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Beef Cattle Sciences

Beef Research Report

2014 Edition

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Beef Cattle Sciences

Beef Research Report

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Beef Cattle Sciences

Beef Research Report

Impact of Previous Exposure to Wolves on Temperament and Physiological Responses of Beef Cattle Following a Simulated Wolf Encounter [1]

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Synopsis

The simulated wolf encounter increased excitability and fear-related stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator.

Summary

One hundred multiparous, pregnant, non-lactating crossbreed beef cows from the Eastern OR Agricultural Research Center - Burns (**CON**; n = 50) and from a commercial operation (**WLF**; Council, ID, n = 50) were assigned to the experiment. However, CON cows were unfamiliar with wolves, whereas WLF cows belonged to a herd that experienced multiple confirmed wolf predation episodes. On d 0, CON and WLF cows were ranked by temperament, BW, and BCS, and allocated to 5 groups (10 CON and 10 WLF cows/group). Groups were individually subjected to the experimental procedures on d 2 (n = 3) and d 3 (n = 2). Within each group, cows were evaluated for temperament, a blood sample was collected, and a data logger was inserted intravaginally to record body temperature at 30 s intervals (pre-exposure assessment). After these assessments, cows were sorted by previous wolf exposure, moved to 2 adjacent drylot pens (10 WLF and 10 CON cows/pen) and subjected to the simulated wolf encounter for 20-min, which consisted of: 1) cotton plugs saturated with wolf urine attached to the drylot fence, 2) continuous reproduction of wolf howls, and 3) 3 trained dogs walked using a leash outside the drylot perimeter fence. Thereafter, WLF and CON cows were commingled and returned to the handling facility for removal of data loggers, temperament evaluation, and blood collection (post-exposure assessment). However, cotton plugs saturated with wolf urine were attached to the handling facility, wolf howls were reproduced during processing, and cows were also exposed for 20 s to the 3 dogs while restrained in the squeeze chute, but immediately before post-exposure assessments. Chute score, temperament score, and plasma cortisol concentration increased ($P \leq 0.01$) from pre- to post-exposure assessment in WLF, but did not change in CON cows ($P \geq 0.19$). Exit velocity decreased ($P = 0.01$) from pre- to post-exposure assessment in CON, but did not change ($P = 0.97$) in WLF cows. In addition, WLF cows had a greater ($P = 0.03$) increase in temperature from pre- to post-exposure assessments compared with CON cows. In conclusion, the simulated wolf encounter increased excitability and fear-related physiological stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator.

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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Introduction

The reintroduction of grey wolves into the Yellowstone National Park allowed wolf packs to disperse into regions outside the Greater Yellowstone Ecosystem, including agricultural lands in Idaho and Oregon (Larsen and Ripple, 2006). As a result, wolves started to inhabit and hunt in livestock grazing areas, which increased the incidence of cattle predation by wolves in both states (Idaho Department of Fish and Game and Nez Perce Tribe, 2013; Oregon Department of Fish and Wildlife, 2013a). The economic and productive implications of predators on livestock systems is often evaluated based on the number of animals injured or killed (Treves et al., 2002; Oakleaf et al., 2003; Breck and Meier, 2004); however, these parameters may not be the only negative impacts that wolf predation causes to beef cattle systems (Kluever et al., 2008; Laporte et al., 2010).

The mere presence of predators alters stress physiology and behavior parameters of the prey (Creel and Christianson, 2008), particularly if the preyed animal was already subjected to similar predation episodes (Boonstra, 2013). More specifically, fear of predation may alter cattle temperament and stimulate adrenal corticoid synthesis (Laporte et al., 2010; Boonstra, 2013), which have been shown to negatively impact health, productive, and reproductive parameters in beef cattle (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012). Based on this rationale, we hypothesized that wolf presence near cattle herds stimulates behavioral and physiological stress responses detrimental to cattle productivity and welfare, particularly in cattle from herds previously predated by wolves. Hence, the objective of this experiment was to evaluate temperament, body temperature, and plasma concentration of cortisol in beef cows previously exposed or not to wolves, and subjected to auditory, olfactory, and visual stimuli designed to simulate an encounter with wolves.

Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (EOARC; Burns, OR). Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Animals and diets

This experiment was conducted using 100 multiparous, pregnant, non-lactating crossbred beef cows from the EOARC Burns (**CON**; n = 50) and from a commercial cow-calf operation (**WLF**; located in Council, ID, n = 50). Both locations occasionally used domestic herding dogs to move cattle across pastures or to the handling facility. The CON cows (age = 5.0 ± 0.1 yr, BW = 1150 ± 13 lbs, BCS = 4.80 ± 0.04, and approximately 6 mo in gestation at the beginning of the experiment) were randomly selected from the EOARC Burns mature cowherd, which is reared and maintained in areas (Burns and Riley, OR) without known wolf establishment or predation episodes (Oregon Department of Fish and Wildlife, 2013a). Hence, CON cows were unfamiliar with wolf presence and predation. The WLF cows (age = 4 yr; BW = 1128 ± 15 lbs; BCS = 4.90 ± 0.06, and approximately 6 mo in gestation at the beginning of the experiment) were randomly selected from the commercial operation, which is located in an area (Council, ID) with established wolf packs (McCall-Weiser Wolf Management Zone; Idaho Department of Fish and Game and Nez Perce Tribe, 2013). Further, WLF cows belonged to a herd that experienced multiple confirmed wolf predation episodes from 2008 to 2012 (USDA-APHIS, Idaho Wildlife Services, Boise, ID; confirmation documents available upon request to corresponding author), although none of the WLF cows were directly predated or injured by wolves. Therefore, WLF cows were considered familiar with wolf presence and predation episodes.

The WLF cows were transported to the EOARC Burns 50 d prior to the beginning of the experiment (d 0). During this period (d -50 to d 0), CON and WLF cows were commingled and maintained in a single meadow foxtail dominated pasture harvested for hay the previous summer, and had ad libitum access to meadow-grass hay, water, and a vitamin-mineral mix (Cattleman's Choice, Performix Nutrition Systems, Nampa, ID). Cows were also individually processed through the EOARC handling facility, but not restrained in the squeeze chute, once a week from d 50 to -2 to acclimate WLF cows to the EOARC personnel and facilities (Cooke et al., 2012).

On d 0, CON and WLF cows were ranked by temperament score (by the same single technician), BW, and BCS, and allocated to 5 groups of 20 cows each (10 CON and 10 WLF cows per group). Each group of 20 cows was maintained on individual meadow foxtail pastures harvested for hay the

previous summer during the experimental period (d 0 to 3), and had ad libitum access to water and the previously described meadow-grass hay and vitamin-mineral mix.

Simulated Wolf Encounter

Due to daylight limitations, 3 groups were randomly selected and received the experimental procedures on d 2, whereas the remaining 2 groups received the experimental procedures on d 3. While an individual group was being subjected to the simulated wolf encounter at the EOARC handling facilities, the other groups remained on their respective pastures. Groups were maintained on pastures that were ≥ 0.3 miles distant from the handling facilities to prevent that cows perceived the simulated wolf encounter model while on pasture.

Pre-exposure assessments. The evaluated group was gathered in its respective pasture and walked to the handling facility, where cows were evaluated for temperament (chute score, exit velocity, and temperament score, by the same single technician; Cooke et al., 2012). Immediately after chute score evaluation, a blood sample was collected and a HOBO Water Temp Pro V2 data logger (Onset Company, Bourne, MA) was inserted intravaginally in each cow to record temperature at 30 s intervals. Each data logger was attached to a controlled internal drug-releasing device (CIDR, Pfizer Animal Health, New York, NY) that did not contain hormones.

Simulated Wolf Encounter. Immediately after the pre-exposure assessments, cows within the evaluated group were sorted by previous wolf exposure and moved to 2 adjacent drylot pens separated by a fence line (10 WLF and 10 CON cows in each pen). Pens were 55 x 55 feet, located 0.05 miles from the handling facility, and had no feed or water source. After being housed in their respective pens, CON and WLF cows were immediately subjected to the simulated wolf encounter for 20-min. More specifically, wolf urine (Harmon Wolf Urine Scent; Cass Creek, Grawn, MI) was applied to 12 cotton plugs (Feminine care tampons; Rite Aid, Camp Hill, PA), and plugs were attached to the drylot fence line every 35-feet (6 plugs /pen) prior to any experimental procedures on d 2 and 3. After cows were settled within each dry lot pen, wolf howls previously recorded from the wolf packs residing in Wallowa County, OR, were continuously reproduced using a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA) located 30 feet from the dry lot pens, whereas cows had no visual contact with the stereo system. Additionally, 3 trained dogs were conducted using a leash by 2 trained technicians outside the drylot perimeter fence. The dogs utilized were 2 adult German Shepherd females (BW = 76 ± 3.3 lbs) to represent adult wolves, and 1 adult Border Collie × Alaskan Malamute female (BW = 49 lbs) to represent a young wolf. The maximum and minimum distances allowed between dogs and cows were 80 and 15 feet, respectively.

Post-exposure assessments. After 20 min of the simulated wolf encounter, WLF and CON cows were commingled and returned to the handling facility for removal of HOBO data loggers, temperament evaluation, and blood collection as in the pre-exposure assessments. However, cows were also subjected to the simulated wolf encounter during processing for post-exposure assessments. While cows were at the dry lot pens, 3 cotton plugs saturated with wolf urine were attached to the walls of the lead chute at 10-feet intervals immediately prior to the squeeze chute, and 1 similar cotton plug was hung inside the squeeze chute (Silencer Chute; Moly Manufacturing, Lorraine, KS). Wolf howls were reproduced throughout the entire processing. Cows were also exposed for 20 s to the same 3 dogs previously used while restrained in the squeeze chute, but before blood collection, HOBO data loggers removal, or temperament evaluation. Dogs were handled via leash by 2 trained technicians in front of the squeeze chute, and remained 15 feet from the restrained cow.

Immediately after the post-exposure assessments, the group was returned to its original pasture, cotton plugs were removed from the handling facility, and the subsequent group was only evaluated after a 30-min interval to prevent residual wolf scent inside the handling facility during the pre-exposure assessment. Further, the wolf howls were not reproduced and dogs were restrained in an enclosed barn during this 30-min interval to prevent unwarranted visual and auditory stimuli prior to the simulated wolf encounter.

Sample analysis

Individual cow temperament was assessed by chute score and exit velocity as previously described by Cooke et al. (2012). Chute score was assessed by a single technician based on a 5-point scale where: 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and continuous struggling.

Exit velocity was assessed immediately by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 6 feet distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, cows were divided in quintiles according to their exit velocity, within CON and WLF cows on d 0 and within group for pre- and post-exposure assessments, and assigned a score from 1 to 5 (exit score; 1 = cows within the slowest quintile; 5 = cows within the fastest quintile). Individual temperament scores were calculated by averaging cow chute score and exit score.

Temperature data from HOBO loggers were processed using the HOBOware Pro software (version 3.3.2; Onset Company). Only data obtained after the end of the pre-exposure assessments (when cows were gathered and moved to the dry lot pens) to the end of the simulated wolf encounter (when cows were commingled to return to the handling facility) were recorded and compiled into 5-min intervals. Hence, cows had 25 min of recorded body temperature; the initial 5 minutes collected prior to the simulated wolf encounter (pre-exposure assessment) and the remaining 20 min collected during the simulated wolf encounter (post-exposure assessments). Blood samples were collected via jugular venipuncture into a commercial blood collection tube (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with sodium heparin. After collection, blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma harvest, and stored at -80°C on the same day of collection. A bovine-specific commercial ELISA kit was used to determine plasma concentration of cortisol (Endocrine Technologies Inc., Newark, CA).

Statistical analysis

Pen within the evaluated group was considered the experimental unit. All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc.; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

Results

The main hypothesis of this experiment was that the mere presence of wolf packs near cattle herds affects temperament and stimulates physiological stress responses known to impair cattle productivity and welfare (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012), particularly in herds previously subjected to wolf predation (Creel and Christianson, 2008; Boonstra, 2013). To address this hypothesis, mature beef cows were subjected to an experimental model designed to simulate a wolf encounter, which was based on wolf scent, pre-recorded wolf howls, and 3 canines physically similar to wolves. Accordingly, wolf scent and recorded howls have been successfully used to mimic wolf presence (Moen et al., 1978; Kluever et al., 2009), given that such stimuli can elicit similar behavioral or physiological responses by prey animals compared with the actual presence of the predator (Kats and Dill, 1998; Apfelbach et al., 2005). Likewise, Kluever et al. (2009) suggested that cattle may acquire a generalized fear response to domestic dogs, perhaps due to the physical and stalking predation characteristics shared among all canids (Nowak, 1999).

It is also important to note that WLF and CON cows originated from different herds, and were reared in different management schemes and environments. Hence, the impact of previous wolf exposure on the temperament and stress-related parameters evaluated herein cannot be completely distinguished from cow source. To address this concern, WLF and CON cows were commingled to receive the same management for 50 d prior to the beginning of the experiment, and were processed weekly to familiarize all cows to personnel and handling facilities. But more importantly, the temperament and physiological parameters evaluated herein are not being directly compared between CON and WLF cows. Instead, these parameters are being evaluated within each cow based on the changes between pre- and post-exposure values. Both herds were also occasionally exposed to herding dogs and reared in areas with large populations of other canids such as coyotes and foxes (Idaho Fish and Game, 2013; Oregon Department of Fish and Wildlife, 2013b). Therefore, differences in temperament and physiological responses between WLF and CON cows following the simulated wolf encounter should be mainly attributed to previous exposure to wolves, and not to interactions with canids in general.

Upon the beginning of the simulated wolf encounter, all WLF groups immediately bunched-up in the farthest corner of the drylot pen, and maintained this disposition during the entire process (Figure 1). Conversely, CON cows remained dispersed within the drylot pen (Figure 1). This behavioral difference suggests that cattle previously

predated by wolves immediately adopt a fear-related protective behavior after perceiving signs of wolf presence, whereas the same outcome may not be observed in cattle unfamiliar with wolves.

Figure 1. Behavioral responses of beef cows during the simulated wolf encounter.

Chute score increased ($P = 0.01$) from pre- to post-exposure assessment in WLF cows but did not change in CON cows ($P = 0.72$), indicating that the simulated wolf encounter increased fear-induced agitation during chute restraining only in WLF cows (Burrow, 1997). Accordingly, WLF cows had a greater ($P < 0.01$) positive change in chute score from pre- to post-exposure assessment compared with CON cows (Table 1). Exit velocity decreased ($P = 0.01$) from pre- to post-exposure assessment in CON cows, which may be associated with fatigue caused by the experimental procedures, but did not change ($P = 0.97$) in WLF cows. Hence, CON had a greater ($P = 0.05$) negative change in exit velocity from pre- to post-exposure assessment (Table 1), suggesting that fear-related responses to the simulated wolf encounter prevented the fatigue-induced decrease in exit velocity of WLF cows. Given that temperament score is based on chute score and exit velocity, this parameter also increased ($P = 0.01$) from pre- to post-exposure assessment in WLF cows but did not change in CON cows ($P = 0.75$), evidencing that the simulated wolf encounter increased excitability only in WLF cows. Thus, WLF cows had a greater ($P = 0.01$) positive change in temperament score from pre- to post-exposure assessment compared with CON cows (Table 1).

Table 1. Temperament measurements and plasma cortisol concentrations of cows previously exposed (WLF) or not (CON) to wolves, and subjected to a simulated wolf encounter.

Item	WLF	CON	*P*-value
Chute Score (1 to 5)			
Pre-exposure	2.27	1.85	0.01
Post-exposure	3.07	1.81	< 0.01
P-Value [1]	< 0.01	0.72	
Change [2]	0.78	-0.06	< 0.01
Exit Velocity (feet/s)			
Pre-exposure	8.10	5.50	< 0.01
Post-exposure	8.09	4.62	< 0.01
P-Value [1]	0.97	0.01	
Change [2]	-0.01	-0.88	0.05
Temperament Score (1 to 5)			
Pre-exposure	2.97	2.08	< 0.01
Post-exposure	3.37	2.05	< 0.01
P-Value [1]	< 0.01	0.75	
Change [2]	0.40	-0.04	0.01
Plasma cortisol (ng/mL)			
Pre-exposure	17.9	13.1	0.04
Post-exposure	23.7	14.6	< 0.01
P-Value [1]	< 0.01	0.19	
Change [2]	5.8	1.5	<0.01

[1] Time comparison within WLF and CON cows.
[2] Calculated by subtracting pre-exposure values from post-exposure values.

Plasma cortisol concentrations increased ($P < 0.01$) from pre- to post-exposure assessment in WLF cows but did not change ($P = 0. 19$) for CON cows,

indicating that the simulated wolf encounter induced a glucocorticoid stress response only in WLF cows (Sapolsky et al., 2000). Accordingly, WLF cows had a greater ($P < 0.01$) positive change in plasma cortisol from pre- to post-exposure assessments compared with CON cows (Table 1). Body temperature increased ($P < 0.01$) for WLF and CON cows during the simulated wolf encounter (Figure 2). This outcome can be attributed to the handling and physical activity that cows endured during the experimental procedures (Mader et al., 2005), in addition to fear-related stress caused by the simulated wolf encounter because increased body temperature is a major component within the neuroendocrine stress response (Charmandari et al., 2005). However, WLF cows had a greater ($P = 0.03$) positive change in body temperature from pre- to post-exposure assessments compared with CON cohorts (0.74 vs. 0.33°F, respectively; SEM = 0.10). Given that WLF and CON cows were handled similarly and walked the same distances during the experimental procedures, this difference detected in body temperature change can be attributed to a greater fear-related stress that WLF cows endured during the simulated wolf encounter.

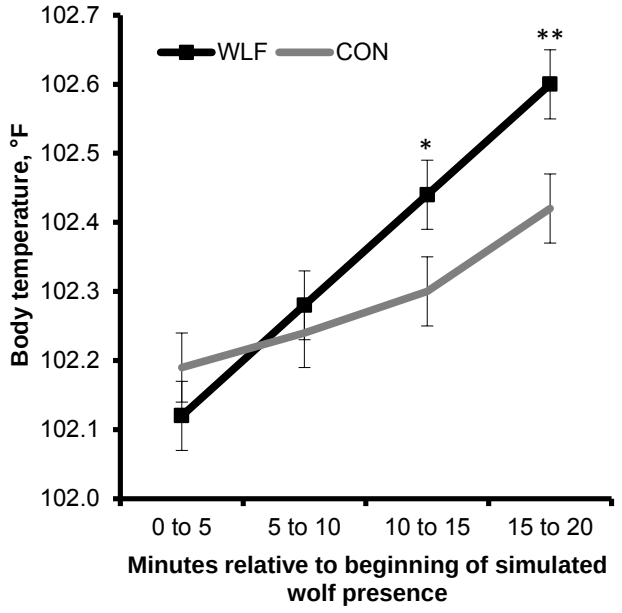

Figure 2. Body temperature of cows previously exposed (WLF) or not (CON) to wolves, and subjected to a simulated wolf encounter. A previous wolf exposure × time interaction was detected ($P < 0.01$). Treatment comparison within time: ** $P = 0.01$, * $P = 0.05$.

Supporting our hypothesis, WLF cows became more excitable and had an increase in plasma cortisol and body temperature following the simulated wolf encounter, suggesting that cows familiar with wolf presence and predation may endure fear-related behavioral and physiological stress responses (Charmandari et al., 2005) when in close proximity with wolves. Conversely, temperament and plasma cortisol concentrations in CON cows were not impacted by the simulated wolf encounter, and the marginal increase in body temperature can be attributed to the handling and physical activity associated with the experimental procedures (Mader et al., 2005). Therefore, wolf presence may not be perceived as a stressor in cows still unfamiliar with predation and interaction with this predator. To our knowledge, no other research has evaluated temperament and physiological stress parameters in beef cows previously exposed or not to wolves, and subjected to a simulated or actual wolf encounter. Hence, results described herein are novel and cannot be properly compared with the limited existing literature within this subject. Nevertheless, Boonstra (2013) described that fear of predation and its behavioral and physiological consequences are based on the anticipatory memory of the attack. Consequently, cows that have not yet been predated by wolves may not experience a fear-related stress response when interacting with wolves for the first time due to the lack of adverse memories from previous predation episodes. In contrast, the behavioral and physiological stress responses detected herein in WLF cows are known to impair performance, reproductive, and health parameters in cattle (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012). These results support the assumption that the impacts of wolf presence and predation on beef cattle systems are not limited to cattle death and injuries, but may also extend to overall productivity and welfare of the herd (Lehmkuhler et al., 2007). Consequently, more research is warranted to directly evaluate the productive and economic consequences that wolves bring to beef cattle operations, including studies with authentic wolf packs, cattle from the same management and genetic background, and assessing cattle performance, reproductive, and health parameters.

Conclusions

Results from this experiment indicate that the simulated wolf encounter increased excitability and fear-related physiological stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator. Therefore, the

presence of wolf packs near cattle herds may negatively impact beef production systems via predatory activities and subsequent death and injury of animals, as well as by inducing stress responses known to impair cattle productivity and welfare when packs are in close proximity to previously predated herds.

Acknowledgements

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Beef Research Report

Beef Cattle Sciences

Influence of Supplement Composition on Utilization of Low-Quality Cool-Season Forage by Beef Cattle [1]

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Synopsis

Mature cattle consuming low-quality, cool-season forage, can use a starch-based energy supplement, along with a source of non-protein nitrogen (**NPN**), to improve nutrient utilization and performance in a manner comparable to supplementation with natural protein.

Summary

Two studies were conducted to evaluate the influence of supplement composition on intake and digestibility of a low-quality (< 6% crude protein; **CP**), cool-season forage by ruminants. Treatments included a non-supplemented control (**CON**), corn, corn with two levels of urea (**LU** = 27% CP; **HU** = 43% CP) and a positive control of soybean meal (**SBM;** 51% CP). In Exp. 1, 5 ruminally cannulated steers were used in an incomplete 5 x 4 Latin square with four 28-d periods. Forage intake and digestibility were not influenced by supplementation ($P > 0.10$); however, forage intake was greater for SBM than HU ($P = 0.01$). Ruminal ammonia (**NH3**) increased with supplementation ($P < 0.01$), increased linearly with urea inclusion ($P < 0.01$), and was greater for HU than SBM ($P < 0.01$). In Exp. 2, 80 late-gestation beef cows were stratified by age, body condition score (**BCS**), and body weight (**BW**) and randomly allotted to treatments. Cow BCS change was improved ($P < 0.01$) with supplementation and with increasing urea inclusion, but did not differ between the HU and SBM treatments ($P > 0.10$). Cow insulin, glucose and non-esterified fatty acids (**NEFA**) were not influenced by supplementation ($P \geq 0.07$) while supplementation increased insulin-like growth factor I (**IGF-I**; $P < 0.01$). These data suggest that a corn-urea based supplement can be utilized as effectively as SBM by ruminants consuming low-quality, cool-season forages as long as the supplements provide comparable intake of CP and energy.

Introduction

Low-quality forages are a vital part of beef cattle diets; nevertheless, forage utilization is typically limited without supplementation (DelCurto et al., 1990a,b; Köster et al., 1996), leading to reduced weight and BCS (DelCurto et al., 1990b; Bohnert et al., 2002). This impaired nutritional status and animal performance often leads to reduced reproductive efficiency (Wiltbank et al., 1962; Bellows and Short, 1978; Hess et al., 2005) when compared with an adequate nutritional state. Consequently, many studies have tried to optimize low-quality forage utilization while maintaining animal performance.

Studies with low-quality, cool-season forages have suggested that forage intake may not be

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increased with protein supplementation (Mathis et al., 2000; Bohnert et al., 2002; 2011). Cool-season forages have a greater proportion of CP as rumen degradable CP (**RDP**) than warm-season forages (Bohnert et al., 2011), suggesting that ruminal NH3 may not limit intake and digestibility to the same extent as with warm-season forages. Consequently, CP supplementation likely does not have the same positive impact on overall energy intake as seen with warm-season forages. Also, little data is available on the effects of supplementing low-quality, cool-season forages with energy-dense supplements containing varying protein concentrations on ruminant performance and forage utilization. We hypothesize that energy supplementation will be more beneficial than protein supplementation for ruminants consuming low-quality, cool-season forages. Therefore, the objectives of these experiments were to evaluate the influence of supplement composition on intake and digestibility of low-quality cool-season forages, as well as cow performance.

Materials and Methods

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 4356).

Experiment 1. Influence of Supplement Composition on Forage Intake and Digestibility in Steers

Five ruminally cannulated steers (1,234 ± 174 lb) were used in an incomplete 5 x 4 Latin square. Treatments consisted of a CON, 3 high energy corn-based supplements with low, moderate and high levels of protein (**CORN** = 0.126 % BW Corn; LU = CORN + 0.009 % BW urea; HU = CORN + 0.017 % BW urea) and a positive control (0.126 % BW SBM). All supplements were formulated to provide similar caloric intakes and the SBM treatment was formulated to provide approximately 100% of the estimated RDP requirement assuming a microbial efficiency of 10%. In addition, the HU supplement was formulated to have the same CP as the SBM supplement; however, a lower than anticipated CP concentration in the corn resulted in the HU supplement having a lower CP concentration than the SBM supplement. The LU supplement was designed to have a CP concentration halfway between that of the Corn and HU supplement. Supplement CP and TDN concentrations were 8, 27, 43, and 51% and 88, 82, 77, and 80%, respectively, for Corn, LU, HU, and SBM. Steers had continuous access to fresh water and chopped fine fescue grass seed straw (4.7% CP).

The 4 experimental periods were 28 d each with 20 d of diet adaptation and 8 d of sampling. On d 23 to 28 fecal grab samples were collected every 12 hr with a 2 hr advancement each day to allow for sampling on each even hour of a 24-hr day. Also, on d 28 ruminal fluid was collected by suction strainer immediately before feeding and at 1, 3, 6, 9, 12, 18, and 24 hr after feeding.

Intake and digestibility data were analyzed as a 5 x 4 incomplete Latin square with the MIXED procedure of SAS. The model included period and treatment and steer was used as the random variable. Contrasts used to partition specific treatment effects consisted of: 1) supplemented vs non-supplemented; 2) linear effect of urea; 3) quadratic effect of urea; and 4) HU vs SBM. Ruminal pH, NH3, and volatile fatty acid (**VFA**) data were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, hour, and treatment x hour. Steer was used as the RANDOM statement to specify variation and steer(period) was used as the subject. The specific term for the repeated statement was hour. Autoregression (AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same contrasts as previously noted were used to partition specific treatment effects.

Experiment 2. Influence of Supplement Composition on Cow Performance

Eighty late-gestation beef cows (1,118 ± 22 lb) were stratified into 4 blocks by age, BCS, and BW and randomly assigned within block to 1 of 5 treatments. Cows were then sorted by treatment, within block, and randomly allotted to 1 of 20 pens (4 cows/pen; 4 pens/treatment). The same treatments as described in Exp. 1 were used. Water and a mineral-salt mix were available free choice. Cows were provided ad libitum access to low-quality (5.0% CP; DM basis) fine fescue grass seed straw. The quantity of SBM supplement provided was calculated to meet 100% of the estimated RDP requirement assuming a microbial efficiency of 10%, while the Corn, LU and HU supplements were provided in amounts estimated to be iso-caloric with the SBM treatment. Each cow was offered 1.8 lb/day of corn, with 59 and 115.2 g/d of urea added for the LU and HU treatments, respectively; SBM was offered to cows at 1.8 lb/day. The nutrient content

of the supplements was the same as described in Experiment 1.

Cow BW and BCS were measured every 14 d until calving and within 24 hr post-calving. Calf BW was also obtained within 24 hr post-calving. Blood samples were collected into 2 commercial 10-mL blood collection tubes via jugular venipuncture at trial onset, d 49, and within 24 hr post-calving.

Cow performance data was analyzed as a randomized block design using the MIXED procedure of SAS. The model included block, treatment, and treatment x block. Blood samples were analyzed using the REPEATED statement with the MIXED procedure of SAS. Model included block, treatment, day, and all resulting interactions. Values were adjusted covariatelty to values obtained at trial onset. Cow(pen) and pen(treatment) were used as the repeated variables, the subject was cow(pen) and appropriate covariate structure was determined by the Akaike information criterion; AR1 was used for insulin and NEFA and compound symmetry (CS) was used for glucose and IGF-I. The same contrasts as previously described were used to partition specific treatment effects. If no treatment x time interactions were detected ($P > 0.05$), treatment means were compared.

Results

Experiment 1. Influence of Supplement Composition on Forage Intake and Digestibility in Steers

Intake and Digestibility. Intake of grass seed straw was not increased with supplementation ($P > 0.10$) but was greater for steers receiving SBM than for HU ($P = 0.01$; Table 1). As designed, CP intake increased with supplementation and increased linearly with increasing urea ($P < 0.01$). However, a lower than expected corn CP concentration resulted in greater CP intake with SBM supplementation than with HU ($P < 0.01$), possibly explaining the increased straw intake with SBM.

Diet and CP digestibility increased with supplementation ($P \leq 0.05$; Table 1), which agrees with previous research, likely because of the greater digestibility of the supplement when compared to the forage. We noted no differences for SBM vs HU or for urea inclusion ($P > 0.10$) on diet digestibility. This agrees with previous work noting ruminal fiber digestibility is not influenced by protein supplementation (Bohnert et al., 2002; Currier et al., 2004). Furthermore, low-levels of energy supplementation typically do not alter fiber digestibility (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997). In contrast, CP digestibility was increased with increasing urea inclusion ($P < 0.01$) while no difference was noted for HU compared with SBM ($P = 0.84$).

Ruminal Fermentation. Ruminal NH3 increased with supplementation ($P < 0.01$), increased linearly with urea inclusion ($P < 0.01$) and was greater for HU compared with SBM ($P < 0.01$; Table 1). Non-supplemented steers had a ruminal NH3 concentration of 1.61 mM, which is within the range of 1.18 to 2.94 mM believed to support optimal growth of rumen microbes in vivo (Slyter et al., 1979). Consequently, it is possible that NH3 was not limiting ruminal fermentation and forage intake in non-supplemented controls.

No treatment effects were seen on total VFA concentration or molar proportions of propionate and butyrate ($P > 0.05$; Table 1). Additionally, the acetate:propionate ratio did not differ between treatments ($P > 0.10$), suggesting similar efficiencies of ruminal fermentation. Nevertheless, the molar proportion of acetate was greater for HU than for SBM steers ($P = 0.01$) while steers supplemented with SBM had greater molar proportions of the branch chain VFA isobutyrate, isovalerate and valerate ($P \leq 0.01$). This was expected, as branch-chain VFA are formed by the fermentation of branch-chain amino acids present in natural proteins such as SBM (Leng, 1973).

Experiment 2. Influence of Supplement Composition on Cow Performance

Pre-calving (within 14 d of calving) BCS change was improved with supplementation ($P < 0.01$; Table 1) and increased linearly with increasing urea supplementation ($P < 0.01$). Likewise, post-calving BCS change was increased with supplementation ($P < 0.01$) and increased linearly with greater urea inclusion ($P < 0.01$). Although results from Exp. 1 suggests that forage intake may have differed between HU and SBM treatments, no differences were noted in pre- or post-calving BCS change for HU compared to SBM cows ($P > 0.10$). Also, calf birth weight increased linearly ($P = 0.04$; data not shown) with increasing urea; no incidences of dystocia were noted.

Plasma IGF-I increased with supplementation ($P < 0.01$; Table 1) suggesting improved nutritional status, with IGF-I appearing to plateau when supplemental protein reached the level corresponding to the LU supplement. Despite differences in animal performance between treatments, no treatment effects were detected for

plasma insulin, glucose, or serum NEFA concentration ($P \geq 0.07$; data not shown).

Conclusions

These results suggest that intake of low-quality, cool-season forage was not limited by RDP. However, the improvement in animal performance with supplementation indicates that both energy and protein were limiting performance. The addition of supplemental energy necessitated the addition of RDP to optimize forage utilization and performance, resulting in similar performance between animals supplemented with natural protein and those receiving an energy dense supplement with added urea. As a result, a starch-based energy supplement, along with a source of non-protein nitrogen, appears to be an acceptable management alternative to sources of natural protein for ruminants consuming low-quality, cool-season forage.

Acknowledgements

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Table 1. Effects of supplement composition in cattle consuming low-quality, cool-season forage

	Treatment[a]						Contrasts[c], *P* =			
	Con	Corn	LU	HU	SBM	SEM[b]	Con vs Supp	L Urea	Q Urea	HU vs SBM
Digestion Study										
Forage intake, % body weight	2.15	2.08	2.17	2.05	2.30	0.071	0.87	0.64	0.10	<0.01
Supplement intake, % body weight	0.00	0.126	0.135	0.143	0.127					
Total intake, % of body weight	2.15	2.21	2.30	2.20	2.43	0.071	0.02	0.85	0.10	<0.01
CP Intake, % of body weight	0.103	0.113	0.143	0.161	0.175	0.0040	<0.01	<0.01	0.13	<0.01
Diet Digestibility, %	33.9	37.0	37.7	36.5	36.9	1.250	0.05	0.76	0.54	0.79
CP Digestibility, %	17.3	20.0	33.3	44.2	43.2	3.584	<0.01	<0.01	0.79	0.84
Ruminal NH_3 mM	1.61	1.50	3.20	4.72	2.96	0.213	<0.01	<0.01	0.69	<0.01
Ruminal pH	6.88	6.81	6.81	6.76	6.88	0.048	0.08	0.38	0.61	0.01
Ruminal Total VFA, mM	134.3	136.4	134.2	135.5	128.0	6.73	0.91	0.93	0.84	0.44
Acetate, mol/100 mol	63.85	63.41	63.22	63.66	61.07	0.672	0.19	0.80	0.71	0.01
Propionate, mol/100 mol	18.06	17.51	18.07	17.99	18.11	0.381	0.63	0.22	0.33	0.75
Isobutyrate, mol/100 mol	1.88	1.85	1.72	1.64	2.38	0.094	0.86	0.06	0.81	<0.01
Butyrate, mol/100 mol	10.86	11.79	11.35	11.48	10.91	0.316	0.15	0.48	0.47	0.22
Isovalerate, mol/100 mol	1.92	2.08	2.25	1.93	3.54	0.204	<0.01	0.51	0.21	<0.01
Valerate, mol/100 mol	3.35	3.22	3.52	3.40	3.94	0.140	0.28	0.36	0.23	0.01
Acetate:propionate ratio	3.56	3.66	3.52	3.58	3.39	0.111	0.84	0.54	0.39	0.16
Cow Performance Study										
Initial BCS	4.76	4.75	4.82	4.62	4.79	0.103	0.86	0.37	0.25	0.21
BCS change										
Precalving	-0.49	-0.32	0.05	0.12	0.26	0.089	<0.01	<0.01	0.17	0.25
Postcalving	-0.63	-0.57	-0.22	-0.05	0.15	0.089	<0.01	<0.01	0.40	0.11
IGF-I, ng/mL	25.0	26.6	39.0	39.4	41.7	2.52	<0.01	<0.01	0.05	0.51

Oregon State University

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Beef Research Report

Beef Cattle Sciences

Influence of Forage Type and CP Supplementation on Utilization of Low-Quality Hay by Beef Cattle [1]

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Synopsis

The data reported here adds to the growing body of evidence that intake of low-quality cool-season forages (**C3**) by ruminants is greater than intake of warm-season (**C4**) forages. Also, our data implies that meadow foxtail (**MF**) hay is better nutritionally than reed canarygrass (**RC**) or tallgrass prairie (**TG**) for beef cattle based on improved intake, digestibility, and ruminal fermentation.

Summary

An in situ study (Exp. 1) was performed using 4 ruminally cannulated steers in a completely randomized design to compare the in situ degradation parameters of 2 low-quality, C3 forages (MF and RC) and a C4 forage (TG). A digestion study (Exp. 2) utilizing 6 ruminally cannulated steers in a 6 × 5 incomplete Latin square design was conducted to compare crude protein (**CP**) supplementation of low-quality C3 and C4 forages. Treatments included MF, RC, and TG hays with and without supplemental CP. In Exp. 1, soluble CP and rate of CP disappearance was similar for MF and RC ($P > 0.05$) with both C3 forages greater than TG ($P < 0.05$). Also, rumen degradable CP (**RDP**) was greatest for MF ($P < 0.05$) with RC greater than TG ($P < 0.05$), while effective degradability of CP was greatest for MF ($P < 0.05$), intermediate for TG ($P < 0.05$), and lowest for RC ($P < 0.05$). In Exp. 2, hay and total intake were increased ($P < 0.01$) with supplementation, for C3 compared with C4, and for MF compared with both RC and TG. Also, dry matter (**DM**) and CP digestibility were increased with supplementation ($P < 0.04$) while not affected by forage type ($P > 0.33$) but were greater for MF compared with both RC ($P < 0.01$) and TG ($P \leq 0.06$). Supplementation increased ($P < 0.01$) ruminal NH3 and total volatile fatty acids (**VFA**), while acetate and propionate were increased ($P < 0.01$) for C3 compared with C4, resulting in a lower acetate:propionate ($P < 0.01$), thereby suggesting improved energetic efficiency

Introduction

Beef cattle in the Intermountain West normally consume low-quality C3 forages (< 7% CP) for extended periods during the annual production cycle (Turner and DelCurto, 1992). In an effort to meet the nutritional needs of these animals, supplemental CP is often provided because it has been shown to increase forage intake (Lintzenich et al., 1995), forage digestibility (DelCurto et al., 1990), and animal performance (Bodine et al., 2001). However, research suggests that CP supplementation of ruminants consuming low-quality C3 forages does not increase forage intake in a manner similar to that observed with C4 forages (Mathis et al., 2000;

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Bohnert et al., 2002; 2011). Therefore, the objective of this experiment was to compare forage intake, digestibility, and ruminal fermentation of ruminants offered low-quality C4 (TG) and C3 hays (MF and RC) with and without supplemental CP in the hope of elucidating the reason(s) for the apparent difference in forage intake response.

Materials and Methods

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 4256).

Experiment 1. In Situ Degradation of low-quality C3 and a C4 forages

Four ruminally cannulated Angus × Hereford steers (1,042 ± 29 lb) were used in a completely randomized design to evaluate the ruminal degradation characteristics of 2 low-quality, C3 forages (MF; RC) and a C4 forage (TG; Table 1). Dacron bags were labeled with a waterproof permanent marker, weighed, and samples of ground forage were added and the bags sealed with an impulse sealer. Bags for each forage source were placed in a bucket containing warm water, transported to the steers, and placed in the rumen. Bags were placed in a weighted polyester mesh bag within the rumen of each steer for incubation periods of 0, 2, 8, 12, 24, 48, and 96 hr. Upon removal, Dacron bags were rinsed under tap water until the rinse water was clear and then dried. The dried samples were weighed, composited by steer, time and forage type, and analyzed for neutral detergent fiber (**NDF**) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). The NDF residue was then weighed and analyzed for CP.

Kinetic variables for NDF and CP digestibility were estimated with SAS (SAS Inst., Inc., Cary NC) using the modified nonlinear regression procedure described by Fadel (2004). Data were analyzed using the MIXED procedure of SAS. The model included forage source as the independent variable. Steer was used as random variable. Means were separated using LSD protected by a significant F-test ($P \leq 0.05$).

Experiment 2. Forage intake and nutrient digestibility of low-quality C3 and a C4 forages with and without supplemental CP

Six ruminally cannulated steers (943 ± 26 lb) were used in an incomplete 6 × 5 Latin square design and housed in individual pens within an enclosed barn with continuous lighting. Steers were provided continuous access to fresh water and the 2 low-quality C3 forages and the single C4 forage used in Experiment 1 (Table 1). Forage was provided daily with feed refusals from the previous day determined before feeding. A trace mineralized salt mix was provided daily. In addition, an intramuscular injection of vitamins A, D, and E was administered to each steer at the onset of the trial to safeguard against deficiency. Treatments were arranged in a 3 × 2 factorial design (3 forages with or without supplemental CP). Soybean meal (**SBM**) was placed directly into the rumen via the ruminal cannula for supplemented treatments. The supplemented treatments were formulated, based on preliminary forage and SBM samples, to provide approximately 100% of the estimated RDP requirement assuming a microbial efficiency of 10%. Experimental periods were 20 d, with intake measured beginning d 13 and concluding d 18.

Samples of forages and SBM were collected d 13 through d18 and orts were collected on d 14 through 19. Forages, SBM, and orts were dried for 48 h and ground. On d 15 through 20, fecal grab samples were collected 2 times/day at 12-hr intervals with a 2-hr increment added between days to shift sampling times. This allowed sampling on every even hour of the 24-hr day. Also, ruminal fluid was collected on d 20 at 0, 3, 6, 9, 12, 18, and 24 hr post-feeding for analysis of pH and ammonia (**NH3**).

Data were analyzed as an incomplete 6 × 5 Latin square using the MIXED procedure of SAS and Satterwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The model included treatment and period as independent variables. Steer was used as random variable. Contrasts used were: 1) supplemented vs not supplemented; 2) C3 vs C4; 3) contrast 1 × contrast 2; 4) MF vs RC; 5) MF vs TG.

Table 1. Feedstuff nutrient content (DM basis) [a]

Nutrient,%	MF	RC	TG	SBM
Exp. 1				
CP	4.6	2.6	5.1	--
NDF	63.6	68.9	77.7	--
Exp. 2				
CP	4.6	2.6	5.2	50.2
NDF	64.1	69.0	77.1	16.1
ADF	33.6	41.5	42.0	5.6

[a] MF = Meadow foxtail hay (cool-season forage); RC = Reed Canarygrass hay (cool-season forage); TG = tallgrass prairie hay (warm-season forage); SBM = soybean meal.

Results

We had a wider range in forage CP than anticipated for the hays used in Experiments 1 and 2, with the greatest CP occurring with TG (5.1 and 5.2%, respectively) and the least occurring with RC (2.6 in both experiments; Table 1).

Experiment 1. In Situ Degradation of low-quality C3 and a C4 forages

The soluble fraction of NDF was greater for MF compared with RC and TG ($P \leq 0.05$) with no difference noted between RC and TG ($P > 0.05$; Table 2). The degradable fraction was comparable between MF and TG ($P > 0.05$) while the proportion of degradable NDF in RC was approximately 22% less than TG and MF ($P \leq 0.05$). The undegradable fraction of NDF was approximately 70% greater ($P \leq 0.05$) for RC compared with TG and MF. Also, though no differences ($P = 0.58$) were noted in the rate of NDF degradation, the effective degradability of NDF was greatest ($P \leq 0.05$) for MF (48.7%) followed by TG (45.2%) which was more digestible than RC (35.6%; $P \leq 0.05$).

The soluble fraction of CP was greatest with RC ($P \leq 0.05$) followed by MF. Also, both C3 forages had a greater soluble fraction than TG ($P \leq 0.05$; Table 2). However, when evaluating the degradable fraction, MF and TG were similar in ruminal degradable CP ($P > 0.05$) but greater than RC ($P \leq 0.05$). Consequently, the undegradable CP fraction was greatest for RC ($P \leq 0.05$) while TG was greater than MF ($P \leq 0.05$). The rate of CP degradation was similar for the C3 forages ($P > 0.05$) which were almost 75% greater than that observed with the C4 forage ($P \leq 0.05$). This agrees with work by Bohnert et al. (2011) in which the CP degradation rate of TG was almost 70% less than that observed with Kentucky bluegrass straw (C3; *Poa pratensis*). The proportion of RDP, as well as the effective degradability of CP, was greatest for MF ($P \leq 0.05$). Also, RC contained a greater proportion of RDP than TG ($P \leq 0.05$). This agrees with a results reported by Bohnert et al. (2011) in which a low-quality C3 forage had approximately 28% greater RDP than a C4 forage with comparable CP concentration. The effective degradability of CP was greater for TG compared with RC ($P \leq 0.05$).

Experiment 2. Forage intake and nutrient digestibility of low-quality C3 and a C4 forages with and without supplemental CP

Hay and total intake were increased with supplementation ($P < 0.01$; Table 3) and were greater for the C3 forages compared with the C4 ($P < 0.01$). These results agree with Bohnert et al. (2011) who reported similar results when comparing CP supplementation of TG with Kentucky bluegrass straw. However, in contrast to Bohnert et al. (2011), we did not note a supplementation × forage type interaction for hay intake ($P = 0.65$). Also, MF had greater hay and total intake than RC ($P < 0.01$) or TG ($P < 0.01$). The differences between the current study and Bohnert et al. (2011) are probably due to differing nutritional quality profiles of the forages and/or the different C3 forage species used.

Digestibility of DM and CP was increased with supplementation ($P \leq 0.03$; Table 3) but not affected by forage type ($P \geq .34$). However, digestibility of DM and CP was greater with MF compared to RC ($P < 0.01$) and digestibility of DM was greater ($P = 0.02$) and CP digestibility tended to be greater ($P = 0.06$) for MF compared with TG.

Supplementation of CP increased ($P < 0.05$) ruminal fluid volume, dilution rate, NH3 (mM), and total VFA concentration compared with no supplementation which agrees with previous work (DelCurto et al., 1990). Likewise, both ruminal fluid volume and dilution rate were greater ($P \leq 0.01$) for C3 compared with C4 which compares favorably with previous data from our research group (Bohnert et al., 2011). These results suggest that supplementation and C3 forages improves production of microbial CP and flow of nutrients to the small intestine, compared with no supplementation and C4 forages, respectively, which should improve the overall nutrient status of the animal. It is of interest to note that even though there was no influence of forage type on total VFA concentration ($P = 0.10$), the molar proportion of the individual VFA acetate was greater with C4 ($P <$ 0.01) while propionate was increased for C3 ($P <$

0.01). This resulted in a lower acetate:propionate ratio for C3 which implies that ruminal fermentation of C3 forage is more energetically efficient to the animal than C4 forage. Similar results were reported in an earlier study from our lab (Bohnert et al., 2011).

When directly comparing the individual forages, MF had a greater ($P < 0.01$) ruminal liquid fill than TG and greater liquid dilution rate ($P < 0.01$) than both RC and TG. Also, MF had a lower acetate molar proportion ($P < 0.01$) than TG and greater propionate than RC and TG ($P < 0.01$). This resulted in a lower acetate:propionate ratio ($P < 0.01$) for MF compared with RC and TG which, as with C3 compared with C4, infers that MF was used in a more energetically manner than either RC or TG.

Conclusions

This research adds to the growing body of evidence that intake of low-quality C3 forages by ruminants is greater than intake of C4 forages. However, it is not evident what specific nutritional quality factors are causing the increased forage intake with C3 compared with C4. Consequently, further research is warranted to help ascertain the indices that will assist nutritionists to better predict forage intake of ruminants consuming low-quality forages. Also, our data indicates that MF is better hay than RC or TG for beef cattle based on improved intake, digestibility, and ruminal fermentation.

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Table 2. Ruminal degradation parameters of two cool-season forages (meadow foxtail and reed canarygrass) and one warm season forage (tallgrass prairie).

Degradation Parameters	Meadow Foxtail	Reed Canarygrass	Tallgrass Prairie	SEM[a]	*P*-Value
NDF					
Fractions, %					
Soluble	7.21^{x}	4.51^{y}	4.74^{y}	0.41	0.006
Degradable	68.7^{x}	55.0^{y}	72.1^{x}	3.5	0.03
Undegradable	24.2^{x}	40.6^{y}	23.1^{x}	3.29	0.02
Kd[b], /h	0.031	0.028	0.026	0.0043	0.58
Effective Degradability, %[c]	48.7^{x}	35.6^{y}	45.2^{z}	0.98	< 0.001
CP					
Fractions, %					
Soluble	28.2^{x}	36.4^{y}	11.7^{z}	0.53	< 0 .001
Degradable	51.1^{x}	21.8^{y}	52.2^{x}	1.41	< 0.001
Undegradable	20.7^{x}	41.9^{y}	36.2^{z}	1.23	< 0.001
Kd[b], /h	.0613^{x}	.0738^{x}	.0388^{y}	0.0078	0.02
RDP, % of CP	62.4^{x}	54.2^{y}	41.4^{z}	1.03	< 0.001
RUP, % of CP	37.6^{x}	45.8^{y}	58.6^{z}	1.03	< 0.001
Effective Degradability, %[c]	79.3^{x}	58.1^{y}	63.8^{z}	1.23	< 0.001

[a] n = 4.
[b] Fractional rate constant.
[c] Calculated as A + {B × [(*K*d/(*K*d + *K*p)]}, where *K*p was the ruminal passage rate, which was set at 2%/h (Hoffman et al., 1993). The units used for *K*d in the equation were per hour.
[x,y,z] Means in a row without a common superscript are different ($P < 0.05$).

Table 3. Intake, digestibility, and ruminal parameters for beef steers consuming low-quality cool-season (C3; Meadow foxtail; Reed canarygrass) and warm-season (C4; tallgrass prairie) grass hays with (+) or without supplemental CP.

								P-Value[b]				
Item	MF	MF+	RC	RC+	TG	TG+	SEM[a]	Con vs Supp.	C3 vs C4	Supp. × Type	MF vs RC	MF vs TG
Intake,% BW												
Hay	1.74	2.25	1.48	2.00	1.46	1.92	0.072	< 0.001	0.004	0.65	< 0.001	< 0.001
Supplement	0.00	0.11	0.00	0.11	0.00	0.11						
Total	1.74	2.36	1.48	2.11	1.46	2.03	0.072	< 0.001	0.004	0.65	< 0.001	< 0.001
CP	0.079	0.160	0.041	0.110	0.076	0.154	0.0038	< 0.001	< 0.001	0.44	< 0.001	0.17
Digestibility, %												
DM	50.0	59.4	37.7	45.1	41.7	47.7	4.23	0.03	0.34	0.73	0.004	0.02
CP	26.8	51.8	-22.5	33.2	11.4	36.9	7.86	< 0.001	0.79	0.27	< 0.001	0.06
NDF	46.2	56.6	36.6	36.9	47.9	48.6	6.80	0.50	0.48	0.70	0.04	0.65
Ruminal Fluid												
Fill, mL/kg BW	232	238	212	240	180	210	12.4	0.04	0.003	0.56	0.48	0.004
Dilution rate, %/h	7.0	10.3	6.1	7.8	6.0	7.5	0.42	< 0.001	0.01	0.20	< 0.001	< 0.001
pH	6.79	6.82	6.80	6.80	6.80	6.70	0.41	0.40	0.10	0.11	0.95	0.14
NH3, mM	0.34	0.67	0.36	0.92	0.40	0.76	0.076	<0.001	0.93	0.54	0.09	0.34
Total VFA, mM	70.2	95.3	78.6	87.6	85.2	97.3	4.34	< 0.001	0.10	0.95	0.33	0.34
Acetate, mol/100 mol	69.6	69.2	69.7	68.8	75.8	75.4	0.50	0.14	< 0.001	0.83	0.71	< 0.001
Propionate, mol/100 mol	15.7	15.2	17.1	17.4	12.9	12.9	0.24	0.75	< 0.001	0.93	< 0.001	< 0.001
Acetate:Propionate	4.5	4.6	4.1	4.0	5.9	6.0	0.11	0.98	< 0.001	0.92	< 0.001	< 0.001

[a] n = 5.

[b] Con vs Supp. = non-supplemented vs supplemented treatments; C3 vs C4 = cool-season vs warm-season forages; Supp. × Type = interaction of upplementation and forage type; MF vs RC = Meadow foxtail vs Reed canarygrass; MF vs TG = Meadow foxtail vs tallgrass prairie.

Oregon State University

BEEF122

Beef Research Report

Beef Cattle Sciences

Effects of Prostaglandin $F_{2\alpha}$ on Reproductive Performance in Dairy Cows [1]

K. G. Younger[2], A. M. Lulay[3], and A. R. Menino, Jr.[4]

Synopsis

Preliminary results of the effects of a single injection of prostaglandin $F_{2\alpha}$ administered to dairy cows 14-d postpartum suggest improved uterine health and reproductive performance.

Summary

The objective of this experiment was to determine the effects of a single injection of prostaglandin $F_{2\alpha}$ (Lutalyse) on uterine health and reproductive performance in dairy cows. Holstein cows from a commercial dairy were randomly assigned to receive an intramuscular injection of either Lutalyse or saline 14-d postpartum. Uterine swabs were performed to evaluate changes in bacterial populations and services per conception and days open were evaluated to determine the effects on reproductive performance due to treatment. Uterine swabbing was performed at 0 and 24 h after injection. Lutalyse treatment tended to decrease uterine bacterial populations, services per conception and days open.

Introduction

During the beginning of the postpartum period, 80 to 100% of lactating dairy cows have bacterial contamination within their uterus (Sheldon et al., 2006). The presence of uterine bacteria may contribute to reduced reproductive performance within these cows. Though most postpartum uterine infections are resolved naturally, at least 20% of infections persist within the cow past 3-wk postpartum (Krause et al., 2014). Metritis most commonly occurs within 10 d of parturition while endometritis can be detected at 21-d postpartum (Sheldon et al., 2009). Development of endometritis in the postpartum dairy cow results in an increased calving interval and reduces probability of conception on the first insemination (Fourichon et al., 2000). The susceptibility to bacterial infection within the uterus can be attributed to a decrease in neutrophil function during this period (Nagahata et al., 2010). The expulsion of uterine contamination is crucial for uterine involution and healthy reproductive performance (Krause et al., 2014). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been found to induce uterine contractility and increase phagocyte activity within the uterine environment (Dhaliwal et al., 2001). An injection of $PGF_{2\alpha}$ would allow defense from bacterial contamination by both neutrophil elimination of bacteria and physical expulsion from the uterus. This would mean that a regimen of postpartum $PGF_{2\alpha}$ injection could result in earlier uterine involution and therefore decreased calving intervals and reduced cases of uterine infection and postpartum complications. The objective of this study was to evaluate the effects of a single intramuscular $PGF_{2\alpha}$ injection administered to d-14 postpartum dairy cows on uterine bacteria populations and reproductive performance including

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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days open and services per conception. We hypothesized that $PGF_{2\alpha}$ injection to dairy cows on day-14 postpartum would reduce uterine bacteria, number of days open and services per conception

Materials and Methods

Cows in this study were from a dairy containing 3000 Holstein cows with 1500 in the milking string. Cows were supplied with water using a free-choice system. The ration fed included corn silage, grass silage, alfalfa, corn, cotton seed and soy bean meal. Postpartum cows were given a 55-d voluntary waiting period before synchronization and artificial insemination. Pregnancy was detected by a veterinarian through rectal palpation and ultrasound at 40 d of gestation. Ovarian structures were also palpated in the event that pregnancy did not occur in order to identify whether inability to conceive was the result of failure of resumption of the estrous cycle.

A total of forty cows at 14-d postpartum were given an intramuscular injection of either $PGF_{2\alpha}$ (Lutalyse) or saline. The first cow was chosen at random for injection of either Lutalyse or saline and subsequent cows were injected with alternating treatments to ensure randomization. Uterine bacteria samples were obtained at 0 and 24 h after injection through rectal palpation and swabbing the inside of the uterine body. For the 0-h sample recovery, the swab was conducted just before the injection was administered. To recover bacteria, the uterus was first palpated rectally, and once the cervix was grasped securely, the vulva was cleaned using paper towels and spread open and the first guarded tube of the swab was inserted into the vagina. This tube was inserted into the external cervical and the second guarded tube was pushed thru the first tube into and through the cervix. Once the second guarded tube was in the uterine body approximately 2 cm, the swab was then pushed through the second guard tube and spun gently on the uterine body. The swab was then retracted back into the second guarded tube and both guarded tubes were removed from the vagina. The swab with the uterine sample was broken off and placed in a tube containing ten ml of Dulbecco's phosphate buffered saline (DPBS). Samples were brought to the lab and a 1/10 dilution was made from the sample collected in the field using DPBS as the diluting fluid. One hundred microliters of each dilution (0 and 1/10) was spread onto each side of a Blood/MacConkey Agar bacteriological culture biplate. The biplates were covered and left facing up for one h at room temperature and placed face down in an incubator at 37°C for 24 h. The numbers of colonies that grew on either side of the biplates were counted and recorded. Services per conception and days open were recovered from breeding records maintained for the cows in the experiment.

Differences in numbers of bacteria between the 0 and 24-h swabbing, services per conception and days open were analyzed using one-way ANOVA with treatment (Lutalyse or saline) as the main effect. All analyses were conducted using the NCSS statistical software program (Number Cruncher Statistical System; 2000, Jerry Hintze, Kaysville, UT).

Results

Changes in uterine bacterial populations did not significantly differ between cows injected with Lutalyse or saline. However Lutalyse treatment reduced total bacteria by 30 cells compared to 3 cells in cows injected with saline (Figure 1).

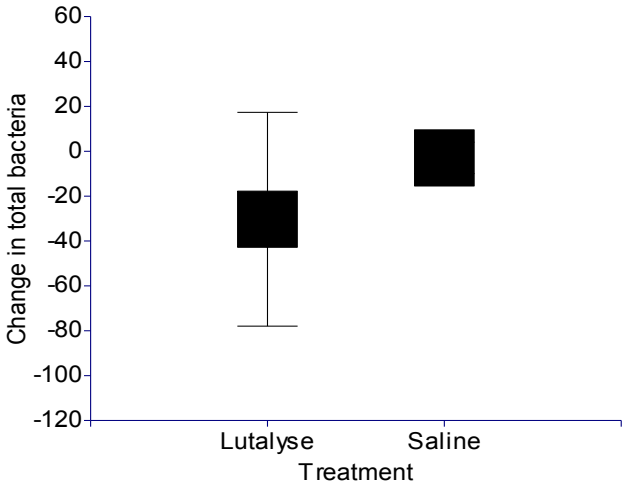

Figure 1. Changes in total uterine bacteria in cows injected with Lutalyse or saline on day-14 postpartum.

The dairy at which this study was performed chose to rebreed cows with exceptional milk production as many times as was financially feasible. It is for this reason some data for cows included beyond 2 or 3 services per conception. However this gave a clearer picture of reproductive performance for each cow. Currently, there is no significant difference in services per conception between cows injected with Lutalyse or saline however cows injected with Lutalyse had 0.2 fewer services per conception (Figure 2).

Number of days open for these cows was also not significant between cows injected with Lutalyse or saline. However, cows injected with Lutalyse had 15 fewer days open compared to cows injected with saline (Figure 3).

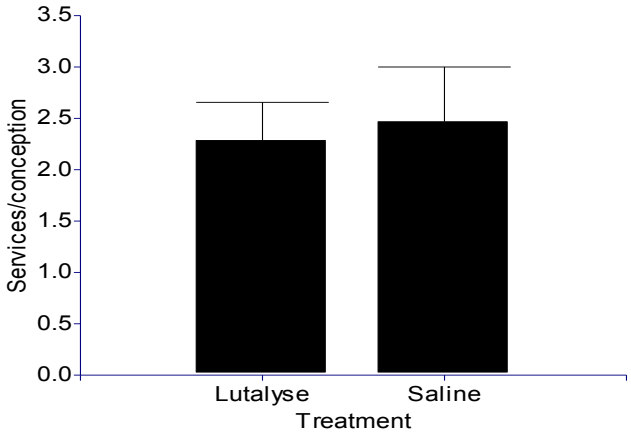

Figure 2. Number of services per conception for cows injected with Lutalyse or saline on day-14 postpartum.

The data presented are preliminary results recovered from a total of 40 cows in the project. The overall project design calls for a total of 200 cows with 100 cows in each treatment. We anticipate that as the animal numbers increase and more uterine and breed data are collected, significant differences in favor of a single injection of Lutalyse at 14-d postpartum will emerge. Lutalyse will likely have a diminishing effect on uterine bacterial load which will improve reproductive performance.

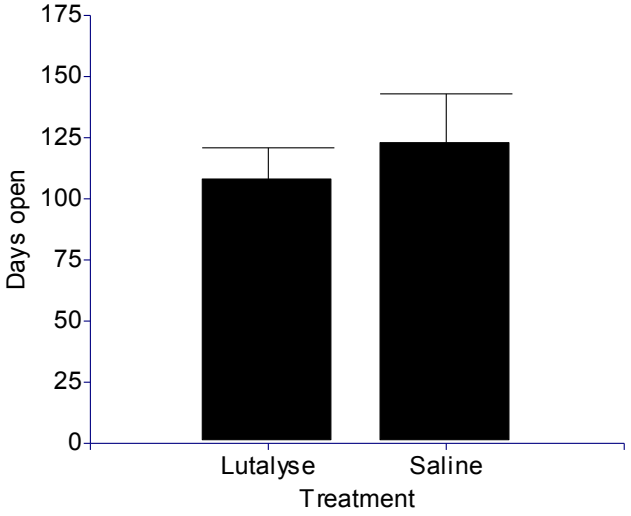

Figure 3. Number of days open for cows injected with Lutalyse or saline on day-14 postpartum.

Conclusions

Preliminary data suggests that Lutalyse administered to dairy cows 14-d postpartum reduces uterine bacteria, services per conception and number of days open. As these are preliminary results, more data must be collected for this experiment to acquire more definitive conclusions. If these results prove to be significant, a single injection of Lutalyse 14 d after parturition would reduce uterine infections and improve reproductive performance in dairy cattle. Dairy producers implementing this protocol would experience reduced costs associated with breeding and using Lutalyse would reduce the overall need for antibiotics.

Acknowledgments

The authors would like to thank the owners and operators of the Konyn Dairy for the generous use of their cows and access to their records for this project. This research was supported by grants from the USDA Animal Health and Disease Fund and the Agricultural Research Foundation.

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Oregon State University

BEEF123

Beef Research Report

Beef Cattle Sciences

Modifying the Hormone Strategy for Superovulating Donor Cows to Reduce Drug Costs without Decreasing the Number of High-Quality Transferable Embryos Recovered [1]

M. K. Gomes[2], A. Snider[3], N. Steigerwald[4], and A. R. Menino, Jr.[5]

Synopsis

This study evaluated two hormone dosing strategies used to superovulate donor cows for embryo transfer. Cows superovulated with the standard dose of 400 mg follicle stimulating hormone (FSH) produced more ova compared to the reduced dose of 200 mg but the reduced dose yielded a greater percentage of transferable embryos.

Summary

The objective of this research was to adjust the hormone doses used in a superovulatory protocol to where drug costs can be reduced while still retaining recovery of a satisfactory number of high quality transferable embryos. We proposed to simply reduce the FSH dose by half and double the gonadotropin-releasing hormone (GnRH) dose. Nineteen crossbred beef cows from the Oregon State University Beef Cattle Ranches were assigned to one of four treatments: 1) 400 mg FSH and 100 µg GnRH, 2) 400 mg FSH and 200 µg GnRH, 3) 200 mg FSH and 100 µg GnRH, or 4) 200 mg FSH and 200 µg GnRH. Embryos were collected non-surgically 7 d after estrus onset and scored for developmental stage and quality. Dose of GnRH had no significant effects on the average numbers of ova, embryos and transferable embryos recovered nor did it have an effect on the percent embryos and transferable embryos recovered. However, twice as many ova were recovered from cows treated with 400 compared to 200 mg of FSH but the percentage of transferable embryos of the total number of ova recovered was greater with 200 compared to 400 mg of FSH. Both high doses of FSH and GnRH increased the number of unfertilized ova recovered. Collectively, these data suggest that although the high FSH dose generated more total ova, many of these were neither fertilized nor transferable.

Introduction

Embryo transfer is an applied reproductive technology that has been used to improve herd genetics and female reproductive efficiency and propagate offspring from elite sire-dam matings (Bó and Mapletoft, 2013). Although the technique is commonly referred to as "embryo transfer", the "transfer" part is only the latter half of the overall procedure where embryos are transferred to timed recipients. The equally, if not more, critical part of an "embryo transfer" is the first half or the embryo collection where in cattle embryos are collected from superovulated donors. Because cows usually ovulate only a single ovum every estrous cycle, donor cows are commonly treated with a 4-day regimen of the pituitary gonadotropic hormone, FSH. Costs associated with FSH are high and with the current standard dosing it costs at least $150 in FSH alone to

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superovulate one donor (Armstrong, 1993). Besides the high cost, overstimulation of the ovaries by FSH occurs reasonably frequently and is counterproductive to both the numbers of total ova and transferrable embryos recovered. The question remains within the standard dosing of FSH is just how much FSH is required to induce an acceptable superovulatory response where a significant number of high quality transferable embryos can be recovered. Often practitioners will administer a second hormone, GnRH, when the donor first exhibits heat. The notion behind this injection is to increase the number of ova ovulated thereby increasing the total number of embryos produced and collected from a donor within a round of superovulation. Despite the frequency of GnRH use in these protocols, a clear benefit of the GnRH injection to improving overall embryo production has not been realized. The lack of a clear response may be due to either the variation associated with superovulatory responses making it difficult to detect differences or insufficient dosing of GnRH. It may well be that in our attempts to increase ovulation rate, we are actually "under-dosing" donors with the current GnRH dosing strategy. Therefore, the objective of this study was to evaluate the number and quality of embryos recovered from donor cows superovulated with reduced dosing of FSH and increased dosing of GnRH. We hypothesized that the reduced dose of FSH and the higher dose of GnRH would yield less total ova but a greater percentage of transferrable embryos.

Materials and Methods

Nineteen crossbred beef cows from the Oregon State University Beef Cattle Ranches were stratified into one of four treatment groups according to age and parity: 1) 400 mg FSH and 100 µg GnRH, 2) 400 mg FSH and 200 µg GnRH, 3) 200 mg FSH and 100 µg GnRH, or 4) 200 mg FSH and 200 µg GnRH. All cows were estrous synchronized and superovulated following the protocol described in Figure 1 with the exception that cows in the 200-mg FSH treatments received eight 25-mg doses twice daily instead of eight 50-mg doses. Estrus detection commenced 24 h after the second injection of Lutalyse and cows were artificially inseminated with one straw of frozen bull semen at 0, 12 and 24 h after onset of estrus. At onset of estrus, cows were injected with 100 or 200 µg of GnRH depending on treatment assignment. Embryos were collected non-surgically seven days after estrus onset and scored for developmental stage and quality using the four rank grading scheme devised by Lindner and Wright (1983).

Differences in the total numbers of ova, embryos, transferable embryos and unfertilized ova and the percent embryos, transferable embryos and unfertilized ova (UFOs) of the total ova recovered were analyzed using analysis of variance (ANOVA) for a 2 X 2 factorial design. Sources of variation in the ANOVA were FSH dose (200 or 400 mg), GnRH dose (100 or 200 µg) and the FSH X GnRH interaction. If significant effects were observed in the ANOVA, differences between means were evaluated using Fisher's least significant differences procedures. All analyses were performed using the NCSS statistical software program (Number Cruncher Statistical System; 2000, Jerry Hintze, Kaysville, UT).

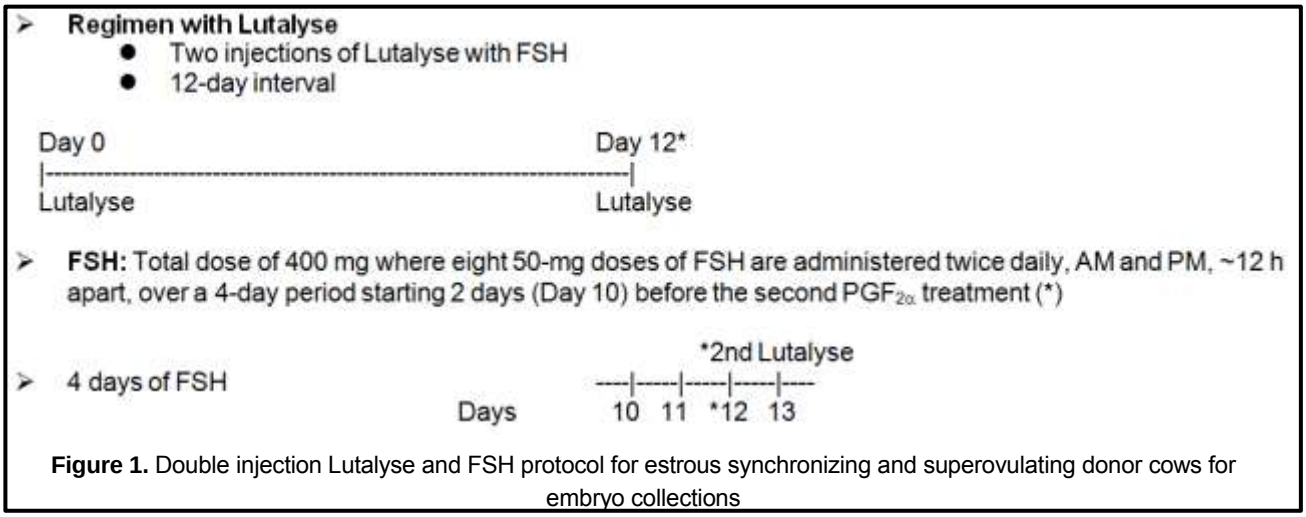

Figure 1. Double injection Lutalyse and FSH protocol for estrous synchronizing and superovulating donor cows for embryo collections

Results

Mean number of ova collected was less (P = 0.08) for cows treated with 200 vs. 400mg of FSH (4.7 ± 2.5 vs. 10.5 ± 1.9, respectively), however no difference (P > 0.10) was observed for cows treated with 100 or 200 µg of GnRH (8.5 ± 2.0 vs. 8.2 ± 2.3, respectively) (Figure 2). The FSH X GnRH interaction was not significant. Mean number of embryos recovered was not different (P > 0.10) between cows treated with 200 vs. 400 mg of FSH (4.1 ± 2.0 vs. 7.8 ± 1.5, respectively) and no difference (P > 0.10) was observed for cows treated with 100 vs. 200 µg of GnRH (7.7 ± 1.6 vs. 4.6 ± 1.8, respectively). The FSH X GnRH interaction was also not significant. Mean number of transferable embryos recovered was not different (P > 0.10) between cows treated with 200 vs. 400 mg of FSH (3.6 ± 1.5 vs. 5.5 ± 1.1, respectively) and no difference (P > 0.10) was observed for cows treated with 100 vs. 200 µg of GnRH (5.8 ± 1.2 vs. 3.4 ± 1.4 respectively). The FSH X GnRH interaction was not significant. Mean number of UFOs collected was less (P = 0.07) for cows treated with 200 vs. 400 mg of FSH (0.6 ± 1.0 vs. 2.8 ± 0.8, respectively) and fewer (P = 0.06) UFOs were recovered from cows treated with 100 vs. 200 µg of GnRH (0.7 ± 0.8 vs. 3.6 ± 0.9, respectively) (Figure 3). The FSH X GnRH interaction was not significant.

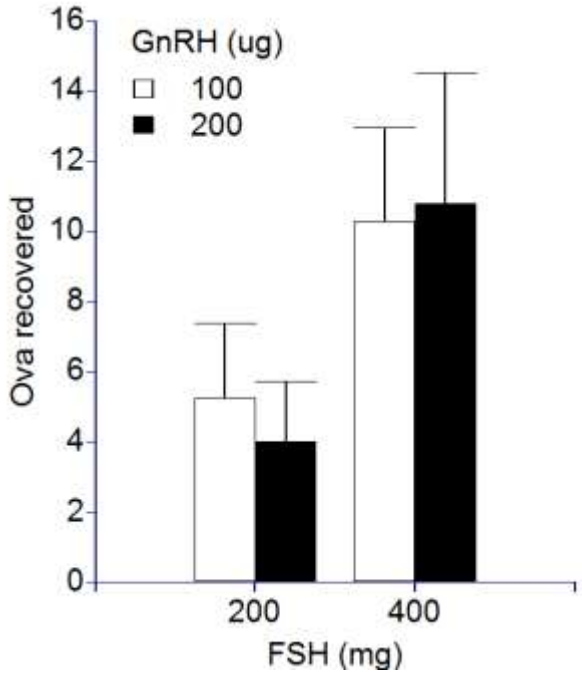

Figure 2. Mean numbers of ova recovered from cows superovulated with two doses of FSH and GnRH.

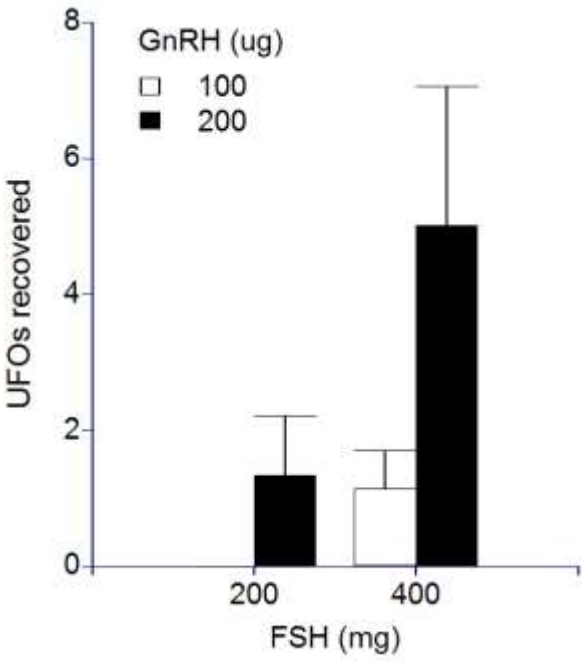

Figure 3. Mean numbers of unfertilized ova (UFOs) recovered from cows superovulated with two doses of FSH and GnRH.

Mean percentages of embryos recovered was not different (P > 0.10) between cows treated with 200 vs 400 mg of FSH (88.6 ± 11.0% vs 72.8 ± 8.4%, respectively) and no difference (P > 0.10) was observed for cows treated with 100 vs 200 µg GnRH (89.0 ± 8.8 vs 72.4 ± 10.3%, respectively). The FSH X GnRH interaction was also not significant. Mean percentage of transferable embryos recovered was greater (P < 0.05) for cows treated with 200 vs 400 mg of FSH (81.6 ± 10.5 vs 52.9 ± 8.0%, respectively), however no difference (P > 0.10) was observed between cows treated with 100 vs 200 µg of GnRH (75.5 ± 8.4 vs 59.1 ± 9.8%, respectively) (Figure 4). The FSH X GnRH interaction was not significant. Mean percentage of unfertilized ova was not different (P > 0.10) between cows treated with 200 vs 400 mg of FSH (11.3 ± 11.0 vs 27.2 ± 8.4%, respectively) and no difference (P > 0.10) was observed between cows treated with 100 vs 200 µg of GnRH (11.0 ± 8.8 vs 27.6 ± 10.3%, respectively). The FSH X GnRH interaction was also not significant.

Conclusions

Although cows superovulated with the standard doses of FSH (400 mg) and GnRH (100 µg) produced more total ova, many were either unfertilized or, if fertilized, not transferrable embryos. These data suggest that the higher dosing

is likely inducing ovulation of poorer quality ova which fail to fertilize and generate competent embryos. Producers involved in embryo transfer may be served better by starting their donors on a reduced dose of FSH then adjusting to a higher dose as the superovulatory response is observed to decrease. The reduced dose not only contributes to a reduced cost but also extends the longevity of a cow to serve as a donor.

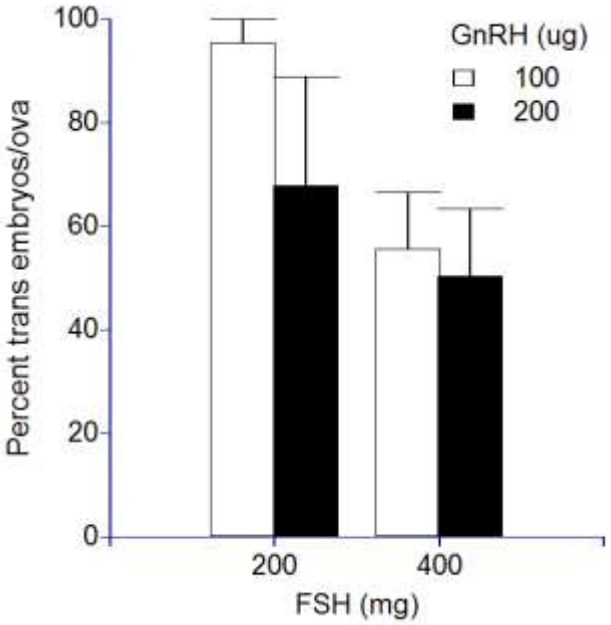

Figure 4. Mean percent transferable (trans) embryos recovered from cows superovulated with two doses of FSH and GnRH.

Acknowledgments

This research was supported by a grant from the Oregon Beef Council.

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Oregon State University

BEEF124

Beef Research Report

Beef Cattle Sciences

Development of a Modified Transfer Medium to Improve Conception Rates in Embryo Recipients [1]

N. Steigerwald[2], M. Gomes[3], A. Snider[4], and A. R. Menino, Jr.[5]

Synopsis

Preliminary results of incubating day-7 cow embryos in a modified transfer medium containing plasmin prior to embryo transfer suggests conception rate is improved in embryo recipients.

Summary

Due to the time and monetary investment in embryo transfer, it is of interest to maximize the resultant pregnancy rates. One reason embryos fail to generate a pregnancy is failure to escape from the embryonic membrane known as the zona pellucida in the "hatching" process. Hatching in the cow occurs on d 10 of pregnancy (where d 0 = onset of estrus) and the uterine enzyme plasmin is known to contribute to this process by partially digesting the zona pellucida. The objective of this research was to determine if culturing embryos in medium containing plasmin prior to embryo transfer would improve hatching rate in vitro and conception rate in embryo recipients. Day 7 cow embryos were incubated for 2 or 16 h in culture medium containing 0, 10, or 100 µg/ml plasmin and either returned to culture in plasmin-free medium to evaluate in vitro hatching or transferred to timed recipients. Hatching rate in vitro was greater for embryos cultured in 100 µg/ml plasmin for 2 h but reduced in embryos cultured for 16 h. Contrary to in vitro results more recipients became pregnant with embryos cultured for 16 h in 100 µg/ml compared to 0 µg/ml plasmin (56 vs. 33%, respectively). These results suggest that culturing embryos in a transfer medium modified with plasmin prior to transfer can improve conception rate. Development of a transfer medium containing plasmin may emerge as a standardized medium to be used in the field for commercial embryo transfer.

Introduction

One cause of early embryonic death is failure to rupture and escape from the zona pellucida in the process known as "hatching". The zona pellucida is a glycoprotein layer laid around the ovum as it develops in the ovary. This layer is important for sperm binding during fertilization and cellular organization in early embryo development but is lost or shed by most species before d 10 of pregnancy. Assisted hatching techniques have been successful in improving pregnancy rates after embryo transfers in many species, and have been studied extensively in human in vitro fertilization. Methods include zona drilling, partial zona cracking, and zona thinning (Hammaded et al., 2011). Many assisted hatching techniques have also been applied to cattle embryos, however these methods require specialized equipment that is impractical for use by veterinarians and technicians who offer mobile embryo recovery and transfer services.

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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Attempts have been made in humans to increase pregnancy rates after embryo transfers by partially digesting the zona pellucida with proteolytic enzymes prior to transfer. Digestion of the zona pellucida with pronase has been shown to enhance post-transfer pregnancy rates of human blastocysts (Fong et al., 1998; Balaban et al., 2002). If a technique for assisted hatching using an enzyme treatment could be refined to eliminate the need for specialized equipment, it could be made available for veterinarians and technicians performing bovine embryo transfer at on-farm locations.

Plasmin is a serine protease that is involved in the events leading to implantation of the early embryo. Plasmin is present in the uterus as inactive plasminogen and is converted to its active form by plasminogen activator (PA) produced by the embryo. Kaaekuahiwi and Menino (1990) demonstrated in cultured cow embryos that zona pellucida thickness was negatively correlated to embryonic PA production and embryos successfully hatching in culture produced more PA than those that did not. Mendoza et al. (unpublished data) reported that cow embryos producing > 0.3 mIU/ml/h PA generated a higher pregnancy rate after transfer than those producing less. The objectives of this experiment were to evaluate in vitro hatching rates of d-7 cow embryos cultured for 2 or 16 h in media containing 0, 10 or 100 µg/ml plasmin and conception rates in recipients. We hypothesized that incubating embryos in medium with plasmin would improve in vitro hatching and conception rates.

Materials and Methods

Donor and recipient cows were housed in a free stall barn with access to pasture when weather permitted. Cows were fed a mixture of grass hay and pasture, and had access to water at all times. In vivo derived bovine embryos were collected at 7 d post-estrus from beef and dairy cows. Cows were estrous synchronized by administering two im injections of 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse®, Pfizer, Inc., New York, NY) 12 d apart, followed by a third im injection of 12.5mg $PGF_{2\alpha}$ on the d 13. Cows were superovulated with twice daily 50-mg injections im of 400 mg porcine follicle stimulating hormone (pFSH; Folltropin®-V, Bioniche, Belleville, ON) on d 10, 11, 12, and 13 after the first $PGF_{2\alpha}$ injection. Cows were heat checked every 12 h after the final FSH injection and artificially inseminated with frozen bull semen at 0, 12, and 24 h after estrus onset. An im injection of 100 µg gonadotropin releasing hormone (GnRH; Fertagyl®, Intervet Inc., Roseland, NJ) was administered at the first breeding. Embryos were recovered by non-surgical uterine flush 7 d after the first breeding and evaluated for morphology and developmental stage microscopically.

Embryos were cultured in 25-µl microdrops for either 2 or 16 h at 39°C in a humidified atmosphere of 5% CO_2 in air in medium containing 0, 10 or 100 µg/ml plasmin. Embryos were removed from the plasmin culture drop, rinsed through two wash drops and placed in a final culture drop of plasmin-free medium. Embryos were returned to culture and observed daily for stage of development.

A subset of embryos designated for embryo transfer were cultured in medium with 0 or 100 µg/ml plasmin for 16 h. Embryos were removed from the plasmin culture drops and loaded into 0.25cc polyvinyl chloride straws. Recipient cows were synchronized with two 5-ml Lutalyse injections on d 0 and 11 of the synchronization program for the embryo donor cows. Embryos were transferred into recipient cows confirmed to be in standing heat on the same day as the donor cows, and confirmed by rectal palpation to have a corpus luteum (CL) on one ovary on the day of transfer. Embryos were transferred into the uterine horn ipsilateral to the CL. Recipients received 100 µg of GnRH at the time of embryo transfer. Rectal palpation was used to diagnose pregnancy in recipient cows at least 38 days after embryo transfer.

Results

Hatching rate in vitro was greater ($P<0.05$) for embryos cultured in 100 µg/ml plasmin for 2 h (Figure 1) but reduced ($P<0.05$) in embryos cultured for 16 h (Figure 2). As we are still in the process of conducting embryo transfers for this project, recipient numbers at this time are limited. To date we have performed 18 transfers with embryos cultured for 16 h in medium containing 0 or 100 µg/ml plasmin for an overall pregnancy rate of 44% (8/18). Contrary to in vitro results where hatching was reduced in embryos incubated for 16 h in medium with 100 µg/ml plasmin, more recipients became pregnant with embryos cultured for 16 h in 100 compared to 0 µg/ml plasmin (56 vs. 33%, respectively). These results suggest that culturing embryos in a medium modified with plasmin prior to transfer can improve conception rate.

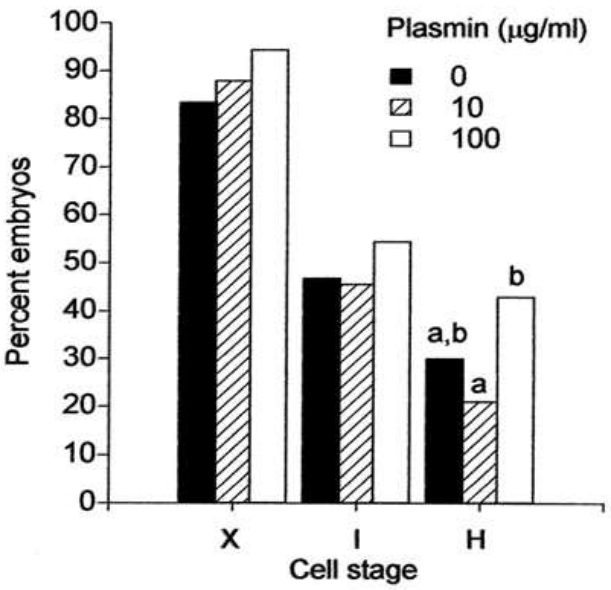

Figure 1. Percent cow embryos developing to the expanded (X), initiating hatching (I) and hatched (H) blastocyst stages following culture for 2 h in medium containing 0, 10 or 100 µg/ml plasmin. [a,b]Percents with different letters differ (P<0.05)..

Conclusions

Preliminary results from these experiments indicate that culturing embryos in plasmin for as little as 2 h increases the percentage of embryos that hatch from the zona pellucida. As the hatching process is a crucial event for establishing pregnancy, assisted hatching techniques such as the one presented have the potential to improve pregnancy rates in embryo transfers. In initial experiments, incubating embryos overnight with 100 µg/ml plasmin increased the success rate of embryo transfers. The objective of future work is to identify a plasmin concentration that improves pregnancy rates after 2 h of culture. Such a protocol would be feasible for veterinarians and embryologists to perform on the farm as part of a mobile bovine embryo transfer service.

Acknowledgments

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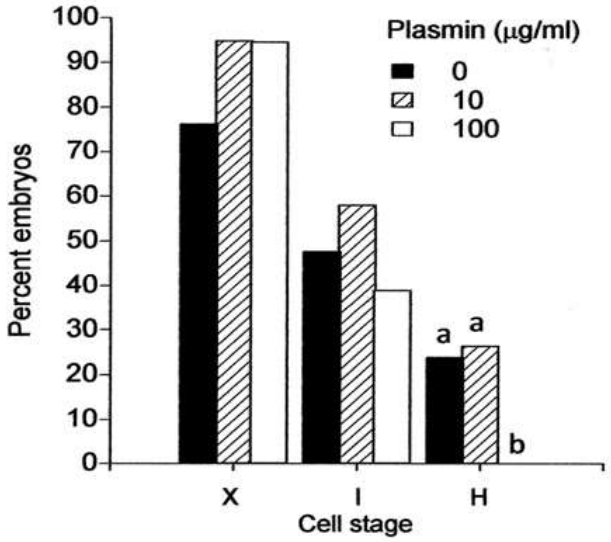

Figure 2. Percent cow embryos developing to the expanded (X), initiating hatching (I) and hatched (H) blastocyst stages following overnight culture in medium containing 0, 10 or 100 µg/ml plasmin. [a,b]Percents with different letters differ (P<0.05).

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Oregon State University

BEEF125

Beef Research Report

Beef Cattle Sciences

Incorporation of Sexed Semen into Reproductive Management of Cow-Calf Operations [1]

R. F. Cooke[2], D. W. Bohnert[2], B. I. Cappellozza[2], R. S. Marques[2], T. DelCurto[3], and C. J. Mueller[3]

Synopsis

Inseminating beef cows with sexed semen may not be a viable option to improve economic returns in cow-calf systems that inseminate and expose the cowherd to a 50-day bull breeding, and subsequently market the calf crop upon weaning.

Summary

The objective of this experiment was to compare reproductive performance and weaning outcomes of beef cows inseminated with sexed or conventional semen. Over 2 consecutive yr, lactating Angus × Hereford cows were assigned to an estrus synchronization + AI protocol. At the time of AI, cows were ranked by parity and assigned to be inseminated with conventional non-sorted semen (**CONV**; n = 454) or with semen sorted for male sperm (**SEXED**; n = 439). Beginning 18 d after AI, cows from both treatments were grouped and exposed to mature bulls for 50 d (1:25 bull to cow ratio). Cow pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography 40 d after AI. Calf birth date, sex, and birth BW were recorded during the subsequent calving season. Cows that were diagnosed as pregnant during the transrectal ultrasonography exam and gave birth during the initial 2 wk of the calving season were considered pregnant to AI. Pregnancy rates to AI and final pregnancy rates (AI + bull breeding) were reduced ($P \leq 0.05$) in SEXED compared with CONV cows. The proportion of male calves born to AI or AI + bull breeding was greater ($P < 0.01$) in SEXED compared with CONV cows. No treatment effect was detected ($P = 0.34$) for weaning rate, whereas SEXED cows had a greater ($P < 0.01$) proportion of steers in the weaned calf crop compared with CONV cows. Steers and heifers from SEXED cows were younger ($P < 0.01$), whereas only SEXED heifers were lighter ($P = 0.05$) at weaning compared with cohorts from CONV cows. Across genders, calves from SEXED cows had reduced ($P \leq 0.01$) weaning age and BW compared with calves from CONV cows. Cows assigned to SEXED had greater ($P = 0.05$) kg of steer weaned/cow exposed to breeding, but reduced kg of heifer weaned/cow exposed to breeding ($P < 0.01$) compared with CONV cows. Across genders, SEXED cows tended ($P = 0.09$) to have reduced kg of calf weaned/cow exposed to breeding compared with CONV cows. In summary, inseminating beef cows with sexed semen reduced pregnancy rates, but increased the proportion of steers weaned and kg of steers weaned/cow exposed to breeding. However, overall kg of calf weaned/cow exposed to breeding was not improved by the use of sexed semen, particularly because of its negative impacts on weaning age and BW of the heifer progeny.

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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Introduction

The major objective of cow-calf systems is to produce 1 calf per cow annually. Therefore, profitability of cow-calf operations is primarily determined by reproductive performannce of the cowherd, which defines the number of calves born and weaned annually (Wiltbank et al., 1961). Economic returns in cow-calf systems can also be increased by adding quality and value to the weaned calf crop, which can be accomplished via breeding strategies such as inseminating the cowherd with sexed semen. More specifically, steers have greater weaning and yearling BW compared with contemporary heifers (Koch and Clark, 1955; Koger and Knox, 1945). In addition, average value/kg of live BW was 10 % greater for feeder steers compared with feeder heifers during the last 5 yr in the U.S. (USDA-Agricultural Marketing Service, 2013). Therefore, we hypothesized that inseminating beef cows with semen sorted for male sperm benefits economic returns in cow-calf operations by increasing the proportion of steers available for marketing after weaning.

Nevertheless, early research demonstrated that sexed semen yield reduced pregnancy rates when compared to conventional semen (Seidel, 2007), which may prevent optimal reproductive performance of the cowherd and annul the potential benefits on calf crop value. However, with recent advances in semen sorting and freezing, some research has suggested that pregnancy rates to sexed semen are improving and reaching comparable results to conventional semen, although additional studies with larger groups of beef cattle are warranted to validate this outcome. Further, no research has assessed the impacts of inseminating beef cows with sexed semen on calf crop performance and overall weaning returns in cow-calf systems. Therefore, the objective of this experiment was to compare reproductive performance and weaning outcomes of lactating beef cows inseminated with sexed or conventional semen.

Materials and Methods

This experiment was conducted over 2 consecutive yr (2011 and 2012) at the Oregon State University (OSU) – Eastern Oregon Agricultural Research Center (EOARC; Burns station and Union station). In 2011, a total of 441 lactating Angus × Hereford cows were enrolled in the experiment (Burns, n = 209 multiparous and 34 primiparous; Union, n = 149 multiparous and 49 primiparous). In 2012, a total of 452 lactating Angus × Hereford cows were enrolled (Burns, n = 196 multiparous and 49 primiparous; Union, n = 160 multiparous and 47 primiparous). Moreover, 345 cows were used in both yr (Burns, n = 194, Union, n = 151), 96 cows were used only in 2011 (Burns, n = 49, Union, n = 47), and 107 cows were used only in 2012 (Burns, n = 51, Union, n = 56). All cows and calves utilized herein were managed as described by Cooke et al. (2012), and cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Animals and treatments. All cows were assigned to an estrus synchronization + AI protocol. Cows received 100 µg of GnRH (Factrel; Zoetis, Florham Park, NJ, USA) plus a controlled internal device release (CIDR) containing 1.38 g of progesterone (Zoetis), followed in 7 d with 25 mg of prostaglandin $F_{2\alpha}$ (Lutalyse; Zoetis) and CIDR removal, followed in 60 h by a second 100 µg injection of GnRH and AI. At the time of AI, cows were ranked by parity and assigned to be inseminated with conventional non-sorted semen (**CONV**; n = 360 multiparous and 94 primiparous; Genex Cooperative, Inc., Shawano, WI, USA) or with semen sorted for male sperm (**SEXED**; n = 354 multiparous and 85 primiparous; GenChoice 90™, Genex Cooperative, Inc.). At the Union station, cows that displayed estrus beginning after the prostaglandin $F_{2\alpha}$ injection and until 24 h before the second GnRH injection were inseminated 12 h after estrus detection (n = 56 for CONV and 51 for SEXED), whereas all other cows were timed-AI at the time of the second GnRH injection (n = 151 for CONV and 147 for SEXED). The CONV semen contained approximately 20 million non-sorted sperm cells per straw, whereas SEXED contained approximately 2.1 million sperm cells per straw with 90 % of these sperm cells expected to be male sperm (Rath and Johnson, 2008). Within each yr and location, cows were inseminated by the same technician with CONV or SEXED originated from the same bull. The Burns station cowherd was inseminated with semen from Club King (1SM00115, Genex Cooperative, Inc.) in 2011 and Upgrade (1SM00121; Genex Cooperative, Inc.) in 2012, whereas the Union station cowherd was inseminated with semen from Chisum (1AN01170; Genex Cooperative, Inc.) during both yr. Beginning 18 d after AI, all cows from both treatments were grouped and exposed to mature Angus and Hereford bulls (age = 5.6 ± 0.4 yr) for 50 d (1:25 bull to cow

ratio). All bulls utilized in this experiment were submitted to and approved by a breeding soundness evaluation (Chenoweth and Ball, 1980) before the breeding season.

Sampling. Cows were evaluated for BCS at the time of AI (Wagner et al., 1988). Blood samples were collected concurrently with AI and 7 d later for determination of plasma progesterone concentration to assess estrus synchronization rate. Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin (158 USP units), placed on ice immediately, and centrifuged at 2,400 × g for 30 min at room temperature for plasma collection. Plasma was stored at −80°C on the same day of collection. Plasma progesterone concentration was determined as described by Munro and Stabenfeldt (1984) with modifications described by Galvão et al. (2004). The intra- and inter-assay CV were 7.8 and 6.0 %, respectively, whereas assay sensitivity was 0.05 ng/mL. Cows with plasma progesterone concentration < 1.0 ng/mL at AI, but ≥ 1.0 ng/mL 7 d later were classified as responsive to the estrus synchronization protocol.

Cow pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka) 40 d after AI. During the subsequent calving season, calf birth date, sex, and birth BW were recorded. Pregnancy loss during gestation was not accounted for in the present experiment because cow pregnancy status was not evaluated after the end of bull breeding. Hence, all cows that gave birth during the calving season were classified as becoming pregnant during the experiment. Calf paternity (AI or bull breeding) was determined according to transrectal ultrasonography and birth date. Only cows that were diagnosed as pregnant during the transrectal ultrasonography exam and gave birth during the initial 2 wk of the calving season were considered pregnant to AI. Calves that died at birth or up to weaning were accounted for as calf loss from birth to weaning. No incidences of dystocia were observed in the present experiment. Calf BW was determined again at weaning, whereas 205-day adjusted weaning BW was calculated according to BIF (2010). Calf weaning value was estimated based on US$/kg of BW within 45.5 kg increments for feeder steers and heifers (from 114 to 386 kg of BW), according to the latest 5-yr U.S. average (2008 to 2012; USDA-Agricultural Marketing Service, 2013).

Statistical analysis. All data were analyzed with cow as the experimental unit and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. All quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). Model statements contained the effects of treatment, parity, yr, location, calf gender (for analyses containing calf parameters), and all resultant interactions. The random statements contained the effect of cow (treatment × location × yr × parity), or cow(treatment × location × yr × parity × calf gender) for analyses containing calf parameters. However, analyses of cow age at AI, as well as quantitative and binary data containing 205-day weaning BW, did not contain the effects of parity in the model and random statements. Pregnancy rates to AI at the Union station were also analyzed using a model statement containing the effects of treatment, parity, yr, AI method (after estrus detection or fixed-time AI), all resultant interactions, and with a random statement containing the effect of cow(treatment × yr × parity × AI method). Results are reported as least square means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

Results

Overall reproductive results

No treatment effects were detected ($P \geq 0.51$) for cow BCS and age at AI, as well as estrus synchronization rate (Table 1). Hence, all treatment effects reported herein were independent of these parameters.

Table 1. Age, BCS, synchronization rate, and pregnancy rates to AI in cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.[1]

Item	SEXED	CONV	SEM	*P*=
Age, yr	5.43	5.47	0.13	0.83
BCS at AI	4.75	4.73	0.03	0.45
Synchronization rate, %	84.6	84.5	1.7	0.96

[1] Parenthesis = number of cows divided by total cows.

Pregnancy rates to AI were reduced in SEXED compared with CONV cows (Table 2), independently if analysis contained all cows exposed

to AI ($P < 0.01$) or only cows that were effectively synchronized to the estrus synchronization protocol ($P < 0.01$). Within the Union station, SEXED cows had reduced ($P \leq 0.05$) pregnancy rates to AI compared with CONV cows independently if cows were inseminated 12 h after estrus detection (53.6 vs. 74.5 % for all cows, SEM = 6.7 %; 57.4 vs. 76.0 % for synchronized cows, SEM = 6.7 %; for SEXED and CONV, respectively) or without estrus detection at fixed-time AI (42.6 vs. 56.3 % for all cows, SEM = 4.0 %; 48.9 vs. 68.5 % for synchronized cows, SEM = 4.2 %; for SEXED and CONV, respectively). Accordingly, previous research reported substantial decreases in pregnancy rates to AI when beef or dairy females are inseminated with sex-sorted semen, either at fixed-time AI or upon estrus detection (Sá Filho et al., 2012; Seidel et al., 1999). These outcomes are mostly attributed to sperm damage associated with the sorting and cryopreservation processes, which reduces the viability and quality of the sorted sperm (Seidel, 2007).

Table 2. Pregnancy rates to AI, bull breeding, final pregnancy rates (AI + bull breeding), and proportion of male calves born from cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.

Item	SEXED	CONV	*P*=
Pregnancy rates to AI, %			
All cows	34.9	56.0	< 0.01
Synchronized cows	40.6	66.1	< 0.01
Pregnancy rates to bull, %	74.6	72.0	0.51
Final pregnancy rates,[2] %	83.5	87.9	0.05
Pregnancies to AI, %	41.6	63.8	< 0.01
Pregnancies to bull, %	58.4	36.2	< 0.01
Proportion of males calves, [3] %			
Pregnancies to AI,	91.2	57.3	< 0.01
Pregnancies to bull,	48.2	52.9	0.38
All pregnancies	65.5	55.3	< 0.01

[1] Within parenthesis, number of cows divided by total cows. Beginning 18 d after AI, cows were exposed to mature Angus and Hereford bulls for 50 d.
[2] Within cows classified as pregnant, the proportion of cows pregnant to AI or bull breeding.
[3] Proportion of males calves in the calf crop sired by AI (pregnancies to AI), bull breeding (pregnancies to bull breeding), or AI + bull breeding (all pregnancies).

Within cows that did not become pregnant to AI, pregnancy rates to bull breeding were similar ($P = 0.51$) between CONV and SEXED cows (Table 2). Given that the bull to cow ratio was approximately 1:25 (36 bulls and 893 cows exposed in the experiment), and a total of 492 cows did not become pregnant to AI (Table 2), the actual bull to non-pregnant cow ratio was 1:14. Hence the number of bulls available to service non-pregnant cows was above the recommended ratio for a 50-day breeding season (Healy et al., 1993; Pexton et al., 1990), which likely contributed to the similar pregnancy rates to bull breeding between CONV and SEXED cows. However, final pregnancy rates (AI + bull breeding) were also reduced ($P = 0.05$) for SEXED compared with CONV cows (Table 2). Within pregnant cows only, SEXED cows had a reduced ($P < 0.01$) proportion of pregnancies to AI and hence greater ($P < 0.01$) proportion of pregnancies to bull breeding compared with CONV cows (Table 2).

Calving results.

Within pregnant cows to AI, the proportion of male calves born was greater ($P < 0.01$) in SEXED compared with CONV cows (Table 2). No differences were detected ($P = 0.38$) in the proportion of male calves from cows pregnant to bull breeding (Table 2). Accordingly, pregnant SEXED cows also had a greater ($P < 0.01$) proportion of male calves at the end of the calving season (AI + bull breeding) compared with pregnant CONV cows (Table 2). Calves from SEXED cows had greater ($P = 0.05$) birth BW compared with calves from CONV cows (Table 3). However, SEXED and CONV cows had similar ($P = 0.19$) kg of calf born/cow exposed to breeding (Table 3). The proportion of male calves born to AI in SEXED cows was in accordance with the expected male to female ratio yielded by the semen sorting process (Rath and Johnson, 2008), which increased the final proportion of male calves born to SEXED compared with CONV cows during calving season (Table 2).

Weaning results – calf parameters.

No treatment effects were detected ($P \geq 0.31$) for calf loss from birth to weaning and weaning rate (Table 3). The proportion of steers weaned was greater ($P < 0.01$) in SEXED compared with CONV cows (Table 3).

A treatment × calf gender interaction was detected ($P < 0.01$) for calf age, BW, and estimated value at weaning. Steers from SEXED cows were younger ($P < 0.01$) compared with steers from CONV cows (Table 4), whereas no treatment effects were detected for steer BW and estimated value at weaning ($P \geq 0.75$). Heifers from SEXED cows were lighter ($P = 0.05$), younger ($P < 0.01$), and had

reduced ($P < 0.01$) estimated value at weaning compared with heifers from CONV cows (Table 4). Across genders, calves from SEXED cows had reduced ($P \leq 0.01$) weaning age and BW compared with calves from CONV cows (Table 3), while estimated calf value at weaning did not differ ($P = 0.24$) between treatments (Table 4).

A treatment × calf gender interaction was also detected ($P < 0.01$) for 205-d adjusted BW and subsequent estimated weaning value. Heifers from SEXED cows also had reduced 205-day adjusted weaning BW ($P < 0.01$) and estimated weaning value ($P = 0.01$) compared with heifers from CONV cows, whereas these parameters were similar ($P \geq 0.78$) among steers from CONV and SEXED cows (Table 4). Across genders, calves from SEXED cows had similar 205-day adjusted BW ($P = 0.25$; Table 3) and estimated weaning value compared with calves from CON cows ($P = 0.27$; Table 4).

Cow-calf production parameters.

A treatment × calf gender interaction was detected ($P < 0.01$) for kg of calf weaned/cow exposed to breeding. Cows assigned to SEXED had greater ($P = 0.05$) kg of steer weaned/cow exposed to breeding, but reduced kg of heifer weaned/cow exposed to breeding ($P < 0.01$) compared with CONV cows (Table 3). Across genders, SEXED cows tended ($P = 0.09$) to have reduced kg of calf weaned/cow exposed to breeding compared with CONV cows (Table 3), whereas estimated calf value/cow exposed to breeding did not differ between treatments ($P = 0.22$ Table 4).

A treatment × calf gender interaction was also detected ($P < 0.01$) for 205-day adjusted kg of calf weaned/cow exposed to breeding. Cows assigned to SEXED had greater ($P = 0.05$) 205-day adjusted kg of steer weaned/cow exposed to breeding, but reduced 205-day adjusted kg of heifer weaned/cow exposed to breeding ($P < 0.01$) compared with CONV cows (Table 3). Across genders, no treatment effects were detected ($P \geq 0.25$) for 205-day adjusted kg of calf weaned/cow exposed to breeding (Table 3) or estimated calf value/cow exposed to breeding (Table 4).

It is important to note that this experiment did not account for any additional costs associated with purchasing sexed semen (Seidel, 2007), which may impact the economical returns of cows inseminated with sexed-semen. Nevertheless, results from this experiment suggest that inseminating beef cows with sexed semen does not improve economic returns in cow-calf operations that market the calf crop upon weaning.

Conclusions

Inseminating beef cows with sexed semen reduced pregnancy rates to AI and final pregnancy rates (AI + 50-day bull breeding), but increased the proportion of steers weaned and kg of steers weaned/cow exposed to breeding. However, overall kg of calf weaned/cow exposed to breeding and estimated calf value/cow exposed to breeding were not improved by the use of sexed semen, particularly because of its negative impacts on weaning age and BW of the heifer progeny. Based on these results, inseminating beef cows with sexed semen may not be a viable option to improve economic returns in cow-calf systems that inseminate and expose the cowherd to a 50-day bull breeding, and subsequently market the calf crop upon weaning.

Acknowledgments

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Table 3. Calf and cow-calf performance parameters from cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.[1]

Item	SEXED	CONV	*P*-value
Calf parameters			
Birth BW, kg	40.4	39.6	0.05
Weaning age, kg	206.4	212.6	< 0.01
Weaning BW, kg	239.3	245.6	0.01
205-day adjusted weaning BW,[2] kg	246.4	247.5	0.56
Cow-calf production parameters [3]			
Kg of calf born per cow exposed to breeding, kg	33.8	35.1	0.19
Calf loss from birth to weaning, %	4.8 (17/367)	6.5 (27/400)	0.31
Weaning rate, %	79.5 (350/439)	82.0 (373/454)	0.34
Proportion of steers weaned, %	66.1 (232/350)	56.2 (210/373)	< 0.01
Kg of calf weaned/cow exposed to breeding, kg			
Steers, kg	130.4	115.2	0.05
Heifers, kg	59.5	85.8	< 0.01
Overall	189.9	201.0	0.09
205-day adjusted kg of calf weaned/cow exposed to breeding,[2] kg			
Steers, kg	133.1	116.7	0.05
Heifers, kg	62.4	86.4	< 0.01
Overall	195.5	203.2	0.25

[1] Within parenthesis, number of cows divided by total cows.
[2] Calculated according to BIF (2010).
[3] Kilograms of calf born and calf weaned per cow exposed to breeding were calculated based on calving rate, weaning rate, and calf BW at birth and weaning.

Table 4. Estimated weaning economical returns, based on original or 205-day adjusted calf weaning BW, from cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.[1]

Item	SEXED	CONV	*P*-value
Original calf weaning BW			
Weaned steers			
Age at weaning, d	210.6	213.0	< 0.01
Weaning BW, kg	250.3	250.9	0.84
Calf value, US$	641.6	643.3	0.75
Weaned heifers			
Age at weaning, d	197.7	212.3	< 0.01
Weaning BW, kg	218.3	238.4	< 0.01
Calf value, US$	529.1	565.3	< 0.01
Overall			
Value per calf, US$	603.2	609.5	0.24
Calf value/cow exposed to breeding, US$	479.0	499.3	0.22
205-day adjusted calf weaning BW[2]			
Weaned steers			
Weaning BW, kg	253.7	253.5	0.95
Calf value, US$	646.3	645.2	0.78
Weaned heifers			
Weaning BW, kg	232.4	239.7	< 0.01
Calf value, US$	553.6	564.7	0.01
Overall			
Calf value, US$	614.7	610.0	0.27
Calf value/cow exposed to breeding, US$	488.3	500.7	0.45

[1] Within parenthesis, number of cows divided by total cows.
[2] Calculated according to BIF (2010).

Oregon State University

BEEF126

Beef Research Report

Beef Cattle Sciences

Effects of Meloxicam Administration on Physiological and Performance Responses of Transported Feeder Cattle [1]

T. Guarnieri Filho[2], R. F. Cooke[2,3], B. Cappellozza[2], M. Reis[2], R. S. Marques[2], and D. W. Bohnert[2]

Synopsis

Meloxicam administration may be a viable strategy to mitigate inflammatory reactions and performance losses elicited by long-distance transportation.

Summary

Eighty-four Angus × Hereford steers were ranked by BW on d -10, and assigned to 21 drylot pens. From d -10 to 0, pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of a corn-based concentrate. On d 0, pens were randomly assigned to transport for 1,440 km in a livestock trailer and receive meloxicam (**MEL**; 1 mg/kg of BW daily; n = 7) or lactose monohydrate (**TRANS**; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or no transport receiving lactose monohydrate concurrently with treatment administration to MEL and TRANS (**CON**; 1 mg/kg of BW daily; n = 7). Upon arrival (d 1), MEL and TRANS steers returned to their original pens for a 21-d feedlot receiving with the same pre-transport diet. Treatments were administered via individual oral drench on d 0 and 1, or mixed with the concentrate from d 2 to 7. Full BW was recorded prior to (d -2, -1, and 0) transport and at the end of experiment (d 20, 21, and 22) for ADG calculation. Daily DMI was recorded from d 1 to 21. Blood samples were collected on d 0, 1, 3, 5, 7, 10, 14, and 21. During the initial 7 d of feedlot receiving, hay and total DMI were reduced ($P \leq 0.03$) in TRANS vs. CON and MEL, similar between CON and MEL ($P \geq 0.26$), whereas concentrate DMI did not differ ($P = 0.16$) among treatments. Mean ADG and G:F were reduced ($P \leq 0.03$) in TRANS vs. MEL and CON, but similar ($P \geq 0.39$) between MEL and CON. Serum NEFA concentrations were greater ($P < 0.01$) for TRANS and MEL vs. CON on d 1. Plasma haptoglobin concentrations were greater ($P \leq 0.03$) for TRANS vs. CON and MEL on d 5, and greater ($P \leq 0.03$) for CON vs. TRANS on d 10. Plasma ceruloplasmin concentrations were greater ($P \leq 0.04$) for TRANS vs. CON on d 3, 5, 7, 10, and 14, greater ($P \leq 0.03$) for TRANS vs. MEL on d 5 and 7, and also greater ($P = 0.05$) for MEL vs. CON on d 3. Hence, meloxicam administration to feeder steers reduced the acute-phase protein response and prevented the performance losses caused by long-distance transportation.

Introduction

Road transport is one of the most stressful events encountered by feeder cattle during their productive lives. Upon long-distance transportation and feedlot arrival, cattle experience inflammatory and acute-phase responses (Arthington et al., 2008; Cooke et al., 2011) that impact feedlot receiving performance by increasing basal metabolism, tissue catabolism, and by reducing DMI and G:F (Johnson, 1997). Hence, strategies that lessen the acute-phase

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response during feedlot receiving, which can be monitored via acute-phase proteins such as haptoglobin and ceruloplasmin (Carroll and Forsberg, 2007), improve productivity of transported cattle (Arthington et al., 2008).

Administration of flunixin meglumine to steers prior to a 24-h road transport and at feedlot arrival alleviated the resultant acute-phase response but did not improve feedlot receiving performance (Cooke et al., 2013a). Perhaps the elimination half-life of flunixin meglumine (less than 8 h; Odensvik and Johansson, 1995) was insufficient to modulate the transport-elicited acute-phase response to an extent that resulted in enhanced cattle performance. Alternatively, meloxicam has an elimination half-life of 28 h (Coetzee et al., 2009) when orally administered to cattle at 1 mg/kg. Accordingly, Van Engen et al. (2014) reported that oral administration of meloxicam to cattle prior to a 16-h road transport reduced transport-induced inflammatory reactions, although authors did not evaluate feedlot receiving performance. Based on this rationale, we hypothesized that oral meloxicam administration prior to transport and during feedlot receiving alleviates the acute-phase response and improves performance of feeder cattle. Hence, the objective of this experiment was to evaluate the effects of oral meloxicam administration on circulating concentrations of cortisol, NEFA, acute-phase proteins, and feedlot receiving performance of transported cattle.

Materials and Methods

Animals and diets. Eighty-four Angus × Hereford steers, weaned 40 d prior to the beginning of the experiment (d -10), were utilized. On d -10, steers were ranked by BW (252 ± 3 kg; initial age 214 ± 2 d) and randomly allocated to 21 dry lot pens (4 steers/pen; 7 × 15 m) in manner which all pens had equivalent average BW. From d -10 to 0, all pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix, which was offered separately from hay at 0800 h. On d 0, pens were randomly assigned to 1 of 3 treatments: 1) transport for 1,440 km in a commercial livestock trailer and oral administration of meloxicam (1 mg/kg of BW; Carlsbad Technologies, Inc., Carlsbad, CA) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving (**MEL**; n = 7), 2) transport for 1,440 km in a commercial livestock trailer and oral administration of lactose monohydrate (1 mg/kg of BW, excipient used in the manufacture of meloxicam tablets; Avantor Performance Materials, Center Valley, PA) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving (**TRANS**; n = 7), or 3) no transport and oral administration of lactose monohydrate (1 mg/kg of BW; Avantor Performance Materials) concurrently with treatment administration to MEL and TRANS steers (**CON**; n = 7).

On d 0 of the experiment. MEL and TRANS steers were commingled and transported at the same time and in the same double-deck commercial livestock trailer (Legend 50’ cattle liner; Barrett LLC., Purcell, OK), while CON steers remained in their respective drylot pens with ad libitum access to alfalfa-grass hay and 2.4 kg/steer (DM basis) of the aforementioned concentrate. Upon arrival (d 1), MEL and TRANS steers returned to their original pens for a 21-d feedlot receiving. All pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of the aforementioned corn-based concentrate during the receiving period, which was offered separately from hay at 0800 h. Water was offered for ad libitum consumption from d -10 to 28, except to MEL and TRANS cattle during transport.

Meloxicam was originally presented in 15 mg tablets, which were ground daily using a commercial food processor (Soho Food Processor; West Bend Housewares, West Bend, WI) to ensure that MEL steers received their exact dose. Lactose monohydrate was administered to TRANS and CON steers to account for potential placebo effects, whereas the CON treatment was included as a non-transport positive control for physiological and performance measurements. On d 0 and 1, meloxicam or lactose monohydrate were manually mixed with 50 mL of 0.9% saline and administered individually to steers via oral drench during handling of MEL and TRANS for truck loading (d 0) or feedlot arrival (d 1). Treatments were mixed with saline within 30 s prior to administration. From d 2 to 7, treatments were mixed daily with the corn-based concentrate according to the total BW of each pen.

Sampling. Individual full BW was recorded and averaged over 3 consecutive days prior to treatment application (d -2, -1, and 0) and at the end of experiment (d 20, 21, and 22) for ADG calculation. Average BW of d -2, -1, and 0 was used to determine meloxicam and lactose monohydrate doses. Individual BW was also collected on d 1,

immediately prior treatment application, to evaluate BW shrink as percentage change from the average BW recorded on d -2, -1, and 0. Concentrate, hay, and total DMI were evaluated daily from d -10 to 21 from each pen by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of steers within each pen, and expressed as kg per steer/d. Total BW gain and DMI of each pen from d 1 to 21 were used for feedlot receiving G:F calculation.

Blood samples were collected on d 0 and 1 immediately before treatment application, and on d 3, 5, 7, 10, 14, and 21 via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without 158 USP units of freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were collected prior to concentrate feeding, except for d 0 when MEL and TRANS cattle were transported after blood collection. All blood samples were placed immediately on ice, centrifuged (2,500 × g for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Plasma concentrations of cortisol, NEFA, haptoglobin, and ceruloplasmin were determined as in Cooke et al., (2013a). The intra- and inter-assay CV were, respectively, 3.8 and 3.4% for cortisol, 4.3 and 6.5% for NEFA, 9.1 and 9.0% for ceruloplasmin, and 6.9 and 7.9% for haptoglobin.

Statistical analysis. Data were analyzed using pen as the experimental unit, with the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment. Data were analyzed using pen(treatment) and steer(pen) as random variables. The model statement used for DMI and G:F contained the effects of treatment, in addition to day, the treatment × day interaction, and average feed intake from d -10 to -1 as covariate for DMI only. Data were analyzed using pen(treatment) as the random variable because DMI was recorded from each pen. The model statement used for blood variables contained the effects of treatment, day, the treatment × day interaction, and values obtained on d 0 as covariate. Data were analyzed using steer(pen) and pen(treatment) as random variables. The specified term for the repeated statements was day, with pen(treatment) or steer(pen) as subject for DMI or blood variables, respectively. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. Results are reported as least square means, as well as covariately adjusted least square means for DMI and blood variables, and were separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effect if no interactions were significant, or according to the highest-order interaction detected that contained the treatment effect.

Results

A treatment effect was detected ($P < 0.01$) for BW shrink from d 0 to 1. As expected, BW shrink was greater ($P < 0.01$) for both TRANS and MEL compared with CON steers, and similar ($P = 0.14$) between TRANS and MEL steers (Table 1). Previous research from our group reported equivalent BW shrink in feeder cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012; Cooke et al., 2013b). No treatment effects were detected ($P \geq 0.13$) on hay, concentrate, and total DMI during the 21-d feedlot receiving (Table 1). Although treatment × day interactions were also not detected for intake parameters ($P \geq 0.15$), TRANS had reduced hay and total DMI during the initial 7 d of feedlot receiving compared with CON ($P \leq 0.03$) and MEL ($P \leq 0.01$) steers (5.43, 5.36, and 5.05 kg/steer daily of hay DMI, SEM = 0.09, and 7.85, 7.71, and 7.40 kg/steer daily of total DMI, SEM = 0.09; respectively). Moreover, hay and total DMI during the initial 7 d of feedlot receiving were similar between MEL and CON steers ($P \geq 0.26$), whereas concentrate intake during this period did not differ ($P = 0.16$) among treatments (2.39, 2.37, and 2.36 kg/steer daily for CON, MEL, and TRANS steers, respectively; SEM = 0.02). No treatment effects were detected for hay, concentrate, and total DMI during the second ($P \geq 0.42$) and third ($P \geq 0.28$) weeks of feedlot receiving (data not shown). These results indicate that all pens readily consumed their daily concentrate allocation, hence their designed meloxicam and lactose monohydrate dose, during the initial 7 d of feedlot receiving. These results also suggest that oral meloxicam administration prevented the decrease in feed intake often observed in transported cattle

during the first week of feedlot receiving (Hutcheson and Cole, 1986; Araujo et al., 2010).

A treatment effect was detected ($P = 0.04$) for ADG during the 21-d feedlot receiving (Table 1). Steers assigned to TRANS had reduced ADG compared with MEL ($P = 0.03$) and CON ($P = 0.01$) steers, whereas ADG was similar between ($P = 0.82$) CON and MEL steers. However, treatment effects detected on ADG were not sufficient to impact ($P = 0.78$) cattle BW at the end of the 21-d feedlot receiving (Table 1). Nevertheless, a treatment effect was detected ($P = 0.03$) for G:F during the 21-d feedlot receiving because TRANS had reduced G:F compared with MEL ($P = 0.05$) and CON steers ($P = 0.01$), whereas G:F was similar ($P = 0.39$) between MEL and CON steers (Table 1). Hence, feedlot receiving performance of MEL was similar to CON and greater than TRANS steers, indicating that oral meloxicam administration prevented the performance losses typically observed in cattle transported for long-distances (Hutcheson and Cole, 1986; Marques et al., 2012; Cooke et al., 2013b).

Table 1. Feedlot receiving performance (21 d) of steers transported for 1,440 km and receiving meloxicam (**MEL**; 1 mg/kg of BW daily; n = 7) or lactose monohydrate (**TRANS**; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or non-transported steers that concurrently received lactose monohydrate (**CON**; 1 mg/kg of BW daily; n = 7).

Item	CON	MEL	TRANS	*P*=
BW, kg				
Initial	259.9	260.4	260.3	0.99
Final	291.5	292.9	287.7	0.78
Shrink, %	-0.71[a]	9.07[b]	9.83[b]	< 0.01
ADG, kg/d	1.50[a]	1.48[a]	1.26[b]	0.03
DMI, kg/d				
Hay	5.90	5.98	5.75	0.19
Concentrate	2.39	2.38	2.38	0.18
Total	8.33	8.34	8.10	0.13
G:F, g/kg	185[a]	177[a]	153[b]	0.03

[1]Within rows, values with different superscripts differ ($P \leq 0.05$).

No treatment effect was detected ($P = 0.89$) for plasma cortisol concentrations during the 21-d feedlot receiving (21.1, 20.6, and 20.4 ng/mL for CON, MEL, and TRANS, respectively; SEM = 1.0). The impact of long-distance transportation on cortisol has been variable, with research studies reporting increased or unaltered circulating cortisol concentrations following transport (Swanson and Morrow-Tesch, 2001). However, previous research from our group reported increased plasma cortisol concentrations during feedlot receiving in cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012; Cooke et al., 2013a; Cooke et al., 2013b). Hence, the lack of treatment effects on plasma cortisol in the present experiment, particularly between CON and TRANS steers, was unexpected and may have hindered proper assessment of meloxicam effects on transport-induced plasma cortisol response. Nevertheless, Van Engen et al. (2014) also did not detect significant differences in plasma cortisol concentrations during feedlot receiving between steers transported for 16 h and orally administered meloxicam or a whey protein placebo prior to transport.

A treatment × day interaction was detected for serum NEFA ($P < 0.01$; Figure 1), given that NEFA concentrations were greater ($P < 0.01$) for TRANS and MEL compared with CON steers on d 1 of feedlot receiving. These results corroborate that stress due to long-distance transport stimulates fat tissue mobilization and increases circulating NEFA concentration in cattle (Marques et al., 2012), whereas oral meloxicam administration did not alleviate this outcome. Newby et al. (2013) also administered meloxicam (0.5 mg/kg of BW) subcutaneously to Holstein cows approximately 24 h after parturition, and reported that serum NEFA concentrations during the initial 12 d of lactation were similar compared with cohorts receiving saline. Hence, meloxicam administration appears not to modulate lipid mobilization in cattle upon stress and nutritional challenges.

A treatment × day interaction was detected for plasma haptoglobin ($P < 0.01$; Figure 1), whereas a tendency ($P = 0.09$; Figure 1) for the same interaction was detected for plasma ceruloplasmin. Corroborating the ADG, DMI, G:F, and physiological differences detected between TRANS and CON steers, previous research from our group also reported that 24-h road transport elicited an acute-phase response that reduced feedlot receiving performance of feeder cattle (Cooke et al., 2012; Marques et al., 2012; Cooke et al., 2013b). Accordingly, circulating concentrations of acute-phase proteins in transported cattle have been negatively associated with receiving ADG (Qiu et al., 2007; Araujo et al., 2010), and such outcome can be attributed to altered basal metabolism, increased tissue catabolism, and reduced feed intake and efficiency during an acute-phase response (Johnson, 1997). The reason why plasma haptoglobin concentrations increased on d 10 of feedlot receiving in CON, but not MEL and TRANS steers, is

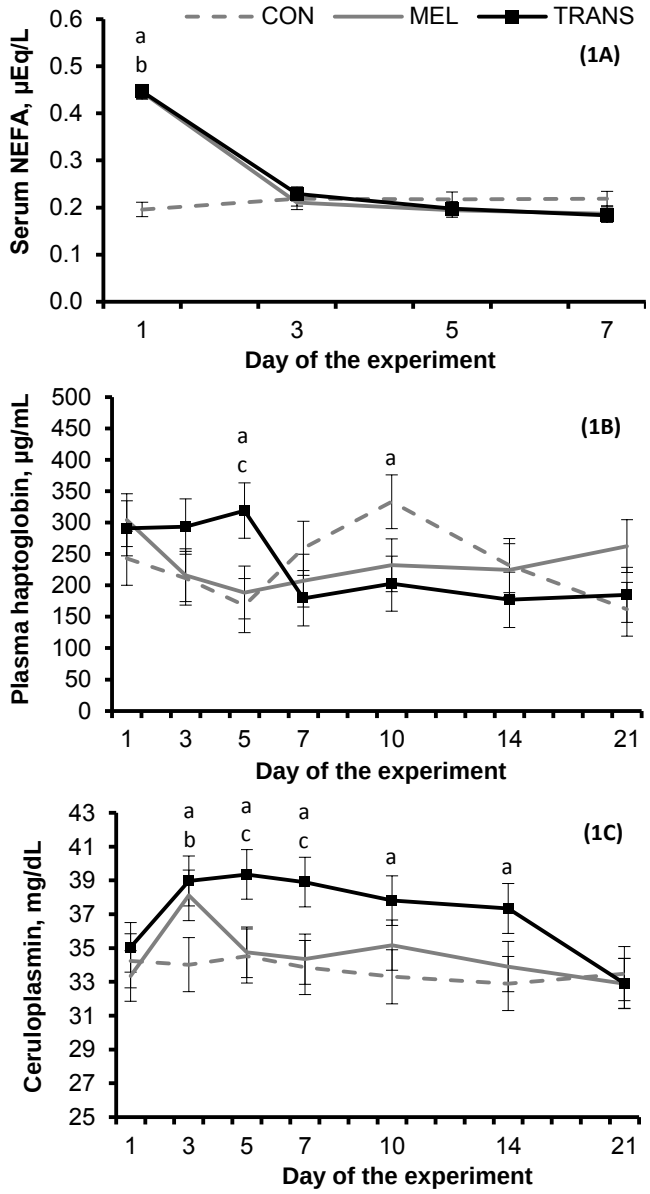

Figure 1. Concentration of serum NEFA (1A), plasma haptoglobin (1B) and ceruloplasmin (1C) in steers transported for 1,440 km and receiving meloxicam (**MEL**; 1 mg/kg of BW daily; n = 7) or lactose monohydrate (**TRANS**; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or non-transported steers that concurrently received lactose monohydrate (**CON**; 1 mg/kg of BW daily; n = 7). Within days, letters indicate treatment differences ($P \leq 0.05$); a = TRANS vs. CON, b = MEL vs. CON, c = TRANS vs. MEL.

unknown. A similar response was not detected for plasma ceruloplasmin, whereas circulating concentrations of acute-phase proteins are typically correlated (Cooke et al., 2009; Araujo et al., 2010). Haptoglobin is also positively associated with morbidity in cattle (Petersen et al., 2004), but no incidence of morbidity or mortality was detected during feedlot receiving. In addition, hay, concentrate, and total DMI of CON were similar ($P \geq 0.42$) compared with MEL and TRANS steers during the second week of feedlot receiving, whereas an inflammatory-induced haptoglobin response is usually accompanied by reduced feed intake (Johnson, 1997; Araujo et al., 2010).

Supporting our hypothesis, meloxicam administration alleviated the acute-phase response elicited by 24-h transport based on differences detected for plasma haptoglobin and ceruloplasmin between TRANS and MEL steers. Meloxicam inhibits cyclooxygenase, an enzyme that regulates synthesis of inflammatory eicosanoids associated with the acute-phase response such as PGE2 (Lees et al., 2004). Accordingly, Van Engen et al. (2014) orally administered meloxicam or a whey protein placebo at approximately 1 mg/kg of BW to steers prior to a 16-h road transport. These authors reported that steers receiving meloxicam had reduced circulating concentrations of biomarkers of stress and inflammation compared with cohorts receiving placebo, including stress-induced neutrophilia, as well as monocyte and lymphocyte counts. However, Van Engen et al. (2014) did not evaluate production parameters to determine if the immunological benefits of oral meloxicam administration to transported cattle would result in enhanced feedlot receiving performance. In the present experiment, feedlot receiving performance of MEL steers was similar compared with CON and greater compared with TRANS. These results indicate that oral meloxicam administration effectively prevented the performance losses caused by road transport, likely by inhibiting the changes in metabolism and feed intake regulated by inflammatory eicosanoids during an acute-phase response (Johnson, 1997; Klasing and Korver, 1997; Lees et al., 2004).

Conclusions

Meloxicam administration to feeder steers prior to road transport, at feedlot arrival, and during the initial week of feedlot receiving (1 mg/kg of BW/administration) reduced the acute-phase protein response and increased ADG, DMI, and G:F during feedlot receiving. Hence, meloxicam administration may be a viable strategy to mitigate inflammatory reactions and performance losses elicited by long-distance transportation.

Acknowledgments

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Oregon State University

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Beef Research Report

Beef Cattle Sciences

Metabolic Imprinting: Impact on Growth and Reproductive Development of Beef Heifers [1]

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Synopsis

Providing a corn-based supplement ad libitum through a creep-feeder for 50 d to nursing heifers, as a method to stimulate a metabolic imprinting process, did not hasten growth, fat accretion, or puberty attainment.

Summary

This experiment compared development of beef heifers receiving metabolic imprinting (**MI**) or not (**CON**) while nursing. Sixty Angus × Hereford heifers (initial age 68 ± 3 d; initial BW 140 ± 3 kg) were utilized. On d 0, heifers were ranked by initial BW, and assigned to pairs in a manner that heifers within each pair had similar BW. Pairs were randomly assigned MI or CON. From d 0 to 51, MI pairs and their respective dams were allocated to 15 drylot pens and had a creep-feeder that allowed heifers to have ad libitum access to a corn-based supplement. The CON heifers and their dams were maintained in an adjacent drylot pen. From d 52 to 111, cows and heifers from both treatments were managed as a single group on a semiarid range pasture. On d 111, heifers were weaned and allocated to 2 pastures according to treatment until d 277, receiving 4.8 kg/heifer daily of alfalfa-grass hay in addition to 2.5 kg/heifer daily of a corn-based concentrate. Full BW was recorded prior to (d -1 and 0) and at the end of imprinting (d 50 and 51), whereas shrunk BW was collected on d 118, 190, and 277. On d 0, 50, 117, 189, and 258, heifers were evaluated for LM depth and backfat thickness via ultrasonography. Blood samples were collected on d 0, 51, 113, 187, and 261 to determine plasma insulin, glucose, and IGF-1 concentrations, as well as every 10 d beginning on d 113 to assess heifer puberty attainment via plasma progesterone. From d 0 to 51, MI heifers had greater ($P < 0.01$) ADG compared with CON cohorts, whereas ADG did not differ among treatments during the subsequent evaluations ($P \geq 0.20$). On d 51, MI heifers had greater ($P < 0.01$) plasma glucose and IGF-I concentrations compared with CON, whereas plasma insulin concentration was greater ($P < 0.01$) for CON compared with MI heifers on d 261. No treatment effects were detected ($P \geq 0.28$) for backfat thickness and LM depth. Heifers receiving MI attained puberty earlier ($P = 0.02$) during the experiment compared with CON heifers, although no treatment effects were detected for heifer age and BW ($P \geq 0.52$) at puberty. Hence, providing a corn-based supplement ad libitum through a creep-feeder for 50 d to nursing heifers did not hasten their fat accretion or puberty attainment later in life.

Introduction

For optimal economic return and lifetime productivity, replacement beef heifers should attain puberty by 12 months of age (Lesmeister et al., 1973). Age at puberty in cattle is greatly influenced by nutritional status and body development (Schillo

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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et al., 1992), including rate of body fat deposition and subsequent circulating concentrations of leptin (Garcia et al., 2002). Accordingly, nutritional alternatives that enhance carcass lipogenesis may hasten puberty attainment in heifers (Williams et al., 2002).

Metabolic imprinting, which is an epigenetic response to a nutritional challenge during early life that permanently alters physiological outcomes in later life (Lucas, 1991; Du et al., 2010), has been shown to promote the physiological mechanisms associated with fat accretion in cattle (Graugnard et al., 2010). McCann et al. (2011) reported that feeding a high-energy supplement to early-weaned beef calves from 100 to 205 days of age enhanced carcass marbling compared with calves weaned at 205 d. Hence, metabolic imprinting may be an alternative to hasten puberty attainment in growing heifers by increasing rate of fat accretion, although this hypothesis has not been tested to date. In addition, research studies evaluating the metabolic imprint concept in beef cattle have utilized early-weaned calves, which prevented proper separation of weaning and nutritional effects (Scheffler et al., 2014), whereas early-weaning may not be a feasible management alternative for many commercial cow-calf systems. One alternative to isolate the metabolic imprinting concept from early weaning is to provide supplements to nursing heifers via creep-feeding. Hence, this experiment compared growth, body composition, physiological parameters, and puberty attainment of nursing beef heifers receiving or not metabolic imprinting via ad libitum access to creep-feeding.

Materials and Methods

Animals and diets. Sixty nulliparous Angus × Hereford heifers (initial age 68 ± 3 d; initial BW 140 ± 3 kg) were utilized in this experiment, which was divided into three phases: imprinting phase (d 1 to d 51), pre-weaning phase (d 52 to d 111), and development phase (d 112 to d 277). On d 0, heifers were ranked by initial BW, and assigned to pairs in a manner that both heifers within each pair had similar initial BW. On d 0, pairs were randomly assigned to: 1) metabolic imprinting (**MI**), or 2) control (**CON**).

During the imprinting phase, MI heifers and their respective dams were allocated to 15 drylot pens (2 cow-calf pairs/pen). Each pen had a creep-feeder that allowed the heifers to have ad libitum access to a grain-based supplement (70% corn, 15% soybean meal, 10% dehydrated alfalfa, and 5% molasses; as-fed basis). The CON heifers and their respective dams were maintained in an adjacent single drylot pen (50 × 100 m). Cows from both treatments received 8.1 kg/cow daily of meadow-grass hay (56% TDN, 65% NDF, 41% ADF, 1.04 Mcal/kg of NEl, 1.07 Mcal/kg of NEm, 0.48 Mcal/kg of NEg, and 8.2% CP) and 5.4 kg/cow daily of mixed alfalfa-grass hay (61% TDN, 42% NDF, 34% ADF, 1.38 Mcal/kg of NEl, 1.30 Mcal/kg of NEm, 0.73 Mcal/kg of NEg, and 19% CP).

During the pre-weaning phase, cows and heifers from both treatments were managed as a single group on a 6,500 ha semiarid range pasture (Ganskopp and Bohnert, 2009). On d 111, heifers were weaned and allocated to 2 meadow foxtail (*Alopecurus pratensis L.;* 6 ha each) pastures harvested for hay the previous summer according to treatment for the development phase. Heifers were rotated between pastures every 10 d, and received 4.8 kg/heifer daily (DM basis) of the same mixed alfalfa-grass hay offered during the imprinting period, in addition to 2.5 kg/daily of a corn-based concentrate (72% corn and 28% soybean meal; as-fed basis).

Sampling. For ADG calculation, heifers were weighted on 2 consecutive days to determine full BW prior to (d -1 and 0) and at the end of the imprinting phase (d 50 and 51), whereas individual shrunk BW (after 16h of feed and water restriction) was collected on d 118, 190, and 277 of the development phase.

On d 0, 50, 117, 189, and 258, heifers were evaluated for LM depth and backfat thickness via real-time ultrasonography. Ultrasound measurements were obtained at the 12th to 13th rib interface using an Aloka 500V (Aloka Co., Ltd., Wallingford, CT) B-mode instrument equipped with a 3.5-MHz, 125 mm general-purpose transducer array (UST-5011U-3.5). A single technician collected images with software from the Cattle Performance Enhancement Company (CPEC, Oakley, KS). Estimates of LM depth and backfat thickness were based on image analysis programming (Brethour, 1994) within the CPEC software.

Blood samples were collected on d 0, 51, 113, 187, and 261 to determine plasma insulin, glucose and IGF-1 concentrations. During the development phase, blood samples were also collected on 10-d intervals to assess heifer puberty attainment via plasma progesterone. Heifers were considered pubertal once plasma progesterone concentrations were ≥ 1.0 ng/mL for 2 consecutive weeks (Perry et al., 1991), and puberty attainment was declared at

the first week of increased progesterone. Age and BW at puberty were determined according to the week of puberty attainment, heifer shrunk BW recorded in the weighing immediately prior to puberty attainment, and subsequent heifer ADG.

Blood samples were collected via jugular venipuncture into commercial blood collection tubes containing 158 USP units of freeze-dried sodium heparin for plasma collection. All blood samples were placed immediately on ice, subsequently centrifuged (2,500 × *g* for 30 min; 4°C) for plasma harvest, and stored at -80°C on the same day of collection. Plasma insulin and progesterone were analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma glucose was determined using a quantitative colorimetric kit (#G7521; Pointe Scientific, Inc., Canton, MI). Concentrations of IGF-I were determined using a human-specific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100 % cross-reactivity with bovine IGF-I, previously validated for bovine samples (Cooke et al., 2012).

Statistical analysis. Growth, body composition, and physiological data were analyzed using the MIXED procedure (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for ADG, age at puberty, and BW at puberty contained the fixed effect of treatment. The model statement used for analysis of blood variables and body composition contained the fixed effects of treatment, day, and the resultant interaction. Body composition and blood variables were adjusted covariately to values obtained on d 0. Data were analyzed using pair(treatment) and heifer(pair) as random variables. The specified term used in the repeated statement for body composition and blood variables was day, the subject was heifer(pair), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means, or covariately adjusted means for body composition and blood variables, and separated using LSD. Puberty attainment was analyzed using survival analysis (LIFETEST procedure of SAS) by regressing the proportion of pubertal heifers on day of the experiment. Differences between treatment survival curves were determined by the Wilcoxon test. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant or according to the highest-order interaction detected.

Results

During the imprinting phase, average intake of the corn-based concentrate by MI heifers was 1,025 ± 128 g/heifer daily. As expected, MI heifers had greater ($P < 0.01$) ADG and during the imprinting phase, which resulted in a tendency ($P = 0.10$) for a greater BW at the end of the imprinting period compared with CON cohorts (Table 1). Based on the average BW during the imprinting period, intake of the corn-based concentrate by MI heifers was 0.83% ± 0.09 of heifer BW. During the pre-weaning phase, ADG was similar ($P = 0.80$) between treatments, whereas weaning BW still tended ($P = 0.10$) to be greater for MI compared with CON heifers (Table 1). During the development phase, ADG remained similar ($P = 0.20$) between CON and MI heifers, although the BW differences disappeared at the end of the development phase ($P = 0.63$). Hence, the metabolic imprinting process adopted herein did not result in hastened growth when heifers from both treatments were managed similarly prior to and after weaning.

Table 1. Body weight and ADG of beef heifers receiving a corn-based supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).[1]

Item	CON	MI	SEM	*P-Value*
Imprinting phase (d 0 to 51)				
BW d 0, kg	103	105	6	0.84
BW d 51, kg	127	143	6	0.10
ADG, kg/d	0.49	0.75	0.03	< 0.01
Pre-weaning phase (d 52 to 111)				
BW d 118, kg	161	175	6	0.10
ADG, kg/d	0.50	0.49	0.03	0.80
Development phase (d 111 to 277)				
BW d 277, kg	292	299	9	0.63
ADG, kg/d	0.82	0.78	0.02	0.20

[1] Values reported on d 0 and 51 are the average of full BW collected on d -1 and 0, and 50 and 51, respectively. On d 118 and 277, shrunk BW was recorded after a 16 h of feed and water restriction. Average daily gain was calculated based on the following recorded BW: Imprinting phase, d 0 and 51; pre-weaning phase, d 51 and 118, development phase, d 118 and 277.

Treatment × day interactions were detected for plasma concentrations of glucose, insulin, and IGF-I ($P < 0.05$; Table 2). On d 51, MI heifers had greater plasma glucose and IGF-I concentrations compared with CON heifers, which can be directly attributed

to the concentrate intake during the imprinting phase (Hess et al., 2005). The similar plasma IGF-I and glucose concentrations between treatments on d 187 and 261 also corroborate with the similar ADG and management of MI and CON heifers (Wettemann and Bossis, 2000). Conversely, plasma insulin concentrations were similar on d 51, but greater for CON compared with MI heifers on d 261, despite similar ADG and dietary management between treatments (Hess et al., 2005).

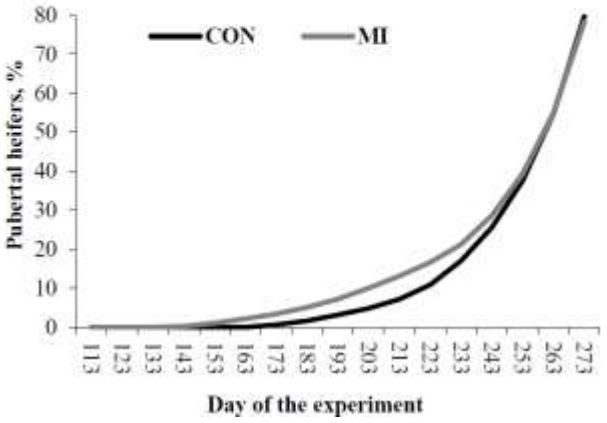
No treatment effects were detected for backfat thickness (P = 0.43) and LM depth (P = 0.28). Heifers receiving MI attained puberty earlier (P = 0.02) during the experiment compared with CON heifers (Figure 1). However, no treatment effects were detected for heifer age (P = 0.52) and BW (P = 89) at puberty attainment, suggesting that treatment effects detected for puberty attainment should be mainly attributed to greater BW of MI heifers at weaning. These results do not support our hypothesis, given that the metabolic imprinting process adopted herein did not impact fat accretion, plasma hormones that regulate puberty attainment such as IGF-I (Jones et al., 1991) during the peripubertal period, nor stimulated heifers to reach puberty at lighter BW or younger age.

In the present experiment, heifers received corn-based supplement ad libitum for 50 d. Conversely, Scheffler et al. (2014) reported that steers fed a high-concentrate diet for 148 d after early weaning produced heavier carcasses with greater marbling scores compared to unsupplemented normal-weaned cohorts. Gasser et al. (2006) also observed that feeding a high-concentrate diet for 10 wk after early weaning hastened puberty attainment in beef heifers. In addition, heifers evaluated herein were still nursing, which likely alleviated concentrate intake during the imprinting period. Accordingly, Gasser et al. (2006) reported that early-weaned heifer calves consumed approximately 2.5 to 3.0% of BW of a high-concentrate diet, whereas average concentrate intake herein was 0.83% of BW. Therefore, research is still warranted to determine if a longer period of imprinting to nursing beef heifers is required to further increase concentrate intake and effectively modulate fat accretion, metabolic responses, and reproductive development

Table 2. Body composition, puberty attainment, and plasma concentrations of glucose, insulin, and IGF-I in beef heifers receiving a corn-based supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).[1]

Item	CON	MI	SEM	*P-Value*
Body composition				
Backfat thickness, mm	3.89	4.00	0.09	0.43
LM depth, mm	46.5	47.6	0.7	0.28
Puberty attainment				
Age at puberty, d	307	300	7	0.52
BW at puberty, kg	262	263	7	0.94
Plasma glucose, mg/dL				
d 51	69.1	75.9	1.6	< 0.01
d 113	66.2	67.	1.6	0.63
d 187	73.7	71.0	1.6	0.26
d 261	75.1	78.5	1.6	0.15
Plasma insulin,				
d 51	2.33	2.34	0.076	0.99
d 113	6.65	5.98	0.076	0.54
d 187	5.06	4.16	0.076	0.41
d 261	9.62	6.16	0.076	< 0.01
Plasma IGF-I, ng/mL				
d 51	51.5	74.0	3.6	< 0.01
d 113	31.8	33.5	3.6	0.75
d 187	64.8	63.5	3.6	0.80
d 261	127.5	123.1	3.6	0.47

Figure 1. Puberty attainment of beef heifers receiving a corn-based supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).



Conclusions

Providing a corn-based supplement ad libitum through a creep-feeder for 50 d to nursing heifers, as a method to stimulate the metabolic imprinting process (Lucas, 1991; Du et al., 2010), did not hasten growth, fat accretion, or puberty attainment. Hence, additional research is still required, including different supplementation lengths, to further evaluate the impact of metabolic imprinting on growth and reproductive development replacement beef heifers.

Acknowledgments

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Beef Research Report

Beef Cattle Sciences

Supplementation Based on Protein or Energy Ingredients to Beef Cattle Consuming Low-Quality Cool-Season Forages: I. Performance, Reproductive, and Metabolic Responses of Replacement Heifers [1]

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Synopsis

Replacement beef heifers consuming a low-quality cool-season forage can equally utilize and benefit, in terms of growth and metabolic parameters, from supplements based on protein or energy ingredients provided as 0.5 % of heifer BW/d at isocaloric and isonitrogenous rates.

Summary

This experiment evaluated the influence of supplement composition on performance, reproductive, and metabolic responses of Angus × Hereford heifers consuming a low-quality cool-season forage (8.7 % CP and 57 % TDN). Sixty heifers were allocated into 15 drylot pens (4 heifers/pen; 5 pens/treatment), and assigned to: 1) supplementation with soybean meal (**PROT**), 2) supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; **ENER**), or 3) no supplementation (**CON**). Heifers received meadow foxtail hay for ad libitum consumption throughout the experiment (d -10 to 160). Beginning on d 0, PROT and ENER were provided daily at 1.30 and 1.40 kg of DM/heifer to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Hay and total DMI were recorded monthly for 5 consecutive days. Blood was collected every 10 d for analysis of plasma progesterone to evaluate puberty attainment. Blood samples collected on d -10, 60, 120, and 150 were also analyzed for plasma urea N (**PUN**), glucose, insulin, IGF-I, NEFA, and leptin. Liver samples were collected on d 100 from 2 heifers/pen, and analyzed for mRNA expression of genes associated with nutritional metabolism. No treatment effect was detected ($P = 0.33$) on hay DMI. Total DMI, ADG, mean concentrations of glucose, insulin, and IGF-I, and hepatic mRNA expression of IGF-I and IGFBP-3 were greater ($P \leq 0.02$) for PROT and ENER compared with CON, and similar between PROT and ENER ($P \geq 0.13$). Mean PUN was greater ($P < 0.01$) for PROT and ENER compared with CON, whereas PROT heifers had greater ($P < 0.01$) PUN compared with ENER. Plasma leptin concentrations were similar between ENER and PROT ($P \geq 0.19$), and greater ($P \leq 0.03$) for ENER and PROT compared with CON on d 120 and 150 ($P = 0.03$). Hepatic mRNA expression of mitochondrial phosphoenolpyruvate carboxykinase was greater ($P = 0.05$) in PROT compared with CON and ENER, and similar between CON and ENER ($P = 0.98$). The proportion of heifers pubertal on d 160 was greater ($P < 0.01$) in ENER compared with PROT and CON, and similar between PROT and CON ($P =$

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0.38). In conclusion, beef heifers consuming a low-quality cool-season forage had a similar increase in DMI, growth, and overall metabolic status if offered supplements based on soybean meal or corn at 0.5 % of BW.

Introduction

Supplementation is often required in heifer development programs based on low-quality forages. Although forages typically represent the main source of energy for forage-fed cattle, and energy is the primary dietary consideration for heifer development, protein is traditionally considered the limiting nutrient in Western U.S. cow-calf operations (DelCurto et al., 2000). Indeed, protein supplementation generally improves digestibility and DMI of low-quality warm-season forages, resulting in increased energy utilization from the forage and cattle BW gain (DelCurto et al., 1990). However, Bohnert et al. (2011) reported that protein supplementation did not increase digestibility and DMI of low-quality cool-season forages. Hence, inclusion of energy ingredients into supplements may be required for optimal growth and reproductive development of replacement heifers consuming low-quality cool-season forages.

Beef heifers, particularly *Bos taurus*, should attain puberty by 12 mo of age to maximize their lifetime productivity (Lesmeister et al., 1973). Energy intake also influences puberty attainment in heifers by other mechanisms besides BW gain, including modulation of hormones known to mediate puberty, such as insulin and IGF-I. Accordingly, Ciccioli et al. (2005) reported that feeding starch-based supplements hastened puberty attainment in beef heifers independently of BW gain. Hence, inclusion of energy ingredients, such as starch, into supplements may further benefit reproductive development of heifers consuming low-quality cool-season forages by favoring circulating concentrations of nutritional mediators of puberty. To test this hypothesis, this experiment compared the effects of supplements based on protein or energy ingredients on performance, plasma metabolites and hormones, expression of hepatic genes associated with nutritional metabolism, and puberty attainment of beef heifers consuming a low-quality cool-season forage.

Materials and Methods

Heifers and diets. Sixty Angus × Hereford weaned heifers (initial age 226 ± 3 d; initial BW 200 ± 2 kg) were used. On d -10 of the study, heifers were ranked by initial BW and age and allocated to 15 drylot pens (5 pens/treatment; 4 heifers/pen), in a manner which all pens had equivalent initial average BW and age. Pens were randomly assigned to receive 1 of 3 treatments: 1) supplementation with soybean [*Glycine max* (L.) *Merr.*] meal (**PROT**), 2) supplementation with a mixture of cracked corn (*Zea mays* L.), soybean meal, and urea (68:22:10 ratio, DM basis; **ENER**), or 3) no supplementation (**CON**). Heifers were offered meadow foxtail (*Alopecurus pratensis L.*) hay for ad libitum consumption during the entire experiment (d -10 to 160). Beginning on d 0, PROT and ENER treatments were fed once daily (0800 h) at a rate of 1.30 and 1.40 kg of DM/heifer, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous (Table 1). Urea was included into ENER to result in isocaloric and isonitrogenous intakes of PROT and ENER. Further, treatment intakes were formulated at 0.50 and 0.54 % of the expected average heifer shrunk BW during the experiment for PROT and ENER, respectively. Average heifer shrunk BW during the experiment was estimated based on initial shrunk BW (d -9) and expected final shrunk BW (d 161). Expected final shrunk BW was projected based on previous research from our group (Cooke et al., 2013), which was conducted at the same research station and using the same cowherd as the experiment described herein.

Sampling. Heifers were weighed on 2 consecutive d to determine both full and shrunk (after 16 h of feed and water restriction) BW at the beginning (d -10 and -9) and end of the study (d 160 and 161). Shrunk BW was used to determine heifer ADG during the study. Blood samples were collected at 10-d intervals throughout the entire experiment (d -10 to 160), starting 4 h after the ENER and PROT treatments were offered, to determine onset of puberty according to plasma progesterone (**P_4**) concentration. Heifers were considered pubertal when plasma P_4 concentration was equal or greater than 1.0 ng/mL for 2 consecutive samplings (Perry et al., 1991), and puberty attainment was declared at the second sampling of elevated progesterone. Blood samples collected on d -10, 60, 120, and 150 were also analyzed for plasma urea N (**PUN**), glucose, insulin, NEFA, IGF-I, and leptin concentrations. Samples were processed and analyzed according to procedures described by Cooke et al. (2012).

Hay and total DMI were evaluated from each pen by collecting and weighing refusals from d 12 to 16, d 53 to 57, d 71 to 75, d 93 to 97, d 112 to 116, and d 143 to 147 of the experiment, which were classified as periods (periods 1 to 6, respectively). Samples of the offered and non-consumed hay were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of heifers within each pen and expressed as kg per heifer/d. Daily intake of NE_m, NE_g, CP, RDP, and starch were estimated based on DMI of each pen, and nutritive value of hay and treatments (Table 1).

On d 100 of the experiment, 2 heifers/pen were randomly assigned for liver sample collection via needle biopsy (Cooke et al., 2008), which began 4 h after supplements were offered. Samples were processed and analyzed via real-time quantitative reverse transcription (**RT**)-PCR for IGF-I, IGFBP-3, pyruvate carboxylase (**PC**), cytosolic phosphoenolpyruvate carboxykinase (**PEPCK-C**), mitochondrial PEPCK (**PEPCK-M**), and cyclophilin mRNA expression, according to Cooke et al. (2008) and Yoganathan et al. (2012).

Table 1. Ingredient composition and nutrient profile of treatments offered during the experiment.

Item	PROT	ENER
Ingredients, % DM		
Cracked corn	--	68
Soybean meal	100	22
Urea	--	10
Nutrient profile, DM basis		
TDN, %	85.4	77.0
NE_m, Mcal/kg	2.02	1.91
NE_g, Mcal/kg	1.37	1.31
CP, %	50.1	45.0
RDP, %	28.3	36.0
NFC, %	33.5	59.0
NDF, %	8.6	9.0
Starch, %	5.4	48.4
Ether extract, %	1.5	2.9
Daily intake		
DM, kg	1.30	1.40
TDN, kg	1.11	1.08
NE_m, Mcal	2.63	2.67
NE_g, Mcal	1.78	1.83
CP, kg	0.65	0.63
RDP, kg	0.37	0.50
NFC, kg	0.44	0.83
NDF, kg	0.11	0.13
Starch, kg	0.07	0.68
Ether extract, kg	0.02	0.04

Statistical analysis. All data were analyzed using pen as experimental unit, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Performance, plasma variables, and gene expression data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement used for BW, ADG, and gene expression contained only the effects of treatment. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. The model statement used for plasma variables contained the effects of treatment, day, the treatment × day interaction, and values obtained on d -10 as covariate. Data were analyzed using heifer(pen) and pen(treatment) as random variables, with day as the specified term for the repeated statement and heifer(pen) as subject. The model statement used for feed and nutrient intake contained the effects of treatment, day, period, and all the resultant interactions. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded daily from each pen, as well as day(period) as the specified term for the repeated statement and pen(treatment) as subject. For both intake and plasma variables, the covariance structure used was first-order autoregressive, which provided the smallest Akaike Information Criterion and hence the best fit for all variables analyzed. Puberty data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). The model statement used contained the effects of treatment, day, and the resultant interaction. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. Results are reported as least square means, or covariately adjusted means for plasma variables, and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were denoted if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to highest-order interaction detected.

Results

No treatment effects were detected ($P = 0.33$) on forage DMI (Table 2). These results support that protein supplementation does not impact DMI of a low-quality cool-season forage (Bohnert et al., 2011), and that supplements based on energy ingredients can be fed at approximately 0.5 % of BW without impacting forage intake (Bowman and Sanson, 1996). Total daily DMI, and estimated daily intake of NE_m, and NE_g were greater ($P < 0.01$) for PROT and ENER compared with CON, and similar

($P \geq 0.41$) between PROT and ENER (Table 2). Estimated daily intake of CP, RDP, and starch were greater ($P < 0.01$) for PROT and ENER compared with CON, whereas ENER had greater ($P < 0.01$) RDP and starch intake, and tended ($P = 0.09$) to have less CP intake compared to PROT heifers (Table 2). Hence, PROT and ENER had greater overall nutrient intake compared with CON heifers. The greater RDP intake of ENER compared with PROT heifers can be attributed to the inclusion of urea into the ENER treatment, and consequent RDP content of treatments (Table 1). In addition, the slightly greater CP intake of PROT compared with ENER, despite similar CP content of treatments can be attributed to the numerical difference in hay intake between PROT and ENER.

Table 2. Performance and puberty parameters of replacement of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).[1]

Item	CON	PROT	ENER	*P* =
DMI, kg/d				
Hay	5.94	5.79	5.51	0.33
Total	5.94	7.10^{b}	6.91^{b}	< 0.01
Daily nutrient intake				
NE_m, Mcal	6.54^{a}	9.00^{b}	8.74^{b}	< 0.01
NE_g, Mcal	3.27^{a}	4.97^{b}	4.87^{b}	< 0.01
CP, kg	0.51^{a}	1.15^{b}	1.11^{b}	< 0.01
RDP, kg	0.35^{a}	0.71^{b}	0.83^{c}	< 0.01
Starch, kg	0.10^{a}	0.17^{b}	0.77^{c}	< 0.01
ADG, kg/d	0.36^{a}	0.76^{b}	0.72^{b}	< 0.01
Puberty on d 160, %	10^{a}	5^{a}	25^{b}	< 0.01

[1] Values with different superscripts differ ($P \leq 0.10$).

A treatment effect ($P < 0.01$) was detected for ADG (Table 2), which was greater ($P < 0.01$) for PROT and ENER compared with CON, and similar between ENER and PROT ($P = 0.52$). These results provide evidence that beef heifers consuming low-quality cool-season forages can equally utilize nutrients provided by supplements based on protein or energy ingredients to support BW gain. Furthermore, differences in CP and RDP intakes between ENER and PROT were minimal and not sufficient to impact heifer ADG.

A treatment effect was detected ($P < 0.01$) for plasma NEFA (Table 3). Mean NEFA concentration was greater ($P < 0.01$) for CON compared with PROT and ENER, and similar ($P = 0.13$) between PROT and ENER. Accordingly, circulating NEFA in cattle was negatively associated with nutrient intake and ADG. However, it is important to note that elevated NEFA is often associated with negative energy balance (Lucy et al., 1991), and heifers from all treatments were in a positive nutritional status based on their ADG (Table 2).

Table 3. Plasma concentrations of NEFA, urea N (**PUN**), glucose, insulin, and IGF-I of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein ingredients (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).[1]

Item	CON	PROT	ENER	*P* =
NEFA, µEq/L	0.412^{a}	0.194^{b}	0.241^{b}	< 0.01
PUN, mg/dL	3.57^{a}	20.07^{b}	17.87^{c}	< 0.01
Glucose, mg/dL	59.3^{a}	65.1^{b}	65.0^{b}	< 0.01
Insulin, µIU/mL	5.20^{a}	6.72^{b}	6.69^{b}	0.02
IGF-I, ng/mL	79.5^{a}	159.4^{b}	149.5^{b}	< 0.01

[1] Values with different superscripts differ ($P \leq 0.10$).

A treatment effect was detected ($P < 0.01$) for PUN (Table 3). During the study, mean PUN was greater ($P < 0.01$) for PROT and ENER compared with CON, whereas PROT also had greater ($P < 0.01$) PUN compared with ENER (Table 3). The greater PUN concentrations of PROT and ENER compared with CON can be directly attributed to their greater CP and RDP intake, and suggest that CON heifers required supplemental CP and RDP. Differences in PUN between ENER and PROT heifers can also be attributed to the slightly greater CP intake of PROT heifers, as well as improved N utilization by ruminal microbes in ENER heifers (Hall and Huntington, 2008).

Mean glucose concentration was greater ($P < 0.01$) for PROT and ENER compared with CON, and similar ($P = 0.91$) between PROT and ENER (Table 3). In agreement, glucose concentration was positively associated with feed intake and BW gain (Vizcarra et al., 1998), as observed herein based on the greater nutrient intake and ADG of PROT and ENER heifers. However, starch is the major dietary precursor for glucose in ruminants; hence, it would be expected that ENER heifers had greater plasma glucose compared to PROT. Nevertheless, Huntington (1997) reported that growing cattle are capable of synthesizing glucose from amino acids.

Supporting this latter rationale, PROT heifers had greater ($P = 0.05$) mRNA expression of liver PEPCK-M compared with ENER and CON, which was similar ($P = 0.98$) between ENER and CON (Table 4). No treatment effects were detected ($P \geq$

0.28; Table 4) for mRNA expression of PC and PEPCK-C, although mRNA expression of these enzymes are modulated by nutrient intake (Cooke et al., 2008) and positively associated with glucose synthesis in cattle (Bradford and Allen, 2005). Nevertheless, circulating NEFA stimulate mRNA expression of PC and PEPCK-C, but no PEPCK-M, to preserve gluconeogenesis in cattle with insufficient nutrient intake (White et al., 2011). Hence, the greater NEFA concentration in CON heifers likely maintained mRNA expression of PC and PEPCK-C similar to that of ENER and PROT.

Treatment effects were detected ($P \leq 0.05$) for plasma insulin and IGF-I (Table 3), as well as mRNA expression of liver IGF-I and IGFBP-3 (Table 4). Mean insulin and IGF-I concentrations were greater ($P < 0.01$) for PROT and ENER compared with CON, and similar ($P \geq 0.21$) between PROT and ENER (Table 3). Expression of liver IGF-I and IGFBP-3 mRNA were also greater ($P \leq 0.05$) in PROT and ENER compared with CON, and similar ($P \geq 0.29$) between PROT and ENER (Table 4). These results corroborate with treatment effects detected for DMI, nutrient intake, and plasma glucose, given that circulating concentration of insulin is positively regulated by nutrient intake and blood glucose (Vizcarra et al., 1998). Availability of energy substrates and circulating insulin positively modulate the expression of liver IGF-I and IGFBP-3 mRNA, and consequent hepatic synthesis of these proteins (Cooke et al., 2008). For these reasons, plasma insulin and IGF-I have been recognized as indicators of nutritional status of cattle (Hess et al., 2005).

Table 4. Expression of hepatic genes associated with nutritional metabolism of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein ingredients (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).[1]

Item	CON	PROT	ENER	*P* =
PC	3.64	2.77	2.66	0.28
PEPCK-C	5.00	4.68	3.92	0.52
PEPCK-M	2.92[a]	4.19[b]	2.90[a]	0.08
IGF-I	3.71[a]	8.31[b]	6.75[b]	0.02
IGFBP-3	1.62[a]	2.46[b]	2.38[b]	0.03

[1] Values with different superscripts differ ($P \leq 0.10$).

A treatment × day interaction was detected ($P = 0.03$) for plasma leptin (Figure 1). Plasma leptin concentrations were similar between ENER and PROT throughout the experiment ($P \geq 0.19$), and greater for ENER and PROT compared with CON on d 120 ($P \leq 0.01$) and 150 ($P \leq 0.03$; Figure 1). Circulating leptin is regulated by body fat content, nutrient intake, and circulating insulin (Houseknecht et al., 1998). Nevertheless, the greater ADG, nutrient intake, and plasma insulin of PROT and ENER heifers compared with CON only resulted in a similar effect on plasma leptin beginning on d 120 of the experiment. The reason for this delay is unknown and deserves further investigation, but may be associated with heifer age and rate of body fat accretion (Houseknecht et al., 1998).

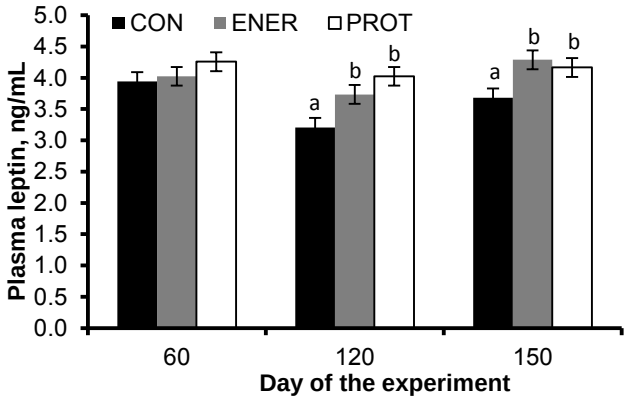

Figure 1. Plasma concentrations of leptin in replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein (**PROT**; n = 5) or energy (**ENER**; n = 5) ingredients. A treatment × hour interaction was detected ($P < 0.01$). Within day, letters indicate differences between treatments ($P \leq 0.03$).

No treatment effects were detected ($P = 0.25$) on puberty attainment (data not shown). However, a greater ($P < 0.01$) proportion of ENER heifers were pubertal at the end of the experiment (d 160) compared with CON and PROT, whereas no differences were detected ($P = 0.38$) between CON and PROT (Table 2). The main hypothesis of the experiment was that replacement beef heifers consuming a low-quality cool-season forage and receiving a supplement based on an energy ingredient would have hastened puberty attainment compared with heifers receiving no supplementation or supplemented with a protein ingredient. This hypothesis was developed based on the premise that energy sources such as corn favor circulating concentrations of insulin, IGF-I, and leptin (Lents et al., 2005), which impact the puberty process by mediating synthesis and activity of GnRH and gonadotropin (Maciel et al., 2004). Indeed, a greater proportion of ENER heifers were pubertal at the end

of experiment compared with PROT and CON, despite the similar ADG and metabolic status between PROT and ENER. Likewise, Ciccioli et al. (2005) reported that heifers receiving a high-starch supplement had hastened puberty attainment but similar ADG compared with cohorts receiving a low-starch supplement. However, these results should be interpreted with caution, because overall puberty attainment herein was lower than expected based on previous work from our research group (Cooke et al., 2012; 2013). The reason for this outcome is unknown, given that ENER and PROT heifers achieved the BW recommended for puberty achievement at 13 mo of age (Patterson et al., 2000). More specifically, % mature BW on d 160 was greater ($P < 0.01$) for ENER and PROT compared to CON (50.7, 62.6, and 65.1 % of mature BW, for CON, ENER, and PROT, respectively), whereas heifer age on d 160 was similar among treatments ($P = 0.97$) and averaged 396 ± 6 d.

Conclusions

Replacement beef heifers offered PROT and ENER had a similar increase in nutrient intake, ADG, and overall metabolic status compared with CON heifers, despite differences in ingredients between treatments. Puberty attainment was enhanced in ENER heifers only, although this outcome should be interpreted with caution due to the reduced number of pubertal heifers across all treatments. Hence, replacement beef heifers consuming a low-quality cool-season forage can equally utilize and benefit, in terms of growth and metabolic parameters, from supplements based on protein or energy ingredients provided as 0.5 % of heifer BW/d at isocaloric and isonitrogenous rates.

Acknowledgments

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Beef Research Report

Beef Cattle Sciences

Supplementation Based on Protein or Energy Ingredients to Beef Cattle Consuming Low-Quality Cool-Season Forages: II. Forage Disappearance in Rumen-Fistulated Steers and Physiological Responses in Pregnant Heifers [1]

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Synopsis

Beef cattle consuming low-quality cool-season forages had similar ruminal forage degradability and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5 % of BW

Summary

Two experiments evaluated the influence of supplement composition on ruminal forage disappearance parameters, performance, and physiological responses of Angus × Hereford cattle consuming a low-quality cool-season forage (8.7 % CP and 57 % TDN). In Exp. 1, 6 steers fitted with ruminal cannulas were assigned to an incomplete 3 x 2 Latin square design containing 2 periods of 11 d each and the following treatments: 1) supplementation with soybean meal (**PROT**), 2) supplementation with a mixture of cracked corn, soybean meal, and urea (**ENER**), or 3) no supplementation (**CON**). Steers were offered meadow foxtail (*Alopecurus pratensis* L.) hay for ad libitum consumption. Treatments were provided daily at 0.50 and 0.54 % of shrunk BW/steer for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. No treatment effects were detected on rumen disappearance parameters of forage DM ($P \geq 0.33$) and NDF ($P \geq 0.66$). In Exp. 2, 35 pregnant heifers were ranked by initial BW on d -7 of the study, allocated into 12 feedlot pens (4 pens/treatment), and assigned to the same treatments as in Exp. 1 for 19 d. Treatments were fed once daily at 1.77 and 1.92 kg of DM/heifer for PROT and ENER, respectively. No treatment effects ($P = 0.17$) were detected on forage DMI. Total DMI was greater ($P < 0.01$) for PROT and ENER compared with CON. Accordingly, ADG was greater ($P = 0.01$) for PROT compared with CON, tended to be greater for ENER compared with CON ($P = 0.08$), and similar between ENER and PROT ($P = 0.28$). Heifers receiving PROT and ENER had greater ($P \leq 0.09$) mean concentrations of plasma glucose, insulin, IGF-I, and progesterone compared to CON, whereas ENER and PROT had similar concentrations of these plasma variables ($P \geq 0.15$). A treatment × hour interaction was detected ($P < 0.01$) for plasma urea N (**PUN**), given that PUN concentrations increased after supplementation for ENER and PROT (time effect, $P < 0.01$), but did not change for CON ($P = 0.62$). In conclusion, beef cattle consuming low-quality cool-season forages had similar ruminal forage disappearance and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5 % of BW.

1. This document is part of the Oregon State University – 2014 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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Introduction

Supplementation is often required in heifer development programs based on low-quality forages. Protein is traditionally considered the limiting nutrient in Western U.S. cow-calf operations (DelCurto et al., 2000), although energy is the primary dietary consideration for female development (Mass, 1987) and forages typically represent the main energy source for forage-fed cattle. Indeed, supplemental protein has been shown to improve digestibility and DMI of low-quality warm0season forages, resulting in increased energy utilization from the forage and cattle performance (DelCurto et al., 1990). However, supplemental protein did not increase forage digestibility and DMI of low-quality cool-season forages Bohnert et al. (2011). Hence, inclusion of energy ingredients into supplements may be beneficial for growth and reproduction of heifers consuming such forages.

After their first breeding season, pregnant heifers still need to grow while maintaining the pregnancy. Energy intake modulates BW gain and circulating concentration of progesterone (**P_4**); a steroid required for pregnancy establishment and maintenance. The hormones associated with the metabolism of energy substrates, particularly starch, increase P_4 concentration by reducing hepatic P_4 catabolism (Cooke et al., 2012) and stimulating ovarian steroidogenesis. Hence, inclusion of energy ingredients into supplements may further benefit reproductive performance of pregnant heifers consuming low-quality cool-season forages by increasing circulating P_4 concentration. However, supplements based on energy ingredients often impair forage digestibility and DMI in cattle (DelCurto et al., 2000). Therefore, 2 experiments compared the effects of supplements based on protein or energy ingredients on ruminal forage disappearance in steers (Exp. 1), and performance and physiological parameters of pregnant beef heifers (Exp. 2).

Materials and Methods

Experiment 1

Steers and diets. Six Angus × Hereford steers (initial shrunk BW 494 ± 11 kg), housed in individual pens (8 × 20 m) and fitted with a ruminal cannula, were assigned to an incomplete 3 x 2 Latin square design containing 2 periods of 11 d each (2 steers/treatment in each period) and the following treatments: 1) supplementation with soybean [*Glycine max* (L.) *Merr.*] meal (**PROT**), 2) supplementation with a mixture of cracked corn (*Zea mays* L.), soybean meal, and urea (68:22:10 ratio, DM basis; **ENER**), or 3) no supplementation (**CON**). Steers were offered meadow foxtail (*Alopecurus pratensis* L.) hay for ad libitum consumption during the entire experiment. The PROT and ENER treatments were provided daily at 0.50 and 0.54 % of steer shrunk BW recorded at the beginning of each period, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Urea was included into ENER to result in isocaloric and isonitrogenous intakes of PROT and ENER. Treatment intake during the experiment averaged at 2.20 and 2.37 kg of DM/steer for PROT and ENER, respectively. Treatments were inserted directly into the ruminal cannula of each steer to ensure readily supplement consumption.

Sampling. Within each period (d 0 to 11), steer shrunk BW was recorded on d 0 after 16 h of feed and water restriction to determine steer initial BW. From d 1 to 7 of each period, voluntary forage DMI was recorded daily by collecting and weighing refusals. From d 8 to 11 of each period, steers were offered 90 % of their voluntary forage DMI determined from d 1 to 7. Immediately before treatments were provided on d 8, Dacron bags containing 4 g (DM basis) of ground dietary hay were suspended into the ruminal ventral sac of each steer, and incubated in triplicates for 0, 1, 3, 5, 8, 12, 24, 36, 48, 72, and 96 h. Before ruminal incubation, all bags were soaked in warm water (39°C) for 15 min. After ruminal incubation, bags were washed repeatedly with running water until the rinse water was colorless, and subsequently dried for 96 h at 50°C in a forced-air oven. The 0-h bags were not incubated in the rumen, but were subjected to the same soaking, rinsing, and drying procedures applied to the ruminally incubated bags. Dried samples were weighed for residual DM determination, and triplicates were combined and analyzed for NDF using procedures modified for use in an Ankom 200 Fiber Analyzer.

Statistical analysis. All data were analyzed using steer as the experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Kinetic parameters of forage DM and NDF disappearance were estimated using nonlinear regression procedures of SAS, as described by Vendramini et al. (2008). Effective degradability of forage DM and NDF were calculated by fixing ruminal passage rate at 0.046/h

(Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979), whereas treatment effects on these parameters were analyzed using the MIXED procedure of SAS (SAS Inst.). The model statement contained the effects of treatment and period as independent variables. Data were analyzed using steer(treatment × period) as the random variable. Results are reported as least square means and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were denoted if $P > 0.05$ and ≤ 0.10.

Experiment 2

Heifers and diets. Thirty-five nulliparous pregnant Angus x Hereford heifers (initial shrunk BW 354 ± 4 kg, initial age = 508 ± 4 d) were utilized in the study. Heifers were concurrently exposed and became pregnant to a fixed-time AI protocol (CO-Synch + controlled internal progesterone-release device) 90 d prior to the beginning of the experiment. Pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) 80 d after AI (d -10). On d -7, all heifers were ranked by initial shrunk (after 16 h of feed and water restriction) BW, and allocated to 12 feedlot pens (4 pens/treatment; 11 pens with 3 heifers and 1 pen with 2 heifers; 8 × 20 m) in a manner which all pens had equivalent initial average shrunk BW. Pens were randomly assigned to receive the same treatments described in Exp. 1. Heifers were offered meadow foxtail hay for ad libitum consumption during the entire experiment (d -7 to 19). Beginning on d 1, PROT and ENER treatments were fed once daily at a rate of 1.77 and 1.92 kg of DM/heifer, respectively, to achieve the same treatment intake as % of initial shrunk BW used in Exp. 1, and to ensure isocaloric and isonitrogenous intakes. The ENER and PROT treatments were not mixed with hay, and were readily consumed by heifers.

Sampling. Heifer shrunk BW was collected prior to the beginning (d -7) and at the end of the study (d 20; after 16 h of feed and water restriction) for ADG calculation. Hay DMI was evaluated daily from each pen from d 1 to 19 by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of heifers within each pen, and expressed as kg per heifer/d. In addition, daily intake/heifer of NE_m, NE_g, CP, RDP, and starch were estimated based on total DMI of each pen, and nutritive value of hay and treatments.

Blood samples were collected immediately prior to and 2, 4, 6, and 8 h after treatment feeding (h 0) on d 13, 15, 17, and 19 of the experiment and analyzed for plasma concentrations of glucose, urea N (**PUN**), insulin, IGF-I, and P_4. Blood samples were also collected on d 0 of the experiment, immediately prior to and 4 and 8 h after hay feeding (h 0) to determine if ENER, PROT, and CON heifers had similar P_4 concentrations prior to the beginning of treatment administration (d 1 to 19). All blood samples were collected via jugular venipuncture into commercial blood collection tubes containing freeze-dried sodium heparin. After collection, blood samples were placed immediately on ice, subsequently centrifuged (2,500 × *g* for 30 min; 4°C) for plasma harvest, and stored at -80°C. Plasma concentrations of P_4 and insulin were determined using Coat-A-Count solid phase ^{125}I RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA). Plasma glucose and PUN were determined using quantitative colorimetric kits (#G7521 and B7551, respectively; Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was only determined in samples collected at 0 and 4 h after feeding, using a human-specific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100 % cross-reactivity with bovine IGF-I.

Statistical Analysis. All data were analyzed using the MIXED procedure of SAS, using pen as experimental unit, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for ADG contained only the effect of treatment. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The model statement used for feed and nutrient intake contained the effects of treatment, day, and the treatment × day interaction. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded from each pen. The specified term for the repeated statement was day and subject was pen(treatment). The model statement used for plasma variables contained the effects of treatment, hour, day, and all the resultant interactions. The model statement for P_4 also contained the average P_4 concentration on d 0 as covariate. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The specified term for the repeated statement was hour(day), whereas heifer(treatment × day) was the subject. For both intake and plasma variables, the covariance structure used was first-order autoregressive, which

provided the smallest Akaike Information Criterion and the best fit for the variables analyzed. Results are reported as least square means, or covariately adjusted means for plasma P_4 concentration, and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies if $P > 0.05$ and ≤ 0.10.

Results

Experiment 1

No treatment effects were detected for ruminal disappearance rate or effective ruminal degradability of hay DM ($P \geq 0.33$) and NDF ($P \geq 0.66$; Table 2), indicating that PROT and ENER did not impact rumen in situ disappearance parameters of a low-quality cool-season forage. Supporting these results, Caton and Dhuyvetter (1997) suggested that ruminal disappearance rate of low-quality forages is not impacted by energy or protein-based supplementation. Nevertheless, supplements based on protein and energy ingredients are often associated, respectively, with improved and decreased ruminal forage digestibility in beef cattle (DelCurto et al., 2000). However, protein supplementation is generally beneficial to forage digestibility when the CP content of the basal forage is less than 8 % (DelCurto et al., 2000), whereas the forage utilized herein had 8.7 % CP (DM basis). Supplements based on energy ingredients can be provided to forage-fed cattle at 0.5 % of BW without major impacts on forage digestibility and intake (Bowman and Sanson, 1996), whereas the ENER treatment was provided at 0.54 % of steer BW.

Table 1. Ruminal in situ disappearance parameters of meadow foxtail hay incubated in forage-fed steers receiving no supplementation (**CON**; n = 4), or supplements based on protein (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4).

Item	CON	PROT	ENER	*P* =
Ruminal disappearance rate, %/h				
DM	2.88	3.36	3.67	0.33
NDF	3.64	4.24	4.06	0.71
Effective degradability, %				
DM	60.7	60.8	60.3	0.95
NDF	55.4	55.5	53.7	0.66

Corn intake above 0.25 % of BW has been shown to impair forage utilization in cattle (Bowman and Sanson, 1996) by reducing ruminal pH, shifting rumen microbes from a cellulolytic population towards an amylolytic population, and decreasing ruminal NH_3 concentration (Caton and Dhuyvetter, 1997). In the present experiment, ENER steers consumed corn at 0.37 % of their BW. However, inclusion of a RDP source into corn-based supplements may offset the negative impacts of corn-based supplements on rumen function and digestibility (Olson et al., 1999). Hence, the inclusion of soybean meal and urea into the ENER treatment, as well as the equivalent intake of CP and RDP by ENER and PROT steers, may also have contributed to the similar ruminal forage digestibility among treatments.

Experiment 2

No treatment effects ($P = 0.17$) were detected on forage DMI (Table 2). This outcome agrees with the lack of treatment effects on ruminal degradability parameters of the forage utilized herein reported in Exp. 1, given that ruminal forage digestibility is positively associated with intake (Allen, 1996). As expected due to the lack of treatment effects on forage intake, as well as treatment design and intake rate, total daily DMI, NE_m, NE_g, CP, and RDP intake were greater ($P < 0.01$) for PROT and ENER compared with CON heifers, and similar ($P \geq 0.18$) between PROT and ENER heifers (treatment effects, $P < 0.01$; Table 2). In addition, estimated mean daily intake of starch was greater ($P < 0.01$) for ENER compared with PROT and CON, and similar ($P = 0.40$) between PROT and CON (Table 2). Hence, PROT and ENER had a similar increase in energy and protein intake compared with CON heifers, although starch was the main energy source provided by ENER.

Table 2. Performance parameters o pregnant beef heifers consuming low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4), or supplements based on protein (**PROT**; n = 4) or energy ingredients (ENER; n = 4).[1]

Item	CON	PROT	ENER	*P* =
ADG, kg/d	0.49[a]	0.89[b]	0.75[b]	0.03
DMI, kg/ d				
Hay	8.60	8.42	8.84	0.17
Total	8.60[a]	10.19[b]	10.50[b]	< 0.01
Daily nutrient intake				
NE_m, Mcal	9.46[a]	12.84[b]	12.89[b]	< 0.01
NE_g, Mcal	4.73[a]	7.06[b]	7.03[b]	< 0.01
CP, kg	0.74[a]	1.62[b]	1.51[b]	< 0.01
RDP. Kg	0.51[a]	1.00[b]	1.12[b]	< 0.01
Starch, kg	0.146[a]	0.239[b]	0.950[b]	< 0.01

[1] Values with different superscripts differ ($P \leq 0.05$).

A treatment effect ($P = 0.03$) was detected for ADG (Table 2). In agreement with the treatment effects observed for DMI and nutrient intake, ADG was greater ($P = 0.01$) for PROT compared with CON, tended to be greater for ENER compared with CON ($P = 0.08$), and was similar between ENER and PROT ($P = 0.28$). These results provide evidence that beef heifers consuming low-quality cool-season forages can equally utilize nutrients provided by supplements based on protein or energy ingredients to support BW gain. Supporting this rationale, similar treatment effects were detected for plasma concentrations of PUN ($P < 0.01$), glucose ($P = 0.04$), insulin ($P < 0.01$), and IGF-I ($P = 0.03$) in the present study (Table 3), which are hormones and metabolites associated with dietary protein and energy metabolism in cattle.

A treatment × hour interaction was detected ($P < 0.01$) for PUN (Figure 1), given that PUN concentrations increased after supplementation for ENER and PROT heifers (time effect, $P < 0.01$), but did not change for CON (time effect; $P = 0.62$). In addition, mean PUN concentration was greater ($P < 0.01$) for ENER and PROT heifers compared with CON, and similar ($P = 0.44$) between ENER and PROT (Table 3). Concentration of PUN is positively associated with intake of CP, RDP, and concentration of ruminal ammonia (Broderick and Clayton, 1997). Therefore, treatment effects detected for PUN can be attributed to the equivalent treatment effects detected for CP and RDP intake (Table 2).

Table 3. Plasma concentrations of urea N (PUN), glucose, insulin, IGF-I, and P_4 of pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4), or supplements based on protein ingredients (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4).

Item	CON	PROT	ENER	*P* =
PUN, mg/dL	4.6[a]	16.3[b]	18.5[b]	< 0.01
Glucose, mg/dL	62.2[a]	66.5[b]	66.6[b]	0.04
Insulin, µIU/mL	2.48[a]	3.65[b]	3.09[ab]	< 0.01
IGF-I, ng/mL	112.9[a]	143.6[b]	137.3[b]	0.03
P_4, ng/mL	6.38[a]	7.79[b]	7.75[b]	0.01

[T] Values with different superscripts differ ($P \leq 0.10$).

Mean plasma glucose concentration was greater ($P = 0.03$) for ENER and PROT compared with CON heifers, and similar ($P = 0.96$) between ENER and PROT (Table 4). Glucose concentration in beef cattle was positively associated with feed intake and rate of BW gain, as observed herein based on the greater nutrient intake and ADG of PROT and ENER compared with CON heifers (Table 2). However, starch is the major dietary precursor for glucose in ruminants; hence, it would be expected that ENER heifers had greater plasma glucose concentrations compared to PROT. Nevertheless, Huntington (1997) indicated that growing cattle are highly capable of synthesizing glucose from amino acids, such as those provided in the PROT treatment or produced by rumen microbes. Mean plasma insulin and IGF-I concentrations were greater ($P \leq 0.08$) for PROT and ENER compared with CON heifers, and did not differ ($P > 0.15$) between PROT and ENER heifers (Table 3).

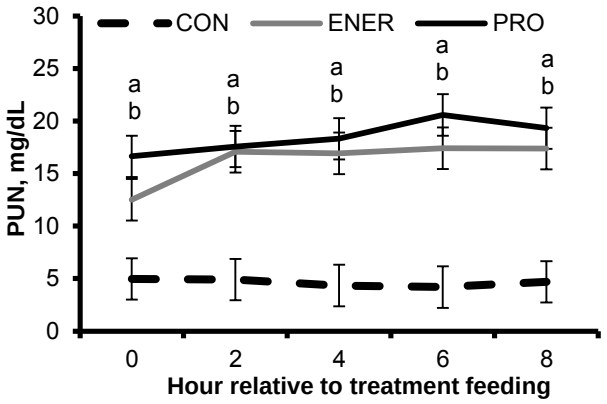

Figure 1. Plasma concentrations of urea N (PUN) in pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4) or supplements based on protein (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4). A treatment × hour interaction was detected ($P < 0.01$) for PUN. Within hour, letters indicate the following treatment differences; a = PROT vs. CON ($P < 0.01$), b = ENER vs. CON ($P < 0.02$).

A treatment effect was also detected ($P = 0.01$) for plasma P_4 concentration. Progesterone concentrations on d 0 were significant covariates ($P < 0.01$) but did not differ ($P = 0.98$) among treatments (6.84, 6.84, and 6.99 ng/mL for CON, ENER, and PROT, respectively; SEM = 0.71), indicating that heifers from all treatment groups had similar plasma P_4 concentration prior to the beginning of treatment administration. Within samples collected on d 13, 15, 17, and 19, mean plasma P_4 concentrations were greater ($P \leq 0.01$) for PROT and ENER compared with CON heifers, and did not differ ($P = 0.93$) between PROT and ENER heifers (Table 3). The main hypothesis of this experiment was that beef heifers consuming a low-quality cool-season forage and receiving a supplement containing an energy ingredient would have greater plasma P_4 compared with unsupplemented or cohorts receiving a supplement

based on a protein ingredient. This hypothesis was developed based on the premise that energy ingredients such as corn favor circulating concentrations of glucose, insulin, and IGF-I (Molento et al., 2002), whereas insulin and IGF-I have been positively associated with circulating P_4 concentration. More specifically, IGF-I is known to stimulate luteal P_4 synthesis (Spicer and Echternkamp, 1995). Insulin also stimulates luteal P_4 synthesis (Spicer and Echternkamp, 1995), and alleviates hepatic P_4 catabolism by CYP2C and CYP3A enzymes (Cooke et al., 2012). In the present experiment, the lack of differences in plasma P_4 concentrations between ENER and PROT heifers, which were greater compared with CON heifers, can be directly attributed to the equivalent treatment effects detected for insulin and IGF-I. Hence, the ENER and PROT treatments utilized herein equally increased plasma P_4 concentrations in pregnant beef heifers consuming a low-quality cool-season forage.

Conclusions

Beef cattle consuming low-quality cool-season forages had similar ruminal forage degradability and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5 % of BW.

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