

2024 North Dakota Livestock Research Report

This is the 13th year I have been the coordinator and editor of this report. This publication aims to report a broad range of findings from researchers across the state to producers and industry personnel in North Dakota and beyond. I hope this report will continue to remind all of us of the quality and breadth of our livestock research and Extension programs in North Dakota.

Our research ranges from discovery research, which could develop into new innovations or technologies to improve production in the future, to more translational research, which provides information that can be used immediately by producers and industry personnel. Both are equally important and often are strongly linked in our research programs. Another important benefit of our research programs is the opportunities for students to gain experience in research methods and the livestock industry, and to become the next generation of animal scientists working in various careers in the industry. Additionally, we aim to have strong linkages between our research and Extension programs to get the information to those across the state who can use it. Please consider participating in Extension events or accessing Extension publications and materials in the coming year.

I want to thank Becky Koch and Deb Tanner for their continued assistance in editing and formatting the reports. Also, thanks to the contributors to the report and to the staff and students who help with livestock research, teaching and Extension activities. Finally, thanks to the funders of the grants that help support the research projects and students/staff working on the projects. We truly appreciate your contributions to our research programs. Without this support, the research and the training of the next generation of animal scientists would not be possible.

If you have any questions about the research in this report, please do not hesitate to contact me or any of the authors of the individual reports. Thanks for your encouragement and support of livestock research in North Dakota.

Kendall Swanson North Dakota Livestock Research Report Editor Professor Department of Animal Sciences Phone: 701-231-6502

email: kendall.swanson@ndsu.edu

Contents

Welcome	.2
Pigs	
Exposing pigs to a ramp and platform during the nursery period improves ease of loading at market weight	.5
Sheep	
Columbia ram performance testing and certification: 2023-2024 Dakota Ram Test	.9
Rambouillet ram performance testing and certification: 2023-2024 Dakota Ram Test	11
Estradiol-induced labor in periparturient ewes: Gene expression changes in maternal reproductive tissues and impact on lamb birth characteristics	15
Cattle	
Discovering performance and value in North Dakota calves: 2023-2024 Dakota Feeder Calf Show Feedout	18
Effectiveness of virtual fence in North Dakota grazing systems	21
Impact of protein source and supplementation frequency on growth performance of backgrounding cattle in an extensive winter grazing system	25
Bale grazing: Can different supplementation strategies enhance beef cattle performance under variable winter conditions?	28
Evaluation of an exogenous enzyme combination in forage-based growing diets fed to growing beef cattle	33
High-forage vs. high-concentrate diets fed to beef heifers during pregnancy and the impacts on feeding behavior and feed efficiency in the dam and morphometric characteristics of the male offspring	37

Continued on page 4

Impacts of limit-feeding high-concentrate diets to beef heifers during the first
180 days of gestation on performance, carcass characteristics and gastrointestinal
tract morphometrics
Matheus S.P. Carlis, German D. Ramirez-Zamudio, Kathryn Slavick, Brooklyn Kuzel, Jennifer L. Hurlbert, Godson Aryee, Justine Kilama, Gabriela Magossi, Rodrigo S. Goulart, Joel S. Caton, Kendall C. Swanson, Samat Amat and Carl R. Dahlen
The influence of gestational body weight gain rate on the development of two generations
of beef cattle
Friederike Baumgaertner, German D. Ramirez-Zamudio, Ana Clara B. Menezes, Isabella M. Jurgens, Mara R. Hirchert, Jennifer L. Hurlbert, Kerri A. Bochantin, Lawrence P. Reynolds, Alison K. Ward, Pawel P. Borowicz, James D. Kirsch, Sheri T. Dorsam, Kendall C. Swanson, Joel S. Caton, Carl R. Dahlen, Kevin K. Sedivec and Wellison J. S. Diniz
Effects of limit-feeding replacement heifers with a high-concentrate or high-forage
diet during gestation on oxygen consumption and mitochondrial function in liver and
jejunum tissues51
Kathryn R. M. Slavick, Jennifer L. Hurlbert, Brooklyn M. Kuzel, Matheus S. P. Carlis, German D. Ramirez-Zamudio, Samat Amat, Kevin K. Sedivec, Kendall C. Swanson and Carl R. Dahlen
Placental vascular development and cell proliferation throughout gestation in beef heifers55 Chutikun Kanjanaruch, Bethania J. Dávila Ruiz, Jennifer L. Hurlbert, Friederike Baumgaertner, Joel S. Caton, Pawel P. Borowicz, Carl R. Dahlen, Lawrence P. Reynolds, Kyle J. McLean, Matthew S. Crouse and Kacie L. McCarthy

Exposing pigs to a ramp and platform during the nursery period improves ease of loading at market weight

Mary L. Kasakamu¹, Jennifer M. Young¹, Ryan S. Samuel², Sarah A. Wagner^{1,*} and Christopher J. Byrd¹

Pigs exposed to a ramp and platform in their pen during the nursery period (3-9 weeks of age) required less time and fewer handler interventions to ascend a semi-trailer loading ramp when they reached market weight (5-6 months of age). Additionally, the presence of the ramp and platform within the pen had no negative effects on pig behavior or performance during the nursery and grow-finish phases of production. Therefore, early and limited exposure to a ramp and platform may be a reliable strategy for reducing novelty associated with loading ramps and improving the welfare of market-weight pigs during the loading phase of transportation.

Summary

Transportation is an essential component of commercial swine production in the United States. Stressors experienced during transportation, including loading and unloading, can result in poor welfare outcomes and economic losses. This study evaluated whether early life exposure to a ramp in the nursery pen (when pigs were approximately 3-9 weeks of age) improved pigs' ability to navigate the ramps required for loading and unloading when they reached market weight (approximately 5-6 months of age). A secondary study objective evaluated whether presence of the ramp in

¹Department of Animal Sciences, North

Dakota State University, 1300 Albrecht

behavior and growth performance during the nursery and grow-finish phases of production.

Experimental pigs (N = 540; 17-21 days of age) housed in 20 pens (27 pigs per pen) were assigned to one of two experimental treatments: 1) access to a ramp in the pen during the nursery phase (RAMP), or 2) a standard pen with no access to a ramp during the nursery phase (CONT) (Fig. 1). After the six-week nursery period (when pigs were approximately 9 weeks of age), ramps were removed from RAMP pens and all pigs were raised under standard conditions until marketing. Nursery behavior (posture, eating, drinking, aggression) and growth performance during the nursery and grow-finish phases were evaluated. No differences in nursery behavior or growth performance were observed (P > 0.05).

At marketing, pigs were loaded in groups of four pen mates onto a semi-trailer and unloaded in mixed treatment groups upon arrival at the processing facility. During loading, time to ascend the ramp to the trailer was quantified, along with the number of trips displayed by any animals and handler electric prod usage. Pigs in the RAMP treatment required less time to ascend the ramp compared to the CONT pigs (P = 0.002). An electric prod was used more frequently with CONT pigs compared to RAMP pigs (P =0.02). During unloading, the total time required for pigs to descend the ramp, as well as the number of trips, turnarounds on the ramp and pigs descending the ramp backwards were quantified. Rattle paddle usage by the handler and the number of pigs dead upon arrival were also recorded. CONT pigs required a shorter time to descend the ramp compared to the RAMP pigs (P = 0.03). Additionally, a greater number of RAMP pigs descended the ramp backwards compared to CONT pigs (P = 0.02). No other treatment differences were observed. In conclusion, exposing pigs to a ramp during the nursery phase improves ease of loading at marketing and has no negative effect on nursery behavior and growth performance throughout the nursery and grow-finish phases.

Introduction

Modern commercial swine production in the United States relies heavily on pig transportation, with most pigs undergoing transportation at least twice during their lives. This is because of increased specialization in the swine industry that has resulted in the separation of the

the nursery pen affected nursery

Blvd., Fargo, ND 58102, USA ²Department of Animal Science, South Dakota State University, Brookings, SD 57007, USA

^{*}Present address: School of Veterinary Medicine, Texas Tech University, Amarillo, TX 79106, USA.

farrowing, wean-to-finish, and marketing phases to different, distinct production sites. Transportation, while essential for the industry, is a known stressor for pigs that can result in injured, non-ambulatory, or dead pigs upon arrival (DOA) to their destination. Approximately 1% of market-weight pigs are injured or lost every year due to transportation (Ritter et al., 2020), which accounts for annual economic losses of approximately \$90 million (Ritter et al., 2020).

Stressors experienced by pigs during the transportation process extend beyond the actual act of moving pigs from one location to another via semi-trailer (Goumon and Faucitano, 2017). The act of loading and unloading can be a major source of stress, likely due to several factors including human handling and the pigs' lack of experience navigating ramps or chutes required for movement onto the semi-trailer. As a result, pigs may perceive the loading and unloading process to be a greater stressor than actual transportation (Rioja-Lang et al., 2019). Therefore, methods for reducing novelty and stress associated with the loading and unloading phases of transportation are needed.

One potential method for reducing stress caused by loading and unloading during the transport process is to modify the nursery housing system by adding a ramp and platform to individual nursery pens. Early exposure to the ramp during the nursery phase may allow the pigs to become familiar with ascending and descending ramps before loading onto a trailer later in life. Animals exposed to increased environmental complexity and stimuli during early life may be better equipped to cope with future challenges encountered in their daily lives (Crofton et al., 2015). While the effects of early life exposure to increased environmental complexity on the welfare of market pigs are largely unknown, previous preliminary work conducted at North Dakota State University suggested that the addition of ramps to nursery pig housing increases speed of loading at marketing and has no negative effects on pig growth (Novak et al., 2020).

The objective of this study was to determine if exposing pigs to a ramp and platform during the nursery phase of production (approximately 3-9 weeks of age) would affect ease of loading and unloading at the time of marketing (approximately 20-24 weeks of age). Additionally, we wanted to determine if the addition of a ramp and platform in the nursery pen would affect pig behavior during the nursery phase and pig growth throughout the nursery and growfinish phases.

Materials and Methods

This study was carried out at South Dakota State University's wean-to-finish facility (Brookings, SD) from March to August 2022. At approximately 21 days of age, 540 weaned piglets were transported to the SDSU wean-to-finish facility. Upon arrival, pigs were placed in 20 pens (21 m^2) in groups of 27 pigs. Each pen had fully slatted concrete floors. One five-space dry feeder and two cup waterers were provided in each pen. Temperatures and ventilation in the facility were continuously adjusted automatically to maintain thermoneutral temperatures in the pens. Artificial lighting was provided in the facility between 8:00 AM and 7:00 PM.

Pigs were assigned to one of two experimental treatments: 1) access to a ramp in the pen during the nursery phase (RAMP; n = 10 pens; Fig. 1A) or 2) a standard pen with no access

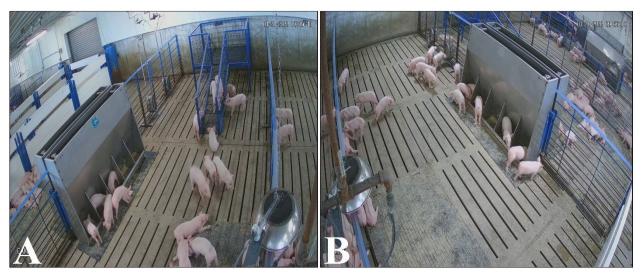


Figure 1. RAMP (A) and CONT (B) pens. The RAMP pen had a platform that pigs could access via a ramp during the nursery phase. The CONT pen had no platform and ramp during the nursery phase.

to a ramp during the nursery phase (CONT; n = 10 pens; Fig. 1B). Pigs in the RAMP treatment had access to a ramp (1.7×0.5 m, 20° angle) and resting platform ($1.1 \times 1.6 \times 0.7$ m) at the top of the ramp. Pigs in the CONT treatment were in a conventional pen without a ramp and platform. At the end of the nursery phase (i.e., on experimental day 40), the ramps were removed from the RAMP pens. All experimental pigs then remained in their conventional pen until they reached market weight at approximately 5.5 months of age.

Feed intake (FI) and body weight (BW) were determined on experimental days 0, 47 (post-nursery phase), and 135 (end of grow-finish phase) for calculation of average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (F:G). Eating, drinking, lying, standing, and aggressive behaviors were determined using video recordings collected on experimental days 1, 2, 3, 6, 19, 26, 33, and 39. On each of those days, observations were conducted for 60 min at 0800, 1200, and 1600 h using a five-minute instantaneous scan sampling method. At each scan sampling interval, the percentage of pigs in a pen performing the behaviors of interest was determined.

The loading process at marketing began on experimental day 138 and took place on six different transport days over a four-week period during August 2022. During each transportation day, four pigs from each pen (20 total groups each transport day; one group of four pigs from each pen) were loaded onto a standard pot-belly semi-truck trailer by the same experimental personnel. Each group of four pigs was required to ascend a covered load-out ramp (6.06 m long; 0.9 m wide, 11.1° incline angle). Two cameras collected the total time taken by each group of four pigs to navigate the load-out ramp. Additionally, the number of times an animal lost its footing (i.e., trips) and the frequency of electric prod usage

were recorded. Data collection began when the front limbs of the first pig in the group stepped onto the ramp and ended when the hind limbs of the last pig in a group stepped onto the trailer. If the experimental handler was not able to move individual pigs or the group up the ramp during the initial 60-sec period of loading, an electric prod was applied to the stopped animal(s) by a second, non-handling experimental personnel, according to approved Transport Quality Assurance (TQA) guidelines (National Pork Board, 2022).

Once the last pig in each group stepped onto the trailer, the truck driver (same individual for all loading days) moved the group of four pigs into one of four upper-level trailer compartments or the lower-level compartment closest to the cab of the semi-truck, where they remained throughout the remainder of the transport process. Each compartment was large enough to hold 14-19 experimental pigs. Experimental treatments were not kept separate from one another within the compartments.

After loading, all experimental pigs were transported approximately 325 km to the processing facility. Upon arrival, pigs were unloaded in variable-sized mixed treatment groups (minimum group size = 1; maximum group size = 15) by facility employees, according to company guidelines. The floor of the trailer was the same as the height of the unloading floor in the facility so no ramp was required for pigs to exit the trailer. However, since the experimental pigs were housed in four upper-compartments in the trailer and one lower-level compartment (i.e., lower-level experimental pigs were required to ascend a ramp to the upper trailer level and then descend the ramp to exit the trailer and enter the facility), the total time taken by each pig to descend the main internal ramp (1.68 m long x 1.0 m wide;

20.8° angle) in the trailer from their transport compartment to the lower level of the trailer was quantified. One camera was mounted at the top of the trailer ramp facing the entrance of the processing facility, and another camera was mounted in the processing facility where the pigs walked off the trailer. Together, the two cameras allowed experimental personnel to calculate unloading time, the number of trips, turnaround attempts by individual pigs and the number of pigs that descended the ramp backwards. Additionally, the number of times a rattle paddle was used by a handler to encourage movement and the number of pigs DOA were collected.

Data were analyzed using the MIXED and GLIMMIX procedures in SAS (v. 9.4; SAS Institute, Inc., Cary, NC, USA). Pen served as the experimental unit for all behavioral and performance measures collected during the nursery and growfinish phases, as well as time to ascend the ramp during semi-trailer loading. Individual pig served as the experimental unit for time to descend the ramp at unloading (since pigs were housed on the trailer and unloaded in mixed groups). A chi-square test was used with the FREQ procedure to determine whether differences in treatment occurred for the incidence of trips (loading and unloading), electric prod application (loading only), rattle paddle application (unloading only), turnarounds on the ramp (unloading only), pigs descending the ramp backwards (unloading only), and DOAs (unloading only). A P-value of less than 0.05 was used as the level of significance in all models.

Results

No differences between treatments were detected for eating (CONT: 2.30 ± 0.13 vs. RAMP 2.20 ± 0.13 %; P = 0.48), drinking (CONT: 0.48 ± 0.06 vs. RAMP 0.53 ± 0.07 %; P = 0.52), aggressive (CONT: 0.79 ± 0.12

vs. RAMP 0.79 ± 0.12 %; P = 0.98) or postural behaviors (lying: P = 0.34; standing: P = 0.60) during the nursey period.

No differences between treatments were detected for BW (58.9 vs. 59.5 ± 0.9 lbs; P = 0.33), ADFI (1.23) vs. 1.23 ± 0.02 lbs/d; P = 0.91), ADG $(0.77 \text{ vs. } 0.77 \pm 0.02 \text{ lbs/d}; P = 0.97),$ or F:G (1.66 vs. 1.58 ± 0.01 ; P = 0.44) on experimental day 47. Similarly, no differences between treatments were detected for BW (253.1 vs.251.8 \pm 2.91.3 lbs; P = 0.95), ADFI (4.76 vs. $4.56 \pm 0.09 \, \text{lbs/d}$; P = 0.16), ADG $(2.16 \text{ vs. } 2.16 \pm 0.04 \text{ lbs/d}; P = 0.77),$ or F:G (2.18 vs. 2.10 ± 0.04 ; P = 0.18) on experimental day 135. Taken together, the presence of the ramp and platform in the nursery pen had no negative effect on pig behavior or performance during their respective measurement periods.

Pigs in the RAMP treatment exhibited a shorter time to ascend the loading ramp compared to CONT pigs (P = 0.002; Fig. 2). Additionally, RAMP pigs required fewer electric prod applications compared to CONT pigs (20 vs. 33 instances; P = 0.02). No treatment differences were observed for trips or turnarounds while ascending the ramp (P > 0.05). Time to ascend the ramp was affected

by transport day (P = 0.004; data not shown), where time to ascend was greater on transport day 1 compared to transport days 3, 4, 5, and 6 (data not shown). Time to ascend the ramp was also greater on transport day 2 compared to transport day 6 (data not shown). The differences in time to ascend the ramp may be due to the experience of the experimental human handler as the experiment went on. However, we are not able to adequately determine the underlying cause of this result. No interaction between time to ascend the ramp and transport day was detected (P > 0.05).

CONT pigs descended the ramp slightly, but not meaningfully, faster than RAMP pigs (12.7 vs. 13.9 seconds; P = 0.03). Additionally, RAMP pigs descended the ramp backwards more often compared to CONT pigs (10 vs. 2 instances; P = 0.02). Our data do not provide a meaningful explanation for this behavior. However, descending the ramp backwards may be an attempt to avoid adverse situations (e.g., unfamiliar handlers) during unloading. The ability of an animal to successfully descend the ramp backwards may be beneficial for reducing ramp-related injuries. No differences in trips, turnaround,

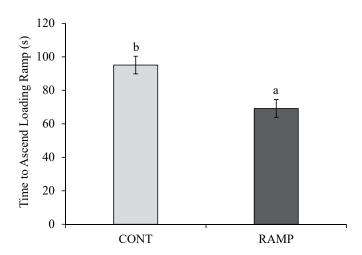


Figure 2. Time required for market-weight pigs to ascend a loading ramp to be transported for market according to experimental treatment. Different superscripts indicate significant differences (P < 0.05).

or rattle paddle application were detected between treatments during the unloading process (P > 0.05).

In conclusion, ramp and platform exposure during the nursery phase of production improves ease of loading at market weight, which is beneficial for the producer (i.e., faster loading) and the pig (i.e., reduced ramp novelty). Ramp exposure had no negative effects on behavior during the nursery period. Similarly, growth performance was not affected by ramp exposure during the nursery or grow-finish period. Future work on this topic should determine best practices for ramp design and exposure timing to improve practicality of application in a commercial system. Additionally, more work is needed to determine the effects of early life ramp exposure on physiological stress during the transportation process and meat quality at marketing.

References

Crofton, E.J., Zhang, Y., Green, T.A., 2015. Inoculation stress hypothesis of environmental enrichment. Neurosci. Biobehav. Rev. 49:19–31.

Goumon, S., and L. Faucitano. 2017. Influence of loading handling and facilities on the subsequent response to pre-slaughter stress in pigs. Livest. Sci. 200:6–13.

Novak, B. L., J. M. Young, D. J. Newman, A. K. Johnson, and S. A. Wagner. 2020. A ramp in nursery housing affects nursery pig behavior and speeds loading of market hogs. Appl. Anim. Sci. 36:574–581.

Rioja-Lang, F. C., J. A. Brown, E. J. Brockhoff, and L. Faucitano. 2019. A Review of Swine Transportation Research on Priority Welfare Issues: A Canadian Perspective. Front. Vet. Sci. 6:36.

Ritter, M. J., C. L. Yoder, C. L. Jones, S. N. Carr, and M. S. Calvo-Lorenzo. 2020. Transport losses in market weight pigs: II. U.S. incidence and economic impact. Transl. Anim. Sci. 4:1103–1112.

Columbia ram performance testing and certification: 2023-2024 Dakota Ram Test

Rachel Gibbs¹, Christopher Schauer¹ and Jaelyn Whaley²

The Dakota Ram Test is a multistate ram performance testing program that evaluates ram wool and growth performance under centralized management. Data generated from this test serves as valuable selection tools to help producers identify rams with superior wool and/or growth performance. Columbia rams that meet the criteria outlined by the Columbia Sheep Breeders Association (CSBA) are eligible for designation as Certified Columbia Rams.

Summary

Columbia sheep producers throughout the northern Great Plains utilize the Dakota Ram Test to generate performance data that can be used for ram selection. This centralized performance test quantifies several economically important and/or heritable traits that producers can evaluate when selecting rams or genetic lineages to retain in their flocks. Fourteen Columbia rams were consigned to the 2023-2024 Dakota Ram Test. Of these, 21% met the CSBA criteria for Certified Columbia Ram designation.

Introduction

The Dakota Ram Test is a 140-day ram performance test that evaluates differences in ram wool and post-weaning growth performance under the same management conditions, nutritional plane, and climate. The CSBA certifies rams that excel in growth performance, carcass quality

¹NDSU Hettinger Research Extension Center

²SDSU Extension Lemmon Regional Center and wool quality through the program initiated in 2017 to promote overall breed improvement.

Procedures

Fourteen spring-born registered Columbia rams were consigned by eight producers and received by the HREC on or before September 17, 2023. Before the test period, rams were evaluated by the Dakota Ram Test committee, and scores for face wool covering and belly wool expansion were recorded. Scores were assigned on a four-unit basis (1-4), with higher scores representing a greater degree of wool covering or expansion. To determine average

daily gain (ADG), initial bodyweight was recorded at the beginning of the testing period (Sept. 21, 2023), every 28 days and at the end of the testing period (Feb. 8, 2024). Rams were shorn, staple length was measured and wool samples were collected on February 9, 2024. Staple length was determined by averaging the length of wool at the shoulder, side and britch, and then was adjusted to estimate 365day staple length (Adj. STL). Wool samples were sent to Texas A&M University for clean-fleece weight and fiber diameter (micron) analyses. Clean-fleece weight was determined from laboratory-scoured clean yield estimates and adjusted to estimate 365-day clean-fleece weight (Adj. CL FL) production. A real-time carcass ultrasound at the end of the testing period estimated ribeye area and fat cover between the 12th and 13th ribs. Ribeye area was adjusted to account for differences in ram bodyweight and reported as inches per 100 pounds. The criteria for CSBA certification as Certified Columbia Rams are in Table 1.

Table 1. CBSA Criteria for Certified Ram Designation

Criteria	Requirement
Fiber Diameter	Within 22.05 & 27.84 microns
A di Ctamba I amath	≥ 4.3 inches if fiber diameter is within 22.05 & 24.94 microns
Adj. Staple Length	≥ 4.8 inches if fiber diameter is within 24.95 & 27.84 microns
Average Daily Gain	≥ 0.80 pounds per day
Adj. Ribeye Area	≥ 1.3 inches per 100 pounds of bodyweight
Face Wool Score	≤3
Belly Wool Score	1
Scrapie Resistance Genotype	RR or QR at Codon 171

Results and Discussion

Fourteen Columbia rams completed the 2023-2024 Dakota Ram Test. Rams averaged 0.84 pounds of gain per day over the 140-day test period and gained an average of 118 pounds. Columbia ram fleeces averaged 9.19 pounds of 365-day adjusted clean wool, a 25.95-micron fiber diameter and a 4.6-inch 365-day adjusted staple length. Average ribeye area was 2.81 square inches, and average fat depth was 0.38 inches. Three rams met all the requirements to qualify for CSBA Certified Columbia Ram designation (Table 2). Of the remaining 11 rams, two rams did not meet the fiber diameter

requirement, one ram did not meet the scrapie genotype requirement (lack of test), four rams did not meet the ADG requirement, eight rams did not meet the adjusted ribeye area requirement, 10 rams did not meet the staple length requirement and one ram did not meet the belly wool score requirement. Despite this, Columbia rams produced in the northern Great Plains are improving, as evidenced by an increased certification rate (21%) in the 2023-2024 Dakota Ram Test. This is a 7% higher certification rate than in 2023, a 14% higher rate than in 2022 and only 1% lower than the highest percentage of certification at the Dakota Ram Test since guidelines were outlined by the CSBA in 2017.

Acknowledgments

The authors express their appreciation to Dave Pearson, Hettinger Research Extension Center shepherd and Dakota Ram Test manager, for his hard work and dedication to the Dakota Ram Test program.

Table 2. Ram Performance and Certification Summary

		Fiber Diameter	Adj. STL	140-d ADG	Adj. REA	Belly Score	Face Score	Codon 171	
Ear Tag	Reg. #	(micron)	(in)	(lb/d)	(in/100lb)	(pt)	(pt)	Genotype	Certified?
Y-4	Y20457	24.05	3.7*	0.99	1.05*	1.00	1.25	QR	N
Y-6	Y20443	24.23	5.0	0.86	1.50	1.00	1.75	RR	Y
Y-5	Y20442	24.35	5.1	1.06	1.30	1.00	1.00	QR	Y
Y-3	Y20456	24.73	4.23*	1.11	0.98*	1.00	1.00	QR	N
Y-9	Y18973	25.13	4.9	0.85	1.54	1.00	1.50	QR	Y
Y-12	Y20446	25.58	4.1*	0.86	1.03*	1.00	1.75	RR	N
Y-1	Y20471	25.63	4.7*	0.88	1.05*	1.00	1.50	QR	N
Y-14	Y19968	25.93	4.7*	0.65*	1.66	1.00	1.00	RR	N
Y-11	Y20445	26.13	5.3	0.64*	1.18*	1.00	2.50	QR	N
Y-10	Y19705	26.23	4.7*	0.76*	1.41	4.00*	1.25	RR	N
Y-13	Y19969	26.78	4.7*	0.72*	1.12*	1.00	1.25	NT*	N
Y-7	Y20441	27.13	4.4*	0.81	1.23*	1.00	1.00	QR	N
Y-2	Y20472	28.23*	4.6*	0.68	0.91*	1.00	1.50	RR	N
Y-8	Y20461	29.28*	4.5*	0.94	1.17*	1.00	1.25	QR	N

Double Line = Fiber diameter separation for staple length requirement

ADG, average daily gain; Adj. STL, adjusted staple length, Adj. REA, adjusted ribeye area

^{* =} Does not meet the certification requirement

Rambouillet ram performance testing and certification: 2023-2024 Dakota Ram Test

Rachel Gibbs1, Christopher Schauer1 and Jaelyn Whaley2

The Dakota Ram Test is a multistate ram performance testing program that evaluates ram wool and growth performance under centralized management. Data generated from this test serves as valuable selection tools to help producers identify rams with superior wool and/or growth performance. Rams are ranked by a productive index, and the top 30% are eligible for designation as Certified Rams as part of the American Rambouillet Sheep Breeders Association (ARSBA) Register of Merit (ROM) program.

Summary

Sheep producers throughout the northern Great Plains utilize the Dakota Ram Test to generate performance data on Rambouillet rams. This centralized performance test measures several economically important and/or heritable traits that producers can evaluate when selecting rams or genetic lineages to retain in their flocks. Forty-two Rambouillet rams completed the performance test at the Hettinger Research Extension Center (HREC) Sept. 21, 2023, to Feb. 8, 2024. Fourteen rams had index scores in the top 30% of the performance test, and eight of them met the additional requirements for ARSBA Register of Merit.

Introduction

The Dakota Ram Test is a 140-day ram performance test that evaluates differences in ram wool and post-weaning growth performance under the same management conditions, nutritional plane and climate. The ARSBA recognizes high-performing Rambouillet rams participating in the Dakota Ram Test as Certified Rams in the ARSBA ROM Program, which can serve as a value-added marketing strategy.

Procedures

Forty-six spring-born registered Rambouillet rams were consigned by 12 producers and received by the HREC on or before Sept. 17, 2023. Before the test period, rams were evaluated for adherence to breed standards by the Dakota Ram Test committee, and scores for face wool, belly wool and wrinkle/skin fold (post-shearing) were collected. Scores were assigned on a four-unit basis (1-4), with higher scores representing a greater degree of wool covering or skin folding. To determine average daily gain (ADG), initial bodyweight

was recorded when the testing period began (Sept. 21, 2024), every 28 days and at the end of the testing period (Feb. 8, 2024). Rams were then shorn, staple length was measured and wool samples were collected on Feb. 9, 2024. Staple length was determined by averaging the length of wool at the shoulder, side and britch, and then was adjusted to estimate 365day staple length (Adj. STL). Wool samples were sent to Texas A&M University for clean-fleece weight and fiber diameter (micron) analyses. Clean-fleece weight was determined from laboratory-scoured clean yield estimates and adjusted to estimate 365-day clean-fleece weight (Adj. CL FL) production. A real-time carcass ultrasound also was performed at the end of the testing period to estimate ribeye area and fat cover between the 12th and 13th ribs. Ram performance was estimated utilizing the approved productive index formula for the ARSBA's ROM program. This index includes adjustments for fiber diameter and fiber diameter variability, with positive scores indicating fleeces with a finer fiber diameter and reduced fiber diameter variability.

Index Score: 60*(ADG) + 4*(Adj. STL up to 5.5") + 4*(Adj. CL FL) +/- Fiber Diameter Adjustment +/-Variability Adjustment

Fiber Diameter Adjustment (-6 to 9 points available): Positive Adjustment when Fiber Diameter < 22 microns = 3*(22 – Fiber Diameter). Negative Adjustment when Fiber Diameter >22 microns = -3*(Fiber Diameter – 22)

¹NDSU Hettinger Research Extension Center

²SDSU Extension Lemmon Regional Center

Variability Adjustment (-5 to 5 points available): Positive Adjustment when Coefficient Variation < 22% = 1.25*(22 – Coefficient Variation). Negative Adjustment when Coefficient Variation > 22% = 1.25*(22 – Coefficient Variation)

Rams were ranked by index score, and the top 30% were

eligible for certification. Additional requirements for certification include: ADG \geq 0.55lb/d, Adj. CL FL \geq 9lb, Adj. STL \geq 4", core fiber diameter \leq 23.77 microns, face wool score \leq 2.7pt, wrinkle/skin fold score \leq 2.5pt, and QR or RR Codon 171 genotype for scrapie resistance.

Results and Discussion

Forty-two rams completed the 2023-2024 Dakota Ram Test following the removal of four rams by the Dakota Ram Test committee due to skeletal abnormalities inconsistent with the breed standard. Ram index scores ranged from 91.57 to 138.76 points and averaged 115.18 points (Table 1). Index scores of rams within

Table 1. Ram Performance Index Score Summary

		140-d	Adj-STL	CL FL	Adjust	ments	Index	Index
Ear Tag	Reg. #	ADG (lb/d)	(in)	(lb)	Dia. (pt)	Var. (pt)	Score (pt)	Ratio
W-2	1000855	1.08	5.3	13.40	-1.20	0.59	138.76	120%
W-13	1000883	1.00	5.2	11.27	2.18	5.00	132.98	115%
W-7	1000877	0.93	5.2	12.19	1.50	5.00	131.70	114%
W-6	1000876	0.92	5.3	13.92	-6.00	5.00	131.35	114%
W-16	1000985	1.04	4.7	14.56	-6.00	-2.97	130.82	114%
W-9	1000879	0.99	5.5	12.02	-5.10	5.00	129.11	112%
W-34	1000909	0.90	5.1	13.87	-6.00	5.00	128.85	112%
W-36	1000911	0.88	5.2	12.95	-3.23	3.94	125.95	109%
W-1	1000854	1.04	5.3	11.81	-5.93	0.94	125.88	109%
W-19	1000864	0.92	5.3	11.71	0.45	2.19	125.83	109%
W-39	1000914	0.86	5.5	11.84	-0.60	5.00	125.63	109%
W-15	1000984	0.92	5.4	12.81	-2.33	-4.25	121.71	106%
W-43	1000967	0.95	5.4	10.82	-3.23	2.28	121.06	105%
W-11	1000881	0.84	4.7	10.90	1.80	4.44	119.41	104%
W-40	1000952	0.74	5.3	0.04	-0.97	5.00	118.06	103%
W-14	1000884	0.91	5.0	0.05	-0.97	2.22	117.83	102%
W-26	1000872	0.76	5.0	0.04	-6.00	5.00	117.09	102%
W-27	1000921	0.83	5.2	0.04	-3.30	2.56	115.36	100%
W-23	1000932	0.97	5.3	0.05	-2.70	2.03	115.10	100%
W-37	1000912	0.85	5.2	0.04	1.13	4.09	114.67	100%
W-12	1000882	0.84	5.0	0.04	-3.08	5.00	114.56	99%
W-44	1000968	0.87	5.1	0.04	-3.83	5.00	114.53	99%
W-35	1000910	0.79	4.6	0.04	4.05	3.84	114.04	99%
W-5	1000875	0.79	5.1	0.04	-2.18	3.66	113.76	99%
W-38	1000913	0.67	5.3	0.04	2.85	4.13	113.47	99%
W-21	1000931	0.90	5.1	0.05	-6.00	1.13	112.17	97%
W-20	1000866	0.72	5.5	0.04	-4.88	-1.50	111.62	97%
W-24	1000929	0.94	4.1	0.04	-3.75	1.22	111.43	97%
W-28	1000922	0.89	4.3	0.04	1.65	2.91	110.03	96%
W-45	1000895	0.87	4.5	0.04	-2.18	3.44	109.56	95%
W-17	1000861	0.81	4.8	0.04	-6.00	5.00	108.99	95%
W-31	1000906	0.77	4.9	0.04	4.50	5.00	107.89	94%
W-25	1000871	0.89	4.7	0.04	-6.00	5.00	102.06	89%
W-41	1000953	0.76	4.1	0.03	-5.63	-0.41	101.84	88%
W-32	1000907	0.63	5.2	0.03	3.60	5.00	101.62	88%
W-10	1000880	0.84	4.1	0.03	-6.00	5.00	99.99	87%
W-18	1000863	0.89	4.0	0.04	-5.40	-1.66	96.03	83%
W-22	1000930	0.57	4.8	0.03	-0.68	2.53	90.41	78%

Double Line = Top 30% Cutoff

ADG, average daily gain; Adj. STL, adjusted staple length, Adj. CL FL, adjusted clean fleece

the top 30% ranged from 119.41 to 138.76 points. Of the rams scoring in the top 30%, eight of 14 met the additional requirements for ARSBA Certified Ram (Table 2). Six of the 14 index-eligible rams were ineligible for certification due to the fiber diameter requirement (five rams) or face wool cover score requirement (one ram). Growth performance was consistent with rams consigned to previous performance tests at the HREC. Rams averaged 97 pounds at the start of the

performance test period and gained, on average, 0.84 pounds per day over 140 days, averaging 216 pounds at the end of the test period. Carcass data presented in Table 3 is not included as part of the productive index but provides producers insight into ram growth and maturity patterns. Rams with larger ribeye areas indicate greater muscling and increased growth patterns, while rams with greater fat cover may indicate greater maturity.

Table 2. Eligibility for Certified Ram Designation

		Codon 171	Index Score	140-d ADG	Adj. STL	Adj. CL-FL	Belly Score	Face Score	Skin Score	Core	
Ear Tag	Reg. #	Genotype	(pt)	(lb/d)	(in)	(lb)	(pt)	(pt)	(pt)	Micron	Certified?
W-2	1000855	RR	138.76	1.08	5.3	13.40	1.00	1.00	1.00	22.40	Y
W-13	1000883	RR	132.98	1.00	5.2	11.27	1.00	1.00	1.00	21.28	Y
W-7	1000877	RR	131.70	0.93	5.2	12.19	1.00	1.00	1.00	21.50	Y
W-6	1000876	RR	131.35	0.92	5.3	13.92	1.00	1.00	1.00	24.60*	N
W-16	1000985	QR	130.82	1.04	4.7	14.56	1.00	1.50	2.00	25.95*	N
W-9	1000879	RR	129.11	0.99	5.5	12.02	1.00	1.50	1.25	23.70	Y
W-34	1000909	RR	128.85	0.90	5.1	13.87	1.00	1.25	1.25	24.15*	N
W-36	1000911	RR	125.95	0.88	5.2	12.95	1.00	1.00	1.00	23.08	Y
W-1	1000854	RR	125.88	1.04	5.3	11.81	1.00	1.00	1.00	23.98*	N
W-19	1000864	RR	125.83	0.92	5.3	11.71	1.00	1.00	1.00	21.85	Y
W-39	1000914	RR	125.63	0.86	5.5	11.84	1.00	1.00	1.00	22.20	Y
W-15	1000984	RR	121.71	0.92	5.4	12.81	1.00	3.00*	1.25	22.78	N
W-43	1000967	RR	121.06	0.95	5.4	10.82	1.00	1.25	1.25	23.08	Y
W-11	1000881	RR	119.41	0.84	4.7	10.90	1.00	1.00	1.50	21.40	Y

^{* =} Does not meet certification requirement

ADG, average daily gain; Adj. STL, adjusted staple length, Adj. CL FL, adjusted clean fleece

Table 3. Ram Growth and Carcass Performance

Ear Tag	Reg. #	REA (sq. in.)	Fat Depth (in.)	Initial BW (lb)	Final BW (lb)	Gain (lb)	140-d ADG (lb/d)
W-2	1000855	3.75	0.52	85	236	151	1.08
W-1	1000854	4.00	0.34	99	245	146	1.04
W-16	1000985	3.72	0.35	100	246	146	1.04
W-13	1000883	3.30	0.27	96	236	140	1.00
W-9	1000879	3.44	0.29	92	230	138	0.99
W-23	1000932	2.81	0.19	83	219	136	0.97
W-43	1000967	3.18	0.27	103	236	133	0.95
W-24	1000929	2.99	0.25	94	226	132	0.94
W-7	1000877	3.75	0.29	109	239	130	0.93
W-6	1000876	3.36	0.41	101	230	129	0.92
W-15	1000984	2.76	0.25	74	203	129	0.92
W-19	1000864	2.84	0.31	79	208	129	0.92
W-14	1000884	3.75	0.37	96	223	127	0.91
W-21	1000931	2.36	0.38	70	196	126	0.90
W-34	1000909	3.04	0.35	113	239	126	0.90
W-25	1000871	2.98	0.33	96	221	125	0.89
W-18	1000863	3.41	0.16	72	196	124	0.89
W-28	1000922	3.52	0.23	85	209	124	0.89
W-36	1000911	2.84	0.35	121	244	123	0.88
W-44	1000968	2.79	0.33	104	226	122	0.87
W-45	1000895	2.62	0.31	106	228	122	0.87
W-39	1000914	3.04	0.29	119	240	121	0.86
W-37	1000912	2.87	0.31	99	218	119	0.85
W-11	1000881	3.12	0.29	97	215	118	0.84
W-10	1000880	4.05	0.21	102	219	117	0.84
W-12	1000882	3.43	0.21	103	220	117	0.84
W-27	1000921	3.16	0.29	86	202	116	0.83
W-17	1000861	2.59	0.33	112	226	114	0.81
W-5	1000875	2.67	0.23	93	204	111	0.79
W-35	1000910	2.68	0.27	80	190	110	0.79
W-31	1000906	2.68	0.29	104	212	108	0.77
W-26	1000872	2.87	0.27	125	231	106	0.76
W-41	1000953	3.33	3.44	106	212	106	0.76
W-40	1000952	2.03	0.21	102	205	103	0.74
W-42	1000966	2.79	0.19	102	205	103	0.74
W-8	1000878	3.61	0.40	93	195	102	0.73
W-20	1000866	2.53	0.21	90	191	101	0.72
W-29	1000904	3.18	0.33	102	201	99	0.71
W-38	1000913	2.74	0.27	102	196	94	0.67
W-32	1000907	2.64	0.23	100	188	88	0.63
W-22	1000930	2.31	0.21	92	172	80	0.57
W-33	1000908	2.85	0.25	118	186	68	0.49

REA, ribeye area; BW, bodyweight; ADG, average daily gain

Estradiol-induced labor in periparturient ewes: Gene expression changes in maternal reproductive tissues and impact on lamb birth characteristics

Bethania J. Davila Ruiz¹, Wellison J.S. Diniz², Priyanka Banerjee², Carl R. Dahlen¹, Pawel P. Borowicz¹, Chutikun Kanjanaruch¹, Alan J. Conley³ and Lawrence P. Reynolds¹

Our study explored the effects of administering estradiol (E2) on the timing of labor in periparturient ewes and the survival and development of their lambs. We focused on how E2 alters gene expression in critical reproductive tissues, including the myometrium, endometrium, cervix and maternal placenta, and its effects on lamb weight and vigor. Our findings indicated that E2 can quickly and effectively induce labor primarily by altering gene expression in the cervix, where E2 promotes inflammation and tissue remodeling necessary for birth. No adverse effects were observed on the offspring. These findings suggest that E2 could provide livestock producers with a reliable method for managing the timing of lambing without increasing the risk of lamb mortality.

Summary

We investigated the effects of exogenous estradiol (E2) on labor initiation in periparturient ewes and its effects on offspring development. Two experiments were conducted: Exp.1 assessed the effect of E2 on birth timing, lamb birth weight and lamb vigor, while Exp.2 examined changes in gene expression in maternal reproductive tissues (myometrium, endometrium, cervix and placenta). Multiparous ewes (139-142 days of gestation)

¹Department of Animal Sciences and Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND USA 58108-6050 ²Department of Animal Sciences, Auburn

University, Auburn, AL USA 36849

³Department of Population Health and Reproduction, School of Veterinary Medicine, UC-Davis, CA 95616

were randomly assigned to receive Silastic® implants containing E2 (300 mg in Exp. 1, 200 mg in Exp. 2, E group) or empty implants (Control, C group). Implants were removed two days after parturition (Exp.1) or 26 hours after treatment (Exp.2). Maternal jugular blood samples were taken to measure E2 and progesterone (P4) concentrations, and tissue samples were collected for gene expression (RNA-Seq) analysis. Results indicated that E2 concentrations in maternal blood were similar between groups before treatment. However, E2 concentration was greater in both experiments in the E compared with the C group after treatment. No differences in P4 concentrations between the E and C groups were observed. In addition, E2 shortened labor (2.84±1.02 in E vs. 7.18 ± 1.1 days in C, P = 0.01)

without affecting lamb characteristics. Furthermore, E2 treatment affected gene expression across all the tissues tested ($P \le 0.10$), with the most potent effects in the cervix, with 8,073 differentially expressed genes in the E compared with the C group. Our findings suggest that E2 induces labor by altering gene expression, particularly in the cervix, where it upregulates genes associated with inflammation and cervical ripening and downregulates genes linked to smooth muscle contraction and tissue integrity. Additionally, E2 had no adverse effects on the offspring, offering livestock producers a potential tool for managing the timing of lambing and improving reproductive outcomes.

Introduction

Parturition is a complex and poorly understood process involving the development of the fetal organs, activation of myometrial contractions, cervical ripening and dilation, fetal membrane rupture, fetal delivery and placental expulsion (Jenkin and Young, 2004). These events should occur synchronously, and each of them is critical for successful delivery (Mesiano, 2001). Among the pathways involved and the various factors identified, the role of steroid hormones, specifically E2 and P4, has emerged as particularly significant in determining the timing of labor onset (Shuler et al., 2018). An increase in estrogenic actions has been

linked with regulating myometrial contractility and cervical ripening, whereas P4 promotes uterine relaxation and inhibits spontaneous contractions and the onset of labor (Pastore et al., 2012; Solano and Arck, 2020).

While most pregnancies end at term, 10% of neonatal losses in U.S. livestock are caused by preterm births (Wu et al., 2006), which frequently have unclear causes. This postpartum loss represents a significant burden on the investment in breeding, nutrition and veterinary care that goes into each birth. Furthermore, managing the timing of lambing is a critical aspect of sheep farming, directly affecting both the ewe's health and the lambs' survival. Traditionally, the unpredictable onset of labor poses challenges for livestock producers, making it difficult to optimize care and resources.

Hormonal interventions that safely and effectively induce labor could allow producers to schedule lambing more precisely. This ability to program delivery would improve animal health outcomes, streamline farm operations, enhance time management and reduce the workload during the busy lambing season. Although E2 is known to be crucial for labor, little is known about how it affects gene expression in reproductive tissues and affects the timing of delivery. Our study aimed to fill that gap by examining the alterations in gene expression of

myometrial, endometrial, cervical and placental tissues after treatment with exogenous E2, as well as the impact on the timing of labor and the characteristics and viability of the offspring.

Experimental Procedures

Two experiments were conducted utilizing the same approach but with varying dosages to evaluate the effect of E2 on birth timing and offspring characteristics (Experiment 1) and gene expression (Experiment 2) on reproductive tissues (myometrium, endometrium, cervix, and placenta). Multiparous Rambouillet ewes between 139 and 142 days of gestation were randomly assigned to either the exogenous estradioltreated group (E), which received six Silastic[®] implants containing 50 mg of E2 in Exp.1 (300 mg/ewe; n=12) and four implants in Exp.2 (200 mg/ewe; n=6); or the control (C) group, which received six empty Silastic® implants in Exp.1 (n=12) and four empty implants in Exp.2 (n=6). Implants were inserted subcutaneously in the axillary region of the ewe and removed two days after parturition (Exp.1) or at the time of slaughter (26 hours after treatment; Exp.2). Maternal jugular vein blood samples were collected one day before and at different time points after treatment in Exp.1 or at 26 hours after treatment in Exp.2 to measure circulating concentrations of E2 and P4 using immunoassay.

In Experiment 1, ewes were treated, and their time to delivery after treatment, live lamb birth weight and lamb vigor were recorded. Lamb vigor was assessed using a scale ranging from 0 to 4, where 0 represents extreme activity and vigor with the lamb standing on all four feet, 1 indicates high activity with the lamb standing on its back legs and knees, 2 represents moderate activity with the lamb active on its chest and holding its head up, 3 indicates weakness with the lamb lying flat but still able to hold its head up, and 4 indicates severe weakness with the lamb unable to lift its head and showing minimal movement. In Experiment 2, ewes were treated and slaughtered 26 h later for tissue collection; cross sections of the myometrium, endometrium, caruncles and cervix were collected for RNA-Seq analysis. After RNA-Seq data quality control and mapping, differentially expressed genes (DEGs) were identified using DESeq2. Statistical significance (P < 0.05 in Exp.1 and $P \le 0.1$ and log2 fold change | 0.5 in Exp.2) was assessed using the MIXED procedure of SAS for both experiments.

Results and Discussion

In Experiment 1, differences were observed in E2 concentrations in maternal blood at various time points, with the E group generally having higher concentrations than the C group, especially at +8 h and

Table 1. Maternal E2 and P4 levels before and after treatment, Exp.1 and Exp.2

					Exp.2		
Hormone	Treatment	Day -1	+8h	Day +1	Post-partum*	Birth +2 d	+ 26h
E2 (pg/ml)	E C p-value	12.18 ± 1.98 16.76 ± 2.25 0.38	192.91 ± 17.32 A 10.97 ± 20.47 B <0.001	108.15 ± 9.25 A 20.31 ± 10.93 B <0.001	92.39 ± 16.06 63.54 ± 19.31 0.26	22.86 ± 3.4 A 1.6 ± 3.43 B <0.001	149.21 ± 55.93 A 30.61 ± 11.73 B <0.01
P4 (ng/ml)	E C p-value	9.31 ± 1.43 8.84 ± 1.73 >0.8	9.1 ± 1.24 9.02 ± 1.46 > 0.96	7.42 ± 1.2 9.6 ± 1.42 >0.18	0.94 ± 0.29 0.64 ± 0.32 >0.48	0.2 ± 0.05 0.2 ± 0.05 =1	6.50 ± 1.42 8.99 ± 1.42 >0.24

^{*}Postpartum samples were taken within 8 hours after parturition. The time from treatment to parturition varied between groups (see table 2). AB Indicates significant differences between E2 and C at a particular time point, P<0.05.

Day +1, which demonstrates the effectiveness of the E2 treatment. The blood concentrations of P4 did not differ between treatment groups, suggesting that elevated E2 alone can trigger labor even without changes in P4 concentrations, as the average delivery time after treatment was 2.84 ± 1.02 d (Table 2).

As shown in Table 2, in Exp.1 timing of parturition was influenced by treatment. The E group significantly reduced the days from treatment to delivery. (the average gestation length in this flock of Rambouillet ewes was 147 d). No differences were observed in lamb birth weight or vigor.

As shown in Fig. 1, E2 treatment affected gene expression in all the tissues tested (p-value ≤ 0.1 and log2 fold change |0.5|). The cervix exhibited the strongest effect, with 8,073 DEGs, including

E2 upregulation of prostaglandinendoperoxide synthase 2 (PTGS-2), prostaglandin E synthase 2 (PTGES2), estrogen receptor 2 (ESR2), and interleukin 6 (IL-6), and downregulation of interleukin 10 (IL-10), alpha-smooth muscle actin (ACTA2), myosin heavy chain 11 (MYH11), gap junction protein Alpha 1 (GJA1), and tissue inhibitors of metalloproteinases (TIMPs). The endometrium had 722 DEGs, including E2 upregulation of OXTR and ESR1 and downregulation of SULT1E1 and TIMPs. The caruncles had a more modest response, with 109 DEGs, while the myometrium was the least responsive to the treatment, with 90 DEGs, including E2 upregulation of *PTGS*-2.

Our findings suggest that E2 causes changes in gene expression in the maternal reproductive tissues, leading to parturition, but without

a decline in circulating levels of P4 in maternal blood. These effects appear to be primarily driven by the alterations in the cervix, where E2 upregulated genes associated with inflammation and cervical ripening, and downregulated genes associated with smooth muscle contraction and tissue integrity.

Furthermore, E2-induced labor did not compromise offspring health, as the lamb birth weight or vigor did not change. These findings are significant for the livestock industry as they offer a potential tool for managing the timing of lambing, which can help reduce the risks associated with unpredictable labor. By controlling birth timing, producers can ensure that lambing occurs under optimal conditions, improving the health outcomes for both ewes and lambs.

Acknowledgments

This research was supported by the USDA NIFA AFRI grant 2021-67015-34277 to Drs. Reynolds and Conley. We also thank the Departments of Animal Sciences at North Dakota State University and Auburn University for their important contributions.

Literature Cited

Jenkin, G. and I. R. Young. 2004. Mechanisms responsible for parturition; the use of experimental models. Anim. Reprod. Sci. 82–83:567– 581

Mesiano, S. 2001. The endocrinology of parturition. Basic science and clinical application. Front. Horm. Res.27:1-18

Schuler, G., Fürbass, R., & Klisch, K. 2018. Placental contribution to the endocrinology of gestation and parturition. Anim. Reprod. 15:822-829.

Pastore, M. B., Jobe, S. O., Ramadoss, J., & Magness, R. R. 2012. Estrogen receptor-β and estrogen receptor-β in the uterine vascular endothelium during pregnancy: Functional implications for regulating uterine blood flow. Semin. Reprod. Med. 30:46-61,

Solano, M. E., and P. C. Arck. 2020. Steroids, pregnancy, and fetal development. Front. Immun. 10:3017.

Table 2. Experiment 1: Time to delivery, lamb birth weight and lamb vigor

Treatment	Days from Treatment to Delivery	Lamb Birth Weight (Kg)	Lamb Vigor (0-4 scale) *
E	$2.84 \pm 1.02 \mathrm{A}$	4.94 ± 0.20	0.3 ± 0.14
C	$7.18 \pm 1.11 \text{ B}$	4.99 ± 0.19	0.09 ± 0.13
p-value	< 0.01	> 0.84	>0.3

^{*} Lamb vigor was assessed using a scale ranging from 0 to 4; see the text for an explanation.

Numbers of differentially Expressed Genes E2 Treatment

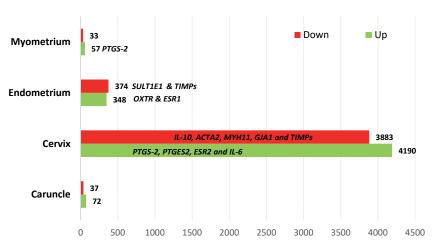


Figure 1. Exp.2: Differentially expressed genes by E2 treatment

 $A\hat{B}$ indicates significant differences between E2 and C, p<0.05.

Discovering performance and value in North Dakota calves: 2023-2024 Dakota Feeder Calf Show Feedout

Karl Hoppe¹, Colin Tobin¹ and Dakota Feeder Calf Show Livestock Committee²

The Dakota Feeder Calf Show is a feedout project to help North Dakota producers identify cattle with superior growth and carcass characteristics. Average difference in profitability between consignments from the top five herds and the bottom five herds was \$205.89 per head for the 2023-2024 feeding period.

Summary

North Dakota cattle producers use the Dakota Feeder Calf Show feedout project to discover the actual value of their spring-born beef steer calves, provide comparisons among herds, and benchmark feeding and carcass performance. Cattle consigned to the feedout project were delivered to the Carrington Research Extension Center livestock unit on Oct. 21, 2023. After a 222-day feeding period with 0% death loss, cattle averaged 1,342.3 pounds (shrunk harvest weight). Feed required per pound of gain was 6.5 pounds (dry matter basis). Overall pen average daily gain was 3.35 pounds. Feed cost per pound of gain was \$0.712, and total cost per pound of gain was \$1.02. Profit ranged from \$338.15 per head for pen-of-three cattle with superior growth and carcass traits to \$14.30 per head. The variability between producers' herds is substantial when discovering the feeding and carcass value of springborn calves.

Introduction

Cow-calf producers need to be price competitive with other meat industry proteins. Controlling increasing production costs with variable returns is challenging. By determining calf value through a feedout program, cow-calf producers can identify profitable genetics under common feedlot management. Substantial marketplace premiums are provided for calves that have exceptional feedlot performance and produce high-quality carcasses.

Cost-effective feeding performance is needed to justify the expense of feeding cattle past weaning. Price premiums are provided for cattle producing highly marbled carcasses. Knowing production and carcass performance can lead to profitable decisions for ranchers raising North Dakota born and fed calves.

This ongoing feedlot project provides producers with an understanding of cattle feeding and cattle selection in North Dakota.

Procedures

The Dakota Feeder Calf Show was developed in 1999 for cattle producers willing to consign steer calves to a show and feedout project. The 2023-24 calves were received in groups of three or four on Oct. 21, 2023, at the Turtle Lake, N.D., weighing station for weighing, tagging, veterinary processing and display. The calves were evaluated for conformation and uniformity, with the judges providing a discussion to the owners at the beginning of the feedout. The number of cattle consigned was 95, of which 82 competed in the pen-of-three contest.

The calves then were transported to the Carrington Research Extension Center for feeding. Prior to transport, calves were vaccinated, implanted with Synovex-S, dewormed and injected with a prophylactic longacting antibiotic.

Calves then were sorted and placed on corn- and distiller grains-based receiving diets. After an eight-week backgrounding period, the calves were transitioned to a diet containing 0.62 megacalorie of net energy for gain (Mcal NEg) per pound finishing diet. Cattle were weighed every 28 days, and updated performance reports were provided to the owners. Cattle were reimplanted with Synovex-Choice on January 19, 2024.

An open house was held on Jan. 31, 2024, where cattle owners could review calves, ponder performance and discuss marketing options.

¹Carrington Research Extension Center ²Dakota Feeder Calf Show Livestock Committee, Turtle Lake, ND

The cattle were harvested on May 31, 2023 (95 head). The cattle were sold to Tyson Fresh Meats, Dakota City, Neb., on a grid basis, with premiums and discounts based on carcass quality. Carcass data were collected after harvest.

Ranking in the pen-of-three competition was based on the best overall score. The overall score was determined by adding the index values for feedlot average daily gain (25% of score), marbling score (25% of score) and profit (25% of score) and subtracting index value for calculated yield grade (25% of score). The Dakota Feeder Calf Show provided awards and recognition for the topranking pen of steers.

Results and Discussion

Cattle consigned to the Dakota Feeder Calf Show feedout project averaged 597.9 pounds on delivery to the Carrington Research Extension Center livestock unit on Oct. 21, 2023. After a 222-day feeding period, cattle averaged 1,342.3 pounds (at plant, shrunk weight). No deaths occurred during the feeding period.

Average daily feed intake per head was 29.4 pounds on an as-fed basis and 21.8 pounds on a drymatter basis. Pounds of feed required per pound of gain were 8.77 pounds on an as-fed basis and 6.51 pounds on a dry-matter basis.

The overall feed cost per pound of gain was \$0.712. The overall yardage cost per pound of gain was \$0.119. The combined cost per pound

of gain, including feed, yardage, veterinary, trucking and other expenses except interest, was \$1.02.

Calves were priced by weight upon delivery to the feedlot. The pricing equation (\$ per 100 pounds = (-0.18403292* initial calf weight, pounds) + 384.5022016) was determined by regression analysis on local livestock auction prices reported for the weeks before and after delivery.

Overall, the carcasses met U.S. Department of Agriculture Quality Grades at 4.2% Prime, 85.2% Choice (including 25.3% Certified Angus Beef), 9.4% Select and 1.1% ungraded., and USDA Yield Grades (YG) at 6.3% YG1, 30.5% YG2, 51.6% YG3 and 11.6% YG4.

Carcass value per 100 pounds (cwt) was calculated using the actual base carcass price plus premiums and discounts for each carcass. The grid price received for May 31, 2024, was \$305.91 Choice YG3 base with premiums: Prime \$25, CAB \$6, YG1 \$6.50 and YG2 \$3, and discounts: Select minus \$12, Standard (ungraded - no roll) minus \$15, YG4 minus \$8, and carcasses heavier than 1,075 pounds or lighter than 650 pounds minus \$20.

Results from the calves selected for the pen-of-three competition are listed in Table 1.

Overall, the pen-of-three calves averaged 428.8 days of age and 1,372.9 pounds per head at harvest. The overall pen-of-three feedlot average daily gain was 3.49 pounds, while weight gain per day of age was 3.22 pounds. The overall pen-of-three

marbling score was 511.5 (average choice, modest marbling).

Correlations between profit and average birth date, harvest weight, average daily gain, weight per day of age and marbling score are shown in Table 2. Average slaughter weight, average daily gain and marbling score had higher correlations to profitability than average birth date, average weight per day of age or yield grade.

The top-profit pen-of-three calves with superior genetics returned \$338.15 per head, while the bottom pen-of-three calves returned \$14.30 per head. The average of the five top-scoring pens of steers averaged \$310.76 per head, while the average of the bottom five scoring pens of steers averaged a loss of \$104.87 per head.

For the pen-of-three competition, average profit was \$228.66 per head. The spread in profitability between the top and bottom five herds was \$205.89 per head.

North Dakota calf value is improved with superior carcass and feedlot performance. Favorable average daily gains, weight per day of age, harvest weight and marbling score can be found in North Dakota beef herds. Exceptional profit per head was a result of extremely high market price in 2024. Feedout projects provide a source of information for cattle producers to learn about feedlot performance and individual animal differences, and discover cattle value.

Table 1. Feeding performance — 2023-2024 Dakota Feeder Calf Show Feedout

Pen of three	Best Three Score Total	Average Birth Date	Average Weight per Day of Age, lbs	Average Harvest Weight, lbs.	Average Daily Gain, lbs.	Average Marbling Score ¹	Ave Calculated Yield Grade	Ave Feeding Profit or Loss/Head
1	2.6693	24-Feb-23	3.04	1392.8	3.59	657.7	3.12	\$307.52
2	2.5229	2-Apr-23	3.46	1464.4	3.84	536.7	3.09	\$315.74
3	2.4768	27-Mar-23	3.36	1438.9	3.65	599.0	3.25	\$276.09
4	2.4753	18-Mar-23	3.11	1364.1	3.60	689.7	4.17	\$338.15
5	2.4740	27-Mar-23	3.20	1370.5	3.43	610.0	3.34	\$316.28
Average Top 5 herds	2.52	20-Mar-23	3.2	1406	3.6	619	3.39	\$310.76
6	2.2560	8-Mar-23	3.45	1545.6	3.86	511.3	3.93	\$332.24
7	2.2499	27-Mar-23	3.02	1295.7	3.37	468.0	2.40	\$199.00
8	2.1691	31-Mar-23	3.42	1451.7	3.67	506.7	3.68	\$278.19
9	2.1152	4-Mar-23	3.06	1381.6	3.42	544.3	3.83	\$271.86
10	2.0682	5-Apr-23	3.30	1386.4	3.54	497.0	3.72	\$259.89
11	2.0625	28-Apr-23	3.25	1287.7	3.40	451.7	2.73	\$161.72
12	2.0472	11-Mar-23	3.07	1364.1	3.52	508.0	3.49	\$199.76
13	2.0255	23-Mar-23	3.44	1488.3	3.53	470.3	4.05	\$324.48
14	2.0104	28-Mar-23	3.39	1450.1	3.53	471.0	3.61	\$239.37
15	1.9268	16-Apr-23	3.20	1308.4	3.36	416.0	2.91	\$162.98
16	1.9223	7-May-23	3.28	1271.8	3.38	493.3	3.22	\$121.61
17	1.8142	19-Mar-23	2.94	1284.5	3.17	419.7	2.75	\$99.56
18	1.6814	3-Apr-23	2.97	1249.5	3.16	386.3	2.46	\$14.30
19	1.6555	21-Apr-23	3.20	1289.3	3.39	483.0	4.07	\$125.88
Average bottom 5 herds	1.80	13-Apr-23	3.1	1281	3.3	440	3.08	\$104.87
Overall average - pens of three	2.14	29-Mar-23	3.22	1,372.92	3.49	511.56	3.36	\$228.66
Standard deviation		18.4	0.2	83.8	0.2	80.1	0.5	93.2
number		19	19	19	19	19	19	19

¹Marbling score 300-399 = select, 400-499 = low choice, 500-599 = average choice, 600-699 = high choice, 700-799 = low prime

Table 2. Correlations between profit and various production measures (pen of three).

	Correlation coefficient
Profit and average birth date	-0.5267
Profit and average slaughter weight	0.8233
Profit and average daily gain	0.8129
Profit and weight per day of age	0.5114
Profit and marbling score	0.7261
Profit and yield grade	0.5735

Effectiveness of virtual fence in North Dakota grazing systems

Miranda Meehan¹, Kevin Sedivec^{2,4}, Zac Carlson³, Josh Wianecki^{1,2}, Chris Byrd¹, Jason Harmon² and Torre Hovick²

Virtual fence is a relatively new technology that has the potential to improve grazing management without physical fences while providing GPS tracking of livestock. For the last year, researchers at North Dakota State University have been evaluating the use of virtual fence to graze rangeland and annual forages. Virtual fence was effective in containing animals in a designated grazing area 92% of the time in both grazing systems. Virtual fence was effective in managing grazing animals. Virtual fences also provided increased management flexibility as they can easily be moved or adjusted to improve grazing distribution, increase harvest efficiency, and enhance wildlife habitat.

Summary

Virtual fence is a relatively new technology that has the potential to improve grazing management without physical fences while providing GPS tracking of livestock. For the last year, researchers at North Dakota State University have been evaluating the use of virtual fence to graze rangeland and annual forages. In both rangeland and annual forage systems, GPS location data was logged at 30-minute intervals, and the number of management cues received by each animal was recorded. This data determined the number of breakouts and time spent outside the designated grazing area. The virtual fence was utilized in two experiments. For the first experiment, virtual fencing was

used to patch graze on rangeland at different grazing intensities to create heterogeneity. Four herds of cow-calf pairs with 19 to 30 pairs per herd grazed rangeland from June 8 to Oct. 20, 2023. Herd size was based on the estimated carrying capacity of each pasture. For the second experiment, yearling heifers grazed annual forage pastures in the fall using four grazing and technology treatments: 1) continuous grazing, 2) strip graze with manual fence, 3) strip graze with automated fence and 4) strip graze with virtual fence. Stocking rates were estimated based on biomass production at the time of grazing. Each treatment was grazed by 8 to 12 head of yearling beef cattle, depending on forage production, from Oct. 6 - Nov. 27 at the Central Grassland Research Extension Center (CGREC) and Oct. 2-16 at NDSU. Virtual fence was effective in containing animals in a designated grazing area 92% of the time in both grazing systems. Virtual fence

was effective in managing grazing animals while providing increased flexibility in management as fences could easily be moved or adjusted to better meet management goals of improving grazing distribution, increasing harvest efficiency and enhancing wildlife habitat. As with any technology, producers must understand how this technology can be integrated into their production system to better enable them to meet their individual management goals.

Introduction

Virtual fence is a relatively new technology that has the potential to improve grazing management without physical fences while providing GPS tracking of livestock. Livestock wear collars that communicate animal location in relationship to a virtual fence boundary via radio and/or cellular tower to a web- or phone-based application. Each animal receives audio and electrical cues, depending on its location in relationship to the virtual fence boundary.

Virtual fence has been shown to be effective in either including or excluding livestock from a designated area with the majority of research reporting 90% or greater success in managing grazing access of livestock (Aaser et al. 2024; Campbell et al. 2018; Campbell et al. 2020). When livestock have an adequate forage supply, the effectiveness of virtual fence increased over the grazing period as animals learned the cues, resulting in reduced electrical

¹Animal Sciences Department, NDSU ²School of Natural Resource Sciences, NDSU

³Danisco

⁴Central Grasslands Research Extension Center, NDSU

cues to be effective (Hamidi et al. 2022; Ranches et al. 2021). There is variability in effectiveness between classes of cattle and individual animals. Boyd et al. (2024) and Utsumi et al. (2023) reported that cows with calves received more audio and electrical cues when compared to dry cows because of the social interactions with uncollared calves.

In addition to enhancing management of grazing animals, virtual fence can be used to improve habitat and wildlife management. The most obvious improvement is the removal of physical fences that inhibit wildlife movements. However, virtual fence also can improve our understanding of habitat use by livestock (Aaser et al. 2024) and manage livestock use of sensitive habitats by either limiting use or restricting use (Campbell et al. 2018; Campbell et al. 2020). To date, there is limited research published on the use of this technology in the United States and no research using it to manage cattle grazing of annual forages.

Experimental Procedure

For the last year, researchers at NDSU have been evaluating the use of virtual fence in grazing systems to manage livestock grazing on both rangeland and cropland to enhance grazing efficiency and livestock production. Prior to the grazing period for both experiments, cattle were fitted with virtual fence collars and went through a fourday training period. Calves were not collared. Across both projects, GPS location data was logged at 30-minute intervals, and the number of management cues received by each animal was recorded. These data determined the number of breakouts and time spent outside of the designated grazing areas.

On rangelands, the team evaluated the use of virtual fence to create heterogeneity of vegetation compared to traditional rotational grazing using fence and seasonlong grazing systems. The virtual fence was managed to patch graze at different grazing intensities. Animals were given access to a quarter, then half, then three quarters of a pasture, leaving one quarter ungrazed (Figure 1). The goal of this project was to enhance conservation benefits to wildlife while benefiting livestock production. To see if this goal is being achieved, the following metrics are being tracked: vegetative structure (wildlife habitat), plant species diversity, wildlife populations, forage production and livestock performance.

This project was piloted in 2023 at the CGREC near Streeter, ND. Virtual fence was used to manage four herds of cow-calf pairs with 19 to30 pairs per herd from June 8 to Oct. 20, 2023 Herd size was based on the estimated carrying capacity of each pasture. During the pilot study, vegetation structure was measured using Robel readings at the end of the grazing period. Livestock performance was

collected by weighing cows and calves at the start and end of the grazing period.

The team is also evaluating strip grazing using different technologies on soil health, animal performance and behavior, and economic viability for cattle producers. We tested three techniques of strip grazing a cover crop: manual fence movement (polywire), automated fence movement, and virtual fence. The objective is to improve harvest efficiency, soil health, and livestock performance in an integrated crop and livestock system. The following parameters are being evaluated: forage production, harvest efficiency, soil chemical and physical properties, and livestock performance.

The strip grazing project was piloted in 2023 at the CGREC and the NDSU campus in Fargo, ND. An annual forage was grazed in the fall using four grazing and technology treatments: 1) continuous grazing, 2) strip graze with manual fence, 3) strip graze with automated fence

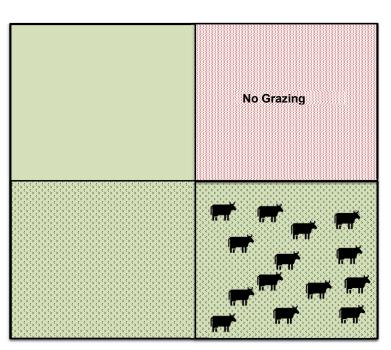


Figure 1. Diagram of patch graze system where cattle are restricted to the southwest quarter, then to the south half, then given access to three quarters, leaving the northwest quarter ungrazed.

and 4) strip graze with virtual fence. There was also an ungrazed treatment at each location. Field size was approximately nine acres, and stocking rates were estimated based on biomass production at the time of grazing. Each treatment was grazed by 8-12 head of yearling beef cattle Oct. 6 - Nov. 27 at CGREC and Oct. 2-16 at NDSU. Each treatment was clipped post grazing to estimate harvest efficiency of the grazing treatments. Livestock performance was also collected by weighing cows and calves at the start and end of the grazing period.

Results and Discussion

One of the greatest concerns from producers looking to adopt virtual fence is its effectiveness in keeping animals in or out of a designated area. Across both studies, we observed 92% containment of grazing animals to designated areas. This is consistent with research conducted in other locations, which reported 90% or greater containment (Aaser et al. 2024; Campbell et al. 2018; Campbell et al. 2020). Similarly, we observed increases in the number of cues animals received following move dates when becoming familiar with the new boundary and at the end of a grazing period when available forage was reduced. Boyd et al. (2024) reported a decline in containment from >94% to 75% as available forage was reduced.

Initial results suggest that we were successful in creating heterogeneity in structure across the pasture (Figure 2) with no negative impacts to cow or calf performance in comparison to the other grazing treatments. Heterogeneity in structure increases the habitat types available for waterfowl and grassland birds, and increases biodiversity of the bird population. Heterogeneity also increases plant species biodiversity, which benefits pollinator populations. These results indicate that virtual

fence can be an effective tool in managing grassland ecosystems for wildlife.

Initial results of using virtual fence to graze annual forages indicate that similar harvest efficiency was achieved across all treatments. However, livestock performance varied between treatments with the virtual fence and manual fence treatments having higher performance (Figure 3). The reduced

performance observed in the autogate treatment is likely due to animal behavior, as the animals did not move through the gate to utilize the last strip of forage. The reduced performance with similar harvest efficiency for cattle on the continuous graze treatment may be because of increased forage waste because of trampling and foraging behavior that resulted in lower quality forage as the grazing period progressed.

Virtual Fencing VOR

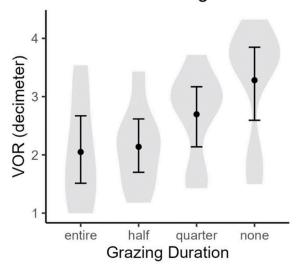


Figure 2. Virtual fence patch grazing structure measured using visual obstruction readings (VOR)

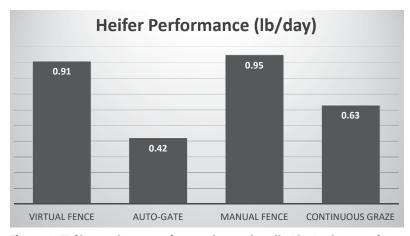


Figure 3. Heifer performance in pounds per day (lbs/day) when grazing late season annual forage with virtual fence, automatic gate, manual fence (polywire) and continuous grazing (no strips).

The initial findings of our research indicate that virtual fence is effective in managing grazing animals, providing increased flexibility in management as fences can easily be moved or adjusted to better meet management goals of improving grazing distribution, harvest efficiency, and wildlife habitat. However, adapting this technology is not without challenges. The cost is likely a barrier to many producers, and more economic information is needed. Economics will be evaluated as the current projects proceed. Additionally, time is needed to learn how to effectively use the technology to manage livestock

to meet individual management goals. As with any technology, producers should understand how this technology can be integrated into their production system to better enable them to meet their individual management goals.

Acknowledgements

The authors acknowledge the North Dakota Agricultural Experiment Station and State Board of Agricultural Research and Education for their financial support. A special thanks to the research technicians at the Central Grasslands Research Extension Center and NDSU Beef Unit for their assistance.

Literature Cited

- Aaser, M. F., S. K. Staahltoft, M. Andersen, A. K. O. Alstrup, C. Sonne, D. Bruhn, J. Frikke, and C. Pertoldi. 2024. Using activity measures and GNSS data from a virtual fencing system to assess habitat preference and habitat utilisation patterns in cattle. Animal. 14:1506.
- Boyd, C. S., R. C. O'Connor, J. Ranches, D.W. Bohnert, J. D. Bates, D. D. Johnson, K. W. Davies, T. Parker, and K. E. Doherty. 2024. Using virtual fence to create fuel breaks in the sagebrush steppe. Rangeland Ecol. Manage. 89:87-93.
- Campbell, D. L. M., S. J. Haynes, J. M. Lea, W. J. Farrer, and C. Lee. 2018. Temporary exclusion of cattle from a riparian zone using virtual fencing technology. Animal. 9:5.
- Campbell, D. L. M., J. Ouzman, D. Mowat, J. M. Lea, C. Lee, and R. S. Llewellyn. 2020. Virtual fencing technology excludes beef cattle from an environmentally sensitive area. Animal. 10:1069.
- Hamidi, D., N. A. Grinnell, M. Komainda, F. Riesch, J. Horn, S. Ammer, I. Traulsen, R. Palme, M. Hamidi, and J. Isselstein. 2022. Heifers don't care: No evidence of negative impact on animal welfare of growing heifers when using virtual fences compared to physical fences for grazing. Animal. 16:100614.
- Ranches, J., R. O'Connor, D. Johnson, K. Davies, J. Bates, C. Boyd, D. W. Bohnert, and T. Parker. 2021. Effects of virtual fence monitored by global positioning system on beef cattle behavior. Transl. Anim. Sci. 5:144.
- Utsumi, S., S. Nyamuryekung'e, A. R. Perea, M. Spetter, A. Cox, L. Macon, R. Estell, and G. C. Duff. 2023. Virtual fencing on rangeland cows during late lactation and following weaning. J. Anim. Sci. 101:3

Impact of protein source and supplementation frequency on growth performance of backgrounding cattle in an extensive winter grazing system

Jaime M. Chambers¹, Grady L. Gullickson¹, Madeliene S. Nichols¹, Yssi L. Entzi¹, Jessie G. Syring¹, Joshua V. Wianecki¹, Lydia E. Trandem¹, Garrett Havelka¹, Kendall C. Swanson¹, Miranda A. Meehan¹ and Zachary E. Carlson¹

The objective of this experiment was to determine the influence of protein source (dried distiller's grains plus solubles or soybean meal) and supplementation frequency (daily or three times weekly) on growth performance of backgrounding cattle in a winter bale-grazing system. Results from the first year of the study suggest that protein source and frequency of supplementation did not influence growth performance. Therefore, the decision for a producer to reduce frequency of supplementation and replace dried distiller's grains plus solubles with soybean meal in backgrounded beef cattle diets should be based on cost and availability.

Summary

Seventy-two Angus-crossed backgrounding calves (initial BW $= 549 \pm 233$) were used in a 44-day winter bale-grazing study that evaluated the differences in growth performance of calves supplemented with dried distiller's grains plus solubles (DDGS) or soybean meal (SBM) either daily or three times weekly. Body weights were collected at day 0, 28, and 44. Cattle had ad libitum access to water, hay, and trace mineralized salt., DDGS or SBM was supplemented (dry matter basis) at an average of 0.75% of their body weight per day so that all calves would receive the same amount of supplement over a seven-day period. There was no difference $(P \ge 0.05)$

in ending body weight, average daily gain or estimated dry matter intake (DMI) between treatments. This suggests protein source and supplementation frequency have minimal effects on backgrounding cattle performance when managed in a bale-grazing system and that choice of supplementation should be based on availability and cost of the protein supplement.

Introduction

Extended grazing systems, such as bale grazing, have become more popular with producers in recent years due to decreased production costs. Animals are able to harvest their own feed while minimizing the cost of purchased feed (Undi and Sedivec, 2022). Much of the research on bale grazing focuses on gestating cows, and limited

research has evaluated the system for backgrounding cattle. Grazing animals usually require supplemental energy and protein to achieve a desired gain (Kunkle et al., 2000) improvement of animal performance, increasing economic return, and(or. Dried distiller's grains plus solubles (DDGS) is commonly used to supplement energy and protein for cattle consuming or grazing forage. Soybean meal (SBM) supplies high concentrations of protein and is a balanced source of essential amino acids, particularly lysine, which is lower in corn and other cereal grains. However, the cost of SBM is high relative to other protein sources. With feed costs making up a large percentage of total production costs, SBM has not been a common feed ingredient in beef cattle diets for several years. With the growing interest in biodiesel, more soybeans are being produced and processed in North Dakota. This growth in soybean production and processing may increase the supply and availability of SBM at potentially lower prices, which could make it useful to producers as an alternative local feedstuff.

The objectives of this study were to evaluate differences in growth performance in backgrounding cattle supplemented with DDGS or SBM daily or three times per week in an extensive winter bale-grazing system.

¹Department of Animal Sciences, North Dakota State University, Fargo, North Dakota, 58108

Experimental Procedure

Twenty-four heifers (initial BW = 482 ± 98 lb) and 48 steers (initial BW = 582 ± 148 lb) Angus-based backgrounding cattle were in a 44-day bale-grazing study on a pasture south of the NDSU Beef Cattle Research Complex in Fargo, North Dakota. Cattle originated from the NDSU Beef Unit. This study was conducted from December 2023 to January 2024.

The study pasture was split into 15 paddocks of 40 ft × 400 ft (0.44 acres) using polywire electric fence. Grass hay round bales were individually weighed and placed in the middle of each paddock with 40 ft between each bale so 10 bales per paddock. Cattle were given access to one bale at a time by moving the polywire electric fence. Cattle were limit-fed a common diet at an estimated 2% of their body weight (dry matter basis) for the five days prior to the initiation and at the completion of the study and weighed on three consecutive days at the beginning and end of the study to equalize gut fill (Watson et al., 2013). Using the average body weight from the first and second days, cattle were stratified and randomly assigned to one of four treatments. The four treatments were DDGS fed daily (DDGS-d), DDGS fed three times per week (DDGS-a), SBM fed daily (SBM-d) and SBM fed three times per week (SBM-a). Each treatment was

replicated three times and randomly assigned to a paddock.

Calves were allowed ad libitum access to water, hay, and trace mineralized salt (American Stockman Big 6 Mineral Salt, NaCl 96-99%, Mn 2,400 ppm, Fe 2,400 ppm, Cu 260-380 ppm, Zn 320 ppm, I 70 ppm, and Co 40 ppm). Cattle that were supplemented daily received 0.75% of BW (dry matter basis), and cattle supplemented three times a week received 1.75% of BW (dry matter basis).

Body weights were collected every 28 days. Weekly feed ingredient samples were collected and ground through a 1-mm screen using a Wiley

Table 1. Nutrient composition of feedstuffs

		DDGS	SBM
Item, %	Hay ¹	Mix ²	Mix ³
DM	90.01	89.92	89.83
CP	9.97	33.24	50.77
Fat	1.94	6.20	1.16
NDF	68.90	47.81	10.95
ADF	38.60	17.80	4.55
Ash	8.73	10.29	1.39
Calcium	0.37	1.72	1.46
Phosphorus	0.22	0.99	0.69
Nitrogen	1.59	5.32	8.12
Sulfur	0.14	0.69	0.40

¹Low-quality grass hay

(DM basis)

Mill (Thomas Scientific, Swedesboro, NJ) and then composited into fourweek composites. Dietary dry matter was determined for every new pallet delivery of feed by sampling ingredients and oven-drying at 60 C for 48 hours. Adjustments were made to supplement fed to calves. Feed weigh-backs were collected over the entire experiment and stored at -4 C for dry matter analysis at a later date. The residue of one bale per paddock was collected and weighed in the spring to estimate residual hay left in the paddock and hay intake.

Data were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). Significance was assigned at $P \le 0.05$ with a tendency assigned between P < 0.10 and > 0.05.

Results and Discussion

There were no protein source \times supplementation frequency interactions (P=0.49) for average daily gain (ADG), ending bodyweight (EBW) or estimated dry matter intake (DMI) (Table 2). There were no differences (P=0.32) due to supplementation frequency for ADG, EBW or estimated DMI. Likewise, there were no differences (P=0.21) due to protein source for ADG or EBW. There was a tendency (P=0.09) for cattle supplemented with SBM to have a greater DMI than cattle supplemented with DDGS.

Table 2. Growth performance and estimated dry matter intake of backgrounding cattle in a bale-grazing system

	Treatment ¹					P-Value			
Item	DDGS-d	DDGS-a SBM-d SBM-a		SEM	TRT ³	FREQ ⁴	TRT x FREQ		
Calves, n	18	18	18	18	-	-	-	-	
Initial BW, lb	531.77	533.32	532.55	531.99	8.8	0.98	0.95	0.90	
Ending BW, lb	629.57	623.4	623.57	621.68	9.6	0.68	0.67	0.82	
ADG, lb/day	2.23	2.05	2.07	2.04	0.11	0.42	0.32	0.49	
DMI ² , lb/day	10.84	10.83	12.19	11.94	0.64	0.09	0.84	0.85	

¹Treatments; DDGS-d: dried distiller's grains plus solubles fed daily; DDGS-a: dried distiller's grains plus solubles fed 3 times per week; SBM-d: soybean meal fed daily; SBM-a: soybean meal fed 3 times per week

²Dried distiller's grains plus solubles; 97% DDGS and 3% limestone (DM basis) ³Soybean meal; 97% SBM and 3% limestone

²Dry Matter Intake: estimated as individual animal intake was not monitored

³TRT; Treatment – DDGS vs SBM

⁴FREQ; Frequency – daily vs 3x per week

These results agree with previous research that suggests protein supplementation can be offered on an infrequent basis to ruminants while still maintaining cattle performance (Huston et al., 1999; Bohnert et al., 2002). Note that we replaced SBM in the diet at the same inclusion level as DDGS, on a dry matter basis, and did not formulate the diets to have balanced metabolizable energy or protein. Other research also suggests that as supplemented crude protein increases, there is a corresponding increase in forage dry matter intake (DMI) when consuming a low-quality forage (Cappellozza et al., 2021). Unlike our results with no difference in DMI, research has shown that as supplement frequency decreases, there is also a decrease in forage DMI. This difference may be because most of the research is on gestating beef cattle and not backgrounding calves, which have differing energy and protein requirements. However,

caution is warranted in our study as hay intake was estimated from the amount of hay residue remaining post-grazing from one bale per paddock.

Our results suggest that a producer can utilize an extended winter bale-grazing system with backgrounding cattle while supplementing DDGS or SBM as few as three times a week. The decision to use SBM rather than DDGS will most likely be made based on supplement cost, transportation cost, and availability.

Acknowledgements

The authors thank the North Dakota Soybean Council for providing financial support. The authors also thank the staff and students at the NDSU Beef Cattle Research Complex, NDSU Beef Unit, and NDSU Nutrition Laboratory.

Literature Cited

- Bohnert, D. W., C. S. Schauer, and T. DelCurto. 2002. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: Cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. 80:1629–37.
- Cappellozza, B. I., D. W. Bohnert, M. M. Reis, K. C. Swanson, S. J. Falck, and R. F. Cooke. 2021. Influence of amount and frequency of protein supplementation to steers consuming low-quality, cool-season forage: intake, nutrient digestibility, and ruminal fermentation. J. Anim. Sci. 99:skab112.
- Huston, J. E., H. Lippke, T. D. A. Forbes, J. W. Holloway, and R. V. Machen. 1999. Effects of supplemental feeding interval on adult cows in Western Texas. J. Anim. Sci. 77:3057–67.
- Kunkle, W. E., J. T. Johns, M. H. Poore, and D. B. Herd. 2000. Designing supplementation programs for beef cattle fed forage-based diets. J. Anim. Sci. 77-1
- Undi, M., and K. K. Sedivec. 2022. Longterm evaluation of bale grazing as a winter-feeding system for beef cattle in central North Dakota. Appl. Anim. Sci. 38:296–304.
- Watson, A. K., B. L. Nuttelman, T. J. Klopfenstein, L. W. Lomas, and G. E. Erickson. 2013. Impacts of a limit-feeding procedure on variation and accuracy of cattle weights1. J. Anim. Sci. 91:5507–5517.

Bale grazing: Can different supplementation strategies enhance beef cattle performance under variable winter conditions?

AS2241-8 • 2024 North Dakota Livestock Research Report

Michael Undi¹, Kevin Sedivec¹ and Jessalyn Bachler²

This study evaluated a molasses-based liquid, alfalfa hay, and corn DDGS as supplements for beef cattle bale grazing grass hay during winter conditions encountered in the northern Plains. Results showed that supplements such as alfalfa hay or molasses-based liquids are effective during mild winters but higher energy supplements such as corn DDGS are required in severely cold winters when cattle require extra energy to maintain or improve performance. Supplementation strategies should consider supplement effectiveness to meet protein and energy requirements, particularly in winter conditions such as those encountered in the northern Plains.

Summary

The 2016 National Academies of Sciences, Engineering, and Medicine (NASEM) model was used to assess the adequacy of a molasses-based liquid, alfalfa hay, and corn DDGS as supplements for beef cattle grazing grass hay grass during a severely cold winter, a moderately cold winter, and a mild winter. All supplementation strategies supplied adequate metabolizable protein (MP) for nonlactating beef cows in environmental conditions encountered. Grass hay did not provide adequate metabolizable energy (ME) to meet requirements across environmental conditions encountered. Molasses-based liquid and alfalfa hay provided adequate ME in a mild winter but not in a

moderately cold or severely cold winter. Corn DDGS supplied ME in excess of cow requirements in all environmental conditions encountered. We concluded that alfalfa hay or molassesbased liquids may be utilized as supplements during mild winters. Supplementation with higher energy feeds such as corn DDGS is required during severe winters where cattle require extra ME to maintain or improve performance.

Introduction

Cattle in the northern Plains are normally kept in open dry lot pens in winter. Alternatively, dry lot use can be minimized by extending the grazing season through strategies such as bale grazing, swath grazing, stockpiling and grazing corn residue. Bale grazing means placing hay bales in a grid pattern on hayfields or pastures for grazing in the fall and winter (McGeough et al, 2018).

Benefits of bale grazing include returning nutrients onto land and minimizing nutrient loss through runoff or leaching. More importantly, lower labor and input costs associated with extended grazing can decrease production costs and potentially enhance profitability of livestock production. Forages utilized for bale grazing are predominantly perennials, mainly grasses and grasslegume mixtures, although straw also may be utilized (McGeough et al, 2018). In situations where cattle are offered low-quality grass hay or straw, supplementation may be required to meet cattle nutrient requirements. Supplementation becomes especially critical during harsh winter conditions, such as those encountered in the northern Plains. Since extended grazing systems are predicated on lower winter feed costs relative to dry lot feeding, supplementation strategies selected for these systems should maintain cost savings while providing targeted amounts of required nutrients. Cost savings can be maintained through strategies that either reduce frequency of supplement delivery to cattle on pasture or eliminate pasture visits for supplementation purposes altogether.

For supplements such as corn DDGS, which have to be delivered to cattle on pasture, supplementation costs can be minimized by decreasing labor and equipment inputs by reducing frequency of supplementation (Wickersham et al., 2008). Less frequent supplement

¹Central Grasslands Research Extension Center, Streeter, ND ²Business & Trades Technology Dept. Williston State College, Williston, ND

delivery often does not negatively impact animal performance and can decrease costs associated with supplementation. Pasture visits for supplementation purposes may be eliminated by supplying highquality forage to complement lowquality forage or treating low-quality forage with molasses-based liquid supplements. Alfalfa hay has been utilized to complement cattle grazing low-quality pastures (Weder et al., 1999). Liquid supplements poured directly onto hay can reduce hay waste, improve hay storage and improve nutrient content of lowquality forage (Walker et al., 2013). Currently, there is limited information on strategies for supplementing cattle in extended grazing systems.

Experimental Procedures

Environmental temperatures (Table 1), feed composition (Table 2) and cow performance data (Table 3) were entered into the 2016 NASEM model to predict ME and MP requirements and supply of nonlactating beef cows during a severely cold winter (10°F), a moderately cold winter (16°F) and a mild winter (25°F). Cow performance data were obtained from a four-year bale grazing study that evaluated a molasses-based liquid, alfalfa hay, corn DDGS as supplements for cows bale grazing grass hay. Liquid supplementation involved pouring the molasses-based liquid supplement, Range-40 (QLF Inc., Dodgeville, WI), onto grass hay bales. Supplementing with alfalfa hay was accomplished by providing one bale of alfalfa hay for every three bales of grass hay fed during each feeding cycle. Corn DDGS was delivered twice weekly to cattle on pasture and fed in bunks to provide 4 lbs corn DDGS/head/day. Bale grazing duration averaged 67 days across the years and grazing generally commenced in mid-November of each year until January except in 2017 when grazing started earlier.

Table 1. Average monthly temperatures during bale grazing.

		Temperature (°F)						
Year	Duration	October	November	December	January	Mean		
2016	Nov. 4 - Jan. 12	-	29.1	6.8	-5.6	10.1		
2017	Oct. 24 - Dec. 28	36.1	27.0	10.9	-	24.8		
2018	Nov. 5 - Jan. 10	-	21.2	30.9	17.8	19.9		
2019	Nov. 14 - Jan. 17	-	26.8	14.9	6.3	16.0		

Table 2. Chemical composition (% DM) of grass hay or grass hay supplemented with a liquid, alfalfa hay or corn DDGS.

	HAY ¹	H-LQS	H-ALF	H-DDG
CP	7.9 ± 0.51	9.0 ± 0.44	10.9 ± 0.82	11.4 ± 0.56
ME, Mcal/lb	3.84 ± 0.64	4.17 ± 2.29	4.01 ± 0.66	4.32 ± 0.59
NDF	66.1 ± 0.69	65.4 ± 0.81	62.4 ± 1.59	60.7 ± 0.43
ADF	47.3 ± 0.96	47.8 ± 3.09	45.1 ± 1.47	42.5 ± 1.16
Ca	0.61 ± 0.04	0.54 ± 0.05	0.89 ± 0.03	0.53 ± 0.04
P	0.11 ± 0.04	0.16 ± 0.02	0.13 ± 0.04	0.24 ± 0.04

¹HAY = grass hay, H-LQS = liquid-treated grass hay, H-ALF = grass hay plus alfalfa hay, H-DDG = grass hay plus corn DDGS.

Table 3. Cow performance data following bale grazing of grass hay, or grass hay supplemented with a liquid, alfalfa hay or corn DDGS.

	DMI lb/d	Initial BW lb	Final BW lb	ADG lb/d	Initial BCS	Final BCS	BCS change
Treatment							
HAY^1	26.5 ^b	1354	1337	-0.25 ^c	5.8	5.7 ^b	-0.08^{b}
H-LQS	26.8 ^b	1356	1363	0.12^{b}	5.8	5.8 ^{ab}	0.04^{a}
H-ALF	26.7 ^b	1363	1370	0.13^{b}	5.8	5.8 ^{ab}	0.03^{ab}
H-DDG	30.1^{a}	1349	1392	0.66^{a}	5.8	5.9 ^a	0.07^{a}
SE	0.31	20.7	21.8	0.05	0.05	0.04	0.04
Year							
2016	26.6 ^c	1302 ^c	1256 ^b	-0.64 ^c	5.5 ^c	5.4 ^c	-0.13 ^c
2017	27.5ab	1362 ^{ab}	1423a	0.95^{a}	5.4 ^d	5.6 ^b	0.22^{b}
2018	28.0^{a}	1400 ^a	1412 ^a	0.16^{b}	6.5 ^a	6.9 ^a	0.39^{a}
2019	27.3 ^b	1357 ^b	1368 ^a	0.18^{b}	5.8 ^b	5.3 ^c	-0.42 ^c
SE	0.25	16.5	18.4	0.05	0.05	0.04	0.04
P-value							
Treatment	< 0.001	0.92	0.09	< 0.001	0.97	0.005	0.004
Year	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment × Year	0.82	0.96	0.87	0.03	0.69	0.75	0.13

¹HAY = grass hay, H-LQS = liquid-treated grass hay, H-ALF = grass hay plus alfalfa hay, H-DDG = grass hay plus corn DDGS.

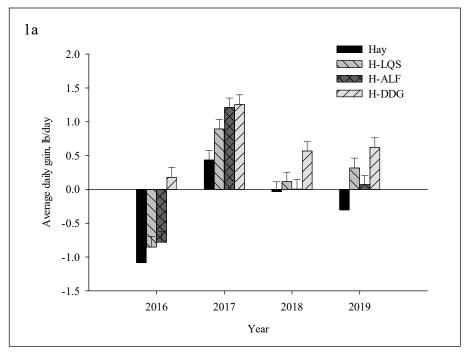
^{a-c}Means with a different letter within column for treatment or year differ ($P \le 0.05$).

Results and Discussion

Cows offered only grass hay lost weight (Figure 1a) and condition (Figure 1b) in moderate and severe environmental conditions encountered during the study. Based on the 2016 NASEM model, grass hay provided adequate MP to meet protein requirements of nonlactating beef cows in the second trimester of pregnancy. Positive MP balance across four years of the study (Figure 2a) suggests that there was no apparent need for protein supplementation. Grass hay, however, did not provide adequate ME to meet daily cow ME requirements across all study years (Figure 2b), suggesting a need for supplementing cattle even in mild weather conditions as occurred in 2017.

Liquid supplementation increased dietary crude protein (CP) concentration above feeding grass hay only. Cows offered liquidsupplemented grass hay gained body weight except in severe environmental conditions (Figure 1a). These cows, however, lost condition except when mild environmental conditions were mild (Figure 1b). Liquid supplementation supplied MP in excess of cow requirements in all conditions encountered (Figure 2a). Liquid supplementation exceeded ME requirements in 2017, which was a mild year but did not supply adequate energy in a moderate year (2019) or in 2016 when severe environmental conditions occurred (Figure 2b). Liquid supplementation may be an option for cattle consuming low-quality grass hay in mild winters but cannot be the sole supplement as winters become severe.

Cows offered grass hay supplemented with alfalfa hay gained body weight (Figure 1a) and condition (Figure 1b) in mild weather but lost weight and condition in severe environmental conditions. Supplementing with alfalfa hay



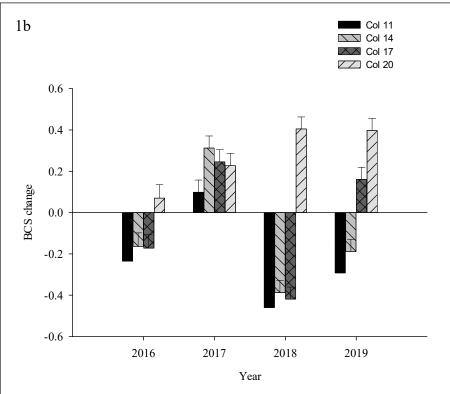
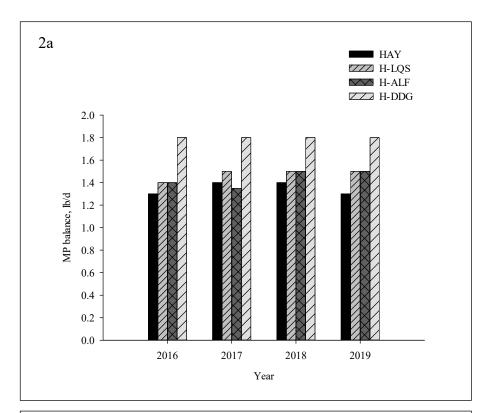


Figure 1: Average daily gains (1a) and BCS change (1b) in cows bale grazing grass hay or grass hay supplemented with a liquid, alfalfa hay, or corn DDGS.

supplied MP in excess of cow requirements in all environmental conditions encountered (Figure 2a). Supplementing with alfalfa hay did not provide adequate ME to meet cow ME requirements in cold winters, such as occurred in 2016 (Figure 2b). The 2016 NASEM model showed that alfalfa hay supplied adequate energy only during mild years but not in years with moderate or severe environmental temperatures (Figure 2b). In mild environmental temperatures such as those in 2017, alfalfa hay supplementation was nearly as effective as liquid supplementation in providing ME to meet cow energy requirements. Although alfalfa hay can effectively meet CP requirements in rations with low-quality roughages, alfalfa hay does not have the caloric density of oilseed meals or other by-product feeds to meet energy needs (DelCurto et al., 2000). In fact, the energy density of alfalfa is similar to that of high-quality grass hay. Weder et al (1999) reported improved animal performance when cattle grazing lowquality forage were offered higherquality alfalfa hay.

Corn DDGS supplementation resulted in positive ADG (Figure 1a) and body condition (Figure 1b) across all environmental conditions encountered. Supplementing with corn DDGS resulted in the highest increase in dietary CP and supplied the highest amount of MP to cows across the years (Figure 2a). Feeding 4 lbs corn DDGS/head/day to cows bale grazing grass hay supplied ME in excess of cow requirements at all environmental temperatures encountered in this study (Figure 2b). Supplementing with DDGS supplied ME that exceeded requirements even in years with severe environmental temperatures such as those in 2016. As a supplement, corn DDGS compares favorably with other supplements such as soybean meal and canola meal since corn DDGS



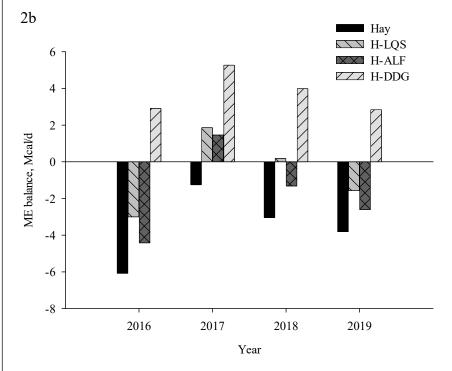


Figure 2. Predicted MP balance (2a), ME balance (2b) in cows bale grazing grass hay or grass hay supplemented with a liquid, alfalfa hay, or corn DDGS.

is a good source of protein, fat, phosphorus and readily digestible fiber (Klopfenstein et al., 2008). The low starch content of corn DDGS makes it a suitable supplement for grazing cattle (Klopfenstein et al., 2008). Among the supplements evaluated, corn DDGS is the only supplement that supplied adequate energy for pregnant beef cows that were bale grazing in all environmental conditions encountered in this study.

Supplementation costs ranged from \$1.33 to \$1.90/head/day for the different strategies. Predictably, bale grazing grass hay alone resulted in the lowest system costs. Minimizing use of purchased hay, reducing transportation costs and grazing hay bales in the fields from which the hay was baled would keep costs of bale grazing low. Liquid supplementation increased grazing costs by \$0.26/head/day over grass hay due to the cost of the liquid supplement. Supplementing with alfalfa hay increased costs by \$0.25/ head/day over grass hay. The highest cost (\$1.90/head/day) occurred when corn DDGS was offered as a supplement, mainly due to the cost of corn DDGS as well as labor required for twice-weekly visits to deliver it to cattle on pasture. Limiting delivery frequency to one visit per week would reduce the cost of corn DDGS supplementation.

Literature Cited

- DelCurto, T., Hess, B. W., Huston, J. E. and Olson, K. C. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. J. Anim. Sci. 77:1-16.
- Klopfenstein, T. J., Ericson, G. E. and Bremer, V. R. 2008. Board invited review: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.
- McGeough, E. J., Cattani, D. J., Koscielny, Z., Hewitt, B. and Ominski, K. H. 2018. Annual and perennial forages for fall/winter grazing in western Canada. Can. J. Plant Sci. 98:247-254.
- National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirement of Beef Cattle. 8th rev. ed. Natl. Acad. Press, Washington, DC.
- Walker, R. S., LaMay, D., Davis, J. R. and Bandyk, C. 2013. Method of feeding a liquid protein supplement with low- to medium-quality hay affects hay waste and cow performance. Prof. Anim. Sci. 29:552-558.
- Weder, C. E., DelCurto, T., Svejcar, T., Jaeger, J. R. and Bailey, R. K. 1999. Influence of supplemental alfalfa quality on the intake, use, and subsequent performance of beef cattle consuming low-quality roughages. J. Anim. Sci. 77:1266-1276.
- Wickersham, T. A., Titgemeyer, E. C., Cochran, R. C., Wickersham, E. E. and Moore, E. S. 2008. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3089-3099.

Evaluation of an exogenous enzyme combination in forage-based growing diets fed to growing beef cattle

Madeliene Nichols¹, Grady Gullickson¹, Yssi Entzie¹, Sarah Underdahl¹, Lydia Hansen¹, Jessica Syring¹, Joshua Wianecki¹, Danielle Jensen¹, Kathlyn Hauxwell¹, Tommy Winders² and Zachary Carlson¹

The objective of this experiment was to evaluate the effects of exogenous fibrolytic enzymes and wheat straw inclusion level on the performance of growing beef steers receiving forage-based growing diets. The results indicate that supplementing exogenous fibrolytic enzymes did not affect growth performance or feed intake but did affect plasma metabolites in a way that suggests greater dietary energy was derived when including exogenous fibrolytic enzymes in the diet. Increasing wheat straw levels decreased average daily gain and dry matter intake.

Summary

Seventy-three crossbred steers were in an 84-day study to evaluate the influence of exogenous fibrolytic enzymes and wheat straw inclusion in forage-based growing diets fed to growing beef steers. Steers were assigned to one of four treatments arranged as a 2 × 2 factorial with two levels of wheat straw inclusion: 5% or 15% of diet dry matter, and two levels of exogenous fibrolytic enzymes: 0 g or 750 g/metric ton of diet (Bovizyme CX; Danisco Animal Nutrition and Health; Wilmington, DE). Body weights and blood samples were collected every 28 days. Individual intake data were collected using an automated feeding system (Insentec Roughage Intake Control, Hokofarm B.V., Marknesse, The Netherlands). Statistical analysis

showed no wheat straw × exogenous fibrolytic enzymes interactions (P \geq 0.18) for ending body weight (EBW), average daily gain (ADG), dry matter intake (DMI), feed-togain ratio (F:G), plasma glucose (GLC), plasma urea nitrogen (PUN) or plasma non-esterified fatty acids (NEFA). Supplementing exogenous fibrolytic enzymes did not affect ($P \ge$ 0.15) growth performance or intake but increased GLC and decreased NEFA concentrations in the blood. Increasing wheat straw inclusion from 5% to 15% negatively affected growth performance, decreasing EBW by 5%, ADG 16%, DMI 8% and F:G 9%.

Introduction

Forages are an integral part of beef cattle diets as they often are the main source of carbohydrates, are the main substrate utilized by ruminal microbes, and provide physical fiber needed to stimulate rumination and reticuloruminal motility (NRC, 2016). However, feeding forage as an energy source may limit the supply of energy and nutrients because of the naturally high cell wall concentration of forages. Cell walls contribute 40% to 70% of forage dry matter, and cell wall digestibility is typically less than 65% in ruminants (NRC, 2016).

The need to enhance cell wall digestibility in ruminants has led to the investigation of exogenous fibrolytic enzymes, which are proteins produced from microbial cells that degrade fiber. Fibrolytic enzymes were originally developed for use in swine and poultry diets to decrease the antinutritional properties of fiber and to degrade the pericarp of grain, but enzymes developed for ruminants have focused on improving fiber digestibility. Although exogenous fibrolytic enzymes have been shown to increase dry matter digestibility (Arriola et al., 2011), stimulate dry matter intake (Beauchemin et al., 1995; Arriola et al., 2011) and improve feed efficiency (Beauchemin et al., 1995; Holtshausen et al.) in beef and dairy cattle, results have been inconsistent. This stems from several factors including, but not limited to, product formulation, inclusion level, delivery method, diet composition and energy status of the target animal (Beauchemin et al., 2003).

The objective of this experiment was to evaluate the effects of exogenous fibrolytic enzymes (Bovizyme[™] CX) and wheat straw inclusion level on the performance of growing beef steers receiving

Department of Animal Sciences, North Dakota State University, Fargo, ND 58102 Danisco Animal Nutrition and Health (IFF), Wilmington, DE 19803

forage-based growing diets. It was hypothesized that 1) steers receiving exogenous fibrolytic enzymes would have greater growth performance and intake than those not receiving exogenous fibrolytic enzymes, 2) steers consuming low-straw diets would have greater growth performance and intake than those receiving high-straw diets and 3) steers receiving the combination of exogenous fibrolytic enzymes and high-straw diet would not differ in growth performance compared to steers fed the no exogenous fibrolytic enzymes and low-straw diet.

Materials and Methods

All procedures were approved by the North Dakota State University Institutional Animal Care and Use committee. Seventy-three crossbred beef steers (633 \pm 42 pounds initial body weight [BW]) were blocked by initial BW (n = 3) and source (n = 2; NDSU Central Grasslands Research Extension Center [n = 38] or NDSU Beef Unit [n = 35]) and randomly assigned to four dietary treatments arranged as a 2 × 2 factorial consisting of two levels of wheat straw: either 5% or 15% of diet dry matter, and two inclusion rates of exogenous fibrolytic enzymes: either 0 g/metric ton or 750 g/metric ton of diet. The study took place at the NDSU Beef Cattle Research Complex. The enzyme product was mixed with the grower supplement and delivered via the total mixed ration (TMR).

Steers were implanted on day –2 with 80 mg of trenbolone acetate and 16 mg of estradiol (Revalor-IS, Merck Animal Health, Summit, NJ). On day 0, steers began receiving their designated treatment diet for ab libitum intake. Individual intake data were collected using an automated feeding system (Insentec Roughage Intake Control, Hokofarm B. V., Marknesse, The Netherlands). Body weights were recorded every 28 days to monitor interim performance.

Plasma samples were collected every 28 days to measure plasma urea nitrogen (PUN), plasma glucose (GLC) and plasma non-esterified fatty acids (NEFA). Net energy for gain (NEg) was calculated using gain and dry matter intake (NRC, 2016).

Data were analyzed as a randomized complete block design using SAS. Significance was set at $P \le 0.05$ and tendency at P > 0.05 and ≤ 0.10 .

Results and Discussion

There were no wheat straw × exogenous fibrolytic enzyme interactions for EBW, ADG, DMI or F:G ($P \ge 0.31$). The inclusion of exogenous fibrolytic enzymes did not influence EBW, ADG, DMI or F:G ($P \ge 0.62$). The results of the current study agree with previous literature suggesting that many factors contribute to the effectiveness of exogenous fibrolytic enzymes to improve animal performance. In dairy cows, Yang (2000) reported that milk yield increased when exogenous fibrolytic enzymes were applied to a barley-based concentrate prior to mixing with the TMR, but these same improvements were not observed when exogenous fibrolytic enzymes were mixed with the entire diet. Beauchemin (1995) reported that ADG, DMI and F:G of growing beef steers was dependent on both the inclusion rate of exogenous fibrolytic enzymes and diet composition; beef steers consuming alfalfa-based diets had the greatest improvements in growth performance, dry matter intake and F:G at moderate levels of exogenous fibrolytic enzyme inclusion while steers consuming timothy hay-based diets had the greatest improvements in growth performance, dry matter intake and F:G at high levels of exogenous fibrolytic enzyme inclusion.

As expected, increasing wheat straw inclusion from 5% to 15% decreased EBW, ADG and DMI,

and increased F:G (P < 0.001). The differences in performance are likely because of changes in fiber and starch concentrations of the diet, resulting in less energy available for cattle fed diets containing more wheat straw. Lesoing (1981) fed growing beef cattle consuming 0%, 10%, 20%, 30% or 40% wheat straw and showed that ADG decreased and feed:gain increased with increasing wheat straw inclusion. Dry matter, neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were 4.6%, 3.3% and 2.5% greater for high-straw treatments as corn silage was replaced with wheat straw (Table 1). Likewise, the concentrations of starch in the high-straw diets were 2.6% less than the low-straw treatments.

No wheat straw × exogenous fibrolytic enzyme interactions ($P \ge$ 0.18) were observed for PUN, NEFA or GLC. The inclusion of exogenous fibrolytic enzymes did not influence PUN (P = 0.15) but did increase GLC and decrease NEFA (P = 0.04and 0.05; respectively). Increased plasma glucose concentration has been observed in other studies when evaluating enzyme supplements, but this occurrence is not consistent. Arriola (2011) reported that plasma glucose increased when lactating dairy cows were supplemented with fibrolytic enzymes when consuming a 33% concentrate diet but not in those consuming a 48% concentrate diet. Holtshausen (2011) reported that insulin concentration increased when dairy cattle consumed a higher level of exogenous fibrolytic enzymes compared to those consuming a lower level. The increase in GLC suggests exogenous fibrolytic enzymes could affect production of gluconeogenic volatile fatty acids The decrease in plasma NEFA could suggest that steers consuming exogenous fibrolytic enzymes were at a higher plane of energy, but this did not translate to an increase in EBW

or ADG. Future research should evaluate the interactions between exogenous fibrolytic enzymes, the host and microbial growth.

Wheat straw inclusion did not affect PUN (P = 0.36) or GLC (P =

0.24), but increasing wheat straw inclusion increased NEFA. This was anticipated as wheat straw is less digestible than corn silage because there is less starch and greater ADF, NDF and lignin in wheat straw (NRC,

2016). The lower digestibility of wheat straw would result in steers consuming high-straw diets to have lower energy intake than steers consuming low-straw diets.

Table 1. Diet composition and nutrient analysis.

	Treatments ¹								
	5% Whe	at Straw	15% Wheat Straw						
Ingredient, %DM	0 g EFE ¹ /MT ²	750 g EFE/MT	0 g EFE/MT	750 g EFE/MT					
Wheat Straw	5	5	15	15					
Corn Silage	35	35	25	25					
DRC ³	20	20	20	20					
DDGS ⁴	20	20	20	20					
Oat Hay	15	15	15	15					
Supplement ⁵	5	5	5	5					
Nutrient Analyses, %									
Dry Matter	68.97	69.28	73.89	73.47					
Ash	8.85	8.84	9.21	9.21					
NDF ⁶	43.48	43.58	46.79	46.89					
ADF ⁷	22.50	22.50	24.99	24.99					
Crude Protein	14.55	14.32	14.05	13.81					
Starch	27.75	27.94	25.18	25.37					
Ca:P	4.50	4.46	4.72	4.56					
NEg, Mcal/kg	1.17	1.17	1.11	1.12					

¹Exogenous fibrolytic enzymes

Table 2. Growth and intake of steers consuming forage-based diets at differing levels of wheat straw and exogenous fibrolytic enzyme inclusion.

	5% Whe	eat Straw	15% Wheat Straw						
	0 g EFE ¹ /MT ²	750 g EFE/MT	0 g EFE/MT	750 g EFE/ MT		P - values			
Steers, n	18	18	18	19	SEM	3 WS × EFE	WS	EFE	
Initial BW ⁴ , lb.	628	633	628	628	5.6	0.62	0.77	0.62	
Final BW, lb.	908	908	858	866	10.9	0.63	< 0.001	0.97	
ADG^5 , lb.	3.34	3.26	2.72	2.84	0.09	0.31	< 0.001	0.82	
DMI^6 , lb/d	19.75	19.60	17.97	18.28	0.37	0.54	< 0.001	0.84	
F:G ⁷	5.91	6.01	6.61	6.44		0.45	< 0.001	0.87	

¹Exogenous fibrolytic enzymes

²Metric ton (MT)

³Dry rolled corn

⁴Dried distiller's grains with solubles

⁵Supplement contained 0.50% (DM basis) urea and formulated to provide 21.1 g/ton of monensin

⁽Rumensin, Elanco Animal Health; DM basis)

⁶Neutral detergent fiber

⁷Acid detergent fiber

²Metric ton

³Wheat straw

⁴Body weight

⁵Average daily gain

⁶Dry matter intake

⁷Feed to gain ratio

Table 3. Plasma metabolites of steers consuming forage-based diets at differing levels of wheat straw and exogenous fibrolytic enzyme inclusion.

	5% Wheat Straw		15% WI	neat Straw				-		
	0 g EFE ¹ /MT ²	FE ¹ /MT ² 750 g EFE/MT 0 g EFE/MT 750 g EFE/M				P- values				
Steers, n	18	18	18	19	SEM	$WS^3 \times EFE$	WS	EFE		
PUN ⁴ , mmol/L	9.17	8.95	9.08	8.62	0.24	0.60	0.36	0.15		
NEFA ⁵ , μmol/ L	316	305	404	347	17.4	0.18	< 0.001	0.05		
GLC ⁶ , mmol/L	4.85	4.93	4.72	4.91	0.06	0.35	0.24	0.04		

¹Exogenous fibrolytic enzymes

Acknowledgements

The authors thank Danisco
Animal Nutrition and Health for their financial support and for donating the Bovizyme™ CX; Merck Animal
Health for donating the Revalor®
-IS; and the staff and students at the
NDSU Beef Cattle Research Complex,
NDSU Beef Unit, NDSU Central
Grasslands Research Extension
Center and the NDSU Animal
Nutrition Laboratory.

References

Arriola, K. G., S. C. Kim, C. R. Staples, and A. T. Adesogan. 2011. Effect of fibrolytic enzyme application to lowand high-concentrate diets on the performance of lactating dairy cattle. J. Dairy Sci. 94: 832-841.

Beauchemin, K. A., D. Colombatto, and D. P. Morgavi. 2003. A rationale for the development of feed enzyme products for ruminants. Can. J. Anim. Sci. 84: 23-36.

Beauchemin, K. A., L. M. Rode, and V. J. H. Sewalt. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75: 641-644.

Holtshausen, L., Y.-H. Chung, H. Gerardo-Cuervo, M. Oba, and K. A. Beauchemin. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. 94: 899-907.

Lesoing, G., I. Rush, T. Klopfenstein, and J. Ward. 1981. Wheat straw in growing cattle diets. J. Anim. Sci. 51: 257-262.

National Academics of Sciences, Engineering, and Medicine. 2016. Nutrient requirements of beef cattle, eighth revised edition. Washington, DC: The National Academies Press.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83: 2512-2520.

²Metric ton

³Wheat straw

⁴Plasma urea nitrogen

⁵Plasma non-esterified fatty acids

⁶Plasma glucose

High-forage vs. high-concentrate diets fed to beef heifers during pregnancy and the impacts on feeding behavior and feed efficiency in the dam and morphometric characteristics of the male offspring

Brooklyn M. Kuzel¹, Kathryn R. M. Slavick¹, Jennifer L. Hurlbert¹, Matheus S.P. Carlis^{1,2}, German D. Ramirez-Zamudio^{1,2}, Sarah R. Underdahl¹, Kevin K. Sedivec³, Samat Amat⁴ and Carl R. Dahlen¹

Feeding pregnant beef heifers a high-concentrate diet reduces feed intake, improves gain: feed ratio and decreases calving ease compared to a high-forage diet without affecting calf vigor at birth or calf body weight and measurements up to 60 days of age.

Summary

The study assessed the impact of feeding a high-concentrate (HC) diet compared to a high-forage (HF) diet to gestating replacement heifers from 15 days pre-breeding through calving. Specifically, the areas of interest evaluated were dam feeding behavior and feed efficiency, calf body measurements and birth weights, and calf body weight to 60 days of age. By design, there was no difference in average daily gain (ADG; P = 0.50) as HF and HC dams were strategically managed for the same targeted ADG of 1 pound/ heifer/day in the first two trimesters of gestation and 1.75 pounds/heifer/

day in the third trimester of gestation; however, the gain:feed ratio was greater in HC dams than HF dams (P < 0.01). Altered feeding events included a greater number of visits and meals in HF dams compared with HC dams (P < 0.01). Time eating per visit was greater in HC dams than HF dams (P < 0.01), but HF dams spent more time eating per meal and per day ($P \le 0.02$) than HC dams. Dry matter intake (DMI) per day was greater in HF dams than HC dams (P < 0.01), but HC dams had greater DMI per visit and DMI per meal (P < 0.01) and an increased eating rate (P < 0.01) compared with HF dams. Additionally, calving ease was greater in HF dams than HC dams (P =0.03). No effect of maternal diet was observed ($P \ge 0.12$) for dam body weight at calving, calf birth weights, calf vigor at birth, or calf body weights and body measurements at 24 h of age. The results may provide support for producers to make management decisions regarding development of pregnant heifers when forages are limited, and alternative feed sources are under consideration.

Introduction

Replacement heifers are crucial to the beef production system as they provide a source of genetic improvement to the herd every year. Nutritional management of heifers during pregnancy is essential because heifers have demands for growth and maintenance while also establishing and maintaining a pregnancy, developing a fetus and producing milk for the calf after parturition (NASEM, 2016). Nutrient partitioning focuses primarily on the basal metabolism and growth of the dam and secondly on fetal development and pregnancy maintenance (Short et al., 1990). This is why proper nutrition of the dam is vital for producing healthy calves. Studies show that maternal nutrition during pregnancy can impact fetal programming (Wu et al., 2004). Fetal/ developmental programming is the phenomenon in which environmental factors affecting the dam can also influence the fetus in utero, leading to molecular and physiological changes with consequences for growth, metabolism and fertility in the offspring's postnatal life (Barker, 2004; Hammer et al., 2023). This experiment evaluated how feeding a high-concentrate diet to the dam throughout pregnancy not only affected feeding behavior and feed efficiency of the dam, but also body

¹Department of Animal Sciences and Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND, USA

²Department of Animal Science, University of Sao Paulo, Pirassununga, SP, Brazil

³Central Grasslands Research Extension Center, North Dakota State University, Streeter, ND, USA

⁴Department of Microbiological Sciences, North Dakota State University, Fargo, ND, USA

measurements and body weights of the calves.

When forage is limited, alternative supplementation could be used in limited amounts to meet nutrient requirements. This experiment utilized two diets: one consisting of 25% concentrate and 75% forage (HF), and the other consisting of 25% forage and 75% concentrate (HC). The diets were fed to gestating beef heifers to target a specific daily gain of 1 pound/ heifer/day in the first two trimesters of gestation and 1.75 pounds/heifer/ day in the third trimester of gestation. The objectives were to evaluate the impacts of developing pregnant beef heifers on a high-forage or high-concentrate diet from 15 days prebreeding through calving on feeding behavior and feed efficiency of the dam and morphometric characteristics of the male calves through 60 d of age.

Procedures

Crossbred Angus heifers (n = 119; initial body weight [BW] 748.9 ± 72.8 lbs.) approximately 13 months of age sourced from the NDSU Central Grasslands Research Extension Center (CGREC) arrived at the North Dakota State University Beef Cattle Research Complex (BCRC) in May 2023. Heifers were fitted with radio frequency identification tags (RFID) and trained to consume feed from the Insentec (Hokofarm Group B.V., Marknesse, The Netherlands) roughage intake control (RIC) bunk system. During the training period, all heifers consumed a common forage diet composed of 65% winter wheat/blended hay, 20% corn silage, 5% corn grain and 10% premix (HF; Table 1). Heifers were blocked by initial BW and randomly assigned to receive either a high-forage diet (HF; n = 60) of 75% forage and 25% concentrate or a high-concentrate diet (HC; n = 59) of 25% forage and 75% concentrate prior to breeding (Table 1). Heifers were grouped by

BW and diet assignment, then placed into one of six pens. The HF heifers remained on the diet provided at the beginning of the experiment throughout gestation. Over four weeks, the HC treatment group was stepped up from the HF diet to a diet containing 75% concentrates. Both HF and HC groups received their final diets 15 days prebreeding throughout gestation. The HC diet was composed of 15% winter wheat/blended hay, 20% corn silage, 55% corn grain and 10% premix (HC; Table 1). Heifers in both HF and HC groups were managed strategically to target BW gains of 1 pound/heifer/day. This was achieved by collecting BW measurements every other week and adjusting individual feed allotments accordingly. In the third trimester of gestation through parturition, feed allocations for pregnant heifers were adjusted to achieve target BW gains of 1.75 pounds/heifer/day.

The roughage intake control (RIC) feeding system controls intake as well as monitors feeding behavior. Each bunk has a scale that monitors how much weight is being taken out as animals are eating. Electronic ear tags allow the system to track each animal

for daily feed consumption, time spent eating and number of visits to the bunk. Further calculations using these variables allowed for a comprehensive evaluation of feeding behavior including visits per day, meals per day, time eating per visit, time eating per meal, time eating per day, dry matter intake (DMI) per day, DMI per visit, DMI per meal and eating rate. Calculations of gain to feed ratio (G:F) and average daily gain (ADG) throughout the experimental period were calculated using BW gains recorded every other week and feed intake data from the Insentec system. Feed intake and feeding behavior variables were averaged across the 266-d collection period that started at breeding and stopped when the first dam calved. Feed efficiency variables were calculated from 15 d prebreeding throughout gestation.

At approximately 14 months of age, heifers were synchronized using a seven-day Select Synch + CIDR protocol (Lamb et al., 2010) and artificially inseminated with male-sexed semen from a single sire in June 2023. At d 35 and d 65 after insemination, transrectal ultrasound

Table 1. Feed ingredient percentages in a high-forage and a high-concentrate diet fed to heifers 15 d prebreeding through gestation 1

	Treat	ment
Item	HF	HC
Ingredient % DM		
Winter wheat/blended hay ²	65	15
Corn silage	20	20
Corn grain	5	55
Premix ³	10	10

¹Feed allotments were delivered to heifers so that targeted ADG was 1 lb/heifer/day gain in the first two trimesters of gestation and 1.75 lbs/heifer/day gain in the third trimester of gestation through parturition. ²Winter wheat was the sole forage used in the diet prebreeding through the second trimester. During the third trimester, a blended winter wheat and winter rye forage was utilized as the forage in both the concentrate and forage diets.

³The premix consists of dried distiller's grain plus soluble, limestone, salt, urea, Monvet 90 Monensin Granule, trace mineral (Feedlot Trace Hubbard), vitamin A, vitamin D, vitamin E and exclusively in the high-concentrate diet, dicalcium phosphate.

was used to determine pregnancy status and fetal sex. Forty-six heifers were confirmed pregnant with male fetuses (HC: n = 22; HF: n = 24) and subsequently maintained on treatment diets through calving in March 2024.

Dams and neonatal calves were weighed at birth prior to suckling. Calves were assigned a vigor score of 1 through 5 (1 = healthy calf and 5 = stillborn) and a calving ease score of 1 through 5 (1 = no assistance required and 5 = cesarean). Dams and calves were then paired in an indoor maternity pen for approximately 24 h. At 24 h, calves were weighed, body measurements were recorded and pairs were returned to group pens. Body measurements included chest circumference, abdominal circumference, crown rump length, shoulder hip length, hip height and hip width (Table 3). Calf BW was collected at d 15 and approximately d 30 and d 60 after birth. At approximately 61 d post-calving, pairs were transported to the CGREC and managed as a single group on pasture until weaning.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS INST. Inc., Cary, NC) with individual animal serving as the experimental unit. Repeated measures were used to evaluate dam feeding behavior and calf BW gain postnatally. No significance was found in the TRT x Day interaction of calf BW gain, so main effects of treatment and day were reported. Dam weight at calving, calf weight presuckling and calf morphometric variables recorded at 24 h were analyzed with the main effect of maternal diet at a single point in time. Results are reported as least square means (LSMEANS) with the standard error of the mean. Significance was considered at P-values ≤ 0.05 and tendencies declared at $0.05 < P \le 0.10$.

Table 2. Feed intake and feeding behavior of gestating beef heifers averaged across the 266-d collection period during gestation.

	Treati	nent ¹		
Item	HF	HC	SE ²	P-value
Feeding Events, per d				
Visits ³	31.67	12.82	0.310	< 0.001
Meals^4	5.84	3.14	0.044	< 0.001
Time Eating, min				
Per visit	5.57	6.68	0.085	< 0.001
Per meal	22.31	21.78	0.218	0.02
Per day	113.16	55.41	0.637	< 0.001
Dry matter intake				
Per day, lb	16.56	14.18	0.051	< 0.001
Per visit, oz	13.80	25.84	0.275	< 0.001
Per meal, oz	52.49	81.36	0.632	< 0.001
Eating rate, oz/min	2.43	4.48	0.022	< 0.001
Measures of feed efficiency ⁵				
ADG, lb	1.28	1.30	0.033	0.50
G:F, lb:lb	0.17	0.21	0.006	< 0.001

¹Treatments were applied to heifers 15 days prebreeding and throughout gestation; HF (n = 24), diet composed of 25% concentrate and 75% forage; HC (n=22), diet composed of 75% concentrate and 25% forage.

Table 3. Weights of calves at birth and 24 h after birth and dams at calving, calf body measurements, and calving ease and vigor score following birth.

	Treatment ¹			
Item	HF	HC	SE	<i>P</i> -value
Weights				
Birth weight, lb	69.8	69.3	2.80	0.87
24-hour weight, lb	72.3	71.1	2.77	0.68
Dam weight at calving, lb	1046.1	1067.7	27.54	0.44
Calf morphometrics				
Chest circumference, in	29.41	29.31	0.411	0.82
Abdominal circumference, in	29.17	28.75	0.625	0.51
Crown rump length, in	30.05	30.97	0.570	0.12
Shoulder hip length, in	13.45	13.24	0.441	0.63
Hip height, in	28.05	27.50	0.340	0.12
Hip width, in	4.50	4.32	0.208	0.39
Ease and vigor score				
Calving ease ²	1.02	1.45	0.187	0.03
Calf vigor ³	1.46	1.64	0.330	0.59

¹Treatments were applied to heifers 15 days prebreeding and throughout gestation; HF (n=24) diet composed of 25% concentrate and 75% forage; HC (n=22) diet composed of 75% concentrate and 25% forage.

²Standard error of the mean.

³Visit is any entry to the bunk detected by electronic ear tag.

⁴Meal is a feeding event that may consist of multiple visits but is bound by a period of 7 minutes with no feeding activity on either side.

⁵Feed efficiency calculations included average daily gain (ADG) and gain:feed ratio (G:F) 15 days prebreeding and through gestation.

²Calving ease score assigned during parturition. 1=no assistance, 2=assisted, easy pull, 3=assisted, difficult pull or mechanical assistance, 4=abnormal presentation, 5=cesarean section.

³Calf vigor score assigned prior to parturition. 1=normal calf, 2=weak calf that nursed without assistance, 3=weak calf assisted to nurse and lived, 4= weak calf assisted to nurse and died, 5=stillborn.

Results and Discussion

By design, there was a strategic effort to keep ADG equal between HF and HC treatments, and results indicated no difference in ADG between HF and HC dams (P =0.50). Dry matter intake (DMI) per visit (P < 0.001) and DMI per meal (P < 0.001) were greater in HC dams compared to HF dams. However, HF dams had greater total DMI per day (P < 0.001) than HC dams, which can be explained by the greater number of visits to the bunk (P < 0.001) and meals (P < 0.001) the HF dams had compared to the HC dams. Although HC dams consumed less total dry matter compared to HF dams, G:F (P < 0.001) was greater in HC dams than HF dams. Seemingly, the nutrientdense concentrate feed comprising the HC diet allowed HC dams to put on more weight while consuming less feed. Time eating per visit (P <0.001) was greater in HC dams than HF dams; however, HF dams spent more time eating per meal (P = 0.02)and per day (P < 0.001) compared with HC dams. Eating rate (P < 0.001) was greater in HC dams than HF dams, being nearly doubled. The HC diet was less bulky than the HF diet, presumably allowing the HC heifers to consume feed faster.

Calving ease was greater in HF dams than HC dams (P = 0.03); however, there was no difference in calf vigor (P = 0.59) between HF and HC calves. Calf BW through 60 d of age was not impacted by the interaction of maternal diet x day (P =0.45; Figure 1). Additionally, maternal gestational diet did not cause differences in BW between calves born to HF and HC dams (P = 0.71). Expectedly, there was an increase in BW in both HF and HC calves with time (P < 0.001). However, BW of HC and HF dams was not impacted at time of calving (P = 0.44; Table 3). There were no differences in calf body measurements at 24 h of age, including chest circumference, abdominal circumference, crown-

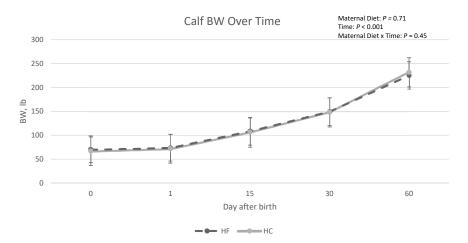


Figure 1. Calf weights at day 0, 1, 15, 30 and 60 of bull calves born to dams fed a HF or HC diet.

rump length, shoulder-hip length, hip height and hip width ($P \ge 0.12$).

These data show that a HC diet can be implemented in feeding replacement beef heifers throughout gestation. Feeding a HC diet improves feed efficiency, indicating heifers are reaching their gains while consuming less feed. The improvement in feed efficiency is highlighted by the equal ADG in HF and HC dams but a greater G:F in HC dams. Depending on the availability and cost of forage and concentrate feeds, limit-feeding concentrates in the diet may be a cost-effective method to reach nutrient requirements for gestating beef heifers. As seen in the results, feeding a HC diet does not impact calf BW or calf body measurements, but there is a decrease in calving ease in HC dams, which may be a concern depending on producer calving systems. Continuing to study effects on male calves later in life is important for further understanding of feeding strategies that may allow producers to make decisions regarding feed efficiency in dams and offspring.

Acknowledgements

The authors thank the United States Department of Agriculture National Institute of Food and Agriculture's Agriculture and Food Research Initiative (award number 2022-67016-37092), the North Dakota Corn Council, Zoetis Animal Health (Parsippany, NJ), and STGenetics (Navasota, TX) for funding and product support. The authors also thank the staff, technicians, and students at the Central Grasslands Research Extension Center and the Beef Cattle Research Complex for their assistance.

Literature cited

Barker, D. J. P. 2004. The developmental origins of well-being. Phil. Trans. R. Soc. B Biol. Sci. 359:1359–1366.

Hammer, C. J., J. S. Caton, C. R. Dahlen, A. K. Ward, P. P. Borowicz, L. P. Reynolds. 2023. Large animal models of developmental programming: Sustenance, stress, and sex matter. Reproduction. 165:F1-F13.

Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. J. Anim. Sci. 88:E181-E192.

Short, R. E., R. A. Bellows, R. B.
Staigmiller, J. G. Berardinelli, E.
E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J.
Anim. Sci. 68:799-816.

Wu, G., F. W. Bazer, T. A. Cudd, C. J. Meininger, and T. E. Spencer. 2004. Maternal nutrition and fetal development. J. Nutr. 134:2169-2172.

Impacts of limit-feeding high-concentrate diets to beef heifers during the first 180 days of gestation on performance, carcass characteristics and gastrointestinal tract morphometrics

Matheus S.P. Carlis^{1,2}, German D. Ramirez-Zamudio^{1,2}, Kathryn Slavick¹, Brooklyn Kuzel¹, Jennifer L. Hurlbert¹, Godson Aryee³, Justine Kilama³, Gabriela Magossi³, Rodrigo S. Goulart², Joel S. Caton¹, Kendall C. Swanson¹, Samat Amat¹ and Carl R. Dahlen¹

This study evaluated the strategy of limit-feeding high-concentrate diets on carcass characteristics and gastrointestinal tract morphometrics in crossbred beef heifers. Limit-feeding replacement heifers with high-concentrate diets can alter body composition and gastrointestinal tract morphometrics, favoring fat and muscle accumulation. Feeding strategies that limit intake but aim for the same daily weight gain, whether using high-concentrate or high-forage diets, can lead to different carcass characteristics and metabolic profiles. Notably, limit-feeding high-concentrate diets can favor fat and muscle accumulation, altering dam efficiency throughout her productive life.

Summary

Traditional heifer feeding strategies typically involve modest rates of body weight (BW) gain and include a relatively low proportion of concentrate feeds. The objective of this study was to evaluate the strategy of limitfeeding high-concentrate diets on performance, carcass characteristics and gastrointestinal tract (GIT)

heifers. The hypothesis was that providing high-concentrate diets in limited amounts, aiming for the same average daily gain (ADG) as high-forage diets, will alter the body composition and GIT morphometrics in beef heifers. Crossbred beef heifers (n = 20) received either a high forage (75% forage:25% concentrate [HF], n = 10) or high concentrate diet (25% forage:75% concentrate [HC] n = 10) starting 85 days (d) before breeding until 180 d of gestation. Heifers were inseminated with male-sexed semen from a single bull. Individual intake data were recorded using the Insentec Roughage Intake Control system. Heifers were weighed, and diet deliveries were adjusted every two weeks to target a gain of 1 lb/d

morphometrics in replacement beef for both groups. Data were analyzed using the MIXED procedure of SAS, with heifer as the experimental unit. No differences between treatments were observed in final BW, ADG or body condition score ($P \ge 0.18$). Heifers receiving the HF diet had a greater (P < 0.05) dry matter intake (DMI), full GIT weight, GIT weight as a percentage of body weight, empty rumen-reticulum weight, total digesta, ruminal pH and cecal pH, and tended ($P \le 0.10$) to have a greater empty abomasum and serum cortisol concentration compared to HC heifers. Conversely, heifers fed the HC diet showed greater (P < 0.05) marbling score, mesenteric fat and ruminal digesta dry matter, number of ruminal papillae, percentage of papillae per absorptive surface area and concentrations of IGF-1 concentrations, and tended ($P \le$ 0.10) to have greater carcass weight, ribeye area and empty large intestine weight. These findings demonstrate that, despite similar gain rates, limit-feeding high-concentrate diets can alter the composition of weight gain, favoring fat, muscle, and GIT morphometry, potentially altering dam efficiency throughout her productive life.

Introduction

Traditional feeding strategies for beef heifers typically involve modest rates of body weight gain

¹Department of Animal Sciences and Center for Nutrition and Pregnancy, North Dakota State University, Fargo,

²Department of Animal Science, University of Sao Paulo, Pirassununga, SP, Brazil

³North Dakota State University, Department of Microbiological Sciences, NDSU Department 7520, Fargo, ND 58108-6050, USA

and a relatively low proportion of concentrated feeds. However, in situations of limited forage availability or high feeding costs, high-concentrate diets fed in a restricted manner can be a viable alternative. This approach aims to increase nutrient utilization efficiency and reduce cattle feeding costs. A study by Loerch (1996), which used a 100% concentrate diet with restricted intake for pregnant Simmental crossbred cows, reported no significant differences in performance and reduced feeding costs compared to feeding high-forage (HF) diets. A key concept when feeding highconcentrate (HC) diets is to provide them in smaller quantities compared to HF diets to achieve targeted daily gains (Brickell et al., 2009; Wathes et al., 2014) due to greater energy content, greater ruminal fermentation potential and digestibility per unit of dry matter (NASEM, 2016).

The literature contains numerous studies, primarily on confined young cattle, reporting that HC diets stimulate ruminal development, increase insulin-like growth factor I (IGF-I) and insulin concentrations, and modify the composition of gain, favoring greater muscle and fat deposition compared to HF diets. Conversely, HF diets are known to increase ruminal volume, lower fat gain and increase DMI compared to HC diets (Diao et al., 2019). However, in these studies, animals typically exhibit different weight gains due to the different compositions of the diets resulting in cattle receiving greater amounts of concentrate generally having greater ADG.

Few studies have evaluated the use of limit-feeding of replacement beef heifers with the same weight gain, particularly regarding performance, carcass characteristics and GIT morphometrics. The objective of this study was to evaluate the strategy of limit-feeding HC diets on performance,

carcass characteristics and GIT morphometrics in replacement beef heifers. The hypothesis was that providing HC diets in limited amounts, aiming for the same ADG as high-forage diets, will alter body composition and GIT morphology in crossbred Angus-based heifers.

Procedures

Twenty crossbred Angus-based heifers (initial body weight [BW] = 727.5 ± 69 lb and age 13) were housed at the NDSU Beef Cattle Research Complex. Heifers were divided into pens equipped with Insentec feeders (Hokofarm Group B.V., Marknesse, The Netherlands) and randomly assigned to one of two treatments: HF group (n = 10) received a diet composed of 75% forage and 25% concentrate, while the HC group (n

= 10) received a diet composed of 75% concentrate and 25% forage. Treatments were fed from 90 d before until 180 d after breeding, through artificial insemination (AI), all heifers to male-sexed semen from a single sire. Pregnancy status was confirmed 28 days post-breeding, and fetal sex was determined 65 days post-breeding to confirm male pregnancies.

The heifers were distributed based on initial weight into two pens (n = 10) with eight feeders each and free water access, and each pen receiving one of the treatment diets. The heifers were weighed bi-weekly and diet deliveries were adjusted to target a gain of 1 lb/d for both groups. The diets were formulated using the Beef Cattle Nutrient Requirements Model (2016 - Version 1.0.37.12).

Table 1. Proportion of ingredients and chemical composition (BCNR prediction) of experimental diets.

Ingredients ¹ , % DM	High concentrate	High forage
Corn silage	20.0	20.0
Distillers grain plus soluble	7.18	7.88
Corn grain	55.0	5.00
Oat hay	15.0	65.0
Limestone	0.90	0.50
Dicalcium phosphate	0.30	0.00
Sodium chloride	0.20	0.20
Urea	0.85	0.85
Monovet 90 Monensin	0.02	0.02
Trace Mineral	0.05	0.05
Vit A	0.20	0.20
Vit D	0.20	0.20
Vit E	0.10	0.10
Chemical composition ² , %DM		
Dry Matter	66.4	67.1
Crude protein	13.4	15.9
Ether extract	3.85	3.13
Ash	4.48	6.64
Neutral detergent fiber	29.1	52.4
Lignin	2.34	4.13
Nonfibrous carbohydrates	50.7	23.4
Starch	47.2	13.2
Total digestible nutrients ²	77.1	63.9
ME ³ , Mcal/kg	0.59	0.48

¹Composition of Monovet 90 Monensin: 200g of monensin in 1 kg.

²Calculated from equations from Weiss et al. (1992).

³ME = metabolizable energy (NRC, 1984)

Performance measures evaluated from AI until d 180 of gestation included DMI and ADG, which were used to calculate gain efficiency (lb gain per lb of feed). Body condition score (BCS; scale of 1–9, with 1 [emaciated] and 9 [obese]) was determined on d 180.

Heifers were harvested at 180 days of gestation at a federally inspected meat processing facility. Hot carcass weight was determined at slaughter, and carcass characteristics including back fat, ribeye area, rib fat, and marbling grade were measured after a 24-h chill.

To evaluate GIT characteristics, the stomach complex (rumenreticulum, omasum and abomasum), the small intestine and the large intestine were separated, and each was weighed without removal of digesta (i.e., full weight). Then the digesta and mesenteric fat were removed, and the stomach complex, small and large intestine, and mesenteric fat were weighed (i.e., empty weight). The empty body weight was determined from subtracting the digesta weight from the final body weight. A sample of rumen wall (approximately 1 cm²) was collected from the ventral cranial sac from each heifer, and papillae were counted. Following papillae counting, 12 papillae were cut from the ruminal wall at their base and arranged on a glass slide for a photo for subsequent morphological evaluation. The evaluated macroscopic morphological variables included the area tissue (AT); number of papillae per cm² of the wall (NPP); height, width and average area of the papillae (AMP); absorptive surface area per cm² of the wall (ASA); and percentage of papillae per absorptive surface area (% papillae/ASA). The absorptive surface area of the wall (ASA) in cm² was calculated as AT + $(NPP \times AMP)/(NPP \times APB)$. The area of the papillae, expressed as a percentage of ASA, was calculated as $(NPP \times AMP)/(ASA \times 100)$ (Ribeiro et al., 2019; Pereira et al., 2020).

Blood samples collected on d 0, 28, 56, 91 and 180 relatives to AI were used to determine serum cortisol, insulin and IGF-I concentration. Seric insulin, cortisol and IGF-I concentrations were determined by chemiluminescence immunoassay by using a commercial kit of Immulite 1000 (Siemens Healthcare Diagnostics Products, Llanberis, UK).

Data were analyzed as a single measure using the MIXED procedure of Statistical Analysis System (SAS, version 9.4, 2018), with heifer as the experimental unit and were considered statistically significant when $P \le 0.05$ and a trend when $0.05 < P \le 0.10$.

Results and Discussion

By design, ADG and final BW were similar between treatments (P = 0.18, P = 0.22; Table 2). Although HF heifers had greater DMI than HC heifers (P < 0.001), tend to have lower empty body weight (P = 0.06)

and there were no differences in feed efficiency (P = 0.30).

Regarding hormone concentrations, heifers fed HF tended to have greater cortisol (P = 0.10) and lower IGF-I (P < 0.001) concentrations compared to heifers fed HC, and no differences in insulin concentration were observed (Table 3).

Heifers fed the HC diet had greater marbling score (P = 0.04) and tended to have greater carcass weight (P = 0.06) and ribeye area (P = 0.07) than heifers fed the HF diet (Table 4).

Heifers fed the HF diet had greater full gastrointestinal tract weight (P < 0.001), gastrointestinal tract weight as a percentage of body weight (P < 0.001), stomach complex full (P < 0.001), empty rumen-reticulum weight (P < 0.05), total digesta weight (P < 0.001) and stomach complex digesta weight (P < 0.001), and tended to have greater empty abomasum weight (P = 0.07; Table 4). Conversely, heifers fed the HC diet had greater mesenteric fat (P = 0.001)

Table 2. Performance of heifers subjected to different diet compositions in Al at 180 days of gestation.

Items ¹	High concentrate	High forage	SEM ²	P-Value
Initial Body weight, lb	905.0	835.0	20.19	0.08
Final Body weight, lb	1,107.0	1,096.5	22.07	0.22
Empty Body weight, lb	982.57	919.32	16.84	0.06
Dry matter intake, lb/day	12.9	16.7	0.04	< 0.001
Average gain, lb/day	1.17	1.34	0.11	0.18
Feed efficiency, ADG/DMI	0.09	0.08	0.01	0.30

¹N. Heifers: Number of heifers, DMI: Dry matter intake, ADG: Average daily gain. ²SEM: Standard error of the mean.

Table 3. Blood metabolite parameters in heifers are subjected to limit feeding high-concentrate diets to beef heifers during the first 180 d of gestation.

Items	High concentrate	High forage	SEM ¹	P-Value
Cortisol, pmol/L	1.44	1.76	0.10	0.10
IGF-I ² , pmol/L	186.2	126.6	4.47	< 0.001
Insulin, pmol/L	5.54	4.80	0.31	0.52

¹SEM: Standard error of the mean.

²IGF-I: Insulin-like growth factor type I.

< 0.001) and tended to have greater empty large intestine absolute weight than heifers fed the HF diet (P = 0.06; Table 4). Furthermore, heifers fed HC had lower ruminal and cecal pH, and greater number of ruminal papillae and percentage of papillae/ASA (Table 4).

These results indicate that, despite the greater DMI for HF heifers, there was no difference in feeding efficiency (FE) due to the similar ADG (Table 2), demonstrating that even with limited intake, the HC diet has better energy efficiency, requiring 3.77 lb less DM to ensure the same performance. This can be attributed to the greater energy in the HC diet per kg of DM, requiring lower DMI to achieve the same energy intake.

However, despite no difference in ADG (Table 2) and BSC (Table 4), the composition of gain was different, as

HC heifers had a greater percentage of the gain represented by muscle development, indicated by the larger ribeye area, and of fat as indicated by mesenteric fat (Table 4). In contrast, HF heifers tended to have a greater portion of their gain represented by digesta and heavier weights of the rumen-reticulum and abomasum directly reflecting in the lower empty body weight (Table 4). Besides the lower digestibility potential of the HF diet, diets with high forage inclusion have longer retention times in the GIT (Allen et al., 2009; Arndt et al., 2014). This is supported by the larger stomach complex digesta, with this value being numerically higher than the DMI for heifers fed the HF diet. Additionally, the larger size of the rumen-reticulum and abomasum contributes to digesta representing a greater proportion of body weight in HF heifers compared to HC heifers.

The reduced ruminal and cecal pH in HC heifers allows us to hypothesize that a greater concentration of short-chain fatty acids (SCFAs) may be present, which could explain the greater number of ruminal papillae, percentage of papillae/ASA and heavier large intestine compared to HF heifers. The greater concentration of SCFAs, especially butyrate, has indicated in several studies as stimulating GIT development, as the butyrate is almost entirely used to energy font by the GIT epithelium (Górka et al., 2018). Further, elevated concentrations of butyrate are associated with cellular proliferation in the ruminant GIT (Górka et al., 2018) and increased IGF-I production (Baldwin VI et al., 2017). These results are consistent with the present study, in which HC heifers had greater concentrations of IGF-I, more ruminal

Table 4. Characterization of gastrointestinal organs and corporeal fat in heifers subjected to limit feeding high-concentrate diets to beef heifers during the first 180 d of gestation.

Items	High-concentrate	High-forage	SEM ¹	P-Value
Body condition score ²	5.35	5.30	0.10	0.71
Carcass, lb	592.6	550.9	11.24	0.06
Back fat, mm	12.2	10.2	0.98	0.31
Ribeye area, mm ²	249.7	229.6	5.61	0.07
Rib fat, mm	5.59	4.47	0.61	0.37
Marbling score	394.0	332.0	15.06	0.04
Gastrointestinal tract Full, lb	206.6	256.7	5.78	< 0.001
Gastrointestinal full tract % of body weight	15.7	21.1	0.47	< 0.001
Gastrointestinal empty tract % of body weight	4.82	4.90	0.13	0.66
Stomach complex, full, lb ²	139.91	195.43	4.45	< 0.001
Rumen-reticulum empty, lb	19.1	20.8	0.53	0.04
Abomasum empty, lb	3.73	5.09	0.51	0.07
Large intestine empty, lb	10.43	9.19	0.42	0.06
Mesenteric fat, lb	34.2	25.7	2.76	< 0.001
Mesenteric % of body weight	3.09	2.33	0.22	0.03
Digesta total, lb	119.6	177.2	4.92	< 0.001
Stomach complex digesta, lb of dry matter	16.60	21.28	0.87	< 0.01
Ruminal pH	6.17	6.84	0.09	< 0.001
Cecal pH	7.02	7.34	0.04	< 0.001
Number of papillae	58.3	46.3	2.96	0.04
%papillae/ASA ²	69.2	61.0	3.15	< 0.001

¹SEM: Standard error of the mean.

 $^{^{2}}$ Body condition score (scale of 1 – 9, with 1 [emaciated] and 9 [obese] (Wagner et al., 1988); Stomach complex includes rumen, reticulum, omasum, and abomasum., %papillae/ASA: Percentage of papillae per absorptive surface area.

papillae and heavier intestinal empty weight compared to HF heifers.

These findings demonstrate that, despite similar gain rates, limit-feeding a high-concentrate diet, compared to a high-forage diet, can alter the composition of weight gain (favoring fat and muscle) and GIT development, potentially altering dam productivity. Additionally, there is a need for further studies evaluating the impact of restricted feeding on ruminal fermentation and fetal development in heifers.

Acknowledgements

The authors appreciate support of this effort from the United States Department of Agriculture National Institute of Food and Agriculture's Agriculture and Food Research Initiative Award # 2021-07185, the North Dakota Corn Council, Zoetis Animal Health (Parsippany, NJ) and STGenetics (Navasota, TX), and the São Paulo Research Foundation (Grant #2023/12386-2).

Literature Cited

- Allen M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317-3334.
- Arndt C., L. E. Armentano, and M. B. Hall. 2014. Corn bran versus corn grain at 2 levels of forage: Intake, apparent digestibility, and production responses by lactating dairy cows. J. D. Sci. 97:5676-5687.
- Baldwin VI R., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the preand postweaning ruminant. J. D. Sci. 87:E55-E65.
- Diao, Q., Z. Rong and F. Tong. 2019. Review of strategies to promote rumen development in calves. Animals. 9:490.
- Górka P., Z. M. Kowalski, R. Zabielski, and P. Guilloteau. 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. J. D. Sci. 101:4785-4800.
- Herrick K. J., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, D. P. Casper, S. C. Moreland, and J. E. Van Eys. 2017. Single-dose infusion of sodium butyrate, but not lactose, increases plasma β-hydroxybutyrate and insulin in lactating dairy cows. J. D. Sci. 100:757-768.

- Loerch S. C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. J. Anim. Sci. 74:1211-1216.
- Pereira M. C. S., J. V. T. Dellaqua, O. A. Sousa, P. F. Santi, L. D. Felizari, B. Q. Reis, and D. D. Millen. 2020. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of Nellore cattle submitted to strategic diets period the adaptation period. Lives. Sci. 234:103985.
- NASEM. 2016. National Academies of Sciences, Engineering, and Medicine. Nutrient requirements of beef cattle model, 8th rev. ed. Washington (DC): National Academic Press.
- Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass Composition in Mature Hereford Cows: Estimation and Effect on Daily Metabolizable Energy Requirement During Winter. J. Anim. Sci. 66:603–612.
- Wathes D. C., G. E. Pollot, K. F. Johnson, H. Richardson, and Cooke. 2014. Heifer fertility and carry over consequences for life time production in dairy and beef cattle. Animal. 8:91-104.

The influence of gestational body weight gain rate on the development of two generations of beef cattle

Friederike Baumgaertner¹, German D. Ramirez-Zamudio^{1,2}, Ana Clara B. Menezes³, Isabella M. Jurgens¹, Mara R. Hirchert¹, Jennifer L. Hurlbert¹, Kerri A. Bochantin¹, Lawrence P. Reynolds¹, Alison K. Ward^{1,4}, Pawel P. Borowicz¹, James D. Kirsch¹, Sheri T. Dorsam¹, Kendall C. Swanson¹, Joel S. Caton¹, Carl R. Dahlen¹, Kevin K. Sedivec⁵ and Wellison J. S. Diniz⁶

Maternal weight gain during early pregnancy in beef heifers affects the growth and metabolic development of the offspring, with observable effects across multiple generations. A moderate increase in the rate of maternal body weight gain during early gestation improves the performance of F1 heifers and elevates their glucose levels. Conversely, lower rates of maternal weight gain increase placental and mammary gland mass, in addition to positively regulating genes in the intestines of F2 fetuses, suggesting a compensatory mechanism.

Summary

A study was conducted to understand how early pregnancy weight gain in beef heifers affects their daughters and granddaughters. One hundred crossbred Angus heifers (F0 generation) were randomly

¹Department of Animal Sciences, and Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND 58108

²Dr. Ramirez-Zamudio is a Visiting Researcher from the Department of Animal Science, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, SP, Brazil 13635-900 ³Present address for Dr. Menezes: Department of Animal Science, South Dakota State University, Brookings, SD, 57007, USA

⁴Present address for Dr. Ward:
Department of Veterinary Biomedical
Science, University of Saskatchewan,
Saskatoon, SK S7N5A2, Canada
⁵Central Grasslands Research Extension
Center, North Dakota State University,
Streeter, ND 58483
Wellison J. S. Diniz
⁶Department of Animal Sciences, Auburn

University, Auburn, AL 36849

a low-gain group (LG; n = 50), fed to achieve a gain of 0.28 kg/day, and a moderate-gain group (MG; n = 50), targeting a gain of 0.79 kg/day. During the first 84 days of gestation, the LG group received a basal total mixed ration (TMR) consisting of 53% hay, 37% corn silage, and 10% DDGS. The MG group received the same ration plus an energy/ protein supplement, provided at 0.58% of body weight per day. After the 84 days, all heifers were fed the same diet. When the daughters (F1 generation) of the F0 heifers were born, they were raised similarly until weaning. At 15 months of age, eight heifers from each group were selected to continue the study. These heifers were inseminated and slaughtered at 84 days of pregnancy to evaluate various aspects, such as maternal body weight, blood glucose and hormone levels, organ weights, and fetal (F2 generation) development. Additionally, gene expression in the intestines of F1 heifers and F2 fetuses

assigned at mating into two groups:

was analyzed. It was found that F1 heifers from the MG group tended to be heavier at birth (P = 0.06) and during post-weaning growth (P =0.07) and had greater blood glucose levels (P = 0.03). F1 heifers from the LG group tended to have heavier placentas at day 84 of their first pregnancy (P = 0.10). Interestingly, F2 fetuses from the LG group showed greater expression of some genes in the intestines (NDUFC1, SDHA, UQCR1, ATP5E, and PPARG; P < 0.05) and heavier mammary glands (P = 0.05). These results show that heifer weight gain during early pregnancy can affect their daughters and granddaughters. This suggests that nutritional management during this period can have long-term effects on the herd, which is essential knowledge for producers seeking to improve the productivity and sustainability of their operations.

Introduction

Proper nutrition for replacement heifers in cow-calf systems is crucial for the farm's long-term success. Pastures are the primary food source, but their quality and availability vary dramatically throughout the year (Drouillard, 2018). Good nutrition is essential in early pregnancy because, during this period, the fetal organs and placenta are forming, which affects calf growth and future performance. Research shows that cow nutrition during this phase can influence the offspring and

the subsequent generation. This phenomenon is called developmental programming (Reynolds et al., 2019).

Previous studies have demonstrated that heifer weight gain and supplementation with vitamins and minerals during pregnancy affect fetal development, including organs, metabolism and placental function (Menezes et al., 2021). However, we still need to learn more about how these affect subsequent generations of beef cattle (Caton et al., 2020; Reynolds et al., 2023).

Therefore, we conducted a study to determine how different rates of heifer (F0 generation) weight gain during the first 84 days of pregnancy affect the growth of their daughters (F1 generation), the reproductive capacity of F1 heifers and the development of the next generation of F2 fetuses. We hypothesized that the rate of body weight gain in replacement heifers during early pregnancy can cause significant changes in the growth and metabolic state of their daughters, and further that these effects may even extend to the granddaughters. Understanding this is important to improving the nutritional management of replacement heifers, which will increase the productivity and sustainability of operations in the long term.

Procedures

One hundred crossbred Angus heifers (F0 generation) were synchronized for estrus using a seven-day Select Synch + CIDR protocol and were artificially inseminated with sexed (X) semen from a single bull. After insemination, the F0 heifers were randomly divided into two dietary treatment groups: a low-gain group, targeting a body weight gain rate of 0.28 kg/day (n = 50), and a moderate-gain group, with a body weight gain rate of 0.79 kg/ day (n = 50) during the first 84 days of gestation. The weight gains of the LG and MG dams were achieved by adjusting the dietary energy

density, with the MG group receiving an energy/protein supplement composed of a mixture of ground corn, dried distillers grains with solubles, wheat bran, fish oil, urea and ethoxyquin.

After the first 84 days of gestation, all F0 dams were managed as a single group and fed a common forage-based diet per the NASEM (2016) recommendations until calving. All F0 dams and their F1 offspring were kept together and received the same nutritional management until weaning. After weaning, the F1 heifers from both dietary treatment groups continued to receive the same dietary management during the prebreeding development period (Table 1). At 15 months of age, a subset of F1 heifers (n = 16; eight from the LG group and eight from the MG group) was selected, artificially inseminated and slaughtered on the 84th day of gestation. The heifers were weighed, and blood samples were collected on the day of insemination and on days 42 and 84 of gestation. Blood was analyzed to measure glucose, nonesterified fatty acids (NEFA), progesterone, insulin, and insulin-like growth factor 1 (IGF-1) concentrations.

At slaughter, carcass weight of the F1 heifers was recorded before and after a 24-hr cooling period. Carcass characteristics, including Longissimus muscle area and subcutaneous fat measured between the 12th and 13th thoracic vertebrae, were evaluated after 24 hr of cooling. Additionally, the following organs and viscera from the F1 heifers were removed and weighed at slaughter: mammary gland, liver (gallbladder removed), heart, lungs, kidneys, pancreas, spleen, small intestine (1 m section of the jejunum), brain and gravid uterus. The uterus was weighed before and after the removal of the conceptus, and the weight of the placenta and fetus (F2 generation) was recorded.

The F2 fetuses were weighed and photographed laterally using the Omni ASH digital videoscope system. Each fetus was dissected, and the following organs were individually weighed: liver, heart, lungs, pancreas, small intestine, rumen, kidneys, spleen, brain, femur, Longissimus dorsi, hindlimb, uterus, ovaries and mammary gland. The recorded fetal images were analyzed using image analysis software for the following measurements: straight crown-rump length, curved crown-rump length, horizontal eye diameter, nose-tocrown distance, body depth (the vertical distance from the backbone to the midventral line) at shoulder level and body depth (the vertical

Table 1. Chemical composition of diet provided to F1 heifers during development until harvest at d 84 of gestation

Chemical Composition	Development Diet1
Dry matter, %	65.6
Ash, %	10.7
Crude Protein, % DM	12.3
Acid Detergent Fiber, % DM	32.9
Neutral Detergent Fiber, % DM	59.1
Ether Extract, % DM	1.44
Calcium, g/kg DM	0.66
Phosphorus, g/kg DM	0.44

 $^{^{1}}$ Proportion of ingredients on dry matter basis: prairie grass hay (70%), corn silage (20%) and premix (10%) with the premix containing dried distillers grains with solubles (5%), ground corn (2.9%), urea (0.87%), limestone (0.4%), dicalcium phosphate (0.55%), monensin (0.02%), vitamin premix (0.01%), mineral premix (0.05%) and NaCl (0.2%).

distance from the backbone to the midventral line) directly behind the navel. Jejunum samples from the F1 heifers and F2 fetuses were collected and stored at -80 C for later mRNA expression analyses. Relative mRNA expression was assessed using RT-qPCR, focusing on genes related to mitochondrial respiratory chain enzymes.

Data were statistically analyzed using the MIXED procedure in SAS 9.4, with significance at $P \le 0.05$.

Results and Discussion

The F1 heifers from the MG group tended to be heavier (P = 0.06) than those from the LG group, with an average body weight of 413.0 ± $4.45 \text{ kg compared to } 401.1 \pm 4.39$ kg in LG heifers during the growth phase. However, slaughter weight and carcass measurements of the F1 heifers were not influenced by their (F0) dams' body weight gain rate during early gestation. Maternal organ and reproductive tract mass of the F1 heifers also did not show significant differences (P > 0.10) due to their dams' rate of body weight gain, except for placental weight, which tended to be greater (P = 0.10) in F1 heifers from the LG group compared to those from the MG group (204.1 g vs. 152.6 g for LG vs. MG; Table 2).

As for the concentrations of NEFA, progesterone, insulin and IGF-1 in the blood of F1 heifers, no effects of their dams' rate of body weight gain during early gestation were observed (P > 0.05; Table 3). However, blood glucose concentrations in F1 heifers were greater (P = 0.03) in the MG (74.1 \pm 1.35 mg/dL) compared to the LG group (69.8 \pm 1.35 mg/ dL; Table 3). These results suggest that the maternal body weight gain rate during early gestation may influence the metabolic status of their F1 offspring, particularly in terms of glucose homeostasis, aligning with findings from previous studies (Wu et al., 2006; Vonnahme et al., 2010).

Table 2. Effect of low and moderate rate of body weight gain in FO dams during early gestation on body weight, carcass characteristics, organ mass, and reproductive tract characteristics of F1 heifers harvested at 84 days of gestation

	Treatment ¹			
Item	LG	MG	SEM	P-value
Live BW, kg	420.6	423.8	10.87	0.84
HCW, kg	218.1	213.2	5.79	0.55
Dressing percentage, %	51.8	51.6	0.51	0.73
Longissimus muscle area, cm2	61.9	61.8	2.69	0.96
Back fat, cm	0.43	0.53	0.077	0.35
Maternal organ mass, g				
Mammary gland	2680.3	2913.5	264.79	0.54
Liver	4383.6	4524.9	114.19	0.40
Lungs	2212.4	2138.5	65.88	0.44
Pancreas	335.3	311.2	18.26	0.35
Kidneys	847.5	863.1	20.27	0.60
Spleen	569.3	547.9	22.09	0.49
Brain	369.0	392.7	13.83	0.21
Heart	1537.4	1526.7	45.08	0.80
Maternal reproductive tract, g				
Gravid Uterus	1568.7	1461.9	58.08	0.21
Empty Uterus	402.1	400.1	14.10	0.92
Placenta	204.1	152.6	21.79	0.10

¹Treatment diets provided to F0 generation from breeding until d 84 of gestation: 1) a basal mixed ration targeting gain of 0.28 kg/d (LG, n = 8), or 2) the basal diet plus an energy/protein supplement to achieve targeted gain of 0.79 kg/d moderate gain (MG, n = 8).

Table 3. Effect of low and moderate rate of body weight gain in FO dams during early gestation on serum metabolites and hormone concentrations of F1 heifers during prebreeding and the first trimester of gestation

	Treati	ment ¹		P-values		
Item	LG	MG	SEM	Treatment	Day	Treatment × Day
Glucose, mg/dL						
Prebreeding ²	66.9	68.4				
d 42 of gestation ³	70.4	75.4	2.34	0.03	< 0.01	0.549
d 83 of gestation ⁴	72.1	78.7				
NEFA, umol/L						
Prebreeding	298.8	211.5				
d 42 of gestation	249.6	207.7	35.87	0.16	0.02	0.46
d 83 of gestation	154.7	157.8				
Progesterone, ng/mL						
Prebreeding	2.06	3.01				
d 42 of gestation	6.56	6.49	0.726	0.71	< 0.01	0.68
d 83 of gestation	6.49	6.27				
IGF-1, ng/mL						
Prebreeding	145.4	146.6				
d 42 of gestation	94.3	106.0	10.13	0.60	< 0.01	0.82
d 83 of gestation	97.7	98.0				
Insulin, uIU/mL						
Prebreeding	5.25	3.15				
d 42 of gestation	6.51	6.02	0.842	0.17	0.04	0.53
d 83 of gestation	4.63	4.32				

 $^{^1}$ Treatment diets provided to F0 generation from breeding until d 84 of gestation: 1) a basal mixed ration targeting gain of 0.28 kg/d (LG, n = 8), or 2) the basal diet plus an energy/protein supplement to achieve targeted gain of 0.79 kg/d moderate gain (MG, n = 8).

²Blood samples were collected prebreeding coinciding with the beginning of estrus synchronization (CIDR insertion – 10 d before artificial insemination).

³Blood samples were collected at the midpoint of the first trimester (d 42 following artificial insemination).

 $^{^4}$ Blood samples were collected at the time of harvest (d 84 ± 0.26 post artificial insemination).

The morphological measurements and organ mass of F2 fetuses were not affected (P > 0.10; Table 4) by the F0 body weight gain rate during early gestation. However, F2 fetuses from the LG group showed an increase in mammary gland mass relative to fetal weight (P = 0.05) and a tendency toward greater absolute mammary gland mass compared with F2 fetuses from the MG group (P = 0.10). Gene expression (mRNA) analysis revealed positive regulation of genes associated with energy metabolism in the small intestine of F2 fetuses from LG compared to those from MG dams (Figure 1). The genes NDUFC1, SDHA, UQCR1 and PPARG were significantly more expressed ($P \le 0.05$), whereas ATP5E also showed a tendency to be more expressed (P = 0.06). These differences in gene expression suggest that the lower maternal weight gain rate during early gestation may prepare the intestines of F2 fetuses for enhanced nutrient absorption and energy utilization, possibly as an adaptive response to lower nutrient availability in utero. This aligns with the concept of developmental programming (Sookoian et al., 2013) and indicates that the maternal nutritional environment can have long-term effects, manifesting across subsequent generations. Although the nutritional intervention was applied to the F0 dams, its effects were evident in the F2 fetuses, highlighting the lasting influence of early gestational nutrition on fetal organ development. This observation builds on the broader understanding of developmental programming as discussed by Reynolds et al. (2019) and Caton et al. (2020).

Conclusion

This study demonstrates that maternal body weight gain rate during the first 84 days of gestation can have lasting effects across multiple generations. Moderate gain results in F1 heifers with better performance and higher glucose

levels. At the same time, lower rate of gain increased the weight of F1 heifer placentas, mammary gland mass and the expression of energy metabolism-related genes in the intestines of F2 fetuses, possibly as an adaptive

response to lower nutrient availability during gestation. These findings underscore the importance of proper nutritional management during early gestation to ensure the healthy development of future generations of beef cattle.

Table 4. Effect of low and moderate rate of body weight gain in FO dams during early gestation on body measurements and organ weights of F2 fetuses harvested at 84 days post-conception

	Treat	ment ¹			
Item	LG	MG	SEM	<i>P</i> -value	
Fetal mass, g					
Body	125.9	117.1	4.09	0.23	
Liver	4.66	4.59	0.268	0.86	
Heart	1.20	1.24	0.077	0.72	
Lungs	4.61	4.20	0.270	0.29	
Small Intestine	2.61	2.42	0.168	0.44	
Pancreas	0.11	0.14	0.017	0.25	
Rumen	4.74	4.54	0.632	0.82	
Kidneys	1.27	1.15	0.081	0.29	
Spleen	0.14	0.12	0.016	0.35	
Brain	4.75	4.67	0.323	0.86	
Femur	0.33	0.32	0.020	0.80	
Longissimus dorsi	1.93	1.78	0.177	0.53	
Hindlimb	3.26	2.70	0.331	0.24	
Ovaries	0.08	0.07	0.010	0.68	
Uterus	0.06	0.06	0.008	0.82	
Mammary gland	0.59	0.53	0.020	0.07	
Fetal mass, % of fetal BW					
Liver	3.70	3.91	0.117	0.21	
Heart	0.95	1.07	0.049	0.10	
Lungs	3.65	3.57	0.126	0.66	
Small Intestine	2.04	2.07	0.103	0.84	
Pancreas	0.09	0.10	0.012	0.35	
Rumen	3.80	3.85	0.491	0.94	
Kidneys	1.01	0.98	0.041	0.58	
Spleen	0.11	0.10	0.010	0.50	
Brain	3.78	4.00	0.226	0.50	
Femur	0.26	0.25	0.012	0.66	
Longissimus dorsi	1.54	1.49	0.106	0.74	
Hindlimb	2.59	2.29	0.240	0.38	
Ovaries	0.06	0.06	0.007	0.99	
Uterus	0.04	0.05	0.006	0.76	
Mammary gland	0.48	0.44	0.016	0.05	
Measurements, cm					
Biparietal distance	3.16	3.13	0.064	0.73	
Crown rump length	13.3	13.0	0.11	0.13	
Curved crown rump length	16.1	15.7	0.26	0.29	
Eye diameter	0.99	0.98	0.025	0.81	
Nose to poll	4.96	4.94	0.067	0.85	
Body depth at front shoulder	4.09	3.92	0.065	0.08	
Body depth behind umbilicus	4.08	3.97	0.096	0.41	

¹Treatment diets provided to F0 generation from breeding until d 84 of gestation: 1) a basal mixed ration targeting gain of 0.28 kg/d (LG, n = 8), or 2) the basal diet plus an energy/protein supplement to achieve targeted gain of 0.79 kg/d moderate gain (MG, n = 7).

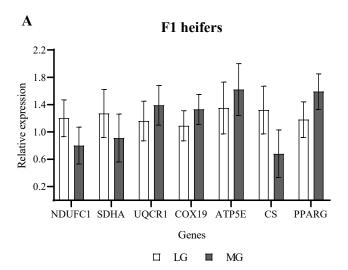
Acknowledgements

We thank the USDA National Institute of Food and Agriculture (Award #2021-67016-33389), the North Dakota Corn Council and the North Dakota Agricultural Experiment Station for financial support. We also extend our appreciation to Zoetis Animal Health for providing synchronization products and to ST

Genetics for their support with sexed semen. Special thanks to the staff at the Central Grasslands Research Extension Center and the Beef Cattle Research Complex for their assistance with animal handling and feeding.

Literature Cited

Caton, J. S., M. S. Crouse, K. J. McLean, C. R. Dahlen, A. K. Ward, R. A. Cushman, A. T. Grazul-Bilska, B. W. Neville, P.



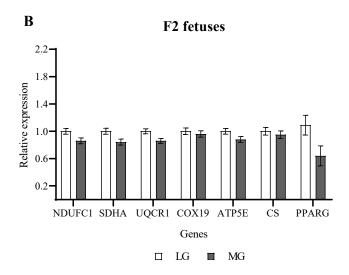


Figure 1. Effect of low and moderate rate of body weight gain in FO dams during early gestation on mRNA expression in the small intestine of F1 heifers (Panel A) and F2 Fetuses (Panel B) harvested at 84 days of gestation. Data are presented as expression relative to the HPRT1 gene. NDUFC1: NADH: ubiquinone oxidoreductase subunit C1; SDHA: Succinate dehydrogenase complex flavoprotein subunit A; UQCR1: Ubiquinol-cytochrome c reductase core protein 1; COX19: Cytochrome c oxidase assembly factor COX19; ATP5E: ATP synthase F1 subunit epsilon; CS: Citrate synthase; PPARG: Peroxisome proliferator activated receptor gamma. Values are least squares means, with error bars depicting standard error.

- P. Borowicz, and L. P. Reynolds. 2020. Maternal periconceptual nutrition, early pregnancy, and developmental outcomes in beef cattle. J. Anim. Sci. 98:skaa358.
- Drouillard, J. S. 2018. Current situation and future trends for beef production in the United States of America A review. Asian-Australas. J. Anim. Sci. 31:1007–1016.
- Menezes, A. C. B., K. L. McCarthy, C. J. Kassetas, F. Baumgaertner, J. D. Kirsch, S. Dorsam, T. L. Neville, A. K. Ward, P. P. Borowicz, L. P. Reynolds, K. K. Sedivec, J. C. Forcherio, R. Scott, J. S. Caton, and C. R. Dahlen. 2021. Vitamin and mineral supplementation and rate of gain during the first trimester of gestation affect concentrations of amino acids in maternal serum and allantoic fluid of beef heifers. J. Anim. Sci. 99:skab024.
- NASEM. 2016. Nutrient requirements of beef cattle. Eighth revised edition. National Academies Press, Washington, DC.
- Reynolds, L. P., P. P. Borowicz, J. S. Caton, M. S. Crouse, C. R. Dahlen, and A. K. Ward. 2019. Developmental programming of fetal growth and development. Vet. Clin. North Am. Food Anim. Pract. 35:229–247.
- Reynolds, L. P., C. R. Dahlen, A. K. Ward, M. S. Crouse, P. P. Borowicz, B. J. Davila-Ruiz, C. Kanjanaruch, K. A. Bochantin, K. J. McLean, K. L. McCarthy, A. C. B. Menezes, W. J. S. Diniz, R. A. Cushman, and J. S. Caton. 2023. Role of the placenta in developmental programming: Observations from models using large animals. Anim. Reprod. Sci. 257:107322.
- Sookoian, S., T. F. Gianotti, A. L. Burgueño, and C. J. Pirola. 2013. Fetal metabolic programming and epigenetic modifications: a systems biology approach. Pediatr. res. 73:531-542.
- Vonnahme, K. A., J. S. Luther, L. P. Reynolds, C. J. Hammer, D. B. Carlson, D. A. Redmer, and J. S. Caton. 2010. Impacts of maternal selenium and nutritional level on growth, adiposity, and glucose tolerance in female offspring in sheep. Domestic animal endocrinology 39:240-248.
- Wu, G., F. Bazer, J. Wallace, and T. Spencer. 2006. Board-invited review: intrauterine growth retardation: implications for the animal sciences. J. Anim. Sci. 84:2316-2337.

Effects of limit-feeding replacement heifers with a high-concentrate or high-forage diet during gestation on oxygen consumption and mitochondrial function in liver and jejunum tissues

Kathryn R. M. Slavick¹, Jennifer L. Hurlbert¹, Brooklyn M. Kuzel¹, Matheus S. P. Carlis^{1,2}, German D. Ramirez-Zamudio^{1,2}, Samat Amat¹, Kevin K. Sedivec³, Kendall C. Swanson¹ and Carl R. Dahlen¹

Results show differing liver tissue metabolism between replacement heifers fed high-concentrate diets and high-forage diets during pregnancy. Diet composition impacts energy use of key organs with the potential to impact whole animal energetic efficiency.

Summary

The study assessed the impact of limit-feeding pregnant replacement heifers with high-concentrate (HC) or high-forage (HF) diets on energy utilization in liver and jejunum of the heifers and their offspring. We hypothesized that the type of diet would influence tissue oxygen consumption and mitochondrial function of the dams and fetuses. Once received at the NDSU Beef Cattle Research Complex, replacement heifers (n = 20; initial body weight [BW] = 749 ± 17 [standard error] lb) were blocked by initial body weight and randomly assigned to either a HC (n = 10) or HF (n = 10) diet targeting BW gains of 1 lb/heifer/day. After an adjustment period, heifers were

fed treatment diets for approximately 85 days before artificial insemination using male sexed semen and remained on their respective dietary treatments until tissue collection. Heifers were euthanized on d 180 of gestation (final BW = 1102 ± 15 [SE] lb), at which time maternal liver, maternal jejunum and fetal jejunum were collected, and tissue oxygen consumption and mitochondrial function were assessed via highresolution respirometry (Oroboros Instruments, Innsbruck, Austria). Fetal jejunum was not found to be influenced by dietary treatments, whereas HF diets tended to increase respiration due to proton leak (L) in the maternal jejunum (P = 0.10). Additionally, maternal liver oxygen consumption was decreased at the nicotinamide adenine dinucleotide + hydrogen (NADH)-linked oxidative phosphorylation (PI) (P = 0.04) and electron transfer capacity (E) respiratory states (P = 0.04) in HC heifers compared to HF heifers. The observed differences between HC and HF heifers in mitochondrial respiration indicate an increased

mitochondrial efficiency and

improved adenosine triphosphate (ATP) synthesis functionality in heifers fed high-forage diets.

Introduction

High-concentrate diets are typically fed to support rapidly growing cattle in finishing feedlots (Terry et al., 2021) to improve growth performance and consumer- desirable carcass characteristics. Many operations in North Dakota rely on pastures and native rangelands. Some supplement with purchased forage, concentrate feeds or cornbased products to meet the nutrient requirements of cattle (Asem-Hiablie et al., 2016). However, feeding highconcentrate diets to replacement heifers retained for calf-rearing needs warrants further research to better understand the impact of limit-feeding with concentrate- or forage-based diets during the first two trimesters of gestation on energy utilization by metabolic organs such as the maternal liver and jejunum, as well as its implications for mitochondrial efficiency and oxygen consumption in maternal and fetal tissues. This research is valuable for producers as it could lead to alternative diet options during times of limited forage availability, such as droughts, and help identify the most cost-effective rations for optimizing heifer growth and offspring outcomes.

¹Department of Animal Sciences and Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND, USA

²Department of Animal Science, University of Sao Paulo, Pirassununga, SP, Brazil

³Central Grasslands Research Extension Center, North Dakota State University, Streeter, ND, USA 58483

The liver and jejunum are key metabolic organs significantly affecting energy utilization and efficiency. Together, the liver and gastrointestinal tract represent 45% to 50% of the animal's total basal energy requirement (Johnson et al., 1990), with the liver utilizing approximately 22% of basal energy requirements, while accounting for less than 2% of overall body weight in cattle (Caton et al., 2000).

Previous studies have shown that ad libitum feeding of high-concentrate diets reduces first-calf dairy heifer lactation and mammary development (Swanson, 1960). However, when introducing limit-feeding strategies to reduce average daily gain to a similar level as when forage-based diets are fed, no influences on heifer lactation and mammary development were reported (Carson et al., 2000).

The goal of this project was to evaluate the influence of feeding HC or HF diets, limit-fed to allow for similar growth rates, on liver and jejunum tissue oxygen consumption and mitochondrial function. Highconcentrate diets typically contain more grains, which have high starch concentrations, and are fermented differently in the rumen with the proportion of propionate relative to acetate increasing. Therefore, we hypothesized that the resulting differences in available energy substrates would influence liver and jejunum mitochondrial function of replacement heifers and their fetal offspring.

Experimental Procedures

The NDSU Beef Cattle Research Complex in Fargo, ND, received 119 crossbred Angus heifers, sourced from the NDSU Central Grasslands Research Extension Center, at approximately 13 months of age. Replacement heifers were blocked by initial body weight (n = 119; initial body weight [BW = 749 ± 17 [SE] lb) and randomly assigned to either

a high-concentrate (HC; n = 59) or high-forage (HF; n = 60) diet targeting BW gains of 1 lb/heifer/day. Heifers were ranked by body weight and sorted into one of six pens and fed individually via an electronic feed bunk (Insentec Roughage Intake Control System, Hokofarm B.V., Marknesse, The Netherlands). All heifers were given an approximate two-week period of adaptation to adjust to the feeding system and facilities. High-concentrate heifers were given adequate time to adjust and step up to their final treatment diet (Table 1), which was implemented 15 days prior to the first day of breeding. Heifers were weighed biweekly, and feed allotments were adjusted based on performance to achieve a target gain of 1 lb/day.

Heifers were subjected to a seven-day select synch + CIDR estrus synchronization protocol (Lamb et al., 2010) and bred via artificial insemination (AI) using male-sexed semen from a single sire. Pregnancy diagnosis was confirmed via transrectal ultrasonography on d 35 following AI, with fetal sex confirmation at d 65 following AI. Heifers (n = 46) of the first breed group continued on their respective

treatment diet through gestation and were used for a different experiment. Heifers that were not pregnant at the first ultrasound (n = 32 HC and n =29 HF) were resynchronized and bred to sexed male semen from a single sire 85 d after beginning to receive their respective treatment diets. The remaining heifers were subjected to a seven-day select synch + CIDR estrus synchronization protocol and bred via artificial insemination using male-sexed semen from a single sire. Pregnancy and fetal sex were confirmed on d 35 and d 65 following AI, respectively. Twenty heifers pregnant by the second insemination with male fetuses were used for the current experiment (n = 10 HC and n= 10 HF).

Heifers were euthanized at 180 days of gestation (final body weight [BW] = 1102 ± 15 [SE] lb) via captive bolt and exsanguination. Maternal liver, maternal jejunum and fetal jejunum (20 mg of each) were collected and placed in a microtube containing chilled preservation media. Tissue samples were transported to the laboratory for high-resolution respirometry analysis and placed in the Oroboros O2k Fluorespirometer (Oroboros Instruments, Innsbruck, Austria) to

Table 1. Feed ingredients of diets delivered to gestating replacement heifers limit-fed either a high-concentrate (HC) or high-forage (HF) diet

Ingredient, % DM	HC	HF	
Corn Silage	20.0	20.0	
DDGS	7.18	7.88	
Corn Grain	55.0	5.0	
Winter Wheat/Blended Hay	15.0	65.0	
Limestone	0.90	0.50	
Salt	0.30	0.20	
Urea	0.8	85	
Monensin	0.02		
Trace Mineral Mix	0.0	05	
Vitamin A	0.2	20	
Vitamin D	0.20		
Vitamin E	0.10		
Total	100.0		

assess tissue oxygen consumption and mitochondrial function utilizing a substrate-inhibitor-uncoupler protocol.

The substrate-uncoupler-inhibitor titration protocol (SUIT) was utilized to assess oxygen consumption focused on the mitochondrial electron transport chain (ETC), which is responsible for ATP production. The ETC stages evaluated in this study included proton leak respiration (L), oxidative phosphorylation capacity (P), NADH-linked oxidative phosphorylation respiration (PI) and electron transfer capacity (E). Leak respiration describes the oxygen consumption utilized to compensate for energy losses, with greater L respiration indicating greater proton leak and heat production. Oxidative phosphorylation capacity is the amount of oxygen needed for mitochondria to produce ATP when abundant substrates are available. NADH-linked OXPHOS respiration evaluates the use of NADH substrates and oxygen through the use of the enzyme glutamate dehydrogenase. Electron transfer capacity measures oxygen consumption of the ETC in a state of overabundant energy substrates.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), with a fixed effect of treatment. Results are reported as least square means and standard errors. For all analyses, heifer was considered the experimental unit, P-values ≤ 0.05 were considered significant and tendencies were considered at $0.05 < P \leq 0.10$.

Results and Discussion

Maternal jejunum was not influenced by maternal dietary treatment. However, high-forage heifers tended to have increased leak respiration ($P \ge 0.10$; Figure 1). Maternal proton leak (L) and oxidative phosphorylation (P) in the

liver tended to be greater (P = 0.10; Figure 2) in HF heifers compared to HC heifers. Additionally, NADH-linked oxidative phosphorylation (PI) and electron transfer capacity (E) were greater (P = 0.04, P = 0.04) in the HF heifers compared to HC heifers. Maternal jejunum from HF

heifers tended (P = 0.10) to have greater oxygen consumption at the L respiratory state, and fetal jejunum oxygen consumption was not influenced ($P \ge 0.51$) by the dietary treatments. In HF heifers, increased oxygen consumption of the liver was noted at every respiratory

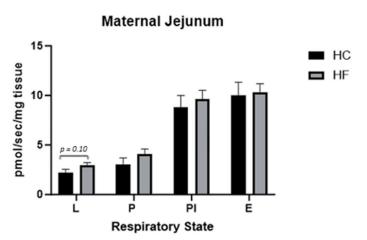


Figure 1. Oxygen consumption in the maternal jejunum of replacement heifers in response to consuming high-concentrate (HC) and high-forage (HF) diets during the first two trimesters of pregnancy. Values are least square means with error bars depicting standard error. No differences in oxygen consumption were observed in the P, PI and E respiratory states; HF high-forage heifers tended to have increased L respiration (P=0.10).

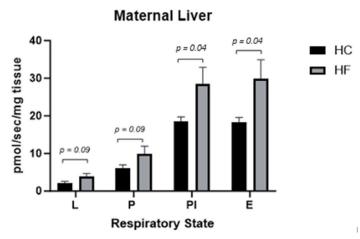


Figure 2. Oxygen consumption in the maternal liver of replacement heifers in response to high-concentrate (HC) and high-forage (HF) dietary treatments. Values are least square means with error bars depicting standard error. Dietary treatment impacted hepatic mitochondrial function. Leak respiration (L) and OXPHOS (P) in the liver tended to be greater in HF heifers compared to HC heifers. Additionally, NADH-linked OXPHOS (PI) and electron transfer capacity (E) were greater in the HF heifers compared to HC heifers.

state evaluated in this study when compared with HC heifers ($P \le 0.09$). These observations could be occurring because of differences in ruminal fermentation and volatile fatty acid concentrations between treatment groups.

Interestingly, no differences between HC and HF heifer maternal or fetal jejunum oxygen were observed (Figure 3). However, research has suggested that increased concentrate inclusion increases rumen papilla surface area and intestinal villi height (Zitnan et al., 2003). The reason for these results could be because intestinal morphology characteristics, including cellular turnover, influence oxygen consumption of the intestine (Sceaffer et al., 2003).

This study found that heifers fed HF diets had increased hepatic efficiency for ATP synthesis and electron transfer capacity compared to heifers fed HC diets. Heifers showed differences and tendencies in how liver and jejunum tissues used oxygen and varied at several mitochondrial respiratory states.

These differences suggest that mitochondrial function adapts to the energy supply and substrates of the available diet. Mitochondrial adaptations might affect energy allocation and result in a collective effect on energetic efficiency at the whole animal level. Although maternal diet did not impact fetal tissues at the evaluated pregnancy stage, further research is being conducted to explore how maternal diet influence fetal growth, organ development and offspring energy utilization of steer calves. Overall, the diet composition fed to replacement heifers impacted energy use of key organs, which has a potential effect on whole animal energetic efficiency.

Acknowledgements

The authors appreciate support of this effort from the United States Department of Agriculture National Institute of Food and Agriculture's Agriculture and Food Research Initiative Award # 2021-07185, Zoetis Animal Health (Parsippany, NJ) and STGenetics (Navasota, TX).

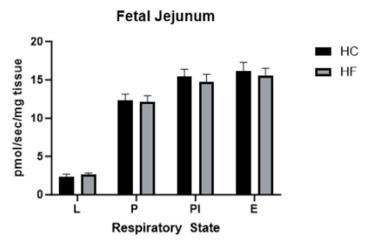


Figure 3. Oxygen consumption in the fetal jejunum from replacement heifers fed high-concentrate (HC) and high-forage (HF) dietary treatments. Values are least square means with error bars depicting standard error. No significant difference in oxygen consumption was noted across evaluated respiratory states between HC and HF treatments ($P \geq 0.51$).

Literature Cited

- Asem-Hiablie, S., C. A. Rotz, R. Stout, and K. Stackhouse-Lawson. 2016. Management characteristics of beef cattle production in the Northern Plains and Midwest regions of the United States. Prof. Anim. Sci.. 32:736-749
- Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567–590.
- Carson, A. F., A. R. G. Wylie, J. D. G. McEvoy, M. McCoy, and L. E. R. Dawson. 2000. The effects of plane of nutrition and diet type on metabolic hormone concentrations, growth and milk production in high genetic merit dairy herd replacements. Anim. Sci. 70:349-362.
- Caton, J. S., M. S. Crouse, L. P. Reynolds, T. L. Neville, C. R. Dahlen, A. K. Ward, and K. C. Swanson. 2018. Maternal nutrition and programming of offspring energy requirements. Transl. Anim. Sci. 3:976-990.
- Caton, J. S., J. J. Reed, R. P. Aitken, J. S. Milne, P. P. Borowicz, L. P. Reynolds, D. A. Redmer, and J. M. Wallace. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87:222–235.
- Johnson, D. E., K. A. Johnson, and R. L. Baldwin. 1990. Changes in liver and gastrointestinal tract energy demands in response to physiological workload in ruminants. J. Nutr. 120:649–655
- Scheaffer, A. N., J. S. Caton, M. L. Bauer, D. A. Redmer, and L. P. Reynolds. 2003. The effect of pregnancy on visceral growth and energy use in beef heifers1. J. Anim. Sci. 81:1853–1861.
- Swanson, E. W. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377–387.
- Terry, S. A., J. A. Basarab, L. L. Guan, and T. A. McAllister. 2021. Strategies to improve the efficiency of beef cattle production. Can J Anim Sci. 101:1-19.
- Zitnan, R., S. Kuhla, K. Nürnberg, U. Schönhusen, Z. Ceresnakova, A. Sommer, M. Baran, G. Greserova, and J. Voigt. 2003. Influence of the diet on the morphology of ruminal and intestinal mucosa and on intestinal carbohydrase levels in cattle. Vet. Med. 48:177–182.

Placental vascular development and cell proliferation throughout gestation in beef heifers

Chutikun Kanjanaruch¹, Bethania J. Dávila Ruiz¹, Jennifer L. Hurlbert¹, Friederike Baumgaertner¹, Joel S. Caton¹, Pawel P. Borowicz¹, Carl R. Dahlen¹, Lawrence P. Reynolds¹, Kyle J. McLean², Matthew S. Crouse³ and Kacie L. McCarthy⁴

The objective of the current study was to characterize placental vascular development and cellular proliferation throughout gestation in beef heifers. By quantifying vascular development (capillary density) and cell proliferation in both maternal and fetal placental tissues, we aimed to elucidate the dynamic changes occurring during this critical period. We found that, as in other ruminants (e.g., sheep), fetal placental vascular development accelerates whereas maternal placental vascular development slows during late pregnancy. These observations will provide a baseline for studies examining how various factors like maternal nutrition affect placental vascular development in cattle.

Summary

This study explored placental blood vessel development in beef heifers by assessing CD31 and CD34 expression, which mark blood vessels, and Ki67 expression, indicating cell proliferation. Throughout a gestation period of approximately 283 days, we collected placental samples from 54 pregnant crossbred Angus heifers at eight gestational stages: days 34 (n=5), 50 (n=5), 63 (n=6), 83 (n=6), 161 (n=7), 181 (n=10), 250 (n=7) and 272 (n=8). Analyses revealed significant differences in capillary area density

(CAD) and capillary number density (CND) between the fetal (cotyledon or COT) and maternal (caruncle or CAR) regions. Early in pregnancy (days 34, 50 and 63), CAR exhibited greater CAD and CND compared to COT (P < 0.01). By days 250 and 272, COT showed a significant increase in CAD (11.528%) and CND (0.512%) compared to CAR (8.373% vs. 0.323%, respectively; P < 0.01). Additionally, Ki67 expression, a marker of cell proliferation, was greater in CAR from days 63 to 250 (P < 0.01). Both CAD and CND displayed cubic increases over gestation in both CAR and COT regions (P < 0.01). COT had a greater proportional increase in CAD (12.43-fold) compared to CAR (2.00-fold), and COT also showed a greater increase in CND (11.23-fold) compared to CAR (3.84-fold). These results indicate that COT vascular development accelerates significantly in late pregnancy, while the

proportional growth of CAR vascular beds is more gradual. Ki67 expression followed a similar cubic increase for both regions (P < 0.01). This study enhances our understanding of placental vascular development and growth in beef heifers, revealing how blood vessel growth changes throughout pregnancy and highlighting the importance of regional differences in vascularization for fetal development.

Introduction

Placental vascular development is crucial for the large increase in placental blood flow that facilitates the exchange of essential substances (nutrients, respirator gases [e.g., O_2 and CO₂] and metabolic wastes) between maternal and fetal circulations, thereby supporting fetal growth and development (Reynolds et al., 2010). This intricate process, known as angiogenesis, involves the formation of new blood vessels in the placenta and is indispensable for maintaining its proper placental function throughout gestation (Reynolds et al., 2010; Caton et al., 2020). In ruminants such as cattle, the placenta comprises distinct cotyledonary (COT) and caruncular (CAR) tissues that originate from the fetus and mother, respectively (Reynolds et al., 2010). Despite its significance, research on placental vascular development in cattle remains scarce. This study aimed to explore placental vascular development in beef heifers by

¹Department of Animal Sciences and Center for Nutrition and Pregnancy, NDSLI

²Department of Animal Science, University of Tennessee, Knoxville, TN ³USDA ARS U.S. Meat Animal Research Center, Clay Center, NE

⁴Department of Animal Sciences, University of Nebraska-Lincoln, Lincoln, NE

analyzing the expression of CD31 and CD34, reliable markers of vascularity that are expressed in capillary blood vessels. We also analyzed Ki67, a marker of cellular proliferation. Ki67 is a nuclear protein associated with cell division and expressed in proliferating cells across various stages of the cell cycle but not in resting cells (Olivera et al., 2001).

We hypothesized that during late gestation, the COT region would exhibit greater percentage increases in CD31 and CD34 due to increased vascularization that is necessary to meet the heightened metabolic demands of the growing fetus. Moreover, Ki67 expression would provide a clear picture of proliferative activity in these tissues, with significant changes reflecting growth processes. By investigating these markers, the study sought to explain the patterns of vascular development and cellular proliferation in placental tissues of beef heifers, enhancing our understanding of fetal development and its implications for beef cattle health and productivity.

Procedures

Fifty-four pregnant crossbred Angus beef heifers, aged 18-24 months, were selected for the study due to their more than 50% Angus genetic makeup. They were fed according to National Academies of Sciences, Engineering, and Medicine (NASEM) guidelines (2016) to ensure optimal nutrition and health. Placental samples were collected at eight gestational stages: days 34 (n=5), 50 (n=5), 63 (n=6), 83 (n=6), 161 (n=7), 181 (n=10), 250 (n=7) and 272 (n=8). Each heifer was sampled only once, and all pregnancies were carried to term, with gestation lengths ranging from 279 to 287 days (average 283±4 days).

Placental and uterine tissues were collected and processed following protocols established in our laboratories. Tissue sections were stained with rabbit anti-CD31 and anti-CD34 antibodies (Abcam) to mark endothelial cells, with DAPI used for nuclear staining and BS1 lectin for identifying COT vs. CAR regions. Tissue sections also were stained for Ki-67 (Abcam) to mark proliferating cells, with DAPI (Life Technologies) used for nuclear staining. Slides were deparaffinized, rehydrated and subjected to antigen retrieval, blocking and antibody incubation steps, then mounted with EverBrite mounting medium (Biotium). Images were captured using a Mica microhub fluorescence microscope (Leica Microsystems) and analyzed with ImagePro-Premiere software (Ver.9.0.1, Media Cybernetics) to measure capillary

area density (CAD) and capillary number density (CND) in different placental regions, including COT, CAR and whole placentome (CAR + COT). The capacity for blood flow in the placenta is measured by CAD, while CND reflects the number of capillaries present and their branching (Reynolds et al., 1990).

Statistical analysis was performed using SAS software. Key placental vascular metrics (CAD and CND) were summarized with descriptive statistics. Generalized linear models (PROC GLM) were used to examine relationships between vascular metrics (CAD and CND) and cell proliferation, gestational age and placental region, while regression

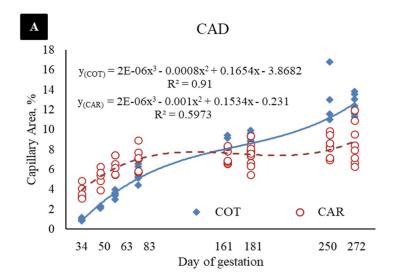
Table 1. Dynamics of capillary area density (CAD) and capillary number density (CND) in beef heifer placenta throughout gestation using expression of CD31 and CD34 as markers of vascularity.

	Days of gestation	Region ¹			
Measurement		COT	CAR	SEM ²	P-value ³
Capillary Area Density, %	34	1.003	3.757	0.556	0.001
	50	2.223	5.244	0.556	<0.001
	63	3.505	6.438	0.507	<0.001
	83	5.546	7.079	0.507	0.04
	161	7.955	7.349	0.469	0.36
	181	8.521	7.518	0.393	0.07
	250	11.528	8.373	0.469	<0.001
	272	12.466	8.623	0.439	<0.001
Capillary Number Density, %	34	0.055	0.081	0.026	0.49
	50	0.068	0.184	0.026	0.002
	63	0.097	0.211	0.024	0.001
	83	0.123	0.231	0.024	0.002
	161	0.231	0.265	0.022	0.28
	181	0.260	0.276	0.018	0.53
	250	0.512	0.323	0.022	<0.001
	272	0.618	0.311	0.020	< 0.001
Ki-67, Labeling Index, %	34	3.302	6.391	3.751	0.56
	50	14.999	18.991	3.751	0.45
	63	16.434	34.649	3.424	< 0.001
	83	21.646	41.611	3.424	< 0.001
	161	27.527	50.220	3.424	< 0.001
	181	29.222	54.482	2.652	< 0.001
	250	38.351	59.265	3.170	< 0.001
	272	27.948	36.837	2.965	0.04

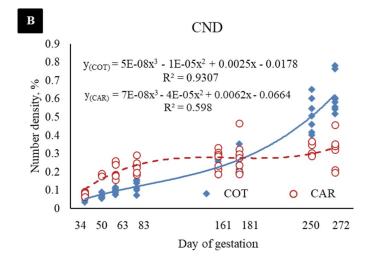
¹CAR = caruncle; COT = cotyledon

²Standard error of the mean

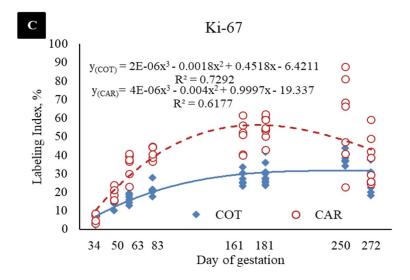
³P-values in bold indicate significant differences between COT and CAR



CAD	Type	R-squared P-value	
СОТ	Linear	0.8926	<.0001
	Quadratic	0.8978	<.0001
	Cubic	0.9100	<.0001
	Exponential	0.6828	<.0001
	Sigmoid	0.8339	<.0001
CAR	Linear	0.485	<.0001
	Quadratic	0.5178	<.0001
	Cubic	0.5973	<.0001
	Exponential	0.3236	<.0001
	Sigmoid	0.4183	<.0001



CND	Type	R-squared	P-value
COT	Linear	0.8642	<.0001
	Quadratic	0.9264	<.0001
	Cubic	0.9307	<.0001
	Exponential	0.8989	<.0001
	Sigmoid	0.9242	<.0001
CAR	Linear	0.5139	<.0001
	Quadratic	0.5499	<.0001
	Cubic	0.5980	<.0001
	Exponential	0.3342	<.0001
	Sigmoid	0.4634	<.0001



Ki-67	Type	R-squared P-value		
COT	Linear	0.6125	<.0001	
	Quadratic	0.7254	<.0001	
	Cubic	0.7292	<.0001	
	Exponential	0.319	<.0001	
	Sigmoid	0.6816	<.0001	
CAR	Linear	0.3108	<.0001	
	Quadratic	0.6138	<.0001	
	Cubic	0.6177	<.0001	
	Exponential	0.0733	<.0001	
	Sigmoid	0.5227	<.0001	
	oiginoid	0.3441	~.0001	

Figure 1. Regressions of (A) Capillary Area Density (CAD), (B) Capillary Number Density (CND) and (C) Cell Proliferation (Ki-67 staining) throughout gestation in beef heifers.

models (PROC REG) were used to analyze growth patterns of CAD and CND, and cell proliferation with gestational age. A P-value of less than 0.05 was considered significant.

Results and Discussion

The study revealed significant insights into placental vascular development throughout pregnancy in beef heifers, focusing on CD31 and CD34 as markers of vascular endothelial cells and Ki67 as a marker of cellular proliferation. Our analysis showed notable variations in CAD and CND across different gestational time points. Early in pregnancy (days 34, 50 and 63), the maternal placenta (CAR) exhibited greater CAD and CND compared to the fetal placenta (COT), indicating that the maternal side initially supports greater vascular development. However, as pregnancy progressed, particularly at days 250 and 272, COT had greater CAD and CND than CAR. This shift highlights the increased demand for vascular development in the fetal placenta during late pregnancy to support the rapidly growing fetus.

Consistent with findings from other studies, such as those by Borowicz et al. (2007) in sheep, which showed greater increases in CAD and CND in COT compared to CAR in late gestation, our results also reveal more pronounced increases in COT. The cubic increase in CAD for both CAR (CAD_(COT) = $2E-06x^3 - 0.0008x^2$ + 0.1654x - 3.8682: R² = 0.9100, P < 0.01) and COT (CAD_(CAR) = $2E-06x^3$ $-0.001x^2 + 0.1534x - 0.231$: R² = 0.5973, P < 0.01) indicates a complex pattern of vascular growth, with COT showing a much larger proportional increase (12.43 fold) compared to CAR (2.00 fold). Similarly, CND displayed a cubic increase, with COT

again showing a greater proportional rise (11.23 fold) compared to CAR (3.84 fold) (CND_(CAR) = 7E-08 x^3 - 4E-05 x^2 + 0.0062x - 0.0664 and CND_(COT) = 5E-08 x^3 - 1E-05 x^2 + 0.0025x - 0.0178; R² = 0.5980 and 0.9307, respectively; P < 0.01). These trends suggest that the fetal placenta undergoes more pronounced vascular growth as gestation progresses, aligning with the increased metabolic needs of the developing fetus.

Ki67 expression, which serves as an index of cellular proliferation, was significantly greater in CAR compared to COT from days 63, 83, 161, 181 and 250 (*P* < 0.01). This indicates greater cellular proliferation in the maternal placenta during early to mid-gestation, possibly reflecting its role in supporting the initial stages of fetal development. Both CAR and COT exhibited cubic increases in Ki67 expression over gestation $(Ki67_{(COT)} = 2E-06x^3 - 0.0018x^2 +$ 0.4518x - 6.4211 and Ki67_(CAR)= $4E-06x^3 - 0.004x^2 + 0.9997x - 19.337; R^2$ = 0.7292 and 0.6177, respectively; P <0.01), suggesting a dynamic pattern of cellular proliferation in response to the changing demands of fetal growth.

In summary, the results of this study underscore the crucial role of both maternal and fetal placental components in supporting optimal fetal growth and development. The early greater vascularity in CAR and the substantial late pregnancy increases in COT highlight the adaptive responses of the placenta to meet the evolving metabolic demands throughout gestation. These findings enhance our understanding of placental physiology in beef heifers and offer foundational insights for further research on placental function and fetal development in cattle.

Acknowledgements

This work was supported by USDA National Institute of Food and Agriculture grant numbers 2021-67015-34277 to Lawrence P. Reynolds, and 2021-67016-33389 and 2022-67016-36479 to Carl R. Dahlen. Mention of a trade name, proprietary product or specific agreement does not constitute a guarantee or warranty by the USDA and does not imply approval to the inclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

Literature Cited

Borowicz, P. P., D. R. Arnold, M. L. Johnson, A. T. Grazul-Bilska, D. A. Redmer, and L. P. Reynolds. 2007. Placental growth throughout the last two-thirds of pregnancy in sheep: Vascular development and angiogenic factor expression. Biol. Reprod. 76:259-267.

Caton, J. S., M. S. Crouse, K. J. McLean, C. R. Dahlen, A. K. Ward, R. A. Cushman, A. T. Grazul-Bilska, B. W. Neville, P. P. Borowicz, and L. P. Reynolds. 2020. Maternal periconceptual nutrition, early pregnancy, and developmental outcomes in beef cattle. J. Anim. Sci. 98:skaa358.

Olvera, M., S. Harris, C. A. Amezcua, A. McCourty, S. Rezk, C. Koo, J. C. Felix, and R. K. Brynes. 2001. Immunohistochemical expression of cell cycle proteins E2F-1, Cdk-2, Cyclin E, p27(kip1), and Ki-67 in normal placenta and gestational trophoblastic disease. Mod. Pathol. 14:1036–1042.

Reynolds, L. P., D. S. Millaway, J. D. Kirsch, J. E. Infeld, and D. A. Redmer. 1990. Growth and in vitro metabolism of placental tissues of cows from day 100 to day 250 of gestation. Reproduction. 89:213–222.

Reynolds, L. P., P. P. Borowicz, J. S. Caton, K. A. Vonnahme, J. S. Luther, D. S. Buchanan, S. A. Hafez, A. T. Grazul-Bilska, and D. A. Redmer. 2010. Uteroplacental vascular development and placental function: an update. Int. J. Dev. Biol. 54:355-66.















