.

Samples of ruminal contents were collected at three week intervals beginning in November, 2004 and ending February, 2005 via a suction strainer (Diamond V, Parma, ID). Three aliquots were collected during each sampling event for analysis of ammonia, methylglyoxal, and VFA (data not reported). Aliquots for analysis of methyglyoxal and ammonia were acidified with 1 mL 6N HCl. All ruminal samples were stored on ice until sampling was complete, transported to the laboratory and frozen (–20^{o} C). An additional aliquot of ruminal fluid was collected for pH determination. A blood sample was collected via coccygeal venipucture into evacuated serum tubes and tested ketone content with a handheld ketone meter. Acidified samples of ruminal fluid were thawed, centrifuged (1,500 x *g*) for 15 minutes, and analyzed for ammonia by the phenol-hypochlorite method (Broderick and Kang, 1980) using 96-well microtiter plate (MRX HD, Dynex Laboratories, Chantilly, VA). Methylglyoxal concentration was determined on acidified ruminal samples by the method of Lodge Ivey et al., (2004).

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) for repeated measures with cow as the experimental unit and compound symmetry covariance structure. The model included effects of sampling day, treatment and their interaction.

Results and Discussion

Supplementation regimen had no effect on ruminal ammonia (P = 0.13), pH (P = 0.99), methylglyoxal (P = 0.63) or blood ketone (P = 0.42). Blood ketone concentrations were different depending on day, (P < 0.01) with detectable ketones increasing as the experiment progressed. Ruminal pH decreased (P < 0.01) over day during the length of the experiment. Ruminal ammonia tended to decrease by day (P = 0.06) with lower values at the completion of the experiment compared to the start. Methylglyoxal increased (P = 0.05) during the duration of the experiment being highest on d 5 and undetectable on d 1. By the end of this experiment the cows were mobilizing body condition indicated by the increase in blood ketone

Mean ruminal ammonia levels for both CON and VAR were low (4.02 vs 2.3 mg/dL). Ruminal NH_3 levels are indicative of the balance between fermentable organic matter and DIP (Horn and McCollum, 1987; Owens et al., 1991). An imbalance of NH_3 and fermentable carbohydrate in the rumen could have a detrimental effect on the microbial population. Satter and Slyter (1974) suggested that 3.6 mM NH_3 N supported maximal microbial growth but that limiting concentrations were perhaps closer to 1.5mM. Russell (1993) demonstrated effects of this phenomenon with *Prevotella rumincola* $B_1$4. When exposed to high concentrations of glucose (50 mM) and low NH_3 (3.6 mM), *P. rumincola* $B_1$4 viable cell number decreased by at least 1,000 fold. This decrease in viability was correlated with an accumulation of methylglyoxal in the supernatant (3 to 4 mM).

In the current study mean levels of methlyglyoxal for the experiment were (0.8 and 1.4 mM ± 0.83 SE) for CON and VAR, respectively did not get as high as levels observed *in vitro* by Russell (1993). However by d 5 mean levels were comparable to those observed *in vitro* (3.7 and 6.9 mM ± 0.12 SE) for CON and VAR respectively. These values indicate that there was an imbalance in fermentable organic matter to ammonia nitrogen in the rumen. It is interesting to note that both treatments had detectable methylglyoxal in the ruminal fluid by d 4 indicating that although the cows on CON treatment were receiving extra DIP it was not enough or was not in the most utilizable form to balance the nutrient content of the rumen. This would support and validate the hypothesis for this study which was when ruminal bacteria experience an imbalance of nitrogen to carbohydrate ratio in the rumen methylglyoxal will be produced to dissipate the imbalance. These data support methylglyoxal concentrations in the rumen as being tool assess nitrogen status of the rumen. This is the first reported attempt to measure methylglyoxal in whole ruminal contents in cows under a commercial management setting. These results indicate that the ruminal microbial population experienced nutrient stress and responded by producing methylglyoxal. These data indicate that methylglyoxal may be a useful tool to assess ruminal nitrogen status. Further research is needed under different dietary conditions to determine the true usefulness of methylglyoxal to assess the effectiveness of protein supplementation strategies.

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EFFECTS OF UNDEGRADABLE INTAKE PROTEIN ON MILK YIELD, DRY MATTER DIGESTIBILITY AND BLOOD UREA NITROGEN IN LACTATING GOATS FED AMMONIATED CORN STOVER

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ABSTRACT: Protein suplementation can improve productivity of ruminants consuming low quality forages by either increased feed intake or by increased efficiency on nutrient usage. An experiment was conducted to evaluate the effects of different levels of UIP on milk yield, dry matter digestibility, and blood urea nitrogen in lactating goats fed ammoniated corn stover. Ten young goats 32.3±2.1 kg initial body weight and 8 to 10 weeks pospartum were used in the experiment. Goats were randomly assigned to one of the three treatments. All goats received a basal diet of 90% ammoniated corn stover (92% DM, 11% CP, 75% NDF) plus 10% alfalfa hay (90% DM, 18% CP, 60% NDF) once a day at 2% BW at 0800 h throughout the trial. Water and salt mineral mix were available at all times. Animals were individually supplemented once daily at 0800 h, receiving 100g of one of the three supplement treatments; low UIP (LUIP; 51% CP, 70% RDP:30% UIP), medium UIP (MUIP; 50.8% CP, 60%RDP:40% UIP), or high UIP (HUIP; 50.7% CP, 50% RDP:50% UIP). On day 19 and day 42 of the treatment period milk production was assessed milking by hand. From day 40 to day 42 fecal collection bags were fitted to the animals to assess total fecal and urine output. On day 43 of the experimental period one blood sample was collected before supplementation and every hour for 6 h after supplementation via jugular venipuncture. Nutrient digestibility, and body weight change were analyzed by analysis of variance for a completely randomized design, while milk yield and blood urea nitrogen where analyzed by split plot analysis of variance. Feed intake was similar (P = 0.43) between treatments while goats in the BP group showed the highest average daily gain (P = 0.5). Milk yield (P = 0.53) and BUN concentration (P= 0.51) were no influenced by supplements. Protein supplementation did not influence nutrient digestibilities between treatments. These results suggest that protein supplementation can diminish body weight loss and support low milk yield in goats consuming ammoniated corn residues.

Key words: goats, protein supplementation, ammoniated straw.

Introduction

Goat population in the Comarca Lagunera (Durango and Coahuila) is around 458 271 heads (SAGARPA, 2004). The main sources of nutrients for most of the herd are native grasses, shrubs, and crop by-products, such as corn stover. Cereal straws are produced in considerable quantities in México, however, due to their low quality represent the most widely underutilized source of energy. Because of their relatively low availability of metabolizable energy crop residues are most efficiently utilized in maintenance rations for non-productive animals (Ward, 1978). However, physical and chemical treatments can improve feed intake and nutrient utilization by ruminants. Use of ammonia gas and urea has attracted considerable attention (Sundstol and Coxworth, 1984) as a mean of adding nitrogen and increasing feed intake and digestibility simultaneously. Hadjipanayiotou et al. (1993) reported an increased nutritive value, voluntary intake, and digestibility in cattle and sheep fed ammoniated crop residues. Because of corn residues might be deficient in protein and energy for lactating ruminants, it will be necessary to supplement these nutrients to prevent deficiencies. An experiment was conducted to evaluate the effects of different quantities of UIP on milk yield, nutrient digestibility, and blood urea nitrogen in lactating goats fed ammoniated corn residues.

Materials and methods

Ten lactating young goats with an average initial body weight of 32.3 ±2.1 kg and 8 to 10 weeks postpartum were used in a completely randomized design to investigate the effect of different quantities of UIP on dry matter digestibility, milk yield, body weight change, and blood urea nitrogen concentration. Corn residue was harvested and grounded in a hammer mill thru a 4 cm screen. Urea (3% measured on air-dry matter) was dissolved in water (0.5 l per kg air-dry straw) and applied on layers (20 cm depth) of known straw weight. The straw was covered with a plastic film, and the edges were sealed with sand. The ensiling period was 15 days at a temperature of 30-35 °C. Before the roughage was offered, it was allowed to aerate for one day to allow for the escape of volatile ammonia.

Feed sub-samples were collected every week and composited for chemical analysis. All goats received a basal diet of ammoniated corn stover (90%; 92% DM, 11% CP, 75% NDF) plus alfalfa hay (10%; 90% DM, 18% CP, 60% FND) once a day at 2% BW at 0800 h throughout the trial. Water and salt mineral mix were available at all times. Animals were individually supplemented once daily at 0800 h, receiving 100 g of one of the three supplement treatments; low UIP (LUIP; 51% CP, 70% RDP:30% UIP), medium UIP (MUIP; 50.8% CP, 60%RDP:40% UIP), or high UIP (HUIP; 50.7% CP, 50% RDP:50% UIP). Goats were weighed in the morning of two consecutive days at the beginning and at the end of the experimental period, to

determine body weight change. Forage intake and refusals were recorded daily and refusals were discarded each morning prior to feeding. Forages were fed following supplemental feeding each morning.

On day 25 and day 41 of the treatment period milk production was assessed milking by hand. From day 36 to day 39 fecal collection bags were fitted to the animals to assess total fecal and urine output. Every 12 h fecal collection bags were removed and replaced on each animal, fecal and urine output was recorded, and thoroughly mixed. Representative sub-samples (10% of total wet weight) were collected and frozen within 1 h after collection at –20 °C for later DM, NDF, and nitrogen determinations. Daily sub-samples were composited by weight (10% wet weight) within goat and treatment. On day 43 of the experimental period one blood sample was collected before supplementation and every hour for 6 h after supplementation via jugular venipuncture. Blood samples were centrifuged at 3000 x g for 20 min at room temperature within 30 min after collection. Serum was harvested and frozen at –20 °C until latter analysis. Serum samples were analyzed for BUN by using an enzymatic procedure (Diagnostic Chemicals Limited, Oxford, Connecticut).

Feed intake, body weight change, dry matter digestibility, and nitrogen retention were analyzed by analysis of variance for a completely randomized design, while milk yield and blood urea nitrogen were analyzed in a split plot analysis of variance. All statistical analysis were performed by using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC).

Results and discussion

Feed intake was similar (P = 0.43) for all goats as shown in table 1, however, a trend to decrease ammoniated roughage intake was observed as the amount of UIP in the supplement increased. Weixian (1995) reported a similar trend in cattle fed ammoniated wheat straw and supplemented with three levels of cottonseed cake. The different quantities of UIP supplemented influenced (P = .05) body weight change in an inconsistent fashion. Goats supplemented with the highest amount of RDP showed the greatest body weight gain with values of 75, 15, and 44 g for treatments LUIP, MUIP, and HUIP respectively. Even though no difference (P= 0.53) was appreciated on milk yield, goats supplemented with the highest amount of UIP produced more milk (433 ml/d) compared with those goats supplemented the lowest quantity of UIP (325 ml/d). These results may indicate animal's priority for milk synthesis and secretion at the expense of body fat reserves. Sparrow et al. (1973) observed the greatest milk yield and also the greatest weight loss in cows receiving a high-protein diet. Serrato-Corona (1998) reported the greatest body weight loss and the highest milk production in postpartum beef cows supplemented with the highest quantity of undegradable intake protein (602 g/d) compared with those animals receiving less amount of UIP and fed sub-maintenance diets. Dry matter and NDF digestibilities were similar (P = 0.29 and 0.43 respectivelly) for all goats as shown in table 2. Effectiveness of protein supplementation in ruminants consuming low digestibility, low protein roughages may be achieved when nitrogen readily degradable to ammonia is fed to satisfy nitrogen ruminal microbes requirements. Protein supplementation may increase forage intake and digestibility when forage protein is less than 7% (Bowman et al., 1995). The failure to improve DM and NDF digestibilities was probably due to the fact that the basal diet contained more than 6% crude protein.

Protein retained in goats receiving different quantities of UIP was similar (P = 0.83) between groups as shown in table 2. Similar nitrogen retention was reported by Serrato et al. (2002) in lactating goats supplemented with different sources of protein. Blood urea nitrogen concentrations were similar (P=0.51) among treatments as shown in Table 1. A positive relationship between increased dietary protein intake and BUN have been reported by several researchers. Blood urea nitrogen is positively correlated with dietary nitrogen in sheep (Preston et al., 1965) and cattle (Preston et al., 1978). Eggum (1970) indicated that at least three factors can influence BUN, namely the quantity and content of protein in the diet, and the time after feeding. Values of BUN found in this trial were in the upper usual normal physiological range, which can be partially explained by the amount of soluble nitrogen applied to the roughage and that in the supplement.

Implications

These results suggest that lactating goats can be maintained with a basal diet of ammoniated corn residues and minimal inputs when good or medium quality forages are not available. Although BUN only responded numerically it can be used as an indicator of nitrogen solubility.

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Table 1. Effects of supplementing increasing quantities of UIP on animal performance and blood urea nitrogen in goats fed ammoniated corn stover.

	Treatment[1]				
Item	LUIP	MUIP	HUIP	P-value	SE[2]
Feed intake, g/día	1132.7	1062.4	942.1	.43	108
Body weight change, g/día	75[a]	15 [b]	44 [a b]	.05	14
Milk yield, ml/día	325	325	433	.53	78
BUN, mEq/L	28.3	23.9	25.5	.51	2.7

[1] LUIP = basal diet + 100 grams of supplement low in undegradable protein (51% CP, 70% RDP:30% UIP) ; MUIP= basal diet + 100 grams of supplement medium in undegradable protein (50.8% CP, 60%RDP:40% UIP); HUIP= basal diet + 100 grams of supplement high in undegradable protein (50.7% CP, 50% RDP:50% UIP).
[2] Standard Error

Table 2. Effects of supplementing increasing quantities of UIP on nutrient intake and retention by lactating goats fed ammoniated corn stover.

	Treatment[1]				
Item	LUIP	MUIP	HUIP	SE[2]	P-value
Intake, g/d					
DM	1040.5	957.9	865.5	99.23	0.43
NDF	747.4	695.6	606.8	76.9	0.42
N	23.6	24.0	23.7	1.7	0.98
Excretion, g/d					
DM	277.0	346.4	265.6	54.6	0.55
NDF	140.7	178.3	122.7	32.5	0.50
N	4.3	5.3	4.2	0.88	0.65
Retained, g/d					
DM	763.5	629.5	618.3	65.9	0.29
NDF	606.7	517.2	501.8	57.4	0.43
N	19.3	18.7	19.7	1.2	0.85
Retention, % of intake					
DM	67.9	59.3	64.0	2.9	0.2
NDF	53.5	48.9	51.9	1.5	0.17
N	1.71	1.78	2.05	0.09	0.07

[1] LUIP = basal diet + 100 grams of supplement low in undegradable protein (51% CP, 70% RDP:30% UIP) ; MUIP= basal diet + 100 grams of supplement medium in undegradable protein (50.8% CP, 60%RDP:40% UIP); HUIP= basal diet + 100 grams of supplement high in undegradable protein (50.7% CP, 50% RDP:50% UIP).
[2] Standard Error

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EFFECTS OF SULFATES IN WATER ON PERFORMANCE OF COW-CALF PAIRS

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ABSTRACT: Data from our laboratory showed water sulfate levels of 3,000 mg/L reduced performance and health of growing steers during summer months. In addition, water averaging 2,600 mg/L in sulfates for cow-calf pairs had little impact on calf growth or milk production, but caused small reductions in cow BW and body condition score (BCS). This experiment was conducted to evaluate the effects of high sulfate water on cow and calf performance, milk production, and reproduction. Ninety-six crossbred, lactating cows (ages 2-13; average calving date of April 14) and their calves were assigned, after stratifying by age, weight, and previous winter management, to one of six pastures (16 cows/pasture). Pastures were randomly assigned to one of two water sulfate levels (three pastures/level). Treatments were low sulfate (LS) water (average 368 +/- 19 mg/L sulfates) or high sulfate (HS) water (average 3,045 +/- 223 mg/L sulfates). The HS water was created by adding sodium sulfate to the LS water. Cows grazed native range and received a conventional mineral supplement ad-libitum from June 3 to August 26, 2004. Water was provided in aluminum stock tanks. Cow 12-h milk production was estimated by the weigh-suckle-weigh method on August 7. Cows were synchronized with a single injection of prostaglandin and bred by natural service. There were no differences in cow weight or BCS change during the trial (P>0.15). Twelve-hour milk production in August was higher (P=0.02) for LS (4.1 kg) than HS (3.4 kg). Calf ADG tended to be higher (P=0.14) for LS (1.16 kg/d) than HS (1.11 kg/d). The percentage of cows that became pregnant during the first 25 days of the breeding season was higher (P=0.06) for LS (81%) than HS (64%), and final pregnancy rates (55-d breeding season) were 92% and 83%, respectively (P=0.20). Sulfate levels averaging 3,045 mg/L in the drinking water of cow-calf pairs during the summer reduced cow milk production and the number of cows bred early in the breeding season.

Key Words: Cows, Sulfates, Water, Performance

Introduction

Our research group continues to evaluate the effects of high sulfate water on cattle, with a goal of defining critical levels of total dissolved solids (TDS) and sulfates in the drinking water. Patterson et al. (2002) reported that water with 3000 mg/L sulfates or greater reduced ADG, DMI, water intake, and gain/feed of growing steers in confinement compared to water with approximately 400 mg/L sulfates. Additional work showed a quadratic decline in ADG, DMI, and gain/feed as sulfates in water for confined steers increased from approximately 400 to 4,700 mg/L (Patterson et al., 2003). These reports also showed that cattle in confinement consuming water with 3000 mg/L sulfates or greater were at a higher risk of polioencephalomalacia (**PEM;** Patterson et al. 2002; 2003). Based on these studies, we have concluded that the critical level of sulfates in the water for growing cattle during the summer months is 3000 mg/L. Since water requirements increase with elevated temperatures (NRC, 1996), this critical level may be different in various environments.

Johnson and Patterson (2004) reported that water with 3,941 mg/L sulfates or greater reduced performance of grazing stocker steers in South Dakota. Few health problems were observed in stocker cattle receiving the high sulfate water over that two-year study. In addition, intermediate levels of sulfates were not tested, so a "critical" level could not be determined. Patterson et al. (2004) reported that water averaging 2,600 mg/L sulfates for cow-calf pairs resulted in reduced cow weights but had little impact on reproduction or calf growth. The objective of this study was to evaluate the effects of sulfates in water averaging 3,000 mg/L for cow-calf pairs grazing native range during the summer on cow and calf performance, milk production, and cow reproduction.

Materials and Methods

The study was conducted from June 3 to August 26, 2004 at South Dakota State University's Cottonwood Range and Livestock Research Station, near Philip, SD. Ninety-six crossbred, lactating cows (ages 2-13 yr; 581 kg) and their calves (average birth date April 14; ages 18–80 days; 82 kg) were assigned, after stratifying by age, weight, and previous winter management, to one of six pastures (16 cows/pasture). Pastures were randomly assigned to one of two water sulfate levels (three pastures/level). Treatments were low sulfate (**LS**) water or high sulfate (**HS**) water. Water was provided daily in aluminum stock tanks (round tanks; approximately 250 cm in diameter).

The LS water was from a rural water system, and the HS water was created by adding sodium sulfate to LS water to a targeted 3,000 mg/L sulfate level. LS water was added to two storage tanks (one provided water for two HS pastures and one provided water for the remaining HS pasture). Sodium sulfate was added to LS water in the storage tanks during the afternoon of each day. Stock tanks were filled the following morning with either LS water or the previously-mixed HS water from the storage tanks. Samples from each water source were taken as stock tanks

were being filled. Water samples were composited weekly and sent to the Water Resource Institute in Brookings, SD for sulfate analysis. A locally available commercial mineral was provided to cows in each pasture ad-libitum (13% Ca; 12% P; 13% salt; 2,000 mg/kg Cu; 8,000 mg/kg Zn).

On June 3 (trial initiation) and August 26 (trial termination), both cows and calves were weighed and cows were assigned a body condition score (**BCS**; 1-9 scale; Richards et al., 1986) by two trained technicians (to the nearest 0.5 of a BCS). Cow-calf pairs were all on LS water and grazed native range prior to trial initiation. Cows and calves were separated and not allowed access to feed or water for approximately 12 h prior to initial weight measurements. At the end of the trial, all cows and calves were placed on LS water for three days prior to final weight measurements. Cows and calves were separated and housed in a drylot without access to feed or water for approximately 12 h prior to final weight measurements.

On August 7, all cows were used to estimate twelve-hour milk production by the weigh-suckle-weigh method (Boggs et al., 1980). In brief, calves were separated from cows at approximately 0800 the day prior to measurements. Calves were returned to dams at 1800, allowed to suckle until content, and again removed. Calves were weighed the following morning at 0600, returned to dams and allowed to suckle until content, and then weighed again. The difference in calf weight prior to and post-suckling was used as an estimate of 12-h milk production. There were two calves in the LS group that did not suckle their dam, so their data were removed from analysis (LS: n = 46; HS: n = 48.

One two-year-old bull was turned into each pasture on July 2. On July 6, cows were given an injection of PGF_{2a} (25 mg i.m. ProstaMate, Phoenix, Scientific, Inc., St. Joseph, MO) to synchronize estrus. Bulls were rotated between pastures within treatment on July 29. Bulls were removed on August 26. Pregnancy was determined by rectal ultrasonagraphy 55 and 88 days following bull turnout. Pregnancies detected at 55 days were determined to be conceived in the first 25 d of the breeding season.

Cow and calf weight and cow body condition score data were analyzed by ANOVA in PROC GLM of SAS (SAS Inst. Inc., Cary, NC) with pasture as the experimental unit. Twelve-hour milk production data were analyzed by ANOVA with animal as the experimental unit. Cow pregnancy rates were analyzed by Chi-Square in PROC GENMOD of SAS, with pasture as the observation and animal as the event within observation.

Results and Discussion

Compiling all weekly water composite sample results revealed the LS water averaged (± standard deviation) 368 ± 19 mg/L sulfates, and the HS treatment averaged 3,045 ± 223 mg/L sulfates. The HS target of 3,000 mg/L was achieved. Patterson et al. (2004) added the sodium sulfate directly to the stock tanks instead of the storage tanks, and they reported that the target sulfate level of 3,000 mg/L was not achieved (average 2,608 ± 408 mg/L). Letting the water set in the storage tanks during the afternoon and overnight after mixing salts may have allowed more of sulfates to go into solution in this experiment.

One cow from the HS treatment died two weeks prior to the end of the experiment. Diagnostics of brain tissue revealed no indication of PEM, but the animal did exhibit high brain sodium levels.

Cow weight change from June 3 to August 26 was not different between treatments (P = 0.17; Table 1). In addition, both groups of cows maintained body condition over the experimental period (P = 0.93; Table 1). Patterson et al. (2004) showed that cows on 2,600 mg/L sulfates had more weight and body condition score loss over the summer than cows on 390 mg/L sulfates. Calves in this study tended to have a lower ADG (P = 0.14) when the cow-calf pair was on HS water (Table 1). This calf weight difference was supported by the HS cows having lower (P = 0.02) 12-h milk production than LS cows (Table 2). Patterson et al. (2004) did not report a significant effect of high sulfate water on calf performance or milk production.

A higher (P = 0.06) percentage of cows on the LS treatment were bred in the first 25 days of the breeding season (81.3%) than were cows on the HS treatment (63.8%). This difference in early-season pregnancy could impact reproduction and weaning weights the following year. Overall pregnancy rates were not different (P = 0.20) between treatments (Table 1).

It is not evident why results varied between this study and those reported by Patterson et al. (2004). The water in the current study was higher in sulfates and more consistent (narrower range) than Patterson el. (2004) reported. In addition, there were more two-year-old cows in the current study (34/96; 5-6/pasture) than in the former study (17/96; 2-3/pasture). Weather patterns and forage conditions are other possible reasons for differences between studies. Indeed, Johnson and Patterson (2004) reported a vegetation type by water quality interaction for ADG in yearling steers.

It is important to note that in the current study treatments were applied in a very specific and rather narrow time frame (one to four months post-calving). If the cattle were exposed to the HS water at different times, influences of physiological state and temperature may cause different responses. For example, at four to six months post-calving, calves would be expected to consume less milk (as a % of BW) and more water, which could make them more directly affected by water sulfates. Finally, the bull to cow ratio used in this study was approximately 1:16. Lower bull to cow ratios could potentially impact reproduction in high sulfate situations.

We conclude that water provided to cow-calf pairs that averaged 3,045 mg/L in sulfates reduced milk production, calf gains, and the percentage of cows bred early in the breeding season.

Implications

High sulfate water had negative impacts on reproduction and calf gains. Grazing cattle receiving high

sulfate water may not have the degree of reduction in gain that cattle in confinement have. Additional work should address whether the effects of high sulfate water on reproduction are due to direct of effects of the water, induced trace mineral deficiencies, or both.

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Table 1. Performance of cow-calf pairs grazing native range and supplied water with low sulfates (average 368 mg/L) or high sulfates (average 3,045 mg/L) during the summer (Least Squares Means)[a]

	Treatment		
Item	Low Sulfate (LS)	High Sulfate (HS)	SEM
Cow initial weight, kg	580	582	7.6
Cow final weight, kg	592	585	9.5
Cow weight change, kg	12	4	7.9
Cow initial body condition score	5.54	5.46	0.088
Cow final body condition score	5.45	5.38	0.122
Cow body condition score change	-0.09	-0.08	0.059
Calf initial weight, kg	82	82	3.1
Calf final weight, kg	180	176	3.7
Calf ADG, kg/d	1.16[b]	1.11[c]	0.019

[a]Trial lasted from June 3 to August 26, 2004 (84 days); Average calving date of April 14.
[b,c]Within a row, means with unlike superscripts differ (P = 0.14).

Table 2. Estimates of twelve-hour milk production using the weigh-suckle-weigh method for cow-calf pairs grazing native range and supplied water with low sulfates (average 368 mg/L) or high sulfates (average 3,045 mg/L) during the summer (Least Squares Means ± SEM)[a]

	Treatment	
Item	Low Sulfate (LS)[a]	High Sulfate (HS)[b]
12-h Milk, kg	4.1 ± 0.22	3.4 ± 0.21

[a]n = 46.
[b]n = 48.

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RUMINAL DEGRADATION OF A CHILE PEPPER BYPRODUCT BY COWS

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ABSTRACT: Six ruminally cannulated Angus cows (635 ± 35 kg) were used in a replicated 3 × 3 Latin square to evaluate ruminal degradation of chile pepper byproduct (**CHILE**; culled chile pods). Cows were randomly assigned to three treatments: 1) corn grain-based diet (**CG**; 66.3% flaked corn), 2) corn silage-based diet (**CS**; 46.5% corn silage), and 3) alfalfa hay-based diet (**AH**; 57.6% alfalfa hay). Diets contained 20% CHILE and were fed twice daily at 1.5% BW/d (DM basis). Periods were 14 d; 10 d for adaptation and 4 d for collections. Dacron bags (10 × 20 cm; pore size 53 ± 10 µm; 18 each for ground CHILE, alfalfa hay standard, and blanks) were incubated in the rumen for 0, 3, 6, 9, 12, 24, 48, 72, and 96 h. In situ degradation of DM and CP for CHILE were separated into fractions A (disappearance at 0 h), B (100%-A-C), and C (residue after 96 h). Rate of degradation (k_d) for fraction B was the slope of the natural logarithms of the residue. Passage rates (k_p) were the slope of log-transformed ruminal concentrations of Yb at 3, 6, 9, 12, 24, 48, and 72 h measured after a dose of labeled CHILE. Fractional pool sizes for CHILE DM were not different ($P > 0.10$) among treatments. Also, fractions A and B for CHILE CP were not different (P > 0.16) among treatments, but fraction C was greater ($P < 0.05$) for CG vs CS. Degradation rates for CHILE DM were greater ($P < 0.05$) for AH (13.2%/h) and lower ($P < 0.05$) for CG (6.6%/h) when compared to CS (10.4%/h). Degradation rates for CHILE CP were not different ($P = 0.79$) for AH (10.0%/h) and CS (9.7%/h), but were lower ($P < 0.05$) for CG (6.0%/h). Passage rates for CHILE were lower ($P < 0.05$) for CG (3.3%/h) when compared CS (4.0%/h), but not different to AH (3.6%/h). Effective degradation (A+B[$k_d/(k_d + k_p)$]) of CHILE CP was similar ($P = 0.50$) for AH (77.5%/h) and CS (78.7%/h), but lower ($P < 0.05$) for CG (70.1%/h). Results indicate that ruminal degradation of CHILE was greater when cows were fed AH and CS than when fed CG. Also, these results imply that degradation of CHILE is similar to other highly digestible fibrous byproducts, and has potential to be incorporated into mixed diets.

Key Words: Culled chile pods, In situ degradation, Cows

Introduction

Byproducts provide an opportunity to reduce feed costs relative to traditional feedstuff commodities for livestock while maintaining performance goals. Chile peppers (*Caspicum anuum*) are a potential byproduct feed for cattle in the southwestern United States. According to National Agricultural Statistics (2004), production of chile peppers exceeds 135,000 tons annually. 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, skins, veins, leaves, seeds, and stalks). Recent research in our laboratory (Cazac et al., 2004) demonstrated that addition of 20% chile pepper byproduct to cattle diets does not negatively impact feed preference. Meija (1995) showed that feeding dairy cows an alfalfa-based diet with 20% chile waste tended to increase feed intake, and Hill and Löest (2003) demonstrated that the energy value of chile pepper byproduct is similar to corn silage. With the exception of these studies, no research has evaluated the digestibility of chile pepper byproducts by cattle. The objective of this experiment was to evaluate the effect of diet type on ruminal degradation of a chile pepper byproduct by cattle.

Materials and Methods

Experimental protocols were approved by the Institutional Animal Care and Use Committee of New Mexico State University.

Experimental Design. Six ruminally cannulated Angus cows (635 ± 35 kg) were used in a replicated 3 × 3 Latin Square. Cows were housed individually in pens (15 × 35 m) where they had partial shading and free access to fresh water. Animals were randomly assigned to one of three diets (Table 1): 1) corn grain-based diet (**CG**), 2) corn silage-based diet (**CS**), and 3) alfalfa hay-based diet (**AH**). Diets contained 20% culled chile pods and were fed twice daily (0700 and 1900) at 1.5% BW/d (DM basis). The chile pepper pods were culled by a processing plant (Biad, Inc., Mesquite, NM) due to inconsistency in size, color, stage of maturity or other factors such as damage to pods. Periods were 14 d, which allowed 10 d for cows to adapt to treatments and 4 d for collection.

In Situ Procedure. Culled chile pods were dried at 55°C, air-equilibrated, and ground (Wiley Mill, Arthur H. Thomas, Philadelphia, PA) to pass a 2-mm screen. Dacron bags (size 10 × 20 cm; pore size 53 ± 10 µm) containing 5 g of substrate were sealed with an impulse heat sealer (Model CD-200; National Instrument Corporation, Baltimore, MD). Fifty-four bags (18 containing ground chile pepper pods, 18 containing ground alfalfa hay, and 18 blanks) were soaked in warm (39°C) water for 20 min and then incubated in the rumen of each cow for 0, 3, 6, 9, 12, 24, 48, 72 and 96 h and removed at a common time. After removal from the rumen, bags were rinsed and then washed (Maytag, Maytag Corporation, NJ) according to procedures described by Mathis et al. (2001). All bags were dried at 55°C and weighed.

Analyses and Calculations. Residue from bags at each incubation time was subsampled and analyzed for DM (100°C for 24 h) and CP (N × 6.25; Flash EA 1112 analyzer, Elantech Technologies, St. Joseph, MI). The extent of ruminal degradation was determined by separating DM and CP into one of three fractions (A, B, and C) determined by their susceptibility to disappearance from the in situ bags. Fraction A was the residue that disappeared at 0 h of incubation, fraction C was the residue remaining after 96 h of incubation, and fraction B was calculated by difference (100%-A-C). Rate of degradation (k_d) was calculated as the slope of the regression of the natural logarithm of DM and CP remaining for fraction B against time.

Table 1. Composition of diets

	Treatment[a]		
Item	CG	CS	AH
Ingredient	---------- % DM ----------		
Alfalfa Hay	10.0	10.0	57.6
Corn Silage	--	46.5	--
Flaked Corn	66.3	20.0	--
Chile Pepper Pods[b]	20.0	20.0	20.0
Wheat Straw	--	--	20.0
Molasses (cane)	1.50	1.50	1.50
Limestone	0.95	0.55	--
Dicalcium P	0.25	0.30	0.30
Salt	0.30	0.30	0.30
Urea	0.70	0.90	--
Nutrient			
NDF	19.6	38.2	54.6
CP	13.9	13.8	14.1
Ca	0.70	0.70	1.15
P	0.34	0.35	0.34

[a]CG = corn grain-based diet; CS = corn silage-based diet; AH = alfalfa hay-based diet.
[b]Nutrient composition: 41 % NDF and 17.5 % CP.

Passage rates (k_p) were determined with Yb-labelled chile pepper byproduct. Dried (55°C) chile pods (300 g) were soaked in 1 L of a 0.3% (wt/vol) $YbCl_3$ solution for 48 h, then washed with water every h for 5 h, and dried at 55°C. Labeled chile was dosed into the rumen via the rumen cannula on d 1 of the 4-d collection period. Rumen samples (250 g) were collected at 3, 6, 9, 12, 24, 48, and 72 h post dosing, dried in a forced-air oven at 55°C, and ground to pass a 2-mm screen. Samples were ashed, solubilized (May and Galyean, 1996), and analyzed for Yb by atomic absorption spectrometry (model 5000, Perkin-Elmer, Norwalk, CT; nitrous oxide-acetylene flame). Ruminal k_p were the slope of log-transformed concentrations of Yb. Effective CP degradation was calculated as $A+B[k_d/(k_d+k_p)]$ as described by Ørskov and McDonald (1979).

Ruminal Sampling. To determine ruminal fermentation characteristics of each dietary treatment, samples of ruminal fluid were collected at 0, 3, 6, 9, and 12 h after feeding on d 14 of each period. Ruminal fluid was strained through four layers of cheesecloth and the pH was measured (Hanna pH meter Model 9042 MP, Van Nuys, CA). A sample of ruminal fluid (10 mL) was acidified with 0.5 mL 6 N HCL in 20-mL polypropylene vials and frozen at -20°C for analysis of NH_3 concentrations using a microplate reader (ELX 808 Ultra Microplate Reader, Bio-Tek Instruments, Inc, Winooski, VT) as outlined by May and Galyean (1996). Also, 10 mL of ruminal fluid was mixed with 2 mL of 25% metaphosphoric acid and frozen until analysis for VFA concentration (May and Galyean, 1996) using gas chromatography (Star 3400, Varian, Walnut Creeck, CA).

Statistical Analysis. Data were analyzed using the MIXED procedure of SAS (v 8.1, SAS Inst. Inc., Cary, NC). For in situ degradation data, the model included period, cow, and diet. Rumen fermentation profiles were analyzed with repeated measures, and the model included period, cow, diet, h, and diet × h. Least square means were separated using pair-wise t-tests if the fixed effects were significant ($P < 0.05$).

Results

Fractional pool size (fractions A, B, C), k_d, and k_p of chile pepper byproduct in the rumen of cows fed CG, CS, and AH are presented in Table 2. Fractions A and B for DM and CP of chile pepper byproduct were not affected ($P > 0.49$) by type of diet offered to cows. However, fraction C for chile pepper DM tended to be greater ($P = 0.10$) for CG and AH versus CS. Also, the C-fraction of chile pepper CP was greater ($P < 0.05$) for CG versus CS, and intermediate for AH. Rates of DM degradation of chile pepper were fastest ($P < 0.05$) for AH, intermediate for CS, and slowest for CG. Similarly, k_d of CP were slower ($P < 0.05$) for CG when compared to AH and CS, but not different ($P = 0.78$) between AH and CS. Passage rates of Yb-labelled chile pepper byproduct were lower ($P < 0.05$) for CG when compared to CS. Effective degradation of chile pepper CP was similar ($P = 0.50$) for AH and CS, but lower ($P < 0.05$) for CG.

For the alfalfa hay standard, fractions A and B for CP were not different among treatments (fraction A = 30.1, 33.3, and 35.1 ± 2.5%; fraction B = 52.4, 52.6, and 18.8 ± 2.5% for CG, CS, and AH, respectively), but fraction C was greater for CG (17.5%) and AH (16.2%) when compared to CS (14.1 ± 0.7%). Crude protein k_d for the standard were slower ($P < 0.05$) for CG (6.5%/h) when compared to CS (13.5%/h) and AH (17.8 ± 0.019%/h).

Ruminal pH was not different ($P = 0.98$) among dietary treatments (Table 3). Ruminal concentrations of NH_3 were lower ($P < 0.05$) for CG and CS when compared to AH. Total concentrations of VFA were similar ($P = 0.32$) for CG and AH, but lower ($P < 0.05$) for CS. However, concentrations of acetate were similar ($P = 0.83$) for CG and CS, but greater ($P < 0.05$) for AH, whereas propionate concentrations were similar ($P = 0.90$) for CS and AH, and greater ($P < 0.05$) for CG. Consequently, ruminal acetate:propionate ratios were lower ($P < 0.05$) for CG versus CS and AH.

Discussion

The results of this experiment indicate that ruminal degradation of chile pepper byproduct (culled chile pods) was lower when cows were fed CG than when fed CS and AH, which is likely due to differences in ruminal fermentation characteristics among the different diets. Although ruminal pH was not different among dietary treatments, lower ruminal acetate:propionate ratios for CG indicate a shift towards non-structural carbohydrate fermentation in the rumen of cows fed CG. Because culled chile pepper pods are a fibrous byproduct (35 to 46% NDF; Hill and Löest, 2003), ruminal fiber digestion may have been impaired among cows consuming CG, impacting the degradation of the DM and CP fraction of culled chile pods.

Table 2. Fractional pool size (A, B, C), degradation rates, and passage rates of culled chile pods in the rumen of cows fed corn grain-, corn silage-, or alfalfa hay-based diets.

	Treatment[a]			
Item	CG	CS	AH	SEM
DM Fraction, %				
A	43.5	46.6	44.7	1.79
B	30.6	29.3	29.2	1.71
C	25.8	24.1	26.1	0.65
DM k_d[b], %/h	6.6[e]	10.4[f]	13.2[g]	1.04
CP Fraction, %				
A	45.6	54.7	52.0	3.20
B	40.1	34.1	34.7	3.39
C	14.2[f]	11.3[e]	13.2[ef]	0.71
CP k_d, %/h	6.0[e]	9.7[f]	10.0[f]	0.72
k_p[c], %/h	3.3[e]	4.0[f]	3.6[ef]	0.24
ED[d] of CP, %	70.1[e]	78.7[f]	77.5[f]	1.27

[a]CG = corn grain-based diet; CS = corn silage-based diet; AH = alfalfa hay-based diet.
[b]k_d = rate of degradation of the B fraction.
[c]k_p = passage rate of Yb-labelled chile pepper byproduct.
[d]ED = effective degradability, A+B[$k_d/(k_d + k_p)$].
[e,f,g]Means within row followed by different superscript letters differ ($P < 0.05$).

Table 3. Ruminal fermentation characteristics of cows fed a corn grain-, corn silage-, or alfalfa hay-based diet.

	Treatment[a]			
Item	CG	CS	AH	SEM
pH	5.9	5.9	5.9	0.018
NH_3, mM	4.7[d]	5.1[d]	10.8[c]	0.9
Total VFA, mM	113.7[c]	93.7[d]	107.5[c]	5.9
Acetate	62.6[d]	63.6[d]	77.0[c]	5.3
Propionate	31.6[d]	15.1[c]	15.4[c]	1.3
Butyrate	14.5	11.5	10.6	1.8
Ac:Pr	2.1[d]	4.7[c]	5.3[c]	0.48

[a]CG = corn grain-based diet; CS = corn silage-based diet; AH = alfalfa hay-based diet.
[b]Ac:Pr = acetate to propionate ratio.
[c,d,e]Means within row followed by different superscript letters differ ($P < 0.05$).

Fractional pool sizes and degradation rates of CP for the alfalfa hay standard used for this experiment were consistent with those reported previously for similar quality alfalfa hay (Mathis et al., 2001). Although statistical comparisons were not conducted, the CP of culled chile pods consistently had a greater fraction A, lower fraction B, but similar fraction C to alfalfa hay standard. Also, k_d for chile pepper CP was consistently lower than that for the alfalfa hay standard. Fractional pool sizes and k_d of CP for chile pepper pods were similar to those reported for corn silage and citrus pulp (NRC, 2001).

Implications

The results of this experiment indicate that ruminal degradation of chile pepper byproduct is greater when cows are fed forage-based diets such as alfalfa hay and corn silage, but lower when cows are fed concentrate-based diets. Also, these results imply that degradation of chile pepper byproduct is similar to other highly digestible fibrous byproducts, and has potential to be incorporated into mixed diets.

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INFLUENCE OF SLICE BALING HAY ON PERFORMANCE OF NEWLY-RECEIVED FEEDLOT STEER CALVES

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ABSTRACT: One hundred and eight beef steers (183.1 ± 1.2 kg initial BW) were used in a completely randomized design to evaluate the effect of baling method on performance and morbidity of newly-received steer calves. A new method of handling alfalfa consists of chopping the alfalfa before baling and after suncuring. Treatments consisted of a 65% concentrate receiving diet containing either 1) ground (Control), or 2) slice (Slice) alfalfa. Steers were fed daily at 0800 at which time animals also received long-stem sudangrass hay the first 7 d. All cattle were monitored daily at 0700 for signs of bovine respiratory disease complex (BRD), including labored breathing, nasal or ocular discharge, anorexia, depression, and lethargy. Animals displaying signs were removed from their pen and were considered morbid if their rectal temperature was ≥ 39.7°C. Steers were weighed on d 0, 16, and 28. Feed, sudangrass hay, or feed plus sudangrass hay intakes were not affected ($P > 0.25$) by treatment. Conversely, ADG from d 0 to 16 was greater ($P < 0.001$) for Slice than Control (0.81, 1.27 ± 0.067 kg/d), and from d 0 to 28 (0.91, 1.23 ± 0.042 kg/d for Control and Slice, respectively). In addition, gain:feed ratio from d 0 to 16 was greater ($P < 0.001$) for Slice than Control (0.25, 0.39 ± 0.021), and from d 0 to 28 (0.24, 0.31 ± 0.013 for Control and Slice, respectively). Moreover, morbidity (44.3, 36.7 ± 3.9%), and retreatment (39.2, 22.2 ± 7.5) tended to be lower ($P < 0.20$) for Slice than Control. Data indicate that Slice alfalfa improves performance of newly-received feedlot cattle.

Introduction

Feedlot cattle may be subjected to a considerable amount of stress during the marketing process and upon arrival at the feedlot. Such sources of stress include weaning (Stookey et al., 1997), marketing and transportation (Loerch and Fluharty, 1999), feed and water deprivation as a result of long hauls (Grandin, 1997), feedlot pens characterized by mud and manure, exposure to a different social dominance order, pathogens (Loerch and Fluharty, 1999) and to a new diet. As a result newly-received cattle typically incur a high incidence of bovine respiratory disease (BRD; Blecha et al., 1984), and low feed intake (Cole, 1996). Low feed intake makes corrections of nutritional deficiencies difficult, which could further compromise immune function (Cole, 1996) and potentially increase susceptibility to infection. Therefore a strategy that improves intake during feedlot receiving might positively impact health and performance.

Roughage source affects intake by changing the energy concentration or by altering rate of ruminal acid production (Galyean and Defoor, 2003). Therefore if a roughage source dilutes energy concentration, an increase in DMI should be expected with animals being fed a concentrate diet. However, if roughage level is above 20%, as on receiving diets, rumen fill will limit intake (Bartle et al., 1994), and weight gain may decrease.

A new method of handling alfalfa is available and consists of chopping alfalfa before baling and after suncuring. It is referred to as slice baling and has been proposed to cause less damage to leaves compared with grinding after baling. Anecdotal information suggests that slice baling alfalfa results in improved quality (greater proportion of leaves), and improved rumen function in feedlot cattle because of fewer fine particles and better uniformity of the stem length. To more closely define the potential value of using slice alfalfa in diets of newly-received cattle, we conducted an experiment to determine if inclusion of slice baled alfalfa during the receiving period would influence feed intake, ADG, feed efficiency and morbidity of beef cattle .

Materials and Methods

One hundred and eight crossbreed steer calves, (183.1 ± 1.2 kg initial BW) were used in a complete randomized design to evaluate effects of baling method on performance and morbidity of newly-received steer calves. Calves were transported 1,014 km from an order buyer facility in Hope, AR to the Clayton Livestock Research Center (CLRC) in Clayton, NM. Steers were processed immediately after arrival, including 1) vaccination with a clostridial antigen (Ultrabac – 7, Pfizer Animal Health); 2) vaccination with an IBR/PI-3-BVD-BRSV vaccine (Bovishield Gold 5 ®(Pfizer); 3) treatment for internal and external parasites (Cydectin®, Fort Dodge Animal Health), and 4) mass medicated (Excede ®, Pfizer). In addition, calves were branded, assigned an individual ear tag, and individual BW was recorded, horns were tipped as needed, and steers calves were surgically castrated. At arrival, calves were individually processed and assigned to one of two treatments: 1) control, ground alfalfa (Control), or 2) Slice alfalfa (Slice). Alternate calves through the chute were assigned to alfalfa treatment, stratified by BW, and assigned to pens (nine steers/pen; six pens/treatment).

Composition of diets is shown in Table 1. Steers calves were fed daily at 0800 at which time animals also received long-stem sudangrass hay the first 7 d. Calves were monitored daily (0700) for signs of bovine respiratory

disease complex (BRD), including labored breathing, nasal or ocular discharge, depression, anorexia, and lethargy. Animals having these signs were removed from their pen for a more thorough examination. When rectal temperature was ≥ 39.7 °C, the animal was medicated with (Baytril100®, Bayer Animal Health). Steer calves were returned to their assigned pen following antibiotic treatment. If rectal temperature decreased within 24-h period but remained ≥39.7°C, the previous sequence of drugs was administered again. If no improvement in health status (continued expression of BRD symptoms and an elevated rectal temperature) was evident within a 24-h period, a second drug sequence was used (Nuflor ® [20 mg/kg of BW]; Schering- Plough Animal Health). The third drug regimen (Albon SR boluses [137.78 mg/kg of BW] and Liquamycin LA 200 [19.84 mg/kg of BW]; Pfizer Animal Health) was administered when no improvement in health (based on previous criteria) was observed 48 h after the second sequence. The animal was considered morbid after the first time it was treated for BRD and was considered retreated if treated two or more times for BRD. The percentage of retreated cattle was defined as the percentage of morbid steers that received medical treatment two or more times.

Body weight and intake measurements were obtained on d 0, 14, and 28. Analysis of variance for a complete randomized design was performed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with pen as the experimental unit.

Results and Discussion

Effects of method of alfalfa baling on performance of newly received-cattle are shown on Table 2. Feed, sudangrass hay, or feed plus sudangrass hay intakes were not affected ($P > 0.25$) by treatment. Conversely, ADG from d 0 to 16 was greater ($P < 0.001$) for Slice than Control (1.27, 0.81 ± 0.067 kg), and from d 0 to 28 (1.23, 0.91 ± 0.042 kg for Slice and Control, respectively). Accordingly, final BW was greater ($P = 0.01$) for Slice compared with Control. In addition, gain to feed ratio from d 0 to 16 was greater ($P < 0.001$) for Slice than Control (0.39, 0.25 ± 0.021), and from d 0 to 28 (0.31, 0.24 ± 0.013 for Control and Slice, respectively). Moreover, morbidity (36.7, 44.3 ± 3.9%) and retreatment (22.2, 39.2 ± 7.5 %) tended to be lower ($P < 0.20$) for Slice than Control.

Animals fed high-concentrate diets typically increase DMI when roughage source dilutes energy concentration. However, when roughage level is above 20%, physical fill limits intake (Bartle et al., 1994). Therefore, a possible explanation for results of this experiment might be that the Control was diluted and Control steers were not able to increase DMI intake to compensate for such dilution due to a ruminal physical fill. However, in order to substantiate this hypothesis, it is necessary to evaluate the alfalfa sources at levels of inclusion that do not limit intake by physical fill. Alternatively, rate of ruminal acid production as a result of roughage physical form might affect various mechanisms, including chewing and rumination with subsequent changes in salivary flow, altered ruminal and intestinal digesta kinetics, and altered site and extent of digestion (Galyean and Defoor, 2003). The increased nutrient availability might have positively affected the immune system which might explain the numeric tendency to reduce morbidity of steers fed the diets containing slice alfalfa as well the improvements in ADG.

Implications

Data indicate that Slice alfalfa can successfully be used as roughage in receiving feedlot diets. Slice alfalfa improved performance and potentially health of newly-received cattle

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Table 1. Composition of experimental diets.

	Alfalfa [a]	
Item	Control	Slice
Ingredients (% DM)		
Ground alfalfa	33.0	0.0
Slice alfalfa	0.0	33.0
Steam flaked-corn	56.13	56.13
Cottonseed meal	2.13	2.13
Cane molasses	5.44	5.44
Yellow grease	1.56	1.56
Limestone	0.47	0.47
Dical	0.62	0.62
Salt	0.27	0.27
Urea	0.58	0.58
Premix [b]	0.79	0.79
Analyzed composition %, DM basis		
CP	14.92	14.58
NDF	27.37	21.81

[a] Control = regular alfalfa hay ground to pass through a 5.08 cm screen; Slice = alfalfa was chopped after removing from the wind row but before being compressed into a baled.

[b] Cotton seed meal-based premix supplied (DM basis): 4.25% vitamin A (30,000 IU/g, 10.40% vitamin E (125,000 IU/g), 13.38% selenium premix (600 ppm), 14.23% ferrous sulfate, 10.19% manganese sulfate, 20.38% zinc sulfate, 21.66% magnesium oxide, 2.34% copper sulfate, 0.21% cobalt carbonate, 0.21% ethylenediamine dihydroiodide, and 2.76% mineral oil.

Table 2. Effects of method of alfalfa baling on performance of Newly-received steer calves.

Item	Alfalfa		SEM	P- value
	Control	Slice		
Steers, no./pen (pen)	9(6)	9(6)		
Initial BW, kg	183.1	183.2	1.72	0.95
Final BW, kg	208.6	217.6	2.11	0.01
Daily DM intake, kg/steer				
Days 0 – 16	2.69	2.68	0.13	0.98
Days 17 – 28	4.67	4.85	0.11	0.25
Days 0 – 28	3.96	4.05	0.11	0.57
Hay Intake, kg/steer				
Days 0 – 7	1.81	1.85	0.02	0.86
Feed plus hay intake, kg/steer				
Days 0 – 16	3.26	3.26	0.13	0.98
Days 0 – 28	3.86	3.94	0.11	0.63
Daily gain, kg				
Days 0 – 16	0.81	1.27	0.07	0.001
Days 17 – 28	1.04	1.17	0.08	0.28
Days 0 – 28	0.91	1.23	0.04	0.001
Gain to Feed Ratio				
Days 0 – 16	0.25	0.39	0.02	0.001
Days 17 – 28	0.22	0.24	0.02	0.45
Days 0 – 28	0.24	0.31	0.01	0.002
Health[a]				
Morbidity, %	44.3	33.7	3.94	0.20
Retreatment, %	39.2	22.2	7.5	0.14

[a] Control = regular alfalfa hay ground to pass through a 5.08 cm screen; Slice = alfalfa was chopped after removing from the wind row but before being compressed into a baled.

[b]An animal was considered morbid if it was treated for bovine respiratory disease. Retreatment was calculated as number of cattle treated two or more times for bovine respiratory disease/total number of cattle treated at least once for bovine respiratory disease.

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EVALUATION OF FEED EFFICIENCY TRAITS IN GROWING BULLS AND RELATIONSHIPS WITH FEEDING BEHAVIOR AND ULTRASOUND CARCASS ESTIMATES

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ABSTRACT: Objectives of this study were to characterize feed efficiency traits and examine phenotypic correlations with performance, ultrasound, and feeding behavior traits in growing bulls. Individual DMI and feeding behavior were measured in Angus (n = 214) and Brangus (n = 26) bulls (initial BW 368.4 ± 46.1 kg) fed a corn silage based diet (ME = 2.78 Mcal/kg) using a GrowSafe feeding system. DMI and 14-d BW were measured for 84 and 91 d in test 1 and 2, respectively, and ultrasound subcutaneous fat depth (BF) measured at start and end of each test. Residual feed intake (RFI) and residual gain efficiency (RGE) were computed as the residuals from appropriate linear regression models involving DMI, ADG and mid-test $BW^{.75}$ (MBW). Partial efficiency of growth (PEG) was computed as ADG divided by DMI for growth. Overall ADG and DMI were 1.44 (SD = 0.26) and 8.51 (SD = 1.10) kg/d in test 1 and 1.73 (SD = 0.28) and 10.07 (SD = 1.35) kg/d in test 2, respectively. RFI was correlated with DMI (0.59) and FCR (0.53), but not ADG or MBW. Bulls with low RFI (< 0.5 SD) consumed 15% less DMI and had 16% lower FCR than bulls with high RFI (> 0.5 SD). PEG was strongly correlated with DMI (-0.51) and RFI (-0.85), but moderately correlated with ADG (0.29) and MBW (-0.25). Bulls with low RFI had favorable PEG (0.36 vs 0.26) compared to bulls with high RFI. RGE was correlated with RFI (-0.61), but was strongly correlated with ADG (0.74). Gain in BF was correlated with ADG (0.31) and DMI (0.29), and tended ($P < 0.10$) to be correlated with RFI (0.11). Feeding duration was not correlated with DMI or ADG, but was correlated with RFI (0.41) and PEG (-0.20). Feeding frequency was not correlated with DMI or ADG, but was correlated with RFI (0.17). Bulls with low RFI had lower ($P < 0.01$) feeding duration and frequency than bulls with high RFI. Compared to other feed efficiency traits examined in this study, RFI will facilitate selection for improved feed efficiency with minimal responses in growth or composition traits.

Key Words: residual feed intake, beef cattle, feeding behavior

Introduction

Cost of feeding animals is the largest expense to the beef industry, particularly the cow/calf enterprise with approximately 65% of the total feed requirements being used to maintain the cowherd (Arthur et al., 2001b). Thus, a large improvement in profitability could be realized by reducing the amount of feed required for maintenance (i.e. improved feed efficiency). The typical efficiency trait measured is feed conversion ratio (FCR), but FCR is negatively correlated with growth and mature size. Selection against FCR would most likely result in increased mature cow size and thereby increased feed requirements for maintenance (Herd and Bishop, 2000).

Arthur et al. (2001b) suggested that residual feed intake, which is the deviation between actual feed intake and predicted feed intake calculated by linear regression of feed intake on growth rate and body size, would be an improved measure of feed efficiency due to independence from growth traits. These researchers have demonstrated that residual feed intake is moderately heritable and genetically independent of growth traits. Herd and Bishop (2000) demonstrated adequate potential for selection against RFI to improve FCR and efficiency of maintenance energy expenditure without increasing mature cow size.

Recent research has shown feeding behavior to be related to feed intake and growth rate (Schwartzkopf-Genswein et al., 2002). Growth rate is negatively correlated to FCR, thus feeding behavior may be correlated with FCR. Schwartzkopf-Genswein et al. (2002) demonstrated that FCR was negatively correlated with feeding duration; therefore, RFI may also be correlated with feeding behavior traits.

Residual feed intake is negatively correlated to carcass lean composition (Herd and Bishop, 2000). Fox et al. (2004) also found that bulls with low RFI had lower subcutaneous fat depth than those with high RFI. The objectives of this study were to characterize feed efficiency traits and examine phenotypic correlations with performance, ultrasound body composition, and feeding behavior traits in growing bulls.

Materials and Methods

Data from two postweaning performance tests conducted at the Beef Development Center in Millican, TX with Angus and Brangus purebred bulls were used in this study. The first test (n = 99 Angus and 16 Brangus) was initiated in June 2004, whereas, test 2 (n = 115 Angus and 10 Brangus) was initiated in November 2004. Bulls were fitted with a radio frequency transponder tag and adapted to the test diet and feeding system for a minimum of 28 d before the start of the tests. The test diet (2.78 Mcal ME/kg DM) consisted of 49% cracked corn, 38.5% corn silage, 5% cottonseed meal, 3% molasses and 4.5% supplement and was fed ad libitum twice daily.

To measure feed intake and feeding behavior traits, bulls were placed into one of two pens each equipped with nine feed bunk units (GrowSafe Systems Ltd., Airdrie, AB). GrowSafe Data Acquisition software was used to record feed intake and feeding behavior data for 84 and 91 d during test 1 and 2. Daily feed intake and feeding behavior traits were computed using GrowSafe Feed Intake Analysis software. Bulls were weighed at 14-d intervals, and ultrasound measurements of subcutaneous fat depth (**BF**) and longissimus muscle area (**LMA**) obtained at the start and end of each test. End-of-test LMA data was not available for test 2, thus LMA data were not considered in the analysis of this study. Hip height (**HH**) and scrotal circumference (**SC**) were measured at the end of each test.

Growth rates of individual bulls were modeled by linear regression of BW against day on test using the regression procedure of SAS (SAS Inst., Cary, NC). These regression coefficients were used to compute metabolic BW (**MBW**; mid-test $BW^{.75}$) during the 84- and 91-d feed intake measurement periods for test 1 and 2, respectively. Moisture analyses of feed ingredient samples (composites of weekly samples) were used to compute average daily DMI from feed intake data.

Four different feed efficiency measures were derived from growth and DMI traits for each bull. Feed conversion ratio (**FCR**) was computed as the ratio of daily DMI to ADG. Partial efficiency of growth (**PEG**) was computed as the ratio of ADG to the difference between actual DMI and predicted DMI for maintenance (Arthur et al., 2001a). The predicted DMI to meet maintenance requirements was calculated as 0.077*MBW ÷ NEm concentrations of the test diets.

Residual feed intake (**RFI**) was computed as the deviation of actual DMI from DMI predicted to meet growth and maintenance energy requirements (Koch et al., 1963). For individual bulls, predicted DMI was derived from a phenotypic regression model of actual DMI on ADG and MBW (Arthur et al., 2001a). This model was fitted separately for each test using the Proc GLM procedure of SAS (R^2 = 0.68 and 0.65 for test 1 and 2, respectively). Within test, RFI was calculated as actual DMI minus predicted DMI. Residual gain efficiency (**RGE**) was computed as the deviation between actual ADG and ADG predicted from MBW and DMI as described by Koch et al. (1963). A separate regression model of ADG on MBW and DMI was fitted for each test (R^2 = 0.47 and 0.43 for test 1 and 2, respectively), and RGE for individual bulls computed as actual ADG minus predicted ADG.

To further characterize RFI, bulls were separated into low, medium and high RFI groups that were < 0.5 SD, ± 0.5 SD, and > 0.5 SD, respectively, from the mean RFI of 0.0 kg/d. RFI SD were 0.62 and 0.80 kg/d for test 1 and 2, respectively. Least squares procedures (PROC GLM of SAS) were used to examine effects of RFI group on performance, feed efficiency, ultrasound and scrotal circumference traits. The statistical model included the fixed effects of RFI group, breed, test and all significant (P > 0.10) interaction terms. The effects of breed on traits examined in this study will not be presented due to limited number of Brangus bulls in the two tests. Phenotypic correlations among traits were determined using PROC CORR of SAS with the partial correlation option used to adjust for fixed effects of breed and test.

Results and Discussion

Summary statistics are presented in Table 1 for the two performance tests. The bulls in test 1 were approximately one mo younger, but had similar initial BW compared to bulls in test 2. Numerically, bulls in test 2 had 20% higher ADG and consumed 18% more feed than bulls in test 1. These differences were likely due to divergent environmental conditions (25 vs 10 °C mean temperature during test 1 and 2, respectively) and(or) genetic background of bulls between the two tests. The phenotypic SD for feed efficiency traits in Table 1 were similar to those reported in previous studies with growing bulls (Arthur et al., 2001a,b; Fox et al., 2004; Schenkel et al., 2004).

Table 1. Summary statistics (mean ± SD) for traits measured during the two postweaning performance tests with Angus and Brangus bulls

Trait[a]	Test 1	Test 2
AGE, d	261.2 ± 25.1	289.6 ± 33.5
Initial BW, kg	368.7 ± 43.4	368.3 ± 48.0
Final BW, kg	489.4 ± 48.3	525.6 ± 58.7
ADG, kg/d	1.44 ± .26	1.73 ± .28
DMI, kg/d	8.51 ± 1.10	10.07 ± 1.35
RFI, kg/d	0.00 ± .62	0.00 ± .80
RGE, kg/d	0.00 ± .19	0.00 ± .22
PEG	0.32 ± .05	0.29 ± .05
FCR, DMI/ADG	6.05 ± .96	5.91 ± .79
Final BF, cm	0.66 ± .19	0.61 ± .19
Final HH, cm	126.2 ± 4.66	127.9 ± 4.34
Final SC, cm	35.0 ± 2.61	37.2 ± 3.03
DUR, min/d	109.4 ± 24.1	121.9 ± 20.9
FREQ, events/d	5.03 ± .66	4.91 ± .55
RATE, g DM/min	80.8 ± 17.1	84.9 ± 18.0

[a]AGE = age at start of test; RFI = residual feed intake; RGE = residual gain efficiency; PEG = partial efficiency of growth; FCR = feed conversion ratio; BF = subcutaneous fat depth; HH = hip height; SC = scrotal circumference; DUR = feeding duration; FREQ = feeding frequency; RATE = Eating rate.

Phenotypic correlations among growth and feed efficiency traits are presented in Table 2. Dry matter intakes were strongly correlated with ADG and MBW, and are consistent with phenotypic and genetic correlations previously reported with growing calves (Arthur et al., 2001a,b; Schenkel et al., 2004; Fox et al., 2004; Nkrumah et al., 2004). Strong phenotypic correlations (> 0.50) were found among the four feed efficiency traits measured in this study. Residual feed intake was strongly correlated with DMI, but not with ADG or MBW, as the use of linear regression to compute this trait forces RFI to be phenotypically independent of its component traits. In this study, RFI ranged from –2.26 (most efficient) to 2.26 kg/d (least efficient). Bulls with

low RFI (< 0.5 SD) consumed 15% less feed than bulls with high RFI (> 0.5 SD) even though there were no differences in ADG and BW between low and high RFI bulls (Table 4). In general, RFI has been shown to be genetically independent of growth and body size (Arthur et al., 2001a,b; Schenkel et al., 2004) in growing bulls.

Residual feed intake was strongly correlated (-0.85) with PEG in a favorable direction. Bulls with low RFI had a higher PEG than bulls with high RFI (0.36 vs 0.26 ADG/DMI for growth). Corresponding phenotypic correlations reported by Arthur et al. (2001b) and Nkrumah et al. (2004) were -0.65 and -0.89 in growing bulls and steers, respectively. Arthur et al. (2001b) reported that the genetic correlation between RFI and PEG was -0.94 suggesting that these two feed efficiency traits are highly related. This is not surprising in that both of these traits attempt to partition variation in feed intake into maintenance and growth components. In this study, PEG was highly correlated with DMI (-0.51), but less so with ADG (0.29) and MBW (-0.25). Arthur et al. (2001b) and Nkrumah et al. (2004) reported similar phenotypic correlations between PEG and these production traits. Thus, responses to selection for PEG may not be as independent of growth and body size as responses to selection for RFI.

Table 2. Partial correlations[a] among growth, feed intake and measures of feed efficiency in growing bulls

Trait[b]	ADG	DMI	RFI	RGE	PEG	FCR
MBW	**0.26**	**0.64**	-0.00	-0.04	**-0.25**	**0.23**
ADG		**0.61**	-0.02	**0.74**	**0.29**	**-0.67**
DMI			**0.59**	-0.05	**-0.51**	0.13
RFI				**-0.61**	**-0.85**	**0.53**
RGE					**0.83**	**-0.92**
PEG						**-0.83**

[a]Correlations in bold are different from zero at $P < 0.05$.

[b]RFI = residual feed intake; RGE = residual gain efficiency; PEG = partial efficiency of growth; FCR = feed conversion ratio; MBW = mid-test metabolic body weight.

In contrast to the lack of a correlation between RFI and ADG, and a weak correlation between PEG and ADG, strong phenotypic correlations existed between ADG and FCR (-0.67) and RGE (0.74). Likewise, strong genetic correlations between FCR and ADG (> -0.50) have been reported in recent studies (Arthur et al., 2001a,b; Schenkel et al., 2004). Residual feed intake was strongly correlated with FCR (0.53) and RGE (-0.61). Bulls with low RFI had more ($P < 0.01$) efficient RGE (0.15 vs -0.13 kg/d) and FCR (5.4 vs 6.4) than bulls with high RFI. The negative correlations between ADG and FCR and RGE suggest that applying selection pressure against these feed efficiency traits may lead to increases in growth rate and cow mature size, and thus increases in feed requirements for cow maintenance (Herd and Bishop, 2000).

Final BF and gain in BF were positively correlated with ADG and DMI (Table 3), which is in agreement with Schenkel et al. (2004). Gain in BF tended ($P < 0.10$) to be correlated to RGE (0.12), but not with FCR or PEG. In contrast, Nkrumah et al. (2004) reported a positive correlation between FCR and gain in BF. Final BF and gain in BF tended ($P < 0.10$) to be positively correlated with RFI. A number of studies have reported positive correlations between RFI and carcass fat traits (Arthur et al., 2001b; Fox et al., 2004; Nkrumah et al., 2004; Schenkel et. al., 2004). In this study, bulls with low RFI had numerically less final BF (0.56 vs 0.61 cm) and gain in BF (0.25 vs 0.30 cm) compared to high RFI bulls, respectively (Table 4). Likewise, Fox et al. (2004) reported that low RFI bulls tended ($P < 0.10$) to have less final BF than high RFI bulls (0.53 vs 0.58 cm, respectively). These results suggest that adjusting RFI for variation in carcass composition may be warranted.

Table 3. Partial correlations[a] among measures of feed efficiency and feeding behavior, scrotal circumference, and ultrasound fat traits in growing bulls

Trait[b]	ADG	DMI	RFI	RGE	PEG	FCR
AGE	-0.06	**0.24**	0.03	**-0.22**	**-0.22**	**0.30**
DUR	0.08	0.13	**0.41**	-0.12	**-0.20**	-0.01
FREQ	0.04	-0.03	**0.17**	-0.00	-0.02	-0.09
RATE	**0.29**	**0.47**	-0.02	0.08	-0.12	0.08
Final HH	**0.24**	**0.37**	-0.08	0.08	-0.05	0.06
Final SC	**0.24**	**0.25**	-0.03	**0.14**	-0.03	-0.07
Final BF	**0.25**	**0.41**	0.10	0.02	**-0.14**	0.04
BF gain	**0.29**	**0.31**	0.11	0.12	-0.05	-0.08

[a]Correlations in bold are different from zero at $P < 0.05$.

[b]RFI = residual feed intake; RGE = residual gain efficiency; PEG = partial efficiency of growth; FCR = feed conversion ratio; AGE = day of age at start of test; DUR = feeding duration; FREQ = feeding frequency; RATE = Eating rate; SC = scrotal circumference; HH = hip height; BF = subcutaneous fat depth.

Age of bulls at the start of the test was phenotypically correlated with DMI, RGE, PEG and FCR, but not RFI (Table 3). Likewise, initial BW was correlated with DMI (0.55), RGE (-0.23), PEG (-0.33) and FCR (0.41), but not RFI. These phenotypic correlations suggest that younger bulls (lighter BW) were more efficient as measured by RGE, PEG or FCR. In contrast, variation in age and BW at the start of the test did not influence RFI. Nkrumah et al. (2004) also found that initial age and BW affected other feed efficiency traits but not RFI. These results suggest that RFI may be less affected by pretest management conditions compared to other feed efficiency traits (Schenkel et al., 2004; Herd and Bishop, 2000).

Final HH and SC were correlated with ADG and DMI (Table 3), which are similar to the results of Schenkel et al. (2004) and Nkrumah et al. (2004). Final SC was not correlated with RFI, PEG or FCR, but was correlated with RGE (0.14), suggesting that selection for improved RGE would tend to increase SC.

Feeding duration and frequency were not correlated with DMI or ADG, however, eating rate was correlated with both DMI (0.47) and ADG (0.29; Table 3). Schwartzkpf-Gensein et al. (2002) reported positive

correlations between feeding duration and DMI (0.38) and ADG (0.14). RFI was positively correlated with both feeding duration (0.41) and frequency (0.17). Bulls with low RFI had lower (P < 0.01) feeding duration and frequency than bulls with high RFI (Table 4). Cammack et al. (2005) reported positive genetic correlations between RFI and feeding duration (0.22) and frequency (0.20) in growing lambs. Feeding duration was correlated with PEG (-0.20), but not RGE or FCR. Feeding frequency and eating rate were not correlated with RGE, PEG or FCR.

Implications

Residual feed intake is a moderately heritable feed efficiency trait that is independent of growth traits. Feeding duration and frequency were phenotypically correlated with RFI, suggesting that feeding behavior traits may be predictive of RFI. Compared to other feed efficiency traits examined in this study, RFI was minimally affected by initial age and BW, suggesting that RFI may be a more robust feed efficiency trait to use in selection programs.

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Table 4. Characterization of performance, ultrasound composition, and feeding behavior traits in growing bulls with low, medium and high residual feed intake (RFI).

Trait[a]	Low RFI (n = 79)	Med RFI (n = 88)	High RFI (n = 73)	SE	P-value
Growth Traits					
Initial BW, kg	364.3	361.9	360.3	6.46	.86
Final BW, kg	505.3	502.7	500.0	7.55	.83
Daily gain, kg/d	1.61	1.61	1.59	0.04	.92
Final hip height, cm	129.6	128.6	128.6	0.59	.24
Feed Efficiency Traits					
Dry matter intake, kg/d	8.52[a]	9.31[b]	10.03[c]	0.15	<.0001
Residual feed intake, kg/d	-0.78[a]	0.03[b]	0.83[c]	0.05	<.0001
Residual gain efficiency, kg/d	0.15[a]	0.01[b]	-0.13[c]	0.02	<.0001
Partial eff. of growth, ADG/DMI for growth	0.36[a]	0.30[b]	0.26[c]	0.01	<.0001
Feed conversion ratio, DMI/ADG	5.39[a]	5.84[b]	6.44[c]	0.11	<.0001
Ultrasound and Scrotal Circumference Traits					
Final subcutaneous fat depth, cm	0.56	0.60	0.61	0.03	.20
Gain in subcutaneous depth, cm	0.25	0.25	0.30	0.03	.25
Final scrotal circumference, cm	36.19	35.58	35.95	0.40	.38
Feeding Behavior Traits					
Feeding duration, min/d	110.9[a]	120.8[b]	131.3[c]	2.90	<.0001
Feeding frequency, events/d	4.83[a]	5.04[b]	5.07[b]	0.08	.02
Eating rate, g DMI/min	80.1	80.0	78.8	2.47	.89

[a]Means with different superscripts in the same row differ (P < 0.05).

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DIETARY ENERGY SOURCE IMPACTS INSULIN SENSITIVITY IN FINISHING STEERS

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ABSTRACT: Angus (n = 8; 210 kg) and 7/8 Wagyu (n = 8; 174 kg) steers were used to test effects of dietary energy source on adipose tissue metabolism and insulin sensitivity. Steers were assigned to either a grain-based (**CORN**) or hay-based (**HAY**) diet to gain .85 kg/d and .72 kg/d, respectively. Steers were fed to similar final BW. At slaughter, subcutaneous (**SQ**) and intramuscular (**IM**) adipose samples were collected. Portions of SQ and IM were cultured with ^{14}C-acetate to quantify fatty acid (**FA**) synthesis, or ^{14}C-glucose to assess the production of $C0_2$ and lactate in the presence of 0 or 500 ng/ml insulin. Remaining samples were used to evaluate glycolytic intermediate concentrations as indicators of glucose flux. Data were analyzed as a split plot with breed, diet, and their interaction in the main plot; and tissue, insulin, tissue by insulin, and their interactions with main plot effects in the subplot. Breed did not affect responses ($P > 0.19$), nor interact with diet ($P > 0.18$). Tissue glucose was 28% greater in steers fed CORN ($P = 0.01$) and 264% greater in IM vs. SQ ($P < 0.01$). Glucose-6-P and fructose-6-P were similar across diets ($P > 0.46$) and tissues ($P > 0.07$). Diet did not impact acetate incorporation into FA ($P = 0.42$). Diet by insulin interactions existed for glucose utilization ($P < 0.05$). Insulin did not increase conversion of glucose to CO_2 or lactate in steers fed HAY, but resulted in a 103% increase in glucose conversion to CO_2 and a 50% increase in glucose conversion to lactate in steers fed CORN. SQ had 37% greater glucose oxidation than IM ($P = 0.04$), and 290% greater acetate incorporation into fatty acids ($P = 0.04$). No tissue differences were observed for glucose conversion to lactate ($P = 0.99$). These results suggest that feeding HAY limited both glucose supply and tissue capacity to increase glucose utilization in response to insulin without altering acetate synthesis to FA. Because SQ consistently utilized more acetate and oxidized more glucose than IM, these results suggest that HAY diets may alter IM metabolism with less impact on SQ.

Key Words: Adipose, Cattle, Insulin

Introduction

Beef carcass value is influenced by the quantity and distribution of adipose tissue. Increasing intramuscular adipose tissue increases carcass quality grade, and thus value, while increasing subcutaneous adipose tissue reduces carcass value due to increased waste. Production systems impact the distribution and quantity of carcass fat. Production systems utilizing high proportions of roughage typically result in lower value carcasses than those using high concentrate strategies (Schaake et al., 1993) and those that increase subcutaneous fat deposition excessively increase production costs while decreasing carcass value (Wertz et al., 2001). Elucidation of the metabolic control of caloric partitioning between subcutaneous and intramuscular adipocytes could lead to development of production solutions that enhance production efficiency and market value. Previous studies have demonstrated substrate preference differences among these fat depots (Smith and Crouse, 1984). Other studies have shown dietary impacts on whole body insulin sensitivity and energy partitioning (Waterman et al., 2003). The objective of this study were to evaluate the effects of different energy sources in steer finishing diets on metabolism and insulin sensitivity in subcutaneous and intramuscular adipose tissues.

Materials and Methods

Eight Wagyu crossbred (7/8 Wagyu or higher) and 8 Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal bermuda grass hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas A&M University Research Center, McGregor. Four steers of each breed type were assigned to receive a high-energy, corn-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 7.5% molasses, 0.96% limestone, 0.56% trace mineral salt, and 0.08% vitamin premix (**CORN**) fed ad libitum for 16 mo. Feeding this diet resulted in an average gain of .85 kg/d. The remaining 4 steers of each breed type were offered coastal bermuda grass hay *ad libitum*, supplemented daily with non-protein nitrogen in a cooked molasses carrier, and an amount of the corn-based diet to gain 0.72 kg/d (**HAY**). The hay-fed steers were fed for 20 mo after weaning. The average initial weights for Wagyu and Angus steers were 174 kg and 210 kg, respectively. Targeted final body weights were 650 kg for steers fed for either 16 mo on corn or 20 mo on the hay-based diet. Although diet and time-on-feed were confounded in the production portion of the trial, steers were fed at to BW constant endpoints. Therefore, it is arguable that retained energy was similar between treatments, and thus differences in time-on-feed were required due to energy concentration differences between diets.

After being fed for their respective time periods, steers in each group were slaughtered on two consecutive days at the Rosenthal Meat Science and Technology Center, Texas A&M University. A section of longissimus dorsi muscle (**LM**) between the 5th and 8th thoracic ribs was

removed immediately following hide removal (approximately 20 min postmortem). The LM section and associated subcutaneous (**SQ**) and intramuscular (**IM**) adipose tissue were immediately placed in 1x Krebs-Henseleit bicarbonate buffer (pH = 7.4) with 5 m*M* glucose at 37°C and transported to the laboratory. Within 20 min, SQ and IM tissue samples were dissected from the LM section. Five g of each tissue sample was immersed in liquid nitrogen, and another 50-100 mg of tissue was used immediately for lipogenesis and glucose metabolism incubations.

Lipogenesis from acetate procedures were followed according to Page et al. (1997). Glucose metabolism was conducted as described by Espinal et al. (1983). Briefly, 50-100 mg of adipose tissue was incubated in either a 10 m*M* sodium acetate, 10 m*M* glucose, 5x Krebs-Henseleit and 20 m*M* HEPES buffer (pH 7.40), and 1 µCi [U-^{14}C] acetate or a 10 m*M* glucose, 5x KHB and 20 m*M* HEPES buffer and 1 µCi [U-^{14}C] glucose for 2 hr at 37°C. Bovine insulin (0 or 500 ng/mL) was also added to those flasks receiving glucose. All reactions were stopped by adding 3 mL of 5% trichloroacetic acid. Measurement of [1-^{14}C] acetate incorporation into fatty acids was conducted as described by Page et al. (1997). Determination [1-^{14}C] glucose carbon into CO_2 was preformed according to Smith (1983), and the remaining glucose carbon recovery in the form of lactate was followed according to the method of Smith and Freeland (1981). It is important to note that this method of lactate recovery also traps pyruvate and other carboxylic acids. All radioactivities were counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA). Glucose, G-6-P, and F-6-P were measured using assay systems described by Bergmeyer (1974), with modifications as conducted by Rhoades et al. (2005).

Data were analyzed as a split plot. Diet, breed, and their interaction served as main plot effects, and were tested using steer nested within breed × diet as the error term. Tissue type, insulin addition (when appropriate), tissue × insulin, diet × insulin, diet × tissue, and diet × insulin × tissue were sub-plot effects, and were tested with residual mean square as the error term. Least square means and estimates of variability are consistent with respect to appropriate error terms. When overall F-tests were significant, means were separated using Fisher's LSD. All analyses were performed using the General Linear Models procedures of SAS v.9 (SAS Institute, Cary, NC, USA).

Results and Discussion

Breed type (Table 1) had no affect on the conversion of glucose to CO_2 (P = 0.19), or lactate (P = 0.58) nor did breed have an affect on acetate incorporation into fatty acids (P = 0.96). Breed did not interact (P > 0.18) with other effects tested. In a companion study, breeds were similar in marbling, although Wagyu were numerically greater, and Angus had greater subcutaneous fat thickness (data not shown).

Tissue glucose concentrations (pooled across tissue type) were 28% greater (P < 0.01) in steers fed CORN (1.52 µmol/g) vs. HAY fed steers (1.19 µmol/g). The concentration of glucose was 264% greater (P < 0.01) in IM tissue (2.13 µmol/g) when compared to SQ adipose tissue (0.58 µmol/g), but no interaction between tissue type and diet was observed for glucose concentration (P = 0.44). These results demonstrate that there was an increased supply of glucose carbon in steers fed a higher concentrate diet. This effect is expected due to the higher production of propionate from starch fermentation associated with such diets (Orskov et al. 1991), and the preferential use of propionate as a glucogenic substrate by ruminants (Danfaer et al., 1995). Additionally, available glucose from either diet was preferentially accumulated in IM depots relative to SQ. This effect is consistent with observations by Smith and Crouse (1984), in which IM adipocyte preferentially utilized glucose as an anabolic substrate.

Table 1. Least squares means for rates of conversion of [U-^{14}C] glucose to CO_2 and lactate (nmol·10^5 cells^{-1}·h^{-1}), and incorporation of [U-^{14}C] acetate (nmol·10^5 cells^{-1}·h^{-1}) into fatty acids of Wagyu and Angus steers.

Item	Wagyu	Angus	SE	*P* > F
CO_2	10.68	14.58	1.98	0.19
Lactate	564.21	681.78	146.95	0.58
Acetate	16.93	17.33	5.99	0.96

Glucose-6-P (0.04 vs. 0.05 ± 0.01 µmol/g for CORN and HAY) and Fructose-6-P (0.06 vs. 0.08 ± 0.02 µmol/g for CORN and HAY) were similar among diets (P > 0.46). There was a tendency (P = 0.07) for increased concentrations of Fructose-6-P in SQ tissue (0.09 µmol/g) vs. IM (0.05 µmol/g; SE = 0.02), however, the low overall concentrations of these intermediates makes this separation unimportant. Due to the lack of accumulation of glycolytic intermediates, it is unlikely that the increased levels of glucose in both the CORN diet and IM adipose tissue is due to an accumulation as a result of decreased pathway flux. This accumulation is more likely the result of enhanced substrate supply (diet effect) or increased uptake by IM.

Diet did not affect (P = 0.42) acetate incorporation into fatty acids, and there were no diet by tissue interactions for this response (P = 0.71). Acetate incorporation rates (nmol·10^5 cells^{-1}·h^{-1}) were 16.46 and 17.80 (SE = 5.99) for CORN and HAY, respectively, pooled across SQ and IM. These rates of acetate incorporation are consistent with values discussed by Smith (1995).

Diet by insulin interactions existed (Table 2) for measures of glucose utilization (P < 0.05). When steers were fed HAY, CO_2 production was similar in the presence or absence of insulin; i.e., the addition of insulin did not increase glucose oxidation. Similarly, lactate synthesis from glucose was not affected by insulin addition when steers were fed a HAY diet. Conversely, the addition of insulin when steers were fed CORN resulted in a 103% increase in glucose conversion to CO_2 and a 50% increase in lactate synthesis from glucose. This data provides substantial evidence that dietary energy source alters insulin sensitivity, as adipose tissues became insulin resistant when cattle consumed a forage-based diet. A mechanism for this effect is suggested by Tardif et al., (2002) who demonstrated that accumulation of ketones interrupted

insulin signal transduction and reduced GLUT-4 migration to cell surfaces. This reduction in insulin sensitive glucose transporter presentation reduces insulin-stimulated glucose uptake, and thus would limit apparent rates of glucose metabolism. Ketone bodies may accumulate under acetate loading, particularly when glucose is limiting (Herdt et al., 1981), and thus, the higher acetate loads anticipated and the reduced glucose concentration demonstrated with HAY vs. CORN diets may have affected this response. Schoonmaker et al. (2003) found greater levels of insulin in steers fed a high-concentrate diet compared to steers fed a high-forage diet during the growing phase. This observation coupled with our results suggests that adipose tissues in steers fed high concentrate diets would not only be exposed to greater circulating insulin but would also be more sensitive to its effects on glucose uptake.

Table 2. Least squares means for rates of conversion of [U-^{14}C] glucose to CO_2 and lactate (nmol·10^5 cells^{-1}·h^{-1}) in adipose tissue from steers fed hay- or corn-based diets incubated with 0 or 500 ng/ml insulin

	Diet					
Item	Hay		Corn			
Insulin	0	500	0	500	SE	*P*
CO_2	8.7^b	10.2^b	10.4^b	21.2^a	1.8	.02
Lactate	278^c	228^c	794^b	1192^a	113	.05

^{a,b,c}Least squares means without common superscript differ ($P < 0.05$).

Neither diet by tissue ($P > 0.71$) nor tissue by insulin ($P > 0.59$) interactions were observed for measured responses ($P > 0.71$). The lack of differential tissue responses to insulin may be due to the level of insulin that was added to the media during the incubation period (McCann and Reimers, 1985). These authors demonstrated that even when insulin sensitivity was altered in heifers, the maximum response to insulin was unaffected.

SQ had a 37% greater ($P = 0.04$) rate of glucose conversion to CO_2, pooled across diets and insulin levels (Table 3). Lactate production was similar ($P = 0.99$) among adipose depots. It is noteworthy, that SQ utilized acetate at a 290% greater ($P = 0.04$) rate for fatty acid synthesis than IM tissue. These data are consistent with previous findings that SQ adipose tissue utilizes more glucose for oxidative metabolism, and uses acetate as its primary substrate for fatty acid synthesis (Smith and Crouse, 1984). These results may provide an alternative explanation for observed tissue differences in glucose concentration. Reduced concentration of glucose in SQ tissue may be due to increased oxidative metabolism of glucose carbon, alone or in conjunction with increased glucose uptake by IM cells.

Overall, these results suggest that feeding HAY limited both glucose supply and tissue capacity to increase glucose utilization in response to insulin without altering acetate synthesis to FA. Because SQ consistently utilized more acetate and oxidized more glucose than IM, these results suggest that HAY diets may alter IM metabolism with less impact on SQ. These results support the hypothesis that high concentrate diets enhance glucose metabolism and increase insulin effects in adipose tissues. Smith and Crouse (1984) established the preferential use of glucose as a substrate in IM fatty acid synthesis, while SQ fat primarily utilizes acetate as substrate. High-concentrate feedstuffs produce a greater proportion of propionic acid than do forage diets (Orskov et al., 1991), and propionate is a preferred glucogenic substrate. Our results support this premise, as CORN fed steers had greater concentrations of glucose in adipose tissues. Conversely, a roughage-based diet provides greater concentrations of acetate. In this study, acetate was much more effectively utilized for fat synthesis by SQ adipose tissue. Acetate load may also inhibit insulin action. In this study, adipose tissues in HAY-fed steers were insensitive to insulin, while insulin had profound effects on adipose from CORN fed steers. These differences could lead to a divergent partitioning of energetic substrate in steers fed different diets, where CORN feeding enhances glucose availability and uptake, and apparently fatty acid synthesis in IM adipose (increased glucose supply, reduced oxidation, similar lactate capture), while HAY feeding reduced glucose availability without altering acetate incorporation in fatty acids. Because SQ used acetate more effectively than IM, this condition would promote SQ deposition over IM.

Table 3. Least squares means for rates of conversion of [U-^{14}C] glucose to CO_2 and lactate (nmol·10^5 cells^{-1}·h^{-1}), and incorporation of [U-^{14}C] acetate into fatty acids (nmol·10^5 cells^{-1}·h^{-1})of SQ and IM adipose tissue from steers

Item	SQ	IM	SE	*P* > F
CO_2	14.59	10.67	1.29	0.04
Lactate	623.20	622.79	79.55	0.99
Acetate	27.26	6.99	5.54	0.04

Reports exist that are consistent with this hypothesis. Propionate is insulinogenic in ruminants (Sano et al., 1995). Choat et al. (2003) reported increased intramuscular fat deposition in steers fed a concentrate diet that generated 39.3% greater propionate. Schaake et al. (1993) found that feeding grain or high-concentrate diets increased intramuscular fat content relative to forage feeding. These findings, especially in light of our observation about diet effects on insulin sensitivity, correspond with increased accretion of intramuscular lipid from glucose (Smith and Crouse, 1984). Although data reported here cannot confirm these hypotheses, further research is certainly warranted in this area.

Implications

The results of this experiment demonstrate diet-mediated differences in insulin sensitivity of adipose tissues in steers. Diet also altered substrate supply. Apparent differences in SQ vs. IM metabolism, and their interaction with diet, provide foundation for a hypothesis regarding diet-mediated regulation of differential adipose tissue

metabolism. Validation of these hypotheses could generate nutritional strategies that alter the rate and site of adipose deposition.

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TEAM TEACHING AGRICULTURAL SCIENCES AT 1994 LAND GRANT INSTITUTIONS

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ABSTRACT: Montana State University – Bozeman, Chief Dull Knife College and Little Big Horn College earned a USDA-CSREES Higher Education Challenge grant in 2003. The primary objective of this project is to develop educational opportunities for 1994 Land Grant Institution students, whom expressed interest in agricultural subjects offered by 1862 Land Grant Universities. Beginning in the summer of 2004, two 4-day introductory range science field-based classes were offered. Instructors from the 1994 colleges co-taught the courses alongside MSU College of Agriculture faculty. These courses were conducted entirely in the field. Students and faculty camped for one of these classes. Student evaluations rated one of the two courses a 4.7 utilizing a Likert-type scale of 1-5 with 5 being maximum. Student evaluations from the class that camped on site stated that the social time with the instructor was just as important as class time. Students also noted that hands-on learning on their reservation lands was valuable. Students responded that it was important for 1862 Land Grant instructors to spend some time working in their reservation environment. Four additional class offerings will be delivered within the next two years. Agricultural instructors from 1862 Land Grant Colleges might consider offering short courses in cooperation with 1994 Land Grant Colleges to increase interest in agricultural careers and student success.

Key words: Education, Field Courses, Native Americans, Agricultural Careers

Introduction

Native Americans are generally under represented at 1862 Land Grant University baccalaureate programs in agriculture and natural resources. Montana Native Americans constituted approximately 9% of Montana's population under age 19, but accounted for only 2% of all students in four-year baccalaureate degree programs (Dell, 2001). Therefore, a Native American of college age in Montana is four times less likely to attend a four year degree program than a non-Native American. Furthermore, once Native Americans are enrolled in four year degree programs, they are much less likely to complete their program than other non-Native students (Dell, 2001). Many Native-American college students also fail to enroll in Agricultural majors, even though the majority of jobs (20%) on some reservations are related to agriculture (Crow Tribe, 2002).

In order to encourage Native American student interest in agriculture and natural resource baccalaureate degree programs, Montana State University – Bozeman (**MSU**; 1862 Land Grant University) and two 1994 Tribal Land Grant Institutions (Little Big Horn College and Chief Dull Knife College) were awarded a USDA-CSREES Higher Education Challenge grant entitled "Transitioning to Excellence in Ag & Natural Resource Baccalaureate Education" in 2003. The objectives of the grant were: to develop five courses to be offered by 1862 University instructors at 1994 College campuses; to educate the 1862 instructors by offering Native American cultural workshops; and to facilitate the transfer of students from 1994 institutions to 1862 institutions. This paper will describe the main ideas from the cultural workshops and discuss the first course offered as a result of this grant.

Materials and Methods

Cultural Workshops

Two Native American cultural workshops were offered to 1862 University instructors, at Little Big Horn College, Crow Agency, MT, and at Chief Dull Knife College, Lame Deer, MT. An important aspect of the workshops was their location on the Crow Indian Reservation, and the Northern Cheyenne Reservation, respectively, so that the 1862 instructors could see first hand the conditions that exist there. Cultural workshops were led by faculty and administrators at the 1994 Colleges, and involved the participation of Native American alumni and students of the 1994 Colleges, as well as students who had attended both a 1994 College and then MSU-Bozeman. The cultural workshops were designed to give 1862 instructors an introduction to Crow, and Northern Cheyenne culture, and emphasized the challenges faced by Native American students in attending the 1862 University.

Major challenges facing Native American students from the Crow, and the Northern Cheyenne Reservations when transitioning to MSU-Bozeman include racism; going from being in the majority racially and culturally on the reservations or when attending the 1994 Colleges, to being a minority at the 1862 University. At Little Big Horn College, 75% of students speak the Crow language as their first language. For many Native American students, English should be considered as second language when attending the 1862 University. The cultural history of going away to school was not a positive experience. Not long ago in their family history, students were forced to

attend boarding school, where their Indian identity was taken away and they were given White names, clothing and indoctrinated with White culture. In addition, physical abuse was prevalent. Alternatively, being a member of the military is looked upon very favorably by a culture where warriors have historically been honored in society. This may explain why a much greater percentage of Native American young people join the military than attend college. Family structure on reservations is also different than in White culture. Relatives that Whites would consider distant, play more important roles in Native American students' lives. Native American culture includes a much larger extended family with whom students have specific responsibilities and roles. This can cause misunderstandings between students and 1862 University instructors. For example, funerals or other family events involving extended family members are vitally important occasions in Indian culture, and students are expected to attend these events. University instructors may not look favorably upon Native students missing class for these family obligations. In addition, following the cultural workshops, it was readily apparent that tribal cultures differ substantially, and having knowledge of a single culture does not necessarily provide insight into challenges faced by all Native American students.

Course Content

The first course offered was entitled "Introduction to Rangeland Ecology and Restoration." A general overview of range ecology was presented in the classroom at both 1994 College locations. Conservation, climate, soils, range sites, hydrology, plant identification, range condition, wildlife, livestock, and planning were the major topics discussed.

Course Structure

After the initial introduction, both classes went to field locations that were previously selected. Instructors from both 1994 and 1862 institutions worked with students on a given range site and completed field sheets for each location. The purpose of each field sheet was to take an inventory of existing conditions, identify problems and suggest solutions. Students at each range site were required to identify plants, texture soils, and determine range condition. Students were rotated from one range site to the next. Group and individual quizzes on plant identification were given to reinforce plant recognition skills. The classes averaged three to four range sites per day, and lasted four days. Classes began at 8:00 am and continued until 5:00 pm. Students were required to work in teams on a range problem they chose. This assignment was due two weeks after the field class was completed.

Seventy percent of the final grade was calculated from individual and group quizzes taken on location. A completely new range site was used for testing purposes on the final day to test students' abilities to apply principles learned. Thirty percent of the grade was based on the written report and oral presentation of their selected problems.

Student Evaluations

An external reviewer administered a student survey in the final hour of each class. The evaluation asked students; 1) Which course activities were the most effective? 2) Would you recommend the class to others? and 3) What improvements should be made? Since there were only two students from the Chief Dull Knife College, only the results from the Little Big Horn College class will be reported.

Results and Discussion

A total of eight students enrolled in the two classes. Six students from Little Big Horn College enrolled in the course, and all students completed the class. Two students enrolled and completed the course from Chief Dull Knife College. Most of the students had very limited exposure to the major concepts of this course, but they performed very well on standardized field tests. This was probably due to continuous field instruction where plant identification mistakes could be reviewed. This was a superior teaching method to a laboratory section of a class where field time is limited to two hours per day for one half of a semester.

The students from the Little Big Horn College class rated the class a 4.7 out of 5 possible. All six students stated they would highly recommend this class to other students. Every student made positive comments about the outdoor class setting. Further questioning revealed that being able to evaluate the Crow Reservation was a large incentive to further their knowledge about grazing and range livestock production. It appears that one of the greatest learning incentives was the fact that students were learning about tribal lands which they may manage in the future. When asked for improvements, most students suggested moving to different vegetative zones to learn more about different areas on the Crow Reservation. A few suggested involving more local landowners. Tribal members participated in on-site course content by speaking to the class about management problems. When we offered students a choice of several problems, all the students selected problems associated with the Tribal ranch. One student suggested offering a more advanced course so they could continue to receive credit.

An area that was not part of the formal survey, but received many comments, related to the fact that all instructors and students camped in a large campsite for four nights. The meals and camp work were contracted. The food was outstanding and the students frequently remarked about what an enjoyable experience it was to eat such good meals. After meals, the group took several excursions to cultural sites such as battle sites, sacred springs and knife sharpening areas, accompanied by a tribal historian. These were new experiences for most of the class and for all of the 1862 instructors. Discussions outside the class structure lead several students to state that the camping helped them to feel comfortable in the class. Three students felt the 1862 instructors should have had more cultural experiences as part of the class. Two students felt that being away from their children was a disadvantage of camping.

Summary and Conclusions

Two range science classes were offered in the summer of 2004 at Little Big Horn College and Chief Dull Knife College in Montana to introduce Native American students to agricultural courses offered at Montana State University – Bozeman. The courses were taught in the field at both locations. Students responded positively to the courses. Faculty from Montana State University – Bozeman received two cultural workshops to improve their understanding of Native American students and the challenges they face in transitioning to the 1862 University. A critical finding of this experience was that offering courses on the reservations was an extremely important learning event for both Native American students and for 1862 University instructors. Native American students indicated their greatest motivation came from learning something about their tribal lands.

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