November, 2008

The Department of Animal Science is pleased to provide you with this 2008 Annual Report summarizing a portion of our research, extension and teaching activities over the past year. The livestock industry is dealing with some difficult challenges including record high input costs and additional repercussions from a depressed national and global economy. Our Department is committed to administering research and extension programs that will assist our clientele in addressing those challenges. Our research activities range from the very basic to the applied. We attempt to utilize our limited resources to help address industry problems using interdisciplinary approaches. In addition, we try to facilitate cooperation and collaboration with other universities, state and federal agencies, and private industry in addressing issues facing animal agriculture. Development of these collaborations and partnerships is more important than ever in addressing these complex issues. As a Land Grant institution working in this manner, we can hopefully contribute to enhanced profitability and the production of high quality, safe and wholesome consumer products.

We hope the information provided in this Annual Report will be useful to you. We welcome your comments and look forward to your continued involvement in our research, extension and teaching programs in the Department of Animal Science at the University of Wyoming.

Sincerely,

Doug L. Hixon
Head and Professor of Animal Science
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ACKNOWLEDGEMENTS

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Burnett Enterprises, Carpenter, WY
The Butcher Block, Laramie, WY
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Department of Defense Medical Research Program
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USDA, APHIS National Wildlife Research Center
USDA-ARS
USDA-CREEES NRICGP
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University of Wyoming Research Office
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Western Feed Supplements
Western Region—Sustainable Agriculture Research and Education (WSARE)
Wyoming Animal Damage Management Board
Wyoming Department of Agriculture—Consumer Health Services Division
Wyoming IDeA Networks for Biomedical Research Excellence (INBRE; NIH-NCRR)
Wyoming Meat Processors Association
Wyoming Wool Growers Association
Zorko’s 7Z Livestock
Z-Tags, Inc.
Z & W Mill
STATEMENT OF PURPOSE

The University of Wyoming encourages its faculty to engage in the discovery of knowledge and new technology when the activities are consistent with their interests, the University’s objective and the needs of the people of Wyoming.

MISSION

The dissemination of knowledge developed or acquired through the University and the provision of leadership and professional assistance in its utilization.

BIOLOGICAL VARIABILITY AND CHANCES OF ERROR

The variability among individual animals in an experiment creates problems in interpreting the results. For example; cattle on treatment X may have had a numerically larger average daily gain than those on treatment Y, but variation in individual animal weight gain within each treatment group may indicate that the difference in weight was not the result of the treatment alone. Statistical analysis attempts to “sort” the normal biological variation within groups of animals from the response attributed to the treatments imposed. From that, researchers can calculate the probability that such differences were from chance (an effect caused by normal biological variability), or produced by treatment (treatment effects).

In the research reports that follow, you will see the notation (P<0.05). That means the probability of the differences resulting from chance is less than five percent. When two averages are said to be “significantly different”, the probability is less than five percent that the difference is from chance – the probability exceeds 95% that the difference results from treatment.

Some papers will report the correlation between two treatments. Correlations are a measure of the relationship between traits. The relationship may be positive (both traits tend to get bigger or smaller together) or negative (as one trait gets bigger, the other gets smaller). The perfect correlation is one (plus 1 or minus 1). If there is no relationship, the correlation is zero. Correlation does not mean cause and effect but rather gives us insight into potential relationships between traits.

In other papers, you may see an average, or mean, given as 2.50 ± 0.10. The first number reported is the average, or mean; 0.10 is the “standard error”. The standard error is a measure of variability, giving a range (2.40 to 2.60 in this case) where we can be 68% certain that the true average, or mean, (with limited numbers of animals) would fall.

Ways of decreasing the variability, and improving the chance of measuring differences due to treatment include: Using several animals per treatment, replicating treatments several times with pens of animals and using similar animals. The statistical analysis allows more valid, unbiased interpretation of the results regardless of the number of animals. In nearly all of the research reported here, statistical analyses are included to increase the confidence you can place in the results.
## Conversion Table

The metric system is the unit of measurement frequently used for reporting of scientific data. To aid in interpretation of these data, conversion factors for common measurement follow:

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<td>4 qt</td>
<td>=</td>
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After being recognized as one of the Outstanding Departments of Animal Science in the U.S. by the *Chronicle of Higher Education* last year (a ranking based on faculty research productivity), we have further strengthened our position with two appointments at the Assistant Professor level. **Dr. Meijun Zhu** is our new food microbiologist and brings considerable post-doctoral experience to this position (60% research, 35% teaching, and 5% service). She started as assistant professor this past August. With Dr. Steve Paisley’s move to the Sustainable Ag Research and Extension Center (SAREC) at Lingle, WY, we received a new Extension Livestock Specialist position. Dr. Paisley will continue as a valuable member of our Department, still delivering beef cattle extension education and performing nutrition/management research benefiting Wyoming’s beef industry. However, being stationed at SAREC will allow Steve to integrate and enhance animal research projects at this new facility. With his relocation we hired **Dr. Scott Lake**, a ruminant nutritionist, into a new Extension Livestock Specialist position (60% extension, 25% research, 10% teaching and 5% service). A Nevada native who received his B.S. and M.S. degrees from the University of Nevada at Reno and his Ph.D. degree in Animal Nutrition from the University of Wyoming, Scott has been in a tenure track teaching and research position at Purdue University for the past three years. Scott will share beef cattle extension responsibilities with Dr. Paisley while assuming up some sheep extension duties. He will also conduct beef research focusing on the cow/calf segment of the industry. Dr. Lake joined us on September 1, 2008.

Two new staff joined us in the Department of Animal Science Office this past year. **Linda Rosa**, a Michigan native and most recently a resident of the Chicago area, moved to Laramie with her husband who accepted a faculty position in the UW Business College. Linda assumed the role as our Accounting Associate, Senior in the Department of Animal Science.  More recently, **Kelli DeCora**, a Laramie native, accepted our Office Assistant, Senior position after Carrie Burke decided to pursue a different employment opportunity. Kelli started in her new position just this past October.

Finally, Mr. **Kelcey Christensen** joined our Department as Meat laboratory Manager this past fall, replacing Shane Thompson who took a position with Cargill Meat Solutions in Colorado. Kelcey, a native of Wright, WY, received his B.S. degree in our ANVS Business Option prior to participating in the 4+1 MBA program in the Business College. Before returning to manage the Meat Laboratory he was an Ag Loan Officer with First National Bank of Wyoming.
Faculty Recognition:

- **Kristi Cammack, Dan Rule, Warrie Means and Doug Hixon** – Recipients of Top Prof Award from Mortar Board Honorary Society (12/2007).
- **Doug Hixon** – Recipient of 2008 Outstanding Faculty Award from UW Alumni/Student Alumni Associations.
- **Min Du** – Received 2008 Young Scientist Award from Western Section, American Society of Animal Science.
- **Paul Ludden** – Received 2008 Outstanding Teaching Award from Western Section, American Society of Animal Science.

Retired Faculty Recognition:

- **Mick Botkin and LeRoy Johnson**, retired faculty members of the Department of Animal Science, each received the Compadre Award from the Wyoming Wool Growers’ Association (12/2007). This award is given to “professionals who have demonstrated a strong commitment with significant contributions to the sheep industry.”

Staff Recognition:

- **Carrie Burke**, Office Assistant, Senior – Recipient of Mortar Board Society’s “Tip of the Cap” Award (4/2008) for going “above and beyond” her assigned responsibilities to assist students.
- **Brandi Brewer**, Accounting Associate – Awarded Honorary Membership in UW Block & Bridle Club in appreciation of her assistance and support of their activities.
- **Brent Larson**, Sheep Unit Manager – Recipient of “Amigo Award” from Wyoming Wool Growers’ Association. This award is given to an industry professional who works directly with producers to adapt innovative technologies to enhance flock and/or resource management.

Graduate Student Recognition –

- **Keith Underwood** - a Ph. D. graduate student working under the direction of Drs. Min Du and Warrie Means in Meat Science, received the 2008 Ellbogen Outstanding Graduate Teaching Award from the UW Graduate School. Keith has accepted a tenure track faculty position at South Dakota State University and will move to Brookings, SD at the end of 2008.
- **Frances Niemela Loehr**, a graduate student working on an M.S. degree with Dr. Steve Paisley, placed 2nd in the Western Section, American Society of Animal Science (WSASAS) Graduate Student Paper competition at the 2008 WSASAS Meetings held in Laramie (6/2008).
- UW Graduate students placed 2nd and 3rd at the 2008 Colorado Nutrition Roundtable Graduate Student Poster Competition last September in Ft. Collins. **Platt Price**, an M.S. student working with Dr. Bret Hess placed 2nd, and **Philipe Moriel**, an intern from Brazil who will initiate an M.S. program with Dr. Hess in January, placed 3rd.
- Gamma Sigma Delta Honorary Society honored **Keith Underwood** as their Outstanding Ph.D. Student and **Marjorie MacGregor** (working with Dr. Steve Horn) as their Outstanding Masters student at their 2008 GSD Awards Brunch last March.
- **John Willford** (Ph.D. student working under the direction of Dr. Larry Goodridge in Food Microbiology; Co-Directed by Bret Hess); **Platt Price; Keith Underwood**; and **Rebecca Cockrum** (an M.S. student working with Dr. Cammack) all were selected as Outstanding Presenters at the 2008 Graduate Student Symposium (April).

**Undergraduate achievements:**

- **Stacia Berry** – Business and Communications Double Option in ANVS major received the Spitaleri Award as Outstanding Graduating Female student at the University of Wyoming; she also gave the Ag College’s 2008 Commencement address.
- **Kassi Bauman**, Cheyenne, WY- Animal Biology Option in ANVS major was selected by Farm Management Company to participate in all-expense paid trip to Deseret Cattle and Citrus in Florida as part of a select group of junior students from across the country to learn about internship & employment opportunities.
- **Gamma Sigma Delta Awards received by ANVS students** – Outstanding Freshman (male) – **Todd Small**, Wheatland, WY; Outstanding Sophomore – **Amy Berry**, Cheyenne, WY; Outstanding Junior – **Travis Allen**, Cheyenne, WY; Outstanding Senior (Female) – **Stacia Berry**, Cheyenne, WY.
- **Animal Science Senior Honor Book Recipients** – **Joanna Hergenreder** (Nunn, CO) and **Stacia Berry**.
- **Academic Quadrathlon Team** won the WSASAS Regional Quadrathlon this past March. They also won the right to participate against the three other regional winners at the 2009 National Cattlemen’s Beef Association Annual Convention in Phoenix in January. Team members include **Lander Nicodemus** from Cheyenne, WY; **Lindsey Noreen** from Bowman, ND; **Rebecca Harrison**, Bakersfield, CA; and **Alison Iroz**, San Diego, CA.
- **2008 Meat Judging Team, Coached by Lynn Franzkowiak**, placed 4th at the 2008 National Western Contest in Greeley, CO. The Team won Beef Grading and Lamb Judging. **Garrett Horton** from Riverton, WY placed 8th overall.

The Meat Judging Team placed 5th overall at the 2008 Ft. Worth Stock Show and Rodeo Contest held at Columbia Packing Company in Dallas, TX in February. UW was 5th in Lamb Judging and 4th in Placings. **Michael Fernandez** from Wray, CO was the high placing UW team member.

UW placed 8th out of 11 teams at the 2008 American Royal Meat Judging Contest held at Nebraska Beef in Omaha, NE in October. The UW Team was 4th in Placings, 5th in Lamb Judging and 5th in Pork Judging. **Garrett Horton** was the high placing UW team member.

- **2008 Livestock Judging Team, coached by Lance Miller**, did not have adequate numbers to compete in the spring semester of 2008 but now has a team that has been working this fall and will travel to the Express Ranches Contest in Oklahoma in
December and then start competing in 2009 with the National Western Stock Show Contest in January.

Research and/or Creative Activities

- Animal Science Faculty received more than $1.4 M in new extramural funding in FY08. Faculty also had more than $1.7 M in continuing active grants obtained in previous years.
- ANSC faculty published or had accepted 35 refereed journal articles as primary authors and another 36 manuscripts as collaborating authors in our most recent annual faculty updates which ended with the 2007 calendar year.
- Research projects are detailed in the bulk of this report.

Extension and Outreach Activities keep our Department connected to producer and consumer clientele throughout the state and region.

- Department continues to administer both a summer meat-breeds Ram Test for the Wyoming Wool Growers’ Association and a winter WY Rambouillet Association Test at the UW Sheep Unit. These tests are excellent Public Relations activities that effectively provide the industry with needed information to enhance genetic improvement in the sheep industry and better position WY Lamb for their branded Mountain States Lamb and Wool Cooperative product. Dr. Bob Stobart and Brent Larson, UW sheep unit manager, direct these activities.

  These Ram Test programs will be further enhanced with the installation of a GrowSafe Feeding System which is currently taking place to allow collection of individual feed intake data for group-fed animals. This will be one of the first such facilities developed for the collection of feed efficiency data in sheep anywhere in the U.S.

- Continue to provide leadership for the educational activities of the Wyoming Beef Cattle Improvement Association (WBCIA). Dr. Steve Paisley administers the WBCIA Feedlot Test and Carcass Evaluation Program which allows WY producers to evaluate retained ownership as a marketing alternative and better positions WY beef for branded product participation. Dr. Paisley also assists with the WBCIA Bull & Heifer Tests as well as their respective sales. These activities are both critical to positioning WY producers to take advantage of industry opportunities. Dr. Scott Lake will also work into these programs as his Extension involvement adds depth to our program.

- Dr. Paisley serves as the WY Beef Quality Assurance Coordinator. This is a program that was designed to reduce the quality and consistency shortfalls in the beef industry that is now more focused on the consumer than in the past. Producers, veterinarians and extension educators can become certified trainers through the program. These activities are funded by the Wyoming Beef Council.
Dr. Warrie Means provides annual leadership to the WY & CO Meat Processors Workshop and assists small meat plants within WY with their HACCP (Hazard Analysis Critical Control Point) Plans allowing them to meet state inspection guidelines.

Extension Specialists in ANSC support educational programming associated with Profitability and Sustainability of Agricultural Systems (PSAS) and Human Nutrition and Food Safety Extension Initiatives within WY Cooperative Extension Service.

Dr. Paisley served as the WY Representative on the four-state Range Beef Cow Symposium (RBCS) Planning Committee that was last held in Ft. Collins, CO in December, 2007 with approximately 800 producers and agribusiness representatives attending this 2 1/2-day symposium that rotates among the four cooperating states every other year. Wyoming will host the 21st RBCS in Dec. 2009 in either Casper or Cheyenne.

The ANSC Department annually hosts the Cowboy Youth Classic each June under the leadership of Lance Miller, Livestock Judging Team Coach and Departmental recruiter. This event includes a Friday afternoon of educational workshops on relevant industry issues for 4-H and FFA youth and their parents. This not only serves as an excellent extension educational opportunity but allows clientele to experience our facilities and meet our people. It has become a fine recruiting tool for potential College of Agriculture students. Approximately 125 young people and their families were served a complimentary Friday evening meal at the 2008 CYC. This year’s event was held on June 20 and 21, 2008 at the Hansen Livestock Teaching Arena. Traditional beef, sheep and swine shows take place on Saturday following the Friday educational events. Youth are required to attend Friday’s educational workshops before they are allowed to show their project animals and compete for prize money in the Saturday show.

Development

Have finalized a scholarship endowment agreement in honor of Gloria and the late Gary Parker of Shamrock Angus, Laramie. This was established by family and friends of the Parkers.

Thanks to the continued contributions of Mayme Schoonover and the state matching program, The Schoonover Scholarship Endowment now provides support for four student scholarships for the 08-09 academic year.

The Department continues to work with the Cowboy Joe Club and the Steer-A-Year Program. By recruiting donors to this program, the ANSC Judging Teams received approximately $5400 in the past year to add to the Riley Judging Team Endowment to help defray travel expenses associated with sponsorship of livestock, meat, and wool judging teams as well as our academic quadrathlon team.

The Seneka Graduate Assistantship pledge was recently funded by Ron and Lynne Pulley of Huntley, WY. This qualifies for a state match and will ultimately fund a graduate stipend in Meat Science & Food Technology.
Lake Research and Extension Program

Scott L. Lake, Assistant Professor, Department of Animal Science

As a new faculty member at the University of Wyoming, I want to focus my research and extension activities towards several relevant issues. Along with a team of scientists and extension educators, we plan on investigating low-input calf and lamb development. More specifically, we hope to develop a systems approach to heifers and ewe lamb development that will reduce input costs while maintaining production levels similar to that of traditional developmental systems. This systems approach also applies toward developing feeder calves, which will likewise reduce input costs, while maintaining the growth and quality that will provide a premium. Therefore, the overall goal of the system will be to manage replacements and feeders together, minimize input costs, and increase productivity or quality. Other focus areas of research and extension will be in the area of multispecies grazing. We hypothesize that cattle and sheep managed together will result in improved pastures, increase plant diversity, and increase the number of productivity per acre. Additionally, we are interested in how interactions between cattle and sheep with wildlife affect range conditions, stocking rate, economics, and productivity of the land. From an extension side, we are interested in developing new programs that focus on current issues, such as budgeting, marketing options, retained ownership programs, niche markets, obtaining Ag loans, and how future generations of ranchers can be sustainable.
Zhu Research Introduction

Meijun Zhu

I am Meijun Zhu, a new hired assistant professor in Food Microbiology at Department of Animal Science. I got my B.S., M.S., and Dr.Sc. in Biochemistry at China Agricultural University in Beijing, China. I obtained my Ph.D. in Meat Science with emphasis in Microbiological Food Safety at Iowa State University at the end of 2004. Since then, I came to the Department of Animal Science at University of Wyoming as a postdoctoral fellow and was recently hired as an assistant professor in Food Microbiology focusing on pre-harvest microbiological safety of meat animals. I have a key interest in studying the mechanism about \textit{E. coli} O157:H7 colonization in the gastrointestinal (GI) tract of beef cattle and the associated bacterial signaling.

Wyoming and surrounding Rocky Mountain States are cattle states. \textit{E. coli} O157:H7 is a major safety threat for beef and its associated products. \textit{E. coli} O157:H7 contamination in beef products results in huge economic losses for the industry and decreases the consumer confidence. Because the fecal shedding of \textit{E. coli} O157:H7 is the major source of \textit{E. coli} O157:H7 contamination, it is extremely important to reduce \textit{E. coli} O157:H7 colonization in the GI tract of beef cattle. To effectively reduce \textit{E. coli} O157:H7 gut colonization, we must understand mechanisms underlying \textit{E. coli} O157:H7 adhesion and gut colonization and associated signaling. We also know that the prevalence in \textit{E. coli} O157:H7 in GI tract is affected by the mucosal immune response, while mucosal immune system differentiates and develops in mid-gestation in ruminant animals, which is largely mediated by the presence/absence of pro-inflammatory cytokines. Therefore, one of my research focuses will study the gut mucosal immune system development in the fetuses of ruminant animals and their impacts on the \textit{E. coli} O157:H7 colonization in the GI tract. \textit{E. coli} O157:H7 colonization in GI tract induces host immune responses. Such response generate stresses to \textit{E. coli} O157:H7 which activates corresponding stress responsive signaling pathways and is essential for the \textit{E. coli} O157:H7 attachment and colonization. I would like to study these signaling pathways and associated mechanisms. Understanding such mechanisms will provide us with molecular targets to reduce or eliminate \textit{E. coli} O157:H7 colonization in the GI tract of beef cattle. Trained as a meat scientist, my research interest also extends to the general safety of meats and their products.
2008 UW Meat Judging Team

Team Members include:
Garrett Horton- Riverton, WY
Elizabeth Griesse- Crawford, NE
James Wheeler- Kailua, HI
Kelsie Speiser- Casper, WY
Michael Fernandez- Wray, CO
Coach Lynn Franzkowiak

The 2008 Meat Judging Team has had a successful, competitive judging year. We began in January where we competed at National Western Stock Show. As a team, we finished 1st in both beef grading and lamb judging, 2nd in overall beef, 4th in placings, 5th in reasons and beef judging and finished 4th overall in the contest. Garrett Horton, of Riverton, WY, placed 8th as an individual overall at NWSS as well. Next, we traveled to the Fort Worth Stock Show and Rodeo, where we finished 4th in placings, 5th in lamb judging and 5th as a team overall in the contest. Our last contest of the spring semester was in Houston, TX, where we finished 5th in pork judging, 6th in placings, and finished 10th overall as a team. Garrett Horton and James Wheeler, of Kailua, HI, both scored a perfect 100 in specifications. Beginning our fall semester, we traveled to Wyalusing, PA to the Eastern Contest, where we finished 8th overall as a team. Next, we traveled to Omaha, NE to the American Royal Contest where we placed 4th in placings, 5th in lamb and pork judging and again finished 8th overall as a team. Our most recent contest was in Plainview, TX where we again finished 8th overall as a team. To complete the 2008 Meat Judging year, we will travel to Dakota City, NE, on November 16th to compete at the International Meat Judging Contest, where we hope to finish very competitively as a team with members of the team having individual accomplishments as well.
UW LIVESTOCK JUDGING TEAM UPDATE

The 2009 University of Wyoming Livestock Judging Team is anxiously awaiting the start of their judging campaign. The team’s first contests will be at the Exposure and Express Ranches Contests the weekend of December 19th and 20th in Oklahoma. At those two contests, team members will be challenged to evaluate high quality cattle classes from a couple of the nation’s most prominent cattle breeders.

Team members and hometowns of the 2009 Livestock Judging Team include Clay Fordyce, Moorcroft, WY; Aurora Lambert, Anchor Point, AK; Chance Marshall, Jackson, WY; Dayna Olson, North Platte, NE; Calvin Schell, Washam, WY; Kodee Schell, Washam, WY; Kurt Sexton, LaPorte, CO; and Kelsie Speiser, Casper, WY. The team practices two afternoons during the week and all day on Saturdays.

Besides the two contests in Oklahoma in December, the team will compete at the following contests in January through March of 2009: National Western Stock Show (Denver, CO), Sioux Empire Farm Show (Sioux Falls, SD), Iowa Beef Expo (Des Moines, IA), San Antonio Livestock Show & Rodeo (San Antonio, TX), Nebraska Cattlemen’s Classic (Kearney, NE), and the Houston Livestock Show & Rodeo (Houston, TX).

The team is being coached by Lance Miller, who can be reached at 307-766-2159 or lrmill@uwyo.edu.
Wyoming Business Council – Agribusiness and University of Wyoming

2008 Winter Ag Expo, January 7 and 8, Wyoming State Fairgrounds, Douglas

The joint committee led by the Wyoming Business Council – Agribusiness Division (Scott Keith), and the University of Wyoming Animal Science Dept. (Dr. Steve Paisley) hosted the second annual Wyoming Winter Ag Expo. The Expo was initiated as an opportunity for Wyoming producers and agriculture-related businesses to showcase their products. The expo combined a wide variety of ag industry businesses, seedstock cattle producers, hay producers, and support industry personnel in one location, while also including educational sessions focusing on hay and livestock production. The two day expo was held at the Wyoming State Fairgrounds Pavilion building in Douglas January 7th and 8th.

The 2007 Winter Ag Expo hosted 41 commercial booth vendors, 19 cattle displays and 6 large equipment vendors. The expo and educational programs were free to the public, with 21 major sponsors, Converse County Tourism Promotion Board included, covering the costs of set-up, advertising, and facility lease. Exhibitor survey results and comments were positive, encouraging the committee to make the Winter Ag Expo an annual event.

Cowboy Youth Classic – June 20,21 2008  Hansen Livestock Teaching Arena

The Cowboy Youth Classic (CYC) is an annual summer workshop and livestock show for Wyoming youth with beef, sheep and swine projects. The 2-day program (Friday - Saturday) includes mandatory educational workshops for both youth and parents, livestock fitting and showing demonstrations, and a jackpot livestock show for all three species.

The CYC is supported by a large number of local sponsors, providing financial support, services, prizes, and in-kind gifts for the event. The 2007 CYC attracted over 100 participants with over 154 cattle, sheep and pigs combined, nearly eclipsing last year’s attendance record.
On June 24-26, the Department of Animal Science hosted the 80th annual Western Section American Society of Animal Science meetings in Laramie. The meetings were coordinated by Dr. Gary Moss, with additional help from Faculty Emeritus Dr. Connie Kercher.

Including the annual Beef Symposium, over 210 faculty, students and research personnel from the Western states, including Canada and Mexico, attended the annual meeting.

2008 Wyoming Beef Cattle Improvement Association

Bull Test – Pingetzer heifer and bull development facility, Shoshoni, WY. Approximately 227 bulls entered into the 2008 test near Riverton, WY. The annual sale is held in conjunction with the WBCIA symposium and sale, the first Saturday in April.

Feedlot Test - Klein Farms, Wheatland, WY. One hundred forty-six were consigned to the annual feedlot performance and carcass evaluation test.

Symposium/Scholarship and Awards Banquet – In conjunction with the WBCIA Bull Sale, the symposium committee hosts an annual educational workshop, followed by awards banquet and benefit auction, generating proceeds to provide three $1,000 to Wyoming students in ag-related college programs.

Wyoming State Fair Carcass Contest and Live Evaluation Program – Sponsored by several groups including the Wyoming State Fair, Wyoming Stock Grower’s Association, Wyoming Farm Bureau and UW Animal Science, the WBCIA organizes and hosts the annual beef carcass contest and live evaluation competition.

Wyoming Supreme Cow Contest – New to the WY State Fair this year, the WBCIA hosted a statewide supreme cow contests, with inaugural entries from Goshen, Platte and Campbell Counties.
Seventy Seven rams from 18 producers were consigned to the Wyoming white-faced ram test last fall. The majority of these rams are purebred Rambouillet rams. At the finish of the test, an index based on their gain performance over the 140 test period along with their wool characteristics is calculated. The top 30% of the purebred Rambouillet according to the index are eligible for registration of merit certification in the Rambouillet Association.

In addition to testing the overall genetic merit of the rams, Brenda Alexander tested a subset of rams for expression of sexual behavior. Behavior testing is not a part of the test and is not included in the index or final report but does provide the producers with libido information regarding their rams.

Eleven producers consigned 102 rams from terminal sire breeds which will be used to produce fat lambs to the Meat Breeds ram test. This test is designed to identify rams with superior growth potential along with increased size of the loin eye muscle. The test runs for 60 days and at the end of the test, the average daily gain (ADG) over the 60 day period along with the loin eye measurement is combined in an index which simplifies evaluation of the genetic potential of these rams.
University of Wyoming Annual Animal Science Research Report
2008

UW SHEEP PROGRAM

Brent Larson, Livestock Manager
Robert Stobart, Associate Professor
Gary Moss, Professor

Summary

The University of Wyoming maintains approximately 120 commercial ewes, 180 purebred ewes (Rambouillet, Suffolk, Hampshire, Columbia) plus rams and replacements. These animals are used extensively to meet needs for Teaching, Research, and Extension/Service. Overriding goals of the sheep program are to: provide students and producers with the knowledge to make informed decisions regarding sheep management; conduct research that will insure long-term viability of the industry; provide superior genetics for breeders; remain cognizant of developments that affect the sheep industry and relate them to students and producers; and evaluate, test, and assist with the development of procedures and presentation of functions that enhance profitability and/or viability of the sheep industry. The Department also hosts annual white-faced and black-faced tests each year for consignor rams. Activities and functions conducted during the past year with these resources are outlined in brief below.

2007-2008 Production

In the spring of 2008, 32 Rambouillet, 20 Columbia, 53 Suffolk, 40 Hampshire, and 105 commercial ewes lambed at the University Experimental Farm. Respective weaning percentages for each of these breeds were 170, 180, 142, 160, and 156%. Also present were rams and replacement ewe lambs.

Use of Animals

Throughout the year sheep present at the experimental farm were used extensively for multiple purposes. Excess animals were shared by individuals conducting discipline oriented or multi-discipline collaborative research studies. Brief descriptions of those uses follow. Note that numbers of animals used may total to more than numbers present because some animals were used for more than one activity.

Research.

- 25 Suffolk ewes. Dr. Kristi Cammack. Effects of elevated dietary nitrate on feed intake, weight, and plasma parameters.
- 10 crossbred rams. Dr. Brenda Alexander. Role of the progesterone receptor in the development of male sexual behavior.
- Wool samples from all ewes and rams. Dr. Bob Stobart. Wool Strength and fiber diameter.
- Ram test animals. Dr. Bob Stobart. Electronic ear tags, Racewell automatic sheep handling system.
- 160 ewes. Dr. Bob Stobart. Electronic ear tags Racewell automatic sheep handling system.

**Collaborative Research.**

- 16 ewes. Dr. Donal Skinner. Department of Zoology & Physiology.
- Wool Samples from all ewes and rams. Dr. Bob Stobart and Dr. Bruce Cameron (Dept of Family and Consumer Science), Wool Color.
- 6 lambs. Dr. Myrna Miller, USDA-ARS research.

**Academics.**

- 11 lambs for fitting and showing. Little “I”.
- 20 lambs. Academic Quadrathalon.
- 2 rams. Management class, vasectomy.
- 60 lambs. Processing and meats class.
- Ewes, lambs and Rams. Sheep production class and Livestock Production class.
- 10 lambs. Contest animals, State Fair.

- 10 lambs. Fitting Contest, State Fair.
- 80 lambs. University, 4-H, FFA judging workouts prior to National Western (300-400 participants).
- 150 fleeces. Wool judging workouts prior to National Western.
- 50 fleeces. Wool judging workouts for 4-H and FFA state contestants.
- 35 hd lambs SAREC.

**Producer events.**

- Host 60 day blackface ram test. --- Consignor rams plus UW rams. 101 hd.
- Host 140 d whiteface ram test (current test is the 47th year) plus field day. --- consignor rams plus UW rams 70hd.

**Sales.**

- 15 lambs: 4-H/FFA club lamb sale.
- 12 lambs: Brad Mills
- 15 ewes: CLA
- 1 ram: Brad Mills
- 1 ram: Collet Fenster

**Other Animals (not produced by UW).**

- 104 ewes from Truman Julian and Brad Boner for: Ram behavior study, Ked study, Blue tongue study, and Fetal Programming.
Effects of High-sulfur Water and Clinoptilolite on Growth Performance and Gene Expression of Steers Fed Forage-based Diets

Kristi Cammack¹, Assistant Professor
Katie L. Kessler¹, M.S. Student
Kathy Austin¹, Research Scientist
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Summary and Implications

Sulfur-induced polioencephalomalacia (PEM), a neurological disorder affecting ruminants, is frequently associated with consumption of high-S water. Identification of a feed supplement that would counteract the negative effects of high-S water would decrease the incidence of S-induced PEM in regions with problematic water sources. The objectives of this study were to 1) determine the effects on performance and gene expression in steers administered high-S water, and 2) determine if clinoptilolite, a clay mineral high in cation-exchange capacity, ameliorates the effects of high-S water consumption. Yearling steers (n=96; 701.5±4.6 lb BW) were randomly assigned to 1 of 4 treatments for a 77 day trial period: low-S water control (566 mg/kg sulfate), high-S water (3,651 mg/kg sulfate), or high-S water plus clinoptilolite supplemented at either 2.5% or 5.0% of diet DM. Feed and water consumption were measured daily, and all steers were weighed days -2, -1, 29, 53, 76, and 77. Plasma and liver samples were collected prior to and at the conclusion of the trial. Morbidity and mortality were higher (P=0.0014) for steers receiving high-S water; however, no differences in animal health were observed among clinoptilolite levels. Dry matter intake was lower (P=0.074) for steers consuming high-S water regardless of level of clinoptilolite. No differences in ADG or feed efficiency were observed. Plasma chemistry analyses showed lower protein (P=0.041) and higher urea N (P=0.050) in high-S water steers at the conclusion of the trial. Mineral analyses showed an interaction (P<0.05) of sample time and treatment in hepatic copper and selenium concentrations. Microarray analyses of selected low-S water and high-S water (0% clinoptilolite) steers revealed differential expression of immune response, cell to cell recognition, and cell surface receptor genes. These results suggest that clinoptilolite does not negate the effects of high-S water, and administration of high-S water decreases herd health, possibly through altered immune function.

Introduction

Dietary S is required for cattle to synthesize the amino acids cysteine and methionine; however, negative effects on production and health have been observed when dietary sulfur exceeds 0.2% DM. (Loneragan et al., 2000). This toxicity can lead to the development of S-induced polioencephalomalacia (PEM). Sulfide, reduced from sulfate, is either absorbed in the rumen or used by the microbes to create proteins. Normally, sulfides absorbed by the rumen are turned into sulfate in the liver and recirculated into the rumen to create essential amino acids, including methionine and cysteine. However, when excess sulfide ions are created due to increased sulfate intake, H₂S gas is produced, which in excess inhibits cytochrome oxidase in the electron transport system. This inhibition causes a reduction in the production of ATP. Cortical neurons are sensitive to this change in ATP, and as a result necrosis occurs in the brain (Karamjeet, 2000).

The objectives of this study were to 1) determine the effects on performance and gene expression in steers administered high-S water, and 2) determine if clinoptilolite, a clay mineral high in cation-exchange capacity, negates the effects of high-S water consumption.

Materials and Methods

Animals and Treatments

This study was conducted at the South Dakota State University Cottonwood Range and Livestock Research Station. Yearling steers (n=96; 701.5±4.6 lb BW) were randomly assigned to 1 of 4 treatments for a 77 day trial period: low-S water control (566 mg/kg sulfate), high-S water (3,651 mg/kg sulfate), or high-S water plus clinoptilolite supplemented at either 2.5% or 5.0% of diet DM, with three pens of eight animals per treatment. Clinoptilolite, a naturally-occurring member of the zeolite clay mineral family, was hypothesized to bind excess hydrogen ions, prevent the formation of excess hydrogen sulfide (H₂S) gas, and avoid S-induced PEM in high-S...
steers. Stock water was mixed using a Dosatron® (Dosatron - North America, Clearwater, FL) with sodium sulfate (Na2SO4) as the S source. Water was administered daily with the morning feeding and in the evening when necessary. All diets contained 50% ground crested wheat grass hay and 45% wheat middling pellets. Diets of the low-S water control and high-S water treatment groups contained 5% ground limestone, which was reduced to 2.5% and 0% with addition of 2.5% and 5.0% clinoptilolite, respectively. Water and feed were provided on an ad libitum basis, and intake was recorded daily to track S consumption (starting day 0). Steers were weighed on days -2, -1, 29, 53, 76, and 77. Weights on days -2 and -1 and days 76 and 77 were averaged to estimate initial and final weights, respectively. Liver biopsies were performed prior to (day 0) and at the end of the trial (day 77) to collect tissue for future gene expression analyses. Biopsies were also performed on steers exhibiting symptoms of PEM at the time of onset. Blood samples were collected on days 0, 58, and 77.

Steers were monitored closely for symptoms of S-induced PEM, including anorexia, ataxia, blindness, lethargy, muscle tremors, diarrhea, stargazing, weight change, and in severe cases recumbency and/or seizure. Steers showing any signs of S-induced PEM were immediately removed from the pen, administered penicillin, thiamine, and dexamethasone in an attempt to relieve symptom(s), and then moved to a small pen with low-S water and free choice hay and wheat middling pellets. Intramuscular (IM) administrations of thiamine and dexamethasone were continued twice daily for 7 days to alleviate symptoms associated with brain edema. Steers that went recumbent, seized, or had worsening symptoms were euthanized. Surviving steers were administered the probiotic Probios® (Bomac Vets Plus Inc., Knapp, MN) after 7 days, and moved to a pasture supplied with low-S water and free-choice crested wheat grass hay.

Brains of euthanized steers were immediately removed and dissected sagitally. Dissected brains, with one portion placed on frozen cold packs and the other preserved in formalin, were delivered to the South Dakota Animal Disease Research and Diagnostic Laboratory in Brookings, SD, for diagnosis of S-induced PEM by the presence of necrotic lesions on the cortical region of the brain which fluoresce under ultraviolet light. Brains were not collected from those steers found dead due to tissue degradation; therefore, diagnosis of S-induced PEM in these animals was based on documented observations of PEM symptoms.

Liver Biopsies

Liver biopsies were performed on days 0 and 77 to collect tissue for copper and future gene expression analyses. Biopsies were also performed on steers exhibiting symptoms of S-induced PEM at the time of onset. To collect liver samples, an incision was made in the animal’s right side into the intercostal space between the 11th and 12th ribs and a 4” 8-gauge bone marrow punch was inserted. The liver was penetrated 3-5 times and negative pressure applied by a 20” precision pump tube and syringe attachment to obtain an approximate 3 g sample. Liver tissue was promptly rinsed in a 1X PBS solution and snap-frozen for copper and future gene expression analyses.

Liver Mineral Analyses

Hepatic tissue from day 0 and day 77 biopsies of 10 randomly selected healthy steers per treatment were sent to the Michigan State University Diagnostic Center for Population and Animal Health for a standard mineral panel, including copper, iron, zinc, selenium, molybdenum, and manganese.

Plasma Analyses

Blood samples were centrifuged for 15 minutes at ≥ 3000 rpm for plasma collection. Plasma samples from blood collected on days 0 and 77 from healthy steers and on day 0 and at onset of symptoms from PEM-afflicted steers, were sent to the Colorado State University Veterinary Diagnostic Laboratory in Ft. Collins, CO, for plasma copper determination. In addition, plasma samples from ten randomly selected control steers (day 77) and from ten PEM-afflicted steers (collected at onset of PEM symptoms) were submitted to the Wyoming State Veterinary Laboratory in Laramie, WY, for standard chemistry panel analyses, including albumin, aspartate aminotransferase, blood urea nitrogen, creatine kinase, gamma glutamyl transpeptidase, globulins, glucose, lactic acid dehydrogenase, phosphorus, total protein, sodium, chloride, and calcium.

Liver RNA Isolation and Gene Chip Screening

Twenty steers were chosen for microarray analyses: 4 control steers; 4 high-S water healthy steers; 4 high-S water PEM-afflicted steers; 4 high-S water + 5% clinoptilolite healthy steers; and 4 high-S water + 5% clinoptilolite PEM-afflicted steers. Samples of hepatic tissue were used for RNA extraction via TRI reagent. The RNA was further purified using an RNeasy kit from Qiagen with Dnase digestion. Purified RNA was screened using the Affymetrix bovine gene chip containing ~24,000 genes at the Montana State University Functional Genomics facility.

Statistics

Performance, plasma chemistry, and liver mineral data were analyzed using the GLM and MIXED procedures of SAS (SAS Inst., Inc., Cary, NC). Differences in least-squares means were tested using a LSD. Signal intensities and flag (present, marginal or absence) call data from the microarrays were normalized and used in analysis of variance (ANOVA) to identify significant differences in expression of genes between steer groups. Genes identified as being significantly differentially expressed (P<0.05) were further analyzed using DAVID (NIAID, NIH) for functional annotation and identification of expression patterns.
Results and Discussion

Morbidity and mortality were higher ($P = 0.0014$) for steers administered high S water. No differences in animal health were observed among clinoptilolite levels. In total, nine cases of PEM were confirmed by presence of cortical lesions. In addition, eight high-S water steers that had previously been exhibiting symptoms consistent with PEM were found dead. The high-S water treatment group had one confirmed and three suspected cases of PEM, the high-S water + 2.5% clinoptilolite had five confirmed cases and four suspected cases, and the high-S water + 5% clinoptilolite had three confirmed cases and one suspected case. Suspected cases exhibited clinical signs of S-induced PEM, but survived the onset of symptoms. No cases of S-induced PEM were observed among low-S water control steers.

No differences in weight change during the 77 day trial were observed; however low-S water control steers had a numerically higher average weight gain (175.7 lb) than steers in high-S water treatment groups (155.0 lb). Dry matter intake was lower ($P = 0.0376$) in high-S water + 2.5% clinoptilolite steers than control steers, but was similar among all other steer groups (Figure 1). No differences in average daily gain or feed efficiency were observed.

There were no differences in plasma copper prior to the trial period among low-S water control and high-S water groups. However, differences were observed on day 58 and day 77 (Figure 2). Plasma copper was lower ($P = 0.04$) in high-S water (no clinoptilolite) steers than control steers on day 58. No other differences in plasma copper were observed on day 58. On day 77, plasma copper was lower ($P = 0.05$) in all high-S water treatment groups than control group; however, plasma copper was not different among high-S water treatment groups. Blood samples were also collected from S-induced PEM steers at the time of death/euthanasia. No differences in plasma copper from this collection were observed among treatment groups.

No differences ($P > 0.10$) were observed in aspartate aminotransferase, creatine kinase, gamma glutamyl transeptidase, globulins, glucose, lactate dehydrogenase, phosphorus, sodium, chloride, or calcium between low-S water control ($n = 10$) and S-induced PEM ($n = 10$) steers. Plasma urea nitrogen was higher ($P = 0.04$) in S-induced PEM steers than in control steers. Plasma urea nitrogen was elevated to 22.6 mg/dl in S-induced PEM steers, compared to 17.2 mg/dl in control steers. Total protein was lower ($P = 0.04$) in S-induced PEM steers than low-S water control steers. Total protein was 7.56 g/dl and 8.26 g/dl in S-induced PEM and control steers, respectively. Additionally, there was tendency for lower albumin ($P = 0.09$) in S-induced PEM steers than control steers. S-induced PEM and control steers had 3.6 g/dl and 4.0 g/dl albumin, respectively.

Mineral analyses showed an interaction ($P < 0.05$) of sample time (day 77 or day 0) and treatment for hepatic copper and selenium concentrations. Hepatic copper was lower on day 0 than on day 77 in low-S water control steers; however, the opposite pattern was observed in high-S water steers. Hepatic selenium was lower on day 0 for both control and high-S water steers; however, the magnitude of increase in day 77 hepatic selenium was greater in control steers. No other differences, aside from time of collection, were observed.

To date, microarray analysis revealed differential expression of genes ($P < 0.05$; minimum fold change of 2) between high-S water (no clinoptilolite) healthy steers and low-S water control steers (44 genes upregulated and 37 genes downregulated in high-S water healthy steers compared to control steers); high-S water (no clinoptilolite) PEM-afflicted steers and high-S water (no clinoptilolite) healthy steers (119 genes upregulated and 176 genes downregulated in high-S water PEM-afflicted steers compared to high-S water healthy steers); and high-S water + 5% clinoptilolite PEM-afflicted steers and high-S water + 5% clinoptilolite healthy steers (1388 genes upregulated and 627 genes downregulated in high-S water + 5% clinoptilolite steers). Although the clinoptilolite was not effective in preventing PEM in high-S water treated steers, further analyses are warranted to determine what effects, if any, clinoptilolite had at the molecular level. Such information may help in assessing the effectiveness, or ineffectiveness, of other potential ameliorators of high dietary S. Functional analyses of differentially expressed genes revealed themes of largely immune response, cell-to-cell recognition, and cell surface receptor function. Upon further analysis, genes will be selected for validation using real-time RT-PCR.

Results from this study suggest that clinoptilolite is not effective in negating the effects of high-S water consumption by roughage-fed feedlot steers. The addition of clinoptilolite did not reduce the incidence of S-induced PEM in steers administered high S water, and also did not prevent hepatic copper depletion. Gene expression analyses of hepatic tissue indicate that immune function genes are affected by high-S water. Differences in expression of selected genes will be confirmed using real-time RT-PCR. Future studies will test the effectiveness of molybdenum in prevention of H₂S production, and hence S-induced PEM, in steers administered high-S water.

References


Figure 1. Average dry matter intake (DMI) of steers in low-S water control, high-S water, high-S water + 2.5% clinoptilolite, and high-S water + 5.0% clinoptilolite treatment groups. Average DMI was lower ($P=0.0376$) in high-S water + 2.5% clinoptilolite steers than control steers, but was similar among all other steer groups.

Figure 2. Average plasma copper concentrations of steers in low-S water control, high-S water, high-S water + 2.5% clinoptilolite, and high-S water + 5.0% clinoptilolite treatment groups on trial days 58 and 77. Plasma copper was lower ($P=0.04$) in high-S water (no clinoptilolite) steers than control steers on day 58. No other differences in plasma copper were observed on day 58. On day 77, plasma copper was lower ($P<0.05$) in all high-S water treatment groups than control group; however, plasma copper was not different among high-S water treatment groups.
**Summary and Implications**

High dietary nitrate has a significant economic impact on livestock production worldwide due to both subacute and acute effects. Nitrate (NO$_3^-$) toxicity is often observed in cattle and sheep populations during periods of drought when forage crops accumulate high levels of NO$_3^-$. In ruminants, NO$_3^-$ is converted to nitrite (NO$_2^-$) in the rumen and reduced to ammonia. The conversion of NO$_3^-$ to NO$_2^-$ exceeds the reduction process when ruminants consume high NO$_3^-$ forages. Nitrite is then absorbed into the blood and oxidizes the ferrous ion in hemoglobin, reducing the ability of blood to carry oxygen to peripheral tissues. Symptoms of subacute NO$_3^-$ toxicity include decreased feed efficiency, reproductive complications, lethargy, weight loss, and even death. The purpose of this study was to confirm individual variation in response to subacute levels of dietary NO$_3^-$ and identify those individuals more and less tolerant to elevated dietary NO$_3^-$. Purebred Suffolk ewes were administered a potassium nitrate (KNO$_3$) supplement (300 mg NO$_3^-$/kg BW daily; n=47) or control supplement (n=8) for an 8 day period. Six NO$_3^-$ tolerant and six NO$_3^-$ intolerant ewes were identified based on performance and symptoms of NO$_3^-$ toxicity. Supplement intake was lower ($P<0.0001$) in NO$_3^-$ treated ewes than in control ewes, indicating that elevated dietary NO$_3^-$ influences feed intake; however, there was no difference in daily gain between the NO$_3^-$ treated ewes and controls ($P=0.9697$). Supplement intake differed between control, tolerant, and intolerant ewes ($P<0.0001$). The average supplement intake of tolerant and intolerant ewes was 82% and 23%, respectively. Daily gain, plasma NO$_2^-$, cortisol, glucose, and vitamin A levels were not different between control, tolerant, and intolerant ewes. Plasma urea nitrogen (PUN) levels were not different between control and intolerant ewes, but PUN levels in tolerant ewes were lower ($P=0.0173$) than in controls.

**Introduction**

More than $340$ million is lost annually by western United States producers due to livestock consumption of toxic plants (Nielsen and James, 1992). Death rates from rapid consumption of high NO$_3^-$ forages (45 – 547 mg NO$_3^-$/kg BW in cattle and 224 - 547 mg NO$_3^-$/kg BW in sheep) range from 7 - 44% in susceptible cattle and up to 42% in susceptible sheep (Harris and Rhodes, 1969). During severe drought conditions, plants do not absorb NO$_3^-$; however, eventual moisture leads to rapid absorption of NO$_3^-$ by plants. Nitrate itself is not toxic to livestock, but high levels of NO$_3^-$ ingestion results in the accumulation of toxic levels of NO$_2^-$ in the blood. Ingested NO$_3^-$ is converted to NO$_2^-$ by rumen bacteria which is further reduced to ammonia. The excess NO$_2^-$ in the blood forms methemoglobin, resulting in a conversion of the ferrous ion of hemoglobin to the ferric form which reduces the ability of blood to carry oxygen to the body (Yaremcio, 1991). Chronic and acute symptoms of NO$_3^-$ toxicity may appear when > 20% of hemoglobin is converted to methemoglobin. The transfer of rumen NO$_2^-$ into the bloodstream is influenced by NO$_3^-$ intake, rate of feed digestion and subsequent NO$_3^-$ release, rate of NO$_2^-$ reduction to ammonia, and absorption of NO$_2^-$ from the rumen. Subacute and/or chronic NO$_3^-$ toxicity can result in lethargy, head pressing, and impaired animal production as evidenced by depressed appetite, reduced or no weight gain, lowered milk production, reproductive complications, and increased susceptibility to infection (Yaremcio, 1991). The long- and short-term effects of subacute NO$_3^-$ toxicity have received little research attention, resulting in frequent misdiagnosis in livestock operations and incorrect or lack of appropriate treatment. Cattle and sheep exhibit similar...
symptoms in response to high levels of NO₃ in forage. However, sheep have a higher tolerance to NO₃ due to their ability to increase the concentration of red blood cells in the blood (Diven et al., 1964), making sheep an ideal model for cattle at the subacute level. The observed variation in susceptibility to toxicity can be partially attributed to the rate and duration of exposure as well as individual tolerance and metabolism levels; however, we hypothesize a genetic component may be responsible for an animal’s ability or inability to tolerate subacute levels of NO₃. In this study, animals that are more or less tolerant to subacute levels of NO₃ intake are identified based on supplement intake, weight change, and demonstration of subacute NO₃ toxicity symptoms.

Materials and Methods

Purebred Suffolk ewes (n=60; initial average BW = 85.7 ± 46.4 kg) were randomly allocated to one of two project start days (contemporary groups) due to time and labor limitations. Within each contemporary group, ewes were randomly allocated to a control (n=5) or elevated nitrate (n=25) diet. The basal diet consisted of bromegrass hay fed at 2.5% of initial BW. A supplement (11.5% total dietary CP) consisting of (92% DM basis) 53.9% soybean meal, 28.7% beet pulp, 10.0% molasses, and 7.4% of vitamins/minerals was fed three times per day (125 g/feeding, as fed basis). Weights were taken on day 1, 3, and 8 of the trial, as well as 4 days after completion of the study.

Liver biopsies were performed on day 3 or 4 and again at the end of the study using modified procedures of Ferreira et al., (1996) to accommodate larger sample extraction. A surgical area of approximately 16 cm² was sheared and sterilized with Lugol’s iodine solution (2% iodine, 4% K+ iodide). A local anesthetic of 8 cc of 2% injectable Lidocaine (VEDCO, St. Joseph, MO) was administered topically and subcutaneously around the incision area. A 1 cm incision was made with a #22 scalpel blade between the intercostal space of the 10th and 11th ribs approximately 9 cm below the processus spinous. A bone marrow biopsy punch (Jorgensen Vet Supply, Loveland, CO) modified with a syringe and tubing was inserted vertically through the intercostal space. The liver was penetrated approximately two to four times with suction to obtain a 1 g sample. Tissue was rinsed with PBS, snap-frozen in dry ice, and stored at -80°C. Incisions were sutured and cleaned with Lugol’s iodine solution, and ewes were given 3 cc of penicillin for precaution against infection. An 11% death rate typically occurs in sheep when performing liver biopsies as opposed to < 0.5% in cattle (Anderson et al., 1962). A total of 121 biopsies were performed in this study, with a 7% death loss due to complications. The RNA obtained from the liver biopsies were used to conduct microarray analyses to determine differentially expressed genes between control, NO₃ tolerant, and NO₃ intolerant ewes. Expression levels of differentially expressed genes will be confirmed using real-time RT-PCR.

Following the initial biopsies, ewes were randomly assigned to one of two NO₃ treatments consisting of 0 (control) or 300 mg supplemental NO₃/kg BW/d. As a precautionary measure, a mixture of 1 – 4% aqueous solution in saline of methylene blue was prepared which was to be injected intravenously based on 5 – 20 mg/kg LW basis. Blood was drawn for analyses at the time of biopsy, 12 hours after nitrate exposure, every 24 hours for the remaining 8 days of the trial, and 4 days after the cessation of treatment. Blood was drawn through the jugular vein and mixed with heparin to prevent clotting. All samples were immediately mixed and put on ice for 1 hour. Samples were then centrifuged for 20 minutes at 1520 × g after which plasma was obtained and stored at -20°C for future analyses.

Nitrate levels in the bromegrass hay and supplement were analyzed using a Standard Range Lab Nitrate Test Kit (L-NTK; NECi, Lake Linden, MI). Hay and supplement samples were dried for 12 h at 60 °C and boiled with activated charcoal for 20 minutes to extract the NO₃. The supernatant was further purified using a 22 guage syringe filter. Provided assay buffer (900 µl) was added to 50 µl of sample. Next 50 µl NADH and 20 µl of YNaR1 were added to each sample and allowed to sit for 20 minutes at room temperature. Finally, 500 µl Color Reagent No. 1 and 500 µl Color Reagent Number 2 were added to the samples and incubated for 10 minutes. All samples were read at 540 nm on a NanoDrop spectrophotometer (Nanodrop Tech., Inc., Wilmington, DE). Plasma samples were also tested for NO₃ levels using the same kit with the omission of the NO₃ reductase and NADH reagents from the assay. Plasma urea nitrogen levels were confirmed using a QuantiChrom™ Urea Assay Kit (DIUR-500; BioAssay Systems, Hayward, CA). Provided reagents A and B were added to 20 µl of samples and standards in equal amounts of 500 µl each and incubated for 30 minutes. Samples were then read at 520 nm on a Beckman Coulter DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). Total hemoglobin was analyzed utilizing a QuantiChrom™ Hemoglobin Assay Kit (DHB-250; BioAssay Systems). Provided reagent (500 µl) was added to a 50 µl sample. After a 5 minute incubation period, samples were read at 400 nm on a NanoDrop spectrophotometer. Blood glucose was determined using a rapid multiassay analyzer protocol (reagent GMRD-002A and standard GMRD-011; 8 m mol/L/144.1 mg/dL) and instrument (Analog Instruments LTD, London, UK). Samples were analyzed using 10 µl of plasma until duplicate results of ± 4.0 mg/dl were reached. Plasma cortisol levels were verified utilizing TKC05 Cortisol RIA Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA). The kit provided tubes previously lined with radiolabeled antibodies. The PBS gel (0.1%; 100 µl) and 1 ml of I²125 were added to each 100 µl of sample. After a 4 hr incubation period, samples were read in a Cobra II autogamma counter (Packard, Downers Grove, IL). All results were compared against internal controls of nonspecific bound and standard samples. Vitamin A levels were confirmed by the University of Michigan’s Diagnostic
Results and Discussion

Nitrate levels in bromegrass hay and basal supplement (without added \( \text{NO}_3^- \)) were within the safe range at < 4 ppm (0.0004% \( \text{NO}_3^- \)). Ewe age, ranging from 2 to 8 yr, had no effect on treatment (\( P = 0.5374 \)) or weight change (\( P=0.2090 \)).

Ewes fed \( \text{KNO}_3 \) treated supplement ingested less supplement (\( P<0.0001 \)) than controls, but daily gain did not differ (\( P=0.9697 \); Table 1). Treated ewes ingested 0.89 ± 0.05% of \( \text{NO}_3^- \), or 19.07 ± 1.17 \( \text{NO}_3^- \) g/d on a DM basis. Supplement intake tended to differ between the two contemporary groups (\( P=0.0571 \)), but there was no treatment by group interaction effect on supplement (\( P=0.2844 \)) or \( \text{NO}_3^- \) intake (\( P=0.0978 \)). Supplement intake differences between the two contemporary groups may be explained by weather pattern differences during treatment. It has been well established that changes in temperature, wind speed, humidity, and precipitation affect feed intake 

Based upon supplement intake, weight change, and symptoms of subacute \( \text{NO}_3^- \) toxicosis, treated ewes were identified as \( \text{NO}_3^- \) tolerant (n=6) and \( \text{NO}_3^- \) intolerant (n=6). Control, tolerant, and intolerant ewes were analyzed for weight change, supplement intake, \( \text{NO}_2^- \) plasma levels, PUN, glucose, cortisol, and vitamin A levels. Tolerant ewes ingested 1.53 ± 0.04% \( \text{NO}_3^- \) daily on a DM basis while intolerant ewes ingested 0.49 ± 0.05% (Table 2). Generally, forages containing acute levels of \( \text{NO}_3^- \) on a DM basis range from 1 to 3% or 10,000 to 30,000 ppm \( \text{NO}_3^- \) (Adams et al., 1992). Wright and Davidson (1969) found that 0.34 to 0.45% \( \text{NO}_3^- \) should be considered toxic while Case (1957) found that forage levels containing 0.70% \( \text{NO}_3^- \) resulted in death. Furthermore, Whitehead (1956) found that 0.92% \( \text{NO}_3^- \) was toxic to ruminants. Interestingly, tolerant ewes were able to tolerate a higher percentage of \( \text{NO}_3^- \) on a DM basis in their diet with no apparent ill effects compared to intolerant ewes that ingested a greatly reduced percentage of \( \text{NO}_3^- \) and yet were noticeably lethargic and had decreased supplement intake. The difference in % \( \text{NO}_3^- \) intake between tolerant and intolerant ewes, in combination with the reports of Adams et al. (1992), Wright and Davidson (1964), Case (1957), and Whitehead (1956), provides further evidence of variation in tolerance to elevated dietary \( \text{NO}_3^- \) in ruminant animals.

Supplement intake (\( P=0.0002 \)) differed between control and tolerant ewes (Table 2). Additionally, intolerant ewes consumed less (\( P<0.0001 \)) supplement than did control and tolerant ewes. Actual \( \text{NO}_3^- \) intake differed (\( P=0.0077 \)) between selected ewe groups (Table 2). Intolerant ewes had a decrease in feed intake, a numerical decrease in daily gain, and were noticeably more lethargic than tolerant and control ewes. Overall, there was no effect (\( P=0.9697 \)) of \( \text{NO}_3^- \) on weight change (Table 2). Nitrate used as a source of non-protein nitrogen in ruminants as an alternative hydrogen sink has been shown to have no affect on weight change in cattle or sheep in most studies (Fann-Bruning and Kaneene, 1993).

While plasma \( \text{NO}_3^- \) levels were not statistically different between control, tolerant, and intolerant ewes, plasma \( \text{NO}_3^- \) was consistently lowest in control ewes and highest in intolerant ewes during the 8 d treatment period (Figure 1). Plasma urea nitrogen levels were not different between intolerant and control (\( P=0.2528 \)) or tolerant (\( P=0.3831 \)) ewes (Figure 2). However, PUN levels were lower (\( P=0.0173 \)) in tolerant ewes than control ewes. Circulating plasma \( \text{NO}_3^- \) and PUN levels are indicators of individual \( \text{NO}_3^- \) metabolism. Though \( \text{NO}_3^- \) tolerant sheep ingested significantly more \( \text{NO}_3^- \) on a DM basis than intolerant ewes, plasma \( \text{NO}_3^- \) and PUN levels did not differ between these two selected groups. Plasma urea nitrogen levels increase over 50% when glomular filtration rate is impaired, indicating impairment of kidney function. Interestingly, tolerant ewes ingested a significantly higher % \( \text{NO}_3^- \) than intolerant ewes and had lower PUN and circulating \( \text{NO}_3^- \) levels. It has been proposed that ruminants produce endogenous levels of \( \text{NO}_3^- \) and \( \text{NO}_3^- \), and endotoxins present due to infection or stress may enhance those levels making individuals more susceptible to nitrate toxicity (Adams et al., 1992). Sheep have the capability to adjust to \( \text{NO}_3^- \), but daily analyses show that at no point during this study were there any differences in blood parameters between tolerant, intolerant, and control ewes. This may indicate that ewes did not adapt to the higher dietary \( \text{NO}_3^- \), and perhaps were not provided adequate time to do so. Furthermore, endogenous levels of \( \text{NO}_3^- \) among treated and control ewes does not fully explain why animals administered \( \text{NO}_3^- \) had the same level of plasma \( \text{NO}_3^- \) and urea concentrations as did controls. It is proposed that \( \text{NO}_3^- \) treated ewes were reducing the \( \text{NO}_3^- \) at different rates, resulting in plasma parameters that were similar among the selected groups. We hypothesize that the cause for individual variation in metabolizing \( \text{NO}_3^- \) is, in part, genetically based, which will be investigated in future gene expression analyses.

Both cortisol and glucose levels were not different between tolerant, intolerant, and control ewes (Figures 3 and 4, respectively). Cortisol levels have been shown to increase when animals are exposed to high levels of \( \text{NO}_3^- \) (Yaremcio, 1991; Zraly et al., 1997). However, measuring cortisol as an indicator of stress does not identify the nature of the actual stressor. It should be noted that cortisol levels were increased on d 0 and d 3. During these two days, animals were being weighed, prepared for a liver biopsy, blood sampled, or exposed to extra handling, such as shearing of the necks, which most likely caused the
observed increases in cortisol. Typically, animals that are stressed release glucocorticoids to maintain balance in the sympathoadrenal system. As a consequence, the increased levels of cortisol stimulate gluconeogenesis which results in elevated plasma glucose. Plasma glucose levels are further increased due to skeletal muscle and adipose tissues preventing the uptake of glucose due to increased circulating glucocorticoids. Increased glucose levels as a result of increased cortisol levels are not directly related to short term stress; however long term stress has been implicated in increased glucose levels (Hadley and Levine, 2007).

Nitrate has been implicated in the reduction of thyroid activity which is responsible for the conversion of carotene to vitamin A in ruminants. Furthermore, the storage of vitamin A from carotene in the liver is greatly reduced, especially by elevated NO$_3^-$ levels (O’Donovan and Conway, 1967). Circulating plasma vitamin A levels were analyzed on d 1, 4, and 7. Results indicated that there were no differences between control, tolerant, and intolerant ewes ($P > 0.1000$; Figure 5). Vitamin A levels may not have been affected due to the subacute exposure of NO$_3^-$. A chronic exposure or an acute dose may have resulted in a more noticeable effect on vitamin A metabolism (Fann and Kaneene, 1993). O’Donovan and Conway (1967) found that ewes grazing a pasture for 5 months with %NO$_3^-$ content on a DM basis (0.05-1.72%) did not result in reduced vitamin A levels.

**Conclusions and Future Aims**

Individual response in ewes to NO$_3^-$ treatment varied as evidenced by differences in performance and behavior. Supplement intake was significantly decreased by increasing dietary NO$_3^-$. Contemporary groups 1 and 2 differed in average supplement intake, which may be attributable to weather patterns or temperature. Further investigation will be required to determine reasons for variation between the two contemporary groups. Lack of differences in plasma NO$_3^-$ levels between control and NO$_3^-$ treated ewes may be due to the NO$_3^-$ being bound in the blood or may be a result of individual threshold levels of NO$_3^-$ intake. This may be further explained with gene chip analyses. These results demonstrate that individual animal performances differ in response to elevated dietary NO$_3^-$ and that reproduction may be affected by short-term exposure to subacute levels of NO$_3^-$ prior to breeding.

Microarray analyses were performed in conjunction with the University of Missouri to identify genes differentially expressed between control, tolerant, and intolerant ewes. Statistical analysis of results is currently undergoing at the University of Minnesota-St Paul. Finally, elevated NO$_3^-$ levels have been associated with female reproductive performance, including hormonal imbalances and reduced implantation rate, potentially resulting in abortion (Yaremciu, 1991; Zraly et al., 1997). Therefore, the effects of subacute levels of dietary NO$_3^-$ on breeding potential is currently being investigated.

**Acknowledgements**

This research was supported in part by a University of Wyoming Agriculture Experiment Station Competitive Grant. We would also like to thank Brent Larson and Ed Van Kirk for their assistance with animal care.

**References**


Yaremciu, B. 1991. Nitrate poisoning and feeding nitrate feeds to livestock. Alberta Agriculture, Food and...
Table 1: Mean production parameters for all ewes¹ (n=55) and selected ewes² (n=18).

¹All ewes in control (n=8) and NO₃³ (n=47) treatment groups.
²Ewes selected as control (n=6), NO₃³ intolerant (n=6), and NO₃³ tolerant (n=6).

**Daily Mean Production Parameters**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>NO₃³</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Supplement (oz; DM)</td>
<td>96.39 ± 7.80</td>
<td>81.79 ± 2.27</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Actual NO₃³ intake (oz; DM)</td>
<td>0.67 ± 0.04</td>
<td>0.33 ± 1.92</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Weight change (lbs)</td>
<td>0.73 ± 1.73</td>
<td>0.33 ± 1.92</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**Daily Mean Plasma NO₂**

Figure 1. Average daily circulating NO₂ plasma levels. There were no differences between control and NO₃³ intolerant (P=0.5778), control and NO₃³ tolerant (P=0.7753), and NO₃³ intolerant and tolerant ewes (P=0.8430).

**Daily Mean Plasma Urea Nitrogen**

Figure 2. Average daily plasma urea nitrogen (PUN) levels. There were no differences between control and NO₃³ intolerant (P=0.2528), and NO₃³ intolerant and tolerant (P=0.3831) ewes; however, PUN levels in control and NO₃³ tolerant ewes did differ (P=0.0173).

**Daily Mean Cortisol**

Figure 3. Average daily cortisol levels did not differ between control and NO₃³ intolerant (P=0.9961), control and NO₃³ tolerant (P=0.9242), and NO₃³ intolerant and tolerant (P=0.9587) ewes.

**Daily Mean Glucose**

Figure 4. Average daily glucose levels did not differ between control and NO₃³ intolerant ewes (P=0.9655), control and NO₃³ tolerant (P=0.9910), and NO₃³ intolerant and tolerant ewes (P=0.9900).

**Mean Plasma Vitamin A**

Figure 5. Average daily vitamin A levels in plasma were not different between control and NO₃³ intolerant (P=0.9962), control and NO₃³ tolerant (P=0.2616), and NO₃³ intolerant and tolerant ewes (P=0.3773).
TRADITIONAL AND SELF-FED CULL COW FEEDING PROGRAMS: EVALUATION OF PERFORMANCE AND ECONOMICS

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Christopher L. Loehr, Graduate Assistant
Steven I. Paisley, State Extension Beef Specialist
Department of Animal Science

Introduction

Cull cows are responsible for a significant portion of annual income for beef cattle producers (15-30%), while also providing a source of meat for the packing industry (Feuz, 1999). The National Cow and Bull Beef Quality Audit (Roebet al., 1999) identified management techniques available to producers to increase the quality and value of beef from cull cows, but research investigating additional income opportunities from cull cows is also important. Historically spring calving herds tend to sell open or other cull cows after weaning, flooding the fall market and creating seasonally low prices. One possibility for optimizing income may be feeding cull cows, taking advantage of relatively efficient gains (Matulis et al., 1987) and postponing the sale of cull cows until the market is less saturated (Yager et al., 1979). The length of the feeding period for cull cows has been suggested as 45 to 60 days to optimize feed conversion to gain (Sawyer et al., 2004), though Wooten (et al., 1979) cautions that while more time on feed improves both fat and lean deposition, initial body condition also contributes, and so deposition is not constant. Boyles (2001) suggests a feeding period of at least 60 days to allow yellow fat from forage diets to be converted to white fat. Corn has been a reasonably priced energy source for several decades, but increasing grain prices, drought conditions in the Midwest and Western High Plains, and rising input costs have impacted cull cow enterprise budgets and potential profitability. Re-evaluating the economic profitability of various feeding strategies has become necessary for determining feasibility of cull cow programs at the production level. Our objective was to evaluate traditional drylot diets versus range-based cull cow finishing programs in performance, carcass traits, and economics. Our hypothesis was that feed performance and carcass traits would be similar across feeding methods, but that self-fed range-based programs may potentially decrease feed and overall costs.

Materials and Methods

Animals

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Seventeen Angus cross cull cows from the University of Wyoming Beef Unit, Laramie, and 55 Angus cross cull cows (average price of $0.43/lb.) purchased from livestock auction in Torrington, WY, were shipped to the James C. Hageman Sustainable Agriculture Research and Extension Center in Lingle, WY. All cows were weighed, body condition scored (BW 1219 ± 44 lb., BCS 4.95 ± 0.16), and had age estimated by dentition. Cows were vaccinated for respiratory and clostridial diseases, dewormed, and assigned to nine pens based on similar (P = 0.12) pen weights, BCS, and age status. Each pen was then randomly assigned to one of three treatments.

Diets

Specific nutritional information of the diets used is summarized in Tables 1 and 2. Animals on all treatments were provided free choice fortified trace mineral salt. Treatments were based on diets available in the High Plains Region in the United States, and included two traditional corn-based diets and one self-fed diet using a controlled intake system (Accuration and Impact Finisher, Purina Mills, St. Louis, Missouri). The corn-based diets used mixed alfalfa and grass hay with barley straw (HAY) or corn silage (SILAGE) as the roughage source to accompany rolled corn and a commercial feedlot supplement containing Rumensin (Elanco, Greenfield, IN). Two step-up diets were formulated to acclimate the cull cows to the final diet over a month-long period. The final ration for both HAY and SILAGE were formulated to provide 0.6 Mcal NE₂/lb. and 11.5% CP. The controlled
intake system (LIMIT) used self-feeders and little or no alfalfa grass mix hay, potentially simulating range-based cull cow finishing programs. The use of the LIMIT diet followed the protocol developed by Purina nutritionists. Animals on the LIMIT treatment required two weeks to adapt to the self-feeder, initially, free-choice hay was provided via round bale feeders, and the limit-fed grain diets were gradually introduced while removing available forage from the pen.

Table 1. Composition of HAY and SILAGE diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>HAY</th>
<th>SILAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient composition, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>9.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Corn Silage</td>
<td>-</td>
<td>20.0</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>5.8</td>
<td>-</td>
</tr>
<tr>
<td>Corn Grain</td>
<td>80.8</td>
<td>68.7</td>
</tr>
<tr>
<td>40-30 FDLT SU</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Nutrient composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>89.2</td>
<td>66.9</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>NE_m, Mcal/kg DM</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NE_e, Mcal/kg DM</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>C:P</td>
<td>1.60</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Table 2. Composition of LIMIT self-fed diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>ACCURATION^a</th>
<th>IMPACT^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient composition</td>
<td>Ad lib</td>
<td>Ad lib</td>
</tr>
<tr>
<td>Mixed Hay</td>
<td>Ad lib</td>
<td>Ad lib</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>40%</td>
<td>70%</td>
</tr>
<tr>
<td>Corn Grain</td>
<td>40%</td>
<td>70%</td>
</tr>
<tr>
<td>Ration</td>
<td>40%</td>
<td>70%</td>
</tr>
<tr>
<td>Nutrient composition</td>
<td></td>
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</tr>
<tr>
<td>CP, min %</td>
<td>32.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Crude Fat, min %</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>C:P</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

^a Contains 130.0 g/T Monensin as Monensin sodium.
^b Contains 227.0 g/T Monensin as Monensin sodium, and 90.0 g/T Tylosin, as Tylosin phosphate.

Measurements and Analysis

Animals were weighed two weeks before the start of the feeding trial, and then on days 1, 2, 29, 60, 90, and 91 of the feeding trial. Both initial and final weights were determined by averaging weights taken on consecutive days. Cows were also implanted with Finaplix-H implants (trenbolone acetate, Intervet, Millsboro, DE) on day 29 to increase feedlot performance as demonstrated by Cranwell (et al., 1996) and Wright (2005). Cattle were harvested on day 94 at Gibbon Packing, L.L.C., Gibbon, NE, with final carcass data collected at the plant on days 95 and 96. Prices of feedstuffs were based on a daily USDA report and averaged across the duration of the study, and are reported in Table 3. Hay, straw, and corn nutrient analyses were completed by SDK Laboratories, Inc. (Hutchinson, Kansas), while Accuration and Impact Finisher data were based on information provided by Purina Mills (St. Louis, Missouri).

Statistical Analysis

Cattle performance, carcass traits, and economic data were analyzed in a completely randomized design using the GLM procedures of SAS (Version 9.1, SAS Inst., Inc., Cary, NC), with pen as the experimental unit. Least squares means are reported, with means separated by least squares procedure when overall P-value <0.05. Proc FREQ and CHISQ were used to interpret USDA quality grade distribution for individual cows.

Results and Discussion

Feedlot Performance

Performance data for cull cows managed under three feeding strategies are summarized in Table 4. Although all treatments achieved similar (P=0.63; 343 lbs, 3.77 lb/d) weight gain during the entire 91 day study, cows assigned to HAY and SILAGE rations attained more rapid gains in the first month (P<0.01), while animals on LIMIT diet had greater gains (P=0.02) in the final period. Average daily gain (ADG) for the first month was greater (P<0.01) for HAY and SILAGE treatments than for LIMIT treatment (5.96 lb/d, 5.83 lb/d, and 2.24 lb/d, respectively), partially due to the adaptation period required for cows on the LIMIT treatment. Cattle assigned to LIMIT diets were initially offered a grass/alfalfa mix hay in round bale feeders, a higher quality hay than recommended in diet transition guidelines, partially explaining the group’s slow adaptation to the limit-fed grain diet. On day 13, the mixed hay was replaced with barley straw and cattle began consuming the self-fed diets. Although DMI for that same period was less (P<0.01) for SILAGE cows than for LIMIT treatment, with SILAGE intermediate (12.5 vs. 10.2 lb/100 lb/head per day (P<0.01; 29.5 vs. 35.9 and 34.5 lb/hd/d for LIMIT, HAY and SILAGE, respectively) and had more efficient gains (P<0.01) for HAY and SILAGE treatments than for LIMIT treatment (9.01 lb/100 lb) than HAY treatment, with SILAGE intermediate (9.91 lb/100 lb). Despite similar total performance and improved feed efficiency, higher total feed costs (Table 3) resulted in LIMIT cattle having higher (P<0.01; $0.91/lb) feed cost/lb of gain than HAY or SILAGE treatments ($0.65/lb and $0.61/lb, respectively).

Carcass Characteristics

Of the 72 cull cows sent to harvest, one cow was injured during transport, was deemed a downer animal, and
did not have carcass data collected. Results of carcass data collection are found in Table 5 for cull cows on the three feeding strategies. Hot carcass weights, 12th rib backfat, and Longissimus area were similar (P > 0.05; 796 lbs., 0.46 in., 11.4 in²) across treatments. These results are expected based on all cows having the same harvest date. Studies by Wooten et al., 1979) and Matulis et al., 1987) indicate that both HCW and backfat increased as days on feed increased, to the end of their studies (108 and 84 days on feed, respectively). Marbling tended (P = 0.08) to be greater for HAY than for LIMIT treatment, with SILAGE intermediate (all Slight; 366, 318, and 356, respectively). Dressing percentage was also higher (P < 0.01) for HAY cattle than for either SILAGE or LIMIT diets (53.2% vs. 51.3% and 51.5% respectively). Fat color and final carcass price were also similar (P = 0.55; 2.80, $105.87/cwt) across treatments.

Economic Analysis

Initial November average value for the cull cows used in this study was $0.43/lb, or $524.08/hd. Feeding cull cows for an additional 91 d on the three strategies increased the average cow value to $845.86 ± 26.48, with no difference between treatments (P = 0.87). Holding cull cows for an additional 91 d demonstrated an average of $321.78/hd increased income. Feed costs for the entire trial period, based on the prices found in Table 3, for the three treatments were greater for LIMIT animals (data not shown, P < 0.01, $306.24/hd) than for those in HAY and SILAGE groups ($216.40/hd and $217.93/hd, respectively). Therefore, while both HAY and SILAGE treatments had the potential to capture $104.61/hd profit by feeding cull cows for 91 d, the use of the LIMIT diet could only augment profit by an average of $15.54/hd.

Table 3. Prices used for estimation of feedstuffs.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Cost, $/unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>$420/ton</td>
</tr>
<tr>
<td>Alfalfa/Grass Hay</td>
<td>$110/ton</td>
</tr>
<tr>
<td>Straw Hay</td>
<td>$55/ton</td>
</tr>
<tr>
<td>Corn</td>
<td>$3.52/bu.</td>
</tr>
<tr>
<td>40% Protein Supplement</td>
<td>$360/ton</td>
</tr>
<tr>
<td>Self-fed rations:</td>
<td></td>
</tr>
<tr>
<td>Accuration</td>
<td>$294/ton</td>
</tr>
<tr>
<td>Impact</td>
<td>$246.60/ton</td>
</tr>
</tbody>
</table>

Conclusions

Despite the initial lag in performance, cattle assigned to the LIMIT treatment respond quickly and eventually achieved similar weight gain compared to cattle assigned to HAY and SILAGE treatments. Cows receiving the silage-based and hay-based finishing rations had similar feed efficiencies and similar feed cost of gains. In comparison, LIMIT cows consumed less feed and had more efficient gains than HAY and SILAGE treatments; however, overall cost of gains were higher, contrary to our initial hypothesis. While there were differences in lean maturity, skeletal maturity and lean color, carcass prices were similar for all treatments.

Implications

As a significant portion of income for the beef producer, cull cow profitability is a key concern for both producers and researchers. Using available resources to add additional value and improve market timing for cull cows may increase profit for the producer. This profit potential is dependent upon the initial body condition, cost and availability of feed, length of feeding period, and final carcass traits. Traditional methods may be more expensive in the future, as grain prices and fuel costs continue to increase, making these strategies less appealing. Range-based methods explored in this study were equally effective, but were more expensive on a per kg gain basis than traditional methods. Further studies in this area should investigate other low-cost feeds, alternative marketing opportunities, and the development of value-added by-products from cull cows to improve overall profitability.

Literature Cited


Table 4. Impact of cull cow feeding and management strategies on feeding performance and costs.

<table>
<thead>
<tr>
<th>Item</th>
<th>HAYa</th>
<th>SILAGEb</th>
<th>LIMITc</th>
<th>SE</th>
<th>P-Valued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pens (8 hd/pen)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pre-trial BW, lb</td>
<td>1211</td>
<td>1248</td>
<td>1202</td>
<td>25.8</td>
<td>0.45</td>
</tr>
<tr>
<td>Initial BCS (1-9)</td>
<td>5.05</td>
<td>4.84</td>
<td>4.95</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>First Month, 29 d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, lb/d</td>
<td>5.97g</td>
<td>5.84g</td>
<td>2.24f</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI, lb-hd^1-d^-1</td>
<td>35.2g</td>
<td>27.1f</td>
<td>34.7g</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G/F, lb/lb</td>
<td>0.170f</td>
<td>0.214h</td>
<td>0.064f</td>
<td>0.013</td>
<td>0.04</td>
</tr>
<tr>
<td>Feed cost/gain, $/lb</td>
<td>0.33f</td>
<td>0.30f</td>
<td>1.30f</td>
<td>0.056</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Second Month, 31 d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, lb/d</td>
<td>2.50</td>
<td>2.66</td>
<td>3.52</td>
<td>0.506</td>
<td>0.37</td>
</tr>
<tr>
<td>DMI, lb-hd^1-d^-1</td>
<td>35.9g</td>
<td>38.2g</td>
<td>24.7f</td>
<td>0.91</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G/F, lb/lb</td>
<td>0.070f</td>
<td>0.069f</td>
<td>0.143g</td>
<td>0.013</td>
<td>0.01</td>
</tr>
<tr>
<td>Feed cost/gain, $/lb</td>
<td>1.06</td>
<td>1.25</td>
<td>0.88</td>
<td>0.272</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Third Month, 31 d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, lb/d</td>
<td>2.69f</td>
<td>3.44f</td>
<td>5.39g</td>
<td>0.492</td>
<td>0.02</td>
</tr>
<tr>
<td>DMI, lb-hd^1-d^-1</td>
<td>36.8g</td>
<td>38.2g</td>
<td>30.0f</td>
<td>1.61</td>
<td>0.02</td>
</tr>
<tr>
<td>G/F, lb/lb</td>
<td>0.074f</td>
<td>0.090f</td>
<td>0.178g</td>
<td>0.013</td>
<td>0.01</td>
</tr>
<tr>
<td>Feed cost/gain, $/lb</td>
<td>1.01</td>
<td>0.79</td>
<td>0.79</td>
<td>0.096</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Total Test, d 91</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, lb^e</td>
<td>1179</td>
<td>1211</td>
<td>1180</td>
<td>27.7</td>
<td>0.67</td>
</tr>
<tr>
<td>Final BW, lb</td>
<td>1513</td>
<td>1569</td>
<td>1519</td>
<td>38.7</td>
<td>0.57</td>
</tr>
<tr>
<td>91 d gain, lb</td>
<td>334</td>
<td>357</td>
<td>339</td>
<td>17.7</td>
<td>0.63</td>
</tr>
<tr>
<td>ADG, lb/d</td>
<td>3.67</td>
<td>3.93</td>
<td>3.73</td>
<td>0.195</td>
<td>0.63</td>
</tr>
<tr>
<td>DMI, lb-hd^1-d^-1</td>
<td>36.0g</td>
<td>34.7g</td>
<td>29.6f</td>
<td>0.86</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G/F, lb/lb</td>
<td>0.102g</td>
<td>0.113g</td>
<td>0.125f</td>
<td>0.005</td>
<td>0.03</td>
</tr>
<tr>
<td>Feed cost/gain, $/lb</td>
<td>0.65f</td>
<td>0.61f</td>
<td>0.91f</td>
<td>0.032</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

a Dry finishing ration consisting of alfalfa/grass hay, straw, and commercial feedlot protein supplement, calculated to provide 0.6 Mcal NE_/lb and 11.5% CP
b Silage-based finishing ration consisting of silage, alfalfa/grass hay, corn and commercial feedlot protein supplement, calculated to provide 0.6 Mcal NE_/lb and 11.5% CP
c Self-fed finishing ration, offered ad-libitum via self-feeders placed in pens

Table 5. Impact of cull cow feeding and management strategies on carcass traits.

<table>
<thead>
<tr>
<th>Item</th>
<th>HAYa</th>
<th>SILAGEb</th>
<th>LIMITc</th>
<th>SE</th>
<th>P-Valued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>24</td>
<td>23^l</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pens (8 hd/pen)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Final live BW^e, lb</td>
<td>1513</td>
<td>1569</td>
<td>1519</td>
<td>38.7</td>
<td>0.57</td>
</tr>
<tr>
<td>HCW, lb</td>
<td>807</td>
<td>805</td>
<td>784</td>
<td>22.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Dressing %</td>
<td>53.2j</td>
<td>51.3i</td>
<td>51.5j</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lean maturity^f</td>
<td>452</td>
<td>434</td>
<td>471</td>
<td>8.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Skeletal maturity^f</td>
<td>576i 560jk</td>
<td>548i</td>
<td>5.9</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Marbling^g</td>
<td>366</td>
<td>356</td>
<td>318</td>
<td>12.8</td>
<td>0.08</td>
</tr>
<tr>
<td>12th rib backfat, in</td>
<td>0.47</td>
<td>0.47</td>
<td>0.44</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>Longissimus area, in^2</td>
<td>11.5</td>
<td>11.6</td>
<td>11.2</td>
<td>0.29</td>
<td>0.58</td>
</tr>
<tr>
<td>Muscling score (1-5)</td>
<td>3.54j</td>
<td>2.84i</td>
<td>3.08ijk</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat color^b</td>
<td>2.62</td>
<td>2.95</td>
<td>2.83</td>
<td>0.21</td>
<td>0.56</td>
</tr>
<tr>
<td>Lean color^c</td>
<td>5.58j</td>
<td>5.58l</td>
<td>6.20bk</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Carcass price, $/cwt</td>
<td>105.54</td>
<td>105.68</td>
<td>106.38</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>Calc. quality grade, % ≥ Utility</td>
<td>70.83</td>
<td>69.57</td>
<td>50.00</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Total cow value, $</td>
<td>852.13</td>
<td>851.13</td>
<td>834.32</td>
<td>26.48</td>
<td>0.87</td>
</tr>
</tbody>
</table>

a Dry finishing ration consisting of alfalfa/grass hay, straw, corn and commercial feedlot protein supplement, calculated to provide 0.6 Mcal NE_/lb and 11.5% CP
b Silage-based finishing ration consisting of silage, alfalfa/grass hay, corn and commercial feedlot protein supplement, calculated to provide 0.6 Mcal NE_/lb and 11.5% CP
c Self-fed finishing ration, offered ad-libitum via self-feeders placed in pens
d Data analyzed as a completely randomized design using GLM of SAS. Least square means are reported, with means separated by least squares procedure when Overall P-value < 0.05.
e Initial and final weights determined by averaging two consecutive day weights.
f A = 100 to 199, B = 200 to 299, C = 300 to 399, D = 400 to 499, and E = 500 to 599.
g Slight = 300 to 399.
h 1 = white; 5 = yellow.
i 1 = bright cherry red; 8 = dark brown.
jk Means with different superscripts differ (P < 0.05).
l During transport to harvest, one cow was injured and arrived as a downer cow, therefore no data was collected.

31
Evaluation of prepartum alternative oilseed meal supplementation on beef cow and calf performance

Frances Loehr, Graduate Research Assistant
Chris Loehr, Graduate Research Assistant
Steve Paisley, Extension Beef Specialist
Travis Smith, University of Wyoming Beef Herdsman
E. Lee Belden, Immunology Professor
Bret Hess, Professor
Department of Animal Science

Introduction

Recently, high plains livestock producers have been hit with high fuel prices, high corn prices, and high overall feed prices related to drought conditions. Corn and oilseed meal prices have been largely impacted by the growing biodiesel and ethanol industry. As corn and soybeans are also heavily demanded for human food and livestock feed use, alternative oilseeds have been analyzed both for potential oil production and livestock feed use. An ideal oilseed will yield high quantity and quality oil for biodiesel production, while also maintaining both protein and fat content for use by livestock. Two feasible options for oilseed production for biodiesel and feed in the high plains of the United States are safflower and camelina.

Safflower seeds can be high in linoleic or oleic unsaturated fatty acids. Linoleic acid is considered an essential omega-6 fatty acid, and is therefore desirable in livestock diets. As an oilseed meal, safflower is of lower nutritive value than the industry standard of soybean meal.

Whole seed camelina, a relative newcomer, contains approximately 38% oil and 27% crude protein, and contains high percentages of omega-3 fatty acids (Lardy, 2008). Feeding the omega-3 fatty acids may have positive impacts on omega-3 levels in meat for human consumption, while also supporting the animal’s immune functions, as suggested by an earlier supplementation study conducted by Lake et al. (University of Wyoming, 2006). One potential concern with feeding camelina is the potentially harmful glucosinolate and tannin compounds, found in low levels in both the seeds and oilseed meal. An additional component of this research is to provide data to support FDA approval for Generally Regarded As Safe (GRAS) classification for camelina as a livestock feed ingredient.

The objectives of this study are to evaluate feeding supplemental camelina meal to pregnant cows, to determine whether alternative oilseeds can perform similarly to traditional soybean meal in cow performance and reproduction, calf performance and health, and in calf passive immunity transfer.

Materials and Methods

Animals

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Ninety-nine pregnant cross-bred Angus cows from University herds were assigned to nine pens to determine the effects of three pre-calving cows fed different oilseeds on cow performance and on calf health and performance. Pens were similar (P=0.78) in cow body weight (1438±145 lb), age (8 years), and body condition score (5.00±0.35). Each pen was then randomly assigned to one of three treatments.

Diets

Animals on all treatments were provided free-choice fortified trace mineral salt. Diets consisted of 25.5 lb/head/day mixed grass hay and one of three supplementation treatments formulated to meet energy, protein, and mineral requirements for cows in late gestation. Supplements consisted of soybean meal and cracked corn (CONTROL), safflower and soybean meals (SAFFLOWER), or camelina meal (CAMELINA).

Measurements & Analysis

At the start of the study period, cows were weighed, body condition scored, and had age verified by herd records. After a 57 day feeding study, and before calving, cows were reweighed and body condition scored. Calving ease and body condition score were noted within 24 hours of calving. Cow weight and condition were also measured at branding (day 49) and at weaning (day 241). Reproductive success was also calculated as % bred as of day 225. Calf birth weight and vigor were noted within 24 hours of birth.
Adjusted 205-day weights were calculated from weaning weights (day 241). Calf death loss was also determined.

**Immunology**

Blood was drawn via venipuncture from cows (14 days before calving) and from calves (within 24 hours of birth) to determine passive immunity transfer success. Calves were also challenged with ovalbumin on day 60, with response measured on day 60, 67, 74, and 81, also by blood collected by venipuncture.

**Statistics**

Data were analyzed in a completely randomized design using the GLM procedures of SAS (Version 9.1, SAS Inst., Inc., Cary, NC) with pen as the experimental unit. Least square means are reported, with means separated by least squares procedure when overall $P$-value < 0.05. CHISQ and Proc FREQ were used to interpret reproductive distribution for individual cows, and death loss by individual.

**Results and Discussion**

Performance data for cows and calves are reported in Table 1, with significant $P$-values noted in note f,g below Table 1, and other non-significant $P$-values listed in this section. All treatments achieved similar ($P = 0.48; 48.4 \text{ lb}, 0.84 \text{ lb/d}$) weight gain and increased ($P = 0.86; +0.17 \text{ BCS}$) body condition during the feeding study. Calving ease and birth weight were similar ($P = 0.45; 1.07 \text{ score and 92.4 lb}$, respectively) across treatments. Cows on CONTROL diet tended ($P = 0.08$) to have lower BCS at calf birth than did animals on the SAFFLOWER treatment, with CAMELINA intermediate (4.93, 5.05, and 5.00, respectively). Body weight and BCS of cows at 49 days postpartum were similar ($P = 0.61; 1481 \text{ lb}, 4.64 \text{ BCS}$) across treatments. Cows bred back at similar rates ($P = 0.23, 90\%$), and death loss of calves was similar ($P = 0.87, 8\%$) across treatments. Adjusted 205-d weaning weights ($P = 0.80; 600 \text{ lb}$) were similar across treatments.

**Immunology**

At the time of publication, calf immune transfer and antigen challenge data are still being processed. No results are available at this time.

**Summary and Implications**

Alternative oilseed meal supplementation yielded similar results to traditional corn and soybean supplementation on cow performance and fertility and on calf weights and death loss.

**References**

Table 1. Impact of three oilseed supplementation strategies on performance of cows and their calves.

<table>
<thead>
<tr>
<th>Item</th>
<th>CON&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Saff&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cam&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SE&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Pens (9 hd/pen)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Pre-trial BW&lt;sup&gt;e&lt;/sup&gt;, lb</td>
<td>1439</td>
<td>1440</td>
<td>1439</td>
<td>25.8</td>
</tr>
<tr>
<td>Initial BCS (1-9)</td>
<td>5.00</td>
<td>4.99</td>
<td>5.01</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Cow Weights</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-trial BW&lt;sup&gt;e&lt;/sup&gt;, lb</td>
<td>1492</td>
<td>1495</td>
<td>1483</td>
<td>26.4</td>
</tr>
<tr>
<td>End-trial BCS</td>
<td>5.15</td>
<td>5.19</td>
<td>5.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Change in BW, lb</td>
<td>52.9</td>
<td>54.4</td>
<td>44.4</td>
<td>6.30</td>
</tr>
<tr>
<td>Change in BCS</td>
<td>0.15</td>
<td>0.20</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Calving BCS</td>
<td>4.93&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td>Branding BW, lb</td>
<td>1338</td>
<td>1347</td>
<td>1352</td>
<td>26.3</td>
</tr>
<tr>
<td>Branding BCS</td>
<td>4.60</td>
<td>4.65</td>
<td>4.68</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>At Calving</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calving Ease</td>
<td>1.06</td>
<td>1.12</td>
<td>1.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Birth Weight, lb</td>
<td>92.7</td>
<td>91.8</td>
<td>94.6</td>
<td>2.62</td>
</tr>
<tr>
<td><strong>Weaning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf 205-adj., lb</td>
<td>595</td>
<td>604</td>
<td>604</td>
<td>10.3</td>
</tr>
<tr>
<td>% Calves lost</td>
<td>9.0</td>
<td>6.0</td>
<td>9.0</td>
<td>-</td>
</tr>
<tr>
<td>Cows Rebred</td>
<td>90</td>
<td>83</td>
<td>97</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control diet consisting of alfalfa/grass hay, and supplement with corn and soybean meal, calculated to meet requirement of cows in late gestation.

<sup>b</sup> Safflower-based diet consisting of alfalfa/grass hay, safflower and soybean meals to provide for requirement of cows in late gestation.

<sup>c</sup> Camelina diet consisting of alfalfa/grass hay, and camelina meal supplement to provide for requirement of cows in late gestation.

<sup>d</sup> Data analyzed as a completely randomized design using GLM of SAS. Standard error is reported, with no treatments having \( P < 0.05 \) from least square means.

<sup>e</sup> Initial and final weights determined by averaging two consecutive day weights.

<sup>f,g</sup> Calving BCS \( P = 0.08 \), trend is noted for means with different superscripts.
AMP-Activated Protein Kinase and Adipogenesis in Sheep Fetal Skeletal Muscle

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Mei J. Zhu, Assistant Professor
K. R. Underwood, Graduate Research Assistant
P. W. Nathanielsz, Professor,
S. P. Ford, Professor
M. Du, Assistant Professor
Department of Animal Science

Summary and Implications

Marbling, or intramuscular fat, is an important factor determining beef quality. Both adipogenesis and hypertrophy of existing adipocytes contribute to enhanced marbling. We hypothesized that the fetal stage is important for the formation of intramuscular adipocytes and AMP-activated protein kinase (AMPK), as a master energy sensor, has a key role in adipogenesis during this stage. The objective of this study was to assess the role of AMPK in adipogenesis in fetal sheep muscle. Non pregnant ewes were randomly assigned to a control (Con, 100% of NRC recommendations, n=7) or over-fed (OF, 150% of NRC, n=7) diet from 60 d before to 75 d after conception when ewes were euthanized. The fetal longissimus dorsi (Ld) muscle was collected at necropsy for biochemical analyses. The activity of AMPK was significantly lower in the fetal muscle of OF fetal muscle compared to Con fetal muscle. In addition, lipid content was also higher in OF fetal muscle. These data show that AMPK activity is inversely related to adipogenesis in fetal sheep muscle, and maternal over-nutrition enhanced adipogenesis of fetal skeletal muscle.

Key Words: AMP-activated protein kinase, sheep, fetus, adipogenesis, skeletal muscle

Introduction

Marbling (intramuscular fat) is a primary criterion for beef quality grading. In addition, marbling is also important for the eating quality of pork and lamb (Hausman et al., 2007; Underwood et al., 2007b). Marbling is correlated with the number of adipocytes in skeletal muscle; however, mechanisms controlling adipogenesis in skeletal muscle remain poorly defined (Hausman and Poulos, 2004; Hausman et al., 2007). Understanding the underlying mechanisms controlling adipogenesis will allow us to develop practical strategies to enhance intramuscular fat accumulation.

Skeletal muscle cells and adipocytes are both derived from mesenchymal pluripotent cells (Artaza et al., 2005; Poulos and Hausman, 2006). Many pluripotent cells exist in fetal muscle of meat animals around mid-gestation, which can differentiate into either myogenic cells or adipogenic cells. In sheep, the differentiation of adipocytes from mesoderm begins in mid-gestation. Early pre-adipocytes proliferation results in an increase in pre-adipocyte numbers. Then in late gestation, the majority of pre-adipocytes differentiate into mature adipocytes (Feve, 2005; Gnanalingham et al., 2005; Muhlhausler et al., 2006).

Adipose tissue growth in later life is due to both hypertrophy of existing adipocytes and hyperplasia due to the generation of new adipocytes from pluripotent cells or pre-adipocytes (Feve, 2005). However, new fat cells generated later in life are mainly located in visceral, retroperitoneal, and subcutaneous fat depots, with few located in the intramuscular fat depot (Faust et al., 1978; Miller et al., 1984; Valet et al., 2002). Thus, intramuscular adipogenesis during the fetal stage is anticipated to have a dominant effect on the number of adipocytes existing within skeletal muscle.

Adenosine monophosphate-activated protein kinase (AMPK) has a central role in energy metabolism (Hardie, 2007). It is switched on by an increase in the AMP/ATP ratio, which leads to the phosphorylation of AMPK at Thr 172 by AMPK kinases (Hawley et al., 2005; Kim et al., 2007). Once activated, AMPK promotes fatty acid oxidation and inhibits lipid synthesis in cells through phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) activity (Carey et al., 2006; Ravnskjaer et al., 2006; Yoon et al., 2006). Thus, it is likely that AMPK is a key player in adipogenesis during fetal muscle development. The objective of this study was to assess the association among maternal over-nutrition, AMPK and adipogenesis of fetal sheep muscle.

Materials and Methods

Care and use of animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. All ewes used in current study were mated with a single ram. Beginning 60 d before conception and continuing to d 75 of gestation (Day of mating = d 0), multiparous Rambouillet/Columbia ewes (parity of ewes were balanced between treatments) were individually fed either a highly palatable concentrated diet at 100% (Con) of NRC
recommendations for energy (NRC, 1985), or 150% (OF) of recommended energy requirements for early gestation. Ewes were housed in individual pens within a temperature (20 °C) controlled room. All ewes were weighed at weekly intervals and rations were adjusted for weekly changes in metabolic BW (BW0.75). Body condition was scored at monthly intervals to evaluate changes in fatness. A body condition score of 1 (emaciated) to 9 (obese) was assigned by 2 trained observers after palpation of the transverse and vertical processes of the lumbar vertebrae (L2 through L5) and the region around the tail head (Sanson et al., 1993).

Immediately prior to necropsy, on d 75, fourteen pregnant ewes (7 Con and 7 OF) were weighed. Sedation was induced by intravenous ketamine (10 mg/kg) and anesthesia was induced and maintained by isoflurane inhalation. Following the blood collection, fetuses were quickly removed and exsanguinated through the cord vein. Fetal longissimus dorsi (Ld) muscle samples were collected from five ewes carrying twin pregnancy in each group. After trimming off surface tissues, a small piece of muscle (1g) was sampled at the anatomical center of the muscle and snap frozen in liquid nitrogen for biological analyses. No difference in body weight was observed between twins and thus one fetus of twin pregnancy was randomly selected for analyses. Though no difference in weight was observed among fetuses of different sexes, the sex of fetuses in each group was balanced. Ewes were given an overdose of sodium pentobarbital (Abbott Laboratories, Abbott Park, IL) and exsanguinated.

Antibodies

Antibodies against phospho-AMPK at Thr 172, phospho-ACC at Ser 79, peroxisome proliferator-activated receptor γ (PPARγ) and horseradish peroxidase linked secondary antibody were purchased from Cell Signaling (Danvers, MA). Anti-β-actin antibody was obtained from Developmental Studies Hybridoma Bank (DSHB, Iowa City, IA 52242).

Immunoblotting analysis

Muscle (0.1 g) powdered in liquid nitrogen was used for immunoblotting analyses as previously described (Zhu et al., 2006; Zhu et al., 2004).

Real-time quantitative PCR (RT-PCR)

The mRNA was extracted from the fetal muscle using TRI reagent (Sigma, St. Louis, MO) and reverse transcribed into cDNA using a kit (Qiagen, Valencia, CA). Reverse transcribed cDNAs were used for real-time PCR analyses by using SYBR Green RT-PCR kit from Bio-Rad (Hercules, CA). Primer sets used were: PPARγ forward, 5'-CCGCATCTCCAGGGGTGC-3', and reverse, 5'-CAAGGAGGCCAGCATCGTGAAAT-3'; PPARα forward, 5'-GGCCGCTGTGATTTACGTT-3', and reverse, 5'-CCATCCCATCGGTAGTACCG-3' (Lomax et al., 2007). Each reaction yielded amplicons between 80 and 200 bp. PCR conditions were as follows: 20 sec at 95 C, 20 sec at 56 C, and 20 sec at 72 C for 35 cycles. After amplification, a melting curve (0.01 C/sec) was used to confirm product purity. Results are expressed relative to 18S rRNA (Lomax et al., 2007).

Oil Red O staining of intramuscular triacylglycerols

Muscle sections were stained with Oil Red O working solution [2:3 mixture of 0.5% (w/v) Oil Red O in 2-propanol and distilled water] for 7 min, and rinsed with PBS to remove excessive Oil Red O dye (Kim and Chen, 2004) and, then, subjected to microscopic observation at 200 x magnification. Two images were captured from each section and 5 sections were examined for each muscle sample. The total area of Oil Red O staining for each image was quantified by using Image J software (NIH, Washington DC) and expressed as the percentage of total image area.

Statistical analysis

Statistical analyses were conducted according to our previous studies in sheep (Zhu et al., 2006; Zhu et al., 2004). Briefly, each animal was considered as an experimental unit. Dietary treatments were considered as the main effect. Data were analyzed as a completely randomized design using GLM (General Linear Model of Statistical Analysis System, SAS, 2000). The differences in the mean values were compared by the Tukey’s multiple comparison, and mean ± standard errors were reported. Statistical significance was considered as P < 0.05.

Results and Discussion

Skeletal muscle cells and adipocytes are both derived from mesenchymal pluripotent cells (Artaza et al., 2005; Poulos and Hausman, 2006). In approximately mid-gestation, fetal skeletal muscle has a large number of pluripotent cells which can differentiate into either myogenic cells or adipogenic cells (Feve, 2005; Gnanalingham et al., 2005; Muhlhauser et al., 2006). Enhancing adipogenesis in fetal muscle is expected to provide sites for fat accumulation in the later life, increasing marbling. Adenosine monophosphate-activated protein kinase has a central role in controlling energy metabolism (Hardie, 2007). Activated AMPK phosphorylates and inhibits the activity of ACC, a key enzyme in lipid synthesis.Activation of AMPK accelerates fatty acid oxidation due to a reduction in malonyl-CoA content (Merrill et al., 1997). We previously demonstrated that AMPK was negatively associated with marbling in beef cattle (Underwood et al., 2007a). Therefore, it is highly possible that AMPK activity is associated with adipogenesis in fetal muscle. Because obesity leads to the reduction of AMPK activity in skeletal muscle (Bandyopadhyay et al., 2006; Sriwijitkamol et al., 2006), we hypothesized that
maternal obesity would inhibit AMPK activity and increase adipogenesis in fetal muscle. In this study, OF ewes developed severe obesity. The weight of OF fetuses was higher than that of Con fetuses weight (374 ± 10 g and 268 ±12 g respectively, \( P < 0.05 \)). These data are consistent with the macrosomal fetuses frequently observed in obese pregnant women (Sahu et al., 2007).

Sheep and cattle are both ruminant animals which makes them physiologically similar. Since the small size of sheep provides convenience and also dramatically reduces experimental costs, we used sheep to study adipogenesis in fetal muscle as affected by maternal nutrition. Marbling is one of the most important traits for beef, but also has importance for lamb and pork (Hausman et al., 2007; Underwood et al., 2007b). Therefore, this study has implications for several meat animal species.

**Down-regulation of AMPK activity in fetal muscle**

Acetyl-CoA carboxylase is a key enzyme regulating lipid metabolism. Its activity is negatively controlled by AMPK through phosphorylation at Ser 79 (Horman et al., 2005; Takekoshi et al., 2006). Therefore, AMPK controls lipid metabolism in cells through phosphorylation of ACC. In addition, AMPK regulates adipogenesis though the exact mechanism is vague (Dagon et al., 2006). Thus, it is likely that AMPK and ACC are involved in adipogenesis within fetal muscle. AMPK phosphorylation at Thr 172 was down-regulated in the skeletal muscle of OF sheep compared to Con sheep (25.4 ± 6.6\%, \( P<0.05 \)) (Fig. 1A). Phosphorylation of ACC was also reduced (36.2 ± 8.1\%, \( P<0.05 \)) in the muscle of OF sheep (Fig. 1B). These data clearly show AMPK activity was down-regulated in OF fetal muscle compared to Con muscle. The activation of ACC due to inhibition of AMPK should promote lipid accumulation in fetal muscle.

**Adipogenesis in fetal muscle**

In fetal muscle through late gestation, there are no mature adipocytes (Casteilla et al., 1987; Lomax et al., 2007). However, the lack of mature adipocytes does not exclude the cells which have progressed through differentiation to the point of being equipped at the molecular level to accumulate triacylglycerols. Indeed, the initial events in adipogenesis start in mid-gestation (Feve, 2005; Gnanalingham et al., 2005; Muhlhauersler et al., 2006). Peroxisome proliferator-activated receptor-\(\gamma\) (PPAR\(\gamma\)) is a key regulator of adipogenesis. The expression of PPAR\(\gamma\) leads to adipogenic differentiation from pluripotent cells and PPAR\(\gamma\) is highly expressed in adipose tissue (Spiegelman et al., 2000). Hence, we measured the PPAR\(\gamma\) mRNA expression to show whether there was enhanced adipogenesis. Indeed, PPAR\(\gamma\) mRNA expression levels were much higher in OF versus Con fetal muscle (Fig. 2A). Furthermore, we also analyzed that PPAR\(\gamma\) protein content by immunoblotting. Two bands of PPAR\(\gamma\) were detected, which might correspond to the two isoforms PPAR\(\gamma1\) and PPAR\(\gamma2\). Again, the PPAR\(\gamma\) content (two bands combined) was higher in OF fetal muscle (Fig. 2B). Because PPAR\(\gamma\) is a marker of adipocyte differentiation, these data indicated enhanced adipogenesis in OF fetal muscle. Of course, PPAR\(\gamma\) is also expressed in skeletal muscle, but the level of expression is very low (Verma et al., 2004; Vidal-Puig et al., 1996). In order to further evaluate adipogenesis in fetal muscle, the content of a pre-adipocyte marker, preadipocyte factor-1 (Pref-1) was analyzed (Kim et al., 2007; Smas and Sul, 1993). Preadipocyte factor-1 is exclusively expressed in pre-adipocytes, not mature adipocytes (Fahrenkrug et al., 1999; Mei et al., 2002). Its expression was higher in OF fetal skeletal muscle compared to Con fetal skeletal muscle, showing a higher number of pluripotent cells had committed to adipogenesis in OF fetuses (Figure 3). Therefore, these data indicated that the adipogenesis was enhanced in OF fetal muscle. Previous studies in pigs and cattle indicate that fetal stage is important for the regulation of genes involved in intramuscular fat accumulation (Cagnazzo et al., 2006; Lehnert et al., 2007). However, to the knowledge of authors, this is the first report showing that over-feeding dams enhances adipogenesis in fetal muscle in important livestock species.

In ruminant animal fetuses, brown adipose tissue is dominant, which rapidly transforms into white adipose tissue within the first week of life (Casteilla et al., 1987; Lomax et al., 2007). PPAR\(\gamma\) is preferably expressed in brown adipose tissue (Lomax et al., 2007). Its expression induces the expression of PPAR coactivator 1\(\alpha\) (PGC-1\(\alpha\)) which further induces the expression of uncoupling protein-1 (UCP-1), a protein conferring the thermogenic function of brown adipose tissue (Lomax et al., 2007). Our data showed that the mRNA expression for both PPAR\(\gamma\) and PGC-1\(\alpha\) was higher in OF fetal muscle than Con fetal muscle (Fig. 4AB), though no difference was observed for UCP-1 mRNA expression. These data further confirmed the enhancement of adipogenesis in OF fetal muscle. Data also indicate that a portion of those developing adipocytes in fetal muscle might be destined to brown adipocytes.

During mid-gestation, adipogenesis in fetal muscle has just been initiated, thus, there are no mature adipocytes available at this stage (Casteilla et al., 1987). Therefore, we did not detect mature adipocytes in fetal muscle. However, the accumulation of lipids in OF fetal muscle was higher than Con muscle (Figure 5), in agreement with the enhanced adipogenesis in OF fetal muscle.

**Conclusion and implication**

AMPK was inhibited in fetal muscle of over-fed ewes and this inhibition was associated with enhanced adipogenesis in fetal muscle. It is likely that adipogenesis in sheep fetal muscle can be enhanced by inhibition of AMPK. These data indicate that maternal nutrient supplementation can be utilized to inhibit AMPK in fetal muscle, which may be a strategy to enhance marbling in beef cattle and other meat animals.

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Figure 1. Phosphorylation of AMP-activated protein kinase (AMPK) and phospho-acetyl-CoA carboxylase (ACC) in fetal longissimus dorsi muscle of ewes fed 100% (Con, □) and 150% (OF, ■) nutrient requirements. Panel A shows representative
phospho-AMPK immunoblots and mean ± SEM; Panel B shows representative phospho-ACC immunoblots and mean ± SEM. (*): Con vs. OF, \( P < 0.05 \). (n=5 per group).

**Figure 2.** Peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) mRNA expression and protein content in fetal *longissimus dorsi* muscle of ewes fed 100% (Con, □) and 150% (OF, ■) nutrient requirements. Panel A shows mean ± SEM of PPAR\( \gamma \) mRNA expression; Panel B shows mean ± SEM of PPAR\( \gamma \) protein content by western blotting. (*): Con vs. OF, \( P < 0.05 \). (n=5 per group).

**Figure 3.** Pre-adipocyte factor-1 (Pref-1) content in fetal *longissimus dorsi* muscle of ewes fed 100% (Con, □) and 150% (OF, ■) nutrient requirements. Mean ± SEM. (*): Con vs. OF, \( P < 0.05 \). (n=5 per group).

**Figure 4.** Peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)), PPAR coactivator-1\( \alpha \) (PGC-1\( \alpha \)) and uncoupling protein-1 (UCP-1) mRNA expression in fetal *longissimus dorsi* muscle of ewes fed 100% (Con, □) and 150% (OF, ■) nutrient requirements. Panel A shows statistical data of PPAR\( \alpha \) mRNA expression; Panel B shows PGC-1\( \alpha \) mRNA expression; Panel C shows statistical data of UCP-1 mRNA expression. (*): Con vs. OF, \( P < 0.05 \). Mean ± SEM. (n=5 per group).

**Figure 5.** Lipid contents in cross-section of fetal *longissimus dorsi* muscle of ewes fed 100% (Con, □) and 150% (OF, ■) nutrient requirements as measured by Oil-Red O staining. (*): \( P < 0.05 \). Mean ± SE. n=5.
Gestational Nutrition Affects Growth, Adipose Tissue Deposition, and Tenderness in Steers

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Summary and Implications

Nutrient restriction of the dam during gestation can have detrimental effects on the progeny. Adipocyte differentiation occurs during mid to late gestation, and therefore nutrition during this time is expected to affect adipose tissue development and resulting carcass composition of steers. Thus, the objective of this study was to examine if cow nutrition by pasture management during mid to late gestation, would affect growth, carcass composition, and tenderness of steer offspring. Fifteen crossbred beef cows were randomly placed on improved pasture (IP, n=8) or native range (NR, n=7) from 120 to 150 through 180 to 210 days of gestation. Then, both groups of cows were placed together and allowed to calve. Steers were weaned at 191 days of age and were fed in a single pen until slaughtered at 15 months of age. Longissimus muscle, adipose tissue, and carcass characteristics were collected. Subcutaneous adipose tissue was fixed, sectioned, stained and used for analyses of cell number and cell diameter. Weight at 315 days of age when steers were placed on a finishing ration did not differ (P=0.71) between treatments. At slaughter, steers born to mothers grazed on IP had heavier live weight (P=0.04) and hot carcass weight (P=0.01) than NR steers. Longissimus muscle area, semitendinosus weight, marbling score, kidney, pelvic and heart fat, and yield grade were similar (P>0.12) between NR and IP steers. Crude fat content (6.00±0.49% vs. 4.82±0.53%, P=0.06) and tenderness (P=0.01) were greater in rib steaks from IP than NR steers. Twelfth rib fat thickness (P=0.05) and adjusted 12th rib fat thickness (P=0.02) were greater for IP steers than for NR steers. Adipose tissue cell number per field tended (P=0.09) to be greater for IP steers than for NR steers. These data show improving pasture quality available to cows during mid to late gestation affects growth and adipose tissue deposition in steers.

Introduction

Arid environments, with extreme variation in precipitation, yield dynamic rangelands that vary in forage production and quality (DelCurto et al., 2000; Grings et al., 2005). Much of the western US is affected by these conditions during various parts of the year. As summer progresses, forage quality decreases with increased plant maturity (Vavra and Raleigh, 1976). Thus, gestating beef cows grazing western rangelands can experience extended periods of low quality forage during late summer into fall, especially in drought conditions (DelCurto et al., 2000). Mid to late gestation is crucial for adipose tissue development and therefore nutrition during this time is expected to affect adipose tissue development and resulting carcass composition of steers (Prior and Laster, 1979). However, direct experimental evidence on gestation nutrition and performance of offspring in a production setting is lacking. It is unclear whether the quality of forage grazed by dams affects the growth performance, fat deposition and meat quality of offspring.

The fetal origins hypothesis states that a stress during gestation, such as nutrient restriction, will cause fetal adaptations which can affect the animal later in life (Barker, 1995). Gestational nutrition is related to altered adiposity (Bispham et al., 2003; Symonds et al., 2003; Underwood et al., 2008a), changes in muscle development (Nordby et al., 1987; Zhu et al., 2004), and growth characteristics of the progeny (Houghton et al., 1990; Beaty et al., 1994; Martin et al., 2007). We hypothesized that grazing of improved pasture in the fall, during mid to late gestation will improve growth performance and meat quality of offspring steers. Therefore, our objective was to test if nutrition by pasture management during mid to late gestation will affect performance and carcass traits of steers.

Materials and Methods

Animals

All animal procedures were approved by the USDA-ARS, Fort Keogh Livestock and Range Research Laboratory (LARRL) and the University of Wyoming Animal Care and Use Committees. Crossbred beef cows located at LARRL outside Miles City, Montana were bred to Angus bulls over a 32-day breeding period to begin
calving at the end of January. Cows were then managed on native range during early to mid gestation. On September 22 when cows were at 120 to 150 days of gestation, cows were allotted randomly to 1 of 2 dietary treatments for 60 days, either native range (NR, n=12) or improved pasture (IP, n=14) consisting of seeded pastures with increased forage production. During the 60 day grazing period, esophageal extrusa samples collected by cows grazing IP varied from 11.1% crude protein (CP) of organic matter (OM) early in the test period to 6.0% CP of OM at the end of the grazing period; whereas, extrusa samples of cows grazing NR ranged from 6.5% CP of OM during early grazing to 5.4% CP of OM at the end of the grazing period. At the beginning of treatment, cows averaged 3.7 ± 1.3 years of age with average BW of 1269 ± 126 lbs and average BCS of 5.6 ± 0.6 (9 point scale; 1 = severely emaciated and 9 = very obese; (Herd and Sprott, 1986)). A total of 8 steers were produced from cows gestating on improved pasture and 7 steers from cows gestating on native range. The NR cows (n=7) that produced steer calves gained 101 ± 9 lbs while the IP cows (n=8) gained 126 ± 9 lbs of BW providing evidence of a disparity in nutritional status of the dams on IP and NR. After the 60 day treatment, all cows were combined onto native range pasture and managed together. Cows were supplemented daily with approximately 4.4 lbs alfalfa hay per cow to meet protein requirements for late gestation. On January 17, (9 days prior to start of calving), cows were weighed and placed into drylot confinement where they were fed an average of 23.6 lbs (11.1% CP, DM basis) ground barley hay per cow on a daily basis. As cows calved, cow-calf pairs were moved to a different lot and were provided ad lib access to ground barley hay. Cow-calf pairs were allowed to bond for several days, and were then transported to native range pastures where they were allowed free grazing and were fed approximately 2.6 lbs barley cake (19.5% CP) and 17.6 lbs alfalfa (22.6% CP) per cow on a daily basis until April 1, when it was predicted that forage was of sufficient quality and availability to satisfy nutritional requirements.

Birth dates of steer calves ranged from January 26 to February 14 (mean date of birth = Feb 5). Steers and their dams were managed as one group on native range until weaning at 191 ± 2.3 days of age. At weaning, steers were weighed and placed in drylot confinement where they were back-grounded in one group on a diet consisting of 80% (DM basis) corn silage, 10% barley hay, 6% barley grain and 4% of a barley-SBM-urea based supplement. The diet contained 13.6% CP and 66.3% TDN (DM basis), and was fed at a rate that resulted in 0.88 ± 0.09 kg ADG. At 315 ± 2.3 days of age, steers were transported to the University of Wyoming research center feedlot near Lingle, Wyoming. Steers were weighed upon entering the feedlot and were placed in a single pen and fed the diets in Table 1 with the diet changed weekly until steers were on the finishing diet. Steers were weighed 1 week after adjustment to the finishing diet and then again after 70 days and at the end of the feeding period (115 days). Weights used were an average of body weights taken two consecutive days before the morning feeding.

**Slaughter**

Steers were transported to Laramie, Wyoming in 2 separate groups 24 hr prior to slaughter. Steers were slaughtered at the University of Wyoming Meat Laboratory as previously described (Underwood et al., 2008c) on 2 separate d within 1 wk. Animals were allotted to slaughter groups randomly with 7 (460.9 ± 4.7 d of age, 534.5 ± 7.2 kg BW) being slaughtered on the first day and 8 (467.0 ± 1.8 d of age, 531.7 ± 9.1 kg BW) being slaughtered 48 hr later. Steers were allowed free access to water with a 24 hour feed withdrawal. Longissimus muscle (LM) samples at 13th rib were collected within 10 min postmortem, snap frozen in liquid nitrogen, and stored at -80°C for biological analysis. The KPH was removed and weighed at slaughter.

**Carcass characteristics**

One trained, experienced technician collected all carcass measurements according to USDA guidelines (USDA, 1997) after a 48-h chill at 32 to 38°F as described previously (Underwood et al., 2008c). Fat thickness was adjusted according to USDA (1997) guidelines. Marbling score was determined by comparison to USDA marbling score standards using the scale where 200 = traces 0, 300 = slight 0, and 400 = small 0 marbling scores.

**Fabrication**

Carcasses were fabricated after a 14 days postmortem storage period (32-38°F) and the whole semitendinosus muscle was dissected and weighed as an estimate of muscle growth (Underwood et al., 2008b; Underwood et al., 2008c).

**Shear Force**

Warner-Bratzler shear force (WBSF) steaks (1.25 in. thick) containing the LM were removed at fabrication (14 d postmortem) from the loin end of the rib for analysis and frozen at -20°F for later analysis. Warner-Bratzler shear force analysis was performed as previously described by Underwood et al. (2008c).

**Proximate Analysis**

Samples for proximate analysis of the LM were removed at fabrication and frozen at -20°F for later analysis. Proximate analysis was performed as previously described by Underwood et al. (2008c).

**Subcutaneous Adipose Tissue Analysis**

A subcutaneous adipose tissue sample (0.5 cm x 0.5 cm) was removed at 48 hours postmortem at the 13th rib. Adipose tissue was frozen at -80°C. Samples were fixed for 24 hr in 10% buffered formalin followed by 24 hour immersion in 30% sucrose (Hulver et al., 2003). Samples were then mounted in OCT compound (Sakura Finetech, Torrance, CA) and sectioned at 16 µm thickness. Sections were stained with Harris Modified Hematoxylin (Fisher Scientific, Fair Lawn, NJ) for 2 min followed by a 5 minute wash with running tap water. Sections were counterstained with 1% Eosin Y followed by 2-min wash with deionized water. Cover slips were mounted over the sections using 42
glycerol. Sections were analyzed for cell diameter using light microscopy with images analyzed using Image J Software (NIH, Bethesda, MD). Cell diameter was measured by averaging the widest diameter and the narrowest diameter of each cell. Twenty fields of view were taken randomly from 5 sections and analyzed for cell diameter. An average of 472 ± 2.4 cells for each animal was measured.

**Immunoblotting Analyses**

Frozen muscle samples (0.1g) were used for immunoblotting analyses as previously described (Zhu et al., 2004). Monoclonal-anti-calpastatin antibody was brought from Affinity Bioreagents (Golden, CO), and anti-Troponin T and anti-actin antibodies were obtained from Developmental Studies Hybridoma Bank (Iowa City, IA).

**Collagen Analysis**

Hydroxyproline concentration in LM tissue hydrolysates was determined colorimetrically (Woessner, 1961). Samples (0.1 g) were analyzed in duplicate (Coefficient of variation≥8.0%) and averaged for hydroxyproline content. Collagen concentration was calculated assuming that collagen weighed 7.25 times the measured weight of hydroxyproline (Maiorano et al., 1993; Field et al., 1996).

**Statistical Analysis**

Animal performance, carcass measurements, immunoblotting analysis, and myofiber analysis were analyzed as a completely randomized design using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). All data was normally distributed according to the Shapiro-Wilk test in SAS. Individual animal was considered as the experimental unit. Data are presented as least squares means ± SEM. Statistical significance was considered when P < 0.05 and trends were considered when P < 0.10.

**Results**

Birth weights, weaning weights and 205 day adjusted weaning weights are reported in Table 2. Birth weights (P=0.46) and adjusted 205 day weaning weights (P=0.44) were similar between treatments, showing improving nutrition during mid to late gestation had no effect on animal growth at a relatively early developmental stage. Low energy diets from 190 days of gestation through term have been shown to decrease the calf birth weights (Houghton et al., 1990). However, another study showed no differences in calf birth weights and 205 day adjusted weaning weights when dams were on a low plane of nutrition during the second trimester of pregnancy, but did show decreased birth weights and BW at 28 days when dams were placed on low plane of nutrition during the second and third trimesters of pregnancy (Freely et al., 2000).

Performance data during the finishing period for steers gestated by cows on IP and NR are reported in Table 2. Steers from both treatments entered the feedlot at a similar (P=0.71) body weight (BW). Steers gestated by cows on NR had lower average daily gain (ADG, P=0.05), lighter total BW gain (P=0.05), and tended to have lighter final BW (P=0.07) than steers gestated on IP. This is in agreement with previous reports that lambs (Nordby et al., 1987) and rat pups (Beermann, 1983) born to dams on low planes of nutrition during gestation were slower growing, had lower ADG, and lighter BW than those from dams on higher planes of nutrition. Steers from dams on IP had heavier BW at slaughter (P=0.04) and heavier HCW (P=0.01) as shown in Table 3. This is inconsistent with our previous data that showed steers gestated by mothers on a low plane of nutrition were similar to controls in BW at slaughter (Underwood et al., 2008a). In our previous study, however, the low plane of nutrition was applied much earlier during gestation (day 31-120 of gestation), which may account for differences in offspring performance. Maternal breed could also be an influence on this as in the previous study animals were Gelbvieh × Angus cows crossed to a South Devon sire and this study used a crossbred herd with other breed influences.

Carcass characteristics of steers from NR and IP are presented in Table 3. Steers from NR and IP had similar LM area (P=0.26) and semitendinosus weights (P=0.19). This is similar to our previous data showing no differences in muscle growth of steers gestated by mothers on a low plane of nutrition during early to mid-gestation (Underwood et al., 2008a). However, Nordby et al. (1987) showed decreased semitendinosus weights of lambs on a low plane of nutrition during gestation. These discrepancies may be due to the difference in gestation time, duration of nutrient restriction, severity of nutrient restriction, and possibly species as these differences in muscle development were in sheep not in cattle.

Fat thickness and adjusted fat thickness at the 12th rib were greater (P≤0.05) for IP carcasses when compared to NR carcasses. This is consistent with our previous data in which steers gestated by mothers on a low plane of nutrition tended to have decreased 12th rib fat thickness and a smaller amount of fat as a percentage of the 9-10-11 rib section (Underwood et al., 2008a). The KPH as a percentage of HCW was similar (P=0.32) between treatments. Marbling score was similar (P=0.12) between treatments, which supports previous findings of steers gestated on a low plane of nutrition (Underwood et al., 2008a). However, the chemical fat content of the LM at the 12th rib tended (P=0.06) to be higher in the IP when compared with the steers born to mothers gestated on NR (data not shown). These data indicate gestational plane of nutrition may alter subcutaneous and intramuscular adipose depots.

Due to the difference in 12th rib fat thickness, we investigated if gestational plane of nutrition in cattle would alter adipocyte number and size using fixed sections stained with Harris Hematoxylin and Eosin Y. Subcutaneous adipose tissue sections of IP steers tended (P=0.09) to have a greater number of cells per field of view using light microscopy (Figure 1). We then examined the mean adipocyte diameter and found no differences (P=0.41) between treatments (Figure 1). These results indicate increased fat thickness of these animals may be due to...
increased number of adipocytes, possibly affected by gestational nutrition.

The WBSF of steers from NR and IP is reported in Table 3. The IP steers had lower (P=0.01) shear force, indicative of more tender meat. Postmortem storage of muscle foods is known to increase tenderness subjectively and objectively (Koohmaraie et al., 1988; Koohmaraie et al., 1991). Increased tenderness during postmortem storage has been attributed to proteolysis of myofibrillar proteins such as troponin-T and other structural proteins (Goll et al., 1983; Koohmaraie et al., 1988; Sentandreu et al., 2002). Calpastatin is associated with tenderness in beef cattle and has been suggested to play a large role in postmortem protein degradation (Morgan et al., 1993; Koohmaraie and Geesink, 2006; Underwood et al., 2008b). However, calpastatin in bovine fetal muscle was increased by low plane of nutrition in a previous study (Underwood et al., 2008a). However, calpastatin in bovine fetal muscle was increased by low plane of nutrition in a previous study (Du et al., 2004). Results from immunoblotting for calpastatin content in LM indicated no differences (P=0.37) between samples from steers born to mothers grazing IP and NR.

Troponin-T is a protein subunit of a myofibrillar protein shown to degrade during postmortem storage yielding a 30 kDa fragment (Ho et al., 1994; Uytterhaegen et al., 1994; Weaver et al., 2008). Thus we used troponin-T as an indicator of postmortem myofibrillar degradation leading to more tender meat. Immunoblotting showed the intact troponin-T protein in samples at 0 d after slaughter and a 30 kDa degradation product of troponin-T at 14 d postmortem. At 14 days postmortem, NR and IP steers were similar (P=0.84) in amount of troponin-T degradation, indicating that difference in WBSF was most likely not due to differences in postmortem proteolysis of myofibrillar proteins. Therefore, we further examined the amount of collagen, to inspect whether the difference in WBSF could be accounted for by a difference in total collagen. Steers born to mothers on NR and IP had similar (P=0.21) amounts of total collagen as measured by hydroxyproline content (Table 3). Similar amounts of total collagen in muscle have been previously demonstrated in beef cattle and sheep of different backgrounds and physiological status (Maiorano et al., 1993; Field et al., 1996, 1997).

In conclusion, nutritional status during mid to late gestation could alter animal performance during the finishing period, subcutaneous adipose tissue deposition, HCW, and Warner-Bratzler shear force in steers finished to slaughter weights. Data indicate the period of mid to late gestation is important for adipogenesis in beef cattle.

References


Figure 1. (Panel A) Number of adipose cells per field of view and (Panel B) adipose cell diameter for steers from cows grazing either native range or improved pasture from 120 to 180 d of gestation. †Indicates a trend for difference (P < 0.10). Values are lsmeans ± SEM.
Table 1. Four step feedlot ration of steers born to mothers on native range and improved pasture from 120 to 180 days of gestation.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Receiving</th>
<th>Step 1 Ration Composition, % DM</th>
<th>Step 2</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Corn</td>
<td>23.5</td>
<td>43.3</td>
<td>60.2</td>
<td>74.9</td>
</tr>
<tr>
<td>Silage</td>
<td>53.7</td>
<td>35.6</td>
<td>20.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>13.3</td>
<td>12.3</td>
<td>11.4</td>
<td>10.6</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>6.7</td>
<td>6.2</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Urea</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Salt</td>
<td>0.8</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Impact Finisher 44</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Chemical Composition

<table>
<thead>
<tr>
<th>Item</th>
<th>Native range</th>
<th>Improved pasture</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>13.2</td>
<td>12.9</td>
<td>12.6</td>
</tr>
<tr>
<td>NDF, %</td>
<td>29.1</td>
<td>25.4</td>
<td>22.3</td>
</tr>
<tr>
<td>ADF, %</td>
<td>15.6</td>
<td>12.7</td>
<td>10.3</td>
</tr>
</tbody>
</table>

1Purina Mills LLC, St. Louis, Missouri. Composition: 44.0% CP, 26.0% NPN, 1.5% fat, 20.0% fiber, 6.0% Ca, 0.9% P, 2.5% salt, 2.5% K, 37450 IU/kg Vit. A.

Table 2. Effects of cows grazing either native range or improved pasture from 120 to 180 d of gestation on birth weight and growth of steers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native range¹</td>
<td>Improved pasture²</td>
</tr>
<tr>
<td>Birth weight, lbs</td>
<td>85.3 ± 4.4</td>
<td>80.6 ± 4.2</td>
</tr>
<tr>
<td>Weaning weight, lbs</td>
<td>533.3 ± 8.2</td>
<td>564.4 ± 7.7</td>
</tr>
<tr>
<td>Adjusted 205 day weight, lbs</td>
<td>576.1 ± 15.4</td>
<td>592.8 ± 13.7</td>
</tr>
<tr>
<td>Finishing Period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight, lbs</td>
<td>782.3 ± 10.4</td>
<td>787.6 ± 9.7</td>
</tr>
<tr>
<td>Final body weight, lbs</td>
<td>1,185.2 ± 18.3</td>
<td>1,234.1 ± 17.0</td>
</tr>
<tr>
<td>Average daily gain, lbs/day</td>
<td>3.28 ± 0.15</td>
<td>3.65 ± 0.14</td>
</tr>
<tr>
<td>Total weight gain, lbs</td>
<td>397.0 ± 17.6</td>
<td>441.4 ± 16.5</td>
</tr>
<tr>
<td>Live body weight at slaughter, lbs</td>
<td>1,146.9 ± 17.0</td>
<td>1,198.2 ± 15.6</td>
</tr>
</tbody>
</table>

¹n = 7.
²n = 8.

Table 3. Carcass and muscle characteristics of steers from cows grazing either native range or improved pasture from 120 to 180 d of gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native range¹</td>
<td>Improved pasture²</td>
</tr>
<tr>
<td>12th rib fat thickness, in</td>
<td>0.44 ± 0.06</td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td>Adjusted 12th rib fat thickness, in</td>
<td>0.49 ± 0.05</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>KPH, % of HCW</td>
<td>3.96 ± 0.25</td>
<td>3.59 ± 0.24</td>
</tr>
<tr>
<td>Longissimus muscle area, in²</td>
<td>11.7 ± 0.3</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>HCW, lbs</td>
<td>726 ± 11</td>
<td>767 ± 10</td>
</tr>
<tr>
<td>Yield grade</td>
<td>3.54 ± 0.18</td>
<td>3.84 ± 0.17</td>
</tr>
<tr>
<td>Marbling score³</td>
<td>420 ± 16</td>
<td>455 ± 15</td>
</tr>
<tr>
<td>Semitendinosus, % of HCW</td>
<td>1.16 ± 0.07</td>
<td>1.20 ± 0.07</td>
</tr>
<tr>
<td>WBSF, lbs</td>
<td>8.28 ± 0.33</td>
<td>6.96 ± 0.31</td>
</tr>
<tr>
<td>Collagen content, μg/mg of LM</td>
<td>19.2 ± 1.9</td>
<td>15.7 ± 1.9</td>
</tr>
</tbody>
</table>

¹n = 7.
²n = 8.
³400 = Small, 300 = Slight, 200 = Traces.
Maternal Protein Supplementation at a Crucial Stage for Muscle and Fat Development Diverts Adipogenesis to Myogenesis in Beef Steers

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P. L. Price, Graduate Research Assistant
L. V. Nicodemus, Graduate Research Assistant
B. W. Hess, Professor
S. I. Paisley, Associate Professor, Beef Extension Specialist
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M. Du, Assistant Professor
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Summary and Implications

Early to mid gestation is as important period for muscle and adipose tissue development in beef cattle, and hence nutrition during this time is expected to affect muscle and adipose tissue development and resulting carcass characteristics of steers. Thus, the objective of this study was to examine if cow nutrition from day 45 through 185 of gestation, would affect muscle growth and adipose tissue in steer progeny. Thirty six crossbred beef cows were randomly placed on a control diet (100% NRC requirements, n=12, C), nutrient restricted (70% of requirements, n=12, NR), or a nutrient restricted diet with protein supplement (NRP, n=12) designed to equal flow of amino acids to the small intestine of C diet from day 45 to 185 of gestation. Then, both groups of cows were placed together, managed to meet requirements and allowed to calve. Calves were weaned at 211 days of age and backgrounded for 14 days. Steers were placed in feedlot and provided a high energy diet for 194 days. Steers were slaughtered at 430 ± 1.7 d of age. Longissimus muscle (LM) tissue and subcutaneous adipose tissue were collected at slaughter. Carcass characteristics were measured at 48 hours postmortem. Subcutaneous adipose tissue and LM was fixed, sectioned, stained and used for analyses of cell number and cell diameter. Live weight, hot carcass weight (HCW), LM area, skeletal maturity, and marbling score were similar (P≥0.23) for C, NR, and NRP steers. Twelfth rib fat thickness and adjusted 12th rib fat thickness of NR steers was less (P≤0.02) than C steers and tended to be less (P=0.08) than NRP steers. Kidney, pelvic and heart fat percentage was lower (P=0.05) for NRP steers compared to C and NR steers. Adipocyte diameter tended to be larger (P=0.10) for NR steers than for NRP steers, and NRP steers tended (P=0.09) to have a greater number of adipocytes per field of view. Steers born to NRP dams had larger semitendinosus muscles than C steers (P=0.008) and NR steers (P=0.07). Muscle fiber diameter was similar (P=0.43) between treatments, but total muscle fiber number in LM area was higher (P=0.02) in NR steers than C steers. These data show maternal nutrition and protein supplementation during gestation affects muscle and adipose tissue development in subsequent beef steers.

Introduction

Gestational nutrient restriction can affect adipose tissue deposition in beef cattle (Underwood et al., 2008c) and sheep (Bispham et al., 2003; Edwards et al., 2005; Ford et al., 2007) and will affect muscle development and carcass composition in sheep (Nordby et al., 1987; Zhu et al., 2004a; Zhu et al., 2006). The differences in adipose tissue development as well as muscle growth and development are hypothesized to be caused by differences at cellular level of the organism as nutrient restriction may decrease secondary myotube number (Zhu et al., 2004a) and increase subcutaneous adipocytes (Underwood et al., 2008c). However, it is still unclear through what mechanisms the changes in muscle and adipose development occur. These changes could be important in livestock production systems as livestock species profitability can be affected by growth and carcass traits.

Myogenesis and adipogenesis occur during early to mid-gestation (Robelin et al., 1993; Lehnert et al., 2007). Maternal nutrition during this stage negatively affects fetal muscle development (Zhu et al., 2004) and offspring performance (Zhu et al., 2006). Maternal nutrient restriction reduces nutrient supply to fetuses, including amino acids (Kwon et al., 2004), and amino acids stimulate myogenesis (Tipton and Ferrando, 2008). Therefore, we hypothesize that protein supplementation to nutrient restricted dams will enhance myogenesis in fetuses, increasing lean mass in offspring experienced maternal nutrient restriction.

Due to the seasonal nature of cow reproduction, maternal nutrient restriction widely exists in pregnant cows in western region due to constant drought which reduces forage production.

Our objective was to evaluate the effects of maternal protein supplementation on the carcass composition of offspring experienced nutrient restriction from day 45 through day 185 of gestation.
Materials and Methods

Animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Cow feeding protocol and diet were described previously (Price et al., 2007). Briefly, 3 and 4 year old cows from the University of Wyoming were estrous synchronized and bred via AI to a single sire on June 6, 2006. Cows and previous calves were allowed to graze summer pasture at the University of Wyoming McGuire Ranch (56 km northeast of Laramie, 2,203 m elevation) until day 33 of gestation. Cows were evaluated for pregnancy via rectal palpation by a licensed veterinarian at 33 day of gestation. Calves were weaned from cows permanently from 50 of the cows (18 triparous and 32 diparous) diagnosed as pregnant. The most uniform cows (n = 42, 18 triparous and 24 diparous) were transported the University of Wyoming Livestock center located in Laramie, WY. Cows were placed in drylots and pen-fed native grass hay (6.2% Crude protein (CP), DM basis) with supplemental protein to provide a diet with 10% CP until initiation of experimental diets. Cows were confirmed pregnant on day 40 of gestation by a licensed veterinarian, and on day 45 of gestation the 36 most uniform cows (12 triparous and 24 diparous) were selected to be individually fed native grass hay plus 1 of 3 supplements from day 45 through 185 of gestation. First the control (C) diet consisted of native grass hay and a soybean meal based supplement formulated for pregnant replacement heifers (1300 lb mature BW) to achieve 0.95 lb/d of BW gain (NRC, 2000), which was estimated to be comparable to a 1.12 lb/d BW gain for non-lactating cows pregnant with their second or third calf. The second dietary treatment was 70% of NE\textsubscript{m} provided by the C diet (NR). The third dietary treatment was 70% of NE\textsubscript{m} provided by the C diet and a ruminally un-degradable protein (RUP) supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden dish meal; DM basis, (Scholljegerdes et al., 2005) designed to provide duodenal essential amino acid flow equal to that of the C diet (NRP,(Scholljegerdes et al., 2004) 

Following the experimental diets cows were co-mingled and fed to meet NRC (2000) requirements for beef cows in late gestation. After calving, calves were vaccinated, branded, and bull calves were castrated. A total of 18 steers were born to the 36 cows on experiment with 4 steers were born to C dams, 8 steers were born to NR dams, and 6 steers were born to NRP dams. Cows and calves grazed summer pasture at the University of Wyoming McGuire Ranch until October 7, 2007.

Calves were weaned at 211 ± 1.6 days of age and then back-grounded for 14 days. Following back-grounding steers were placed in the feedlot for 194 days.

Slaughter

Steers were transported to Laramie, Wyoming in 2 separate groups 24 hr prior to slaughter. Steers slaughtered at the University of Wyoming Meat Laboratory as previously described (Underwood et al., 2008b) on 2 separate days within 1 week. Animals were allotted to slaughter groups by age and live weight with 8 being slaughtered on the first day and 10 being slaughtered 48 hours later. Steers were allowed free access to water with a 24-h feed withdrawal. Longissimus muscle samples were collected within 10 minutes postmortem, snap frozen in liquid nitrogen, and stored at -80°C for biological analysis. The KPH was removed and weighed during slaughter before final trimming and inspection of the carcass.

Carcass characteristics

One trained, experienced technician collected all carcass measurements as described by USDA (1997) after a 48-h chill at 2 to 4°C as described previously (Underwood et al., 2008b). Marbling score was determined by comparison to USDA marbling score standards using the scale where 200 = traces 0, 300 = slight 0, and 400 = small 0 marbling scores. The KPH weight from slaughter was used to determine the percentage of KPH to HCW.

Fabrication

Carcasses were fabricated after a 14 to 21 day postmortem storage period (32-38°F) and the semitendinosus muscle was dissected and weighed as an estimate of muscle growth (Underwood et al., 2008b).

Subcutaneous Adipose Tissue and Longissimus Muscle Analysis

A subcutaneous adipose tissue and longissimus muscle (LM) sample (0.4 in × 0.4 in) was removed at 72-hours postmortem at the 13\textsuperscript{th} rib. Adipose tissue was fixed for 24 hours in 10% paraformaldehyde in phosphate buffered saline (PBS), followed by a 24-hour immersion in 70% ethanol then a change of solution to fresh 70% ethanol and storage in 70% ethanol until samples could be embedded. Prior to embedding adipose tissue was immersed in 2 changes of 95% ethanol for 12 hours followed by 2 changes of 100% ethanol for 12 hours. The tissue was then trimmed to 0.2 in × 0.2 in. Tissue samples then went through two 12 hour incubations in histological grade xylene (Surgipath Medical Industries, Inc., Richmond, IL) followed by a 12 hour incubation in a 50% xylene and 50% paraffin infiltration medium (Surgipath Medical Industries, Inc., Richmond, IL) at 50°C for 12 hours. Tissue was then incubated in 3 changes of paraffin infiltration medium at 50°C for 12 hours. Samples were then mounted in Paraplast Xtra tissue embedding medium (McCormick Scientific, St. Louis, MO) allowed to cool for 30 minutes on a refrigerated plate and sectioned at 10 μm thickness using a MICROM HM310 microtome (MICROM Inc., Walldorf, Germany). Sections were then incubated in a water bath for 1 minute, placed on Gold Seal Rite-On microslides (Gold Seal Industries, Portsmouth, NH) and dried on Lab-Line slide warmer (Lab-Line Instruments Inc., Melrose Park, IL) for 45 minutes. Sections were deparaffinized using two 3 minute incubations in xylene followed by 3 minute incubations in graduated ethanol (100%, 95%, 70%). Section were then washed for 3 minute with running tap water and stained with Harris Modified Hematoxylin (Fisher Scientific, Fair Lawn, NJ) for 15
seconds followed by a 3-minute wash with running tap water, then a 3 minute incubation in Scott solution and a final 3-minute wash with running tap water. Sections were counterstained with 1% Eosin Y for 1 minute followed by 2 brief rinses in water. Tissue sections were then washed with 70% ethanol, 95% ethanol, two 3 minute washes in 100% ethanol, and two 3 minute incubations in xylene. Cover slips were mounted on the sections using Cytoseal XYL mounting medium (Richard-Allen Scientific, Kalamazoo, MI). Sections were visualized using Leica DMLB light microscope (Leica Microsystems, Wetzler, Germany) with the QCapture imaging program (Quantitative Imaging Corporation, Tucson, AZ) and images acquired using a MicroPublisher 3.3 RTV camera (Quantitative Imaging Corporation, Tucson, AZ) at 40 × magnification. Images were analyzed using Image J Software (NIH, Bethesda, MD) and the analyze particle function was used after adjusting the picture threshold to show the cell membranes in black, cell interior in red, and background space in grey. Cell diameter was calculated from the area of each cell given by the Image J software assuming cells were circular using the equation 1. Twelve fields of view were taken randomly from 6 sections and analyzed for cell diameter. An average of 2118 ± 90 adipocytes and 3068 ± 121 muscle cells for each animal was measured. 

The live BW and HCW of all treatments were similar (P=0.24) showing no differences in body weight at the end of the feeding period (Table 2). Steers born to NR dams had 43% less (P=0.02) subcutaneous fat at the 12th rib as compared to C steers and had 32% less (P=0.07) 12th rib fat than NRP steers. Additionally, NR steers had 41% less (P=0.01) 12th rib fat thickness when adjusted for total carcass external fat thickness as compared to C steers and 24% less (P = 0.08) KPH than NRP steers. This is similar to our previous result where we found steers nutrient restricted from day 31-125 of gestation showed decreased 12th rib fat thickness (Underwood et al., 2008a). Also, decreased fat thickness of steers on a lower plane of nutrition during mid to late gestation has been reported (Underwood et al., 2008c). There was no difference (P=0.43) in KPH as a percentage of HCW between NR and C steers. This data is similar to previous work in steer that reported no differences in KPH fat when dams were nutrient restricted or on a low plane of nutrition during gestation (Underwood et al., 2008a; Underwood et al., 2008c). Our data is in contrast to recent studies in sheep which showed an increased adiposity of fetuses and wether lambs that were nutrient restricted during gestation (Bispham et al., 2003; Ford et al., 2007). However, NRP steers had 25% (P=0.04) less KPH as a percentage of HCW than C steers and 19% (P=0.05) less KPH as a percentage of HCW than NR steers. This may be due to the RUP supplement in these animals which may negatively affect adipogenesis at a crucial stage for visceral adipose tissue development.

To further investigate the difference in 12th rib fat thickness adipose tissue samples were taken at the 12th rib and paraffin embedded to evaluate cell diameter and number. Subcutaneous adipose tissue sections of NRP steers tended (P=0.09) to have a greater number of cells per field of view using light microscopy (Table 3) when compared to the NR steers and were similar (P=0.37) to the C steers. Nutrient restricted steers tended (P=0.10) to have larger adipocytes than NRP steers. Both NR and NRP steers had similar (P=0.27) adipocyte diameter when compared with C steers. These results indicate decreased 12th rib fat thickness of the NR steers may be due to a decreased number of adipocytes, probably affected by low gestational nutrition.

Steers born to NRP dams had 15% larger (P=0.007) semitendinosus expressed as percentage of HCW than C steers and had 8% larger (P=0.07) semitendinosus as a percentage of HCW than NR steers. A study which showed that calves born to cows grazing on low quality range in Nebraska and supplemented with protein during pre and postpartum stages had similar LM area and growth characteristics (Stalker et al., 2006). These data show protein supplementation during a crucial stage for myogenesis enhanced skeletal muscle development. As to the knowledge of authors, this is the first report showing that maternal protein supplementation enhanced skeletal muscle development in steer offspring.

We investigated if gestational plane of nutrition in cattle could alter muscle fiber number and size using paraffin embedded sections of LM. Muscle fiber diameter was similar (P=0.43) across all treatments. The total number of muscle fibers in the LM calculated from the paraffin embedded sections of NR and NRP steers was increased by 23% (P=0.05) and 21% (P=0.06), respectively when compared to C steers (Table 3). Since muscle fiber numbers are determined during the fetal stage (Picard et al.,
1994), these data suggest that myogenesis in fetal muscle was enhanced by maternal nutrient restriction and nutrient restriction with protein supplementation.

In conclusion, gestational nutrition from day 45 to 185 of gestation can alter animal subcutaneous adipose tissue deposition, visceral adipose tissue deposition, and muscle growth. Data indicate mid-gestation is important for adipogenesis and muscle development in beef cattle. Additionally, protein supplementation when nutrient restricted during gestation can increase myogenesis and decrease adipogenesis in beef cattle.

References


Table 1. Ingredient and diet composition for cows on control, nutrient restricted, or nutrient restricted with protein supplement diet.

<table>
<thead>
<tr>
<th>Ingredient 1, % as fed</th>
<th>C</th>
<th>NR</th>
<th>NRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native grass hay</td>
<td>86.6</td>
<td>86.6</td>
<td>77.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.3</td>
<td>8.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Dical</td>
<td>2.4</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.4</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Premix 2</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>-</td>
<td>-</td>
<td>9.5</td>
</tr>
<tr>
<td>Feather meal</td>
<td>-</td>
<td>-</td>
<td>3.4</td>
</tr>
<tr>
<td>Blood meal</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
</tr>
</tbody>
</table>

| Diet Composition       |          |           |           |
| DM, %                  | 92.3     | 92.3      | 92.6      |
| CP, % of DM            | 10.0     | 10.0      | 17.1      |
| NDF, % of DM           | 62.7     | 62.7      | 58.8      |
| IVDMD, %               | 47.2     | 47.2      | 50.9      |

- 127.2 of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.
- 68.3% KCL, 27.6% FeSO₄, 3.1% ZnO, 0.6% MnO, 0.4% CuSO₄.
- Actual DMI from d 48 through 163 of gestation.

Table 2. Carcass and muscle characteristics of steers from dams on a control, nutrient restricted, or nutrient restricted with protein supplement diet from day 45 to 185 of gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight, lbs</td>
<td>C¹</td>
<td>NR²</td>
</tr>
<tr>
<td>Hot carcass weight, lbs</td>
<td>1249 ± 49a</td>
<td>1295 ± 33a</td>
</tr>
<tr>
<td>Fat thickness, in</td>
<td>0.47 ± 0.06a</td>
<td>0.27 ± 0.04bc</td>
</tr>
<tr>
<td>Adjusted fat thickness, in</td>
<td>0.53 ± 0.06a</td>
<td>0.31 ± 0.04bc</td>
</tr>
<tr>
<td>LM area, in²</td>
<td>13.4 ± 0.7a</td>
<td>13.6 ± 0.5a</td>
</tr>
<tr>
<td>KPH, % HCW</td>
<td>3.05 ± 0.25a</td>
<td>2.88 ± 0.17a</td>
</tr>
<tr>
<td>Yield Grade</td>
<td>3.30 ± 0.28d</td>
<td>2.65 ± 0.19d</td>
</tr>
<tr>
<td>Skeletal Maturity</td>
<td>124.1 ± 3.1a</td>
<td>119.5 ± 2.1a</td>
</tr>
<tr>
<td>Lean Maturity</td>
<td>147 ± 20d</td>
<td>180 ± 14d</td>
</tr>
<tr>
<td>Marbling Score</td>
<td>444 ± 53a</td>
<td>444 ± 37a</td>
</tr>
<tr>
<td>Semitendinosus, lbs</td>
<td>5.38 ± 0.33b</td>
<td>5.62 ± 0.22abc</td>
</tr>
<tr>
<td>Semitendinosus, % HCW</td>
<td>1.253 ± 0.047b</td>
<td>1.345 ± 0.033abc</td>
</tr>
</tbody>
</table>

Table 3. Muscle fiber and adipocyte diameter and number of steers from dams on a control, nutrient restricted, or nutrient restricted with protein supplement diet from day 45 to 185 of gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle fiber diameter, μm</td>
<td>C¹</td>
<td>NR²</td>
</tr>
<tr>
<td>Muscle fiber number in LM area, in 10000 fibers</td>
<td>13.89 ± 1.12bc</td>
<td>17.09 ± 0.89a</td>
</tr>
<tr>
<td>Adipocyte diameter, μm</td>
<td>99.0 ± 3.2bc</td>
<td>100.8 ± 2.2d</td>
</tr>
<tr>
<td>Adipocyte number per field of view</td>
<td>171 ± 16de</td>
<td>168 ± 11c</td>
</tr>
</tbody>
</table>

Table 1. Ingredient and diet composition for cows on control, nutrient restricted, or nutrient restricted with protein supplement diet.

<table>
<thead>
<tr>
<th>Ingredient 1, % as fed</th>
<th>C</th>
<th>NR</th>
<th>NRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native grass hay</td>
<td>86.6</td>
<td>86.6</td>
<td>77.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.3</td>
<td>8.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Dical</td>
<td>2.4</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.4</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Premix 2</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>-</td>
<td>-</td>
<td>9.5</td>
</tr>
<tr>
<td>Feather meal</td>
<td>-</td>
<td>-</td>
<td>3.4</td>
</tr>
<tr>
<td>Blood meal</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
</tr>
</tbody>
</table>

| Diet Composition       |       |       |       |
| DM, %                  | 92.3  | 92.3  | 92.6  |
| CP, % of DM            | 10.0  | 10.0  | 17.1  |
| NDF, % of DM           | 62.7  | 62.7  | 58.8  |
| IVDMD, %               | 47.2  | 47.2  | 50.9  |

- 127.2 of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.
- 68.3% KCL, 27.6% FeSO₄, 3.1% ZnO, 0.6% MnO, 0.4% CuSO₄.
- Actual DMI from d 48 through 163 of gestation.

Table 2. Carcass and muscle characteristics of steers from dams on a control, nutrient restricted, or nutrient restricted with protein supplement diet from day 45 to 185 of gestation.

Table 3. Muscle fiber and adipocyte diameter and number of steers from dams on a control, nutrient restricted, or nutrient restricted with protein supplement diet from day 45 to 185 of gestation.

- a,b Means within row lacking common superscript differ (P < 0.05).
- c,d Means within row lacking common superscript differ (P < 0.10).
The Effects of Co-ensiling Wet Distiller’s Grains Plus Solubles With Corn Silage on Growth Performance of Bred Beef Heifers During Late Pregnancy

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Ronald Lemenager, professor, Department of Animal Sciences, Purdue University
Lori Snyder, assistant professor, Department of Agronomy, Purdue University
Scott Lake, assistant professor, Department of Animal Science, University of Wyoming

Research for this project was conducted at Purdue University

Summary and Implications

The objective of this study was to evaluate the effects of co-ensiling wet distiller’s grains (WDG) with corn silage on growth performance of bred heifers during the last trimester of gestation. Ninety-six commercial Angus, bred heifers (two year-old; 522 ± 49.1 kg of initial BW; 5.3 ± 0.1 initial BCS) were blocked by weight and randomly assigned to one of four diets on a 62-d trial; 1) A corn silage and soybean meal control (CON), 2) Corn silage co-ensiled with wet disstiller’s grains with solubles (3:1 corn silage:WDG on a DM basis; CO-EN), 3) Corn silage mixed with dry distiller’s grains with solubles (DDG) added at feeding time (CS+DDG) and, 4) Corn silage mixed with WDG added at feeding time (CS+WDG). All the diets were formulated to be iso-caloric and iso-nitrogenous and to meet NRC requirements of two year-old heifers in the third trimester of gestation. Cows were weighed and body condition scored on two consecutive days at the beginning and the end of the trial. Initial and final BW was corrected for fetal weight according to day of gestation. By design, there was no difference in initial BW (P = 0.39) and initial BCS (P = 0.36) between treatments. Heifers fed the CS+DDG diet had decreased DMI (P < 0.01) compared to all other diets. Heifers fed the CO-EN treatment had a greater ADG (P = 0.03) and tended to have a greater G:F (P = 0.06) compared to other treatments. Heifers fed the CO-EN treatment had greater change in BW (P = 0.03) compared to the CON and the CS+DDG treatments, while the CS+WDG treatment was intermediate. There was no significant differences in BCS (P = 0.35), final BCS (P = 0.40), or final BW (P = 0.14) due to dietary treatment. Results from this study suggest that co-ensiling corn silage with WDG creates a viable feedstuff for growing heifers and improves growth performance compared to a traditional corn silage diet or the addition of either WDG or DDG at the time of feeding. Additionally, co-ensiling provides extended shelf life and increased feeding flexibility for smaller production units.

Introduction

The beef industry serves as one of the most important value-added enterprises in the U.S. with over a million farms and ranches benefiting directly from the sales of cattle (NCBA, 2006). In 2002, gross receipts from the sale of cattle and calves totaled over $45 billion and accounts for over 21% of all agricultural receipts. This makes the beef sector the single largest agricultural enterprise in the U.S. (USDA, 2006). It has been estimated that although the U.S. beef industry has less than 10% of the world’s cattle population, it provides nearly 25% of the world’s beef supply (USDA, 2002). Interestingly, small and medium-sized beef producers (less than 200 cows) account for 96.5% of the beef cow operations and 67% of the U.S. beef cow inventory (USDA, 1997).

Despite increased consumption and growth within the industry, production agriculture is at a crossroads. Government subsidies given to the bio-fuel industries have contributed to the growth in the corn-based ethanol industry which, in turn, has resulted in future corn prices of over $4/bushel. The ramifications of the shift towards ethanol production are far reaching. The sudden increase in corn prices during the fall of 2006 has placed a heavy burden on beef producers. Small and medium-sized producers currently are not capable of utilizing commodity feeds with limited ‘shelf-life’, like wet distiller’s grains (WDG), and this places them at a severe disadvantage compared to larger operations. The increasing cost of traditional feed grains (especially corn) which have been traditionally used in beef production has the potential to drive them out of business.

Garcia and Kalscheur (2004) reported successful storage and co-ensiling of WDG with corn silage, soybean hulls, and wet beet pulp. The challenge is that WDG are naturally low in pH and may inhibit the fermentation process,
especially in residues lacking readily fermentable carbohydrate sources (IBC, 2005). Furthermore, how the ensiling process of the mixed ingredients affects the rate of oxidation of the feed (spoilage at the face of the open silo structure and in the feed bunk) is not known. Additionally, questions regarding performance of animals fed these mixtures, maximal inclusion rates to determine optimal end-product quality, and how these mixtures fit into small to medium-sized farm operations have not been answered. Therefore, the objectives of the current study are to evaluate the effects of co-ensiling corn silage and WDGS on performance of heifers during the third trimester of gestation.

Materials and Methods

Ninety six, two year-old, commercial Angus heifers (initial BW of 522 ± 49.1 kg; initial BCS of 5.3 ± 0.1) in their last trimester of gestation were blocked according to BW and BCS (24 pens with 4 heifers per pen; 6 replicates per treatment) and assigned in a completely randomized design, to one of four diets (Table 1): 1) a control diet (CON) consisting of corn silage plus soybean meal; 2) co-ensiled corn silage with WDG added at 25% DM basis (CO-EN); 3) corn silage plus 25% DDG added at mixing (CS+DDG); and 4) corn silage plus 25% WDG added at mixing (CS+WDG).

Diets were formulated to meet requirements (NRC, 1996) for Angus heifers during the last trimester of gestation and to be iso-caloric and iso-nitrogenous (Table 2). The diets were offered on a limited-fed basis, once daily, at 0900 with free access to water throughout the 62-d trial. At the beginning and the end of the trial, animal’s BW and BCS were recorded on two consecutive days. Initial and final BW were corrected for day of gestation with the following equation (Ferrell et al., 1976):

\[ GU = 743.9e^{0.02000-0.0000143t} \]

Where, GU stands for Gravid uterus (fetus, fetal membranes, fetal fluids and uterus), \( e \) is a constant and \( t \) is the status of pregnancy in days.

<table>
<thead>
<tr>
<th>Table 1. Ingredient composition of diets fed to heifers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diets</strong> (% of DM)</td>
</tr>
<tr>
<td><strong>Ingredient</strong></td>
</tr>
<tr>
<td>Corn Silage(^4)</td>
</tr>
<tr>
<td>Soybean meal</td>
</tr>
<tr>
<td>Co-ensiled (^4)</td>
</tr>
<tr>
<td>DDG(^4)</td>
</tr>
<tr>
<td>WDG(^5)</td>
</tr>
<tr>
<td>Mineral premix (^5)</td>
</tr>
</tbody>
</table>

\(^4\) CON = control (corn silage with soybean meal), CO-EN = co-ensiled, CS+DDG = corn silage plus DDG added at mixing, CS+WDG = corn silage plus WDG with solubles added at mixing.

\(^5\) DDG = Dry distillers grains with solubles. WDG = Wet distillers grains with solubles.

\(^6\) 70% CaCO₃, 11.5% inorganic mix, 18.5% NaCl.

Table 2. Composition of diets (DM basis) fed to heifers

<table>
<thead>
<tr>
<th>Ingredient</th>
<th><strong>CON</strong></th>
<th><strong>CO-EN</strong></th>
<th><strong>CS+DDG</strong></th>
<th><strong>CS+WDG</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>NEg, Mcal/Kg (^2)</td>
<td>1.06</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
</tr>
<tr>
<td>CP, %</td>
<td>12.2</td>
<td>12.5</td>
<td>12.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Prot. Sol., % CP</td>
<td>44.7</td>
<td>41.6</td>
<td>38.3</td>
<td>34.5</td>
</tr>
<tr>
<td>aNDF, %</td>
<td>38.4</td>
<td>37.7</td>
<td>39.7</td>
<td>37.8</td>
</tr>
<tr>
<td>ADF, %</td>
<td>21.7</td>
<td>19.2</td>
<td>21.6</td>
<td>20.1</td>
</tr>
<tr>
<td>DM, %</td>
<td>38.3</td>
<td>36.1</td>
<td>38.8</td>
<td>40.5</td>
</tr>
</tbody>
</table>

\(^2\) CON = control (corn silage with soybean meal), CO-EN = co-ensiled corn silage with wet distiller’s grains 3:1 (DM basis), CS+DDG = corn silage plus dry distiller’s grains with solubles added at mixing, CS+WDG = corn silage plus wet distiller’s grains with solubles added at mixing.

Results and Discussion

By design, there was no difference in initial BW (\( P = 0.39 \)) and initial BCS (\( P = 0.36 \)) between treatments (Table 3). Heifers fed the CS+DDG diet had decreased DMI (\( P < 0.01 \)) compared to all other diets. Heifers fed the CO-EN treatment had greater ADG (\( P = 0.03 \)) than those fed the CON and CS+DDG diets. The CO-EN fed heifers also tended to have greater G:F (\( P = 0.06 \)) compared to those fed the CON and CS+WDG. Similar results were reported by Larson et al. (1993) and Ham et al. (1994) when evaluating WDG in finishing steers and by Klopfenstein et al. (2007) when comparing WDG to corn-based diets. Heifers fed the CO-EN treatment had greater overall gain in BW (\( P = 0.03 \)) compared to the CON and the CS+DDG treatments, while the CS+WDG treatment was intermediate. There was no significant differences in BCS (\( P = 0.35 \)), final BCS (\( P = 0.40 \)), or final BW (\( P = 0.14 \)) due to dietary treatment.

The increased performance (ADG and BW change) observed with heifers fed the CO-EN treatment compared to CON and CS+DDG treatments may be due, in part, to differences in DMI. It is interesting to note, however, that there were no differences in performance between heifers
fed the CO-EN and CS+WDG diets, but there was a tendency ($P < 0.06$) for the CO-EN heifers to be more efficient.

Results from this study suggest that WDG co-ensiled with corn silage have equal or greater feeding value when fed to heifers in the last trimester of gestation compared to corn silage based diets supplemented with soybean meal, DDG or WDG at feeding time. This creates an opportunity for smaller production units to utilize the WDG in their feeding management plans.

### Literature Cited


### Table 3. Effect of treatments on performance of Angus heifers during the last trimester of gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>CO-EN</th>
<th>CS+DDG</th>
<th>CS+WDG</th>
<th>SEM</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, lbs</td>
<td>17.3*</td>
<td>17.8*</td>
<td>15.4*</td>
<td>17.9*</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>Initial BW, lbs</td>
<td>1.149</td>
<td>1.149</td>
<td>1.156</td>
<td>1.154</td>
<td>4.2</td>
<td>0.39</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>5.43</td>
<td>5.36</td>
<td>5.33</td>
<td>5.27</td>
<td>0.09</td>
<td>0.36</td>
</tr>
<tr>
<td>ADG, lbs</td>
<td>1.8b</td>
<td>2.32*</td>
<td>1.96b</td>
<td>2.10ab</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>G:F</td>
<td>0.106</td>
<td>0.130</td>
<td>0.127</td>
<td>0.117</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Final BW, lbs</td>
<td>1.263</td>
<td>1.293</td>
<td>1.278</td>
<td>1.283</td>
<td>11.9</td>
<td>0.14</td>
</tr>
<tr>
<td>Final BCS</td>
<td>5.62</td>
<td>5.73</td>
<td>5.54</td>
<td>5.48</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>Change in BW, lbs</td>
<td>113.6b</td>
<td>143.3*</td>
<td>121.3b</td>
<td>129.7ab</td>
<td>9.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Change in BCS</td>
<td>0.19</td>
<td>0.38</td>
<td>0.21</td>
<td>0.21</td>
<td>0.11</td>
<td>0.35</td>
</tr>
</tbody>
</table>

1 CON = control (corn silage with soybean meal), CO-EN = co-ensiled corn silage with wet distiller’s grains plus solubles 3:1 (DM basis), CS+DDG = corn silage plus dry distiller’s grains with solubles added at mixing, CS+WDG = corn silage plus wet distiller’s grains with solubles added at mixing.

2 Means within a row lacking a common superscript differ ($P < 0.05$).
Nutrition

Natural Source Vitamin E Supplementation and Reproductive Efficiency in Beef Cows

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Ronald P. Lemenager, Professor of Animal Sciences, Purdue University
Scott L. Lake, assistant professor, Department of Animal Science, University of Wyoming

Research for the project was conducted at Purdue University

Summary and Conclusions

The objective of this study was to determine the effect of supplemental natural-source vitamin E (NSVE) on reproductive efficiency in beef cows. In a two year study, one hundred twenty-seven Angus-cross beef cows (n = 77 in year one, n = 50 in year two; initial BW = 607 kg; initial BCS = 5.2) were randomly assigned to one of two isocaloric dietary supplements: 1) corn-based supplement (CON) or 2) corn-based supplement formulated to contain 1000 IU/d NSVE (NAT). Supplementation began 6 wk prepartum and continued until the breeding season. Cow BW and BCS were measured throughout the study to evaluate changes in energy balance. Cow blood samples were collected at calving to determine α-tocopherol concentration and weekly beginning four weeks postpartum to determine progesterone concentrations and return to estrus. Cows were synchronized using CO-Synch + CIDR® and bred by AI based on heat detection. Non-responding cows were time bred (AI) 66 h after prostaglandin injection. Cows returning to estrus following AI were bred by natural service. Dietary supplement did not affect (P > 0.10) change in BCS or BW. Cows supplemented with NSVE had greater (P < 0.001) concentrations of α-tocopherol at calving than CON cows. Dietary supplement did not affect (P = 0.79) the percentage of cows cycling before the breeding season; however, supplementation of NSVE tended to increase first service (P = 0.09; NAT 55%, CON 41%) and overall (P = 0.09; NAT 90.6%, CON 80%) pregnancy rates. Numerically, NSVE supplementation increased second service conception rates (P = 0.23) by 15% and first plus second services combined (P = 0.15) by 12% when compared to CON cows. These data suggest that while supplementing NSVE does not improve resumption of cyclicity, it may improve first service and overall conception rates. Further investigation is needed to elucidate the mechanisms associated with improved conception rates in cows supplemented with NSVE in absence of improved cyclicity.

Introduction

The overall goal of cow-calf producers is to optimize pounds of calf weaned per cow exposed. Calves born earlier in the season will wean heavier and are thus more profitable than those born later in the season. Cows must be bred to calve within a condensed time period to produce a uniform group of calves at weaning. The average gestation length for beef cows is 281 d with an estrus cycle length of 21 d (Aiello, 1998), leaving only 84 d for the cow to undergo uterine involution, resume cyclicity, and become pregnant. Therefore, beef producers need to utilize production and management practices that will improve reproductive efficiency through enhanced postpartum rebreeding.

Supplementation of vitamin E has been shown to improve reproductive parameters in beef and dairy cows; however, there is a paucity of research involving the effects of vitamin E in reproducing beef cows. Supplementation of vitamin E has improved conception rates in primiparous beef heifers by as much as 50% compared to heifers receiving no vitamin E supplementation (Laflamme and Hidiroglo, 1991). Campbell and Miller (1998) reported a reduction in number of days to resumption of estrus from 70 to 50 in dairy cows receiving 1000 IU vitamin E per day. Similarly, Baldi et al. (2000) reported a decrease in the number of days to conception from 111 to 84 and total number of inseminations required for conception from 2.2 to 1.3 in dairy cows due to vitamin E supplementation at 2000 IU/d compared with 1000 IU/d.

As an antioxidant, vitamin E can prevent oxidation of lipids within cell membranes and increase cellular integrity (Machlin, 1991). Vierk et al. (1999) suggested that luteolysis of the corpus luteum (CL) is due to accumulation of toxic oxidative species, and vitamin E may suppress these oxidants, thus enhancing the maintenance of the CL, allowing for adequate progesterone secretion to maintain pregnancy.

The vitamin E requirement for beef cows has not been well established due its interrelationships with other dietary components, although Machlin (1991) stated that levels of
15 to 60 IU per kg DM were sufficient for young calves. Dairy cows are supplemented with 500 to 2,000 IU per day, leading some researchers to suggest that supplementation of 1000 IU of vitamin E per day to beef cows may improve reproductive performance (Franklin, 1998).

We hypothesized that 1000 IU vitamin E per day will improve reproductive efficiency in beef cows through a decrease in postpartum interval; therefore, our objectives are to evaluate the effects of natural source vitamin E (NSVE) on reproductive efficiency by investigating possible decreases in postpartum interval and improved overall conception rates.

Materials and Methods

Experimental Design

All protocols for this study were approved by the Purdue Animal Care and Use Committee. In a two-year study, 127 two- and three-year-old Angus-cross beef cows (n = 77 in year one, n = 50 in year two; initial BW = 607 ± 7 kg; initial BCS = 5.2 ± 0.14; 1 = emaciated, 9 = obese; Wagner et al., 1988) were blocked by age, BW, and BCS into one of two supplemental dietary treatments. Beginning an average of 6 wk prepartum, cows were given ad libitum access to hay and water. Cows were fed corn silage once daily and given a corn-based supplement containing either no added vitamin E (CON) or 1000 IU NSVE · cow⁻¹ · d⁻¹ (NAT; Vitamin E 405 Natural Source, d-α-tocopheryl acetate, ADM Alliance and Nutrition, Inc., Quincy, IL) as a top dress. Supplementation was provided until the beginning of the breeding season. At an average of 75 d postpartum, cows were synchronized using the CO-Synch + CIDR® protocol. An intravaginal controlled internal drug release device (CIDR, Pfizer Animal Health, New York, NY) and GnRH (100 µg, i.m.; Cystorelin, Merial, Iselin, NJ) were administered to cows. Seven days later, the CIDR was removed and prostaglandin F₂α (PGF₂α, 25 mg, i.m.; Lutalyse®, Pfizer Animal Health, New York, NY) was administered. Cows were monitored for signs of estrus behavior twice daily and those detected in estrus were bred by AI using the am/pm rule where cows detected in estrus in the morning are bred that evening and those detected in estrus in the evening are bred the following morning. Cows not exhibiting estrus by 66 h post-CIDR removal were bred (AI) and given GnRH (100 µg, i.m.). Cows were placed with a bull 14 d after AI. Pregnancy and fetal age were determined by ultrasonography 90 d after AI. First, second, first plus second combined, and overall conception rates were determined in relation to the AI date.

Sample Collection

Blood samples were collected via the coccygeal vein into 5-mL Vacutainer tubes (Becton, Dickson and Co., Franklin Lakes, NJ) 24 h after parturition for analysis of α-tocopherol concentration and weekly beginning 4 wk postpartum until breeding for analysis of progesterone concentration to determine days to resumption of estrus. Blood samples were immediately refrigerated for 8 h, centrifuged at 939 × g for 20 min, and serum was collected and stored at -20°C.

Sample Analysis

Serum samples were analyzed for vitamin E as α-tocopherol by HPLC. Briefly, in a 13 × 100 mm glass tube, 250 µL of serum was dissolved in 250 µL ethanol containing butylated hydroxytoluene (0.1 mg/mL) and 20 µL of δ-tocopherol (100 µM) as the internal standard and vortexed. Hexane (1 mL) was added and samples were centrifuged (3 min, 1200 × g). The hexane layer was removed, the extraction was repeated, and the hexane layers were combined and dried under nitrogen flow at 37°C. The residue was dissolved in 400 µL ethanol, filtered, transferred to a 300 µL auto sampler vial, and injected into the HPLC for analysis of vitamin E. Tocophers were separated by isocratic HPLC at 0.8 mL/min using a reverse phase MD-150 column (150 cm × 3.2 mm, 3 µm particle size; ESA, Inc., Chelmsford, MA). The column was equilibrated in ammonium acetate (0.2 M) in a mixture of methanol:ammonium acetate (90:10 V/V, pH 4.36). The tocopherol isoforms were eluted over a 15-min period. Monitoring was performed with an electrochemical ESA CoulArray® detector (ESA, Inc., Chelmsford, MA) with potentials set at 200, 400, 600, and 800 mV. Identification and quantification of vitamin E were accomplished by comparison of retention time and peak areas with the internal standard.

Progesterone concentrations were measured using RIA (Coat-A-Count In-vitro Diagnostic Test Kit, Siemens Corp., Tarrytown, NY). Briefly, 100 µL of serum and 1 mL iodinated (¹²⁵I) progesterone were added to progesterone antibody-coated tubes. Tubes were incubated at room temperature for 3 h, decanted, and counted for one minute in a gamma counter (Cobra® II Auto-gamma® Counting Systems, Packard Instrument Co., Meriden, CT). Circulating progesterone concentrations were determined from the logit-log representation of the standard curve. Cows exhibiting circulating progesterone concentrations greater than 1.0 ng/mL were considered cycling. The intra- and inter-assay coefficients of variation were 5% and 2.1%, respectively.

Statistical analyses

Initial and final BW and BCS and changes were measured using the GLM procedures (SAS Institute, Cary, NC). Percentage of cows cycling prior to the breeding season and conception rates were determined using the CATMOD procedures (SAS Inst. Inc., Cary, NC). The model included the effects of maternal dietary supplement, year, age, and all possible interactions. No interactions (P ≥ 0.12) were detected; therefore, only main effects of supplement are presented. All means presented are least squares means of each group and greatest SEM are reported. Significance was declared at P < 0.05.
Results

The effects of NSVE on cow BW and BCS measurements and circulating α-tocopherol concentrations are presented in Table 1. By design, there were no differences in initial BW (P = 0.62) or BCS (P = 0.60). Natural source vitamin E supplementation did not affect changes in cow BW (P = 0.69) or BCS (P = 0.88) throughout the study.

Table 1. Effects of natural source vitamin E supplementation on cow BW, BCS, and serum α-tocopherol concentration

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplements¹</th>
<th>SEM²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, lbs</td>
<td>CON 1,331</td>
<td>14.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Final BW, lbs</td>
<td>NAT 1,343</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>BW Change, lbs</td>
<td>1,292</td>
<td>8.47</td>
<td>0.69</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>5.29</td>
<td>0.14</td>
<td>0.60</td>
</tr>
<tr>
<td>Final BCS</td>
<td>5.13</td>
<td>0.13</td>
<td>0.81</td>
</tr>
<tr>
<td>BCS Change</td>
<td>-0.11</td>
<td>0.10</td>
<td>0.88</td>
</tr>
<tr>
<td>α-tocopherol, µg/mL</td>
<td>2.15</td>
<td>0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3.34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Dietary supplement NAT formulated to contain 1000 IU natural source vitamin E · cow⁻¹ · d⁻¹; CON contained no additional natural source vitamin E.
² Greatest SEM presented.
³ Serum α-tocopherol concentration measured 24 h after parturition.

Circulating concentrations of α-tocopherol were greater (P < 0.001) in NAT compared with CON cows 24 h postpartum.

Table 2. Effects of natural source vitamin E supplementation on resumption of estrus and conception rate

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplements¹</th>
<th>SEM²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to estrus³</td>
<td>60.3</td>
<td>2.29</td>
<td>0.52</td>
</tr>
<tr>
<td>Cycling, %⁴</td>
<td>48.4</td>
<td>7.09</td>
<td>0.79</td>
</tr>
<tr>
<td>Conception Rate, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Service</td>
<td>40.6</td>
<td>6.79</td>
<td>0.09</td>
</tr>
<tr>
<td>Second Service</td>
<td>36.7</td>
<td>9.12</td>
<td>0.23</td>
</tr>
<tr>
<td>First + Second</td>
<td>64.0</td>
<td>6.40</td>
<td>0.15</td>
</tr>
<tr>
<td>Overall</td>
<td>80.0</td>
<td>4.83</td>
<td>0.09</td>
</tr>
</tbody>
</table>

¹ Dietary supplement NAT formulated to contain 1000 IU natural source vitamin E · cow⁻¹ · d⁻¹; CON contained no additional natural source vitamin E.
² Greatest SEM presented.
³ Average number of days for cows to resume estrus following parturition.
⁴ The percentage of cows resuming estrus prior to the breeding season.

The effects of NSVE supplementation on resumption of estrus prior to the breeding season, as well as conception rates, are presented in Table 2. There was no difference in days to resumption of estrus (P = 0.52) or the percentage of cows returning to estrus prior to the breeding season (P = 0.79) due to vitamin E supplementation. There was a tendency for higher first service (P = 0.09), first plus second service combined (P = 0.15), and overall (P = 0.09) conception rates in NSVE cows compared with cows supplemented the CON treatment. Age of cow affected (P = 0.01) resumption of estrus, with fewer two-year-old (first parity) heifers (37%) resuming cyclicity before the breeding season than three-year-old (second parity) cows (62%). First service conception rates were greater (P = 0.01) in year two (63%) compared with year one (33%).

Discussion

Circulating serum α-tocopherol concentrations were 2.15 and 3.34 µg/mL in CON and NAT cows, respectively. Wichtel et al. (1996) and Hidiroglo et al. (1992) suggested that serum concentrations less than 2 µg/mL are deficient. Vitamin E is stored in all body tissues, but depletion rates vary between tissues and small amounts can be maintained in the body for long periods of time (McDowell, 1989). Therefore, the lack of response in resumption of estrus and postpartum interval in this study may be attributed to the CON cows having sufficient circulating α-tocopherol concentrations. Alternatively, supplementation of NSVE above 1000 IU/d may improve these reproductive parameters by increasing circulating α-tocopherol concentrations above 4 µg/mL, which Hidiroglo et al. (1992) considered adequate.

Although dietary supplement did not affect postpartum interval prior to the breeding season, more three-year-old (second parity) cows resumed cyclicity before the breeding season compared with two-year-old (first parity) heifers. This difference in postpartum interval between two- and three-year-old cows is in agreement with previously reported data (Strauch et al., 2001; Renquist et al., 2006). Postpartum interval is increased in two-year-old (first parity) heifers due to greater nutrient demands, a greater incidence of dystocia, and longer uterine involution compared with multiparous beef cows (Bellows et al., 1982; Renquist et al., 2006).

Previous research involving vitamin E supplementation on postpartum interval has been inconclusive. Campbell and Miller (1998) reported a decrease in the number of days to resumption of estrus in dairy cows due to vitamin E supplementation. Likewise, Harrison et al. (1984) also reported that postpartum interval was decreased in dairy cows when vitamin E was supplemented; however, the authors also reported the incidence of metritis, as well as cystic ovarian disease, were not affected by vitamin E supplementation, further complicating the insight into potential mechanisms of vitamin E on uterine health and postpartum interval. In the present study, adequate concentrations of vitamin E in CON cows likely explains the lack of significant differences in postpartum interval prior to the breeding season.

Laflamme and Hidiroglo (1991) reported similar increases to the present study with regards to overall
conception rate with vitamin E supplemented heifers having greater overall conception rates than non-supplemented heifers by 50%. While the results of the present study are not as drastic, NAT cows had a tendency to increase first service and overall conception rate when compared with CON cows. The effect of vitamin E on conception rates could be due to the role of vitamin E in preventing early embryonic death and fetal resorption as demonstrated in rats by Evans and Bishop (1922). Rats reared on vitamin E deficient diets showed normal ovarian behavior but had increased fetal resorption by the second day of gestation. Early embryonic death has been associated with premature luteolysis of the CL. Prostaglandin F2α is responsible for lysis of the CL which in turn induces ovulation, and is derived from arachidonic acid through the cyclooxygenase pathway (Murdoch et al., 1993; Mattos et al., 2000). A vitamin E deficiency may result in increased PGF2α concentrations through enhanced phospholipase A or cyclooxygenase activity within the cyclooxygenase pathway (Panganamala and Cornwell, 1982) which may lead to early embryonic death. Vierk et al. (1998) demonstrated that as an antioxidant, α-tocopherol in ewes may salvage the CL from apoptosis by suppressing oxidative stress, thus possibly explaining the tendency for first service and overall conception rates to be improved with NSVE supplementation in the present study.

The original hypothesis investigated decreases in postpartum interval and post-breeding effects of NSVE; however, postpartum interval before the breeding season were not affected by supplementation of NSVE, leading the authors to believe that NSVE affects reproductive efficiency through post-breeding mechanisms. Further research is needed to elucidate the mechanisms that affect conception rates when resumption of cyclicity is not affected.

**Implications**

These data suggest that supplementation of NSVE at 1000 IU/d could improve first service and overall conception rates in beef cows without affecting energy balance. It is possible that a more beneficial response in reproductive efficiency might be seen when levels greater than 1000 IU/d of NSVE are supplemented.

**Literature Cited**


Nutrition

Conception Rates of Beef Cows Fed a Supplement with Rumen-protected Fat

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Eduardo P. Goncalves¹, Research Intern and Undergraduate, UNESP, Botucatu, Brazil
Venerand Nayigihugu¹, Research Scientist
Glen Aines⁵, Dairy Enterprise Sales Manager
Eliot Block⁵, Senior Manager
Bret W. Hess⁴, Professor
¹Department of Animal Science
²ARM & HAMMER® Animal Nutrition, Division of Church &Dwight Co., Inc.

Summary and Implications

Recent studies from the University of Wyoming with fat supplementation showed reduction in conception rate in postpartum beef cows, differing from a study conducted in Brazil which showed an increase in conception rate when cows were fed a rumen-protected fat. Our objective was to supplement beef cows a rumen-protected fat to provide the same amount of linoleic acid utilized in the study from Brazil. Primiparous and multiparous lactating beef cows (n = 102; initial BW = 1143 ± 139lb) were synchronized, inseminated and received a control (3.9 lb/d) or a rumen-protected fat (3.0 lb/d) supplement for 40 days. Body weight gain (P=.54) did not differ between treatments. First conception rate (P=.84) was 50.9% cows fed the Control supplement and 47.2% for the cows fed the supplement containing rumen-protected fat. Rumen protected fat can be used as a supplement for postpartum beef cows if it is economically feasible.

Introduction

Extensive details of our experimental results have been described in a review by Hess et al. (2005). It was concluded that supplementing cracked high-linoleate safflower seeds decreased first service conception rates (Hess, 2003) because fewer cows had functional corpora lutea (Grant et al., 2003), which may be the related to an increase in prostaglandin F2α (PGF2α; Grant et al., 2005) or perturbations in the insulin-like growth factor-I (IGF-I) system (Scholljegerdes et al., 2004a). Subsequent χ² analysis of data from experiments in which young beef cows were fed cracked high-linoleate safflower seeds during early lactation revealed that pregnancy rate was reduced (P=.06) from 93.6% for cows fed control diets to 80.4% for cows fed high-linoleate safflower seeds (Hess et al., 2008).

Recent observations from experiments conducted with Megalac-E® are not consistent with observations described above. For example, Lopes et al. (2007) noted that pregnancy rate to timed AI increased from 45.6% to 56.5% if Nelore cows were fed 100 g of Megalac-E® from the beginning of estrous synchronization through 30 day after breeding. Based on personal communications, intestinal supply of 18:2n-6 was increased ~35 g/d for cows fed Megalac-E®. It is hypothesized that the discrepancy between our previous observations and those reported by Lopes et al. (2007) is attributable to cows fed Megalac-E® having twice as much 18:2n-6 available at the small intestine as cows fed high-linoleate safflower seeds. Such an increase in metabolizable linoleic acid may inhibit PGF2α production by competing with arachidonic acid for binding of the key enzyme, cyclooxygenase (Staples et al., 1998). Our objective was to supplement beef cows a rumen-protected fat to provide the same amount of linoleic acid utilized in the study from Brazil.

Materials and Methods

General

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Primiparous (n = 45) and multiparous (n = 57) lactating beef cows (initial BW = 519 ± 63kg) were synchronized (CO-Synch) with an intravaginal progesterone device (CIDR®, Pfizer Animal Health, US) + injection of 2 mL (100 µg; i.m) of GnRH on day 0. On day 7, the intravaginal progesterone device was removed and 5 mL (25 mg; i.m) of prostaglandin (Lutalyse®, Pfizer Animal Health, US) was administered. Sixty six hours later a 2 mL (100 µg; i.m) injection of GnRH was administrated, and cows were inseminated.

Diet and Sampling

On day 0, cows were randomly assigned to one of two dietary treatments (each age group- served as a block): The Control groups were fed a beet pulp-based supplement at 3.9 lb.cow⁻¹.d⁻¹ whereas fat-supplemented cows were offered a hand-fed supplement containing rumen-protected fat (3.0 lb.cow⁻¹.d⁻¹), formulated to
deliver Megalac-R® at 250 g cow⁻¹ d⁻¹ (estimated to provide between 34 to 41 g/d of 18:2n-6). Supplements were formulated to provide equal quantities of protein and energy (Table 1). During the synchronizing period (day 0 to the day of insemination), cows had free access to bromegrass hay (DM basis: 66.5% NDF, 43.9% ADF, 0.97% N, 59.0% IVDMD). On day 7, cows after insemination were transported to native pasture (DM basis: 53.8% NDF, 30.1% ADF, 2.0% N, 59.0% IVDMD), where they remained on their respective block (according to age of cows) and were fed supplements for 30 days. Blood samples were obtained from either the coccygeal artery or vein into commercial blood collection tubes (Vacutainer, 10mL; Becton Dickinson, Franklin Lakes, NJ) and into 10mL, EDTA-coated, glass Vacutainer tubes (Becton, Dickson and Co., Franklin Lakes, NJ) 30 days after breeding. Plasma and serum harvested were centrifuged at 1300 x g for 30 minutes. Serum and plasma obtained were frozen at -20°C until the laboratory analyses. Serum was used to determine pregnancy diagnosis (BioTracking LLC, Moscow, ID) and plasma will be used to evaluate fatty acids concentrations.

<table>
<thead>
<tr>
<th>Table 1 – Ingredients of diets fed to the cows.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Beet pulp</td>
</tr>
<tr>
<td>Plain white salt</td>
</tr>
<tr>
<td>Ranch-O-Mineral</td>
</tr>
<tr>
<td>MgOx</td>
</tr>
<tr>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>Molasses</td>
</tr>
<tr>
<td>Megalac-R®</td>
</tr>
</tbody>
</table>

Statistical

Data were analyzed as randomized complete block design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC).

Results and Discussion

Body weight gain did not differ (P=.54) between treatments, indicating that the supplements provided the same amount of energy and protein to the animals.

First conception rate was not affected (P=.84) by treatment, with 50.9 and 47.2% conception rates for cows on the Control and rumen-protected fat treatments, respectively. Unlike previous experiments published by our laboratory, the present study did not show a reduction in conception rate in postpartum cows fed fat. Nevertheless, results of the present experiment are consistent with observations presented in a review of the literature in which Hess et al. (2005) noted that supplementing fat to cows during the postpartum period did not affect pregnancy rates.

One possible explanation for different responses between the present study and Lopes et al. (2007) could be due to differences in circulating IGF-1 concentrations between the breeds used in the two studies. Many studies have shown that Bos indicus cows have higher IGF-1 concentrations compared to Bos taurus cows (Simpson et al., 1997; Spicer et al., 2002; Roberts et al., 2005). It was demonstrated that IGF-1 can increase follicular steroidogenesis (Webb et al., 1992; Spicer and Echtornkamp, 1995; Washes et al., 1995), anticipate ovulation (Gong et al., 2002; Castañeda-Gutiérrez et al., 2005), and increase pregnancy rates (Castañeda-Gutiérrez et al., 2005). Furthermore, Thomas et al. (1997) observed that cows (Brahman x Hereford) fed fat supplements had higher follicular fluid concentrations of IGF-1 compared to a non-fat supplement. Other factors that were not reported but should be considered include cow body condition score, as well as pasture quality and forage intake.

Conclusions

Present results are consistent with literature in which fat supplementation to postpartum beef cows did not affect first conception rate. Rumen-protected fat can be used as a supplement for postpartum beef cows if it is economically feasible compared to other energy sources.

References


Figure 1. Daily cost per cow for the Control and Megalac-R® based on the prices of feedstuffs in September 2008.
University of Wyoming Annual Animal Science Report
2008

Nutrition

Effects of Crude Glycerin on Feedlot Performance and Carcass Characteristics of Market Lambs

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Clint Rusk, Professor, Department of Animal Sciences, Purdue University
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Scott Lake, Assistant professor, Department of Animal Science, University of Wyoming

Research for the project was conducted at Purdue University

Summary and Implications

The objectives of this study were to determine the effects of feeding crude glycerin on feedlot performance and carcass characteristics of market lambs. Forty-eight Southdown x Suffolk lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers) were blocked by weight and assigned randomly to one of four dietary treatments (1 ewe and 1 wether/pen; 8 pens/treatment): 1) 25% dried distiller’s grains with solubles (CON), 2) 15% glycerin (15GLYC), 3) 30% glycerin (30GLYC), and 4) 45% glycerin (45GLYC). Crude glycerin (approximately 90% glycerin) replaced corn in the diet on a one to one basis. Weights were taken every 21 days to monitor BW change. Lambs were harvested when wethers reached an approximate 12th rib fat depth of 0.51 centimeters. Lambs fed CON and 15GLYC diets had greater DMI (P < 0.001), ADG (P < 0.001), G:F (P < 0.001), and had fewer days on feed (P < 0.001) compared with both the 30GLYC and 45GLYC treatments. No differences were detected in final body weight (P = 0.76), HCW (P = 0.78), LM area (P = 0.44), body wall thickness (P = 0.41), flank streaking (P = 0.24), or leg score (P = 0.21) due to dietary treatment. Lambs fed CON and 15GLYC diets also had greater dressing percentage (P = 0.01), 12th rib fat depth (P = 0.002), and yield grade (P = 0.003) compared with both the 30GLYC and 45GLYC diets; however, lambs on the 30GLYC and 45GLYC treatments tended to have greater LM ether extract (P = 0.09) compared with lambs on the CON and 15GLYC treatments. These results imply glycerin can be added at up to 15% DM in the diet of market lambs without decreasing feedlot performance or carcass characteristics.

Introduction

The drastic increase in ethanol and biodiesel production has led to elevated prices of traditional feedstuffs; leaving livestock producers searching for alternative feeds to lower production costs and maintain performance. Production of biodiesel in the U.S. over the next decade is expected to yield an estimated 1.4 billion pounds of glycerin. The price of crude glycerin is expected to drop from $0.20-0.25 cents per pound to $0.05 cents per pound, potentially providing a cost-effective alternative energy feed resource for livestock.

In ruminant animals, glycerol can be rapidly converted to propionic acid and readily absorbed through the rumen wall (Kijora et al., 1997). Of the primary volatile fatty acids, propionate is the only one which is directly gluconeogenic. Feeding glycerol increased ruminal propionate and subsequently increased circulatory glucose concentration in cattle (Chung et al., 2007; Trabue et al., 2007). Similarly, total organic matter digestibility was not influenced due to varying levels of glycerol in diets containing low levels of starch (Schröder and Südekum, 1999). Schröder and Südekum (1999) found that feeding glycerol decreased the acetate:propionate ratio and stimulated water intake, both of which were beneficial to transition dairy cows. Johns (1953) reported that adding glycerol to sheep rumen contents resulted in the formation of propionic acid. DeFrain et al. (2004) reported that substitution of corn with glycerol resulted in similar plasma glucose concentrations in dairy cattle, suggesting that glycerol has the potential to act as an energy substitute for ruminant animals. Our hypothesis was that glycerol may act as a viable energy substitute in finishing lamb rations. Therefore, our objectives were to determine feedlot performance and carcass characteristics of feedlot lambs.
Materials and Methods

General
All protocols for this study were approved by the Purdue Animal Care and Use Committee. The experiment was conducted from May through September 2007 at the Purdue University Animal Science Research and Education Center. Forty-eight crossbred lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers) were blocked by weight and randomly assigned (1 ewe and 1 wether/pen; 8 pens/treatment) to one of four dietary treatments (Table 1): 1) corn-based feedlot ration (CON), 2) CON diet with 15% crude glycerin (approximately 90% glycerol) added to replace corn (15GLYC), 3) CON diet with 30% crude glycerin added to replace corn (30GLYC), and 4) CON diet with 45% crude glycerin added to replace corn (45GLYC).

Laboratory Analysis
Weights for the lambs were collected every 21 days to monitor change in BW. Wether lambs were harvested when they obtained an approximate back fat thickness of 0.51 cm and the respective paired ewe lamb was removed from the study and returned to the Purdue University flock. Wether lambs were harvested at the Purdue University Meats Laboratory where HCW, flank streaking scores, LM area (taken by tracing the muscle at the 12th rib), and leg score conformations were evaluated 24 h after harvest and recorded. A 12th rib sample was also taken at this time, frozen in liquid nitrogen and stored for later ether extract analysis.

Table 1. Composition of diets fed to finishing lambsa (DM %)

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>15GLYC</th>
<th>30GLYC</th>
<th>45GLYC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa-grass hay</td>
<td>10.8</td>
<td>10.7</td>
<td>10.6</td>
<td>19.9</td>
</tr>
<tr>
<td>Corn</td>
<td>59.9</td>
<td>24.5</td>
<td>8.1</td>
<td>–</td>
</tr>
<tr>
<td>DDGS</td>
<td>25.1</td>
<td>25.0</td>
<td>25.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Mineral</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Gluten</td>
<td>–</td>
<td>1.2</td>
<td>3.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Soyahulls</td>
<td>–</td>
<td>19.5</td>
<td>17.9</td>
<td>–</td>
</tr>
<tr>
<td>Crude Glycerin</td>
<td>–</td>
<td>14.9</td>
<td>30.6</td>
<td>44.7</td>
</tr>
</tbody>
</table>

a Dietary Treatments: 1) corn-based feedlot ration (CON), 2) CON diet with 15% crude glycerin (approximately 90% glycerol) added to replace corn (15GLYC), 3) CON diet with 30% crude glycerin added to replace corn (30GLYC), and 4) CON diet with 45% crude glycerin added to replace corn (45GLYC).

Statistical Analysis
Data was analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was tested against the dependent variables. The model statement included the effect of treatment on specific characteristics to calculate the variations from the mean. The lsmeans statement was used to compute the adjusted means for the different treatments.

Results and Discussion
The effects of crude glycerin on feedlot performance of market lambs are presented in Table 2. Lambs fed the CON and 15GLYC treatments had greatest DMI (P <.0001), ADG (P <.0001), G:F (P <.0001) and fewer days on feed (P <.0001, 105 ± 37 d) compared with both the 30GLYC and 45GLYC treatments. The lambs on the CON and 15GLYC treatments finished an average of 28 d earlier than those on the 30GLYC treatment and an average of 63 d earlier than the lambs on the 45GLYC treatment. The increased number of days on feed when glycerol is fed at levels greater than 15% could be a significant economic disadvantage. Similar results for ADG and feed efficiency observed in this study for the 15GLYC lambs were reported when 10% glycerin was added to the finishing diet of Angus-cross steers (Pyatt et al., 2007). Similarly, Schröder and Südekum (1999) reported no difference in DMI when glycerol was fed at 10% of DM as a replacement for fermentable starch in the diet of dairy cows.

The effects of crude glycerin on carcass characteristics of market lambs are presented in Table 3. No differences were detected in final BW (P = 0.76), HCW (P = 0.78), 12th rib LM area (P = 0.44), body wall thickness (P = 0.41), flank streaking (P = 0.24), or leg score (P = 0.21) due to dietary treatment. However, lambs on the CON and 15GLYC treatments had the greatest dressing percentage (P = 0.01), back fat thickness (P = 0.002), and yield grade (P = 0.003) compared to the 30GLYC and 45GLYC treatments. However, lambs fed the 30GLYC and 45GLYC treatments tended to have greater ether extract (P = 0.09) compared with the lambs fed the CON and 15GLYC treatments.

Though the lambs on the CON and 15GLYC treatments outperformed the lambs on the 30GLYC and 45GLYC, it was determined that glycerin can be used by the tissues as an energy source and it can be used to replace a portion of dietary corn in the lamb finishing diets.

Implications
These results suggest that crude glycerin (90%) can be added at up to 15% of the DM in finishing diets of lambs without decreasing feedlot performance or carcass characteristics.

Literature Cited
Table 2. Effects of dietary treatment on finish lamb performance

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>15GLYC</th>
<th>30GLYC</th>
<th>45GLYC</th>
<th>SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs, no.</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<td></td>
</tr>
<tr>
<td>Initial Wt, lbs</td>
<td>63.9</td>
<td>63.3</td>
<td>64.4</td>
<td>64.2</td>
<td>0.23</td>
<td>0.89</td>
</tr>
<tr>
<td>Start Wt, lbs</td>
<td>74.1</td>
<td>72.3</td>
<td>73.9</td>
<td>71.7</td>
<td>0.15</td>
<td>0.64</td>
</tr>
<tr>
<td>End Wt, lbs</td>
<td>120.8</td>
<td>118.8</td>
<td>124.4</td>
<td>120.4</td>
<td>3.70</td>
<td>0.76</td>
</tr>
<tr>
<td>DMI, lbs</td>
<td>6.2d</td>
<td>6.44c</td>
<td>5.64d</td>
<td>4.70e</td>
<td>0.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ADG, lbs/d</td>
<td>0.32c</td>
<td>0.25d</td>
<td>0.21c</td>
<td>0.15f</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Feed efficiency, G:F</td>
<td>0.12c</td>
<td>0.08d</td>
<td>0.08de</td>
<td>0.06e</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Days</td>
<td>82.67e</td>
<td>82.50e</td>
<td>110.83d</td>
<td>145.67c</td>
<td>7.89</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% crude glycerin (approximately 90% glycerol) added at the expense of corn (15GLYC), CON diet with 30% crude glycerin added at the expense of corn (30GLYC), and CON diet with 45% crude glycerin added at the expense of corn (45GLYC).

b Greatest SEM is presented.

c,d Means within a row lacking a common superscript differ

Table 3. Effects of dietary treatment on carcass characteristics of finishing lambs

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>15GLYC</th>
<th>30GLYC</th>
<th>45GLYC</th>
<th>SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
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</tr>
<tr>
<td>Hot Carcass Wt, lbs</td>
<td>71.4</td>
<td>71.7</td>
<td>70.1</td>
<td>66.2</td>
<td>2.87</td>
<td>0.78</td>
</tr>
<tr>
<td>Dressing Percentage</td>
<td>57.7c</td>
<td>58.5c</td>
<td>55.5d</td>
<td>55.3d</td>
<td>0.8</td>
<td>0.01</td>
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</table>

a Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% crude glycerin (approximately 90% glycerol) added at the expense of corn (15GLYC), CON diet with 30% crude glycerin added at the expense of corn (30GLYC), and CON diet with 45% crude glycerin added at the expense of corn (45GLYC).

b Greatest SEM is presented.

c,d Means within a row lacking a common superscript differ
IN VITRO DIGESTIBILITY OF BROMEGRASS HAY AS AFFECTED BY ADDITION OF CRUDE GLYCERIN

Venerand Nayigihugu, Research Scientist, Assistant
Platt L. Price, Research Assistant
Bret W. Hess, Professor
Department of Animal Science

Summary and Implications

An in vitro experiment was conducted to determine the effect of adding different levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay. Triplicate in vitro filter bags containing 0.02 oz bromegrass hay (7.19% CP, 39.83% ADF, 69.15% NDF; DM basis) or crude glycerin (25 w/w% glycerol) replacing bromegrass at 7.5, 15, or 30% of the substrate were incubated for 2, 4, 8, 12, 24, and 48 h. At each time point, a 10-mL of ruminal fluid was acidified with 7.2 N H$_2$SO$_4$ and frozen for later VFA and NH$_3$ analyses. After 48 h incubation, bags were rinsed in tepid distilled water and dried in a 131°F forced air oven for 48 h to determine apparent DMD. Bags were then rinsed in NDF solution to determine true DMD and rumen degradable protein (RDP). Inclusion of crude glycerin in the substrate linearly decreased (P≤0.05) apparent DMD at 0, 4, 8, 24, and 48 h after incubation, but the rate of apparent DMD did not differ (P=0.23) among treatments. True DMD did not differ (P=0.65) among treatments at any of the incubation time points, although the rate of true DMD decreased (P=0.05) as crude glycerin levels increased. Addition of crude glycerin did not affect (P=0.20) extent or kinetics of NDF disappearance. After 8 and 12 h of incubation, RDP decreased (P=0.04) linearly primary due to reduced RDP for the 30% crude glycerin treatment. Although fraction A was similar (P=0.16) among treatments, fraction B was greater and fraction C was less with the inclusion of 30% crude glycerin. Crude glycerin addition did not affect total (P=0.42) or molar proportions (P=0.44) of individual VFA or NH$_3$ concentrations (P=0.98) in the in vitro fluid. Replacing up to 30% of dietary forage with crude glycerin (25 w/w% glycerol) may have minimal impacts on ruminal digestibility and fermentation in ruminants.

Introduction

Glycerol, a liquid substance of sweet taste and high energy concentration (Fisher et al., 1971; 1973; Sauer et al., 1973), is a co-product of the biodiesel industry. Crude glycerin may have benefits for ruminant animals, because glycerol can be converted to glucose by the liver (Krebs et al., 1966) and kidneys (Krebs and Lund, 1966) providing energy for cellular metabolism. However, previous studies on ruminal metabolism of glycerol have indicated that glycerol is extensively fermented in the rumen (Kijora et al., 1998). Khalili et al. (1997) reported that glycerol addition of 0.59 oz/ lb of barley silage decreased the molar proportion of acetate and increased molar proportions of propionate and butyrate in the rumen of dairy cows. Schröder and Südekum (1999) concluded that crude glycerin can substitute up to 10% of readily fermentable DM in mixed diets fed to ruminants without compromising digestibility. Limited information is available on level of crude glycerin inclusion in forage-based diets. Therefore, the objective of this experiment was to evaluate the effect of adding various levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay.

Materials and Methods

An in vitro experiment was conducted to determine the effect of various levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay. Treatments included bromegrass hay without crude glycerin or crude glycerin (25 w/w% glycerol) replacing 7.5, 15, and 30% of the hay substrate. Treatment mixtures were thoroughly blended by hand to produce homogenous samples. Bromegrass mixtures were then analyzed for DM and ash (AOAC, 1990), N (LECO model FP-528 Nitrogen Determinator, LECO, St. Joseph, MI), ADF and NDF (Ankom 200, ANKOM Technology, Fairport, NY). Residues from ADF and NDF were then analyzed for total N, as previously described, to estimate acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN), respectively. The difference between NDIN and ADIN was computed to estimate B$_1$ fraction. In vitro filter bags (Ankom F57; 25 μm pore size; ANKOM Technology, Fairport, NY) were pre-rinsed in acetone for 5 min to remove surfactants that may inhibit microbial digestion and triplicate bags containing 0.02 oz of each treatment mixture were incubated in 1 of 4 jars (ANKOM Technology, Fairport, NY) randomly assigned to 1 of 3 Daisy® incubators (ANKOM Technology, Fairport, NY) resulting in 3 replications per treatment. A set of filter bags for each treatment were submerged in a non-inoculated media, which constituted the 0 h time point. The remaining in vitro filter bags were placed into corresponding jars that contained pre-warmed McDougall’s buffer that was inoculated with ruminal fluid (2:1 ratio) collected from 2 cows consuming bromegrass hay. After 2, 4, 8, 12, 24, and 48 h of incubation, triplicate filter bags and 1 blank bag

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were serially removed from each jar and immediately frozen.

At each sampling point, 0.3 oz of in vitro fluid was collected and acidified with 7.2 N H₂SO₄ and frozen for later VFA and NH₃ analysis. In vitro fluid was analyzed for VFA concentration (Goetsch and Galyean, 1983) using a Hewlett-Packard 5850 gas liquid chromatography (Hewlett Packard, Avondale, PA) equipped with a 59-in x 0.2-in (i.d.) column (Nukol, Supelco, Bellafonte, PA). The initial oven temperature was 230°F and final temperature was 302°F with the rate of 46°F/min. Hydrogen was used as a carrier gas with a column flow rate of 0.7 oz/min. Injector and flame ionization temperature were 485°F. In vitro NH₃ concentrations were determined by the phenol-hypochlorite procedure (Broderick and Kang, 1980).

Filter bags were thawed, rinsed in tepid water until rinse water was clear, and dried in a 131°F force-air oven for 48 h to determine apparent DMD. Filter bags were then rinsed in NDF solution to correct for microbial contamination for determination of true DMD. Residues were then analyzed for total N to estimate RDP (Mass et al., 1999).

Rate of NDF disappearance was calculated using non-linear regression (Martens and Loften, 1980). The non-linear model of Orskov and McDonald (1970) was used to calculate protein fractions A, B, and C. All resulting data were then subjected to ANOVA using the GLM procedures of SAS (SAS Institute, Cary, NC) for a randomized complete block design. The block effect was incubator and the treatment effect was glycerol level. Fermentation pattern data were analyzed as a split-plot design. Treatment effects were tested using jar within treatment as the error term (error a). The time effect and treatment × time interaction were tested using the residual error term (error b). Single degree of freedom orthogonal contrast procedures were used to evaluate treatment differences (Steel and Torrie, 1980).

**Results and Discussions**

Chemical composition of bromegrass-crude glycerin substrates is presented in Table 1. Südekum (2007) reported similar total tract digestibilities when 15% of different purity glycerol was added to a 40:60 forage to concentrate ration. In the current experiment, apparent and true DMD increased across incubation time (Table 2) which is indicative of microbial digestion. Inclusion of crude glycerin in the substrate linearly decreased (P≤0.05) apparent DMD after 0, 4, 8, 24, and 48 h and a quadratic decrease (P=0.02) was observed after 8 h of incubation. Crude glycerin did not affect (P=0.23) rate of apparent DMD. Although there was a numerical advantage of adding 30% crude glycerin to bromegrass hay, in vitro true DM disappearance did not differ (P=0.65) among treatments at any incubation time point. Rate of true DM disappearance decreased (P=0.05) as crude glycerin level increased. Addition of crude glycerin did not affect (P≥0.27) extent or kinetics of NDF disappearance (data not shown). Schröder and Südekum (1999) fed sheep 48, 78, 131, or 185 g/d of glycerol (DM basis) in a low starch, concentrate diet and found either no effect or positive effects on digestibility of OM, starch, and cell-wall components. They further reported, however, that feeding the same levels of glycerol in high-starch concentrate diets resulted in a decrease in cell-wall digestibility but did not affect digestion of OM or starch.

At the 8 h time point, RDP increased linearly (P=0.04) from 0 to 30% crude glycerin treatment (Table 3). Addition of crude glycerin increased (P≤0.01) RDP at the 12 and 24 h incubation points. A quadratic (P=0.01) response was observed at 12 and 24 h of incubation points. Addition of crude glycerin did not affect (P=0.16) fraction A. Fraction B increased (P=0.003) linearly from 0 to 30% crude glycerin. Fraction C exhibited a quadratic (P=0.03) response because it increased with the addition of 7.5% crude glycerin and then decreased when crude glycerin replaced 30% of the substrate. Treatment × time interactions were not observed for NH₃ (P=0.70) or total VFA (P=0.16). In contrast to results reported by Khalili et al. (1997) and Schröder and Südekum (1999), crude glycerin addition did not affect NH₃ (P=0.98), total VFA (P=0.42) or molar percentages of individual VFA (P≥0.44), (Table 4).

We concluded that replacing up to 30% of dietary forage with crude glycerin (25 w/w% glycerol) may have minimal impacts on ruminal digestibility of forage-based diets.

**Implications**

Crude glycerin may be included in diets of ruminants without affecting ruminal fermentation. However, additional studies are necessary to determine the optimum level of crude glycerin inclusion in forage-fed diets of ruminants.

**Literature Cited**


Khalili, H., T. Varvikkos, V. Toivonen, K. Hissa, and N. Suvitie. 1997. The effects of adding glycerol or unprotected free fatty acids or a combination of two on


Table 1. Chemical composition of bromegrass hay with various levels of crude glycerin.

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
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<th>15</th>
<th>30</th>
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<td>91.65</td>
<td>87.79</td>
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Table 2. Apparent and true DM digestibility of bromegrass hay with various levels of crude glycerin.

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\(^{1}n = 12\)

Table 3. Rumen degradable protein and protein fractions of bromegrass hay with various levels of crude glycerin.

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<th>Incubation time, h</th>
<th>Concentration of glycerol, %</th>
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<td>Fraction C</td>
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\(^{1}n = 12\)
Table 4. Ruminal ammonia (NH$_3$) and volatile fatty acids (VFA) produced from bromegrass hay with various levels of crude glycerin

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<tr>
<th>Concentration of glycerol, %</th>
<th>NH$_3$, mg/dL</th>
<th>Total VFA, mM</th>
<th>SEM$^1$</th>
<th>P</th>
<th>Orthogonal contrasts</th>
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</thead>
<tbody>
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<td></td>
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<td>0.2</td>
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<td>8.5</td>
<td>7.2</td>
<td>7.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.22</td>
<td>0.97</td>
<td>1.41</td>
<td>0.89</td>
<td>0.2</td>
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<tr>
<td>Valerate</td>
<td>0.59</td>
<td>0.57</td>
<td>0.53</td>
<td>0.56</td>
<td>0.01</td>
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<tr>
<td>Isovalerate</td>
<td>1.01</td>
<td>1.02</td>
<td>0.86</td>
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<td>0.03</td>
</tr>
<tr>
<td>Acetate: Propionate</td>
<td>4.6</td>
<td>4.3</td>
<td>4.4</td>
<td>4.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

$^1 n = 12$
Nutrition

SITE AND EXTENT OF NUTRIENT DIGESTION IN LAMBS FED WHOLE CANOLA, BROWN MUSTARD, OR CAMELINA SEEDS

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Summary and Implications

The objective of this experiment was to compare site and extent of nutrient digestion in lambs fed diets containing whole canola, brown mustard, or camelina sativa seeds. Four black-face wether lambs (170.8 ± 9.3 lb. body weight) fitted with ruminal, duodenal, and ileal canulae were used in a 4 × 4 Latin square design experiment. Experimental diets consisted of 18% ground (1 in.) bromegrass hay, 65.2% cracked corn, 15% soybean meal, and 1.8% limestone (as-fed basis, Control) with oil seeds replacing enough of the soybean meal to provide 3% added fatty acid from each of the whole oil seeds. A 7 day adaptation period was followed by 3 days of duodenal, ileal, fecal, and ruminal sampling. Intake of organic matter (OM) was greater (P<0.0001) for lambs fed camelina than Control with canola being intermediate and lambs fed brown mustard not differing from lambs fed camelina and canola. Site and extent of OM digestibility did not differ (P=0.217) among treatments. Total (P=0.124) and molar proportions (P≥0.185) of individual volatile fatty acids (VFA) did not differ among treatments. Intake of nitrogen (N) was greater (P<0.0001) for lambs fed camelina and brown mustard compared with Control; lambs fed canola were intermediate. Nonetheless, ruminal ammonia (NH₃) concentrations were not affected (P=0.461) by dietary treatment. Digestibility in the small intestine (% entering) of N was less (P=0.017) for lambs fed camelina than lambs fed any of the whole oil seeds. Expressed as a percentage of intake, total tract neutral detergent fiber (NDF) digestibility was greater (P=0.038) for lambs fed camelina and canola than the Control with brown mustard supplemented lambs being similar to all treatments. The feeding value of whole camelina sativa and brown mustard seeds are comparable to whole canola seeds when included in finishing lamb diets.

Materials and Methods

General

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Four wether lambs (170.8 ± 9.3 lb. body weight) were fitted with ruminal, duodenal, and ileal canulae. After allowing time for recovery from the surgeries, lambs were maintained in individual metabolism crates (4.6 × 2 ft.) under continuous lighting in a climate controlled room where they had free access to water.

Diets and Sampling

Following the design of a 4 x 4 Latin square experiment, lambs were assigned to 1 of 4 dietary treatments. The Control diet contained no supplemental fat and consisted of ground (1 in.) bromegrass hay, cracked corn, soybean meal, molasses, limestone, urea, and salt...
Oilseeds were added to replace enough of the soybean meal to provide 3% added fatty acids from canola, brown mustard, or camelina whole oilseeds. Due to a recent report indicating that oilseeds do not need to be processed to improve diet digestibility (Price et al., 2008), all seeds were fed whole for the present experiment. All dietary treatments were formulated to be isonitrogenous. Lambs were offered 25% of their daily ration at 0530 and 75% at 1730. As an external marker of digesta flow, 2.5 g of titanium dioxide (TiO2) was dosed intraruminally immediately before each feeding (Myers et al., 2006). Each seven day adaptation period was followed by 2 days of duodenal and ileal sampling and 1 day of ruminal sampling. Duodenal and ileal samples were collected beginning at 0500 on day 8 of each period with the collection of 150 mL of duodenal and ileal digesta repeated every 4 hours. Collection times were advanced by 2 hours on day 9 so that digesta was collected every hour of a theoretical 24-h clock. Beginning at 0500 on day 10, 500 mL of ruminal digesta was collected every 2 hours for 12 hours. Ruminal pH was immediately measured on whole rumen contents using a combination electrode (Orion Research Inc., Boston MA). A 10 mL aliquot was strained through 4 layers of cheesecloth, placed in a 12 mL conical vial with 0.10 mL of 7.2 N H2SO4, and stored in the freezer for subsequent VFA and NH3 analysis.

Table 1. Ingredient composition of diets fed to lambs.

<table>
<thead>
<tr>
<th>Ingredients, % of DM</th>
<th>Control</th>
<th>Canola</th>
<th>B.M.</th>
<th>Camelina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromegrass hay</td>
<td>17.38</td>
<td>16.37</td>
<td>16.34</td>
<td>16.37</td>
</tr>
<tr>
<td>Cracked corn</td>
<td>61.12</td>
<td>61.12</td>
<td>61.12</td>
<td>61.12</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>14.46</td>
<td>2.98</td>
<td>1.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Canola</td>
<td>-</td>
<td>11.78</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brown Mustard</td>
<td>-</td>
<td>-</td>
<td>13.41</td>
<td>-</td>
</tr>
<tr>
<td>Camelina</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.46</td>
</tr>
<tr>
<td>Salt</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Urea</td>
<td>0.00</td>
<td>1.01</td>
<td>1.08</td>
<td>1.05</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.88</td>
<td>1.88</td>
<td>1.88</td>
<td>1.88</td>
</tr>
<tr>
<td>Molasses</td>
<td>4.10</td>
<td>4.10</td>
<td>4.10</td>
<td>4.10</td>
</tr>
</tbody>
</table>

1Supplemental whole oil seeds were added so that the diets contained 3% added fatty acids (as-fed) as canola, brown mustard (B.M.), or camelina.

Laboratory Analysis

Beginning 2 days before and throughout the collection period, samples of all feedstuffs were taken on a daily basis for laboratory analysis. Duodenal and ileal samples were frozen at -20°C, lyophilized (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY), and composited within lamb for each collection period for analysis of TiO2 (Myers et al., 2004). Feed and digesta samples were analyzed for (dry matter) DM and ash (AOAC, 1990), N (Leco Corp., Henderson, NV), and NDF (Ankom Technology, Fairport, NY). Acidified ruminal fluid samples were prepared (Goetsch and Galyean, 1983) and analyzed for VFA concentrations using a Hewlett-Packard 5890 GLC (Hewlett-Packard, Avondale, PA) equipped with a 15-m x 0.53-mm (i.d.) column (Nukol, Supelco, Bellafonte, PA). The initial oven temperature was 110°C and final temperature was 150°C with a ramp of 8°C/min. Ruminal NH3 concentration was determined by the phenyl-hypochlorite procedure (Broderick and Kang, 1980).

Calculations and Statistical Analysis

Digesta flow was calculated by dividing the amount of TiO2 dosed by the concentration of TiO2 in duodenal and ileal samples. Nutrient flow was calculated by multiplying nutrient concentration by digesta flow. Digestion data were analyzed using the GLM procedures of SAS (Version 8.0, 1998, SAS Inst., Inc., Cary, NC) for a Latin square. After a significant preliminary F-test, Fisher’s LSD was used to separate treatment means. Time series data were analyzed as a split-plot design. Treatment effects were tested using animal x period x treatment as the error term. Time and treatment x time interactions were tested using the residual error term. No interactions (P=0.088 to 0.999) were detected for time course data; therefore, only the main effects were reported.

Results and Discussion

Intake of OM was greater (P<0.001) for lambs fed camelina than Control with canola being intermediate, and lambs fed brown mustard did not differ from lambs fed camelina and canola (Table 2). Nitrogen intake was greater (P<0.001) for lambs fed camelina and brown mustard compared with Control; lambs fed canola were intermediate. These differences in intake may be attributable to analysis of grab samples of soybean meal taken during the trial period differing from the initial analysis of soybean meal used to formulate diets. The differences in N intake; however, did not affect (P = 0.461) ruminal NH3 concentrations (Table 3), which have been shown to increase with an increase in dietary CP (Pritchard and Males, 1985). Additionally, site and extent of OM digestibility did not differ (P>0.217) among treatments. This is consistent with Kucuk et al. (2004) who reported no differences in OM digestibility with increasing levels of soybean oil in a high-concentrate diet fed to lambs. Digestibility of N in the small intestine (% entering) was less (P=0.017) for lambs fed Control than lambs fed any of the whole oilseeds, suggesting that protein quality was improved by replacing soybean protein with protein from the oilseeds. In contrast, Aldrich et al. (1997) noted that small intestinal N digestibility was not affected by supplementing the diets of steers with canola seeds. The current study used a high-concentrate diet fed to lambs, whereas Aldrich et al. (1997) utilized a high-roughage diet for their control. Total tract digestibility of N was greater (P=0.002) for lambs fed camelina than Control with canola being intermediate, and lambs fed brown mustard did not differ from the other fat-supplemented treatments. Intake of NDF was greatest (P<0.001) for canola followed by camelina, brown mustard, and Control. Supplementing fat did not affect (P=0.762) ruminal fiber.
digestibility, which was expected because diets were formulated to provide 3% added fat to avoid detrimental effects on ruminal fiber digestibility. Total tract NDF digestibility was greater \( P=0.038 \) for lambs fed canola seed and camelina than the Control with brown mustard supplemented lambs being similar to all treatments. In contrast, total tract NDF digestibility of forage-based diets was not affected if steers were fed whole canola seeds (Leupp et al; 2006) or heifers were fed whole flaxseed (Scholljegerdes and Kronberg, 2007).

Ruminal pH was not affected \( (P=0.206) \) by dietary treatments (Table 3). In feeding dairy cows whole camelina seeds and camelina meal, Hurtaud and Peyraud (2007) detected a lower pH for camelina treatments before feeding and similar ruminal pH 3 hours after feeding. Leupp et al. (2006) reported a lower ruminal pH in steers fed canola seeds. Similar to other reports published by our laboratory (Kucuk et al., 2004; Atkinson et al., 2006); however, adding 3% dietary fat did not affect \( (P=0.124) \) total ruminal VFA concentrations. Molar proportions of individual VFA did not differ \( (P≥0.185) \) among dietary treatments, which was also reported by Kucuk et al. (2004) who fed lambs high-concentrate diets containing 3.2% soybean oil.

In conclusion, although some variations in nutrient digestibility were noted, our overall hypothesis that nutrient digestibility of camelina and brown mustard would be comparable to canola held true. The feeding value of whole *Camelina sativa* and brown mustard seeds were comparable to whole canola seeds when included in finishing lamb diets. Oilseed crop growers should be able to market brown mustard and camelina as a dietary supplement for lambs.

References


Table 2. Intake and site and extent of organic matter (OM), nitrogen (N), and neutral detergent fiber (NDF) digestion in lambs fed whole canola, brown mustard, or camelina seeds.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Canola</th>
<th>Brown mustard</th>
<th>Camelina</th>
<th>SEM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake, g/d</td>
<td>1181.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1235.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1236.78&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1245.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Digestibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent ruminal, % of intake</td>
<td>55.13</td>
<td>50.59</td>
<td>51.00</td>
<td>57.27</td>
<td>4.0</td>
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<tr>
<td>Small intestine, % entering</td>
<td>49.55</td>
<td>54.35</td>
<td>54.84</td>
<td>53.65</td>
<td>3.2</td>
<td>0.653</td>
<td></td>
</tr>
<tr>
<td>Large intestine, % entering</td>
<td>24.10</td>
<td>19.21</td>
<td>21.51</td>
<td>18.78</td>
<td>6.5</td>
<td>0.931</td>
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<tr>
<td>Lower tract, % entering</td>
<td>61.81</td>
<td>64.10</td>
<td>64.46</td>
<td>63.59</td>
<td>2.4</td>
<td>0.871</td>
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<td>Total tract, % of intake</td>
<td>83.13</td>
<td>82.42</td>
<td>82.96</td>
<td>84.43</td>
<td>0.6</td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intake, g/d</td>
<td>17.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Digestibility</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine, % entering</td>
<td>67.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9</td>
<td>0.017</td>
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<tr>
<td>Large intestine, % entering</td>
<td>9.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>Lower tract, % entering</td>
<td>70.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.01&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>76.61&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>79.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2</td>
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<tr>
<td>Total tract, % of intake</td>
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<td>69.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.82&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>Intake, g/d</td>
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<td>339.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>323.75&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Ruminal, % of intake</td>
<td>58.92</td>
<td>60.21</td>
<td>56.49</td>
<td>60.67</td>
<td>3.0</td>
<td>0.762</td>
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<tr>
<td>Lower tract, % entering</td>
<td>11.39</td>
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<td>25.28</td>
<td>29.60</td>
<td>5.3</td>
<td>0.177</td>
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<tr>
<td>Total tract, % of intake</td>
<td>64.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.04&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>72.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5</td>
<td>0.038</td>
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<sup>1</sup>n = 4
<sup>a,b,c,d</sup>Within the same row, means with unlike superscripts differ (P < 0.05).

Table 3. Ruminal pH, ammonia (NH<sub>3</sub>), and volatile fatty acids (VFA) in lambs fed whole canola, brown mustard, or camelina seeds.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary treatment</th>
<th>Control</th>
<th>Canola</th>
<th>Brown mustard</th>
<th>Camelina</th>
<th>SEM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.87</td>
<td>6.09</td>
<td>6.04</td>
<td>5.87</td>
<td>0.08</td>
<td>0.206</td>
<td></td>
</tr>
<tr>
<td><strong>NH&lt;sub&gt;3&lt;/sub&gt;, mM</strong></td>
<td></td>
<td>8.27</td>
<td>8.05</td>
<td>9.29</td>
<td>10.07</td>
<td>0.9</td>
<td>0.461</td>
<td></td>
</tr>
<tr>
<td><strong>Total VFA, mM</strong></td>
<td></td>
<td>133.00</td>
<td>127.40</td>
<td>132.80</td>
<td>146.40</td>
<td>4.8</td>
<td>0.124</td>
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</tr>
<tr>
<td>Acetate</td>
<td></td>
<td>54.9</td>
<td>57.9</td>
<td>55.5</td>
<td>52.8</td>
<td>1.4</td>
<td>0.191</td>
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<tr>
<td>Propionate</td>
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<td>29.3</td>
<td>28.9</td>
<td>31.8</td>
<td>32.6</td>
<td>2.4</td>
<td>0.636</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td>11.9</td>
<td>9.3</td>
<td>8.5</td>
<td>10.4</td>
<td>1.3</td>
<td>0.339</td>
<td></td>
</tr>
<tr>
<td>Isobutyrate</td>
<td></td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
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<td>0.1</td>
<td>0.135</td>
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<tr>
<td>Valerate</td>
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<td>0.9</td>
<td>1.0</td>
<td>1.2</td>
<td>0.2</td>
<td>0.292</td>
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<tr>
<td>Isovalerate</td>
<td></td>
<td>1.8</td>
<td>2.0</td>
<td>2.2</td>
<td>2.4</td>
<td>0.3</td>
<td>0.695</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>n = 4
Reproductive Biology

Differential Gene Expression in Oviducts Collected During Proestrus from Ewes Fasted During the Luteal Phase of the Estrous Cycle

Kathleen J. Austin¹, Senior Research Scientist
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¹Department of Animal Science
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Summary and Implications

Short-term fasting during the luteal phase of the estrous cycle perturbs circulating concentrations of progesterone and estradiol, causing a delay in the onset of the surge release of LH (Alexander, 2006). Although ovulation rate is not affected by fasting in this model, number of lambs born is decreased. Fasting may affect fertility by decreasing ova quality and/or altering the oviduct/uterine environment. The objective of this study was to elucidate differences in gene expression in the oviduct near the time of expected ovulation. Estrous cycles were synchronized with PGF2α. Control ewes were given ad libitum access to good-quality grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through d 11 of the estrous cycle (d 0 = first day of estrus). On d 12 all ewes were treated with 10 mg PGF2α, and fasted ewes were returned to ad libitum feed. Oviduct tissue was collected from fasted and fed ewes during the expected peri-ovulatory period (72 hr following PGF2α and realimentation). At 72 hr, ewes were euthanized and mid sections of oviduct were dissected and snap-frozen for RNA analysis. Purified RNA was screened using the Affymetrix bovine gene chip containing ~24,000 genes at the Montana State University Functional Genomics facility. Although the RNA was ovine in origin, there is at least an 80% presence/identity when screening bovine chips with ovine RNA. Genes differentially expressed on the microarray chip were analyzed using DAVID (NIAID, NIH) for functional annotation and identification of expression patterns, and genes of interest were confirmed using semi-quantitative real time RT-PCR. Differential gene expression was observed in oviducts of the fasted ewes compared to control ewes. Affected intracellular processes include ADP ribosylation, transcription, transport, phosphorylation, ubiquitinylation, apoptosis, proteasomal degradation, GTP binding, signal transduction, carbohydrate transport and metabolism, regulation of transcription, and cell adhesion. Some genes found to be up-regulated in the oviducts of fasted ewes by microarray analysis include ARF-1, ARF-3, RhoGDP inhibitor, ataxin, ubiquitin, proteasomal subunit 8, HSD17B1, and a wide array of zinc finger proteins (P < 0.05). Genes down regulated by microarray analysis include, but are not limited to, TNFα, MX1, MDA5, and IRF 7 (P< 0.05). The mRNA for ARF 1 (P = 0.056) and ARF 3 (P = 0.029) were found to be up regulated by real-time PCR as well. These results imply a role for nutrition in molecular mechanisms regulating the environment of the oocyte at the site of fertilization which may impact overall fertility.

Introduction

Fasting during the luteal phase of the estrous cycle alters the circulating levels of estrogen and progesterone and delays the surge release of LH (Alexander, 2006). The number of lambs born to fasted ewes was decreased when compared to control ewes while ovulation rates were not effected (Kiyma, 2004). Our lab has predicted that affected molecular mechanisms in the oviduct, uterus and/or follicles may be responsible for this decreased fertility. Therefore, we examined the molecular environment of the oviduct, specifically changes in gene expression which may affect fertility of ewes fasted during the luteal phase of the preceding estrous cycle.

Materials and Methods

Ewes, Treatment and Tissue Collection

Estrous cycles were synchronized with PGF2α and randomly allotted to control or fasted groups (n= 9 ewes per group). Control ewes were fed grass hay ad libitum while feed was withheld from the fasted ewes on d 7 through 11 of the estrous cycle. On d 12 all ewes were treated with 10 mg of PGF2α and returned to feed. Oviduct tissue was collected from all ewes 72 h following PGF2α and realimentation. Tissue was removed from the mid section of the oviduct and snap frozen for RNA analyses.
**RNA Isolation**

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

**Gene Chip Screening**

Ten µg of total RNA was sent to the LMPC and Functional Genomics Core at Montana State University for labeling and hybridization to bovine gene chips following the procedures of Affymetrix. Data were analyzed using DAVID (NIAID, NIH) to obtain functional annotation. Expression analysis was confirmed using Real- Time RT-PCR.

**Real- Time RT-PCR**

RNA was converted to cDNA using the IScript cDNA synthesis kit from Biorad. Fifteen µL of master mix consisting of 12.5µl of SYBR green supermix (BioRad), 1µL each of forward and reverse primer and 0.5 µL H2O were added to 10 ul cDNA and the IQ5 was programmed to run 40 cycles of 95°C for 30 sec., 60°C for 30 sec followed by melting curve analysis. Primer pairs were designed using PRIMER 3 software with GAPDH as an internal reference gene. Data were analyzed using the GLM procedures of SAS.

**Figure 1.** The estrous cycle of the ewe adapted from McDonald’s Veterinary Endocrinology and Reproduction illustrating the fasting model employed in the current studies. The diagram shows the period of fasting, PGF2α treatment, and the decline in the levels of progesterone in control ewes (yellow line).

**Results and Discussion**

Based on gene chip analysis, cellular processes affected by fasting include ADP ribosylation, transcription, transport, phosphorylation, ubiquitinylation, apoptosis, proteasomal degradation, GTP binding, signal transduction, carbohydrate transport and metabolism, and cell adhesion. Genes upregulated (P < 0.05) in the oviduct of fasted ewes included ARF-1, ARF-3, RhoGDP inhibitor, Ataxin, ubiquitin, HSD17 B1 and a wide variety of zinc finger proteins. (Figure 3). Genes downregulated (P < 0.05) in the oviducts of fasted ewes included TNF alpha, MX1, MDA5, and IRF 7 (Figure 3). Real- time PCR was used to confirm that mRNAs for ARF 1 and ARF 3 were upregulated (P < 0.05; figure 2) while levels of mRNA for IGF1, IGF2, BP1, BP3 and BP6 in the oviduct did not differ among fasted and control ewes.

Fasting during the luteal phase of the estrous cycle alters progesterone metabolism and delays the subsequent ovulatory surge release of LH and decreases conception rate. These results imply nutrition regulates the environment of the oviduct at the site of fertilization. Upregulation of ARFs 1 and 3 may imply fasting alters secretory pathways and intracellular transport mechanisms (Boman, 2000; Stearns, 1990). Future studies will involve genetic analyses of endometrium and follicles to identify genes/gene products which may alter reproductive efficiency in farm animal species.

**References**


Figure 3. A subset of genes up or down regulated by Affymetrix microarray analyses. A FC above one indicates upregulation while a FC value less than one implies downregulation (P < 0.05). Microarray analyses were performed at the Montana State LMPC and Functional Genomics Core Facility comparing RNA collected during proestrus from ewes fasted during the luteal phase of the estrus cycle (n = 3) to ewes which were not fasted (n = 3) and were fed normally during the luteal phase of the estrus cycle.
Reproductive Physiology

DIFFERENTIAL EXPRESSION OF THE IGF-I GENE IN ENDOMETRIUM OF EWES FASTED DURING THE LUTEAL PHASE OF THE ESTRUS CYCLE

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ABSTRACT: Short-term fasting during the luteal phase of the estrous cycle perturbs circulating concentrations of progesterone and estradiol causing a delay in the onset of the surge release of LH. Although ovulation rate is not affected by fasting in this model, numbers of lambs born are decreased. Fasting may affect fertility by decreasing ovulation quality and/or altering the endometrium/oviduct environment. The objective of this study was to elucidate differences in gene expression in the endometrium near the time of expected ovulation in fed and fasted ewes. Estrous cycles were synchronized using PGF$_2$α. Control ewes were given ad libitum access to grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through d 11 of the estrous cycle (d 0 = first day of estrus). On d 12 all ewes were treated with PGF$_2$α, and fasted ewes were returned to ad libitum feed. Endometrial tissue was collected from fasted and fed ewes during the expected periovulatory period (72 h following PGF$_2$α and realimentation of fasted ewes). At 72 h, ewes were euthanized and sections of endometrium were dissected and snap frozen for RNA analysis. Semi-quantitative Real Time PCR was used to examine gene expression within the endometrium of ewes which had ovulated. Data was analyzed using GLM procedures of SAS. Gene expression for IGF-I was up-regulated ($P < 0.01$) in the endometrium of fasted compared to fed ewes. Gene expression for IGF-II, IGFBP1, IGFBP3, IGFBP6, estrogen receptor α, estrogen receptor β, and progesterone receptor did not differ ($P \geq 0.4$) among fasted and fed ewes. Although estrogen can stimulate IGF-I synthesis, serum concentrations of estrogen did not differ ($P = 0.7$) between groups at 0, 24, 48, and 72 hours following realimentation. Fasting during the luteal phase of the estrous cycle preceding proestrus influences uterine expression of IGF-I which may influence peri-implantation embryo survival.

Key Words: Fasting, Endometrium, Sheep, IGF

Introduction

Limited feed resources can decrease reproductive efficiency to an extent dependent on the degree (Mackey et al., 2000) and reproductive status (Smith, 1988) at the time of feed restriction. Short-term fasting of mature ewes during diestrus results in increased serum concentrations of progesterone and a delayed pre-ovulatory surge release of LH (Alexander et al., 2007).

Short-term fasting during the luteal phase of the estrous cycle decreased serum concentrations of FSH (Alexander et al., 2007) and affected numbers of small and medium sized follicles. Although the numbers of large follicles did not differ (Alexander et al., 2007), estradiol was decreased in fasted ewes, during the 24 h period prior to the anticipated surge-release of LH. Serum concentrations of insulin and IGF-I were decreased by fasting, while serum concentrations of GH remained similar among fasted and fed ewes (Kiyma et al., 2004). Although ovulation rate did not differ between fasted and fed ewes (Kiyma et al., 2004), numbers of lambs born tended to be decreased in fasted ewes (unpublished observation). Therefore, the objective of this study was to determine if feed withdrawal during the luteal phase preceding proestrus cause changes in mRNA expression in the endometrium which may detrimentally affect embryo survival.

Materials and Methods

Animals

Estrous cycles of 39 mature (≥3 yr old) Western White-Faced ewes in moderate body condition (BCS= 5 to 7) were synchronized with two 100 mg doses of PGF$_2$α (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI) on d 1 and 10 of the estrous cycle. Estrous behavior was monitored in the presence of two vasectomized rams at 0600 and 1800 for 4 d following the second injection of PGF$_2$α. Twenty ewes with synchronized estrous cycles were selected and randomly allotted to control or fasted (n=10/group treatments) groups. Ewes were housed by treatment in separate, adjacent pens. Control ewes were given ad libitum access to grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through 11 of their estrous cycle (d 0 = first day of estrus). Ewes in both groups had ad libitum access to water. On d 12, all ewes were treated with 100 mg of PGF$_2$α, and fasted ewes were returned to ad libitum feed. Blood samples were collected by jugular venipuncture prior to administration of PGF$_2$α and at 24, 48, and 72 hours post administration. Serum samples were analyzed for concentrations of estradiol. Endometrial tissue was collected from fasted and fed ewes.
during the expected periovulatory period (72 hr following PGF-α and realimentation of fasted ewes). At 72 hr, ewes were euthanized and sections of endometrium were dissected and snap frozen for RNA analysis.

**RNA Isolation and cDNA synthesis**

Total Cellular RNA was isolated from endometrial tissue. Concentration of RNA was determined using a NanoDrop spectrophotometer and 10 μg was further purified using RNEASY (Qiagen Inc; Santa Clara, CA) with on-column DNase digestion. Approximately 2.0 μg of RNA was mixed with 4 μL reverse transcription buffer (5X) and 1 μL of iScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 minutes at 25 °C, 30 min at 42 °C, 5 min at 85 °C and held at 4 °C. The cDNA was diluted with 100 μL nuclease free water and stored at -20 °C until quantitative PCR was performed.

**Semi-Qualitative Real Time PCR**

Diluted cDNA (10 μl) was used as a template for semi- qualitative Real Time PCR amplification in 25 μl reactions consisting of 12.5 μL SYBR Green Supermix (Bio-Rad Laboratories), 0.5 μL H2O and 1 μL each forward and reverse primer. Ovine GAPDH, IGF-I, IGF-II, IGFBP1, IGFBP3, IGFBP6, estrogen receptor α (ERα), estrogen receptor β (ERβ), progesterone receptor (PR), ADP ribosylation factor-1 (Arf1), hydroxysteroid dehydrogenase-17-beta-1 (Hsd17β1), pre-proghrelin (PPG), and tumor necrosis factor alpha (TNFα) primers were designed using Primer 3 software to generate ~100bp amplicons. Semi-quantitative RT-PCR was performed using 40 cycles of 95 °C for 30 sec and 62 °C for 30 sec. Following amplification, cDNAs were melted (melting curve analysis) to ensure quality of amplification by incubating RT-PCR products for 10 sec at each step with increase in temperature by 0.5 °C from 55 °C to 95 °C in each cycle. Messenger RNA levels were quantified and reported relative to GAPDH.

**Statistical analysis**

All mRNA data were analyzed by GLM procedures of SAS (Version 9.0). Type III sums of squares were used to examine mean differences in the average fold change of mRNA expression within the endometrium of fasted as compared to fed ewes.

**Results and Discussion**

At 72 hr following administration of PGF-α, some ewes had not ovulated, as serum concentrations of estradiol remained elevated. Ewes selected for mRNA analysis had ovulated by 72 hrs, and serum concentrations of estradiol were low. Since numbers of large follicles and ovulation rate were unaffected, early embryo survival must be affected by fasting. Fasting may affect embryo quality, but may also alter the uterine environment adversely affecting embryo survival.

Expression of mRNA for IGF-I was up-regulated (P < 0.01) in the endometrium of fasted compared to fed ewes (Fig. 1). Gene expression for IGF-II, IGFBP1, IGFBP3, IGFBP6, ERα, ERβ, PR, Arf1, Hsd17β1, PPG, and TNFα did not differ (P ≥ 0.4) among fasted and fed ewes (Table 1). Although estrogen can stimulate IGF-I synthesis, serum concentrations of estrogen did not differ (P = 0.7) among treatments, nor was there a time by treatment interaction (P = 0.54; data not shown). Differences in serum concentrations of estrogen may be masked by the limited number of blood samples collected.

Nutritional influences on reproduction may affect reproductive efficiency indirectly through the liver IGF-I system or affect IGF synthesis directly in the reproductive tissues (Roberts et al., 2001). While mRNA levels of IGF-I within the endometrium were up-regulated in fasted ewes, serum concentrations of IGF-I was decreased and concentrations did not return to levels comparable to those of control ewes until 72 hr following realimentation (Kiyma et al., 2004), suggesting differential regulation of liver and tissue IGF synthesis.

Receptors for IGF-I are found in several tissues throughout the body. IGF-I and IGF-II stimulate hormone synthesis and secretion in ovarian granulosa and theca cells (Jones and Clemmons, 1995). The IGFs are also responsible for stimulation of DNA synthesis and cell replication, by affecting the cell cycle (Jones and Clemmons, 1995). Depending on the location of the embryo within the reproductive tract IGF may contribute to the preparation status of the endometrium for embryo implantation and survival. The decrease in serum concentration of IGF-I may contribute to the decreased numbers of follicles in fasted ewes (Alexander et al., 2007).

IGF-I is under the direct influence of estrogen and acts as a mediator for some of the actions of estrogen on the uterus (Stevenson et al., 1994). In the present study there were no differences in serum concentrations of estradiol, yet IGF-I was up-regulated in the endometrium of fasted ewes. In other studies decreased serum concentrations of estradiol were reported in fasted compared to fed ewes during the 24 h before the anticipated surge-release of LH (Alexander et al. 2007). Discrepancy in results could be attributed to the decreased sampling frequency in the present study.

In conclusion, fasting during the luteal phase of the estrous cycle preceding proestrus influences uterine expression of IGF-I which may detrimentally affect peri-implantation embryo survival.
Figure 1. Expression of IGF-I mRNA within the endometrium of fed and fasted ewes which had ovulated. \( P < 0.01 \)

Table 1. Relative concentrations of mRNA present within endometrium of fasted as compared to fed ewes using semi-quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-II</td>
<td>0.84</td>
<td>0.52</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>1.13</td>
<td>0.92</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>1.25</td>
<td>0.50</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>0.91</td>
<td>0.40</td>
</tr>
<tr>
<td>Estrogen receptor α</td>
<td>1.03</td>
<td>0.93</td>
</tr>
<tr>
<td>Estrogen receptor β</td>
<td>1.60</td>
<td>0.47</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>1.28</td>
<td>0.47</td>
</tr>
<tr>
<td>Arf1</td>
<td>0.91</td>
<td>0.72</td>
</tr>
<tr>
<td>HSD17β1</td>
<td>1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>PPG</td>
<td>1.48</td>
<td>0.21</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.88</td>
<td>0.70</td>
</tr>
</tbody>
</table>

\( ^a \) Fold change represents up or down-regulation of mRNA in fasted compared to fed ewes.

Literature Cited


Expression of Appetite Regulatory Genes, Receptors and Hormones in Obese Dams and their Fetuses

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Summary and Implications

More than half of Americans are overweight or obese with obesity levels reaching epidemic proportions. Studies indicate that maternal body mass index (BMI) and incidence of obesity in progeny are correlated. Maternal obesity may affect body weight of offspring by influencing expression of hypothalamic appetite regulating hormones. Agouti-related protein (AgRP) and neuropeptide Y (NPY) have been identified as appetite stimulators while pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) inhibit appetite. Leptin, originating in adipocytes, signals energy balance and decreases food intake when hypothalamic receptors are occupied. Estrogen receptor beta (ERβ) inhibits appetite whereas estrogen receptor alpha (ERα) has no known influence on appetite. The ratio of ERβ to ERα can regulate appetite through differential expression of NPY and AgRP. The objective of this study was to determine the expression of appetite regulatory genes in control and obese dams and determine if dam BMI affects expression of appetite regulators in the developing fetus. Multiparous, Western whitefaced ewes were weighed and individually fed 100% (control; n = 3) or 150% (obese; n = 3) of NRC requirements from 60 d prior to mating until d 75 of gestation. On d 75 of gestation, ewes were weighed and euthanized. Body weight increased 50% in the obese ewes and 7% in the control ewes during this time period. Fetuses were collected and weighed, and hypothalamic tissue of the dams and fetuses was isolated. Results were analyzed using the GLM and CORR procedures of SAS (SAS Inst. Inc., Cary, NC). There was no effect of sex or sex by treatment interaction (P > 0.05) on gene expression in the fetuses. Ratio of ERβ to ERα in ewes was higher (P < 0.05) in obese ewes versus control ewes, and also tended to differ (P < 0.10) in fetuses from obese ewes. Additionally, control dams had a higher (P < 0.05) expression of ERα than obese dams. No other differences in gene expression were observed. Dam and fetal CART gene expression were negatively correlated (P < 0.05), with CART expression upregulated in the dam but downregulated in the fetus. No other correlations between dam and fetal gene expression were significant. Overall, we propose that the effect of maternal obesity on the ratio of ERβ to ERα in the fetus may affect the propensity for obesity in offspring by influencing the appetite stimulators NPY and AgRP.

Introduction

Obesity has reached pandemic proportions in developed countries in the past two decades. In 2005 a survey showed that 23.9% of Americans were obese (BMI > 31), and 3% were morbidly obese (BMI > 40). Obesity has been associated with deleterious risks such as type II diabetes, high blood pressure, coronary heart disease, and stroke. The increased incidence of obesity has increased interest in how maternal appetite regulation effects fetal programming. Recent studies have shown a positive relationship between maternal BMI and the incidence of obesity in progeny. Furthermore, studies have shown that appetite regulatory neuropeptides expressed within the hypothalamus may be altered through the manipulation of maternal diet during critical developmental periods of gestation. Orexigenic neuropeptides such as NPY and AgRP and anorexogenic neuropeptides such as CART and POMC are appetite regulatory genes in the arcuate nucleus of the hypothalamus that may influence appetite regulation in the developing fetus (McMillen et al., 2005; Arora and Anubhiti, 2006). The hormone leptin, secreted by
adipocytes, has also been shown to aid in energy balance and appetite regulation. As receptors in the hypothalamus are occupied, leptin signals increased energy balance which leads to decreased food intake. The ratio of ERβ to ERα has been shown to influence appetite through the differential expression of NPY and AgRP (Mühlhäusler et al., 2004). The expression patterns of NPY, AgRP, CART, and POMC are similar in the fetal sheep during late gestation compared to adult sheep; however, leptin receptor expression levels are different suggesting differing roles of leptin before and after birth (McMillen et al., 2005). Leptin expression before birth may be conserved for thermogenesis as compared to the adult animal where expression levels play a role in energy balance. The objective of this study was to determine the expression of appetite regulatory genes in control and obese dams and determine if dam BMI affects expression of appetite regulators in the developing fetus.

Materials and Methods

Animal Protocol
Multiparous Western whitefaced ewes were individually weighed and fed 100% (control; n = 3) or 150% (fat; n = 3) in accordance to NRC requirements on a DM basis to meet the maintenance of an early pregnant ewe from 60 d prior to mating until d 75 of gestation. Rations were calculated on a metabolic body-weight basis (weight0.75) and were adjusted weekly based on weight gain or loss. The diet consisted of pelleted beet pulp (79.7% TDN, 93.5% DM, and 10.0% CP) and a mineral supplement (51.43% sodium triphosphate, 47.62% potassium chloride, 0.39% zinc oxide, 0.06% cobalt acetate, and 0.50% ADE vitamin premix). On d 75 of gestation, ewes were weighed, and blood was collected by venipuncture from the jugular vein. Ewes were anesthetized with Ketamine (22.2 mg/kg BW, i.v.) and maintained under isofluorane inhalation (2.5%). Following fetal blood collection, ewes euthanized by castrated rams. Ewe and fetus weights were only significantly affected in all but one gene, CART, which could explain why neuropeptide expression did not differ among treatment in fetuses or in ewes. Relative expression in the ewes was different between control and fat fetuses or in control and fat ewes was not different. However, there was a negative correlation in expression between the dam and fetus in both the control and fat diets (P = .027; P = .035), respectively (Figure 2). Bergen et al., (1999) showed that three different species of mature male mice fed high fat diets for 98d resulted in a significant decrease in orexigenic neuropeptide, NPY, relative gene expression in two of the breeds with significant effect of diet on body weight. Interestingly, anorexigenic neuropeptide, POMC, relative expression levels were only significantly increased in only one of the affected orexigenic mice breeds. It was concluded that there was little relationship between body weight and appetite regulator neuropeptide mRNA expression. However, Dube et al., (2007) states that obesity due to genetic factors are often associated with hyperphagia and increased NPY expression in rodents, which may explain why only one mouse breed had decreased NPY levels and increased POMC levels in response in increased body weight. A similar study in 4 d fasted castrated rams by Adam et al. (2002) determined that fasting affected expression of NPY, AgRP, and leptin receptor, but CART and POMC expression did not differ. The study cited used male sheep and probes from rats and hamsters in all but one gene, CART, which could explain why neuropeptide expression levels differed from our study in which pregnant ewe tissues were utilized and all but one primer was of ovine origin.

Differences between control and obese diets showed ERα relative expression in the ewes (P = .038), but there was no difference in the fetuses (P = 0.1). ERβ expression did not differ among treatment in fetuses or ewes (P = 0.06; P = 0.1), respectively (Figure 3).
There was no difference in leptin receptor between control and obese fetuses ($P = 0.8$) or ewes ($P = 0.2$), but there was a trend for a negative correlation of leptin receptor expression between fat fetuses and dams ($P = 0.09$; figure 4). Therefore, as expression of maternal leptin receptor levels increase, expression of fetal receptors tend to decrease. Gastrointestinal signals such as gastric distention and cholecystokinin send satiety signals via the vagus nerve to the hindbrain. Leptin regulates body weight by enhancing hindbrain sensitivity to satiety signals by filled receptors in the arcuate nucleus of the hypothalamus (Cummings et al., 2005). Leptin receptor is highly expressed in the arcuate nucleus of the hypothalamus and increases in circulating leptin concentrations during periods of increased food intake result in a decrease in orexogenic neuropeptide expression (McMillen et al., 2006). There were no differences in expression of ER$\alpha$ in male and female fetuses ($P = 0.345$) or in control or fat diets ($P = 0.124$; Figure 5).

Male and female fetuses whose dams were fed either control or fat diets did not have an effect on ER$\alpha$ expression ($P = 0.938$). Sex of fetuses as well as control and fat diets did have an effect on ER$\beta$ expression ($P = 0.056$); ($P = 0.055$), respectively. No difference in sex of fetuses whose dams were fed control or fat diets was detected ($P = 0.629$; figure 5).

Figure 1. Relative gene expression levels of AgRP and NPY of dams and fetuses fed control or fat diets.

Figure 2. Relative gene expression of CART or POMC between dams and fetuses fed control or fat diets.
Figure 5. The sex by treatment interaction on relative gene expression levels of ERα and β on sex in control and obese fetuses.

Conclusions and Future Aims

Overall, these results indicate that maternal nutrition does not affect fetal appetite programming at this stage of development. However, Mühlhäusler et al., (2004) found that appetite regulatory neuropeptides, NPY, AgRP, POMC, and CART, are fully functional in the sheep at 110 d gestation. It is possible some species are able to conserve nutrients available due to a genetic predisposition or a “thrifty phenotype” that once had an adaptive advantage to a poor diet (Armitage et al., 2004). This is a possible explanation as to why certain species of mice express more or less amounts of appetite regulators in response to increased body weight.

References


Reproductive Physiology

Increased Circulating Progesterone Levels in the Offspring of Nutrient Restricted Ewes

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Summary and Implications

Undernutrition can frequently occur in pregnant ruminants maintained on range. We have reported that fetuses of ewes nutrient restricted from 28-78 d of gestation exhibited increased oxidative base lesions within DNA of day 78 fetal oogonia compared to controls. Such lesions in fetal oogonia could affect oocyte/follicular/corpora lutea (CL) function later in life. Progesterone secretion by the CL is vital for initiation and maintenance of cyclicity and pregnancy. This study examined the impact of maternal nutrient restriction during early gestation on progesterone secretion during an estrous cycle of female offspring. Dams were fed either a nutrient restricted (NR: 50% National Research Council (NRC) recommendations) or control (C: 100% NRC) diet from 28 to 78 days of gestation and then both groups were fed 100% of requirements to lambing. Female lambs from NR and C dams were commingled in a single group and fed 100% NRC from weaning through the time of testing. During October and November, yearlings born from NR (n=7) and C (n=7) dams were monitored daily for estrus and blood sampled every three days from standing estrus (d 0) to the subsequent estrus. This procedure was repeated at 3 yrs of age. NR and C ewes were of similar BW both at 1 (62.4 ± 3.1 kg) and 3 (75.8 ± 2.6 kg) yrs of age. Estrous cycle lengths were similar across treatment groups (19.1 ± 0.2 d). P4 area under the curve was 20% higher in NR vs. C offspring at 1 yr of age (P = 0.05), and 23% higher at 3 yrs of age (P = 0.08). Maternal diet-induced increases in systemic progesterone in female offspring of NR vs. C ewes could indicate differences in either CL progesterone secretion, incidence of multiple ovulations and/or progesterone clearance. Regardless of mechanism, these data provide evidence for the persistent in utero programming of progesterone levels in offspring induced by differences in early gestational maternal nutrition. These results, along with a growing body of other data, indicate the important of maternal nutrition during the first half of gestation.

Introduction

Progesterone is one of two primary steroid hormones controlling female reproductive function. Progesterone is essential for normal regulation of reproductive cyclicity, initiation and maintenance of pregnancy, and may also influence changes in energy metabolism associated with cyclicity and pregnancy. The primary source of progesterone secretion in females is the corpus luteum (CL), a structure formed on the ovary following ovulation of an oocyte.

Undernutrition during early gestation may be a common feature of ruminants maintained on range due to seasonal fluctuations in forage quality and quantity. Much attention has been paid in the past to maternal nutrition in late gestation, when fetal demands are greatest due to rapid fetal body growth. However, because placental development (which will later impact nutrient/gas transfer efficiency to the rapidly growing late gestation fetus) and the initial differentiation of fetal organs occurs during early gestation, more attention has been given recently to nutrient needs of pregnant animals during the first half of pregnancy.

We have previously reported that fetuses of ewes nutrient restricted from 28-78 d of gestation exhibited increased oxidative base lesions within DNA of day 78 fetal oogonia (precursor cells to future oocytes) compared to controls (Murdoch et al., 2003, Reprod. Biol. Endocrinol. 1:6). Such lesions in fetal oogonia could affect oocyte quality later in life, thus impacting the follicle and eventual CL associated with that oocyte. Therefore, this study examined the impact of maternal nutrient restriction during early gestation on progesterone concentrations throughout the estrous cycle of female offspring in order to begin to examine whether maternal nutrient restriction may impact reproductive function in offspring.
Materials and Methods

**Animals.** Mature, multiparous ewes were bred by a single intact ram. Beginning at d 28 of pregnancy, dams were fed either 50% of nutrient recommendations (nutrient restricted, NR; NRC, 1985) or 100% of nutrient recommendations (control, C). At d 78 of gestation, NR dams were realimented to 100% of nutrient recommendations so that both NR and C dams continued throughout gestation receiving 100% of recommendations. Female lambs from NR and C dams were commingled in a single group and fed 100% of recommendations from weaning through the times of testing. Following the final test period, all ewes were bred by a single intact ram maintained in the same pen as the ewes for several weeks.

**Sample collection.** During October and November, yearlings born from NR (n=7) and C (n=7) dams were monitored daily for estrus and blood sampled every three days from standing estrus (d 0) to the subsequent estrus. This procedure was repeated at 3 yrs of age. Blood was collected via jugular venipuncture into evacuated blood collection tubes containing sodium heparin as an anticoagulant. Plasma was collected following centrifugation at 4 ºC and stored frozen until time of hormone analysis.

**Hormone Analysis.** Progesterone concentrations were analyzed using a commercially available radioimmunoassay kit (Siemens Healthcare Diagnostics, Deerfield, IL)

**Statistics.** To estimate overall progesterone levels throughout the estrous cycle, area under the curve (AUC) was calculated for each ewe by trapezoidal approximation using the progesterone concentration at first estrus as a baseline (Prism 4, GraphPad Software Inc.). Differences were determined by t-test for peak progesterone (determined as day 12 of estrous cycle) and AUC.

**Results**

NR and C ewes were of similar BW both at one (62.4 ± 3.1 kg) and three (75.8 ± 2.6 kg) years of age. During both years, estrous cycle lengths were similar across treatment groups averaging 19.1 ± 0.2 d. Progesterone AUC was higher in NR vs. C offspring at one year of age (P = 0.05) and peak progesterone was also higher in NR vs C ewes (P=0.03) (Fig. 1) At three years of age, progesterone AUC tended to be higher in NR vs C ewes (P = 0.08) and peak progesterone remained higher in NR vs C ewes (P=0.03) (Fig. 2). Following testing at three years of age, all ewes conceived and carried a successful pregnancy to term. Twinning rate was not different between three-year old NR and C ewes.

**Figure 1.** Progesterone concentrations throughout an estrous cycle of offspring of nutrient restricted (NR) vs control (C) ewes at one year of age.

**Figure 2.** Progesterone concentrations throughout an estrous cycle of offspring of nutrient restricted (NR) vs control (C) ewes at three years of age.
Conclusions

Maternal diet-induced increases in systemic progesterone in female offspring of NR vs. C ewes could indicate differences in either CL progesterone secretion, incidence of multiple ovulations and/or progesterone clearance. Since all ewes conceived successfully and carried pregnancies to term following testing at three years of age, apparent reproductive success was not altered at this later age. Furthermore, no difference in twinning rate indicates number of ovulations was unlikely to be responsible for the differences observed in progesterone concentrations throughout the non-pregnant cycle at either age. Regardless of mechanism, these data provide evidence for the persistent in utero programming of progesterone levels in offspring induced by differences in early gestational maternal nutrition. However, since the differences between NR and C offspring appears to lessen with age, perhaps there is the flexibility for physiologic systems to recuperate over time from the effects of nutrient deprivation during fetal development. If reproduction can be altered for years after birth by maternal nutrition during early gestation, this highlights the importance of maternal nutrition throughout the entire pregnancy.
Reproductive Physiology

Effects of Early Gestational Undernutrition on Fetal Growth, Organ Development, and Placentomal Composition in the Bovine

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Department of Animal Science

Summary and Implications

Multiparous beef cows bred to a single sire and gestating female fetuses (n= 30) were fed to either meet NRC recommendations (control; C) or fed below NRC recommendations (68.1 % of NEm and 86.7 % of MP recommendations; nutrient restricted, NR) from day 30 to 125 of gestation. On day 125 of gestation 10 C and 10 NR cows were necropsied and the remaining 5 NR cows were realimented to achieve similar BW and BCS with the remaining 5 C cows by day 190 of gestation and both groups were necropsied at d 245 of gestation. At necropsy fetal weights and organ weights were recorded, placenta diameters were recorded and all placentomes were separated into caruncular (CAR) and cotyledonary (COT) components and each component pooled. Fetal weights at day 125 of gestation was 948 ± 14 g (n=10) for C cows, however, fetal weights of NR cows fell into two distinct groups: NR non intrauterine growth restriction (IUGR) cows had fetal weights similar to C cows (974 ± 20 g, n=6), while fetal weights of NR IUGR cows were reduced (773 ± 23 g, n= 4; P<0.01). Fetal brain weight as a percentage of fetal weight was increased (P<0.01) and fetal heart weight as a percentage of fetal weight tended (P=0.08) to be increased in the NR IUGR fetuses compared to fetuses from the other two groups. Nutrient restricted IUGR cows exhibited reduced (P<0.01) COT weights and tended (P=0.07) to have reduced total placenta surface area compared to NR non IUGR and C cows. On day 245 of gestation, fetal weights and CAR weight were similar for NR and C cows, COT weights, however, were reduced (P<0.01) in NR vs. C cows. Decreased fetal growth in NR IUGR cows on day 125 of gestation was associated with decreased COT weights and reduced placental area. The return of NR cows to a BW and BCS similar to that of C cows during the second half of gestation resulted in similar fetal weights of NR and C cows by day 245 of gestation. Thus, a bout of fetal IUGR, which has been linked to postnatal insulin resistance, obesity and cardiovascular disease may go undetected if cows undernourished during early gestation receive feed supplementation in the second half of gestation to assure normal birth weight.

Introduction

In response to maternal under nutrition, fetal intrauterine growth restriction (IUGR) can occur (McMillen et al., 2001; Vonnahme et al., 2003). In response to IUGR, there is an adaptive fetal response which maintains growth of organs and tissues important to survival while at the same time reducing the growth of other less important organs and tissues (Hales and Barker, 1992). This is especially true during early gestation, which is a time period for organ and tissue development and placental vascularization in the mammalian conceptus (Ford, 1995; Reynolds and Redmer, 1995). IUGR fetuses have a significantly increased risk of developing obesity, type II diabetes, hypertension and cardiovascular diseases in postnatal life (Poore and Fowden, 2002;Ford et al., 2007). Offspring that have been IUGR during gestation have fewer total nephrons compared with non IUGR offspring (Gopalakrishnan et al., 2005) and this reduced nephron number has been correlated to increased blood pressure (Gilbert et al., 2005). Further, offspring that have experienced IUGR during early gestation exhibit altered growth rates and carcass quality when compared to non IUGR offspring (Ford et al., 2007). We hypothesized that restriction of nutrient intake from d 30 through d 125 of gestation in cattle would decrease maternal BW and BCS and reduce the growth and development of the fetus, with a resulting asynchrony in organ development.

Materials and Methods

This study was conducted at the University of Wyoming and all procedures were approved by the University of Wyoming Animal Care and Use Committee. Unsuckled multiparous Angus X Gelvieh cows (n = 116) with an initial BW of 1259 ± 139 lbs and initial body condition score (BCS) of 5.4 ± 0.7 (BCS scale 1-9) were synchronized using a controlled intravaginal drug release device (CIDR, Pfizer, Exton PA) for 7 days and upon removal of the CIDR, 25 mg of PGF2α (Lutalyse, Pharmacia & Upjohn Co, Kalamazoo, MI) was administered. Cows were then checked for estrus every 12 h and artificially inseminated using semen from a single sire ~ 12 h after the onset of estrus. Cows were blocked by BW, BCS and age at breeding and assigned to one of two diets adjusted for BW0.75. Control cows (C) were fed native grass hay (12.1 % CP, 70.7 % IVDMD) fortified with vitamin and
minerals according to NRC (2000) for a mature cow to gain 0.72 kg/d for the first 125 d of gestation. Nutrient restricted (NR) cows were fed millet straw (9.9 % CP, 54.5 % IVDMD) to provide 68.1 % of the NE\textsubscript{m} and 86.7 % of the MP requirement (NRC 2000) from d 30 to d 125 of gestation and 50 % of the vitamins and minerals provided to the control cows. Cow BW was recorded every 14 d and rations were adjusted for changes in cow BW throughout the experiment. On d 80 of gestation, cows were ultrasonoused (Aloka 500 with a 5-MHz linear probe, Aloka, Wallingford, CT) to confirm pregnancy and determine fetal sex. A randomly chosen subsample of thirty cows (15 C and 15 NR) that were gestating female fetuses was selected for necropsy during gestation. After d 125 of gestation C cows continued to be fed the same native grass hay to maintain a BCS of 5.75. The NR cows were realimented by feeding the millet straw and a supplement (79.6 % cracked corn, 6.1 % soybean meal, 5.3 % sunflower meal, 4.2 % cane molasses, 2.6 % safflower seed meal, and 1.6 % dried skim milk; 13.2 % CP and 77.6 % IVDMD) on a DM basis and vitamins and minerals were fed at the same level as fed to C cows. The supplement and straw fed to NR cows after d 125 of gestation supplied 2.15 Mcal more NE\textsubscript{d} than the control diet so NR cows would achieve a BCS equal to C cows by d 220 of gestation.

Ten C and ten NR cows were assigned to necropsy at d 125 of gestation. The remaining cows (5 C and 5 NR) were necropsied on d 245 of gestation. On the day of necropsy all cows were stunned by captive bolt and exsanguinated. The gravid uterus was immediately removed. Fetal weight, crown rump length (CRL), and abdominal circumference were recorded. Fetal brain, heart, liver, and kidneys were collected and weights recorded. The remaining abdominal organs were removed and the eviscerated fetus was weighed to obtain a fetal empty carcass weight.

All placentomes from each cow were counted and the greatest and least diameters of each at the fetal-maternal interface were recorded and averaged to provide an average placental diameter. The average diameter was used to calculate a surface area for each placetome and from this, the sum of all placental surface areas within a uterus were calculated along with the average surface area per placetome. Placentomes were then removed from the uterus, and separated into caruncular and cotyledonal components by gentle traction and pooled by tissue type, and total caruncular, and total cotyledonal tissue weights determined. The total cotyledon weight was divided by the total caruncular weight to provide a ratio of fetal and maternal contributions to the placetome.

**Glomerulus Quantitation**

The number of glomeruli in the left kidney of each fetus on d 245 of gestation was determined using a modification of the acid maceration/mechanical dissociation method of Damadian et al. (1965) and routinely conducted on our laboratory (Gilbert et al., 2007). Glomeruli were counted in duplicate using a light microscope. Independent counts were performed by two experienced individuals, and the values averaged to obtain the final number of glomeruli.

**Statistical Analysis**

Upon evaluation of the raw fetal data from the necropsies conducted on d 125 of gestation, it was clear that there were two distinct groups of fetuses within the NR group. There was a group of 4 NR fetuses that were markedly smaller than the remaining 6 NR fetuses and C fetuses. Further, these small fetuses exhibited increased brain and heart weight as a percentage of fetal weight, as well as a decreased abdominal circumference when compared to the other 6 NR fetuses. This reduced fetal weight and asymmetric organ growth is indicative of IUGR as reported by others (McMillen et al., 2001). Cows in the NR group on d 125 were thus analyzed as two distinct groups (NR IUGR, n = 4; NR non IUGR, n = 6).

Maternal BW and BCS at 30 d of gestation, BW and BCS change during nutritional treatment, and biweekly BW and BCS measurements during realimentation, fetal weight at necropsy, age of cow, fetal organ weights, and placental characteristics were analyzed within d 125 or d 245 of gestation using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment in the model statement. Absolute glomerulus number per kidney and glomeruli per gram of tissue in the left kidney on d 245 of gestation was analyzed using the GLM procedure. Means were separated using the LSMEANS option of SAS. Effects were considered significant at \( P \leq 0.05 \) and tendencies at \( P \leq 0.10 \).

**Results**

Body weight and BCS of the 30 cows utilized in this study were similar at 30 d of gestation regardless of the nutritional groups (C and NR) to which they were assigned averaging 1292 ± 40 lb. and 5.5 ± 0.3 respectively. At necropsy on d 125, C cows had gained 53 ± 9 lb. BW \((P<0.001)\), while NR non IUGR and NR IUGR cows had lost 95 ± 15 and 104 ± 20 lb. BW, respectively \((P<0.0001)\). Further, C cows had gained 0.2 ± 0.1 BCS units, while NR non IUGR and NR IUGR cows had lost \( P<0.01 \) 0.3 ± 0.1 and 0.4 ± 0.1 BCS units, respectively; \( P<0.01 \) at necropsy on d 125. The remaining five C cows gain 90 ± 15 lb. and 0.1 ± 0.1 BCS units from d 125 to 245 of gestation. The five NR cows necropsied at d 245 of gestation lost 106 ± 18 lb. and 0.3 BCS units from d 30 to 125 of gestation, but during realimentation the NR cows reached a similar BW to C cows by d 150 of gestation; however it took until d 195 of gestation for BCS to become similar for C and NR cows. For the cows necropsied at 125 d of gestation, the NR IUGR cows were younger than the NR non IUGR cows \((3.5 ± 0.3 \text{ vs.} 5.0 ± 0.6 \text{ yrs of age, respectively; } P<0.05)\), with the C cows being intermediate in age \((4.3 ± 0.5 \text{ yrs})\).

Fetal size and weights, as well as fetal organ weights at d 125 of gestation are shown in Table 1. Fetal weight and fetal empty carcass weight were similar between C and NR non IUGR groups. In contrast, fetal weight and empty carcass weight were markedly reduced \((P < 0.01)\) in NR IUGR fetuses compared with fetuses from the other two groups. Additionally, abdominal circumference was reduced \((P <
0.01) in the NR IUGR compared with the C and NR non IUGR fetuses on d 125 of gestation. Brain and heart weight were increased ($P < 0.01$) in NR non IUGR fetuses compared with C fetuses, and were reduced ($P < 0.01$) in NR IUGR fetuses. Further, liver weights of NR IUGR fetuses were less ($P < 0.01$) then those of the C and NR non IUGR groups. The weights of the fetal kidneys were not influenced by maternal dietary intake. Fetal brain weight corrected for fetal weight was increased ($P < 0.01$) in the NR IUGR fetuses compared to fetuses from the other two groups. Fetal heart weight corrected for fetal weight also tended ($P = 0.08$) to be increased in NR IUGR fetuses compared to C fetuses. Fetal liver and total kidney weight as a percentage of fetal weight were not influenced by maternal dietary intake.

Fetal measurements and organ weights of female fetuses at d 245 of gestation are shown in Table 2. Fetal size and weights were similar between NR and C fetuses at d 245 of gestation. Fetal organ weights at d 245 of gestation were also not influenced by maternal dietary intake from d 30 to d 125 of gestation. All other fetal organ weights corrected for fetal weight were similar between C and NR fetuses at d 245 of gestation.

Despite similarities in kidney weight, absolute glomerular number and glomeruli per gram of tissue in the left kidney on d 245 of gestation were markedly reduced ($P < 0.05$) in NR versus C fetuses (1,968,533 ± 191,716 and 30,102 ± 2,770 versus 2,874,667 ± 286,888 and 44,484 ± 1,462 glomeruli, respectively; Figure 1).

Placental characteristics at d 125 and d 245 of gestation are shown in Table 3. Caruncular weight was similar for C and NR cows regardless of fetal IUGR status at d 125 and d 245 of gestation. Cotyledonal weight and cotyledonal weight divided by caruncular weight were reduced ($P < 0.01$) in NR IUGR fetuses compared to NR non IUGR and C fetuses at d 125 of gestation. At d 245 of gestation cotyledonal weight and cotyledonal weight divided by caruncular weight were still reduced ($P < 0.01$) in NR compared to C cows. Neither the number of placentomes nor average placenta surface area per placenta was influenced by maternal nutrient intake at d 125 of gestation. Total placenta surface area tended ($P = 0.07$) to be reduced in NR IUGR cows compared to C and NR non IUGR cows at d 125 of gestation. At d 245 of gestation the number of placentomes tended ($P = 0.07$) to be decreased, while the average surface area per placenta tended ($P = 0.09$) to be increased in NR cows compared to C cows. This resulted in a total placenta surface area that was similar for NR and C cows at d 245 of gestation.

**Discussion**

This is the first study in the cow that the authors are aware of demonstrating a significant decrease in fetal growth during mid gestation, in response to a global dietary restriction during early gestation. Interestingly, this fetal growth reduction occurred in some cows (NR IUGR group), but not in others (NR non IUGR group). Further, this reduction in fetal weight in the NR IUGR group was associated with asymmetric organ development characteristic of IUGR (McMillen et al., 2001; Vonnahme et al., 2003).

It is accepted that IUGR fetuses have a significantly increased risk of developing obesity, type II diabetes, hypertension and cardiovascular diseases in postnatal life (Poore and Fowden 2002; Gilbert et al., 2005). We have previously reported that ewes subjected to a 50% nutrient restriction from day 28 to 78 of gestation exhibited IUGR at midgestation (Vonnahme et al., 2003). Further, when allowed to lamb, ewes on this nutrient restriction protocol produced offspring who exhibited increased adiposity, altered growth rates, glucose dysregulation, hypertension and altered carcass quality when compared to offspring from ewes fed a control diet throughout gestation (Gilbert et al., 2005; Ford et al., 2007).

The reason for reduced fetal growth in only a portion of our nutrient restricted cows (NR IUGR group) may be a result of the less severe dietary restriction imposed on the cows on this study (68 % of the NE$_{m}$ and 87 % of the MP requirement) than in our previously published sheep studies (Vonnahme et al., 2003; Ford et al., 2007), or the younger age of the cows in the NR IUGR group than the NR non IUGR group. This could indicate that younger cows are more susceptible to nutrient restriction then older cows. Support for this hypothesis is the fact that young beef cows continue to grow from 3 and 4 yrs of age, before exhibiting a decreased growth rate from 4 to 7 years of age (Arango et al., 2002).

Early gestational nutrient restriction in the ewe followed by realimentation to a similar BW and BCS produced offspring with similar birth weights (Ford et al., 2007). Similarly, nutrient restriction during the first trimester of gestation in cattle, followed by normal management produced calves with similar birth weights (Long et al., 2007). This presents a problem for producers, as they have no way of confirming IUGR during gestation based on calf size at birth.

Similar to the fetal weights observed during late gestation, individual organ weights and measurements were similar between NR and C cows on d 245 of gestation. This, however, does not eliminate the possibility that organ development was compromised in fetuses gestated by NR-realimented cows. On both d 125 and d 245, kidneys of fetuses from NR and C cows were of similar size and weight, and the ratio of kidney weight to fetal weight was similar across the two dietary groups. In contrast, absolute glomerular number, and glomeruli per gram of tissue were markedly reduced in the left kidney of NR fetuses on d 245 when compared to age matched controls. Maternal nutrient restriction of ewes during the first half of gestation results in fetuses with reduced glomerulus number relative to kidney weight at 135 d of gestation in male fetuses (Gilbert et al., 2007) and offspring at 6 mo of age with fewer total nephrons compared with offspring of ewes fed adequate nutrition (Gopalakrishnan et al., 2005). Further, reduced nephron numbers of male offspring from ewes nutrient restricted 50% during the first half of gestation were correlated to increased blood pressure at 9 mo of age (Gilbert et al., 2005).

Impaired placental growth has been associated with IUGR in sheep and pigs (Mellor, 1983; Schoknecht et al., 1994). In this study we report decreased cotyledonal tissue...
weight at d 125, only in NR IUGR cows exhibiting fetal IUGR and at d 245 of gestation in NR vs. C cows. The decreased total placental surface area in the NR IUGR fetuses at d 125 of gestation compared to the NR non IUGR and C groups could account, in part, for the observed decrease in fetal growth due to a reduction of maternal to fetal transport of nutrients. In ewes, decreased cotyledonary weight and vascularization are associated with fetal IUGR (Reynolds et al., 2006).

These data demonstrate that a prolonged nutrient deprivation during early gestation in younger cows can result in fetal IUGR. However, after realimentation of these early gestational nutrient deprived cows experiencing IUGR to NRC recommendations in late gestation, fetal size and organ weights were found to be similar to that of C cows during late gestation. These data suggest accelerated fetal growth by IUGR fetuses during late gestational maternal nutrient supplementation, which would be expected to result in normal birth weights. Since most ruminants in the United States are exposed to periods of inadequate nutrition during gestation, especially early gestation (DelCurto et al., 2000), this could be altering the quality of offspring born. The changes in the structure and/or function of fetal organs and tissues resulting from a bout of early maternal nutrient restriction may have lasting effects on body composition, growth efficiency and health status of offspring in postnatal life.

References


### Table 1. Measurements and selected organ weights of female conceptuses on d 125 of gestation from control or nutrient restricted cows.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NR nonIUGR</th>
<th>NR IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal wt, g</td>
<td>947.6 ± 14.2(^a)</td>
<td>973.8 ± 20.1(^a)</td>
<td>772.6 ± 22.5(^b)</td>
</tr>
<tr>
<td>Fetal Carcass wt, g</td>
<td>730.4 ± 12.4(^a)</td>
<td>749.9 ± 16.0(^a)</td>
<td>604.1 ± 19.6(^b)</td>
</tr>
<tr>
<td>Crown Rump Length, cm</td>
<td>27.4 ± 1.2</td>
<td>27.4 ± 1.6</td>
<td>25.6 ± 1.9</td>
</tr>
<tr>
<td>Abdominal Circumference, cm</td>
<td>22.0 ± 0.4(^a)</td>
<td>22.3 ± 0.5(^a)</td>
<td>19.9 ± 0.6(^b)</td>
</tr>
<tr>
<td>Brain wt, g</td>
<td>19.5 ± 0.3(^b)</td>
<td>20.8 ± 0.4(^a)</td>
<td>17.9 ± 0.5(^c)</td>
</tr>
<tr>
<td>Brain wt, g/ fetal wt, g</td>
<td>0.0206 ± 0.0003(^a)</td>
<td>0.0211 ± 0.0005(^a)</td>
<td>0.0231 ± 0.0006(^b)</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>7.6 ± 0.2(^b)</td>
<td>8.4 ± 0.2(^a)</td>
<td>6.8 ± 0.3(^c)</td>
</tr>
<tr>
<td>Heart wt, g/ fetal wt, g</td>
<td>0.0080 ± 0.0002(^d)</td>
<td>0.0085 ± 0.0003(^d)</td>
<td>0.0088 ± 0.0003(^e)</td>
</tr>
<tr>
<td>Liver wt, g</td>
<td>35.3 ± 1.3(^a)</td>
<td>35.4 ± 1.7(^a)</td>
<td>24.2 ± 2.1(^b)</td>
</tr>
<tr>
<td>Liver wt, g/ fetal weight, g</td>
<td>0.037 ± 0.002</td>
<td>0.036 ± 0.002</td>
<td>0.032 ± 0.002</td>
</tr>
<tr>
<td>Total kidney wt, g</td>
<td>7.7 ± 0.4</td>
<td>7.8 ± 0.5</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>Total kidney wt, g/ fetal wt, g</td>
<td>0.0080 ± 0.0004</td>
<td>0.0078 ± 0.0005</td>
<td>0.0088 ± 0.0006</td>
</tr>
</tbody>
</table>

\(^1\) NR= nutrient restricted, IUGR= intrauterine growth restriction, female fetuses from cows fed 68% of NRC NEm recommendations and 87% of MP recommendations from d 30 to d 125 of gestation. Control cows received 100% of NRC recommendations.

\(^{a,b,c}\) means within a measurement differ \(P<0.01\)

\(^{d,e}\) means within a measurement differ \(P<0.10\)

### Table 2. Measurements and organ weights from female conceptuses on d 245 of gestation from control or nutrient restricted cows.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal wt, kg</td>
<td>26.1 ± 0.9</td>
<td>26.8 ± 0.9</td>
</tr>
<tr>
<td>Fetal Carcass wt, kg</td>
<td>20.0 ± 0.7</td>
<td>20.7 ± 0.7</td>
</tr>
<tr>
<td>Crown Rump Length, cm</td>
<td>78.8 ± 1.2</td>
<td>79.1 ± 1.2</td>
</tr>
<tr>
<td>Abdominal Circumference, cm</td>
<td>69.2 ± 1.9</td>
<td>68.3 ± 1.9</td>
</tr>
<tr>
<td>Brain wt, g</td>
<td>163.9 ± 5.6</td>
<td>152.5 ± 5.6</td>
</tr>
<tr>
<td>Brain wt, g/ fetal wt, g</td>
<td>6.3 ± 0.4</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>178.6 ± 16.3</td>
<td>184.2 ± 16.3</td>
</tr>
<tr>
<td>Heart wt, g/ fetal wt, g</td>
<td>6.7 ± 0.5</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>Liver wt, g</td>
<td>594.3 ± 43.8</td>
<td>599.0 ± 48.9</td>
</tr>
<tr>
<td>Liver wt, g/ fetal weight, g</td>
<td>22.6 ± 1.0</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td>Total kidney wt, g</td>
<td>128.3 ± 8.0</td>
<td>122.5 ± 8.0</td>
</tr>
<tr>
<td>Total kidney wt, g/ fetal wt, g</td>
<td>4.9 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
</tbody>
</table>

\(^1\) NR= nutrient restricted, female fetuses from cows fed 68% of NRC NEm recommendations and 87% of MP recommendations from d 30 to d 125 of gestation. After d 125 NR cows received in excess of NRC recommendations. Control cows received 100% of NRC recommendations.
Table 3. Placental characteristics on d 125 and d 245 of gestation from control or nutrient restricted cows.¹

<table>
<thead>
<tr>
<th></th>
<th>d 125 necropsies</th>
<th>d 245 necropsies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NR nonIUGR</td>
</tr>
<tr>
<td>Caruncular wt, g</td>
<td>395 ± 22</td>
<td>341 ± 28</td>
</tr>
<tr>
<td>Cotyledonary wt, g</td>
<td>337 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>309 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cot wt, g / Car wt, g</td>
<td>0.86 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td># of placentomes</td>
<td>86 ± 8.</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>average placentome surface area, mm</td>
<td>9.8 ± 0.7</td>
<td>10.0 ± 0.9</td>
</tr>
<tr>
<td>Total placentome surface area, mm</td>
<td>791 ± 29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>829 ± 37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

¹ NR= nutrient restricted, IUGR= intrauterine growth restriction, female fetuses from cows fed 68 % of NRC NEm requirements and 87 % of MP requirements from d 30 to 125 of gestation. After d 125 NR cows received in excess of NRC requirements. Control cows received 100 % of NRC requirements.

<sup>a,b</sup> means within a measurement differ P < 0.01

<sup>c,d</sup> means within a measurement differ P < 0.10
Reduced Angiogenic Factor Expression in Cotyledonary (COT) Arteries of Overnourished, Obese Ewes at Midgestation

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Summary and Implications

In the sheep, maternal:fetal exchange takes place through 75-120 button-like structures connecting the vascularized chorionic villi with the uterine wall. These structures called placentomes, are comprised of maternal caruncular (CAR) and fetal cotyledonary (COT) components. We have recently established an obese ewe model to study the mechanism(s) whereby maternal obesity at conception and throughout pregnancy effects fetal growth and development. We recently reported a reduced COT vascularity (~50%) in obese ewes by day 75 of gestation, which was consistent with reduced angiogenesis in that tissue and was the focus of this study. Multiparous ewes carrying twin fetuses were assigned to a control (C, 100% of NRC recommendations; n=10) or obesogenic (OB, 150% of NRC; n=10) diet from 60 days before conception to day 75 of gestation, at which time COT arteries were collected from 5 ewes/group and snap frozen on liquid nitrogen. The remaining ewes in each group were maintained on their respective diets and allowed to lamb. For those animals necropsied at day 75 of gestation, OB ewes and their fetuses were ~50% and ~30% heavier (P<0.05), respectively, than C ewes and fetuses. Interestingly, for ewes allowed to lamb, lambs from both dietary groups exhibited similar birth weights. Vascular endothelial growth factor (VEGF), angiopoietin (ANG) 1, ANG-2, fibroblast growth factor (FGF)-2 and placental growth factor (PLGF) mRNA levels were reduced (P<0.05) 2 fold or more in COT arteries of OB versus C ewes, while no treatment differences were observed in VEGF receptors FLT-1 and KDR, or ANG receptor Tie-2 expression. Hypoxia inducible factor (HIF)-1α showed a trend towards a difference (P=0.09) between the two groups with a 1.92 fold reduction in OB vs. C ewe COT arteries. COT arterial protein expression of VEGF, PLGF and FGF-2 were then quantified by Western-blot, and were reduced (P<0.05) 30%~50% in OB ewes when compared to C ewes. This data are consistent with the concept that decrease COT artery angiogenic factor expression in OB ewes at midgestation results in the observed decrease in COT vascularity and thus nutrient delivery to the fetus, slowing fetal growth, and reducing lamb birth weight.

Introduction

A progressive increase in human obesity has become a common threat all over the world. In the United States, ~61.9% of adult women > 20 years old are classified as overweight with a body mass index (BMI, body weight correlated by height) of 25.0-29.9, including 33.3% who are clinically obese (BMI > 30.0) (U.S. Department of Health and Human Services, 2007). Of specific interest here, the prevalence of obesity in women of child bearing age has reached 28.6% in the current U.S. population (U.S. Department of Health and Human Services, 2007). Clinical studies reveal that maternal obesity exposes mothers to a high risk of preeclampsia, and cesarean delivery (Morin and Reilly, 2007), and exposes their offspring to a high risk of metabolic disorders such as type II diabetes, obesity and hypertension, cardiovascular diseases and even neuronal disorders in later life (Buckley et al., 2005; Godfrey et al., 1998; Wu et al., 2006; Ray et al., 1997). However the mechanisms, whereby maternal obesity effect placental vascular development, leading to changes in fetal growth and development, are still unclear.

In the sheep both CAR and COT tissues are richly endowed with blood vessels that interdigitate at the fetal:maternal interface to facilitate nutrient and gas exchange. Stegeman (1974) reported that capillary area density (capillary area as a proportion of total tissue area) in fetal COT tissues increases 9.4 fold from day 50 to day 135 of gestation (sheep gestation length: ~148 days), while in maternal CAR tissues a 2.8 fold increase was observed. Blood flow is a function of blood velocity and
capillary area, and thus capillary area density is positively related to nutrient supply to the fetus (Smith and Kampine, 1990). Following implantation, placental and uterine angiogenesis accelerate the development of COT and CAR capillary beds that are crucial in the movement of maternal oxygen and nutrients into the fetal compartment (Aron and Anthony, 2003). Angiogenic VEGF and its receptors FLT-1 and KDR, ANG-1 and ANG-2, and their receptor Tie-2, FGF-2, PLGF are known to play the central role in increasing placental vasculogenesis and angiogenesis (Aron and Anthony, 2003). More specifically, Borowicz et al. (2007) reported that in normal sheep pregnancy both COT and CAR tissues exhibited an increased mRNA expression of VEGF, FLT-1, ANG-2, FGF-2 and HIF-1, indicating the significance of the angiogenic factors and receptors in increasing placental vascularity. Nitric oxide (NO) is also reported to mediate VEGF and/or FGF-2 initiated angiogenesis (Zheng et al., 2006), and therefore, endothelial nitric oxide synthase (eNOS) is down regulated by VEGF and FGF-2.

Materials and Methods

Animal care and tissue collection

This study was conducted at the University of Wyoming. All procedures were approved by the University of Wyoming Animal Care and Use Committee. Multiparous ewes carrying twin fetuses were assigned to a control (C, 100% of NRC recommendations; n=10) or obeseogenic (OB, 150% of NRC; n=10) diet from 60 d before conception to 75 dGA, at which time COT arteries were collected from 5 ewes/group and snap frozen on liquid nitrogen. The remaining ewes in each group were maintained on their respective diets and allowed to lamb.

At day 75 of gestation, each ewe was weighed immediately before necropsy. Ewes were sedated with Ketamine and maintained under isoflurane inhalation general anesthesia (2.5%). Fetal body weight was measured and recorded. From each placenta, two placentomes were selected at random for immunohistochemical localization of VEGF, PLGF and FGF2. Only the smallest arteries that entered the COT tissues (0.5–1.0mm in diameter) were collected and snap frozen in liquid nitrogen, and stored at -80°C until the time of experiment. Numbers, weights, types of all placentomes, total caruncular, and total cotyledonary tissue weights from each placenta were determined. All placentomes were classified as type A as previously described (Vonnahme KA et al., 2006).

Total RNA extraction and single-strand DNA synthesis

COT arteries were pulverized in liquid nitrogen. 0.05-0.1g power of each COT artery sample was used for total RNA extraction using classic Trizol reagent method. Total RNA then purified through a QIAGEN RNeasy mini kit (QIAGEN, Inc., Valencia, CA) according to the respective protocols. Purified RNA was determined qualification by another RNA denaturing electrophoresis, and UV absorption at 260/280nm as well as total RNA concentration measured by a Nano-drop-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE). Total RNA from each tissue sample was adjusted to the same concentration for the single-strand DNA synthesis to reduce inaccuracy. Single-strand DNA was synthesized using a Promega Improm-II™ Reverse Transcription System (Promega BioSciences, San Luis Obispo, CA) according to the kit protocol. Single-strand DNA UV absorption at 260/280nm and concentration was measured.

Real-time PCR

Genes that were selected in Real-time PCR experiment are listed in Table1. All Real-time PCR reactions were conducted through a Bio-Rad IQ5 Realtime-PCR Reaction System (Bio-Rad Laboratories, Inc., Hercules, CA). Reactions for each gene were run in duplicate. 18s RNA were used as a loading control at each run.

Primers

VEGF and PLGF primers were designed through the online software Primer 3 and confirmed conservative by NCBI blast. FLT-1, KDR, ANG-1, ANG-2, Tie-2, NOS3 primers are designed according to Redmer et al. (2005) and the primers quality were confirmed using both Primer 3 and NCBI blast tool. HIF-1α primers and 18s RNA primers were designed according to Johnson et al. (2006) and Lo’pez-André et al. (2005), respectively, and confirmed quality using both Primer 3 and NCBI blast.

Total Protein Extraction and Western-blot

0.08-0.1g of each pulverized COT artery sample was homogenized in a polytron homogenizer, with ice-cold lysis buffer. Then the homogenates were sonicated and clarified by centrifugation. After centrifugation, the supernatant was mixed with SDS sample loading buffer and heated at 95°C for 5min. A standard SDS-PAGE was run to separate proteins following transferring separated proteins to nitrocellulose membrane and blocking with first and second antibodies dissolved in 5% and 2% skim milk, respectively. Membrane was then visualized using ECL™ Western blotting detection reagents and exposed to film. The film was then scanned into a computer and the specific binding bands density was quantified by ImageQuant TL software (Amersham Bioscience, Piscataway, NJ). Specific binding bands density was normalized according to the density of a reference sample as well as β-Actin content in the same samples.

Antibodies

First anti-VEGF antibody (A-20, Mouse IgG, sc-152) was purchased from Santa Cruz Biotecnology, Inc. (Santa Cruz, CA). First anti-PLGF antibody (Rabbit IgG, ab9542) was from Abcam, Inc. (Cambridge, MA). First anti-FGF-2 antibody (Rabbit IgG, AB-33-NA) was from R&D Systems (Minneapolis, MN). First anti- β-Actin antibody (Mouse IgG, A-1978) was from Sigma-Aldrich.
Inc. (St. Louis, MO). Second anti-Mouse IgG (#7076) and second anti-Rabbit IgG (#7074) were both from Cell Signaling Technology, Inc. (Danvers, MA)

Statistical analysis

Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System) (SAS 9.1, 2002-2003, SAS Institute Inc., Cary, NC). Means separation was performed using LSMEANS. Means ± SEM was considered significantly different when P<0.05, and P values between 0.05 and 0.1 were considered trends.

Results and Discussion

Ewes on the OB diet increased their body weight by ~30% from diet initiation to mating (71.56 ± 3.23 and 92.84 ± 2.97kg, respectively; P<0.05) and increased an additional ~13% in body weight from mating to necropsy on day 75 of gestation (102.22 ± 2.41kg). In contrast, C ewes exhibited a modest nonsignificant increase in body weight from diet initiation to necropsy (68.32 ± 2.91 and 72.18 ± 3.27kg, respectively; P=0.10). Fetuses from OB ewes were ~30% heavier than fetuses gestated by C ewes averaging 245 ± 7 vs. 188 ± 8g, respectively. Total placental weight, total placental number, total weight of CAR and COT tissues did not exhibit any significant differences across the two dietary groups (Table 1). However, ewes on the OB diet exhibited a 68.26% reduction (P<0.05) of vascular area density in COT tissues comparing to that of C ewes (0.44±0.0008 vs.1.39% ± 0.0035%, respectively). COT blood vessel diameter was not different between OB ewes and C ewes (Table 1).

Vascular endothelial growth factor, PLGF, FGF-2, ANG-1 and ANG-2 mRNA levels in OB ewe COT arteries decreased 2.8 fold, 2.3 fold, 2.2 fold, 4.3 fold and 4.2 fold, respectively compared to mRNA levels expressed in C ewe COT arteries (P<0.05, Figure 2). HIF-1α mRNA expression exhibited a reduction of 1.92 fold in OB ewe COT arteries versus that of C ewes, but did not reach significance (P=0.09). No significant differences were detected in eNOS, FLT-1, KDR and Tie-2 mRNA expression in COT arteries between the two dietary groups.

Those angiogenic factors that exhibited a significant diet difference in COT artery mRNA expression level, and for which a commercially available antibody, which cross-reacted with ovine could be found, were chosen for protein quantification via Western-blot. Only VEGF, PLGF and FGF-2 protein levels were quantified in COT arteries. Vascular endothelial growth factor, PLGF and FGF-2 protein expression in COT arteries of OB ewes were 41%, 32% and 50% lower (P<0.05) than those in COT arteries of C ewes (Figure 3). The observed band of VEGF was a dimer that was at ~42kDa. The observed bands for PLGF were a PLGF-1 dimer (56kDa, less expressed) and a PLGF-2 dimer (65kDa, dominant form). The decreased protein levels of VEGF, PLGF and FGF-2 detected via Western-blot in OB vs. C COT arteries was consistent with their altered mRNA expression seen from Realtime-PCR.

We also looked into endothelial localization of VEGF, PLGF and FGF-2 in cotyledonary blood vessels. However, ewes on the OB diet exhibited a reduction of 1.92 fold on their fractional COT vascularity at mid-gestation, and fetal growth throughout the second half of gestation using an established OB ewe model. In OB ewes, reduced COT vascularity was associated with the decreased expression of selected angiogenic factors, but not their receptors or eNOS. These data are also consistent with those of Redmer et al. (2005) in an overnourished adolescent ewe model. These researchers reported a reduction of VEGF, ANG-1 and ANG-2, KLT-1 but not receptor KDR or Tie-2 at mRNA levels, and decreased placental proliferation of adolescent ewes receiving a high dietary intake compared to a moderate diet intake group at day 81 of gestation. This compensatory mechanism may reduce the nutrient and oxygen supply to the rapid developing fetuses and lead to a decreased fetal growth rate during the second half of gestation, which correlates with the observation that the fetuses from OB ewes developed macrosomia compared to C ewe fetuses at mid-gestation, but that lambs from both dietary group weighed the same at the time of parturition. Hence, further studies are needed to measure the expression pattern of selected angiogenic factors and their receptors in the second half of gestation probably at different time points, and determine their effects on fetal growth. However, the mechanism of how the excessive nutrient supply triggers the decrease of angiogenic factor expression, and vascularity at midgestation is still not clear.

References


Stegeman JHJ. Placental development in the sheep and its regulation to fetal development. Bijdragen Tot De Dierkunde (Contrib Zool) 1974; 44:3-72.

Table 1. Measurements taken at day 75 of gestation and parturition from C and OB ewes.

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Lamb Wt (kg)</th>
<th>Total Plac No.</th>
<th>COT BV Diameter (u)</th>
<th>Total CAR Wt (g)</th>
<th>Total COT Wt (g)</th>
<th>COT VAD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.32±0.47</td>
<td>44.3±2.7</td>
<td>36.64±2.72</td>
<td>115.09±9.76</td>
<td>541.85±30.90</td>
<td>1.39±0.35^a</td>
</tr>
<tr>
<td>Obese</td>
<td>6.00±0.30</td>
<td>46.7±2.6</td>
<td>43.54±6.55</td>
<td>108.93±7.45</td>
<td>507.63±33.81</td>
<td>0.44±0.08^b</td>
</tr>
</tbody>
</table>

Plac = placentome; BV = blood vessel; Wt = weight; VAD = vascular area density; BV = blood vessel. COT tissues were all collected from type A placentomes. Please refer to Vonnahme et al. (2006) for more information on placental differentiation (24). Ewes utilized for the measurements were 5/dietary group.

^a Means±SEM with different superscripts differ, P<0.05.

Figure 1. Realtime-PCR quantification of (A) selected angiogenic factors and (B) receptors of selected angiogenic factors and eNOS mRNA levels in COT arterial tissue from both OB (n=8) and C (n=8) ewes at d75 of gestation. **Means ± SEM differ (P<0.05). * Trend towards difference (P=0.09).
Figure 2. Western-blot measurement of selected protein expression in COT arterial tissue of both OB (n=10) and C (n=10) ewes at day 75 of gestation. (A) Representative Western-blot to VEGF, PLGF and FGF-2; (B) Statistical analysis on Western-blot result of the three proteins in (A) **Means ± SEM differ (P<0.05).
EFFECT OF RU486 ON DEVELOPMENT OF TESTICULAR STEROIDOGENESIS AND RAM SEXUAL BEHAVIOR

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Valerie A. Uthlaut, Graduate Student
Brenda M. Alexander, Assistant Professor

Summary and Implications

Progesterone influences the development and expression of male sexual behavior in rodents and may be important for the expression of sexual behavior in rams. Masculinization and/or defeminization of the central nervous system in sheep occur between d 60 and 70 of pregnancy. A second phase of testosterone-responsive sexual development occurs at 6 to 8 weeks of age in ram lambs. To determine if progesterone influences adult sexual behavior during this developmental period, twin born male lambs (n = 10) were used in this study. One of each twin was treated with 10 mg of the progesterone receptor antagonist mifepristone (RU486; n = 5) and his co-sibling was treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. Sexual behavior and serum concentrations of testosterone were evaluated at 9 mo of age. Lamb BW were similar at the end of RU486 treatment (8 wk of age), and did not differ during behavior testing at 9 mo of age. Change of weight, however, tended to be greater in RU486 treated ram lambs. Testes were measured by a scrotal tape at the end of the treatment period and during behavior testing at 9 mo of age. Although testicular circumferences and BW did not differ, serum concentrations of testosterone were less at 9 mo of age in rams treated with RU486. Sexual behavior was evaluated for 30 min at three different times by placing the rams with two estrous ewes. Behavior was classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick, and nudge) or consummatory (mount attempt, mount, and ejaculation) behavior. Expression of investigatory behavior was decreased at the first exposure to estrous ewes in RU486 treated rams, but not in subsequent tests. Consummatory behavior was similar among treatment groups at all observations. Blocking the progesterone receptor at 6 – 8 weeks of age influences steroid production in the yearling ram, but a robust influence of progesterone on the expression of sexual behavior remains to be determined.

Introduction

Typical breeding practices for food-animal species utilize limited numbers of males to inseminate large numbers of females. Therefore, it is critical that libido (sexual interest or motivation), mating competence (ability to inseminate females) and fertility (semen quality) of males is adequate to insure reproductive success. Libido in rams is highly variable and is influenced by developmental (Roselli et al., 2003) and environmental (Price, 1987) factors.

Progesterone is named for its progestational role in maintaining pregnancy in mammals, and is traditionally regarded as a “female hormone.” The facilitory and inhibitory effects progesterone exerts on female reproductive behavior are well documented. Progesterone is a precursor for both androgen and estrogen synthesis. In the male, androgens are necessary for the development of secondary sex characteristics and testosterone is considered the primary male sex hormone. However the role of testosterone in the expression of male-typical behavior has been overstated since there is little correlation between plasma testosterone concentrations and male behavior (reviewed in: Andersen and Tufik, 2006). Testosterone is aromatized to estradiol 17β in specific hypothalamic nuclei and is considered the centrally active hormone in the male (reviewed in: Resko et al., 1999). Progesterone receptors are also present in behaviorally relevant nuclei of the male brain, and progesterone receptor knock-out male mice exhibit sexual-behavior deficits (Phelps et al., 1998).

The physiological significance of progesterone in the male, outside of its role as a precursor for androgen production, is not well understood. The progesterone receptor is upregulated in the hypothalamus of male fetuses during brain sexual differentiation (Roselli, et. al., 2006) and may play a role in the development of central pathways necessary for the expression of adult sexual behavior in rams. A second phase of testosterone-responsive sexual development occurs in male sheep during 6 to 8 weeks of age (Orgeur and Signoret, 1984). Progesterone acting through its receptor may affect the expression of sexual behavior either directly by altering neural development or indirectly through changes in testosterone production of the testes. The objective of the current study was to evaluate
the role of progesterone, acting at its receptor, in post-natal development of ram sexual behavior.

Materials and Methods

Animal care and use was approved by the University of Wyoming internal animal care and use committee. Twin born male lambs (n = 10) 4 wk of age were used for this study. One sibling of each pair was treated with 10 mg of the progesterone receptor antagonist mifepristone (RU486; n = 5) with his co-sibling treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. At the end of the treatment period lambs were weaned and fed a forage-based diet which supported moderate growth for 7 mo. Body weights and scrotal circumference were collected weekly during the treatment period and at 9 mo of age.

Rams at 9 mo of age were individually exposed to ewes in estrus on three occasions separated by approximately 14 days. Rams were confined in a pen (2.4 m x 4.7 m) with two ewes in estrus. Behavior was monitored by digital camera for 30 minutes during each exposure period. Behavior was quantified by manually viewing the digital recording. Behaviors were classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick and nudge) or consummatory (mount attempt, mount and ejaculation).

A single blood sample was collected by jugular venipuncture at 9 mo of age for analysis of serum concentrations of testosterone. Blood samples were allowed to clot overnight at 4°C. Serum was separated by centrifugation at 1500 g for 20 min, and stored at -20°C. Concentrations of serum testosterone were determined in a single radioimmunoassay.

Behavior was summarized as investigatory or consummatory and analyzed using GLM methods of SAS (Ver. 9.1, Cary, NC). Effects of treatment were tested as the main effect for behavior with time and treatment by time interactions tested as subplot effects. Animal within treatment was used as the error term for treatment effects. Treatment effects of BW, scrotal circumference and serum concentrations of testosterone were analyzed by analysis of variance using GLM procedures of SAS (Ver. 9.1, SAS Inst. Inc., Cary, NC).

Results

Lamb weights and testicular circumference did not differ when treatments were initiated at 4 wk of age, nor did they differ (P > 0.4) at 8 wk of age following RU486 treatment (21.3 ± 1.7 kg; 14.6 ± 0.8 cm). Rams weighed 66.4 ± 3.2 kg at 9 mo of age with an average testicular circumference of 33.2 ± 0.4 cm. Neither weight nor testicular circumference varied (P > 0.15) among treatment groups. Although BW was not different at the time of RU486 treatment or at 9 mo of age, change in weight tended to be greater (P = 0.08) in rams treated with RU486 as lambs (Fig. 1).

Although BW and testicular circumference did not differ among treatment groups, serum concentrations of testosterone were greater (P = 0.06) in control rams than rams treated with RU486 as lambs (Fig. 2).

Expression of investigatory behavior was decreased (P = 0.03) in RU486 treated rams compared to control rams at the first exposure to estrous ewes, but not (P ≥ 0.4) in subsequent tests (Table 1). Consummatory behavior was similar (P ≥ 0.2) among treatment groups at all observations (Table 1).

Discussion

Differences in mating behavior exist among individuals of all species studied. Mating performance of rams is important for the profitability of the sheep industry. Stallflug et al. (2006) indicated twice as many poor-performing rams were needed to obtain breeding results equal to a single high-sexually performing ram. With nearly 30% of rams classified as non-performers (Fitzgerald and Perkins, 1991), the importance of ram sexual behavior is well recognized.

Testicular androgen production increases in male sheep fetuses between d 35 and 70 of pregnancy. Masculinization of external genitalia occurs early in that developmental period with masculinization and/or defeminization of the central nervous system occurring later in the period of sexual differentiation. Expression of progesterone receptor mRNA, but not androgen or estrogen receptor, was greater in the hypothalamii of male than female sheep fetuses at 64 d of gestation (Roselli et al., 2006) suggesting that expression of the progesterone receptor may be important in sexual differentiation of the male brain.

Figure 1. Change in BW (kg) from 8 wk to 9 mo of age in control and ram lambs treated with RU486 from 4 to 8 wk of age *(P = 0.08)
A second phase of testosterone-responsive sexual differentiation occurs in rams between 6 and 8 wk of age. This period coincides with the expression of intense sexual play by the male lamb (Orgeur and Signoret, 1984). Although progesterone receptors have not been quantified in the hypothalamus of lambs at this developmental period, progesterone acting through its receptor may affect the expression of play behavior and alter expression of adult sexual behavior. Progesterone receptor knock-out mice show greater androgen-receptor immunoreactivity in the medial preoptic area than WT males (Schneider et al., 2005). Therefore, altering activity of the progesterone receptor may affect biological change in the male through altered expression of the androgen receptor. In conclusion blocking the progesterone receptor at 6–8 wk of age enhanced growth, and decreased testosterone production, but a robust influence on the expression of sexual behavior remains to be determined.

Table 1. Expressed sexual behavior expressed during 30 min exposure to estrous ewes.

<table>
<thead>
<tr>
<th>TRT</th>
<th>Test</th>
<th>Invest*</th>
<th>SE</th>
<th>Consum*</th>
<th>SE</th>
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<td>24.0</td>
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<tr>
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<td>16.6</td>
<td>4.9</td>
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<td>11.4</td>
<td>4.9</td>
<td>7.4</td>
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<tr>
<td>RU486</td>
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<td>10.7</td>
<td>4.9</td>
<td>3.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Figure 2. Serum concentration of testosterone (ng/mL) in control and RU486 treated ram lambs *(P = 0.06).*


Reproductive Physiology

Increased Macrophage Migration Inhibitory Factor (MIF) in the Pancreas of Fetuses Gestated by Overnourished, Obese Ewes at Midgestation

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Summary and Implications

Altered fetal development as a result of maternal obesity has been shown to impact postnatal health. Since maternal obesity is progressively increasing in the United States, an increased knowledge of the impacts of maternal obesity on fetal growth and development is urgently needed, especially since maternal obesity has been linked to type 2 diabetes, a disease which is increasing in children and young adults. Macrophage Migration Inhibitory Factor (MIF) was recently identified as a glucose-dependent pancreatic β-cell product, which increases insulin secretion in an autocrine fashion, and thus may play an important role in facilitating increased fetal insulin secretion in response to circulating glucose. Altered pancreatic levels of MIF would alter fetal pancreatic function and thus might be expected to impact offspring health and body composition after birth. The pregnant sheep is a preferred biomedical model for human pregnancy. Ewes were assigned to a control (C, 100% of National Research Council (NRC) recommendations, n=5) or obesogenic (OB, 150% of NRC, n=6) diet from 60 days before conception to 75 days of gestation when animals were euthanized and the gravid uterus recovered. OB ewes increased (P<0.05) their body weight by ~50% while C ewes increased their body weight only ~7% from diet initiation until necropsy. Fetuses from OB ewes were heavier than those from C ewes (374 ± 10 vs. 268 ± 12 g, P<0.05). Although all organs were heavier (P<0.05) in fetuses from OB vs. C ewes, only pancreatic weight was increased relative to fetal body weight (0.05 ± 0.01 vs. 0.12 ± 0.01 %, P<0.05). Concentrations of both glucose and insulin were elevated (P<0.05) in the blood of OB vs. C ewes (65.5 ± 6.6 vs. 52.1 ± 3.5 mg/dL and 25.0 ± 9.0 vs. 4.8 ± 1.6 uIU/ml, respectively) and their fetuses (40.7 ± 4.2 vs. 26.2 ± 2.3 mg/dL and 5.8 ± 0.8 vs. 1.6 ± 0.1 uIU/ml, respectively) on day 75. Plasma cortisol was also greater (P<0.05) in OB ewes and fetuses when compared to C ewes and their fetuses. Fetal pancreatic tissue sections were incubated with guinea pig anti-porcine insulin or rabbit anti-MIF antibodies at 4°C overnight, then with fluorescent labeled 2° antibodies: Rhodamine labeled goat anti-guinea pig or AlexaFluor 488 labeled goat anti-rabbit for 60 min at 22° C. By evaluating immunostaining of both insulin positive cells and MIF positive cells, we determined that MIF is largely expressed within the cytoplasm of insulin positive beta cells. The protein level of MIF in pancreatic tissue was quantified via western blot using a specific polyclonal antibody to MIF. The pancreatic MIF protein level was increased (P<0.05) in fetuses of OB ewes compared with fetuses from C ewes. The increased pancreatic MIF expression in fetuses from OB ewes would be expected to facilitate insulin release and thus glucose uptake by fetal body tissues.

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Introduction

The National Health and Nutrition Examination Survey (NHANES 1999 - 2000) reported the overweight people make up about 65% of the U.S. population compared with 56% in the older NHANES study conducted from 1988-1994; further. Similarly, individuals considered to be obese (BMI ≥ 30 kg/m2) constituted about 31% of subjects in the 1999-2000 study, compared with 23% in the older NHANES study(1988-1994). (www.cdc.gov/nchs/products/pubs/pubd/hestats/obese/obse99.htm).

The World Health Organization (WHO) has declared that obesity is one of the top ten adverse health risks in the world (www.who.int/nut/obs.htm). Overweight and obesity are associated with poor health and metabolic syndromes such as type 2 diabetes and cardiovascular disease. Pancreatic β-cells are the major cell responsible for insulin secretion. Insulin secretion by β-cells is carefully regulated by autocrine, paracrine and endocrine factors in a very precise manner. Proper β-cells function is
crucial to ensure that under normal conditions, plasma glucose concentration remain within a relatively narrow physiological range. Both obesity and type-2 diabetes are associated with increased insulin resistance. Under normal conditions, the pancreatic islet β-cells increase insulin release in response to elevated blood glucose after a meal, which results in the placement of that glucose into body tissues for growth, storage and metabolism. A reduced ability of insulin to remove glucose from the blood stream in a timely manner is referred to as insulin resistance. For obesity linked type-2 diabetes, β-cells are unable to compensate fully for the decreased insulin sensitivity of body tissues, and responds by increasing insulin secretion. Macrophage Migration Inhibitory Factor (MIF) was recently identified as a glucose-dependent pancreatic β-cell product, which increases insulin secretion in an autocrine fashion, and thus may play an important role in facilitating increased fetal insulin secretion in response to insulin resistance and therefore affecting the postnatal health of the fetuses. Thus, MIF level in prenatal life become a very important issue associated with potential diabetes and other diseases in terms of offspring health. However, no studies have been conducted in this area so far. The objective of this study was to evaluate the impact of maternal obesity on fetal development and MIF involved pancreatic function at midgestation.

Materials and Methods

Animals. From 60 days before conception (First day of mating = day 0) to day 75 of gestation or term, multiparous ewes were fed either a control (C; 100% of National Research Council (NRC) recommendations, n=10) or an obesogenic (OB, 150% of NRC recommendations, n=11) diet on a metabolic BW basis (BW^0.75). One-half of the ewes were necropsied on day 75 and the remaining animals were continued on their diets and allowed to lamb. Immediately prior to necropsy, on day 75, each ewe was weighed and a sample of blood was collected via jugular venipuncture and plasma and serum frozen at 22°C until assayed for glucose and insulin, respectively. Under anesthesia, fetal blood was obtained from paraumbilical sites were blocke

Glucose was analyzed using the InfinityTM (ThermoTrace Ltd, Cat. # TR15498; Melbourne, Australia) colorimetric assay modified in the following manner; plasma was diluted 1:5 in dH2O, and 10µL of diluted plasma was added to 300µL reagent mix. All samples were run in triplicate. The intra-assay and inter-assay CVs are 3% and 5%, respectively. Insulin and cortisol were measured by RIA in accordance with manufacturer recommendations. When using ovine serum, the intra-assay CV is < 3 %, while the inter-assay CV is < 5%.

Immunohistochemistry. Five 5µm sections were obtained from paraffin-embedded blocks of fetal pancreatic tissue maintaining at least 50 µm between sections. Paraffin embedded sections were then deparaffinized and hydrated by routine methods before the antigen retrieval procedure. Nonspecific antigenic sites were blocked by a 5-min incubation in 1.5% normal goat serum (Vector Laboratories, Burlingame, CA) in PBS with 0.1% Triton X (Union Carbide Corp, Somerset, NJ) and 0.05% Tween 20 (Bio-Rad Laboratories Inc., Hercules, CA) before the sections were incubated with guinea pig anti-porcine insulin (Dako, Carpinteria CA, 1:500) and rabbit anti-MIF (generously provided by Dr. Ji Li in University of Wyoming, 1:200) antibodies at 4°C overnight, then with fluorescent labeled 2° antibodies Rhodamine labeled goat anti-guinea pig (Millipore, Billerica, MA 1:500) and AlexaFluor 488 labeled goat anti-rabbit (Invitrogen, Carlsbad, CA, 1:500) for 60 min at 22°C. Images were visualized using an Olympus BX50 microscope and captured digitally using a Retiga EXiFast camera. Pictures at 400 X magnification were taken using QED Imaging software (Media Cybernetics, Silver Spring, MD) for several fields of view for each section.

Dot blotting assay. Triplicate 2 µl samples from each extracted SDS-PAGE protein sample were spotted onto a nitrocellulose membrane (0.45µm; Bio-Rad Laboratories Ltd) and air dried under a hood. Then membranes were blocked with 5% nonfat milk powder in TBST (50 mM Tris-HCl, pH7.6, 150 mM NaCl, 0.05% Tween-20,) for 2 h. Blocked membranes were then incubated overnight at 4°C with guinea pig anti-porcine insulin primary antibody (Dako, Carpinteria CA, 1:1000, Cat# A0564). At the end of the primary antibody incubation, the membranes were washed with TBST three times, for 10 min each. After that, membranes were incubated with horseradish peroxidase-conjugated anti-guinea pig secondary antibody (Chemicon, Temecula, CA, 1:5000, Cat # AP193P) for 1 h at RT. After three 15 min washes, membranes were visualized using ECLTM Western blotting detection reagents (Amersham Bioscience) and exposure to film (MR, Kodak, Rochester, NY). The density of each dot was quantified using an Imager Scanner II (Amersham Bioscience) and ImageQuant TL software (Amersham Bioscience).
ice-cold buffer containing 137 mM NaCl, 50 mM Tris-HCl, 2% SDS, 1% Triton -100 solution, 10% glycerol, 2.5 mM EDTA, 1 mM CaCl2, 1 mM MgCl2, 2 mM Na3VO4, 100 mM NaF, pH 7.4. Each pancreatic homogenate was mixed with an equal volume of 2x standard SDS sample loading buffer. A Hoefer mini-gel system was used for casting gels and running electrophoresis. Gradient gels of 5-20% were used for SDS-PAGE separation of proteins. After electrophoresis, the proteins on the gels were transferred to nitrocellulose membranes in a transfer buffer containing 20 mM Tris-base, 192 mM glycine, 0.1% SDS and 20% ethanol. Membranes were incubated in a blocking buffer consisting of 5% non-fat dry milk in TBST (0.1% Tween-20, 50 mM Tris-HCl, pH 7.6, and 150 mM NaCl) for 1 h. Membranes were incubated overnight in primary antibodies with 1:500 dilution for rabbit anti-MIF (generously provided by Dr. Ji Li in University of Wyoming) and 1:1000 dilution for mouse anti-β-tubulin antibody (Sigma-Aldrich, St. Louis, MO) in TBST with 2% non-fat dry milk. After the primary antibody incubation, membranes were washed three times for 10 min each with 15 ml of TBST. Membranes were then incubated with horseradish-peroxidase-conjugated anti-rabbit and anti-mouse secondary antibody (cell signaling, Danvers, MA) at 1:1000 dilution for 1 h in TBST with gentle agitation. After three 15 min washes, membranes were visualized using ECL Western blotting reagents (Amersham Biosciences) and exposure to film (MR; Kodak, Rochester, NY). Density of bands was quantified by using an Imager Scanner II and ImageQuant TL software.

**Determination of Effects of the Model on Mid-gestational Glucose Tolerance.** Jugular venous blood samples (3 mL) were collected from lambs born to C and OB ewes at two month postnatal age. These samples were placed in heparin and sodium fluoride coated tubes (2.5 mg/mL; Sigma) to establish baseline values of glucose and insulin at 15 and 5 min before iv administration of glucose (0.25 g/kg body weight) via an indwelling catheter. Blood samples were collected at 2, 5, 10, 15, 30, 60, and 120 min after the injection. Catheters were carefully flushed with saline following the glucose infusion and each blood sampling. Blood samples were placed on ice until centrifuged at 3000 x g for 10 min and plasma stored at -80°C until subsequent analysis.

**Statistics.** Data were analyzed as a completely randomized design using GLM (General Linear Model of Statistical Analysis System) (Institute, 2000). Mean separation was performed using LSMEANS. Means ± SEM were considered different when P<0.05 unless otherwise stated. Data are presented as means ± SEM and are considered different when P<0.05). In the day 75 necropsy study, n = 5 for C animals and n = 5 for OB animals unless otherwise stated. For the 2 month old lamb study, n = 7 for both groups.

**Results**

Fetal body weight and Crown Rump Length from overnourished, obese ewes were greater (P<0.05) than C ewes on day 75 (NOT PRESENTED). Most fetal tissue weights from OB ewes were greater (P<0.05) than those from C ewes, but only pancreatic weight was increased (P<0.05) as a percentage of fetal weight. (Table 1). Glucose, insulin and cortisol concentrations were greater (P<0.05) in both fetal and maternal blood of OB ewes when compared to C ewes (Figure 1). Insulin concentrations in fetal pancreatic tissue of OB ewes was also greater (P<0.05) than that of C ewes (Dot Blot; NOT PRESENTED). By evaluating immunostaining of both insulin positive cells and MIF positive cells, we determined that MIF is largely expressed within the cytoplasm of insulin positive β-cells. The protein level of MIF in pancreatic tissue was quantified via western blot using a specific polyclonal antibody to MIF (Figure 2). The pancreatic MIF protein level was increased (P<0.05) in fetuses of OB ewes compared with fetuses from C ewes. Clear differences were observed in responses of the two groups of lambs to the glucose challenge at two months of age (Figure 3). Lambs from OB ewes demonstrated lower baseline glucose levels at rest and a smaller AUCg than lambs of C ewes, but higher insulin AUCi level (P<0.06), which means the OB lambs tend to secrete more insulin to respond a similar dose of glucose compared with C lambs which accelerated glucose uptake by body tissues.

**Conclusions**

We have demonstrated in this study that maternal obesity results in both maternal and fetal hyperglycemia and hyperinsulinemia and increased cortisol level by mid gestation in the sheep model, which was associated with an increase in fetal weight. Both pancreatic weight and pancreatic weight per unit fetal weight increased markedly in fetuses gestated by obese mothers. MIF expression in pancreas is increased in fetuses from OB ewes. Increased cortisol level may be responsible for increased MIF expression in pancreas in that secretion of MIF is stimulated by glucocorticoids, and cortisol is one of the most important ones. The increased pancreatic MIF expression in fetuses from OB ewes would be expected to potentiate insulin release and thus glucose uptake by fetal body tissues. From the postnatal study, OB lambs tend to secrete more insulin to respond a similar dose of glucose than C which accelerate glucose uptake by tissues, which is consistent with the prenatal study. Failure of the pancreas to return to a normal cellular composition and function postnatally could lead to obesity, altered insulin secretion and diabetes in offspring.
Table 1. D78 fetal tissue and organ weights (g) from control ewes or ewes

<table>
<thead>
<tr>
<th>Tissues and Organs</th>
<th>Control ewe wt (g)</th>
<th>Fat ewe wt (g)</th>
<th>C ewe wt/unit fetal wt (%)</th>
<th>OB ewe wt/unit fetal wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>7.15 ± 0.23</td>
<td>10.12 ± 0.35*</td>
<td>2.69 ± 0.11</td>
<td>2.70 ± 0.069</td>
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<tr>
<td>Kidney</td>
<td>1.37 ± 0.09</td>
<td>2.09 ± 0.09*</td>
<td>0.51 ± 0.35</td>
<td>0.56 ± 0.12</td>
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<tr>
<td>Adrenal</td>
<td>0.055 ±0.004</td>
<td>0.075 ± 0.017*</td>
<td>0.021 ± 0.002</td>
<td>0.020 ± 0.005</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.14 ± 0.02</td>
<td>0.47 ± 0.03*</td>
<td>0.05 ± 0.01</td>
<td>0.12 ± 0.01*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.32 ± 0.04</td>
<td>0.44 ± 0.03*</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>6.84 ± 0.36</td>
<td>9.81 ± 0.32*</td>
<td>2.55 ± 0.08</td>
<td>2.63 ± 0.11</td>
</tr>
<tr>
<td>Liver</td>
<td>15.92 ± 0.99</td>
<td>24.26 ± 0.97*</td>
<td>5.93 ± 0.22</td>
<td>6.46 ± 0.15</td>
</tr>
<tr>
<td>Testis</td>
<td>0.069 ±0.015</td>
<td>0.077±0.005</td>
<td>0.024 ± 0.005</td>
<td>0.021 ± 0.002</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.019 ±0.003</td>
<td>0.026±0.004</td>
<td>0.008 ± 0.001</td>
<td>0.007 ± 0.001</td>
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<tr>
<td>St. muscle</td>
<td>0.49 ± 0.01</td>
<td>0.65 ± 0.05*</td>
<td>0.20 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Ld muscle</td>
<td>2.40 ± 0.16</td>
<td>3.46 ± 0.20*</td>
<td>0.97 ± 0.02</td>
<td>0.95 ± 0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>2.56 ± 0.14</td>
<td>3.27 ± 0.18*</td>
<td>0.96 ± 0.05</td>
<td>0.87 ± 0.04</td>
</tr>
</tbody>
</table>

*Means ± SEM within a row and measurement (Weight or Wt/unit fetal wt) differ (P<0.05)

Figure 1. Maternal and fetal blood glucose, insulin and cortisol levels. *Mean±SEM within a hormone and condition (maternal or fetal) differ (P<0.05).
Figure 2. Western blot quantified MIF protein expression level in pancreatic tissue. *Mean ± SEM differ (P<0.05)

Figure 3. Two month old lambs glucose (Panel A) and insulin (Panel B) responses to a glucose challenge. *Mean±SEM differ (P<0.05); +Mean±SEM differ (p<0.06)
Maternal Obesity and Overnutrition Increase Ewe Placental Fatty Acid Transport at 75 days of Gestation

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Summary and Implications

Placental FA transport is important for fetal growth and development. FA transport across the placenta is mediated by the fatty acid transporters (FATP, 1 and 4) as well as fatty acid translocase (FAT)/CD36, a multi-ligand putative FA transport protein. Little information is available regarding the specific impact(s) of maternal obesity on placental FA transport. The objective of this study is to determine the impact of maternal obesity on placental FA transport and the expression of FATP1, FATP4 as well as FAT/CD36 in cotyledonary (COT) tissue at midgestation. Results indicated that, at 75 dG, twin fetuses of OB ewes were heavier than C ewes (234 ±7 vs. 186 ±7 g; P < 0.05). Maternal leptin levels were higher in OB ewes than C ewes (1.29 ±0.06 and 1.49 ±0.17 vs. 1.00 ±0.09 AU, respectively); no difference was observed for FAT/CD36 in COT tissues. OB ewes exhibited elevated leptin concentrations in association with altered net placental transport of FA into the fetal compartment, particularly critical n-3 and n-6 long chain polyunsaturated FA. This increase in FA transport was associated with enhanced expression of FATP1 and FATP4 in COT tissue.

Introduction

In the USA, pre-pregnancy obesity increased from 13.0% in 1993-1994 to 22.0% in 2002-2003, a net increase of 69.3% (Kim et al., 2007). More importantly, a shift towards progressively higher gestational weight gain appears evident, resulting in excessive nutrient uptake during pregnancy (Siega-Riz et al., 2004). Recent evidence suggests that high pre-pregnancy BMI is associated with enlarged fetal body weight, newborn adiposity and complications for offspring in later life. Placental long chain fatty acid (LCFA) transport from the maternal to fetal circulation is important for fetal growth and development (Schaiff et al., 2007). Fatty acid transport proteins are a family of transmembrane proteins that enhance LCFA uptake (Hanebutt et al., 2008). FATP1 and FATP4 expressed in the human term placenta (Larque et al., 2006). Peroxisome proliferator-activated receptors (PPAR) are highly expressed in placenta (Wang et al., 2002) and they are essential for placenta development and fatty acid metabolism and transport (Schaiff et al., 2007). However, up to now, FATPs in the placenta of ruminant animals are poorly studied. We hypothesized that maternal obesity alters the expression of FATPs in placenta which changes cross-placenta transport of fatty acids.

Materials and Methods

Care and use of animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. From 60 days before conception to day 75 of gestation, multiparous Rambouillet/Columbia ewes were placed in individual pens and fed either a highly palatable diet at 100% (control, C; n=5) of National Research Council (NRC) recommendations or 150% (obesogenic, OB; n=5) of NRC recommendations on a metabolic BW basis (BW^{0.75}). Ewes were weighed at weekly intervals so that individual diets could be recalculated, with rations adjusted for weight gain, and body condition scored at monthly intervals to evaluate changes in fatness as previously described (Ford et al., 2007).

Tissue collection

On the day of necropsy, ewes were sedated with Ketamine, anesthetized by isofluorane inhalation and exanguinated while remained under general anesthesia. All placentomes present in the uteri of both C and OB ewes were classified as type A using the criteria described previously (Vonnahme et al., 2006). At each necropsy, maternal and fetal blood samples were collected from five twin bearing ewes in each dietary group under isofluorane anesthesia for fat acid profile analysis by GC-MS. After euthanasia, placental COT tissue was separated from CAR tissue, frozen in liquid nitrogen and stored at -80 °C for Western blot and real time RT-PCR analysis.
Fatty acid analysis
GC-MS was used to analyze maternal and fetal plasma fatty acid profile (Hsieh et al., 2008).

Leptin analysis
Leptin was measured by multi-species leptin RIA kit from Linco Research (Missouri, USA) following manufacturers instructions.

Western blotting analysis
Protein extractions were separated by 10% SDS-PAGE gels and transferred to nitrocellulose membranes for immunoblotting analyses with rabbit anti-FATP1(Cat# sc-25541, FATP4 (Cat# sc-25670) or CD36 (Cat# sc-9154) antibody (Santa Cruz Biotech, INC). Band density was normalized according to the β-tubulin content (Zhu et al., 2007a; Zhu et al., 2007b).

Real-time reverse transcript PCR (RT-PCR) analysis
Total RNA was extracted using Tri®Reagent (Sigma) and cleanup with RNeasy Mini kit (Qiagen, Cat#74104). cDNA was synthesized with SuperScriptTM III first-strand synthesis for RT-PCR kit (Invitrogen, Cat#18080-051). Real-time RT-PCR was conducted on Bio-Rad iQ5 machine. Primer sets for FATP1, CD36, FABP1, FABP3, FABP4, FABP5, FABPpm and PPARγ are synthesized by Invitrogen. β-tubulin was used as the housekeeping gene.

Statistics
Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System) (SAS, 2000). Means separation was performed using LSMEANS. Means ± SEM were considered different when P < 0.05.

Results and Discussion
Maternal plasma leptin concentrations are markedly higher in OB ewes compared to C ewes at day 75 of gestation (Figure 1). This plasma leptin concentration is highly correlated with body condition scores of pregnant ewes (Figure 2). In maternal plasma, the omega-3 LCPUFA 20:5 n-3 (EPA, eicosapentaenoic acid) and 22:6 n-3 (DHA, docosahexaenoic acid) are decreased in the overnourished ewe group, while the n-6 parent fatty acid, 18 carbon linoleic acid (18:2) nearly doubled as a percentage of fatty acids. Stearic acid also dropped in the overfed group (Fig 3). In fetal plasma, again, the long chain n-6 PUFA precursor, linoleic acid (18:2, LA) was elevated, as well as the 22 carbon LCPUFA [22:4 n-6 (arachidonic acid) and 22:5 n-6 (DPA, docosapentaenoic acid)] are markedly increased in the fetus of OB ewes (Fig 4). The pattern is consistent with the hypothesis that the higher maternal plasma LA increased of LA in the fetal plasma, which in turn fueled increases in the n-6 LCPUFA. Since n-6 LCPUFA has pro-inflammatory effects and the placenta is known to possess high levels of cyclooxygenases and lipoxygenases (Xu et al., 2007), enhanced n-6 LCPUFA might be partially responsible for the inflammation responses in OB maternal and fetal circulations (Zhu et al., 2008). We also found that 16 carbon mono and diene FA dropped in overfed group, but the physiological significance of such a change is unclear. Western analysis indicated that both FATP1 and FATP4 were higher in over-nourished ewes, but CD36 did not differ between dietary groups (Fig 5). Consistently, both FATP1 and CD36 mRNA expression are elevated in obese ewes (Fig 6). However, FABP1, 3, 4, 5, and plasma membrane FABP expression level did not differ between Control and over-nourished ewes, though they are all expressed in sheep placentomes (Fig 7).

PPARs are a group of the ligand-activated nuclear receptor superfamily and function as transcription factors regulating the expression of genes related to lipid metabolism and adipogenesis (Duttaroy, 2004). PPARγ is known to be essential for placental development (Xu et al., 2007) and uptake of fatty acids (Schaiff et al., 2005). The expression of PPARγ was markedly higher in over-nourished ewes (Fig 8). Consistent with mRNA expression, PPARγ protein content was also higher in the placenta of obese ewes (Fig 8). Activation of PPARγ enhances the uptake of fatty acids by increasing the expression of FATP1 and FATP4 (Schaiff et al., 2007).

Placental uptake of fatty acids from the maternal circulation is used both for placental metabolism and also for delivery to the fetal circulation (Schaiff et al., 2007; Xu et al., 2007). Besides regulating fatty acid transport, PPARγ also changes fatty acid metabolism in the placenta. The change in fatty acid composition in fetal circulation of OB sheep might reflect the superimposed changes in both metabolism and fatty acid transport in the placenta, which warrants further studies.

Conclusion
OB ewes exhibited elevated leptin concentrations in blood in association with enhanced placental transport of LCFA into the fetal compartment. Overfeeding results in a drop in n-3 LCPUFA in the maternal plasma, and a concomitant rise in n-6 PUFA in maternal and fetal plasma. OB treatment enhanced the expression of FATP1, FATP4 and CD36 in COT tissue. FABP1, FABP3, FABP4, FABP5 and FABPpm are expressed in sheep placentomes. However, their mRNA expression level did not differ between control and OB ewes. PPARγ mRNA and protein level are higher in COT tissue of OB ewes, which may lead to the increase in FATP1 and FATP4 expression and enhance fatty acid uptake.

Acknowledgement
The authors would like to thank Dr. Myrna Miller and Mr. Ryan Gustafson for assistance with animal care and tissue collection. This project was supported by University of Wyoming INBRE P20 RR016474-04 and by National Research Initiative Competitive Grant no. 2008-35203-
References
Fig 5. Relative Content of FATP1, FATP4 and CD36 protein in COT on 75dG. ■ control fed ewes; □ obese over-nourished ewes. Mean ± SEM; **: p<0.01; n=5 in each group.

Fig 6. mRNA content of FATP1 and CD36 in COT on 75dG. ■ control fed ewes; □ obese over-nourished ewes. Mean ± SEM; **: p<0.01; *: p<0.05; n=5 in each group.

Fig 7. mRNA content of fatty acid binding proteins in COT on 75dG. ■ control fed ewes; □ obese over-nourished ewes. Mean ± SEM; n=5 in each group.

Fig 8. Protein and mRNA content of PPARγ in COT tissue of control and OB ewes. ■ control fed ewes; □ obese over-nourished ewes. Mean ± SEM; n=5 in each group.
Maternal Obesity and Over-Nutrition Altered System Type A Amino Acid Transporters in Ewe Placentomal Cotyledonary Tissues on 75days of Gestation

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Abstract

Fetal development, especially skeletal muscle development, requires large amount of amino acids which are obtained from mothers through the placenta. Therefore, the capacity of the placenta to transfer nutrients has dramatic impact on fetal skeletal muscle development and the growth performance of offspring. However, the impact of maternal obesity on placental AA transport remains poorly defined. There are three sub-types of the placental Na⁺-dependent system A transporter, SNAT1, 2 and 4 which mediate neutral AA transport. SNAT2 is ubiquitously expressed in mammalian tissues and is likely responsible for the majority of placental system A activity. The objective of this study is to examine the impact of maternal obesity and over-nutrition on the fetal: maternal (F:M) AA ratio and placental protein abundance for SNAT2. In this study, Nonpregnant ewes were randomly assigned to a control (C, 100% of NRC recommendations) or obesogenic (OB, 150% of NRC) diet from -60 to 75 days of gestation (dG). Under isoflurane anesthesia, maternal and fetal blood samples were collected for AA analysis by HPLC from five twin bearing ewes in each dietary group. After euthanasia, placental COT tissue was separated from CAR tissue, and frozen in liquid nitrogen. Fetuses from OB ewes were ~26% heavier (P<0.05) than those from C ewes at 75 dG (234± 7 vs. 186±7g). Blood concentrations of Asn, Thr, Cit, Arg, Tau, Tyr, Trp, Val, Phe, Leu and Orn were higher (P<0.05), or tended to be higher (Met and Lys, P<0.10) in OB than C ewes. In contrast, F:M ratios, for Asn, Ser, Glu, His, Gly, Thr, Cit, Arg, b-Ala, Tau, Ala, Tyr, Trp, Met, Val, Phe, Leu, Orn and Lys were reduced (P<0.05) in OB compared to C ewes. SNAT2 content in COT tissue was reduced in OB when compared to C ewes (0.5±0.1 vs. 1.0± 0.2 Arbitrary Units; P<0.05). Maternal obesity in pregnancy reduced expression of placental SNAT2 protein and efficiency of placental AA transport in ewes, providing a mechanism whereby fetuses may mitigate excessive delivery of AA under conditions of maternal obesity and over-nutrition. Decreased AA transport to the fetus may play a role in altered fetal cellular structure and function.

Introduction

In ruminants, individual placentomes, composed of a fetal portion (cotyledon, COT) and maternal portion (caruncle, CAR), are responsible for nutrient exchange between mother and fetus. The increasing prevalence of overweight and obese women of childbearing age is a growing public health concern. The impact of maternal obesity on placental AA transport, which is essential for normal fetal development, remains poorly defined. There are three sub-types of the placental Na⁺-dependent system A transporter, SNAT1, 2 and 4 which mediate neutral AA transport. SNAT2 is ubiquitously expressed in mammalian tissues and is likely responsible for the majority of placental system A activity. The objective is to examine the impact of maternal obesity and over-nutrition on the F:M AA ratio and placental abundance for SNAT1 and SNAT2.

Materials and Methods

Care and use of animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. From 60 days before conception to day 75 of gestation, multiparous Rambouillet/Columbia ewes were placed in individual pens and fed either a highly palatable diet at 100% (control, C; n=5) of National Research Council (NRC) recommendations or 150% (obesogenic, OB; n=5) of NRC recommendations on a metabolic BW basis (BW⁰.⁷⁵). Ewes were weighed at weekly intervals so that individual diets could be recalculated, with rations adjusted for weight gain, and body condition scored at monthly intervals to evaluate changes in fatness as previously described (Ford et al., 2007).

Tissue collection

On the day of necropsy, ewes were sedated with Ketamine, anesthetized by isoflurane inhalation and exanguinatied while under general anesthesia. All placentomes present in the uteri of both C and OB ewes were classified as type A using the criteria described previously (Vonmahne et al., 2006). At each necropsy, maternal and fetal blood samples were collected under isoflurane anesthesia, for AA analysis by HPLC from five twin bearing ewes in each dietary group. After euthanasia, placental COT tissue was separated from CAR tissue,
frozen in liquid nitrogen and stored at -80 °C for Western blot and Real time RT-PCR analysis.

**Western blotting analysis**

Protein extractions were separated by 10% SDS-PAGE gels and transferred to nitrocellulose membranes for immunoblotting analyses with goat anti-SNAT2 antibody (Santa Cruz Biotech. INC). Band density was normalized according to the β-tubulin content (Zhu et al., 2007a; Zhu et al., 2007b).

**Real-time reverse transcript PCR (RT-PCR) analyses**

Total RNA was extracted using TriReagent (Sigma) and cleanup with RNase Mini kit (Qiagen, Cat#74104). cDNA was synthesized with SuperScript™ III first-strand synthesis for RT-PCR kit (Invitrogen, Cat#18080-051). Real-time RT-PCR was conducted on Bio-Rad iQ5 machine. Primer sets used in RT-PCR are synthesized from Invitrogen.

**Statistics**

Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System) (SAS, 2000). Means separation was performed using LSMEANS. Means ± SEM were considered different when P < 0.05.

**Results**

Fetuses from OB ewes were 26% heavier (P < 0.05) than those from C ewes on day 75 of gestation (234 ± 7 vs. 186 ± 7g; mean ± SEM). Maternal blood concentrations of Asn, Thr, Cit, Arg, Tau, Tyr, Trp, Val, Phe, Leu and Orn were higher (P < 0.05), or tended to be higher (Met and Lys, P < 0.10) in OB than C ewes (Table 1). In contrast, F: M ratios, for Asn, Ser, Gln, His, Gly, Thr, Cit, Arg, b-Ala, Tau, Ala, Tyr, Trp, Met, Val, Phe, Leu, Orn and Lys were reduced (P < 0.05) in OB compared to C ewes (Table 2). Real time RT-PCR analysis showed that SNAT1 and SNAT2 mRNA were significantly increased in OB ewes (Figure 1). Western blotting analysis indicated that SNAT2 content in COT tissue was reduced in OB when compared to C ewes (Figure 2), showing differential regulation of SNAT mRNA and protein expression.

**Discussion and Conclusion**

The fetal stage is crucial for skeletal muscle development, since there is no net increase in muscle fiber numbers after animals born (add Zhu et al, 2004 biology of reproduction). Given that we raise animals mainly for their lean, impairment in fetal skeletal muscle development will have long-term negative effect on the growth performance of offspring by reducing lean/fat ratio (Zhu et al., 2006b). Proper fetal muscle development requires large amounts of amino acids which are obtained from mother through placenta. Therefore, the capacity of placenta to transfer amino acids has profound impact on fetal skeletal muscle development. But surprisingly, few studies were conducted on cross-placental amino acid transport in agriculturally important animals.

Major factors affecting cross-placenta nutrient delivery include the activity and location of amino acid transporter systems, effects of changes in placental surface area, uteroplacental blood flows, and maternal concentrations of amino acids (Regnault et al., 2002). In our previous studies in pregnant sheep (Zhu et al., 2006a, 2007), we observed that maternal under-nutrition altered placental vascularity and nutrient transport. These studies raised a question: what will happen to cross-placental nutrient delivery in over-nourished ewes? Therefore, we conducted the current study which indicated that maternal over-nutrition in pregnancy reduced expression of placental SNAT2 protein and efficiency of placental AA transport in ewes. Such reduction in placental AA transport may be a mechanism whereby fetuses may mitigate excessive delivery of AA under conditions of maternal obesity and over-nutrition, providing a protective mechanism for the fetus. In combination of our previous studies showing that maternal under-nutrition enhances placental efficiency in delivery nutrients, these data clearly show that a mechanism exists in the placenta which alters its own function in order to ensure proper delivery of nutrients to fetuses.

Placental Na-dependent system A transporters, SNAT1, 2 and 4 mediate neutral AA transport. However, up to now, most studies only confirmed the existence and expression of SNATs in placenta, and the regulation of these SNATs is poorly studied (Jones et al., 2006; Desforges et al., 2006; Novak et al., 2006b; Novak et al., 2006a). Therefore, the remaining question is how the placenta senses maternal nutrition status and regulates its own function? Mechanisms regulating AA transporters in placenta are unclear. Since the placenta is crucial for proper fetal development, understanding such mechanisms will allow us to manipulate nutrient delivery to fetuses, to ensure optimal fetal skeletal muscle development and enhancing offspring growth performance.

**Acknowledgement**

The authors would like to thank Dr. Myrna Miller and Mr. Ryan Gustafson for assistance with animal care and tissue collection. This project was supported by University of Wyoming INBRE P20 RR016474-04 and by National Research Initiative Competitive Grant no. 2008-35203-19084 from the USDA Cooperative State Research, Education and Extension Service.

**References**


Table 1. d75 maternal and fetal plasma AA profile from C and OB fed ewes

<table>
<thead>
<tr>
<th></th>
<th>Control (n=5)</th>
<th>OB (n=5)</th>
<th>P-value</th>
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<tr>
<td>ASP</td>
<td>4.6±0.7</td>
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<td>GLU</td>
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<tr>
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<td>163±9</td>
<td>NS</td>
</tr>
<tr>
<td>HIS</td>
<td>31.1±2.6</td>
<td>34.3±2.9</td>
<td>NS</td>
</tr>
<tr>
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<td>499±31</td>
<td>374±13</td>
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<td>THR</td>
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<td>CIT</td>
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<td>ARG</td>
<td>50.5±3.6</td>
<td>89.4±10</td>
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<td>β-ALA</td>
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<td>LYS</td>
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<td>0.10</td>
</tr>
</tbody>
</table>

Mean ± SEM; **: p<0.01; *: p<0.05; †: p=0.06; n=5 in each group.

Fig. 1. Maternal and fetal mRNA expression of toll-like receptors, macrophage markers and pro-inflammatory cytokines in preplacental COT tissues at 75 dG.
Table 2. Day75 F:M plasma AA ratio from Control and OB fed ewes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OB</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>2.8 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>GLU</td>
<td>0.6 ± 0.1</td>
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<td>NS</td>
</tr>
<tr>
<td>ASN</td>
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<tr>
<td>HIS</td>
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<tr>
<td>GLY</td>
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<tr>
<td>ARG</td>
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<tr>
<td>β-ALA</td>
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<td>LYS</td>
<td>3.3 ± 0.5</td>
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<td>0.010</td>
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</table>

Figure 1. System A amino acid transporters, SNAT1 and SNAT2 mRNA expression relative to β-tubulin in ewe placentomal COT tissues on day 75 of gestation. (n=5 per group*: P<0.05; **: P<0.01)

Figure 2. SNAT2 protein content in ewe COT tissues on day 75 of gestation. Upper panel shows representative immunoblot; lower panel shows group means±SEM (n=5 per group*: P<0.05; **: P<0.01)
Research Briefs

Wool Research
Bruce Cameron¹, Robert Stobart², Angus McColl³

¹University of Wyoming Department of Family and Consumer Science
²University of Wyoming Department of Animal Science
³Yocom McColl Testing Labs Inc, Denver CO

Our objectives are to identify and quantify the physical characteristics of wool that will add value at the farm gate level, assist with preparation that would increase value and to identify new technologies that enable faster, more accurate and less costly methods to characterize the physical description of the wool.

Color of wool, can we select for it?

Background: The U.S. sheep industry has experienced a decline in numbers for the past 60 years. Income from sheep is based almost entirely on wool and market lambs. Income derived from wool sales is subject to worldwide price structuring. Currently, approximately 70 percent of the U.S. wool clip is exported due to the lack of domestic-processing capability. This requires competition with countries that for decades have used wool color as a selection criterion in the production of wools for export. It has been shown that yellow fleeces suffer a significant price discount in international markets. Wool color is an important characteristic that influences value. As far as color is concerned, superior wool is generally a creamy white color. The discoloration of the wool may for example, limit the dyeing potential of that wool. Clean-color specification is becoming increasingly more important for wool marketing.

Purpose Statement: To improve the color characteristics of domestic wools, one question that needed to be addressed; were there genetic differences among animals regarding fleeces produced to be more susceptible to yellowing propensity and if so, was their sufficient variation to permit selection against yellowing. This study was conducted to collect preliminary data on the propensity to develop yellow discoloration in wool of male and female Rambouillet sheep.

Summary of Findings: The study indicated that there were differences in the yellowing propensity of the Rambouillet breed and that it could be possible to select sheep based on this data to improve wool color. There was no correlation between fiber diameters and the yellowing propensity observed in this study

Conclusions: There is potential for economic gain if wool does not have a propensity to develop yellow discoloration. This study implies that there is a genetic predisposition for wools to develop a yellow color, there appears to be sufficient variability that selection against the propensity to yellow would decrease the incidence of natural yellowing in fleeces.

Applications: To increase economic return from wool it must be able to compete with wools produced internationally that already exhibit a desired color. Selecting for animals that do not have the propensity to produce off colored wool would help the producer to supply the types of wools necessary to compete in the international market whereby increasing the desirability of these kinds of wools.

Use of NIRS (Near Infra-red Spectroscopy) to establish residual grease and ash content of scoured wool samples.

Background: Wool producers, warehouseman and end-users need timely, accurate information concerning the product they are marketing. Current procedures are accurate, but the time lag involved between shearing and having objective information on the resulting wool clip for marketing purposes is too long in today’s marketing environment. A system which would significantly reduce the time necessary to produce the necessary information is desperately needed. NIRS has shown to be the system which will reduce this time lag. Traditionally, calculation of Wool Base and VM Base has been and still is being ascertained by lengthy, labor intensive and costly methods. Determination of Wool Base and Vegetable Matter Base requires washing, drying, and several chemical tests in order to arrive at the final weight. Wool Base and Vegetable Matter Base are the two determinants of various commercial yield calculations. The introduction of near-infrared spectroscopy (NIRS) to determine alcohol-extraction content and mineral matter content (ash) has significant implications in the testing field. There are several issues associated with the current wool-testing processes that will have a direct impact on
the viability of large scale use of the current test methods. There is a concern among wool testing laboratories about occupational health and safety issues; the current test methods not only impact worker safety and health but also have a detrimental effect on the environment. Wool testing laboratories are also concerned about how to improve service of testing to the industry while attempting to keep costs in line.

**Purpose Statement:** NIRS is currently used by IWTO certified labs to determine ethyl alcohol-extractable matter and ash. The ethyl alcohol-extractable matter test is extremely slow, very labor intensive, has OH&S risks plus utilizes a chemical that is costly to buy. The ash content test method is also very slow, labor intensive and poses an environmental risk due to the smoke produced and also utilizes materials that are very costly to buy and have limited life spans. Another test, estimating the residual grease of commercially scoured wool can be conducted by NIRS. This test is similar to the ethyl alcohol-extraction test in that it is very slow, labor intensive and OH&S risks because a highly dangerous, toxic solvent is used. These tests are very labor intensive, currently it requires approximately two hours per sample for ethyl alcohol extraction, the glassware needed for each sample costs $250 per unit. Mineral matter determination requires that the sample be burned and then placed in a muffle furnace for 3 to 4 hours, this requires a significant quantity of natural gas and produces a by product that is released into the atmosphere. Each crucible or container, which is used for each sample costs over $35 and have a limited lifespan. The use of NIRS technology would reduce this time to minutes, plus reduce the need for costly solvents and the associated hardware necessary to conduct the tests. The health and welfare of the laboratory workers would also be enhanced. Calibration of the instrument is crucial to provide accurate, repeatable results. Calibration must be carried out with samples that are similar to samples which will be measured by the instrument in the future.

**Summary of Findings:** We have measured many samples and have had difficulty getting repeatable results, presentation of the sample to the instrument has been a problem and we are currently looking at several methods that would allow the sample to be presented with a uniform surface. We believe this lack of uniformity in presentation is leading to the problems of repeatable measurements. We are continuing to evaluate samples for inclusion in the calibration and validation steps.

**Conclusions:** It has been difficult to calibrate the instrument with the methods we have been using to measure the scoured wool samples. We are attempting to standardize how the samples are presented to the instrument for subsequent measurement. We will continue to evaluate our presentation methods and when we are confident that we are getting repeatable results, will continue to measure samples to include in the calibration and validation data sets.

**Applications:** Once the NIRS instrument is calibrated, it will decrease the turnaround time from acquisition of a sample to having the report returned to the owner.

**Effects of aflatoxicosis on fertility in male and female mice and subsequent differential gene expression in tolerant mice.**

**Kristi M. Cammack, Kathleen J. Austin and Rebecca R. Cockrum**

Effects of chronic aflatoxicosis on reproduction have been reported in many livestock species, rodents, and in humans. Within males exposed to chronic levels of aflatoxin, variation in the impact on fertility varies among individuals. The objective of our first experiment is to confirm the genetic variation in fertility of male mice exposed to aflatoxin B1, identify males more or less tolerant to aflatoxicosis, and identify differentially expressed genes associated with high fertility in aflatoxin-treated male mice. Mature males were initially mated with two females to confirm that they were fertile. Fertile males were administered 50 µg kg\(^{-1}\) BW aflatoxin B1 in corn oil (treated; n = 9) or corn oil alone (control; n = 9) for 45 d. Males were placed with females (1:4) from d 35-45 and females were monitored daily for post coital plugs to determine mating success. Males were euthanized on d 46 and testes collected for sperm analysis and gene expression analysis. Based on sperm counts, mating test data, and TUNEL staining, testes from 3 aflatoxin tolerant, 3 aflatoxin intolerant, and 3 control male mice were selected for gene expression analyses. These experiments will be used to identify genes and provide insights into mechanisms of tolerance associated with high fertility following exposure of male mice to aflatoxin. While much of the research has focused on reproduction in males and the effects of aflatoxicosis on spermatogenesis, research in females is limiting and has primarily focused on reports of anatomical abnormalities of the mother and fetus. The objective of the second study was to better understand the effects of aflatoxin exposure on the maternal
gonads (uterus and ovary), and on fetal development through gene expression analysis. Female mice were paired with males (4:1) for 1.5 cycles (~5 d). The females were then removed and placed two per cage for the duration of gestation (approximately 17-19 d). During this time, females were administered 0.1 mg kg\(^{-1}\) aflatoxin B1 in corn oil daily for 14 d (treated; \(n = 12\)) or corn oil alone (control; \(n = 12\)). This dose of aflatoxin B1 has been previously reported as detrimental without causing maternal mortality. Female mice were killed by cervical dislocation. Maternal tissues, including ovaries, kidneys, and liver, and placental buttons were weighed, fixed, and frozen for future analyses. The fetuses were weighed, measured (crown-rump) and examined for abnormalities. Fetal liver was collected, fixed and frozen. Tissues collected from these experiments will be used for microarray analysis to provide insight into the pathogenesis of aflatoxin B1 and its effects on fertility. Additionally, genetic differences that may infer tolerance or intolerance to aflatoxin B1 in mice (male or female) will be examined.

**Impacts of Different Ewe Selection Criteria and Early Gestational Undernutrition on Fetal Growth Through Changes in Placentomal Morphology, Vascularity and Efficiency.**

*Stephen P. Ford.*

**Background:** Rangelands of the High Plains and Intermountain West of the United States experience marked fluctuations in quality and quantity of forages. For this reason, gestating ewes on rangeland pastures often experience prolonged bouts of undernutrition. Growth rates and carcass characteristics of young ruminants are known to vary considerably even when the genetics and nutritional management are constant. Considerable evidence suggests indicating that maternal nutritional status during certain critical periods of gestation impact their offspring in postnatal life.

**Purpose Statement:** This study investigated if markedly different multigenerational ewe management systems could alter the impacts of early to mid-gestational maternal undernutrition on fetal growth and offspring quality.

**Summary of Findings:** Ewes subjected to a nomadic existence and limited nutrition throughout the year from Baggs, WY (Baggs ewes) maintained normal fetal weights, as well as normal circulating glucose and essential amino acid concentrations, and normal organ weight and composition when subjected to nutrient restriction [NR, 50% National Research Council (NRC) requirements] from day 28 to 78 of gestation. In contrast, ewes of similar breeding, size, body weight, and age from the University of Wyoming flock (UW ewes), selected and accustomed to a sedentary lifestyle and above adequate nutrition, exhibited a 30% decrease in fetal weight, under the same NR. In contrast to the Baggs ewes, growth restricted fetuses of UW ewes exhibited reduced circulating glucose and essential amino acid concentrations, enlarged hearts, reduced kidney nephron numbers and fewer muscle fibers in several skeletal muscles than fetuses from control fed (CF, 100% NRC requirements) UW ewes.

The ability of NR Baggs ewes to maintain normal fetal weights and organ composition in the face of maternal nutrient restriction was associated with an early conversion from Type A placentomes to larger and more vascular Types B, C, or D placentomes by day 78 of gestation. In the sheep, 75 to 120 placentomes (individual placental units) are distributed over the placental surface, and are the sites of nutrient and oxygen delivery from mother to fetus. The progressively increasing size and vascularity of placentomes as they advance from A to B, C and then D placentomes is closely related to an increasing placental transport capacity, and nutrient delivery to the fetus. Placentomal conversion in the ewe normal occurs only in late gestation, when fetal growth and nutrient requirement are increasing exponentially. NR Baggs ewes convert their placentomes early in gestation to alleviate the impact of maternal nutrient restriction on the fetus. In contrast to Baggs ewes, the uteri of both NR and CF UW ewes contained only Type A placentomes on day 78 on gestation.

When NR UW and Baggs ewes were returned to a control diet from day 79 to term, size, viability and lamb birth weights were similar. At 2 months of age, however, male lambs born to NR UW ewes exhibited greater (\(P<0.05\)) glucose and insulin concentrations in response to an iv glucose bolus than CF UW ewes (i.e. insulin resistant). By 8 months of age, while these same lambs again exhibited greater (\(P<0.05\)) levels of glucose to an iv glucose bolus, insulin concentrations was markedly depressed (\(P<0.05\)), suggesting pancreatic failure. Further,
male lambs from NR UW ewes ate more, were fatter and had markedly higher blood pressures at 9 months of age than male lambs from CF UW ewes. At slaughter on day 280, male lambs from NR UW ewes exhibited markedly increased intra-abdominal fat and a reduced skeletal muscle mass than CF UW lambs. We observed no differences in postpartum fatness, insulin sensitivity or pancreatic function, blood pressure or carcass quality between lambs from NR and CF Baggs ewes.

Conclusions: We hypothesize that due to their harsh environment and reduced level of nutrition, Baggs ewes developed the ability to convert their placentomes to more efficient types in early gestation, alleviating the impacts of maternal undernutrition on fetal growth and offspring quality.

Applications: These data strongly suggest that environment/nutrition can have marked impacts on ewe productivity in a relatively short period of time. We are currently evaluating differences in placentomal gene expression patterns as placentomes progress from type A through type B, C, and D stages to determine potential differences between Baggs and UW ewes. Discovery of gene expression differences by ewe type (Baggs vs. UW) will provide insight into the signal which the undernourished Baggs ewe utilizes to convert type A placentomes during early gestation to more efficient forms (B, C, and D). This knowledge may lead to our ability to alter placentomal type, and thus optimize placentomal efficiency. Supported in part, by grants from the University of Wyoming BRIN P20 RR16474 and INBRE P20 RR16474-04, and NIH HD 21350.

Finally! An Effective Replacement for Ectrin® WDL for Sheep Ked Control

Jack Lloyd, Robert Stobart, Will Reeves, Greg Johnson, Rodney Kott, and Hayes Goosey

1University of Wyoming Department of Entomology
2University of Wyoming Department of Animal Science
3USDA, ABADRL
4Department of Animal and Range Science, Montana State University.

Background: The sheep ked, Melophagus ovinus, is probably the most serious insect pest affecting sheep in the United States. This blood feeding insect pest causes: reduced weight gains; reduced production of fleece; reduction in quality of fleece; defective pelts; and back loss.

Of particular concern is the damage to pelts of market lambs. Feeding by the sheep ked is responsible for a condition in the pelt known as “cockle.” The cockle defect is a nodule or deposition of dense fibrous material in the hide resulting from an allergic reaction to the salivary secretions of the sheep ked. This blemish cannot be softened; the nodules will not accept a dye leading to an unevenly dyed pelt; and the hide cannot be sueded to mask the defect. The result is an inferior leather product, unacceptable to the garment industry, which results in significant loss of income to the producer.

In 1983, Dr. Jack Lloyd, in cooperation with Fermenta Animal Health, demonstrated that the insecticide, Ectrin® WDL eliminated the sheep ked from flocks in Wyoming. Although treated flocks remained ked free, most sheep producers treated again in the spring following shearing because the treatment was so inexpensive. With the cooperation of the Wyoming Dept. of Agriculture, a Wyoming state label was secured for treatment of sheep with Ectrin® WDL (= water dispersible liquid). The following year neighboring Rocky Mountain States applied for state labels, and a year afterward, Fermenta Animal Health developed a national label.

Unfortunately, Ectrin® WDL is no longer available because the active ingredient, fenvalerate, is no longer licensed in the United States. Other commercial insecticide formulations have not been as efficacious as Ectrin® WDL, and flocks have become heavily infested once more.
In 2006, Dr. John Riner, who was with Fermenta Animal Health when Ectrin® WDL was developed for sheep ked control, expressed his belief that Ectrin® WDL was efficacious because it was a water base formulation, and that the active ingredient fenvalerate, was no more effective against sheep ked than other commercially available pyrethroid insecticides. Dr. Riner, who is now with KMG Company, subsequently provided us with a water base formulation of the pyrethroid insecticide permethrin, Permectrin® WS (= water soluble).

In addition to the Permectrin® WS, the PYthon® ear tag, which was efficacious in earlier studies with biting gnats and mosquitoes, was evaluated. Although Lloyd and co-workers have evaluated numerous cattle insecticide ear tags for sheep ked control, none had provided the desired degree of efficacy.

Purpose Statement: the objective of the study was to determine whether Permectrin® WS and PYthon® ear tags eliminated sheep ked from treated sheep. Sheep were examined by whole body count prior to treatment, held in isolation, and examined again 6 weeks following treatment. The six week period represents approximately two life cycles of the sheep ked.

Summary of findings: Our standard method of evaluating control of sheep ked was employed in the study. Two groups of ked-infested sheep were located in Wyoming and Montana. The sheep were assigned to four pens of 7 animals in Wyoming and 6 animals in Montana. The four pens received (1) one PYthon® ear tag each, (2) Permectrin® WS as a pour-on (a single line down the backline), (3) Permectrin® WS as a low volume (10 cc/animal) spray applied to the underside of the animal (according to the Ectrin® WDL label), and (4) no treatment (control).

The PYthon® ear tag and Permectrin® WS low volume spray eliminated sheep keds from treated sheep at both locations. The results with the pour-on were variable, we believe because there was run-off of chemical with this mode of application. The same problem had occurred earlier with Ectrin® WDL. We believe that the insecticide could be applied to the back as a fine spray to prevent runoff. Complete coverage of the body is unnecessary as the active ingredient is very lipophilic and moves through the fleece and over the skin.

Conclusions: Both the PYthon® ear tag and Permectrin® WS low volume spray effectively controlled sheep ked, and again sheep producers will be able to control the sheep ked in their flocks.

Applications: The PYthon® ear tag is commercially available and approved for biting gnat control on sheep in both Wyoming and Montana. Sheep ked will easily be added to the list of targeted pests on the label. Other states, if they desire, should be able to easily develop a state label. Permectrin® WS is currently labeled for sheep ked control in the United States, although it is not currently being marketed. Further cooperation with KMG should bring this product to market, and make it available to sheep producers.

Repellents to Protect Sheep from Culicoides sonorensis, a Biological Vector of Bluetongue Virus.

Dr. Jack Lloyd¹, Dr. Robert Stobart², Dr. Will Reeves³, and Dr. Greg Johnson⁴

¹University of Wyoming Department of Entomology
²University of Wyoming Department of Animal Science
³USDA, ABADRL
⁴Department of Entomology, Montana State University

Background: In the summer of 2007, sheep producers in northern Wyoming and southern Montana experienced an increase in cases of a viral disease known as “bluetongue” in their flocks. Bluetongue is a costly disease that is untreatable. Producers whose flocks develop this disease are quarantined and experience moderate losses of mature sheep and lambs. The greatest economic loss to the producer is in the loss of weight and condition of the affected sheep and reductions in flock fertility. Bluetongue is also of international concern; a recent memorandum from Europe indicated that as of October 20, 2008, all transport of ruminant livestock (sheep, cattle, goats), has been banned due to bluetongue disease.
Since bluetongue virus is transmitted by a biting gnat, Dr. Jack Lloyd proposed two approaches to reduce or eliminate feeding by gnats on sheep. The first was the use of an insect repellent spray, specifically directed toward the ventral or underside of the animal. Dr. Lloyd and his students developed a spray race for treating sheep in this manner in the late 1970’s. At that time they were targeting another insect pest, the sheep ked. It seemed that this approach might also work for the biting gnat because behavioral studies from California and Colorado indicated that the species of gnat of concern tended to feed on the underside of the body.

The second approach was the use of an insecticide ear tag, specifically the PYthon insecticide cattle ear tag, which is approved for use on cattle. The PYthon ear tag (9.5 g.), manufactured by Y-Tex Corporation in Cody, Wyoming, is simply inserted in one ear of the sheep. In earlier work at the University of Wyoming, Dr. Lloyd and his colleagues found that these ear tags, applied to calves, significantly reduced mosquito attack for several weeks.

**Purpose Statement:** The objective of the study was to determine whether the repellent spray treatment or the PYthon insecticide ear tags significantly reduced feeding on sheep by the biting gnat, *Culicoides sonorensis*. The treatments were evaluated on a weekly basis to determine the residual effectiveness of the treatments. A number of additional insecticide-repellent treatments were evaluated.

**Summary of Findings**

Groups of 4 sheep received each experimental treatment. Repellent sprays were applied to the ventral side of each animal by a small hand sprayer. The PYthon tag was applied to one ear using a Y-Tex Ultra Tagger according to label directions. Weekly following treatment, experimental sheep were restrained while a feeding tube with unfed *Culicoides sonorensis* was held on the skin in the area of the axilla for 15 minutes. Following feeding, total numbers of females and numbers of engorged females were recorded.

**Conclusions**

The most effective treatments were the PYthon ear tag at one tag per sheep and an oil solution of 2.0% permethrin/2.0% piperonyl butoxide as a ventral spray at 10 ml per animal. Both treatments significantly (statistically) reduced feeding rates for a period of 5 weeks following treatment.

**Applications**

Based on our data the PYthon insecticide ear tag has received state label status in both Wyoming and Montana. The tag is available in most livestock supply stores. Other states should have no difficulty acquiring a state label if they so wish.

It is expected that approval of the oil solution, also a product of Y-Tex Corp., will be through EPA by next spring, and will be available nationally. The oil solution application will be much less expensive than the ear tag.

To illustrate the importance of blue tongue disease in Europe we received the following message from Dr. Mike Fletcher at Y-Tex Corporation, “To my knowledge yours is the only data on control of Culicoides on sheep with PYthon tags. That data has been sent to authorities in Europe and used as justification for a study to be done in the coming season. I would say yes your work has had world-wide implication.”
Field Test of the Coyote Lure Operative Device for Delivery of Oral Contraceptives.

Marjorie J. MacGregor, Graduate Research Assistant and Steven W. Horn, Professor.

Coyotes (*Canis latrans*) have and continue to be significant predators of livestock, mainly domestic sheep. Managing coyote predation remains a challenge. A variety of non-lethal population control methods including reproductive inhibitors are currently being tested. The primary constraint of an efficient, inexpensive biological control method is developing a species-specific method of delivery. The purpose of this study was to test the efficacy of the Coyote Lure Operative Device (CLOD) for delivery of oral reproductive inhibitors in Southeastern Wyoming in conjunction with enhanced sensory cues and winter weather variables. One hundred and ninety CLODs were activated during the study period, November 1, 2007 – April 3, 2008. DNA analysis confirmed activations by 66 coyotes (*Canis latrans*), 90 red fox (*Vulpes vulpes*) and 7 unknown canid species. Preference for activations by CLODs with Karo® syrup when used in conjunction with a lure (Fatty Acid Scent) was significant ($F_{3, 1052}=6.18$, $p=0.000$). No correlations were found to exist between number of activations and weather patterns. This field research shows that oral reproductive inhibitors can be delivered to free ranging coyotes during the winter, via the CLOD. The data shows that the CLOD is not a species-specific delivery device; rather, it is a canid specific delivery device.

Anticancer Effects of a Pegylated Curcumin

Caitlin J. Murphy, Huadong Tang, Edward A. Van Kirk, Kellee D. Sundstrom, Dale D. Isaak, Kathleen J. Austin, Brenda M. Alexander, Robbie M. Schamber, Youqing Shen, and William J. Murdoch

Curcumin is a polyphenolic extract of the curry spice tumeric. Anticancer modes of curcumin action have been attributed to cell-cycle arrest and apoptosis. It appears that curcumin is pharmacologically safe. Unfortunately, parenteral applications for curcumin have been limited by poor solubility in aqueous injection vehicles. Polyethylene glycol (peg) is a hydrodynamic biocompatible polymer. Conjugation to peg is a promising technology for improving the compliancy of lipophilic drugs. Prospective anticancer properties of a novel pegylated curcumin were assessed. Hydroxyl groups of the aromatic end-rings of curcumin were esterified with low molecular weight chains of peg. Dose inhibitions in metabolic activities (MTT assay) of human ovarian (SKOV-3, OVCAR-3), breast (MDA-MB-468), and colon (KM-12) cancer cell lines exposed to pegylated curcumin were greater than or equivalent to native curcumin. Follow-up in vitro experiments using SKOV-3 cells indicated that the proliferation marker cyclin D was undetectable (immunoblot) by 8 h and that apoptosis (chromatin condensation) occurred between 24 and 48 h after treatments. Tumor burdens in athymic mice bearing intraperitoneal SKOV-3 xenografts were reduced by an intravenous injection of pegylated curcumin. These results indicate that injectable water-soluble polymers of curcumin are suitable for clinical applications in cancer therapy.
Adaptation of Annual Legumes as Winter Annuals on the Central High Plains for use in Integrated Crop and Livestock Systems

Steve Paisley, Extension Beef Cattle Specialist, Christopher Loehr, Graduate Research Assistant UW, Frances Loehr, Graduate Research Assistant UW, Jim Krall, Director of Research, Jerry Nachtman Research Associate II, SAREC

Common dryland, or rain-fed, agronomic practices on the high plains often consist of a wheat/fallow crop rotation. Wheat/fallow consists of one year of growing wheat followed by one year in which the ground is tilled and/or sprayed with herbicide, but no crop is actually grown. Fallow is used to build sufficient soil moisture for the subsequent wheat crop. A considerable amount of annual moisture comes during the winter months and it would be beneficial to establish a crop there to take advantage of this moisture, as well as to reduce wind erosion. Additionally, traditional cropping systems may have to undergo serious changes in the near future, as milder winters and warmer summers impact agriculture here on the northern Great Plains. At the same time, the price of land, fuel, and inputs continues to increase. The purpose of this project is to investigate possible alternatives to the traditional wheat/fallow crop rotation by adding a forage component composed of a legume crop in place of traditional fallowing. This added crop has the potential to improve nitrogen status in the soil, thus reducing inputs and possibly increasing economic returns in the form of harvestable forage, while reducing soil erosion from both water and wind. The main focus of this project is to test winter survivability and forage production of several legumes species. Winter survivability is of concern as these crops would be sown into wheat stubble in the fall, when they would germinate, followed by harvesting the subsequent spring or summer before another rotation of wheat. Species currently being examined are: toni lentil, indianhead lentil, common hairy vetch, namoi wolly pod vetch, morava vetch, rasina vetch, Laramie medic, and Austrian winter pea. Many of these species’ growth has not been studied in southeastern Wyoming. Additional legumes include the recently established Laramie medic and newly acquired Phrygia medic from Australia. Crops are being grown under irrigation at the James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), near Lingle, Wyoming. Forage analysis will be conducted on these crops along with an economic analysis of this system versus a common wheat fallow operation. Table 1 shows some preliminary data collected from this project as the average weight in pounds collected from two 2.56 ft sections of row cover.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Winter Trial</th>
<th>Summer Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austrian Winter Pea</td>
<td>1.56</td>
<td>2.73</td>
</tr>
<tr>
<td>Indianhead Lentil</td>
<td>0</td>
<td>1.16</td>
</tr>
<tr>
<td>Toni Lentil</td>
<td>0</td>
<td>0.90</td>
</tr>
<tr>
<td>Morava Vetch</td>
<td>0</td>
<td>2.34</td>
</tr>
<tr>
<td>Rasina Vetch</td>
<td>0</td>
<td>1.85</td>
</tr>
<tr>
<td>Namoi Wolly Pod Vetch</td>
<td>0</td>
<td>1.91</td>
</tr>
<tr>
<td>Hairy Vetch</td>
<td>3.00</td>
<td>0.84</td>
</tr>
<tr>
<td>Laramie Medic</td>
<td>2.29</td>
<td>*</td>
</tr>
</tbody>
</table>

* Laramie Medic was not planted in the summer trial.

Samples were collected from two 2.56 ft sections of row cover.
Evaluation of N-3 Fatty Acid Supplementation and Length of Time on Grass on N-3 Fatty Acid Deposition in Tissues of Grazing Beef Cattle
Daniel C. Rule, Paul Kenehan, and Bret W. Hess

Grass-fed beef is re-emerging as a product of focused interest in many parts of the U.S. Issues related to grass-fed beef include its composition of fatty acids, which is said to be “rich in omega-3 (n-3) fatty acids and conjugated linoleic acid (CLA). The hypothesis of the current research is cattle grazing grasses will consume and subsequently deposit n-3 fatty acids at different rates during the grazing season because associative interactions between the consumed grass and the ruminal environment will decrease ruminal biohydrogenation; a period of time during the grazing season will, therefore, allow for maximal absorption of supplemental n-3 fatty acids. To test this hypothesis, the following objectives are being pursued. Objective 1. Determine the change in concentration of n-3 fatty acids in muscle and fat biopsies during the grazing season early, mid, and at harvest in cattle grazing irrigated pasture (alfalfa; wheat grass; bromegrass). Objective 2. Determine the effect of supplementation of long-chain n-3 fatty acids (EPA and DHA, common in fish oil) on concentrations of these fatty acids during grazing early, mid and finally at harvest in the cattle. The meat of grass-fed beef typically is very low in fat, which is its most important attribute. Furthermore, fatty acid profiles of grass-fed beef, which indicate relatively high proportions of n-3 fatty acids and conjugated linoleic acid, are being exploited as a marketing tool because both of these types of fatty acids have several important health benefits when consumed in the appropriate quantities. However, because of the low fat concentration of grass-fed beef, the concentrations of these fatty acids are very low indeed. Producing a meat product truly “rich” in n-3 fatty acids and conjugated linoleic acid underscores the promotional thrust of grass-fed beef producers. The rationale for the current ongoing study stems from the problem that feeding these types of fatty acids, which are unsaturated, are converted to saturated fatty acids by enzymes produced by ruminal microorganisms (biohydrogenation), so the amounts deposited in the animal's tissues are normally quite low. The published literature indicates that concentrations of n-3 fatty acids and CLA in muscle of grass-fed beef are variable, and could be high-enough to approach a significant intake by consuming this beef. The nature of the variation is uncertain, but we have shown that n-3 fatty acid levels in native grasses decrease as the grazing season progresses. It has also been documented that loss of n-3 fatty acids, such as EPA and DHA, is not very high in the ruminant animal; whereas, other studies have shown substantial losses when fed to grass-fed cattle. Thus, we are addressing the question: could an interaction occur between components of the forage at different times of the grazing season and the ruminal environment so that degradation of polyunsaturated fatty acids like the n-3 fatty acids is minimized? This study is being conducted at SAREC with 36 LowLine Angus steers. The trial was begun June 1, 2008 and grazing of the irrigated pasture was completed on about October 1, 2008. Biopsies of subcutaneous fat tissue and muscle were obtained on July 15 and again on September 11. The steers are currently being fed on harvested forage. The trial is expected to be completed on December 5, 2008 with fatty acid analyses to follow with expected completion in early spring, 2009.

Evaluation of New Techniques to Enhance the use of Artificial Insemination on farm.
Robert H. Stobart¹, Brent Larson¹, Harvey Blackburn², and Phil Purdy²

¹Department of Animal Science, University of Wyoming, Laramie, WY, 82071.
²USDA-ARS National Animal Germplasm Program (NAGP), Fort Collins, CO, 80521

Background: Artificial insemination (AI) has played a seminal role in facilitating genetic improvement in dairy, beef and swine industries. The technology has not been routinely employed by the sheep industry and as a result genetic improvement has not been fully realized. The University of Wyoming and the USDA-ARS National Animal Germplasm Program (NAGP) have recognized this void and are collaborating to address the problem.

Purpose: We have taken a two pronged approach to our research. We recognized that there is a need for the NAGP to be able to transport and cryopreserve ram semen which was collected in remote locations for genebank
development. This approach can be applied in breeding programs wishing to introduce new genetics into flocks or even enabling a commercial enterprise to collect semen for a customer and cryopreserve it in a laboratory at a distant location.

The ability to cryopreserve semen samples is the first aspect of increasing the use of superior genetics; the second is an inexpensive method to artificially inseminate sheep. To date laparoscopic insemination has produced the most repeatable results but this method is expensive. Furthermore, the procedure requires a high level of expertise to achieve acceptable levels of fertility. Therefore, the purpose of our research has been to develop methods of semen handling and AI that is simple, inexpensive, and effective and will enable the exchange of genetics across the country.

Summary of findings: Our first experiments focused on the ability to collect and hold semen at 5 ºC (41 ºF) for up to 48 hours prior to freezing. Semen was collected from rams in and out of the breeding season, cryopreserved immediately after collections or after holding for 24 or 48 hours at 5 ºC. Sperm quality was evaluated after thawing for motility and cell membrane integrity (live sperm). These analyses demonstrated that holding time did not impact post-thaw sperm quality (Table 1). These results strongly suggest semen can be collected, diluted and shipped overnight.

Because the analyses of the semen were purely in vitro the next experiments were performed to determine the fertility of semen which had been held for up to 24 hours prior to cryopreservation. The estrous cycles of ewes were synchronized and laparoscopic inseminations were performed using semen that was fresh, cryopreserved immediately after collection (T0), or held for 24 hours at 5 ºC and then cryopreserved (T24). This experiment was replicated over two years, except the T0 treatment was excluded in year two. The results of these experiments demonstrated that there were no differences in cryopreservation treatment (T0 vs. T24) importantly indicating that holding time does not decrease the quality/fertilizing potential of the sperm (Table 2). Furthermore, the fertility trial also demonstrated that holding time was not deleterious in terms of prolificacy (number of lambs born per ewe lambing).

We have now started to explore an alternative method of non-surgical AI. Non-surgical techniques have not been consistently successful due to the inability to traverse the cervix of the ewe. Therefore, we are focusing on creating an alternative method to AI sheep in a fast economical manner using the semen holding methodologies and the semen cryopreservation methodologies we developed. Our technique has resulted in 47% of ewes inseminated with fresh and 10% of the ewes inseminated with frozen semen (24 hours old at the time of cryopreservation) lambing. Our conclusion to date is that the AI technique works but is still in need of fine-tuning in order to increase fertility. We now believe the key to increasing fertility with this technique, and with semen that has been held prior to freezing, is dependent upon proper timing of insemination. Therefore, future research will investigate alternative methods of ewe estrous synchronization for use with frozen-thawed semen.

Conclusions: The results demonstrate that frozen thawed ram semen held at 5ºC for 24 hours prior to cryopreservation is as effective in fertilizing ewes as that frozen immediately after collection. Achieving fertility using semen cryopreserved in this manner is important because it will enable the NAGP to successfully collect samples from around the U.S. and freeze them in the laboratory in Fort Collins, CO. It also affords sheep producers the ability to perform on farm collections and processing at a distant laboratory.

Applications: This information will help the sheep industry because it demonstrates a protocol for collecting, freezing and inseminating frozen-thawed ram semen which can be utilized by breeders. Furthermore, the creation and adaptation of these techniques will enable a greater exchange of genetically superior rams and performance data thus resulting in increased flock improvement and improved breeding value accuracies which will benefit the industry as a whole.
Table 1. Characteristics of frozen-thawed ram sperm held for 0, 24, or 48 hours at 5°C prior to freezing. Samples from six rams per season were collected but the same rams were not used in both seasons.

<table>
<thead>
<tr>
<th>Sperm parameter</th>
<th>Autumn holding time</th>
<th>Spring holding time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Live sperm (%)</td>
<td>28</td>
<td>35</td>
</tr>
</tbody>
</table>

Superscripts indicate significant differences within that row and season.

Table 2. The fertility rate (%) and prolificacy (lambs born per ewe lambing) of ram sperm that was inseminated fresh or after cryopreservation. Cryopreserved sperm were frozen immediately after reaching 5 °C (T0) or after incubation at 5 °C for 24 h (T24).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Fertility % (#)</th>
<th>Prolificacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fresh</td>
<td>25.0 (27)</td>
<td>1.14 ± 0.19</td>
</tr>
<tr>
<td>1</td>
<td>T0</td>
<td>20.1 (35)</td>
<td>1.27 ± 0.20</td>
</tr>
<tr>
<td>1</td>
<td>T24</td>
<td>16.0 (35)</td>
<td>1.72 ± 0.20</td>
</tr>
<tr>
<td>2</td>
<td>Fresh</td>
<td>37.4 (33)</td>
<td>1.45 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>T24</td>
<td>45.3 (29)</td>
<td>1.20 ± 0.17</td>
</tr>
</tbody>
</table>

Central Ram Tests, inclusion of individual rams feed efficiency.
Robert H. Stobart and Brent Larson.

**Background:** Central ram tests are currently the only accurate method of establishing true genetic merit of rams, however use of EPD’s is becoming more prevalent with increasing data to improve their accuracy values. The University of Wyoming has conducted a central ram test for 47 years, over that period, as technology has improved, more data relating to an individual ram’s growth rate and carcass characteristics and fleece production has occurred allowing a better estimation of genetic potential. We now can use ultrasound to estimate how large a loin eye muscle is and how much backfat that animal has accumulated. However the one important characteristic we have not been able to identify is feed efficiency. The industry has seen increasingly higher costs to finish lambs, which directly impacts return to the producer. If we had a method of determining individual animal feed efficiency, we could then start selecting for those animals along with the other parameters we are currently utilizing.

**Purpose:** The GrowSafe System was originally developed for the cattle industry, as this industry has a population base that could make this system economically viable to sell while providing a system that greatly enhances the
ability to identify animals that convert feed to lean tissue more efficiently than others. Cattle and Sheep are basically similar, they are both ruminants, meaning they can utilize grass, forbs, browse and cereal grains as their major source of nutrients. The bulk of the animals entering the food chain in the United States is fed for a period of time in a feedlot. With the overwhelming impact of higher grain prices, efficiency of converting grains into lean tissue has become extremely important. Historical methods of determining individual animal intake, and consequently its feed efficiency is very expensive, time consuming and labor intensive. Whereby the GrowSafe Feed Intake and Behavior Monitoring System provides a researcher with electronic animal identification, comprehensive intake measuring, feeding behavior monitoring and a fully integrated central management system. The system continuously monitors the individual feeding behaviors and intake of animals with minimal disruption to typical behaviors. GrowSafe Feed Intake and Behavior Monitoring System capabilities include:

- Proprietary GrowSafe ID reading technology -
- Accurate feed disappearance measurement and high volume data acquisition
- Portability
- Wireless
- Automatic System Auditing - Each day the system automatically audits the total feed supplied to the bunk and the amount of feed assigned to individual animals providing unparalleled data accuracy and integrity

The system measures and/or calculates:
- Duration and intake for every individual feeding event up to one second accuracy (feeding event criterion is user defined)
- Head down and in to out duration during this event up to one second accurate
- Feed disappearance during events up to one second accurate
- Feeding frequency over user selected time interval
- Automatic calculation of feed supply
- Feeding rate throughout feeding event
- Animals standing at the bunk not consuming feed
- Time of the event, one second accurate
- Intervals between events
- Number of animals feeding simultaneously
- Social hierarchy - “who feeds besides who” and “who feeds first”

We have currently purchased a “Growsafe” system and we will be collecting data from the current ram test

**Summary:** We will have the capability of estimating feed efficiency of growing rams as well as evaluating the offspring from rams who have had their feed efficiency estimated. This technology will reveal new directions in research, such as investigating a number of research projects whereby different feedstuffs and nutrient levels can
be evaluated for their contributions to efficiency. Additionally the area of feeding behavior may lead to improved feeding systems that reduce the overall costs associated with group feeding animals.

Conclusions: The recent development of GrowSafe technology allows researchers to collect feed intake and feeding behavior data on individual animals in typical commercial production environments. The use of this system allows researchers to measure feed intake and feeding behavior variables on individual animals in commercial production environments. The system adds to the knowledge of how we can improve the efficiency of converting feedstuffs to high quality protein

Applications: To identify animals inherently more efficient in converting feedstuffs to high quality protein and utilize the genetics of these animals to increase performance and decrease costs associated with feeding animals. Identify feedstuffs that may assist with increasing the feed efficiency of ruminants, increasing returns to producers.

**Correlation between production traits and reproductive performance in white-faced yearling rams.**  
*PI: Valerie A. Uthlaut, Graduate Student and Brenda M. Alexander, Assistant*

Of the 196,000 rams in the United States, approximately 23% of these rams are expected to be non-performers (Fitzgerald and Perkins, 1991). This results in a loss of 13.5 million dollars to U.S. sheep producers annually. The objective of this study is to increase the profitability of sheep producers by eliminating non and low sexually performing rams from producer flocks. Thirty-three white faced rams ranging in age of ten months to one year were tested to evaluate their sexual behavior. Concurrently, these rams were tested for performance traits on the Wyoming Ram Test. To determine sexual performance, each ram was individually exposed to two ewes in estrus for a period of 30 minutes. To eliminate human interference, ram behavior was recorded using a digital camera and analyzed at a later time. Ram sexual behavior was categorized as: anticipatory (ano-genital sniffs, flehmen response, fore-leg kicks and nudges) and consummatory (mount attempts, mounts and ejaculations) behavior. Rams were tested a maximum of three times from January to March. Rams exhibiting consummatory behavior were not re-tested. For production traits, each ram was assigned an index ratio according to his performance on the Wyoming Ram Test. Out of the 33 rams tested, 100% exhibited anticipatory behavior while only 24% went on to exhibit consummatory behavior. Although a high proportion of rams were inactive at initial exposure to estrus ewes, sexual behavior is a learned, as well as innate, process and a proportion of the rams that were inactive may become satisfactory breeders. This research is in its first year of a three year study.