NDSU Central Grasslands Research Extension Center

2016 Annual Report

Range - Forage - Livestock

NDSU | NORTH DAKOTA AGRICULTURAL EXPERIMENT STATION

NDSU | EXTENSION SERVICE

Table of Contents

Overview

3 **Summary of the Year**

4 **Weather for the 2015 - 2016 Crop Year**

47 **Staff and Advisory Board**

Articles

6 **Performance of Beef Cattle Managed in Two Overwintering Environments**
Jessalyn Bachler, Stephanie Becker and Michael Undi

10 **Bale Grazing Poor-quality Grass Hay With and Without Supplementation**
Jessalyn Bachler, Stephanie Becker and Michael Undi

13 **Extending the Grazing Season With Corn Residue and Cover Crop**
Stephanie Becker, Michael Undi, Ryan Odenbach and Hal Weiser

16 **Impacts of Bale Grazing on Herbage Production, Forage Quality and Soil Health in South-central North Dakota**
Fara Brummer, Kevin Sedivec, Mary Berg, Chris Augustin, Penny Nester, Sheldon Gerhardt, Jackie Buckley, Ashley Stegeman and Dennis Whitted

21 **Land Management Strategies to Control Kentucky Bluegrass Invasion**
Megan Endreson and Ryan Limb

26 **Quality of Hay from Road Right of Ways in North Dakota**
Miranda Meehan, Kevin Sedivec, Fara Brummer and Carl Dahlen

29 **Effects of Maternal Nutrition on Fructose and Expression of the Fructose Transporter *GLUT5* in Bovine Tissues and Fluids from Days 16 to 50 of Gestation**
Nathaniel Greseth, Matthew Crouse, Kyle McLean, Mellissa Crosswhite, Nicolas Negrin Pereira, Alison Ward, Larry Reynolds, Carl Dahlen, Bryan Neville, Pawel Borowicz and Joel Caton

34 **Effects of Maternal Nutritional Status on Nutrient Transporter Expression in Bovine Utero-placental Tissue on Days 16 to 50 of Gestation**
Matthew Crouse, Kyle McLean, Mellissa Crosswhite, Nicolas Negrin Pereira, Alison Ward, Lawrence Reynolds, Carl Dahlen, Bryan Neville, Pawel Borowicz and Joel Caton

40 **The Use of Testicular Fine-needle Aspiration, Histology and Immunohistochemistry for Determining Bull Fertility at an Early Age**
Nicolas Negrin Pereira, Pawel Borowicz, Bryan Neville, Jordan Flaten and Carl Dahlen

43 **The Effect of Vitamin A Treatment on Testicular Development in Young Peri-pubertal Bulls**
Nicolas Negrin Pereira, Pawel Borowicz, Jordan Flaten, Bryan Neville and Carl Dahlen

Summary of the Year

Welcome to the 2016 CGREC Annual Report

2016 was busy and included highs and lows.

The year started with new research projects involving beef nutrition, cattle genetics and cattle reproduction; however, we terminated our long-term project studying grazing intensity – a study that Bob Patton began in 1989. We ended the research year with two new projects conducted by Michael Undi studying late-season grazing strategies using bale grazing and corn residue options.

We said goodbye to numerous staff members in 2016, with Bob Patton retiring in March. Bob had a wonderful career at the CGREC that began in 1987. Bob started one of the few "true" long-term studies in the northern Plains looking at long-term grazing intensity research in the Missouri Coteau region of North Dakota. He conducted this study from 1989 through 2015, a span of 27 years.

This project let us better understand the impacts of grazing intensity on plant community dynamics, water movement and plant uptake, climate change and invasive cool-season grasses on plant community function. Good luck to Bob and Janet in their next chapter of life's journey! We thank them for their dedication to range science and furthering our knowledge of grassland function and grazing management.

We said goodbye to Bryan Neville, a former beef scientist at the center and the center's director from 2012 to 2016. Bryan brought a tremendous amount of knowledge to the center in beef nutrition and livestock systems, introducing new research to the center revolving around livestock nutrition, reproductive physiology and a systems approach to grazing management. Good luck to Bryan and Tammi, and we wish Bryan well in his new career.

Lastly, we said goodbye to Fara Brummer, the center's area livestock systems specialist with the NDSU Extension Service. Fara started her position at the CGREC in April 2014, bringing a missing link to the center in terms of Extension outreach to our clientele. Fara enjoyed engaging our local ranchers and county agents, and conducting numerous demonstration projects that looked at the entire system (land, livestock, water and people). Fara went back to Oregon with her husband, Colin, and two children (Grace and Faith) to work at Oregon State University.

Our newest staff member, Jessalyn Bachler, began work on May 15 as our herdsman technician. I began as the interim director in October. Follow our quarterly newsletter (www.ag.ndsu.edu/CentralGrasslandsREC/newsletter) and the 2017 Annual Report for our new hires in 2017.

The CGREC became home to numerous graduate student and a Fulbright program student in 2016. Our graduate students included Megan Endreson, advised by Ryan Limb, range scientist in the School of Natural Resource Sciences at NDSU; Kayla Chilcoat, advised by Joel Caton, animal scientist in the Animal Sciences Department, NDSU; Nicolas Negrin Pereira, advised by Carl Dahlen, Extension livestock specialist, and Pawel Borowicz, animal scientist, in the Animal Sciences Department, NDSU; and Felipe Alves Correa Carvalho Da Silva, advised by Carl Dahlen. Articles summarizing these students' projects may be found in this year's report and the 2015 report.

We had the privilege of hosting Friederike Baumgaertner, a Fulbright program student from Germany. Friederike ("Rike") is studying livestock systems and reproduction under the guidance of Carl Dahlen.

The center held numerous tours in 2016, including the 35th annual field day in July. We invite you to our 2017 annual field day on July 10 from 4 to 7 p.m., followed by a supper, good conversation and a visit with Trent Loos, radio host of Loos Tales.

The CGREC continues to address our original mission of conducting research and outreach on range and grassland science, forage management and applied beef cattle systems production. We have been developing new infrastructure, hiring new graduate students and working closely with the NDSU Main Station scientists (Range Sciences, Animal Sciences, Soil Science and Plant Sciences) to start new range, forage, wildlife and pollinator, soil health and beef cattle research in 2017.

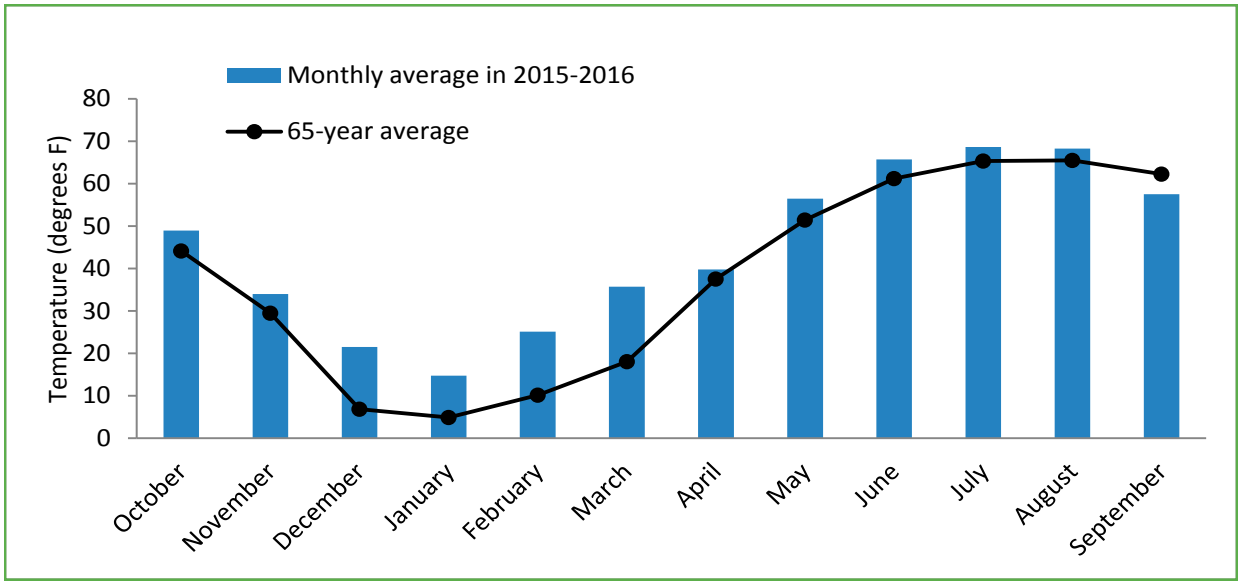
We hope to continue serving you for many years to come. You always are welcome to stop by the center.

Kevin Sedivec, Interim Director

Central Grasslands Research Extension Center
4824 48th Ave. SE, Streeter ND 58483

(701) 424-3606 / Fax: (701) 424-3616
www.ag.ndsu.edu/CentralGrasslandsREC

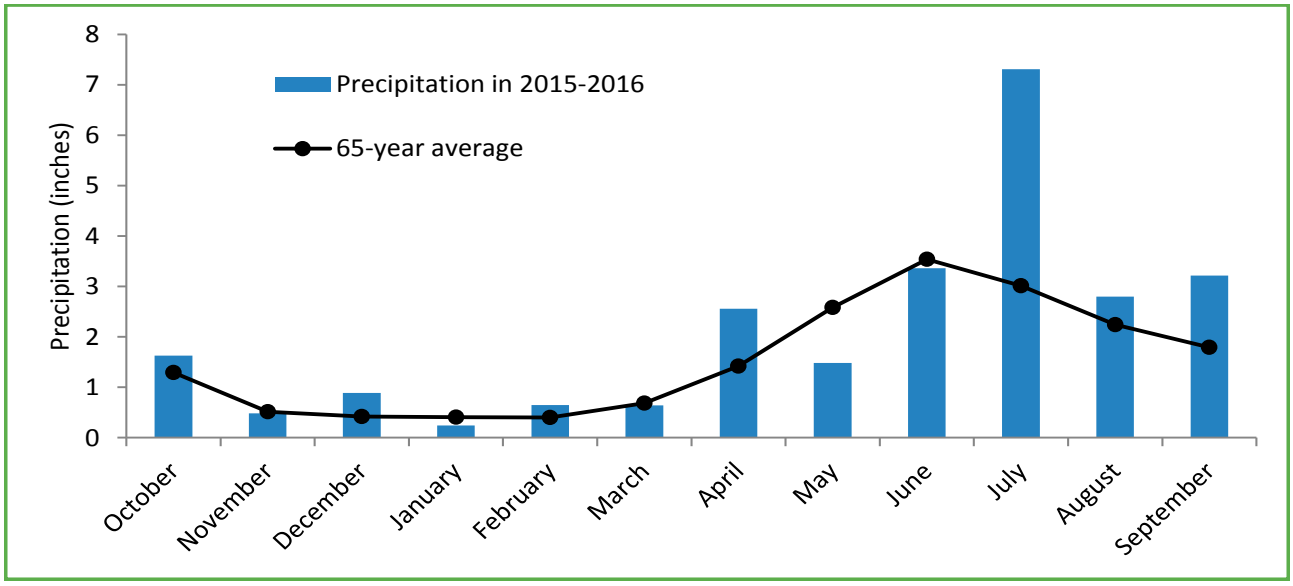
Monthly Temperatures for the 2015 - 2016 Crop Year



Month	Maximum temperature degrees F	Minimum temperature degrees F	Average temperature degrees F	Long-term[1] average temperature degrees F	2015 - 2016 deviation from long-term[1] average degrees F
October	90	27	49.0	44.1	4.8
November	64	3	34.0	29.4	4.6
December	55	-9	21.5	6.8	14.7
January	40	-18	14.8	4.9	9.8
February	63	-6	25.2	10.1	15.0
March	72	2	35.7	18.1	17.6
April	70	16	39.8	37.6	2.2
May	86	26	56.5	51.5	5.0
June	89	42	65.8	61.2	4.6
July	92	44	68.7	65.4	3.3
August	88	44	68.3	65.5	2.8
September	83	36	57.6	62.3	-4.8

[1] 65 years: 1951 - 2016	Last spring frost: May 15, 2016 (26°F) First fall frost: Oct. 6, 2016 (29°F) 144 frost-free days	65-year average last spring frost: May 13 65-year average first fall frost: Sept. 22 Long-term average: 132 frost-free days

Monthly Precipitation for the 2015 - 2016 Crop Year



Month	Precipitation[1] inches	Long-term[2] average precipitation inches	Deviation from long-term[2] average inches	Accumulated precipitation inches	Accumulated long-term[2] average inches	2015-2016 percent of long-term[2] average	Snow inches
October	1.63	1.29	0.34	1.63	1.29	126	0
November	0.48	0.51	-0.03	2.11	1.80	117	3
December	0.89	0.42	0.47	3.00	2.22	135	14
January	0.24	0.41	-0.17	3.24	2.63	123	5
February	0.65	0.40	0.25	3.89	3.03	128	6
March	0.64	0.68	-0.04	4.53	3.71	122	5
April	2.56	1.42	1.14	7.09	5.13	138	0
May	1.48	2.58	-1.10	8.57	7.72	111	0
June	3.36	3.54	-0.18	11.93	11.25	106	0
July	7.31	3.01	4.30	19.24	14.27	135	0
August	2.80	2.24	0.56	22.04	16.51	134	0
September	3.22	1.79	1.43	25.26	18.30	138	0
Total	25.26	18.30	6.96	25.26	18.30	138	33

[1] Rain and melted snow

[2] 65 years: 1951-2016

Performance of Beef Cattle Managed in Two Overwintering Environments

Jessalyn Bachler, Stephanie Becker and Michael Undi

Central Grasslands Research Extension Center - NDSU, Streeter

Allowing beef cattle to harvest their own forage reduces reliance on inputs such as labor and machinery required for forage harvest and potentially can decrease production costs. This study assesses performance of beef cattle kept on pasture or in dry lot pens during the winter in North Dakota. Preliminary results suggest that feed quality may be more important than housing in determining cattle performance in the winter. Further, weather events such as blizzards will not necessarily hinder bale grazing when producers take proper precautions to ensure that animals have access to water, feed and shelter.

Summary

This study was conducted to assess the performance of pregnant beef cows kept on pasture or in dry lot pens during the winter in North Dakota. Non-lactating, pregnant Black Angus cows (n = 32; body weight = 1,321 ± 150 pounds; body condition score = 5.6 ± 0.31), were divided into two groups and kept on pasture or in dry lot pens during the winter. Cattle in both housing scenarios were offered the same Conservation Reserve Program (CRP) hay (7.5 percent crude protein [CP]; 51.7 percent total digestible nutrients [TDN]), free choice.

Two-day body weights were taken at the start and end of the study. Two observers assigned cow body condition scores (BCS) using a 9-point system (1 = emaciated, 9 = obese) at the start and end of the study. Despite heavy snow accumulations from three blizzards in December, cows were able to graze for 70 days before termination of the study.

Keeping cows on pasture or in dry lot pens during the North Dakota winter had no effect ($P > 0.05$) on final body weight (BW), daily gain or BCS. Whether on pasture or in dry lot pens, cows lost body weight and condition, which indicates that the CRP hay fed did not provide adequate nutrients to meet animal requirements of non-lactating beef cows.

Preliminary results suggest that feed quality may be more important than housing in determining animal performance during the winter. Further, weather events such as blizzards will not necessarily hinder bale grazing, but producers need to take precautions to ensure that animals have access to water, feed and shelter.

Introduction

Winters in North Dakota are characterized by cold temperatures, low wind chills, freezing rain and snow. A large portion of winter (40 to 70 days) averages 0°F, although extreme minimum temperatures of -60°F have been recorded (Enz 2003).

The majority of beef cows in the Northern Plains are housed in open dry lot pens during the winter (Asem-Hiablie et al. 2016) and are exposed to these extreme winter conditions. In dry lots, cattle are fed mechanically harvested feeds such as hay and silage.

Kevin Sedivec

Winter feed costs, resulting from labor, machinery and energy required to provide feed, water and bedding to cattle kept in dry lots, make up more than 60 percent of total feed costs for most beef cow-calf operations. Because total feed costs account for approximately 60 percent of cow-calf production costs (Taylor and Field 1995), beef producers are interested in reducing winter feed costs by extending the grazing season.

Extending the grazing season by keeping cattle on pasture for a significant period of time during the winter allows animals to harvest their own food and decreases reliance on inputs such as machinery and energy required to harvest forage (D'Souza et al. 1990). By maximizing the use of grazed grass, the cheapest feed resource for ruminants (Hennessy and Kennedy 2009), extending the grazing season can

decrease production costs and enhance profitability of livestock production (D'Souza et al. 1990; Hennessy and Kennedy 2009).

Strategies for extending the grazing season such as swath grazing, bale grazing and stockpiling have been evaluated (D'Souza et al. 1990; Willms et al. 1993; Volesky et al. 2002; McCartney et al. 2004; Jungnitsch et al. 2011; Kelln et al. 2011; Baron et al. 2014). The economic benefits from these strategies accrue mainly from cost reductions of feeds and feeding, labor, fuel, machinery maintenance and repair, and manure removal.

Environmentally, keeping cattle on pasture returns nutrients directly onto the land and allows for optimal nutrient capture by growing plants (Jungnitsch et al. 2011; Kelln et al. 2011). Depositing manure directly on pastures avoids nutrient accumulation in one place, minimizing nutrient loss to the environment through runoff or leaching (Kelln et al. 2012; Bernier et al. 2014).

Extending the grazing season must be assessed against benefits to the animal as well as to the producer. The majority of beef producers in North Dakota still overwinter cattle in dry lots. Low adoption rates of extended-grazing strategies by producers may be attributed to limited local information on animal performance in extended-grazing systems, especially bale grazing, as well as data on the economics of extended grazing under North Dakota winter conditions.

Kevin Sedivec

Participants at a winter grazing conference in North Dakota (www.ag.ndsu.edu/centralgrasslandsrec/winter-grazing-workshop-held-nov-4-5) identified the need for more locally generated information on extended grazing strategies. Therefore, this study was conducted to assess performance of pregnant beef cows managed in two overwintering environments (pasture or dry lot) under south-central North Dakota winter conditions.

Procedures

This study was conducted from Nov. 4, 2016 to Jan. 12, 2017 at the Central Grasslands Research Extension Center, Streeter, N.D. Non-lactating pregnant Black Angus cows (n = 32; BW = 1,321 ± 150 pounds; BCS = 5.6 ± 0.31) were assigned to one of four groups of similar total body weight and were kept on pasture or in dry lot pens.

Two-day body weights were taken at the start and end of the study. Two observers assigned BCS using a 9-point system (1 = emaciated, 9 = obese; Wagner et al. 1988) at the start and end of the study. Animal handling and care procedures were approved by the NDSU Animal Care and Use Committee.

Michael Undi

Bale Grazing. Historically, the bale grazing site was cropland in a corn and small-grain rotation. In the two years prior to the start of this study, the site was planted with cool-season cover crops, mainly rye, turnips and other brassicas. In 2016, the site was burned down with 2,4-D and Round-up in late April, after which meadow brome was planted in early May.

The field was divided into eight three-acre paddocks using four-strand, high-tensile wire electric fencing. One water tank was placed between two paddocks. Wind breaks were placed in each paddock.

In early fall, round CRP hay bales (7.5 percent CP; 51.7 percent TDN) were placed in each paddock in two rows approximately 50 feet apart. Cows were allotted four bales in one grazing session; access to new bales was controlled using portable electric fencing.

Cows were moved to a new set of four bales when the depth of waste feed remaining across the diameter of each bale was less than 4 inches. Cows within each treatment moved at different paces. Cows had *ad libitum* access to fresh water, mineral supplement and salt blocks.

Dry Lot. Two groups of cows were kept in two dry lot pens. Each pen contained a two-bale hay feeder and a Richie water tank. Pens were bedded with straw as needed throughout the study. Dry lot cows were fed the same CRP hay (7.5 percent CP; 51.7 percent TDN) as the bale-grazed cows. Like the bale-grazed cows, dry lot cows had *ad libitum* access to fresh water, mineral supplement and salt blocks.


Michael Undi

Results

Initial cow BW and BCS were similar ($P > 0.05$) between treatments (Table 1). Keeping cows on pasture or in dry lot pens did not influence ($P > 0.05$) final BW or daily gains (Table 1). No difference ($P > 0.05$) in BCS change was found between cows kept on pasture and those in dry lot pens (Table 1). Whether on pasture or in dry lot pens, cows lost weight and BCS.

Table 1. Performance of cows kept on pasture or in dry lot pens in the winter.

	Housing			
	Pasture	Dry lot	SE	*P*-value
Initial BW, lbs.	1,316	1,327	38.1	0.87
Final BW, lbs.	1,264	1,279	38.7	0.79
Daily gain, lbs./day	-0.66	-0.74	0.077	0.63
Initial BCS	5.6	5.8	0.08	0.17
Final BCS	5.4	5.5	0.09	0.31
BCS change	-0.24	-0.27	0.05	0.68

Discussion

This study was marked by three blizzards, which led to huge snow accumulations. Despite snow depths being greater than 20 inches in some places, cows were able to bale graze for 70 days before the termination of the study. The study was terminated after it became impossible to access water points. Strategies for extending the grazing season should be accompanied by a contingency plan for feed and water supplies in case grazing becomes impossible.

Here are some interesting observations from blizzard events of 2016:

- First, despite windbreaks, not all cows sought shelter during the blizzards. Some simply would stand on the leeward side of the bales, while other cows did not seek shelter at all and continued to graze.
- Secondly, when water troughs were cleared of snow after each blizzard and re-filled, not all cows visited the water troughs immediately, as anticipated. However, we saw a "catch up" period of several days following blizzards when water intake increased, as noted by more frequent filling of water troughs.

Events such as blizzards can prevent or drastically reduce access to water, requiring pastured cows to utilize snow as a source of water. Animals can survive on snow, as shown in beef calves (Degen and Young 1990a) and pregnant beef cows (Degen and Young 1990b).

Cows in both housing scenarios lost body weight and condition during the course of the study, which was probably a function of the quality of hay offered to cows. The hay was low in energy, protein and phosphorus (P) content and supplied approximately 57, 95 and 60 percent of the energy, protein and P, respectively, required by cows in mid-gestation (National Research Council 1996). As such, these cows would have benefitted from some form of supplementation.

Keeping cows on pasture or in dry lot pens did not influence animal performance in this study because both housing scenarios provided similar protection from the elements, particularly wind. Windbreaks used in this study seemed to be effective in ensuring that both groups of cows had adequate protection.

Many producers in the Northern Plains use windbreaks to protect cattle from harsh winter weather (Asem-Hiablie et al. 2016). Using windbreaks minimizes convective heat loss, thereby reducing the use of endogenous reserves (Olson and Wallander 2002). However, using windbreaks may not improve overall performance because time spent behind windbreaks is time spent not feeding or foraging (Olson and Wallander 2002).

The smaller-size dry lot pens would be expected to give dry lot cows a competitive energy expenditure advantage over cows on pasture. Animals on pasture spend more energy walking in search of food and water or shelter and more time eating and foraging for food than housed animals (Osuji 1974).

Extra muscular activities, over and above those observed indoors, might increase maintenance energy requirements of animals on range by 25 to 50 percent (Osuji 1974). However, this might not apply in bale-grazing situations where animals do not travel long distances to feed.

Keeping cattle on pasture or in dry lot pens in winter must be assessed against benefits to the animal, as well as financial benefits to the producer. Extending the grazing season reduces feed costs significantly because animals harvest their own food (D'Souza et al. 1990). Several studies (D'Souza et al. 1990; Willms et al. 1993; McCartney et al. 2004; Jungnitsch et al. 2011; Kelln et al. 2011; Baron et al. 2014) have shown economic advantages of extending the grazing season associated with reducing costs of feeds and feeding, labor, fuel, machinery maintenance and repair, and manure removal.

Conclusions

Preliminary results from this study suggest that feed quality may be more important than housing in determining cattle performance in overwintering environments. Further, weather events such as blizzards will not necessarily hinder bale grazing, but producers should take precautions to ensure that animals have access to water, feed and shelter.

Acknowledgments

Technical assistance provided by Dwight Schmidt, Rodney Schmidt, Thomas Mittleider and Rick Bohn is gratefully acknowledged.

Literature Cited

Asem-Hiablie, S., Rotz, C.A, Stout, R., and Stackhouse-Lawson, K. 2016. Management characteristics of beef cattle production in the Northern Plains and Midwest regions of the United States. Prof. Anim. Sci. 32: 736-749. http://dx.doi.org/10.15232/pas.2016-01539.

Baron, V.S., Doce, R.R., Basarab, J., and Dick, C. 2014. Swath-grazing triticale and corn compared to barley and a traditional winter feeding method in central Alberta. Can. J. Plant Sci. 94: 1125-1137.

Bernier, J.N., Undi, M., Ominski, K.H., Donohoe, G., Tenuta, M., Flaten, D., Plaizier, J. C., and Wittenberg, K.M. 2014. Nitrogen and phosphorus utilization and excretion by beef cows fed a low quality forage diet supplemented with dried distillers' grains with solubles under thermal neutral and prolonged cold conditions. Anim. Feed Sci. & Technol. 193: 9-20.

Degen, A.A., and Young, B.A. 1990a. Average daily gain and water intake in growing beef calves offered snow as a water source. Can. J. Anim. Sci. 70: 711-714.

Degen, A.A., and Young, B.A. 1990b. The performance of pregnant beef cows relying on snow as a water source. Can. J. Anim. Sci. 70: 507-515.

D'Souza, G.E., Marshall, E.W., Bryan, W.B., and Prigge, E.C. 1990. Economics of extended grazing systems. Am. J. Alternative Agric. 5 (3): 120–125.

Enz, J.W. 2003. North Dakota topographic, climatic, and agricultural overview. www.ndsu.edu/fileadmin/ndsco/documents/ndclimate.pdf

Hennessy, D., and Kennedy, E. 2009. Extending the grazing season. Livestock. 14: 27-31. doi: 10.1111/j.2044-3870.2009.tb00233.x

Jungnitsch, P., Schoenau, J.J., Lardner, H.A., and Jefferson, P.G. 2011. Winter feeding beef cattle on the western Canadian prairies: Impacts on soil nitrogen and phosphorous cycling and forage growth. Agric. Ecosyst. Environ. 141: 143-152.

Kelln, B.M., Lardner, H.A., McKinnon, J.J., Campbell, J.R., Larson, K., and Damiran, D. 2011. Effect of winter feeding system on beef cow performance, reproductive efficiency, and system cost. Prof. Anim. Sci. 27: 410-421.

Kelln, B., Lardner, H., Schoenau, J., and King, T. 2012. Effects of beef cow winter feeding systems, pen manure and compost on soil nitrogen and phosphorous amounts and distribution, soil density, and crop biomass. Nutr. Cycl. Agroecosyst. 92: 183–194.

McCartney, D., Basarab, J.A., Okine, E.K., Baron, V.S., and Depalme, A.J. 2004. Alternative fall and winter feeding systems for spring calving beef cows. Can. J. Anim. Sci. 84: 511-522.

National Research Council. 1996. Nutrient requirements of beef cattle. 7th Revised Edition. National Academy Press, Washington, D.C.

Olson, B.E., and Wallander, R.T. 2002. Influence of winter weather and shelter on activity patterns of beef cows. Can J. Anim. Sci. 82: 491-501.

Osuji, P.O. 1974. The physiology of eating and the energy expenditure of the ruminant at pasture. J. Range Manage. 27 (6): 437-443.

Taylor, R.E., and Field, T.G. 1995. Achieving cow/calf profitability through low cost production. Range Beef Cow Symposium. University of Nebraska, Lincoln. http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1198&context=rangebeefcowsymp

Volesky, J.D., Adams, D.C., and Clark, R.T. 2002. Windrow grazing and baled-hay feeding strategies for wintering calves. J. Range Manage. 55: 23-32.

Wagner, J.J., Lusby, K.S., Oltjen, J.W., Rakestraw, J., Wetteman, R.P., and Walters. L.E. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603-612.

Willms, W.D., Rode, L.M., and Freeze, B.S. 1993. Winter performance of Hereford cows on fescue prairie and in drylot as influenced by fall grazing. Can. J. Anim. Sci. 73:881-889.

Bale Grazing Poor-quality Grass Hay With and Without Supplementation

Jessalyn Bachler, Stephanie Becker and Michael Undi
Central Grasslands Research Extension Center - NDSU, Streeter

When bale grazing, ensuring that animals have adequate nutrition is important. For cows receiving poor-quality feed, this can be achieved by supplementation using methods that minimize labor and energy costs. This study examines methods of supplementing cows bale grazing poor-quality hay. Preliminary results suggest that poor-quality grass hay offered to overwintering pregnant cows in early to mid-gestation may not contain adequate energy, protein and phosphorus (P) to meet animal requirements. Further, alfalfa hay or liquid supplements may not provide the extra nutrients required to meet this shortfall.

Summary

This study was conducted to investigate methods of supplementing cows bale grazing poor-quality hay during the winter in North Dakota. Non-lactating pregnant Black Angus cows (n = 64; body weight = 1,312 ± 142 pounds; body condition score = 5.6 ± 0.31) were assigned to one of eight groups of similar total body weight and kept on pasture in the winter. The following bale grazing treatments were examined: a) poor-quality hay, b) poor-quality hay supplemented with alfalfa hay, c) poor-quality hay supplemented with corn dried distiller's grains with solubles (DDGS) and d) poor-quality hay treated with a liquid supplement.

Two-day body weights (BW) were taken at the start and end of the study. Two observers assigned body condition scores (BCS) using a 9-point system (1 = emaciated, 9 = obese) at the start and end of the study. Despite heavy snow accumulation resulting from three blizzards, cows were able to bale graze for 70 days before the termination of the study.

Supplementing cows bale grazing poor-quality hay did not significantly influence ($P < 0.05$) final BW or BCS. However, daily gain and BCS change were greater ($P < 0.05$) when cows were supplemented with DDGS. Cows supplemented with alfalfa or liquid supplement lost weight and body condition, which might indicate that these supplements did not supply adequate energy to meet animal demands. Preliminary results suggest a need for higher-energy supplements in winter.

Introduction

Beef cattle in the Northern Plains typically graze poor-quality forages in the winter (Marshall et al. 2013). Poor-quality forages are generally low in energy, protein and minerals, and can impair rumen microbial function, leading to poor forage intake and digestion (Köster et al. 1996). Utilization of poor-quality forages can be improved through supplementation, which is especially important at critical times such as summer plant dormancy or fall and winter months (Caton and Dhuyvetter 1997).

Michael Undi

Effective supplementation requires regular supplement intake at levels that do not vary significantly on a daily basis (Garossino et al. 2003). Cost-effective supplement delivery methods minimize feed costs by delivering supplement to grazing cattle less frequently (Schauer et al. 2005; Canesin et al. 2014) or eliminating pasture visits altogether (Klopfenstein and Owen, 1981; Undi et al. 2001).

Although the majority of producers in the Northern Plains keep cattle in dry lot pens in the winter (Asem-Hiablie et al. 2016), producers are interested in extending the grazing season by keeping cattle on pasture in the winter.

The adoption of extended grazing is driven by winter feed cost reductions associated with moving away from dry lots (Kelln et al. 2011). Supplementation techniques that minimize or eliminate pasture visits in extended grazing systems will further the goal of minimizing winter feed costs.

This study was conducted to investigate strategic methods of supplementing cows bale grazing poor-quality hay in winter.

Procedures

This study was conducted from Nov. 4, 2016 to Jan. 12, 2017 at the Central Grasslands Research Extension Center, Streeter, N.D. Non-lactating pregnant Black Angus cows (n = 64; BW = 1,312 ± 142 pounds; BCS = 5.6 ± 0.31) were assigned to eight groups of similar total body weight and kept on pasture in the winter. The cows were pregnancy-checked prior to the start of the study to eliminate open cows. Cows were treated with IVOMEC (Ivermectin) pour-on during sorting.

The bale grazing site was a 26-acre field that, historically, was cropland with a corn and small-grain rotation. In the last two years before the start of this study, the site was planted to cool-season cover crops, mainly rye, turnips and other brassicas.

The site was burned down with 2,4-D and Round-up in late April, after which meadow brome was planted in early May. The field then was divided into eight three-acre paddocks using four-strand, high-tensile wire electric fencing. One water tank was installed between two paddocks. The site was mowed prior to bale placement to reduce the possibility of cows grazing standing forage.

In early fall, round Conservation Reserve Program (CRP) hay bales (7.5 percent crude protein [CP]; 51.7 percent total digestible nutrients [TDN]) were placed in each paddock in two rows 50 feet apart. Net wrap was removed prior to feeding. Bales were placed on their sides to reduce waste and the loss of liquid supplement.

Cows were allotted four bales at a time, and access to new bales was controlled using portable electric fencing. Cows were moved to a new set of bales when the depth of waste feed remaining across the diameter of each bale was less than 4 inches. Wind breaks were placed in each paddock. Cows had *ad libitum* access to fresh water, mineral supplement and salt blocks.

Bale Grazing Treatments. Bale-grazed cows were assigned to one of four treatments as follows: a) poor-quality hay (control), b) poor-quality hay supplemented with alfalfa hay, c) poor-quality hay supplemented with corn DDGS and d) poor-quality hay treated with a liquid supplement. Poor-quality hay was obtained from a CRP field of mixed prairie grasses that had not been harvested for several years.

Cows supplemented with DDGS were fed 4 pounds/head/day DDGS twice a week. For the liquid supplement treatment, approximately 9 gallons of liquid supplement (Quality Liquid Feeds Inc. [QLF]) was poured onto upright bales. This amount of liquid supplement was calculated to increase hay protein content by approximately 3 percentage points. Bales were allowed to sit upright after pouring until the supplement had seeped through, after which the bales were flipped on their sides.

Cows had *ad libitum* access to water. Cows on the control, alfalfa hay and liquid-supplemented hay treatments were fed a 6-12+ mineral supplement (CHS Inc., Sioux Falls, S.D.) because these diets were low in P. All cows were offered a salt block.

Two-day body weights were taken at the start and end of the study. Two observers assigned BCS using a 9-point system (1 = emaciated, 9 = obese; Wagner et al. 1988) at the start and end of the study. Animal handling and care procedures were approved by the NDSU Animal Care and Use Committee.

Results

Initial cow BW and BCS were similar ($P > 0.05$) among cow treatments (Table 1). Supplementation did not influence ($P > 0.05$) final body weights or BCS significantly, compared with the control (Table 1). However, cows supplemented with DDGS maintained body weight and condition, while control cows and cows supplemented with alfalfa hay or liquid supplement lost body weight and condition (Table 1).

Table 1. Animal performance of cows bale grazing poor-quality grass hay and poor-quality grass hay supplemented with alfalfa hay, a liquid supplement or DDGS.

		Supplementation				
	Control[1]	Alfalfa hay[2]	QLF[3]	DDGS[4]	SE	*P*-value
Initial BW, lbs.	1,316	1,301	1,314	1,316	51.4	0.99
Final BW, lbs.	1,264	1,269	1,277	1,357	52.4	0.25
Gain, lbs./day	-0.74[b]	-0.45[b]	-0.52[b]	0.58[a]	0.145	<0.001
Initial BCS	5.6	5.6	5.5	5.5	0.10	0.67
Final BCS	5.4	5.4	5.4	5.5	0.09	0.21
BCS change	-0.24[b]	-0.17[b]	-0.16[b]	0.07[a]	0.083	0.003

[1]Grass hay, [2]Grass hay + alfalfa hay, [3]Liquid supplement-treated hay, [4]Grass hay + DDGS

[a,b]Means in the same row followed by a different letter differ significantly ($P < 0.05$).

Discussion

This year, the first year of the study, was marked by three severe blizzards, which led to heavy snow accumulations. Despite snow depths of more than 20 inches in some places, cows were able to bale graze for 70 days before the termination of the study.

Poor-quality grass hay offered to cows was low in energy, protein and P, and supplied 57, 95 and 60 percent, respectively, of the nutrients required by cows at this stage of pregnancy. Body weight and condition losses in cattle supplemented with alfalfa hay or liquid supplement suggest that these supplements did not provide the extra nutrients, particularly energy, required to meet requirements of cows in mid-gestation in winter. Further evaluation of supplements will be conducted in this multiyear study.

Acknowledgments

Technical assistance provided by Dwight Schmidt, Rodney Schmidt, Thomas Mittleider, Rick Bohn, Tom Lere (QLF) and Curt Lahr (QLF) is gratefully acknowledged.

Literature Cited

Asem-Hiablie, S., Rotz, C.A, Stout, R., and Stackhouse-Lawson, K. 2016. Management characteristics of beef cattle production in the Northern Plains and Midwest regions of the United States. Prof. Anim. Sci. 32: 736-749.

Caton, J.S., and Dhuyvetter, D.V. 1997. Influence of energy supplementation of grazing ruminants: Requirements and response. J. Anim. Sci. 75:533 -542.

Canesin, R.C., Berchielli, T.T., de Vega, A., Reis, R.A., Messana, J.D., Baldi, F., and Páscoa, A.G. 2014. Reducing supplementation frequency for Nellore beef steers grazing tropical forages. Sci. Agric. 71(2): 105-113.

Garossino, K.C., Ralston, B.J., McAllister, T.A., and Olson, M.E. 2003. Measuring individual free-choice protein supplement consumption by wintering beef cattle. Can. J. Anim. Sci. 83: 21–27.

Kelln, B.M., Lardner, H.A., McKinnon, J.J., Campbell, J.R., Larson, K., and Damiran, D. 2011. Effect of winter feeding system on beef cow performance, reproductive efficiency, and system cost. Prof. Anim. Sci. 27: 410-421.

Klopfenstein, T., and Owen, F.G. 1981. Value and potential use of crop residues and by-products in dairy rations. J. Dairy Sci. 64: 1250-1268.

Köster, H.H., Cochran, R.C., Titgemeyer, E.C., Vanznat, E.S., Abdelgadir, I., and St. Jean, G. 1996. Effect of increasing degradable intake protein on intake and digestion of low quality, tall grass-prairie forage by beef cows. J. Anim. Sci. 74: 2473-2481.

Marshall, C.L., Fensterseifer, S.R., Arias, R.P., Funston, R.N., and Lake, S.L. 2013. The effect of winter protein supplementation during the third trimester on cow and subsequent calf performance. Proc. West. Soc. Am. Soc. Anim. Sci. 64: 103-105.

Schauer, C.S., Bohnert, D.W., Ganskopp, D.C., Richards, C.J., and Falck, S.J. 2005. Influence of protein supplementation frequency on cows consuming low-quality forage: Performance, grazing behavior, and variation in supplement intake. J. Anim. Sci. 83:1715-1725.

Undi, M., Kawonga, K.C., and Musendo, R.M. 2001. Nutritive value of maize stover/pasture legume mixtures as dry season feed for sheep. Small Ruminant Res. 40: 261-267.

Wagner, J.J., Lusby, K.S., Oltjen, J.W., Rakestraw, J., Wetteman, R.P., and Walters. L.E. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66: 603-612.

Extending the Grazing Season With Corn Residue and Cover Crop

Stephanie Becker[1], Michael Undi[1], Ryan Odenbach[2] and Hal Weiser[3]

[1]Central Grasslands Research Extension Center- NDSU, Streeter
[2]Stutsman County Soil Conservation District, Jamestown, N.D.
[3]Natural Resources Conservation Service, Jamestown, N.D.

Methods of supplementing grazing cattle in winter should aim to reduce winter feed costs, which are the single highest annual cost in a cow-calf operation. Methods that eliminate pasture visits in winter can reduce labor and fuel costs. This four-year study will evaluate methods of supplementing cows grazing corn residue.

Summary

The study evaluates the performance of cows grazing corn residue alone, corn residue plus protein/energy tubs, and corn residue plus cover crop. The cover crop was established successfully in 2016, the first year of this project. While the corn residue/cover crop biomass was sufficient to graze beef cows, the grazing study was abandoned due to a blizzard.

Introduction

The abundance of corn residue in North Dakota gives beef producers a readily available feed resource to graze cattle in winter. Corn residue is a poor-quality feed, low in protein and minerals, and with limited feed intake and digestion when fed as a sole feed. The nutrient supply to cows grazing corn residue can be improved by targeted supplementation.

Supplementation methods that reduce labor and fuel costs by eliminating pasture visits are of interest to beef producers. Providing grazing animals with feeds that possess complementary characteristics can eliminate pasture visits.

Poor-quality/good-quality feed combinations such as corn residue and alfalfa hay (Klopfenstein and Owen 1981) or corn residue and creeping forage legumes (Undi et al. 2001) have been shown to improve performance in beef cattle (Klopfenstein and Owen 1981) and sheep (Undi et al. 2001). Cover crops intercropped into corn can be grazed in combination with corn residue after corn harvest.

The benefits of cover crops in improving cropping systems and agricultural sustainability are well-documented (Magdoff and van Es 2009; Blanco-Canqui et al. 2012; Wortman et al. 2012). Cover crops increase soil organic matter, reduce soil erosion, improve soil physical and biological properties, increase nutrient cycling, suppress weeds, improve soil water availability, supply nutrients to the following crop and break pest cycles (Magdoff and van Es 2009; Blanco-Canqui et al. 2012; Wortman et al. 2012). Some cover crops are able to break into compacted soil layers, allowing the following crop's roots to develop more fully (Magdoff and van Es 2009).

An additional benefit of cover crops, which normally receives the least consideration, is the importance of cover crops as a source of livestock feed. For poor-quality feeds such as corn residue, cover crops will improve animal performance by supplying nutrients that are low in residue because intercropping corn residue with forage legumes will improve corn residue quantity and quality (Alford et al. 2003).

This study will evaluate methods of supplementing cows that graze corn residue for part of the winter. Methods being evaluated include the use of protein/energy tubs and cover crops as supplements.

Procedures

This study is being conducted on a 90-acre field planted to corn. The field has been divided into nine 10-acre paddocks to examine the following supplementation strategies: a) grazing corn residue (control), b) corn residue plus protein/energy tubs and c) corn residue plus cover crop.

In the first year, a cool-season cover crop (triticale, winter rye, oats, peas, yellow clover, crimson clover and brassicas) was intercropped at 38 pounds/acre into standing corn at the


Michael Undi

V6 to V7 stage. The cover crop was established successfully and monitored throughout the growing season. Each component of the corn residue and individual crop in the cover crop mixture was sampled for nutrient composition.

The grazing portion of this study due to start after the corn harvest was terminated due to a series of blizzards in December. The study will be continued to assess cow performance, impacts on soil health and the economics of corn residue grazing and supplementation.

Results

Intercropping a cover crop into corn did not influence the corn yield; the yield averaged 43.7 ± 1.7 and 45.8 ± 1.7 bushels/acre for corn and corn-cover crop plots, respectively. The nutrient composition of corn residue is shown in Table 1.

Components with the highest nutrient content are the grain and the leaf. The husk is low in protein but has a good energy profile, while the cob is poor in protein and energy. The nutrient composition of the cover crop is shown in Table 2 (next page). All cover crops have high crude protein (CP) content, but rapeseed and radish have extremely low dry-matter content.

Discussion

The cover crop was established successfully and monitored throughout the growing season. At the end of corn harvest, the corn residue/cover crop biomass was sufficient to graze

beef cows. Planting a cover crop when the corn was well-established minimized competition for nutrients; hence, intercropping did not impact corn yield negatively.

In cereal-legume mixes, agronomic features such as fertilizer application, sowing time and the proportion of crop mixture are basic determinants of competition among component crops (Belel et al. 2014). Where constituent crops are arranged in rows, the degree of competition is determined by the comparative growth rates, growth duration and proximity of roots of the diverse crops (Belel et al. 2014).

The cereal component in a cereal-legume intercrop has a faster growth rate, a height advantage and a more widespread rooting system that gives it an upper hand in competition

Table 1. Nutrient composition in percent of dry matter (%DM) of whole corn and corn residue components.

			Component				
	Whole plant	Residue[1]	Grain	Leaf	Husk	Cob	Stalk
CP[2]	9.0	3.0	10.3	9.2	5.7	3.6	3.5
TDN	76	57	90	56	62	15	50
NDF	35.1	75.1	7.9	70.5	75.3	87.7	81.0
ADF	18.9	44.8	1.7	45.8	36.5	44.0	53.9
Ca	0.2	0.1	0.04	0.7	0.2	0.07	0.2
P	0.2	0.05	0.2	0.1	0.2	0.04	0.07
K	0.5	0.6	0.2	1.0	1.1	0.34	1.4
Mg	0.2	0.2	0.1	0.3	0.2	0.05	0.2
S	0.1	0.04	0.1	0.1	0.02	0.03	0.04

[1] Whole plant minus grain

[2] Crude protein (CP), total digestible nutrients (TDN), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium

Table 2. Nutrient composition of individual crops in the cover crop mix.

	Rapeseed	Radish	Winter peas	Oats	Winter rye	Triticale
DM[1], %	17	9	63	79	67	66
Nutrient composition (% of DM)						
CP	21.8	20.1	18.5	10.3	10.9	11.0
TDN	75	73	61	60	61	64
NDF	18.0	25.1	38.0	62.6	56.4	49.2
ADF	16.5	20.2	35.7	37.2	35.7	32.2
Ca	1.6	1.5	2.1	0.3	0.2	0.2
P	0.4	0.4	0.4	0.3	0.3	0.3
K	2.9	3.9	2.9	1.6	1.1	1.1
Mg	0.3	0.3	0.4	0.2	0.1	0.1
S	0.5	0.4	0.6	0.1	0.1	0.1

[1] Dry matter (DM), crude protein (CP), total digestible nutrients (TDN), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg) and

with associated legumes (Belel et al. 2014). Other studies have reported that intercropping corn with red clover and ryegrass (Scott et al. 1987) or red clover and vetch (Baributsa et al. 2008) does not impact corn yield negatively.

This year, the first year of the study, was marked by three blizzards that led to huge snow accumulations. As a result, the grazing study failed to take off. The study will continue for three more years.

Acknowledgments

We thank Dwight Schmidt and Rodney Schmidt for technical assistance.

Literature Cited

Alford, C.M., Krall, J.M., and Miller, S.D. 2003. Intercropping irrigated corn with annual legumes for fall forage in the High Plains. Agron. J. 95: 520-525.

Baributsa, D.N., Foster, E.F.F., Thelen, K.D., Kravchenko, A.N., Mutch, D.R., and Ngouajio, M. 2008. Corn and cover crop response to corn density in an interseeding system. Agron. J. 100: 981-987.

Belel, M.D., Halim, R.A., Rafii, M.Y., and Saud, H.M. 2014. Intercropping of corn with some selected legumes for improved forage production: A Review. J. Agric. Sci. 6 (3): 48-62.

Blanco-Canqui, H., Claassen, M.M., and Presley, D.R. 2012. Summer cover crops fix nitrogen, increase crop yield and improve soil–crop relationships. Agron. J. 104: 137-147.

Klopfenstein, T., and Owen, F.G. 1981. Value and potential use of crop residues and by-products in dairy rations. J. Dairy Sci. 64: 1250-1268.

Magdoff, F., and van Es, H. 2009. Building soils for better crops: Sustainable soil management. 3rd ed. Handbook Series Book 10. Sustainable Agriculture Research and Education (SARE).

Scott, T.W., Mt. Pleasant, J., Burt, R.F., and Otis, D.J. 1987. Contributions of ground cover, dry matter, and nitrogen from intercrops and cover crops in a corn polyculture system. Agron. J. 79: 792-798.

Undi, M., Kawonga, K.C., and Musendo, R.M. 2001. Nutritive value of maize stover/pasture legume mixtures as dry season feed for sheep. Small Rum. Res. 40: 261-267.

Wortman, S.E., Francis, C.A., and Lindquist, J.L. 2012. Cover crop mixtures for the Western Corn Belt: Opportunities for increased productivity and stability. Agron. J. 104: 699-705.

Impacts of Bale Grazing on Herbage Production, Forage Quality and Soil Health in South-central North Dakota

Fara Brummer, Kevin Sedivec, Mary Berg, Chris Augustin, Penny Nester, Sheldon Gerhardt, Jackie Buckley, Ashley Stegeman and Dennis Whitted

North Dakota State University Extension Service

Bale grazing is used to reduce feed and labor costs as well as improve manure distribution. The effects of bale grazing on forage production and quality and soil health is surveyed in this two-year study on four North Dakota ranches. Grass production varied, but forage quality was improved by bale grazing. Soil nutrient parameters were enhanced by bale grazing as compared with the control.

Summary

The effect of bale grazing on grass production six months after treatment varied based on ranch site location in our demonstration trials conducted in 2015 and 2016. The overriding variables that appear to affect grass production are distance between bales and stocking rate intensity (density and duration of time). Grass production was greater on the bale-grazed treatment, compared with the control treatment (no bales on site), 15 feet from the bale center; however, no difference was found within the zone 0 to 10 feet from the bale center six months after treatment.

Bale grazing enhanced grass crude protein and phosphorus content six months after treatment from the bale center out to 10 feet. Although bale grazing did not enhance total grass biomass production from within the 0- to 10-feet zone from the bale center, it increased grass crude protein content within this zone. Bale grazing also enhanced grass phosphorus content within the 0- to 5-feet zone of the bale center.

Soil nitrates, phosphorus and potassium at the 0- to 6-inch soil profile increased on the bale-grazed treatment at all distances from the bale edge six to nine months after treatment, with no increase on the control sites. The percent of organic matter at the same soil depth increased up to 1.4-fold at the bale-grazed sites, compared with the control sites.

Our field trials demonstrated that the added urine, feces and hay waste within the 10-feet zone of the bale center did not impact herbage production (no benefits or negative effects); however, these nutrients did enhance forage quality. Herbage within the 5-feet zone of bale center also had an enhanced phosphorus content, a direct result from the added urine and feces. This additional phosphorus is beneficial in meeting the requirements of grazing livestock, as well as removing excess phosphorus from the soil. Soil nutrient parameters were enhanced significantly at the bale grazed sites at the 0- to 6-inch soil profile from bale edge out to 12.5 feet, when compared with the control.

Introduction

Bale grazing is the practice of allowing livestock access to hay bales in a hayfield or improved pasture to reduce labor and feed delivery costs (Lardner et al. 2008). Livestock growers in the northern Great Plains practicing this technique also are interested in improving soil health and forage production through manure distribution while maintaining adequate livestock performance. Recently published data have shown a positive relationship between bale grazing and nitrogen capture, as well as forage growth (Jungnitsch et al. 2010, Kelln et al. 2012); however, local producer concerns in our region prompted the need for further applied research.

This project was conducted on four ranches in North Dakota to examine winter hay bale grazing effects on herbage production and nutritional quality six and 18 months after treatment. Parameters measured included: herbage production, nutritional quality, soil nutrient content, cow body condition and system costs.

Because bale grazing introduces higher nitrogen and phosphorus into a system, grazing on native pastures is not recommended. Therefore, this project was conducted on improved pastures planted to domesticated cool-season grasses. Herbage production, nutritional quality and soil nutrient content are presented in this report.

Procedures

Four ranches were selected on different ecological sites — claypan, thin loamy, loamy and shallow gravel — in south-central North Dakota. Sites consisted of improved, cool-season grass pastures/hay. Three of the sites had not been bale grazed previously.

Four bales of similar hay type were selected randomly per ranch to represent the bale-grazing (BG) treatment in September 2015. Bale grazing on all sites occurred from January through March 2016. Four control sites without bales

(C) were selected systematically on the same soil series, slope and plant community directly outside the bale-grazed area and sampled using the same protocol as the bale-grazed sites. See Figures 1 and 2 for project layout design and description.

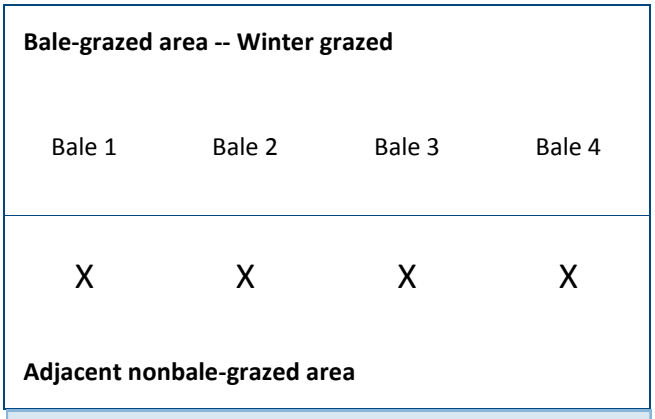


Figure 1. Example of bale-grazed study area showing a smooth brome grass pasture split into bale grazing treatment and the parallel nonbale grazing treatment (control), with "X" representing a corresponding sample location.

Herbage production was collected during peak production for cool-season grasses in North Dakota and before summer grazing occurred. Vegetation was clipped for biomass in late June or early July at four distance points (0, 5, 10, 15 feet) along each cardinal direction (16 total plots) from the bale center after cattle had grazed the bales in 2016 (Figure 2).

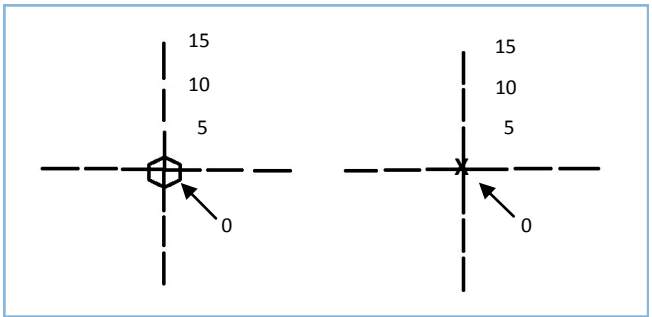


Figure 2. Example of collection locations from bale center and control center, 5, 10 and 15 feet from center for herbage production and soil nutrient content.

Grasses and forbs were separated and composited by plant form from all cardinal directions per bale distance point (four composited samples per bale distance). Hay residue was sampled at the same points and similarly composited to determine waste post-grazing, and to test for a possible relationship with herbage regrowth and quality.

Herbage samples were weighed, oven dried at 150°F and reweighed for moisture content. Wet chemistry nutritional analysis on the grass component was conducted at the North Dakota State University Animal Science Nutrition Lab. Analysis included crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, calcium (Ca), magnesium (Mg) and phosphorus (P).

Soil samples were collected pretreatment in September 2015 and again 12 months later in 2016, or six to nine months after the bale-grazing treatment. Soil cores were collected at 0- to 6-inch and 6- to 24-inch depths from the same four bale treatment sites and four control sites that were used for herbage production. Soil parameters collected included penetrometer (compaction), electrical conductivity, Haney soil health calculation, nitrate, phosphorus, potassium, pH and organic matter.

Results and Discussion

Herbage Production. We found no difference ($P > 0.1$) in total grass biomass production among samples from the bale center, and 5 and 10 feet from the bale center on hay/pasture land that was bale grazed or on similar control hay/pasture land sites six months after treatment. However, bale grazing enhanced ($P < 0.1$) grass production 15 feet from the bale center (Table 1).

Table 1. Grass production at the on winter-grazed bales vs. no winter grazing six months after treatment (collected before grazing in late June/early July at peak production).

	Bale center	5 feet from center	10 feet from center	15 feet from center
Treatment	Pounds/acre[1]			
Bale grazed	5,274[a]	5,320[a]	4,613[a]	8,604[b]
Control	5,358[a]	5,823[a]	5,888[a]	6,160[a]

[1] Herbage production by treatment and distances from bale with the same letter (a, b) are not significantly different ($P > 0.1$).

When bales were placed close together (less than 15 feet), as seen at the Napoleon study site, the bale-grazed site produced from 21 to 172 percent less herbage, depending on distance from the bales, than the control site (Table 2). Because of the close bale spacing, manure and waste are naturally more prominent, as seen in the high residue levels (Table 2). Plus, stock density may have been lower than recommended, leaving a high level of residue on the ground.

Table 2. Grass production and hay residue remaining six months after treatment at different distances from the bale when bales were grazed in early winter (January – March).

Location	Bale distance average (feet)	Parameter	Bale center	5 feet from center	10 feet from center	15 feet from center
			lbs./acre	lbs./acre	lbs./acre	lbs./acre
Tuttle	25 to 30	Residue from bale	28.5	16.0	7.1	NC[1]
		Bale-grazed production	2,860	3,620	5,083	NC
		Control production	3,103	3,740	6,779	NC
Wing	10 to 50[2]	Residue from bale	18.7	36.5	44.6	14.2
		Bale-grazed production	9,196	10,749	3,202	9,604
		Control production	5,695	8,423	5,789	6,125
Napoleon	15	Residue from bale	87.2	140.6	79.2	71.2
		Bale-grazed production	5,587	3,366	2,727	7,199
		Control production	8,775	7,865	7,432	8,679
Fort Rice	50	Residue from bale	69.0	71.2	9.8	11.6
		Bale-grazed production	3,454	3,544	7,440	9,009
		Control production	3,859	3,264	3,551	3,677

[1] NC = Not collected
[2] Spread unevenly throughout the field

When bales were placed 50 feet apart at the Fort Rice study site, bale grazing had no effect on grass production up to 5 feet away from the bale center (Table 2). However, bale grazing increased grass production by 109 to 145 percent at 10 and 15 feet from the bale center, respectively. This open spacing pattern reduces selection, more evenly distributes cattle and leaves less residue if cattle are forced to clean up the hay. This spacing causes higher levels of residue close to the bales but distributes manure more evenly away from the bales, helping explain bale grazing's positive effect on herbage production.

When the bales were placed 25 to 30 feet apart at the Tuttle study site, we found no difference between the bale-grazed sites and control sites (Table 2). Herbage production 10 feet away from the bale showed trends toward higher herbage production on the bale-grazed site, but without data from the 15-foot location, we were unable to determine if this production trend would continue to increase.

The Wing study site was the only location to show increased herbage production from bale grazing within the first 5 feet around the bale, with an increase of 28 to 61 percent. This site also showed a reduction in herbage production at 10 feet from the bale, that area with the greatest level of residue on the ground (Table 2).

However, where residue was low, as seen at 15 feet away from the bale, the bale-grazing site had an increased herbage production of 56 percent. This study site had bales spread irregularly, ranging from 10 to 50 feet. This uneven distribution of bales may have created uneven feeding patterns and increased the pecking order, creating these positive and negative impacts due to bale grazing within the same unit.

Forage Quality. Our demonstration trials exhibited that bale grazing increases ($P < 0.1$) the crude protein content of the grass portion of the vegetation six months after treatment (late June/early July) at the bale center out to 10 feet (Table 3). Grass crude protein content was greater ($P < 0.1$) than the control at the bale center, and 5 and 10 feet from the bale, but not ($P > 0.1$) at 15 feet from bale center.

These findings indicate that benefits from bale grazing occur throughout the zone within 10 feet of the bales. This benefit

Table 3. Grass quality parameters on winter-grazed bales vs. no winter grazing six months after treatment (collected before grazing in late June/early July at peak production).

Treatment	Bale center	5 ft. from center	10 ft. from center	15 ft. from center
		Crude protein (%) content[1]		
Bale grazed	17.2^{ax}	17.3^{ax}	15.9^{ax}	13.0^{bx}
Control	9.8^{ay}	9.8^{ay}	10.2^{ay}	10.9^{ax}
		Phosphorus (%) content[1]		
Bale grazed	0.30^{ax}	0.30^{ax}	0.27^{ax}	0.27^{ax}
Control	0.23^{ay}	0.23^{ay}	0.22^{ax}	0.24^{ax}
		Calcium (%) content[2]		
Bale grazed	0.48	0.44	0.41	0.38
Control	0.41	0.42	0.39	0.39
		Neutral detergent fiber (%) content[2]		
Bale grazed	61.7	60.9	62.4	64.4
Control	64.2	64.4	63.7	64.1
		Acid detergent fiber (%) content[2]		
Bale grazed	34.2	33.4	33.7	35.2
Control	33.9	34.7	33.7	34.1

[1] Nutritional parameters by treatment and distances from bale with the same letter (a, b) within row (treatment) are not significantly different ($P > 0.1$), and with same letter (x, y) within columns (between treatments) are not significantly different ($P > 0.1$).

[2] No differences ($P > 0.1$) were found between treatments or among distances.

is a result of added nitrogen from urine and fecal material concentrated within this 10-feet zone.

Grass phosphorus content was not ($P > 0.1$) different between bale treatment distances or among control distances (Table 3). However, the bale-grazing treatment increased ($P < 0.1$) grass phosphorus content when compared with the control at the bale center and 5 feet from the bale center six months after treatment (Table 3).

No differences ($P > 0.1$) in NDF, ADF or calcium content of the grass component were found between the bale-grazed and control sites six months after treatment (Table 3). Within our demonstration trials, bale grazing had no effect on NDF, ADF or calcium content within the 15-feet zone six months after treatment.

Soil Nutrient Content. Nitrates (NO_3-N), phosphorus and potassium increased on the bale-grazed treatment at all distances from the bale edge (2.5 feet from bale center), six to nine months after treatment (Table 4, next page), at the 0- to 6-inch soil profile. In contrast, these nutrients did not change significantly on the control sites at the same soil depth. On average across the distances from bale edge, nitrates increased six-fold, phosphorus 2.4-fold and potassium 2.8-fold at the 0- to 6-inch profile.

The percentage of organic matter increased 1.3-, 1.3- and 1.4-fold six to nine months after treatment at the bale edge (2.5 feet from center), 5 and 10 feet from bale edge; respectively (Table 4). In contrast, organic matter on the control site increased 1.1-fold after one year.

The Haney soil health calculation increased at all distances from the bale edge and on the control from 2015 to 2016. Because the control had a similar positive trend, compared with the bale-grazing treatment, the increase occurred due to environment or climatic effects and not due to the bale-grazing treatment during our sampling period (Table 5).

In contrast to the Haney soil health calculation, pH tended to decrease on all treatments and control sites (Table 5). Thus, the pH decline was not related to the bale-grazing treatment. Electrical conductivity (EC/salts) appears to have increased six to nine months after the bale-grazing treatment at all distances from the bale center (Table 5).

The control sites actually had a decline in EC from 2015 to 2016. Although we found, on average, a 25 percent increase in EC levels on the bale grazed treatment, EC levels remained under 0.5 mmhos/cm – an extremely low level.

Table 4. Soil nutrient parameters in the 0- to 6-inch profile on winter-grazed bales in 2015 (pretreatment) and 2016 (six to nine month post-treatment).

	NO_3-N (lbs./ac)		Phosphorus (ppm)		Potassium (ppm)		Organic matter (%)	
Distance from bale edge	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Bale edge: 2.5 feet from center	11.4	74.0	11.3	30.0	366.7	888.9	3.9	5.2
7.5 feet from center	12.2	92.2	8.9	22.8	336.6	1047.5	3.9	4.9
12.5 feet from center	14.8	65.6	10.1	20.7	334.5	1007.3	4.1	5.6
Control (no bale grazing)	29.6	18.4	9.7	9.9	292.4	408.7	4.2	4.6

Table 5. Haney soil health calculation, pH and electrical conductivity (EC) in the 0- to 6-inch profile on winter-grazed bales in 2015 (pretreatment) and 2016 (six to nine month post-treatment).

	Haney soil health calculation (range: 1 to 50+)		pH		EC (mmhos/cm)	
Distance from bale center	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Center	19.7	38.8	7.6	7.0	0.31	0.44
5 feet from center	19.4	37.0	7.6	7.0	0.35	0.48
10 feet from center	19.2	35.7	7.6	7.1	0.32	0.41
Control (no bale grazing)	20.5	34.6	7.5	6.9	0.29	0.19

Although EC increased following the bale grazing treatment, the EC levels were still very low.

This project has provided insight on the impacts of bale grazing on herbage production, forage quality and soil nutrient composition when studying different scales of bale distribution and stocking densities. Because we had only one study site per bale spacing patterns, more work should be conducted to address this question of bale spacing and stocking density to further verify our findings and help explain the positive impacts bale grazing may create.

Conclusion

The effects of bale grazing on herbage production varied by ranch location; however, the distance between bales was the variable with the most impact on production. Residue and manure appeared to be a limiting factor affecting forage production where bales were spaced at 15 feet or less. The open spacing pattern of bales at 40 to 50 feet apart appeared to better distribute cattle and minimize hay residue.

Bale grazing positively affected crude protein and phosphorus content of grass growth during the growing season following the bale grazing treatment; however, the bale-grazing treatment had no effect on ADF, NDF or calcium content.

Bale grazing increased soil nitrate, phosphorus and potassium levels, irrelevant of distance from the bale edge. Bale grazing did not change pH or improve the Haney soil health calculation during the growing season following treatment.

A follow-up year is planned to determine if improvements may be seen 18 to 20 months after treatment on areas that were impacted negatively and if the positive benefits are retained for two growing seasons on the other sites.

Literature Cited

Jungnitsch, P., J.J. Schoenau, H.A. Lardner and P.G. Jefferson. 2011. Winter feeding beef cattle on the western Canadian prairies: Impacts on soil nitrogen and phosphorus cycling and forage growth. Agric., Ecosyst. Environ. 141: 143-152.

Kelln, B., H. Lardner, J. Schoenau and T. King. 2012. Effects of beef cow winter feeding systems, pen manure and compost on soil nitrogen and phosphorus amounts and distribution, soil density, and crop biomass. Nutr Cycl Agroecosyst. 92: 183-194.

Lardner, H., J. Schonenau and B. Kelln. 2008. Low-cost winter feeding systems for cow-calf producers. Final Report, Saskatchewan Agriculture Development Fund, ADF Project #20040529.

Land Management Strategies to Control Kentucky Bluegrass Invasion

Megan Endreson and Ryan Limb

North Dakota State University School of Natural Resource Sciences, Fargo

Early-intensive and patch-burn grazing are being tested as management strategies for the control of Kentucky bluegrass (Poa pratensis), an invasive exotic perennial grass. Preliminary results suggest that both early-intensive and patch-burn grazing can increase forage quality and production in Kentucky bluegrass-invaded sites. However, livestock performance under these management strategies should be considered because one may be more sustainable than the other. Treatments will continue for the next several years to make detailed comparisons and conclusions regarding the effects of our various grazing treatments.

Introduction

Land fragmentation, fire suppression and outdated land management strategies among other land use changes, have allowed and amplified the spread of invasive species (D'Antonio and Vitousek 1992). Once established, invasive species displace natives and alter ecosystem processes such that novel ecosystems with no historical precedent are created (Hobbs et al. 2009). Kentucky bluegrass, an exotic cool-season perennial grass, is one such invader in the Northern Great Plains. Its invasion has reduced diversity and altered plant community structures and functions (Toledo et al. 2014).

Kentucky bluegrass begins growth in early spring before native species, spreads tillers quickly and develops a thick sod that suppresses germination of later-emerging species (Toledo et al. 2014). Although its forage quality is high in the spring during active growth, it goes dormant during the summer, which decreases its forage value (Hockensmith et al. 1997). As Kentucky bluegrass abundance increases, rangeland forage production shifts to an earlier period and forage quality is lost throughout the growing season. Furthermore, plant communities dominated by Kentucky bluegrass experience less annual forage production, compared with historic native communities (Toledo et al. 2014).

Without appropriate management, a Kentucky bluegrass invasion will result in uniform rather than diverse plant communities across the landscape, with impaired forage quality and production (Toledo et al. 2014). Grazing intensity, in particular, can have important impacts on the structure and composition of the plant community by affecting forage utilization, and therefore, forage production (Bryan et al. 2000). Thus, early-intensive grazing should suppress actively growing Kentucky bluegrass which will reduce competition for later-emerging native species when grazing is absent.

Historically, grassland plant communities of the Great Plains were influenced by disturbances, namely fire, grazing and their interaction (Samson et al. 2004). The variabilities and interaction of fire and grazing, coupled with the inherent climate variability, resulted in a structurally and compositionally diverse plant community (Fuhlendorf and Engle 2004). However, the composition of the Northern Great Plains grassland ecosystem changed dramatically as European colonization replaced bison with domestic cattle and suppressed natural wildfires (Samson and Knopf 1994).

Thatch created by Kentucky bluegrass.

Preliminary research on the effects of prescribed burns on Kentucky bluegrass invasions provide some indication that fire may be an effective management tool. In the Kansas tallgrass prairie, a four-year study with annual burns experienced a reduction in Kentucky bluegrass cover from 30 to 7 percent (Abrams 1988). A short-term study in South Dakota found a similar decrease in Kentucky bluegrass the first year following a prescribed burn. However, Kentucky bluegrass cover returned to pre-treatment levels two years after fire (Bahm et al. 2011). Therefore, additional disturbance is necessary to maintain the benefits of burning in the year following prescribed fire.

Patch-burn management has yet to be studied for its potential to control Kentucky bluegrass, but it does promote structural

and compositional diversity of the plant community (Fuhlendorf and Engle 2001). In the southern tallgrass prairie, a study found that patch-burn management suppressed the invasion of a different exotic species. Furthermore, the use of prescribed fire is associated with an increase in forage quality, while patch-burn grazing has the potential to increase livestock weight gains (Limb et al. 2011).

The objectives of this study are to determine if: (1) early-intensive grazing followed by summer rest can shift the balance of the plant community toward native species, (2) patch-burn grazing will reduce Kentucky bluegrass and promote native species abundance and diversity, and (3) livestock weight gains will differ between management strategies.

Steers grazing a pasture invaded by Kentucky bluegrass. *Megan Endreson*

Procedures

This study is being conducted at the Central Grasslands Research Extension Center in Stutsman County northwest of Streeter, N.D. Various grazing experiments have occurred on the study site in previous years, but the site received only light summer grazing in 2009 and 2010 prior to the initiation of management strategies associated with this study.

In 2011, three of 12 pastures of roughly 30 to 40 acres each were assigned season-long grazing while another three were assigned early-intensive grazing. In 2014, patch-burn grazing was assigned to three of the 12 pastures with the remaining assigned as idle pastures not to be grazed. Livestock are not rotated among pastures, and each pasture receives the same treatment each year.

Season-long grazed pastures receive moderate stocking rates between 0.96 and 1.85 animal unit months per acre (AUMs/acre) and involve grazing the cattle mid-May through August. Early-intensive grazed pastures receive the same stocking rate to achieve a similar grazing pressure as season-long grazed pastures. However, early-intensive grazing accomplishes this in a shorter period of time by removing cattle after 1.2 months.

Patch-burn grazed pastures incorporate the same stocking rate and length of grazing as season-long grazed pastures but incorporate a patch-burn treatment. Beginning in 2014, one-fourth of each pasture has been burned in late fall after a heavy frost or early spring after snow melt such that, after four years, each patch-burn-assigned pasture will have been burned in its entirety.

Changes in the plant community are monitored by sampling the relative canopy cover (see Glossary, page 25) of all plant species, litter, bare ground, rock, and fecal pat in 20 0.5- x 0.5-meter frames along 40 meter transects at four locations within each pasture. Annual forage production is determined by clipping standing forage at the peak of production in late July from within three caged grazing exclosures at four locations within each pasture. Clipped samples are harvested, oven-dried and weighed.

Livestock weight gain is monitored by weighing cattle before and after they are placed on their respective grazing treatment pastures. Livestock weight gain data for 2016 has yet to be analyzed and is not included in the results of this report.

These sampling procedures differ from those conducted on the early-intensive and season-long grazed pastures from 2009 to 2013, so results prior to initiation of the patch-burn grazing treatment cannot be compared.

Prescribed fire on one of the patch-burn grazed pastures. *Megan Endreson*

Results

In 2011, vegetation sampling indicated the dominance of Kentucky bluegrass at our study site; it was present on 90 percent of sampled sites and its relative canopy cover averaged about 30 percent. Patch-burn grazing was initiated in 2014, so vegetation sampling for this project officially

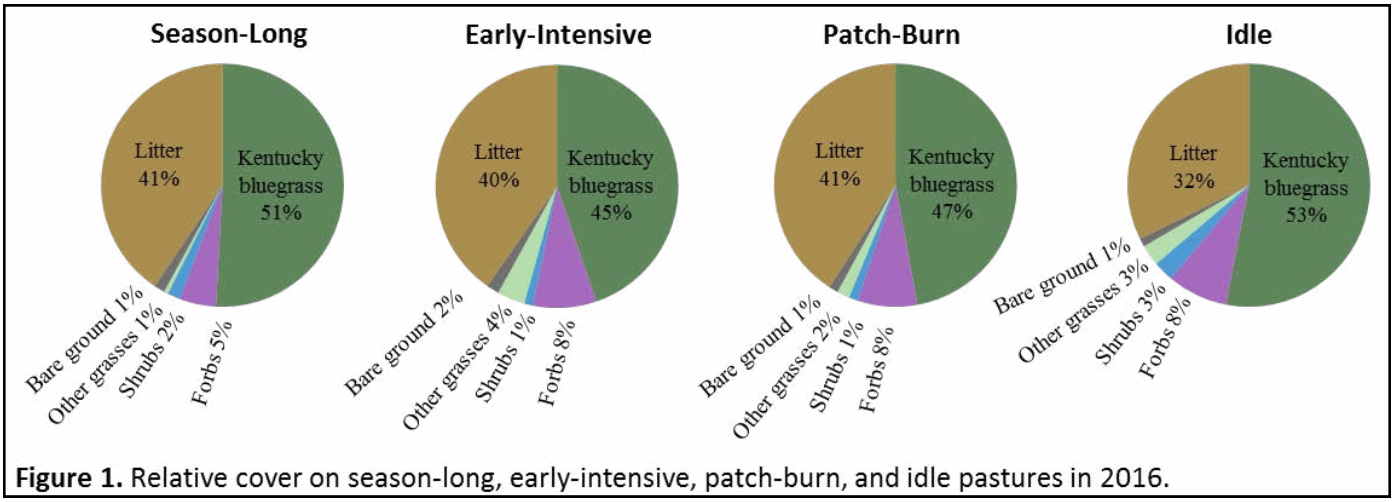


Figure 1. Relative cover on season-long, early-intensive, patch-burn, and idle pastures in 2016.

began in 2015. Procedures and data collection varied slightly between 2015 and 2016, which prevents analysis between those study years. Here, we present preliminary results with data collected in 2016.

Relative Canopy Cover. Relative canopy cover in 0.5- x 0.5-m frames was sampled between July 5 and Aug. 3 in 2016. Figure 1 details the average relative canopy cover on season-long, early-intensive, patch-burn and idle pastures for 2016. Richness, evenness, and diversity analyses (see Glossary) for 2016 are detailed in Figure 2. Plant species richness was the highest for early-intensive (S=35, SE=0.35) and patch-burn grazed pastures (S=34.42, SE=4.17) while richness was significantly lower in season-long (S=25.75, SE=2.43) and idle pastures (S=31.38, SE=4.68). Diversity and evenness were the highest in early-intensive grazed pastures followed by idle, patch-burn, and then season-long grazed pastures.

Annual Forage Production. Annual forage production sampling occurred during peak-production in mid-August 2016. Table 1 details the average annual production of

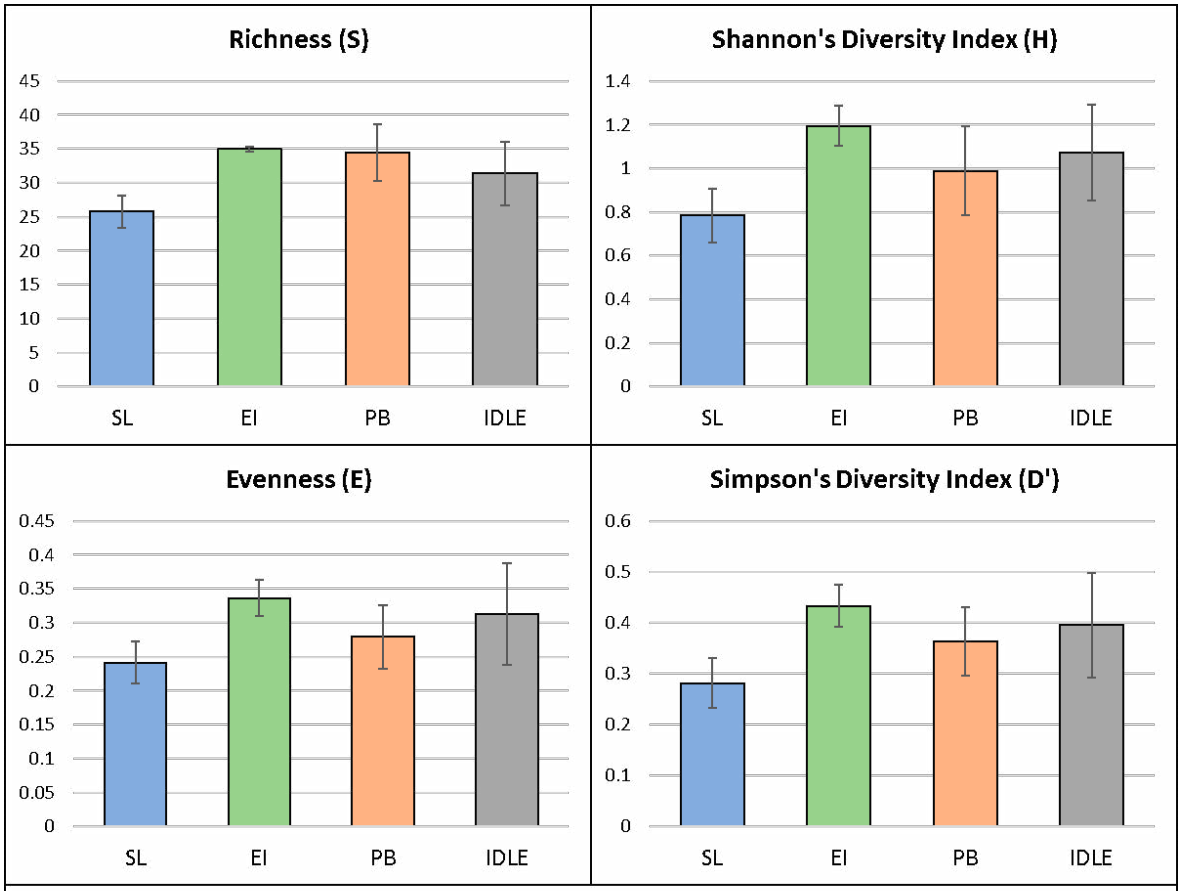


Figure 2. Richness, Evenness, Shannon's Diversity, and Simpson's Diversity indices on season-long (SL), early-intensive (EI), patch-burn (PB) and idle pastures in 2016. *Error bars represent Standard Error of Mean.

Table 1. Annual biomass production on season-long, early-intensive, patch-burn and idle pastures in 2016 ($P \leq 0.05$).

Average annual biomass production (lbs./acre)			
Season-long	**Early- intensive**	**Patch- burn**	**Idle**
5,875 ± 571	6,748 ± 546	5,379 ± 378	4,625 ± 175

season-long, early-intensive, patch-burn and idle treatments in 2016 ($P \leq 0.05$). The control produced significantly less annual forage production than all grazing treatments, while early-intensive grazed pastures produced the highest average.

Discussion

Kentucky bluegrass invasion is creating homogeneous plant communities with reduced forage quality and production in the Northern Great Plains (Toledo et al. 2014). Our results are consistent with expectations that any grazing management strategy results in more structural and compositionally diverse plant community than in ungrazed pastures. Because Kentucky bluegrass gains a competitive advantage by emerging earlier than native species, we expect early-intensive grazing to shift the balance toward later-emerging native species.

Preliminary results from 2016 suggest that our early-intensive grazing treatment has increased diversity and production of the plant community. However, livestock performance should be considered because livestock weight gains may suffer. As 2016 data is analyzed and the project continues, we can make conclusions regarding impacts of our grazing treatments on livestock weight gains and suggest any potential need for nutritional supplements.

Because patch-burn management is associated with increased structural and compositional diversity of the plant community, we expect diversity and forage production in our patch-burn grazed pastures to increase throughout the course of this project. Furthermore, we expect grazing after burning to enhance the benefits of patch-burning, which could have an additive effect on forage quality and production.

Cattle weight gains also are expected to increase, as suggested in previous research (Limb et al. 2011). Preliminary results from 2016 on relative canopy cover and production suggest that patch-burn grazing has increased the quality and production of forage but has not surpassed that of early-intensive grazing yet.

At this stage of the project, Kentucky bluegrass remains dominant in each pasture. If our grazing management procedures were ceased, the abundance of Kentucky bluegrass would increase quickly without disturbance. Furthermore, the effects of patch-burn grazing will become clearer as additional patches are burned.

At this time, only two patches within each pasture have burned. Treatments and monitoring will continue during the next several years. 2018 will mark the first full cycle of our patch-burn procedures in patch-burn grazed pastures. At that time, we expect to make detailed comparisons and conclusions regarding the different effects of our various grazing treatments.

Peak production on a patch-burn grazed pasture.

Literature Cited

Abrams, M.D. 1988. Effects of burning regime on buried seed banks and canopy coverage in a Kansas tallgrass prairie. Southwestern Naturalist 33:65-70.

Bahm, M.A., T.G. Barnes and K.C. Jensen. 2011. Herbicide and fire effects on smooth brome (*Bromus inermis*) and Kentucky bluegrass (*Poa pratensis*) in invaded prairie remnants. Invasive Plant Science and Management 4:189-197.

Bryan, W.B., E.C. Prigge, M. Lasat, T. Pasha, D.J. Flaherty and J. Lozier. 2000. Productivity of Kentucky bluegrass pasture grazed at three heights and two intensities. Agronomy Journal 92:30-35.

D'Antonio, C.M. and P.M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23:63-87.

Fuhlendorf, S.D. and D.M. Engle. 2001. Restoring heterogeneity on rangelands: Ecosystem management based on evolutionary grazing patterns. Bioscience 51:625-632.

Fuhlendorf, S.D. and D.M. Engle. 2004. Application of the fire-grazing interaction to restore a shifting mosaic on tallgrass prairie. Journal of Applied Ecology 41:604-614.

Hobbs, R.J., E. Higgs and J.A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution 24:599-605.

Hockensmith, R.L., C.C. Sheaffer, G.C. Marten and J.L. Halgerson. 1997. Maturation effects on forage quality of Kentucky bluegrass. Canadian Journal of Plant Science 77:75-80.

Limb, R.F., S.D. Fuhlendorf, D.M. Engle, J.R. Weir, R.D. Elmore and T.G. Bidwell. 2011. Pyric-herbivory and cattle performance in grassland ecosystems. Rangeland Ecology & Management 64:659-663.

Samson, F. and F. Knopf. 1994. Prairie conservation in North America. BioScience 44:418-421.

Samson, F.B., F.L. Knopf and W.R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. Wildlife Society Bulletin 32:6-15.

Toledo, D., M. Sanderson, K. Spaeth, J. Hendrickson and J. Printz. 2014. Extent of Kentucky bluegrass and its effect on native plant species diversity and ecosystem services in the Northern Great Plains of the United States. Invasive Plant Science and Management 7:543-552.

Glossary

Canopy cover – a vertical projection of the outline of each plant. The portion of a framed area covered by all individuals of a species is the relative canopy cover of the species.

Richness – the number of species in a sample representative of the ecological community.

Abundance – the number of individuals of a species in a sample that represents its relative representation in the ecological community.

Evenness – a measure of the relative abundance of all species in the sample.

Diversity – a measure used to describe the composition of the ecological community that takes into account the number of species (richness) and the relative abundance of each (evenness). Diversity is often calculated in two ways:


Janet Patton


Rick Bohn

Shannon's diversity index – a measure of diversity calculated from a mathematical formula that includes richness and evenness and considers all species in the sample. Higher values indicate a more diversity with higher richness and evenness, whereas lower values indicate a less diverse ecological community. Index values are rarely greater than 4.

Simpson's diversity index – the probability that two randomly sampled individuals are of the same species. The index is a measure of diversity also calculated from a mathematical formula that includes richness and abundance, but is weighted more towards the most abundant or dominant species. Index values range from 0 to 1 with higher values indicating less diversity (in the sense of evenness) as a single or few species have increased abundance. A value of 0, on the other hand, would indicate infinite diversity.

Quality of Hay from Road Right of Ways in North Dakota

Miranda Meehan[1], Kevin Sedivec[2], Fara Brummer[3] and Carl Dahlen[1]

[1]Department of Animal Sciences, NDSU
[2]School of Natural Resource Sciences, NDSU
[3]Central Grasslands Research Extension Center, NDSU

Hay harvested from forages growing in road ditches commonly is used as feed for beef cattle, yet little is documented regarding the nutrient content of ditch hay, the amount of ditch hay harvested or the intended use of ditch hay in North Dakota. Extension agents collected 182 ditch hay samples from 36 counties across North Dakota, and samples were analyzed to reveal factors contributing to variation in nutrient quality and recommendations for balancing quality and quantity of forage harvested. The results of this project revealed factors influencing ditch hay quality and management practices that can be implemented to improve hay quality, while reinforcing the importance of testing forage quality.

Summary

Extension agents engaged producers in 36 counties throughout North Dakota to collect a total of 182 samples of hay harvested from road rights of way (ditch hay). Samples were classified based on the county where the hay was produced, and the cutting date, whether the hay was rained on, type of binding material used, target species for feeding the hay, and whether the hay was going to be fed on the ground, in a hay feeder or as part of a total mixed ration (TMR) were reported. Each hay sample was analyzed for concentrations of dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro organic matter digestibility (IVOMD), calcium (Ca) and phosphorus (P). Samples were bound with plastic twine (40.6 percent), net wrap (40 percent) or sisal twine (19.4 percent). Producers primarily planned to feed ditch hay to cattle (about 90 percent), with the remaining hay produced for horses, sheep and bison. Producers intended to feed bales using a bale feeder (63.9 percent), directly onto the ground (36.8 percent) or with a TMR (11.6 percent). The mean nutrient content value for samples was 91.4 percent DM, 10.8 percent ash, 8.5 percent CP, 65.1 percent NDF, 52 percent total digestible nutrients (TDN), 0.61 percent Ca and 0.2 percent P. Crude protein content was impacted by the cutting day ($P < 0.01$), with forage harvested early in the year having greater concentrations, compared with those harvested later in the year. Rain during the interval from cutting to baling reduced the TDN content by 2 percentage points ($P = 0.01$). Results highlight the variability observed in ditch hay nutrient content and reinforce the importance of testing individual feeds to ensure appropriate delivery of nutrients to different classes of livestock.

Introduction

Hay from road ditches commonly is harvested and used as feed for beef cattle and other livestock. In some cases, the forages being harvested from ditches are very high quality, while in others, the hay is harvested well after dedicated hay fields, and optimizing quality may not be a top priority.

In addition, different types of roads (federal, state, county, township, etc.) may have specific regulations about the time of year forages must be cut and/or removed from the ditches. These and other factors may influence hay quality. Little is known about the nutrient content of ditch hay, the amount of ditch hay harvested or the intended use of ditch hay in North Dakota.

Procedures

A total of 182 hay samples were collected from road rights of way and hay lots in 36 counties across North Dakota (Figure 1). Samples were collected from July 15 through Oct. 31, 2015. County Extension agents were critical to the success of this effort, with agents from 29 counties volunteering to aid in collecting and characterizing samples.

Hay samples were composed of hay collected from five or more bales from each sampling location. Samples were collected using a Penn State Forage Probe. Samples were classified based on the county where hay was produced, cutting date, whether hay was rained on, type of binding material used, type of plant species present in the hay and type of road adjacent to the ditches.

Additional information regarding the miles of ditch hay baled by producers, percentage of hay inventory represented by ditch hay, target species for feeding the hay, and whether hay was going to be fed on the ground, in a hay feeder or as part of a total mixed ration also was collected.

Each hay sample was analyzed for concentrations of dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro organic matter digestibility (IVOMD), calcium (Ca) and phosphorus (P).

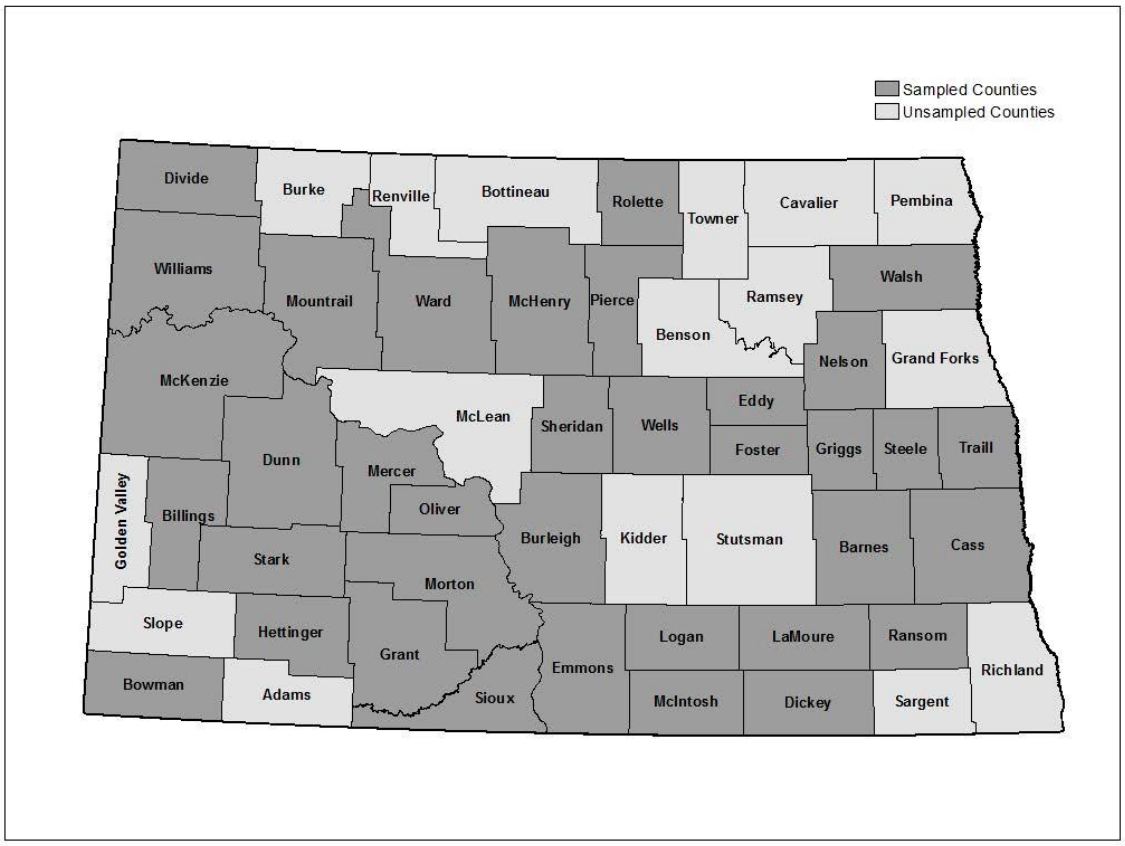


Figure 1. Location of North Dakota counties from which road right-of-way hay samples were collected in 2015.

Following analysis, individual reports of the nutrient content of sampled hay, along with appropriate feeding recommendations, were distributed to participating producers.

Means and standard errors of nutrient content of samples were determined. A general linear model (GLM) and single factor univariate analysis of variance using PROC GLM SAS (Ver. 9.2, SAS 2002) was used to determine the impacts of cutting date, rain, road type and region on nutrient content. Mean separations test was performed using the LSMEANS procedure with the Tukey adjustment.

Results and Discussion

Plastic twine (40.6 percent) and net wrap (40 percent) were the bale binding materials most often used, with a smaller proportion of bales being bound with sisal twine (19.4 percent). A majority of the hay sampled was going to be fed to cattle (about 90 percent), with the remaining proportion split among horses, sheep and bison. The feeding method indicated most often was using a bale feeder (63.9 percent), followed by feeding on the ground (36.8 percent) and feeding with a TMR (11.6 percent).

Variability also was observed in the analyzed nutrient content of the forages (Table 1). Some samples tested were extremely high quality, whereas the quality of other samples was poor.

Unpaved roads in several parts of North Dakota are experiencing heavy traffic related to oil-field activities, and dust has the opportunity to collect on standing and cut forages from ditches adjacent to these heavily used roads. Dust contamination was noted in several of the samples taken for the core oil-field counties, with ash content (essentially inert, indigestible material) reaching a maximal value of 37 percent, compared with our average value of 10.8 percent.

The cutting date of samples ranged from June 10 to Sept. 10. The concentration of crude protein in samples was impacted by the cutting date, with forage harvested early in the year having greater concentrations of crude protein, compared with those harvested later in the year (Figure 2). This trend was expected because the protein content of standing forages decreases with plant maturity during the course of the growing season.

Across North Dakota, road ditches consist of cool-season introduced grass species, the most common being smooth bromegrass. These species initiate growth early in the spring and reach peak production in early July. To achieve the best

Table 1. Nutrient content of ditch hay samples.

Item	Average	Minimum	Maximum
Dry matter (DM)	91.4	83.7	95.6
Ash	10.8	6.8	37.0
Crude protein (CP)	8.5	5.9	17.0
Neutral det. fiber (NDF)	65.1	35.2	53.6
Total dig. nutrients (TDN)	52.0	34.8	58.5
Calcium (Ca)	0.61	0.28	1.44
Phosphorus (P)	0.20	0.10	0.35

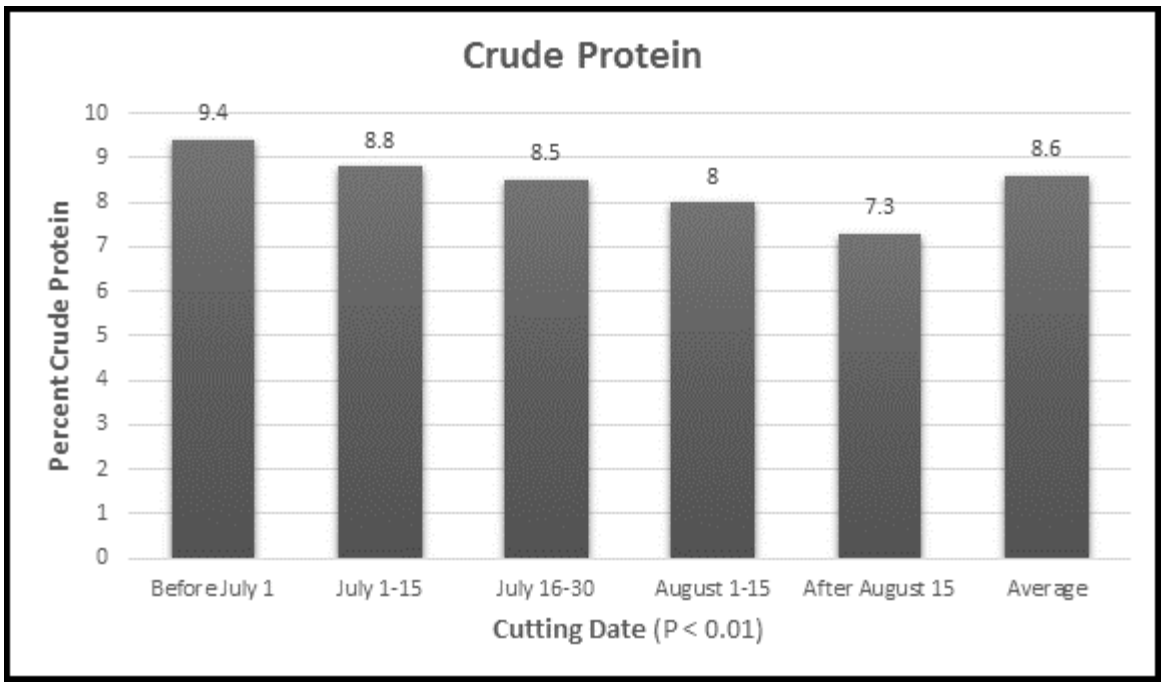


Figure 2. Concentrations of crude protein in ditch hay samples by cutting date.

combination of quality and quantity, this would be the optimal time to harvest; however, specific regulations regarding the timing of ditch hay harvest for roads under certain jurisdictions may prohibit harvesting forage of optimum quality.

Approximately 25 percent of the samples submitted were rained on during the interval from cutting to baling. The number of days the forages were wet in the swath ranges from one-half a day to more than 14 days, with four days being the average. Rain was associated with an increased ash content, and the TDN content of samples that had been rained on was 2 points less than those that had not had rain fall on them.

To understand the variation in forage quality across the state, samples were divided to represent eastern and western regions. The region did not impact the protein content of the forages, but acid detergent fiber and TDN were impacted.

Samples from the eastern region had greater TDN (via reduced ADF), compared with samples from the western region. Variations in soil type, temperature, moisture and species composition between regions all likely contributed to the differences observed.

Individual reports of the nutrient content of sampled hay allowed producers to incorporate the hay into a ration while ensuring the nutritional requirement of their livestock were being met. In addition, these producers have an increased awareness of factors influencing hay quality and management practices that can be implemented to improve hay quality.

The results of this project allowed us to understand the variation in quality of ditch hay within a single year's harvest and factors contributing to that variation. The largest factor influencing hay quality is cutting date. To achieve the best combination of quality and quantity, early July is the optimal time to harvest. The variation in the results reinforces the importance of testing nutrient contents of individual feeds to ensure appropriate delivery of nutrients to different classes of livestock.

Acknowledgments

Thank you to Karla Ryan, Danielle Steinhoff, Paige Brummond, Yolanda Schmidt, Mark Miller, Katelyn Hain, Brad Brummond, Annie Mae Kelly, Kelcey Hoffmann, Megan Minten, Angie Johnson, Brian Zimprich, Breana Kiser, Nicole Wardner, Tim Becker, Lindsay Maddock, Joel Lemer, Alicia Harstad, Kelsie Egeland, Ashley Stegeman, Sheldon Gerhardt, Crystal Schaunaman, Craig Askim, Katie Wirt, Jackie Buckley, Rick Schmidt, Duaine Marxen, Becky Buchmann and Kurt Froelich for collecting forage samples. In addition, thank you to Jim Hennessey of the Mountrail County Ag Agency for assisting with forage sample collection.

Financial support for this work was provided by the NDSU Extension Service.

Effects of Maternal Nutrition on Fructose and Expression of the Fructose Transporter *GLUT5* in Bovine Tissues and Fluids from Days 16 to 50 of Gestation

Nathaniel Greseth[1], Matthew Crouse[1], Kyle McLean[1], Mellissa Crosswhite[1], Nicolas Negrin Pereira[1], Alison Ward[1], Larry Reynolds[1], Carl Dahlen[1], Bryan Neville[2], Pawel Borowicz[1] and Joel Caton[1]

[1]Department of Animal Sciences, NDSU
[2]Central Grasslands Research Extension Center, NDSU

The objectives of this study were to determine the effects of maternal nutritional status on fructose concentration in maternal and fetal fluids and the mRNA expression of the fructose transporter GLUT5 in maternal and fetal tissues on days 16, 34, and 50 of gestation. The expression of GLUT5 was not influenced by maternal nutritional status; however, the concentration of fructose in amniotic fluids was influenced by day of gestation and maternal nutritional status.

Summary

We tested the hypothesis that the concentration of fructose in maternal and fetal fluids, and expression of *GLUT5* in utero-placental tissues, would be influenced by day of gestation and maternal nutritional status. Angus-cross heifers (n = 46, about 15 months of age; average initial body weight [BW] = 716 pounds) were estrus synchronized, bred via artificial insemination (AI) and ovariohysterectomized on day 16, 34, or 50 of their respective gestations (n = 6 to 9/day). Some heifers (n = 6) were not bred to serve as nonpregnant (NP) controls and were ovariohysterectomized on day 16 of the synchronized estrous cycle. Immediately after AI, heifers were assigned randomly to one of two treatment groups: Control (CON) received 100 percent of the National Research Council (NRC, 2000) requirements to gain 1 pound per heifer daily, and restricted (RES) received only 60 percent of the CON diet. Tissues collected included: caruncular tissue from the uterine horn ipsilateral to the corpus luteum (PC), from the uterine horn contralateral to the corpus luteum (NPC), inter-caruncular tissue from the uterine horn ipsilateral to the corpus luteum (PIC) and from the uterine horn contralateral to the corpus luteum (NPIC), as well as chorioallantoic tissue (FM). Fluids collected included: maternal serum, histotroph collected from horn ipsilateral to the corpus luteum (P histotroph) and from the horn contralateral to the corpus luteum (NP histotroph), allantoic fluid and amniotic fluid. Fetal membranes, allantoic fluid and amniotic fluid were not collected in NP heifers due to the lack of the presence of fetal tissues and fluids in NP animals. Serum fructose concentrations were greater ($P < 0.01$) in nonpregnant heifers, compared with pregnant heifers. Concentrations of fructose in P histotroph and NP histotroph were greater ($P < 0.01$) on day 50, compared with days 16 and 34. Amniotic fluid was influenced by a day × treatment interaction, with day 34 RES being greater ($P = 0.04$) than day 50 CON and RES heifers. Expression of *GLUT5* was greater on day 34 in PC ($P = 0.02$) and NPC ($P < 0.01$). In FM, day 16 was greater ($P = 0.04$), compared with days 34 and 50 of gestation. These results indicate that the expression of GLUT5 is not influenced by maternal nutritional status; however, concentrations of fructose in amniotic fluids are influenced by maternal nutritional status and day of gestation.

Introduction

First-service AI rates in beef cows are approximately 90 percent (Bridges et al., 2013); however, by day 30, only 50 to 60 percent are viable embryos in beef cows. Furthermore, fetal growth is vulnerable to maternal dietary nutrient deficiencies during the first trimester of gestation (Wu et al., 2004).

Currently, fetal undernutrition occurs in grazing livestock worldwide (Wu et al., 2004). Early in gestation, trans-placental exchange has yet to be established; therefore, nutrients must be transported to the conceptus via nutrient transporters in the uterus and developing placenta, such as the fructose transporter *GLUT5*.

Fructose is the most abundant hexose sugar in fetal blood and fetal fluids of ungulates (Kim et al., 2012), and maternal undernutrition has been implicated in altered fructose transport (Zhang et al., 2015). Having an understanding of how maternal nutrition affects the mRNA expression and supply of fructose to the conceptus could lead to future research that may directly influence the flux of fructose from the maternal to fetal systems in early gestation.

This research utilized a newly developed technique to ovariohysterectomize cattle without slaughter to allow for an accurate analysis of fetal growth and development, as well as utero-placental tissues and fluids on days 16, 34 and 50 of early gestation in beef heifers.

In this study, we tested the hypothesis that the concentration of fructose in maternal and fetal fluids, along with the relative mRNA expression of *GLUT5* in maternal and fetal tissues, would be influenced by day of gestation and maternal nutritional status.

Procedures

All animal procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (IACUC numbers A14053 and A16049). Crossbred Angus heifers (n = 49, about 15 months of age; average initial BW = 716 pounds) were exposed to the 5-day CO-Synch + CIDR estrus synchronization protocol. Six heifers were not inseminated to serve as nonpregnant controls, but received an ovariohysterectomy on day 16 of the subsequent synchronized estrous cycle. The remaining heifers (n = six to nine/day of gestation/treatment) were bred by AI to a common sire at 12 hours after observed estrus and ovariohysterectomized at days 16, 34, and 50 of gestation.

Immediately following the ovariohysterectomy, maternal caruncular (PC) and inter-caruncular tissue (PIC) were collected from the uterine horn ipsilateral to the corpus luteum, along with caruncular (NPC) and inter-caruncular tissue (NPIC) collected from the uterine horn contralateral to the corpus luteum. Also, fetal membranes (chorioallantois) were obtained.

Fetal membranes were collected only on days 16, 34, and 50 of gestation due to nonpregnant controls not having fetal membranes. All tissues were frozen immediately in liquid nitrogen-cooled isopentane and stored at minus 112 F.

Serum samples were obtained via jugular venipuncture on the day of ovariohysterectomy, and blood constituents were separated by centrifugation and stored at minus 20 F. Histotroph was obtained from the uterine horn ipsilateral to the corpus luteum (P histotroph) and from the uterine horn contralateral to the corpus luteum (NP histotroph) for pregnant and NP heifers.

Allantoic and amniotic fluids were collected on days 34 and 50 of gestation due to the limited presence of fetal fluids on day 16. Histotroph, allantoic and amniotic were snap-frozen in liquid nitrogen-cooled isopentane and stored at minus 20 F immediately after being obtained.

Fructose concentrations among fluid samples were determined by utilizing a colorimetric assay kit. Expression of *GLUT5* was determined by first isolating and purifying RNA from collected tissue samples, followed by real-time quantitative PCR (qPCR) to determine differences in mRNA expression of the fructose transporter in each tissue relative to a NP endometrium sample.

Results and Discussion

Concentrations of fructose in maternal serum were greater ($P < 0.01$) in NP heifers, compared with pregnant heifers (Table 1). In P histotroph, fructose concentrations were greater ($P < 0.01$) on day 50, compared with days 16 and 34 (1.34, 0.18, and 0.63 millimolar [mM], respectively; SEM = 0.22).

Fructose concentrations also were greater ($P < 0.01$) on day 34 + 50, when compared with day 16 (Table 1). In NP histotroph, day 50 (0.97 mM) was greater ($P = 0.01$) than days 16 and 34 (0.10, and 0.36 mM, respectively; SEM = 0.20).

Additionally, fructose concentrations were greater ($P = 0.02$) in NP histotroph on days 34 + 50, compared with day 16 (Table 1). Fructose concentrations in amniotic fluid were influenced by a day × treatment interaction, where day 34 RES (3.60 mM) was greater ($P = 0.04$) than the day 50 CON and RES treatments (2.57, and 1.55 mM, respectively; SEM = 0.29). Furthermore, day 34 and 50 CON (3.30 and 2.57 mM, respectively) were greater than day 50 RES (1.55 mM; SEM = 0.29).

In PC, a main effect of day was observed; day 34 was greater (44.40-fold greater than NP; $P = 0.02$; Table 2) than day 16 and day 34 (4.79 and 6.49-fold greater than NP, respectively; SEM = 10.88).

In NPC, the expression of *GLUT5* was greater ($P < 0.01$) on days 34 and 50, compared with day 16 (44.19, 36.60 and 3.22 -fold greater than NP, respectively; Table 2). Furthermore, the relative expression of *GLUT5* in pregnant heifers was greater ($P = 0.02$), compared with NP heifers.

A main effect of day was observed in FM where day 16 was greater ($P = 0.04$) than day 50 (80.17 and 27.39, respectively; SEM = 13.82; Table 2). Also, day 16 was greater, compared with day 34 + 50 ($P = 0.03$). The results show that as pregnancy advances, fructose and mRNA expression of *GLUT5* changes significantly among various tissues and fluids measured in this study.

The low fructose concentration found in maternal serum is expected because fructose is not a main physiological fuel for the dam. The cause of the NP heifers having a greater fructose concentration in serum samples could be due to fructose being utilized by the conceptus. The greater mRNA expression of *GLUT5* in pregnant heifers, compared with NP

Table 1: Fructose concentrations mM in Serum (maternal serum), P histotroph (histotroph from the horn ipsilateral to the corpus luteum), NP histotroph (histotroph from the horn contralateral to the corpus luteum), Allantoic (allantoic fluid) and Amniotic (amniotic fluid) on days 16, 34 and 50 of gestation.

			Day of Gestation[1]						*P* - values[2]					
Fluid[3]	Trt[4]		NP	16	34	50	Trt[5]	SEM[6]	NP vs. P	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
Serum	CON		0.13	0.08	0.075	0.071	0.07							
	RES		-	0.08	0.085	0.094	0.09	0.016	< 0.01	0.79	0.92	0.97	0.35	0.82
		Day[7]		0.08	0.080	0.083								
P Histotroph	CON		0.14	0.12	0.58	1.03	0.58							
	RES		-	0.23	0.69	1.64	0.86	0.310	0.09	< 0.01	0.03	< 0.01	0.27	0.65
		Day		0.18[h]	0.63[h]	1.34[g]								
NP Histotroph	CON		0.01	0.11	0.45	0.67	0.41							
	RES		-	0.08	0.26	1.27	0.54	0.277	0.13	0.02	0.02	0.01	0.58	0.33
		Day		0.10[h]	0.36[h]	0.97[g]								
Allantoic	CON		-	-	5.53	5.07	5.30							
	RES		-	-	4.56	4.83	4.69	0.76	-	-	0.95	0.90	0.44	0.64
		Day		-	5.04	4.95								
Amniotic	CON		-	-	3.30[ab]	2.57[b]	2.94							
	RES		-	-	3.56[a]	1.55[c]	2.55	0.29	-	-	-	< 0.01	0.21	0.04
		Day		-	3.43	2.06								

[1]Number of days after AI.

[2]Probability values for the effect of day, treatment and day × treatment on the concentration of fructose. Contrast statements comparing pregnant vs. nonpregnant concentration (NP vs. P), day pre-implantation vs. day post-implantation (day 16 vs. 34 + 50) and day post-attachment comparison (34 vs. 50).

[3]Fluids evaluated for fructose concentrations mM include maternal serum (Serum), histotroph flushed from the horn ipsilateral to the corpus luteum (P histotroph), histotroph flushed from the horn contralateral to the corpus luteum (NP histotroph), allantoic fluid (Allantoic) and amniotic fluid (Amniotic).

[4]CON = Heifers fed a TMR that meets 100 percent of NRC requirements to gain 0.45 kg daily. RES = Heifer restricted to 60 percent of CON diet.

[5]Mean fructose concentration of treatment groups across day of gestation within fluid.

[6]Average SEM for day × treatment interaction. Day 16 CON n = 7, day 16 RES n = 7, day 34 CON n = 6, day 34 RES n = 9, day 50 CON n = 7, day 50 RES n = 7.

[7]Mean fructose concentration across treatment within day of gestation.

[a-c]Means within fluid without common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without common superscript differ in main effect of day ($P \leq 0.05$).

heifers in NPC, may be explained by the conceptus's increasing need of fructose, which is partially supplied by maternal blood concentrations.

The consistent concentration of fructose in P histotroph and NP histotroph may be explained by the availability of fructose for transport into the uterine lumen. Fructose concentrations were found to be less than 1 mM in maternal circulation; therefore the total available fructose to be transported to the conceptus from the maternal system is low.

The placenta is a site of the conversion of glucose to fructose (Kim et al., 2012), which plays a role in the consistently high fructose concentration and the increase in fructose concentration found in fetal fluids compared with maternal fluids. This conversion of glucose to fructose indicates the

Table 2: Relative mRNA expression of *GLUT5* in PC (pregnant caruncle), PIC (pregnant inter-caruncle), NPC (non-pregnant caruncle), NPIC (non-pregnant inter-caruncle) and FM (fetal membranes from days 16, 34, and 50 of gestation as a fold change in relation to nonpregnant heifer samples set to 1.

			Day of Gestation[1]					*P* - values[2]					
Tissue[3]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. P	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
PC	CON		6.61	22.06	9.07	12.58							
	RES		2.98	66.74	3.91	24.54	15.33	0.24	0.06	< 0.01	0.02	0.35	0.20
		Day[7]	4.79[h]	44.40[g]	6.49[h]								
PIC	CON		1.46	3.60	6.11	3.72							
	RES		3.59	3.82	1.20	2.87	2.36	0.36	0.58	0.97	0.85	0.66	0.31
		Day	2.52	3.71	3.66								
NPC	CON		4.24	36.44	53.54	31.41							
	RES		2.21	51.93	19.66	24.60	10.39	0.02	< 0.01	0.36	< 0.01	0.43	0.07
		Day	3.22[h]	44.19[g]	36.60[g]								
NPIC	CON		25.23	38.42	49.57	37.74							
	RES		14.29	27.02	6.99	16.10	16.90	0.17	0.46	0.82	0.74	0.13	0.57
		Day	19.76	32.72	28.28								
FM	CON		59.78	43.59	33.40	45.59							
	RES		100.57	46.63	21.38	56.19	19.54	-	0.03	0.30	0.04	0.52	0.44
		Day	80.17[g]	45.11[gh]	27.39[h]								

[1]Number of days after AI.

[2]Probability values for the effect of day, treatment and day × treatment on the mRNA expression of *GLUT5*. Contrast statements comparing pregnant vs. nonpregnant expression (NP vs. P), day pre-implantation vs. day post-implantation (day 16 vs. 34 + 50), and day post-attachment comparison (34 vs. 50).

[3]Tissues evaluated for mRNA expression of *GLUT5* include caruncular tissue collected from the uterine horn ipsilateral to the corpus luteum (PC), caruncular tissue collected from the uterine horn contralateral to the corpus luteum (NPC), inter-caruncular tissues collected from the uterine horn ipsilateral to the corpus luteum (PIC), inter-caruncular tissue collected from the uterine horn contralateral to the corpus luteum (NPIC), and chorioallantois (FM).

[4]CON = Heifers fed a TMR that meets 100 percent of NRC requirements to gain 0.45 kg daily. RES = Heifer estricted to 60 percent of CON diet.

[5]Mean *GLUT5* mRNA expression of treatment groups across day of gestation within tissue.

[6]Average SEM for day × treatment interaction. Day 16 CON n = 7, day 16 RES n = 7, day 34 CON n = 6, day 34 RES n = 9, day 50 CON n = 7, day 50 RES n = 7.

[7]Mean *GLUT5* mRNA expression across treatment within day of gestation.

[a-c]Means within tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

essentiality of fructose for the growth and development of the conceptus. Furthermore, this consistently high concentration could be explained by the conceptus's hypoxic environment.

Vascularization of the fetal membranes is limited up to day 35 of gestation, which results in an oxygen-poor environment for the conceptus due to a lack of a transport of oxygen via a shared blood supply. Glucose thrives in a hyperoxic environment, while fructose thrives in a hypoxic environment.

This information is emphasized by our observed results in fetal fluids and FM. Fructose concentration decreased from day 34 to 50 (numerically in allantoic), while relative

expression of *GLUT5* decreased from day 16 to 50, indicating a decreased need of fructose transport.

These decreases could be due to vascularization intensifying after day 35, resulting in an increase in oxygen for the conceptus's environment, thereby decreasing the concentration of fructose (3.43 to 2.06 mM in amniotic fluid from day 34 to 50, respectively) and increasing the need of glucose (results from our lab not shown; 1.46 to 1.68 mM glucose in amniotic fluid from day 34 to 50, respectively).

In amniotic fluid, fructose concentrations differed between day 34 RES and day 50 CON and RES, as well as between day 50 CON and RES. We interpret these data to imply that a compensatory mechanism may be in action when examining the greater fructose concentration found in day 34 RES, compared with day 50 CON and RES.

Organogenesis takes place throughout the first 50 days of gestation, with most of the fetal organs having significantly developed by day 50. At this time, the conceptus could have a greater need of fructose. Therefore, a compensatory mechanism may have been in action, resulting in a greater amount of fructose being made available to the conceptus for day 34 RES to maintain a viable pregnancy.

When examining the greater concentrations found in day 50 CON, compared with day 50 RES, we interpret these data to imply that the conceptus could have a lower need of fructose at this time, which is supported by the decrease in fructose concentration observed from day 34 to 50 in amniotic fluid and the increased placental development and vascularization on day 34, compared with day 50.

This potential decreased need of fructose may have resulted in the lack of the aforementioned compensatory mechanism. Therefore, the greater concentration of fructose found in day 50 CON could be explained by the day 50 CON receiving 100 percent of NRC requirements, while the day 50 RES received only 60 percent of requirements.

In PC and NPC, relative *GLUT5* mRNA expression was greater on days 34 and 50, compared with day 16. We interpret these data to imply that the increase could be due to the critical period for maternal recognition (days 15 to 16) already passing (Senger, 2012), resulting in an increase in expression to compensate for the nutritional needs of the developing conceptus.

The FM had high mRNA expression of *GLUT5* relative to NP. We interpret this data to imply that the conversion of glucose to fructose may have an impact on the mRNA expression of *GLUT5* in FM.

When examining the main effect of day seen in FM, the fold change relative to NP decreases from 80.17 at day 16 to 27.39 -fold greater than NP by day 50. We interpret these data to imply that sugars such as fructose are highly important in supplying energy for the elongation of the conceptus on days 12 to15 to ensure maternal recognition of pregnancy to occur by days 15 to 16 (Senger, 2012).

In conclusion, these data partially support our hypothesis that day of gestation and maternal nutritional status would impact mRNA expression of *GLUT5* in utero-placental tissues and fructose concentration among maternal and fetal fluids. In partially keeping with our hypothesis, we found that day of gestation, but not a 40 percent global nutrient restriction, affects the relative expression of *GLUT5* in PC, NPC and FM.

In addition, day of gestation, and not a 40 percent global nutrient restriction, affects fructose concentration in histotroph. Furthermore, maternal nutritional status and day of gestation affect fructose concentration in amniotic fluid.

With the establishment of these data, future research can be aimed at increasing efficiency of maternal and fetal nutrition. Specifically, providing improvements to the dam's nutrition at certain points of gestation allows the conceptus to receive an appropriate amount of fructose throughout early gestation, which it needs for proper growth and development. Applications such as this may result in increased reproductive efficiency and, ultimately, aid in supporting the increasing need of food by the growing world population.

Literature Cited

Bridges, G.A., M.L. Day, T.W. Geary and L.H. Cruppe. 2013. Deficiencies in the uterine environment and failure to support embryonic development. J. Anim. Sci. 91:3002-3013

Elliot, I. Meat output must double by 2050. Feedstuffs. Accessed 8/5/2016: http://feedstuffsfoodlink.com/story-meat-ouput-must-double-by-2050-71-66920

Kim, J., G. Song, G. Wu and F.W. Bazer. 2012. Functional Roles of Fructose. PNAS. 109:E1619 – E1628.

Senger P.L. 2012. Early Embryogensis and Maternal Recognition of Pregnancy. In: Pathways to Pregnanncy and Parturition. Current Conceptions Inc. Redmond, Ore. P. 282.

Wu, G., F.W. Bazer, T.A. Cudd, C.J. Meininger and T.E. Spencer. 2004. Maternal nutrition and fetal development. J. of Nutrition. 9:2169-2172.

Zhang, S., T.R.H. Regnault, P.L. Barker, K.J. Botting, I.C. McMillen and C.M. McMillan, C.T. Roberts and J.L. Morrison. 2015. Placental adaptation in growth restriction. Nutrients. 7:360-389.

Effects of Maternal Nutritional Status on Nutrient Transporter Expression in Bovine Utero-placental Tissue on Days 16 to 50 of Gestation

Matthew Crouse[1], Kyle McLean[1], Mellissa Crosswhite[1], Nicolas Negrin Pereira[1], Alison Ward[1], Lawrence Reynolds[1], Carl Dahlen[1], Bryan Neville[2], Pawel Borowicz[1] and Joel Caton[1]

[1]Department of Animal Sciences, NDSU
[2]Central Grasslands Research Extension Center, NDSU

The objectives of this study were to determine the effect of a 40 percent global nutrient restriction on the messenger ribonucleic acid (mRNA) expression of nutrient transporters known for their roles in transporting arginine (CAT-1, CAT-2 and CAT-3) and glucose (GLUT1) across the uterine endometrium and fetal membranes to the fetus from days 16 to 50 of gestation. The results indicate that a 40 percent global nutrient restriction only affects mRNA expression of arginine transporter CAT-2 and not any other transporter investigated.

Summary

We hypothesized that maternal nutrition and day of gestation would impact mRNA expression of nutrient transporters GLUT1, CAT-1, CAT-2 and CAT-3 in beef heifers. Crossbred Angus heifers (n = 49) were synchronized, bred via artificial insemination (AI), assigned to nutritional treatment (CON = 100 percent of requirements for 1 pound/day of gain and RES = 60 percent of CON) and ovariohysterectomized on days 16, 34 or 50 of gestation (n = six to nine/day). Nonpregnant (NP) controls were not bred and ovariohysterectomized on day 16 of the synchronized estrous cycle (n = 6). The resulting arrangement of treatments was a 2 × 3 factorial + 1. Caruncle (CAR), intercaruncular endometrium (ICAR) and fetal membranes (FM) were obtained from the pregnant uterine horn immediately following ovariohysterectomy. For NP controls, only CAR and ICAR were obtained. The relative expression of the glucose transporter GLUT1 and cationic amino acid transporters CAT-1, CAT-2 and CAT-3 was determined for each tissue utilizing NP-CAR and NP-ICAR tissue as the baseline. For FM, NP endometrium served as the baseline. We found no interaction of day and treatment in FM for any genes ($P \geq 0.05$). Expression of GLUT1 and CAT-1 showed a day effect, being greater ($P < 0.05$) in FM on days 34 and 50, compared with day 16. In CAR, we found no day × treatment interaction, and CAT-3 expression tended ($P = 0.06$) to be greater in CON vs. RES heifers. Additionally, expression of GLUT1, CAT-1 and CAT-2 in CAR were greater ($P < 0.01$) on day 16, compared with days 34 and 50, day 34 compared with day 50, and days 16 and 34 compared with day 50, respectively. In ICAR, CAT-2 showed a day × treatment interaction, being greater ($P = 0.01$) on day 50 CON, compared with all other groups. Transporter CAT-3 tended ($P = 0.09$) to be greater in day × treatment in ICAR on day 16 CON, compared with all other days and treatments. The expression of GLUT1 was greater ($P < 0.01$) in ICAR on day 16 than all other days. Arginine transporter CAT-1 was greater ($P < 0.01$) in ICAR on days 34 and 50, compared with day 16. These results partially support our hypothesis and indicate that day was a more influential factor for mRNA expression of utero-placental glucose and cationic amino acid transporters than maternal nutritional status in heifers during early pregnancy.

Introduction

Fetal growth is vulnerable to maternal dietary nutrient deficiencies during the first trimester of gestation (Wu et al., 2004). During the first 50 days of gestation, organogenesis is taking place. This time period of gestation is a critical developmental window with significant cellular and tissue differentiation. Nutritional influences may alter the mammalian phenotype through affecting gene regulatory mechanisms involved in DNA synthesis and replication, thus "imprinting" potential susceptibilities to chronic disease and metabolic issues into the genome (Waterland and Jirtle, 2004).

Currently, fetal undernutrition occurs in grazing livestock worldwide (Wu et al., 2004). Maternal undernutrition has been implicated in fetal growth restriction and altered placental growth, reduced amino acid and glucose transport, and increased apoptosis and autophagy, which overall can yield decreased fetal growth during gestation (Zhang et al., 2015).

Before the establishment of hemotrophic nutrition, the placenta is developing and the fetus begins to utilize increasing quantities of glucose and amino acids (Groebner et al., 2011). Thus, the expression of glucose and amino acid transporters in the utero-placenta becomes essential to the viability of the conceptus.

Therefore, we studied the utero-placental glucose transporter GLUT1 (SLC2A1), which is present in most tissues

throughout the body and is ubiquitous across species. The amino acid transporters investigated are CAT-1, CAT-2 and CAT-3 (SLC7A1, SLC7A2 and SLC7A3), whose substrates are cationic amino acids such as arginine and lysine, which are directly linked to angiogenesis and cell proliferation. In this experiment, we tested the hypothesis that mRNA for glucose and cationic amino acid transporters in utero-placental tissues would be expressed differentially due to day of gestation and maternal nutritional status.

Procedures

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Crossbred Angus heifers (n = 49, about 15 months of age; average initial body weight [BW] = 722 pounds) were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol. Six heifers were not inseminated to serve as nonpregnant (NP) controls but received ovariohysterectomy for tissue collections on day 16 of the synchronized estrous cycle. The remaining heifers (n = six to nine/day of gestation/treatment) were bred by AI to a common sire at 12 hours after observed estrus and ovariohysterectomized at days 16, 34 or 50 of gestation.

Heifers were housed at the NDSU Animal Nutrition and Physiology Center. Heifers were acclimated to individual bunk feeding for two weeks before the beginning of the trial.

Immediately following AI, heifers were assigned randomly to one of two treatment groups. Control heifers (CON) received 100 percent of National Research Council (NRC, 2000) requirements for 0.45 kilogram per day (kg/d) gain to reach 80 percent of mature BW at first calving. Restricted heifers (RES) were placed on a 40 percent global nutrient restriction, which was accomplished by reducing total diet delivery 60 percent of the control delivery.

The diet was delivered via total mixed ration (TMR) and consisted of grass hay (73.4 percent neutral detergent fiber [NDF], 8 percent crude [CP]), corn silage (55.6 percent NDF, 6.3 percent CP), alfalfa haylage, (48.9 percent NDF, 16.4 percent CP), grain supplement, (32.6 percent NDF, 24.1 percent CP) and dried distillers grains (53.4 percent NDF, 31.3 percent CP), on a dry-matter (DM) basis.

Immediately following ovariohysterectomy (McLean et al., 2016), utero-placental tissues (caruncle, CAR; intercaruncular endometrium, ICAR; fetal membrane [chorioallantois], FM; cotyledon, COT; intercotyledonary placenta ICOT; and amnion, AMN) were obtained from the uterine horn containing the conceptus, as previously described (Grazul-Bilska et al., 2010, 2011). Fetal membranes also were collected only from heifers that were bred due to a lack of FM in NP controls.

On day 50 of gestation, FM was split into COT and ICOT. Amnion was collected only on day 50. Once collected, all tissues were frozen in liquid nitrogen-cooled isopentane and stored at minus 112 F.

The RNA was extracted from each tissue and purified. The level of mRNA expression of each transporter within the tissue was established using polymerase chain reaction (PCR) to determine differences in mRNA expression of the transporters across days of early gestation.

Results and Discussion

The mRNA expression of glucose transporter GLUT1 was greater ($P < 0.01$) in AMN, compared with COT and ICOT (0.67 vs. 0.24 and 0.29, respectively; Table 1). Arginine transporter CAT-1 mRNA expression was greater ($P = 0.02$) in AMN when compared with COT and ICOT (0.30 vs. 0.22 and 0.17, respectively; Table 1).

Cationic amino acid transporter CAT-2 mRNA expression was greater ($P = 0.05$) in AMN, compared with ICOT (3.27 vs. 0.82, respectively). The level of expression of CAT-3 was greater ($P < 0.01$) in AMN, compared with COT and ICOT (7.64 vs. 0.73 and 2.75, respectively).

Table 1. Relative expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2* and *CAT-3* in AMN, COT and ICOT on day 50 of gestation using NP endometrium as a baseline value set to 1.

Gene[1]	AMN[2]	COT[3]	ICOT[4]	SEM[5]	*P*-value[6]
GLUT1	0.67[a]	0.24[b]	0.29[b]	0.07	< 0.01
CAT-1	0.30[a]	0.22[b]	0.17[b]	0.03	0.02
CAT-2	3.27[a]	1.42[ab]	0.82[b]	0.66	0.05
CAT-3	7.64[a]	0.73[b]	2.75[b]	1.38	< 0.01

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2* and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.
[2]Amnion taken on day 50 of gestation.
[3]Cotyledons taken from fetal membranes on day 50 of gestation.
[4]Intercotyledonary tissue (fetal membrane tissue not including cotyledons; taken from fetal membranes on day 50 of gestation).
[5]Average SEM was used within gene; AMN n = 11, COT n = 14, ICOT n =14
[6]Probability values for the effect of tissue on level of expression of individual genes.
[a-b]Means within gene without a common superscript differ by tissue ($P \leq 0.05$).

We found no day × treatment interaction or main effect of treatment for any gene in FM (P > 0.05). Expression of GLUT1 was greater (P = 0.04) on day 50 of gestation, compared with day 16 (0.38 vs. 0.15, respectively; Table 2). Cationic amino acid transporter CAT-1 expression was greater (P < 0.01) on days 34 and 50, compared with day 16 (0.23 and 0.22 vs. 0.05, respectively; Table 2). The mRNA expression of CAT-2 tended to be greater (P = 0.09) on day 50 of gestation, compared with day 16.

We also found no day × treatment interaction (P ≥ 0.05) on the mRNA expression of GLUT1, CAT-1, CAT-2 or CAT-3 in CAR. Expression of CAT-3 showed a tendency (P = 0.06) to be greater across day of gestation in CON vs. RES (2.60 vs. 1.16; Table 3). Expression of GLUT1 was greater (P < 0.01) on day 16 of gestation, compared with days 34 and 50 (2.89 vs. 0.85 and 1.14 respectively).

The mRNA expression of CAT-1 was greater (P < 0.01) on day 34, compared with days 16 and 50 (5.22 vs. 1.47 and 0.51 respectively; Table 3). Additionally, mRNA expression of CAT-1 tended to be greater (P = 0.09) in the post-implantation (days 34 and 50) vs. implantation (day 16). Expression of cationic amino acid transporter CAT-2 was greater (P = 0.02) on day 34, compared with day 16 of gestation (14.67 vs. 4.36, respectively). In addition, CAT-2 mRNA expression showed a tendency (P = 0.06) to be greater in pregnant vs. NP heifers.

The expression of CAT-2 showed a day × treatment interaction (P = 0.01) being greater, with day 50 CON heifers having greater expression, compared with days 16 and 50 RES and day 34 CON heifers (Table 4). The cationic amino acid transporter CAT-3 tended (P = 0.09) to be greater in day 16 CON, compared with all other days and treatments.

Table 2. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2* and *CAT-3* in fetal membranes (FM) due to CON and RES dietary treatments from days 16 to 50 of gestation in beef heifers using NP endometrium as a baseline value set to 1.

			Day of Gestation[2]					*P* – values[3]		
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	Day	Trt	Day × Trt
GLUT1	CON		0.11	0.25	0.38	0.25	0.08	0.04	0.70	0.90
GLUT1	RES		0.19	0.27	0.38	0.28				
		Day[7]	0.15[h]	0.26[gh]	0.38[g]					
CAT-1	CON		0.04	0.22	0.22	0.16	0.17	< 0.01	0.70	0.99
CAT-1	RES		0.05	0.24	0.23	0.17				
		Day	0.05[h]	0.23[g]	0.22[g]					
CAT-2	CON		0.42	0.84	1.97	1.08	0.66	0.09	0.87	0.82
CAT-2	RES		0.24	1.16	1.57	0.99				
		Day	0.33	1.00	1.77					
CAT-3	CON		0.08	3.94	5.20	3.07	2.21	0.39	0.61	0.57
CAT-3	RES		2.38	0.93	3.02	2.11				
		Day	1.23	2.43	4.11					

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2* and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.
[2]Day of Gestation = number of days after AI. Each gene of interest expression value is reported as a fold change in relation to NP endometrium level of gene expression.
[3]Probability values for the effect of day, treatment and day × treatment on the level of expression of individual genes.
[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet.
[5]Mean gene expression of treatment group across day of gestation within tissue and gene of interest.
[6]Average SEM used within gene. d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7
[7]Mean gene expression across treatment within day and gene of interest.
[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).
[g-h]Means within row lacking common superscript differ in main effect of day ($P \leq 0.05$).

Table 3. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in caruncular CAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

			Day of Gestation[2]					*P* - values[3]					
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. Preg	16 vs. 34 and 50	34 vs. 50	Day	Trt	Day × Trt
GLUT1	CON		2.40	0.93	1.38	1.57	0.44	0.21	< 0.01	0.47	< 0.01	0.77	0.23
GLUT1	RES		3.38	0.76	0.89	1.67							
		Day[7]	2.89[g]	0.85[h]	1.14[h]								
CAT-1	CON		1.08	5.24	0.53	2.28	1.03	0.21	0.09	< 0.01	< 0.01	0.78	0.90
CAT-1	RES		1.85	5.20	0.49	2.51							
		Day	1.47[h]	5.22[g]	0.51[h]								
CAT-2	CON		5.76	14.37	7.98	9.37	3.63	0.06	0.02	0.04	0.02	0.77	0.89
CAT-2	RES		2.95	14.97	7.58	8.50							
		Day	4.36[h]	14.67[g]	7.78[gh]								
CAT-3	CON		1.29	2.23	4.28	2.60	1.09	0.44	0.24	0.11	0.20	0.06	0.90
CAT-3	RES		0.45	0.99	2.05	1.16							
		Day	0.87	1.61	3.16								

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.
[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.
[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.
[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet
[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.
[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7
[7]Mean level of expression across treatment within day and gene of interest.
[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).
[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

The mRNA expression of GLUT1 was greater (P < 0.01) on day 16 of gestation compared with day 34 (2.11 vs. 0.75). Arginine and Lysine transporter CAT-1 was greater (P < 0.01) on days 34 and 50, compared with day 16 (14.62 and 11.13 vs. 0.58, respectively). Additionally, CAT-1 mRNA expression was greater in ICAR (P < 0.01) in pregnant, compared with NP heifers (8.78 vs. 1, respectively).

Fertilization rates for first-service AI are approximately 90 percent in beef heifers (Bridges et al., 2013); however, by day 30 of gestation, only 50 to 60 percent of heifers maintain a viable pregnancy. Moreover, Thatcher et al., (1994) indicated that up to 40 percent of all embryonic loss occurs before day 40 of gestation in sheep and cattle.

Glucose and amino acids, specifically arginine, are crucial for proper energy metabolism and growth, and are key regulators of mTOR, which is linked to angiogenesis and cell proliferation, causing increased fetal growth and mitigating apoptosis (Tan and Miyamoto, 2016).

The expression of all transporters investigated was greatest on day 50 in AMN, compared with COT and ICOT. Amniotic fluid contains the nutrient reserve from which the conceptus draws to meet its energetic and growth requirements prior to transplacental exchange. The reported data further demonstrate the increased emphasis on transport of nutrients across the AMN to provide nutrients to the conceptus.

Table 4. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in intercaruncular ICAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

			Day of Gestation[2]					*P* - values[3]					
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. Preg	16 vs. 34 and 50	34 vs. 50	Day	Trt	Day × Trt
GLUT1	CON		2.44	0.63	1.77	1.61	0.39	0.43	< 0.01	0.10	< 0.01	0.26	0.42
GLUT1	RES		1.77	0.87	1.08	1.24							
		Day[7]	2.11[g]	0.75[h]	1.43[gh]								
CAT-1	CON		0.65	13.86	9.94	8.15	2.59	< 0.01	< 0.01	0.13	< 0.01	0.56	0.89
CAT-1	RES		0.51	15.37	12.33	9.41							
		Day	0.58[h]	14.62[g]	11.13[g]								
CAT-2	CON		6.83[ab]	2.31[c]	7.78[a]	5.64	1.43	0.04	0.88	0.56	0.22	0.68	0.01
CAT-2	RES		3.10[bc]	6.08[abc]	3.17[bc]	4.11							
		Day	4.97	4.19	5.48								
CAT-3	CON		9.69	1.55	5.53	5.59	1.89	0.09	0.12	0.27	0.13	0.45	0.09
CAT-3	RES		3.87	4.25	5.05	4.39							
		Day	6.78	2.90	5.29								

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.
[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.
[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.
[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet.
[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.
[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7
[7]Mean level of expression across treatment within day and gene of interest.
[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).
[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

Before the establishment of transplacental exchange, nutrient transporters are the only method of supplying nutrients to the conceptus. Therefore, evaluating the concentration of nutrients in the maternal and fetal fluids (serum, histotroph, and allantoic and amniotic fluids) is of interest to determine whether nutrient restriction during early gestation affects nutrient concentrations in maternal and fetal fluids or nutrient transport capacity, thereby altering the abundance of nutrients available for transport to the conceptus.

We interpret these data to imply that a 40 percent global maternal nutritional restriction may affect the mRNA expression of some (CAT-2) but not all utero-placental nutrient transporters investigated in this study. The effects of day of gestation on the mRNA expression of glucose and cationic amino acid transporters reflect the changing nutrient supply and demand curve necessary for proper conceptus growth. Moreover, additional knowledge in this area will facilitate increased efficiencies of beef cattle production and contribute to meeting the projected world food demands.

Acknowledgments

We thank the North Dakota State Board of Agricultural Research and Education and the U.S. Department of Agriculture's National Institute of Food and Agriculture and Agriculture and Food Research Initiative (2016-67016-24942) for research support. We also express appreciation to personnel at the NDSU Animal Nutrition and Physiology Center, NDSU Animal Science Nutrition Laboratory and Animal Science Reproductive Physiology Laboratory, and Animal Science graduate students Jordan Hieber and Evan Knutson for their assistance in completing this project.

Literature Cited

Bridges, G.A., M.L. Day, T.W. Geary and L.H. Cruppe. 2013. Deficiencies in the uterine environment and failure to support embryonic development. J. Anim. Sci. 91:3002-3013. doi:10.2527/jas.2013-5882

Crouse, M.S., K.J. McLean, L.P. Reynolds, C.R. Dahlen, B.W. Neville, P.P Borowicz and J.S. Caton. 2015. Nutrient transporters in bovine utero-placental tissues on days 16 to 50 of gestation. Proc. West. Sec. Amer. Soc. Anim. Sci. 66: 44-47.

Grazul-Bilska, A.T., P.P. Borowicz, M.L. Johnson, M.A. Minten, J.J. Bilski, R. Wroblewski, D.A. Redmer and L.P. Reynolds. 2010. Placental development during early pregnancy in sheep: vascular growth and expression of angiogenic factors in maternal placenta. Reproduction. 140:165-174. doi: 10.1530/REP-09-0548

Grazul-Bilska, A.T., M.L. Johnson, P.P. Borowicz, M. Minten, J.J. Bilski, R. Wroblewski, M. Velimirovich, L.R. Coupe, D.A. Redmer and L.P. Reynolds. 2011. Placental development during early pregnancy in sheep: cell proliferation, global methylation, and angiogenesis in the fetal placenta. Reproduction. 141:529-540. doi: 10.1530/REP-10-0505

Groebner, A.E., I. Rubio-Aliaga, K. Schulke, J.D. Reichenbach, H. Daniel, E. Wolfe, J.J.D. Meyer and S.E. Ulbrich 2011. Increase of essential amino acids in the bovine uterine lumen during preimplantation development. Reproduction. 141: 685-695. doi: 10.1530/REP-10-0533

McLean, K.J., C.R. Dahlen, P.P. Borowicz, L.R. Reynolds, B.W. Neville and J.S. Caton. 2016. A technique to ovario-hysterectomize cattle for use in gestational research. Proc. Midwest Sec. Amer. Soc. Anim. Sci. 94:143

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Tan, V.S.P., and S. Miyamoto. Nutrient-sensing mTORC1: Integration of metabolic and autophagic signals. 2016. J Cell Cardiol. In Press, Corrected Proof doi:10.1016/j.yjmcc.2016.01.005

Thatcher, W.W., C.R. Staples, G. Danet-Desnoyers, B. Oldick and E.P. Schmitt. 1994. Embryo health and mortality in sheep and cattle. J. Anim. Sci. 74 (Suppl 3):16-30.

Waterland, R.A., and R.L. Jirtle. 2004. Early nutrition, epigenetic changes at transposons and imprinted genes, enhance susceptibility to adult chronic diseases. Nutrition. 20: 63-68. doi:10.1016/j.nut.2003.09.011

Wu, G., F.W. Bazer, T.A. Cudd, C.J. Meininger and T.E. Spencer. 2004. Maternal nutrition and fetal development. J. of Nutrition. 9:2169-2172.

Zhang, S., T.R.H. Regnault, P.L. Barker, K.J. Botting, I.C. McMillen, C.M. McMillan, C.T. Roberts and J.L. Morrison. 2015. Placental adaptation in growth restriction. Nutrients. 7:360-389. doi: 10.3390/nu7010360

Bryan Neville

The Use of Testicular Fine-needle Aspiration, Histology and Immunohistochemistry for Determining Bull Fertility at an Early Age

Nicolas Negrin Pereira[1], Pawel Borowicz[1], Bryan Neville[2], Jordan Flaten[1] and Carl Dahlen[1]

[1]Department of Animal Sciences, NDSU

[2]Central Grasslands Research Extension Center, NDSU

The objective of the study was to assess the use of different techniques to allow for early, low-cost, reliable and low-invasive methodologies for the determination of Sertoli cell populations and germ cells in the bull. The techniques are: fine-needle aspiration with a fine needle (FNA-F), fine-needle aspiration with a gross needle (FNA-G), classic histology cuts and immunohistochemistry (androgen receptor expression by fluorescence). A significant correlation exists between histology and immunohistochemistry measurements, which positions the immunohistochemistry technique as a novel, very specific and reliable methodology for precocious determination of future fertility in young bulls.

Summary

Testicular parenchyma samples from 14 young peri-pubertal bulls were taken for the assessment of four different techniques: fine-needle aspiration using two different needle calibers or by open cut for histology (hematoxylin/eosin) or immunofluorescence (androgen receptor expression). For fine -needle aspiration, we used two different needle calibers attached to a syringe: 22G (FNA F) or 16G (FNA G). Once the needle was inserted in the testicular parenchyma, a vacuum was produced inside the syringe by pulling back the plunger. A smear was obtained by expelling the sample onto a glass slide. Then it was air-dried, fixed with 70 percent alcohol and stained with hematoxylin/eosin. Tissue samples were cut into 4- by 4-millimeter (mm) pieces using a microtome blade, fixed in a 10 percent formaldehyde solution, embedded in paraffin and cut into sections 5 micrometers (µm) thick using a microtome. Samples were stained with hematoxylin-eosin for histology or incubated sequentially with mouse monoclonal antibody to androgen receptor and goat-antirabbit (IgG CF633) fluorescent stain. To evaluate slides, images were taken at 200 times (FNA and histology) or 100 times magnification (immunohistochemistry) using an epifluorescence microscope equipped with a camera. Within each image, four to six seminiferous tubules within the five randomly chosen fields were selected for determination of Sertoli cell number and germ cell number. The ratio of germ to Sertoli cells was calculated and the CORR procedure of SAS was used to determine correlation of this ratio among each respective evaluation technique. Differences were considered significant at $P < 0.05$. A correlation coefficient of 0.58 ($P<0.001$) was observed among the technique of histology (5.27±0.41) and AR expression through fluorescence (4.44±0.60) for germ cells/Sertoli cell ratio. No significant correlations were found among FNA techniques and histology or immunohistochemistry cell ratios. Expression of AR by fluorescence in Sertoli cells represents a new, highly specific technique for precocious detection of more potential fertile bulls as young peri-pubertal calves.

Introduction

Fertility in the bull can be defined as the ability to produce viable calves. This characteristic of the bull is key in beef farm profitability. In the U.S., more than 95 percent of the herds rely on natural service. An early and accurate fertility prediction technique in the bull could have a great impact on profitability of beef operations (Wiltbank et al., 1986).

A bull breeding examination has proven to be a reliable and cost-effective technique to detect those animals that are satisfactory potential breeders and those not apt for their use (Barth et al., 2002). The inclusion of semen analysis has improved the effectiveness of this examination, but a significant range of different fertility levels still occurs within those bulls classified as apt for breeding (Chenoweth and McPherson, 2016).

Researchers have demonstrated for several years the importance that the final establishment of the Sertoli cell population has in determining future daily sperm production in most mammals (Berndston et al., 1987; P.J. O'Shaughnessy et al., 2011). The relationship between the size of the Sertoli cell population and number of germ cells has been used as a tool for determining fertility in different mammal species (Berndston et al., 1987). Determining the size of the Sertoli cell population in the bull at an early age can be a powerful tool in detecting those individuals with a potential higher sperm capacity.

The fine-needle aspiration technique has been used for several years in human and stallion testicles, providing a reliable, low-cost, diagnostic, low-invasive tool with minimal complications for cytology studies in the testis (Aridogan et al., 2003; Leme et al., 2012).

Expression of AR has been described in specific cells within the mammalian testis, such as in Leydig and peritubular myoid cells being expressed exclusively in Sertoli cells within the seminiferous tubules but not being expressed by germ cells (O'Hara et al., 2015).

Hardly any information is available about the application of different techniques for the determination of Sertoli and germ cell populations in the male calf and their potential use for detecting higher daily sperm producers later in adult life as bulls. The aim of this study was the comparison of four different techniques for determining Sertoli and germ cell population sizes in the peri-pubertal bulls: FNA F (22G needle), FNA G (16 G needle), classic histology cuts (hematoxylin/eosin stain) and immunofluorescence (AR expression).

Procedures

Fourteen Aberdeen Angus and Shorthorn bull calves (287±3.3 days of age, 683±29 pounds) from the North Dakota State University Beef Unit were used in the study. Previous to castration, bulls were restrained and given epidural anesthesia.

The scrotum skin was opened using a scalpel and the testicles exposed. Plexus pampiniform, testicular artery and vas deferens were crushed and severed using an emasculator to prevent bleeding.

Testicular tissue samples were obtained for evaluation via: 1) fine-needle aspiration with a fine needle (FNA F) using a sterile 22-gauge, 1¼-inch needle, 2) fine-needle aspiration with a gross needle (FNA G) using a sterile 16-gauge, 1¼-inch needle, 3) histology cut (stained with hematoxylin and eosin) and 3) immunofluorescence (androgen receptor expression).

For the FNA techniques, needles were connected to a 5- or 10-milliliter (ml) syringe and gently inserted perpendicularly into the testicular parenchyma. Once fully inserted in the parenchyma, the plunger was pulled back to produce a vacuum inside the syringe.

The needle was moved backward and forward within the testis two or three times for approximately four seconds. Once the needle was outside the testicle, a syringe filled with air was reattached to the needle and the plunger pressed to expel the sample onto a glass slide.

A smear was produced by sliding a second glass slide at an angle, extending the sample on the glass surface. The slide then was air-dried, fixed in 70 percent alcohol and stained with hematoxylin-eosin.

For histological examination, 4- by 4-mm testis parenchyma samples from the same region of each testis were taken and placed in a 10 percent formaldehyde fixative solution, embedded in paraffin and cut in 5-µm-thick sections using a microtome (Leica Biosystems Inc., Buffalo Grove, Ill.). Slides for histology were stained with hematoxylin-eosin.

Immunohistochemistry sections were submerged in sodium citrate buffer and placed in an antigen retriever (2100 Retriever, Aptum Biologics, UK) and incubated sequentially using mouse monoclonal antibody to androgen receptor (ab9474, abcam, Cambridge, Mass.) at 4 C overnight with agitation, and then stained with goat-antirabbit IgG CF633 fluorescent stain.

For each slide or tissue section, images were taken at 200 times magnification (FNA smears and histology slides) or 100 times magnification (immunohistochemistry slides) using a Zeiss Imager M2 epifluorescence microscope equipped with Zeiss piezo automated stage and AxioCam HRm camera (Carl Zeiss International, Jena, Germany). Image analysis (Image-Pro Plus, Media Cybernetics Inc., Bethesda, Md.) was performed for images of five randomly chosen fields. Within each image, four to six seminiferous tubules were selected randomly for Sertoli and germ cells individual cell counts using the Image-Pro Plus image analysis software (Media Cybernetics Inc., Rockville, Md.).

The germ cell to Sertoli cell ratio was calculated by dividing the total number of germ cells by the total number of Sertoli cells within each tubule. The mean of all ratios were determined for each testicle and analyzed using the correlation procedure of SAS (SAS Inst. Inc., Cary, N.C.). Significant differences were considered when $P < 0.05$.

Results and Discussion

The different ratios between total germ cells and Sertoli cells were determined for each technique and presented in Table 1.

A correlation of 0.58 was observed ($P = 0.001$) between germ-to-Sertoli cell ratios obtained via histology and ratios obtained via immunohistochemistry (Table 2). In addition, a correlation of 0.69 was observed in germ-to-Sertoli ratio obtained via FNA F and FNA G techniques ($P = 0.001$). No correlations were found, however, in germ-to-Sertoli cell ratios between samples obtained via FNA F and FNA G techniques (3.60±0.47 and 3.59±0.39, respectively) and histology (5.27±0.41) or AR expression by fluorescence (4.44±0.59)

Table 1. Testicular cytology determination in young peri-pubertal bulls using four different techniques.

Trait	Technique			
	FNA F[1] (Mean±SEM)[3]	FNA G[2] (Mean±SEM)	Histology (Mean±SEM)	Immuno-histochemistry (Mean±SEM)
Ratio (germ cells/Sertoli cell)	3.605[a] ±0.468 n=12	3.598[a]±0.393 n=13	5.271[b]±0.409 n=14	4.440[b]±0.599 n=14

[1]FNA F = fine-needle aspiration with a fine needle
[2]FNA G = fine-needle aspiration with a gross needle
[3]SEM = standard error of the mean

The difference found between the ratios obtained by FNA and histology contrasts with the findings of other authors (Aridogan et al., 2003), who obtained a 0.9 correlation in human patients between fine-needle aspiration and histology techniques. To our knowledge, this is the first time that a specific immunohistochemistry fluorescent method against AR has been used in peri-pubertal bulls as a specific predictive tool of potential fertility in the adult individual. AR expression through immunofluorescence has shown to be a specific and novel useful tool for determining Sertoli cell populations in the young bull.

Table 2. Correlation among four different techniques for testicular cytology determination in young peri-pubertal bulls.

Technique		FNA-F[1]	FNA-G[2]	Histology	Immunohistochemistry
FNA-F[1] (n = 22)	Pearson correlation	1.000	0.686	-0.008	-0.217
	P - value	-	0.001	0.971	0.331
FNA-G[2] (n = 22)	Pearson correlation	0.686	1.000	0.175	-0.202
	P - value	0.001	-	0.434	0.366
Histology (n = 27)	Pearson correlation	-0.008	0.175	1.000	0.581
	P - value	0.971	0.434	-	0.001
Immunohistochemistry (n = 28)	Pearson correlation	-0.217	-0.202	0.581	1.000
	P - value	0.331	0.366	0.001	-

[1]FNA-F = fine-needle aspiration with a fine needle
[2]FNA-G = fine-needle aspiration with a gross needle

Literature Cited

Aridogan I.A., Bayazit Y., Yaman M., Ersoz C. and Doran S. 2003. Comparison of fine-needle aspiration and open biopsy of testis in sperm retrieval and histopathologic diagnosis. Andrologia 35, 121-125.

Barth A.D. and Waldner C.L. 2002. Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. Canadian Veterinary Journal 43: 274-284.

Berndston W.E., Igboeli G. and Parker W.G. 1987. The numbers of Sertoli cells in mature Holstein bulls and their relationship to quantitative aspects of spermatogenesis. Biology of Reproduction 3 7, 60-67.

Chenoweth P.J. and McPherson F.J. 2016. Bull breeding soundness, semen evaluation and cattle productivity. Animal Reproduction Science 169: 32-36.

Leme P.D. and Papa F.O. 2012. How to perform and interpret testicular fine needle aspiration in stallions. Journal of Equine Veterinary Science. Vol 30, N 10.

O'Hara L. and Smith L.B. 2015. Androgen receptor roles in spermatogenesis and infertility. Best Practice & Research Clinical Endocrinology & Metabolism 29 (595-605).

O'Shaughnessy P. J. and Fowler P.A. 2011. Endocrinology of the mammalian fetal testis. Reproduction 141, 37-46.

Wiltbank J.N. and Parish N.R. 1986. Pregnancy rate in cows and heifers bred to bulls selected for semen quality Pregnancy rates in cows and heifers bred to bulls selected for semen quality. Theriogenology. Volume 25, Issue 6, June 1986, 779-783.

The Effect of Vitamin A Treatment on Testicular Development in Young Peri-pubertal Bulls

Nicolas Negrin Pereira[1], Pawel Borowicz[1], Jordan Flaten[1], Bryan Neville[2] and Carl Dahlen[1]

[1]Department of Animal Sciences, NDSU
[2]Central Grasslands Research Extension Center, NDSU

The establishment of the final number of Sertoli cells in the bull calf testicle around puberty is one of the key determining factors of the animal's adult fertility. Vitamin A administration did not have any significant effect in testicular development in peri-pubertal bull calves.

Summary

Fourteen Angus and Shorthorn bull calves between 11 and 12 months of age were assigned randomly to one of two treatments: 1) Intramuscular injection of 1 million International Units (IU) of vitamin A (Vit A) or 2) no treatment (Control). Scrotal circumference (SC) was measured in all bull calves at the time of the treatment application and 11 days later at castration. Testes and epididymis were dissected and weighed, and samples of parenchyma were collected from each testicle. Samples were fixed in formaldehyde, embedded in paraffin and cut. Slides were deparaffinized with successive washes of xylene and alcohol. After antigen retrieval and blocking with 10 percent normal goat serum, sections were incubated sequentially with mouse monoclonal antibody to androgen receptor and goat-antirabbit (IgG CF633) fluorescent stain. Samples were examined under a fluorescent microscope and the images captured with a digital camera and processed using Image-Pro Plus software. Effects of treatment on SC, testicular weight, testicular parenchyma weight and epididymis weight, number of Sertoli cells, germ cell, and ratio of germ cells to Sertoli cells within the seminiferous tubule (ST) were analyzed using the ANOVA procedure of SAS. No differences were observed among treatments for SC (1.63±0.26 vs. 2.17±0.17), testicular weight (228.169±17.002 vs. 221.792±24.983), testicular parenchyma weight (grams [g]) (141.02±11.82 vs. 131.46±14.49), epididymis weight (g) (12.01±1.10 vs. 9.43±1.01), number of Sertoli cells/ST (45.56±3.14 vs. 48.04±2.18), number of germ cells/ST (217.29±26.83 vs. 156.04±16.75) and the ratio of germ cells/ Sertoli cell (5.03±0.93 vs. 3.30±0.43) for vitamin A and control, respectively. These results suggest that vitamin A has no positive effect on fertility when administered in peri-pubertal bulls.

Introduction

More than 95 percent of our beef herds rely on natural service as the main breeding system. Very little information is available about interventions administered during the peri-pubertal period in the bull and their ramifications on adult fertility.

Sperm production in the bull is influenced early in life through the establishment of the definite number of Sertoli cells in the testicle, which nourish developing sperm before puberty, when they stop replicating (Sharpe et al., 2003). Bulls with a larger Sertoli cell population have greater daily sperm production (DSP), testicular weight and SC, compared with bulls having fewer Sertoli cells (Berndston et al., 1987).

Recent findings showed that the proportion of Sertoli cell numbers was higher in bulls that produced good-quality semen with higher numbers of viable spermatozoa after thawing in comparison with bulls that had lower proportions of Sertoli cells (Rajak et al., 2016). Sertoli cell proliferation has a specific window of time that extends from the fetal stage at midgestation up to six to 10 weeks in the newborn calf (Moura et al, 1997).

Recent findings suggest that influencing the final number of Sertoli cells in the bull testis is possible once differentiated (Zakhidov et al., 2015). Several factors have been described as having an effect on Sertoli cell replication, such as thyroid hormone (T3), testosterone (T), follicle-stimulating hormone (FSH), insulin growth factor (IGF-I) and vitamin A through its active compounds retinoic acid (RA) and retinol (RE) (Lucas et al., 2014).

Retinoic acid also has been reported to interact with transforming growth factor beta (TGF-β) in various other tissues, not limited to the testis, affecting cell proliferation and differentiation (Cupp et al., 1999).

Retinoic acid receptors RARα and RARβ have been discovered in rat Sertoli cell nucleus (Livera et al., 2002), and the fundamental role of RA in spermatogenesis has been known for several years, where animals deficient in vitamin A are sterile with a complete halt in spermatogenesis. This was demonstrated in genetically engineered mice lacking RA receptors, showing a delay in Sertoli cell differentiation, progressive spermatogenic degeneration and infertility (Nicholls et al., 2013). Retinoic acid in the rat had a

detrimental effect in the fetal testis and a beneficial effect on the neonatal rat testis (Livera et al., 2000).

Most of the studies performed on Sertoli cell proliferation and differentiation have been done on species such as rats, sheep, humans and pigs but very little information is available on the effects exerted by vitamin A on Sertoli cell populations in the bull.

Procedures

Fourteen Aberdeen Angus and Shorthorn bull calves (287±3.3 days, 683±29 pounds) from the North Dakota State University Beef Unit were used in the study. Ten days prior to castration, bulls were assigned randomly to one of two treatments; 1) intramuscular injection of 1 million IU of vitamin A (Vitamin AD Injectable, Durvet Laboratories, Blue Springs, Mo.; Vit A); or 2) no treatment (Control).

Testicle scrotal circumference (SC) was measured in each bull at the time of treatment administration and also at the time of castration 11 days later. All bulls were castrated using the open method with a scalpel following application of epidural and local anesthesia. Once the testicles were removed, the epididymis was dissected and detached from the testicles. Testicles and epididymis were weighed separately.

The tunica albuginea was dissected from each testicle and the testicular parenchyma was weighed. Samples for histological examination (4 by 4 millimeters [mm]) from one region of each testis parenchyma were taken, fixed in a 10 percent formaldehyde solution, embedded in paraffin and cut into sections 5 micrometers (µm) thick using a microtome (Leica Biosystems Inc., Buffalo Grove, Ill.).

Samples were submerged in sodium citrate buffer and placed in an antigen retriever (2100 Retriever, Aptum Biologics, UK) and incubated sequentially using mouse monoclonal antibody to androgen receptor (ab9474, abcam, Cambridge, Mass.) at 4 C overnight with agitation, and then stained with goat-antirabbit IgG CF633 fluorescent stain.

For each tissue section, images were taken at 100 times magnification using a Zeiss Imager M2 epifluorescence microscope equipped with Zeiss piezo automated stage and AxioCam HRm camera (Carl Zeiss International, Jena, Germany). Image analysis (Image-Pro Plus, Media Cybernetics Inc., Bethesda, Md.) was performed for images of five randomly chosen fields. Within each image, four to six seminiferous tubules were selected randomly for Sertoli and germ cell individual cell counts using the Image-Pro Plus image analysis software (Media Cybernetics Inc., Rockville, Md.).

The ANOVA procedure of SAS (SAS Inst. Inc., Cary, N.C.) was used to analyze differences in scrotal circumference, testicular weight, testicular parenchyma weight and epididymis weight between treatments. The number of Sertoli cells and germ cells per seminiferous tubule (ST) were counted and means calculated for each testicle.

The ratio of germ cells to Sertoli cells was calculated by dividing the total number of germ cells by the total number of Sertoli cells within each tubule. Total means were obtained for each testicle and analyzed using the same statistical procedure. Significant differences were considered when $P < 0.05$.

Table 1. Effect of vitamin A on testicular development in young beef bulls.

	Vitamin A		Control		
Item	**Mean**	**SEM**	**Mean**	**SEM**	***P* Value**
No. bulls	8		6		
Difference in SC (cm)	1.63	0.26	2.17	0.17	0.13
Testicular weight (g)	228.17	17.00	221.79	24.98	0.83
Testicular parenchyma weight (g)	141.02	11.82	131.46	14.49	0.61
Epididymis weight (g)	12.01	1.10	9.43	1.01	0.11
Sertoli cells/ST	45.56	3.14	48.04	2.18	0.56
Germ cells/ST	217.29	26.83	156.04	16.75	0.10
Ratio (germ cells/Sertoli cell)	5.03	0.93	3.30	0.43	0.1

SEM = standard error of the mean; SC = scrotal circumference; ST = seminiferous tubules.

Results and Discussion

No differences were observed between treatments (Table 1) for measures of scrotal circumference (P=0.13), and weights of the testicles (P=0.83), parenchyma (*P*=0.61) or epididymis (P=0.11; Table 1). In addition, no differences were observed between treatments in the number of Sertoli cells per ST (*P* = 0.44), number of germ cells per ST (*P* = 0.20) or the ratio of germ cells/Sertoli cell (*P* = 0.16).

This preliminary experiment indicated that the administration of vitamin A in young peri-pubertal bulls did not have a beneficial effect in testicular development. The lack of effects of vitamin A on the different testicular cell types could be the reflection of an already differentiated and stable Sertoli cell population because previous studies indicate that the end of Sertoli cell division occurs before 30 to 40 weeks of age in the bull (Rawlings et al., 2008).

The lack of information about precise time frames for obtaining beneficial effects on testicular development in the bull by the application of different treatment and management strategies highlights the need for further research.

Literature Cited

Berndston W.E., Gaius Igboeli and Parker W.G. 1987. The numbers of Sertoli cells in mature Holstein bulls and their relationship to quantitative aspects of spermatogenesis. Biology of Reproduction 3 7, 60-67.

Cupp A.S., Dufour J.M., Kim G., Skinner M.K. and Kwan Hee Kim. 1999. Action of retinoids on embryonic and early postnatal testis development. Endocrinology. Vol. 140.

Livera G., Rouiller-Fabre V., Durand P. and Habert R. 2000 Multiple effects of Retinoids on the development of Sertoli, Germ and Leydig cells of fetal and neonatal rat testis in culture. Biology of Reproduction 62, 1303-1314.

Livera G, Rouiller-Fabre V, Pairault C, Levacher C and Habert R._ 2002. Regulation and perturbation of testicular functions by vitamin A. Reproduction 124, 173-180.

Lucas T.F.G., Nascimento A.R., Pisolato R., Pimenta M.T., Lazari M.F.M. and Porto C.S. 2014. Receptors and signaling pathways involved in proliferation and differentiation of Sertoli cells. Spermatogenesis 4 e28138. Landes Bioscience.

Moura A.A. and Erickson B.H. 1997. Age-related changes in peripheral hormone concentrations and their relationships with testis size and number of Sertoli and germ cells in yearling beef bulls. Journal of Reproduction and Fertility 111, 183-190.

Nicholls P.K., Harrison C.A., Rainczuk K.E., Vogl A.W. and Stanton P.G. 2013. Retinoic acid promotes Sertoli cell differentiation and antagonizes activing-induced proliferation. Molecular and Cellular Endocrinology 377:33-43.

Rajak S.K., Thippeswamy V.B., Kumaresan A., Shankar Layek S., Mohanty T.K., Gaurav M.K., Chakravarty A.K., Datta T.K., Manimaran A. and Prasad S. 2016. Testicular citology indicates differences in Sertoli cell counts between “good freezer” and “poor freezer” bulls. Indian Journal of Experimental Biology. Vol. 54, 17-25.

Rawlings N., Evans ACO, Chandolia R.K. and Bagu E.T. 2008. Sexual maturation in the bull. Reproductive Domestic Animals 43, (295-301).

Sharpe R.M., McKinnell C., Kivlin C. and Fisher J.S. 2003. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction 125, 769-784

Zakhidov S.T. and Marshak T.L. 2015 Experimental evidence of profileration and reproduction of highly differentiated Sertoli cells. Biology Bulletin Vol.42 N°4 (287-295).

Central Grasslands Research Extension Center on July 6, 2016

CGREC Staff

Bryan Neville	Director*/Animal Scientist
Michael Undi	Animal Scientist
Fara Brummer	Area Extension Specialist / Livestock Systems
Dwight Schmidt	Farm Manager
Sandi Dewald	Administrative Secretary
Rodney Schmidt	Herdsman
Rick Bohn	Range Technician
Stephanie Becker	Animal Science Technician
Jessalyn Bachler	Livestock Technician
Karen Schmidt	Office Maintenance
Janet Patton	Research Assistant

*Kevin Sedivec began serving as interim director in Oct. 2016.

Permanent Staff - 2016

Summer Staff - 2016

Summer Staff

Cheyenne Dockter
Courtney Frieson
Megan Gross
Jessi Lagein
Christy Sperling

Graduate Students

Megan Endreson, Range Science, NDSU

Advisory Board

Gary Aichele, Steele
Jon DeKrey, Tappen
Jay Doan, Sterling
Tim Faller, Mandan
Charlotte Heim, Bismarck
Richie Heinrich, Medina
Mark Holkup, Bismarck
Deb Lachenmeier, Streeter
Martin Marchello, Bismarck
Tom Moss, Steele
Darrell Oswald, Wing
Krista Reiser, Washburn
Arlyn Scherbenske, Steele
Charlie Stoltenow, Fargo
Robert Weigel, Kintyre

Advisory Board meetings are held following the Field Day in July and on the second Thursday of November.

NDSU Central Grasslands Research Extension Center

4824 48th Ave. S.E.
Streeter, ND 58483

701-424-3606
www.ag.ndsu.edu/CentralGrasslandsREC

Photos by Rick Bohn, Kevin Sedivec, Michael Undi and Megan Endreson