

THE EFFECTS OF NUTRITION AND REPRODUCTIVE STRATEGIES ON
PERFORMANCE OF BEEF CATTLE GRAZING NATIVE SHORTGRASS RANGE IN
WESTERN KANSAS

by

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Abstract

Cattle grazing dormant native range (< 7% crude protein; CP) require supplementation of additional protein to sustain body weight (BW) and body condition score (BCS). Daily delivery of these supplements is an economic burden to cattle producers faced with challenging economic circumstances. Supplementing cows infrequently (as little as once/week) has produced equivalent BW and BCS changes compared to daily delivery. Dried distiller's grains with solubles (DDGS) provides more ruminally-undegradable protein (RUP; 50-60%) compared to traditional oilseed-meal supplements (i.e. soybean meal) that are >50% ruminally-degradable protein (RDP). Therefore, our objective was to evaluate the effects of supplementation frequency on performance, reproductive success, eating behavior, and subsequent calf performance of spring-calving cows supplemented with DDGS. No differences in ending BW ($P = 0.69$) and BCS ($P = 0.49$), or changes in BW and BCS over the supplementation period ($P = 0.82$ and 0.70 , respectively) were observed among cows supplemented every d, every 3 d, or every 6 d. Calf BW at birth, weaning weight (WW), and average daily gain (ADG) were similar among treatments ($P = 0.19$, 0.12 , and 0.10 , respectively). First-service conception rate (FSCR) and final pregnancy rate (PR) were also not affected by supplementation frequency ($P = 0.62$ and 0.76 , respectively).

The development of replacement heifers is a large expense for cow-calf producers. Improved breeding and heifer development strategies aimed at ensuring the success of replacement females have been developed but reproductive failure still remains a problem. The stress associated with breeding and handling procedures may decrease reproductive success. Therefore, the objective was to determine if intramuscular administration of flunixin meglumine (1.1 mg/kg BW) 14 days post-breeding would improve FSCR and PR in non-transported replacement heifers. Under the conditions of our study, flunixin meglumine did not improve ($P = 0.87$) first service conception rate above that of control heifers (41.2% and 42.3%, respectively). Final pregnancy rate also was not different between treatments and averaged 81.8% ($P = 0.40$).

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Chapter 1 - Review of Literature – Protein Supplementation of Beef Cattle

Introduction

Spring-calving cows grazing dormant native forage during the winter are often deficient in one or more nutrients. Cow nutrient requirements during gestation are greatest during the last trimester, while the forage base is typically low-quality (< 7% CP). Supplementation strategies may be implemented to correct deficiencies of specific nutrients and ensure cow productivity. Supplementing protein may improve forage digestibility; whereas supplementing energy may increase net energy intake. Changes in delivery frequency affect the ruminal environment and metabolic activity. In addition, the use of grain co-products as an inexpensive alternative to conventional feedstuffs may prove more economical. Combined, these considerations can result in more economical and efficient winter supplementation methods for cow-calf producers.

Protein vs. Energy Supplementation

Each grazing situation is unique in terms of nutrient availability. Disregarding measures of forage quality, standing forage dry matter, and failure to implement an appropriate winter supplementation program may negatively affect cow BW and BCS. A forage supply below 7% CP is typically incapable of meeting total cow nutrient requirements (Mathis, 2003). Maintaining acceptable production responses under these circumstances is most often achieved through protein supplementation (Mathis, 2003).

Dormant native range typically supplies sufficient potential energy; however it is often poorly digestible due to the high degree of lignification that accompanies advanced plant maturity. Lignin is a structural plant carbohydrate that is bound to substrate normally utilized by ruminal microbes. Energy supply to ruminal microbes and ruminal nitrogen (N) availability are decreased under these conditions. When ruminal nitrogen concentrations are low the population of fiber-digesting bacteria decreases which hinders ruminal function and results in incomplete forage digestion (Olson and Harty, 2007). Supplemental crude protein provides ruminal microorganisms the N required for optimum efficiency which will improve forage digestion and make additional carbohydrates available (DelCurto et al., 1990b; Heldt et al., 1998; Olson and Harty, 2007).

DelCurto et al. (1990b) evaluated protein supplementation with ruminally-cannulated steers fed low-quality winter-harvested native range (2.9 % CP). These authors found that steers supplemented with medium- and high-protein supplements ($\geq 28\%$ CP) experienced 30% greater neutral detergent fiber (NDF) digestion and increased total dry matter digestibility (DMD) compared to non-supplemented steers.

Conversely, when forage quality is high but quantity of that forage is relatively scarce, energy supplementation is required to maintain performance (Mathis, 2003). Cereal grains provide the energy required, but the rapidly fermenting nature of these supplements causes rumen pH to become more acidic. Fiber-digesting bacteria cannot tolerate an acidic environment and amyolytic species dominate the population (Olson and Harty, 2007). In addition, the substitution effect results in energy intake from supplementation replacing energy intake from the forage base. This can be helpful in maintaining energy intake; however, forage digestibility is immensely reduced.

Chase and Hibberd (1987) examined substitution effects when cows were supplemented with high-energy feedstuffs. Ground corn was substituted for cottonseed meal at 0, 1, 2, or 3 $\text{kg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ in a series of isonitrogenous supplements supplying approximately 256 g CP/d. At low levels of supplementation, corn only comprised a small portion (approximately 10%) of total dry matter intake (DMI). However at high levels, not only did corn account for approximately 50% of total DMI but DMI had also decreased by almost 4.0 kg compared animals fed low levels of corn. Digestible organic matter intake (DOMI) across all 4 treatments was relatively similar, indicating that the inclusion of corn (or any similar energy supplement) will not improve and may even decrease utilization of low-quality forage.

When energy supplementation is warranted, high-fiber feedstuffs are a viable alternative. Normally, negative fermentation characteristics that are typically associated with high-starch feedstuffs can be avoided while still supplying ample energy (Mathis and Sawyer, 2007). Bodine et al. (2001) observed an increase in ADG with a high-fiber supplement (wheat middlings/soybean hulls-based) versus a protein supplement (cottonseed meal-based) on beef heifers fed limited intakes of high-quality bermudagrass (10-15% CP). Martin and Hibberd (1990) supplemented mature beef cows and first-calf heifers fed low-quality grass hay (4.1% CP) with increasing levels of soybean hulls. Peak forage intake occurred at 1 $\text{kg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ of supplementation and declined slightly with increased supplementation.

Heldt et al. (1999) also compared protein and energy supplementation with ruminally-cannulated steers consuming a low-quality prairie hay diet (5.7% CP). These authors observed that RDP improved organic matter digestibility (OMD) by 10% and TDOMI by 27% compared to non-supplemented steers. Conversely, increasing energy supplementation in the form of starch decreased ruminal ammonia (NH_3) concentrations and forage digestibility.

Supplementing cows grazing low-quality forage with soybean meal significantly improved forage DMI and total DMD compared to supplementation with cracked barley (i.e. a high-energy feedstuff; Kartchner, 1980). Additionally, Larson et al. (2009) observed sustained long-term effects on steer calf progeny of protein-supplemented versus non-supplemented dams. Steer calves from supplemented dams had significantly better marbling scores and a greater proportion graded Choice or better.

Collectively, these studies demonstrated that supplementation of high-starch feedstuffs to grazing livestock consuming low-quality forage was detrimental to forage utilization. Instead, it is clear that a proper winter feeding strategy should focus on protein supplementation.

Site of Protein Digestion

The crude protein content of feedstuffs is comprised of RDP and ruminally-undegradable protein (RUP; NRC, 2000). Ruminally-degradable protein is the fraction broken down into NH_3 and actively incorporated into microbial cell protein (MCP), which will eventually be passed into the small intestine, digested, absorbed, and utilized by the animal. Additionally, the free NH_3 produced by ruminal microbes can be transported across the ruminal wall, packaged as urea in the liver, and maintained in the blood stream. Urea may be re-circulated to the rumen where it undergoes degradation to NH_3 and CO_2 and can be used as a source of N by ruminal microbes. In contrast, RUP escapes ruminal microbial degradation and can be absorbed from the small intestine and directly utilized by the animal without undergoing ruminal fermentation. The differences in digestion and/or absorption site may affect the utility of various protein sources for animals fed low-quality forage diets.

When forage quality is low, the use of high-RDP supplements is the most widely accepted method of improving overall DMI and DMD (Bohnert et al., 2002b). Ruminal microorganisms require specific nutrients for growth, many of which result from degradation of

RDP (Allison, 1969; Russell and Hespell, 1981). Thus, supplemental RDP stimulates ruminal microorganisms and enhances digestion of low-quality forage diets.

Increasing supplementation of RDP improved digestion of low-quality forage (Mathis et al., 2000). When ruminally-fistulated steers were consuming bromegrass hay (5.9% CP) or a forage-sorghum hay (4.3% CP), forage OMI, TOMI, and TDOMI linearly increased with increasing levels of supplemental RDP (0 to 0.124% of BW). Additionally, total-tract OM digestion and NDF digestion of the lowest quality forage (sorghum hay) was significantly improved with increasing RDP supplementation, although this same improvement was not noted for steers consuming bromegrass hay.

Bandyk et al. (2001) examined the effect of site of supplemental protein digestion by infusing casein either ruminally or post-ruminally (RDP or RUP, respectively) to steers fed low-quality native hay (3.4% CP) for 7 d without supplementation. During a 10-d collection period, cattle supplemented ruminally averaged a 3 kg/d increase in forage intake. Cattle supplemented post-ruminally experienced a drastic decline in forage intake for the first 4 d of supplementation, only to increase forage intake at roughly half of the magnitude of the ruminally-infused steers. These data were interpreted to indicate that the change in forage intake for post-ruminally-infused steers may be due to satisfying ruminal microbes need for N through increased urea N recycling.

The role of supplementing RDP or RUP to steers consuming low-quality forage and the resulting effects on urea recycling was evaluated in two separate studies (Wickersham et al., 2008; Wickersham et al., 2009). Increasing levels of RDP supplement (from 61 g/d to 183 g/d) increased TDOMI, as well as urea production, amount of urea entering the gut, and recycled urea utilization. The same trends were observed with increasing RUP supplementation as well (from 62 g/d to 186 g/d). The highest levels of RDP and RUP supplementation resulted in similar numerical values for TDOMI, while supplementation with RDP improved forage OMI and NDF digestion compared to that for RUP supplementation. In contrast, supplementation of RUP tended to increase N retention as a proportion of N absorbed compared to RDP supplementation. The amount urea transferred to the gut as a proportion of total N intake was also greater for RUP supplementation than for RDP supplementation (69 and 45%, respectively).

The effect of increasing RUP supplementation was also evaluated by Lents et al. (2000) who observed no improvement in cow BW or BCS when increasing levels of RUP (53 g/d, 106

g/d, and 159 g/d) were supplied with a static amount of RDP from a soybean meal-based supplement to mature cows grazing native range post-calving (February to May). A complicating factor in that study was that forage quality improved during the course of the trial (from 3.9% to 13.5% CP). Therefore, it is possible that the increased protein intake from the forage base was sufficient to meet or exceed cow requirements and negated any potential benefits of RUP supplementation.

Sletmoen-Olson et al. (2000a, 2000b) evaluated increasing supplemental RUP (53, 233, 412 g/kg supplement DM, respectively) in two companion studies using pregnant cows supplemented with static RDP (211 g/kg supplement DM) and consuming ad libitum cool-season hay (5.8% CP). Forage OMI and total OMI were not affected by increasing supplemental RUP. However, cow BCS was greater when cows were supplied with moderate- and high-RUP compared to low-RUP supplementation. This may be a function of the increased MP supply observed in moderate- and high-RUP supplemented cows (95 and 314 g/d, respectively) compared to low-RUP supplemented cows (-104 g/d). Concurrently, plasma NEFA concentrations were higher in low-RUP cows compared to moderate- and high-RUP cows. Higher NEFA concentrations have been related to negative energy balance (Erfle et al., 1974) and increased body fat mobilization (Blauwiekel and Kincaid, 1986).

No improvements in ADG, BCS status, or pregnancy rate were observed in cows supplemented with a static amount of RDP (0.09 % BW) and increasing amounts of RUP (0.05%, 0.07%, and 0.09% BW, respectively) when grazing low-quality warm-season pastures (2.3% CP; Bailey et al., 2011). Additionally, cows fed the low-RUP level (0.05% BW) and fed low-quality warm-season hay (2.1% CP) had greater forage DMI, total DMI, and total digestible DMI compared to moderate- or high-supplemented cows. Conversely, high-supplemented cows (0.09% BW) had a greater total-tract DMD compared to low-supplemented cows (52.4% vs. 51.8%), which may be attributable to greater supplement intake.

Bohnert et al. (2002b) concluded that RUP supplements can be beneficial to ruminants grazing low-quality forages. These authors hypothesized that some portion of high-RUP supplements is available to the ruminal microbes, even though a majority of the CP bypasses ruminal degradation. Depending on the amount of RDP supplied, this can be adequate to maintain ruminal microbial function.

Rusche et al. (1993) examined supplementation of RDP (a soybean-meal based supplement) or RUP (a corn gluten meal/blood meal-based supplement) fed to mature cows consuming low-quality prairie hay (6% CP). These researchers found that diets high in RUP increased ADG when fed at 150% of NRC (1984) requirements for CP compared to a high-RDP diet also formulated to meet 150% of NRC requirements for CP. Supplying additional protein post-rationally may have increased urea recycling, and satisfied the ruminal demand for NH_3 . Additionally, greater flow of basal amino acids (AA) to the small intestine may have been directed toward body tissue gain (Rusche et al., 1993).

Sawyer et al. (2012) evaluated the efficiency of high-RDP and high-RUP supplements in ruminally-cannulated cows. A 50:50 blood and feather meal mixture represented a high-RUP supplement while cottonseed meal served as the high-RDP supplement. Total retained N as a proportion of intake was not different when comparing RDP- and RUP-based supplements. Total-tract NDF digestibility and DMD also were similar between treatments. Under these conditions, utilizing a RUP can be as effective as RDP in improving forage digestion.

Differences between RDP- and RUP-based supplements were also evaluated by Mulliniks et al. (2012). Over a 4-year study encompassing 333 cows, hand-fed cottonseed meal was compared to a self-fed supplement formulated with 50% of DM as a blood and feather meal mix (i.e. feedstuffs high in RUP). Even with the inherently lower supplement intake observed with the self-fed supplement, cow BW, BCS, and reproductive success were not different between treatments. High-RUP supplements had a greater efficiency compared to cottonseed meal (1.32 kg BW spared/kg supplement fed vs. 0.57 kg BW spared/kg supplement fed, respectively) when intake was evaluated. This improved efficiency with high-RUP supplementation and lower required cow intake could culminate in reduced cow costs per year.

The infusion of casein, either ruminally or post-rationally, yielded significantly different metabolic responses in wethers consuming bromegrass hay (5% CP; Swanson et al., 2004). Rumen:abomasum infusion ratios were 100:0, 67:33, 33:67, 0:100. Total retained N as a proportion of intake significantly increased with a shift toward abomasal infusion with the most effective ratio being 33:67. The infusion ratios were chosen to be similar to the relative RDP and RUP fractions of commonly-used protein supplements and may warrant inclusion of RUP (at $\geq 33\%$) into a grazing livestock scheme to improve retained N. However, it is important to note

that all wethers were supplemented with soybean meal during the infusion periods, possibly confounding the effects of additional RDP infusion.

Researchers also evaluated several combinations of RDP and RUP supplementation in the form of infused casein in beef steers (Wickersham et al., 2004). Six levels of RDP (ranging from 0 to 1.45 g CP) were used in a factorial arrangement with 2 RUP levels (0 and 0.87 g CP). When steers were infused strictly post-ruminally, TDOMI, digestible NDF intake, and hay organic matter OMI all increased significantly compared to strictly ruminal infusion. Conversely, equal amounts of RDP and RUP infusion yielded greater improvements in these measures compared to RDP or RUP supplementation alone. These results were interpreted to suggest that supplementation with a feedstuff that provides moderate amounts of both RDP and RUP could prompt the most significant improvements in forage utilization, BW, and BCS status.

Level of Protein Supplementation

Protein supplements vary in crude protein content. A protein supplement exceeding 30% CP should be provided to maximize both intake and digestibility of low-quality forages (Heldt et al., 1998). Beaty et al. (1994) varied the crude protein content of the protein supplement while maintaining a 2 kg/hd feeding rate. The greatest BCS losses occurred when low-protein supplements (approximately 10% CP) were fed. Utilizing supplements above 30% CP limited BCS losses during the winter grazing period for spring calving cows to one-half of a score.

DelCurto et al. (1990a) examined the effects of varying CP levels on cow BW and BCS changes using low (13% CP), moderate (25% CP), and high (39% CP) protein supplements. The supplements were isocaloric mixtures of soybean meal and dry-rolled sorghum grain and fed at 0.5% BW/d. Cows receiving the 25 or 39% CP supplements gained weight during the second trimester of gestation, while the cows receiving the 13% CP supplement lost weight during the same period. All cows lost weight during the final trimester until 48 h postpartum; however, the 39% CP supplement minimized BW and BCS losses during this period.

Differences in cow protein requirements pre- and postpartum are a major factor in selecting a supplement that is > 30% CP. Accelerated fetal growth and an increased fetal demand for glucose during the third trimester result in the greatest nutritional requirements for the cow during gestation. Cows fed high-protein supplements were better able to meet these requirements, and it has been postulated that increased CP content may provide precursors for

gluconeogenesis (DelCurto et al., 1990a). Although cows fed high-protein supplements lost BW and body condition during the third trimester, those losses were minimized and the additional BW and body condition gained pre-partum allowed for mobilization of body reserves, likely resulting in increased milk production, and improved calf growth (DelCurto et al., 1990a).

Mathis et al. (1999) varied soybean meal intake based on BW to cows grazing low-quality winter range. A range of allotted intakes (from 0.1% - 0.5% BW/day) showed a distinct plateau in BCS change. At 0.1% BW/day, BCS loss was approximately 1.5 scores. This loss decreased by one-half score for every 0.1% increase in intake. At 0.3% of BW and beyond, BCS was static. Since soybean meal is mostly comprised of RDP, this potentially demonstrates that the RDP requirement was attained and any additional RDP supplementation was unnecessary.

Köster et al. (1996) evaluated the effects of different amounts of RDP supplementation to mature, non-pregnant cows grazing native tallgrass-prairie. Protein, in the form of casein, was infused ruminally from 0 to 720 g/d in 180 g increments. Forage OMI, TOMI, and TDOMI all increased with the first level of supplementation (180 g/d). The addition of more RDP continued to increase these measures, but at a decreasing rate. The diminishing response to additional RDP may demonstrate that the RDP requirement for this class of cattle was met under these conditions and the potential to improve forage intake with RDP supplementation is limited (Köster et al., 1996).

Infrequent Protein Supplementation

Delivering protein supplements infrequently has become a common practice among cattle producers. The potential savings in labor and fuel are accompanied by BW and BCS changes that are similar to daily supplementation (Wettemann and Lusby, 1994; Bohnert et al., 2002b; Schauer et al., 2005). When mature ewes are supplemented less frequently (as infrequently as once/week), the net N ingested must remain static when compared to daily supplementation in order to be effective (Krehbiel et al., 1998). In this scenario, the excess N supplied on the day of supplementation exceeds the capability of the microbes to incorporate the available N into microbial crude protein (MCP). Thus, the excess N is converted to NH_3 , transported across the ruminal wall and packaged in the liver as urea (due to the potential toxic effects of accumulated NH_3). The rumen then becomes more permeable to urea due to the concentration gradient that forms between the capillary bed surrounding the rumen and the rumen itself, resulting in greater

N recycling to the rumen via saliva or by profusion of the ruminal wall (Krehbiel et al., 1998; Marini & Van Amburgh 2003). The increased N recycling results in greater retained N as a proportion of intake (Bohnert et al., 2002b).

Wickersham et al. (2008) ruminally infused casein into steers consuming low-quality hay daily or every 3 d. On non-supplementation days, ruminal ammonia levels decreased due to a lack of dietary CP. As a result, 42.1% of MCP came from N that had been previously stored as urea and recycled to the rumen, compared to only 22.8% for steers supplemented daily. Researchers also observed greater retained N, urea production, and recycling for steers supplemented 3 d/wk compared to steers supplemented daily. These data may indicate that livestock supplemented infrequently are more dependent on urea recycling than those supplemented daily.

Farmer et al. (2004) evaluated ruminal NH_3 concentrations of steers consuming low-quality hay (5.3% CP) and supplemented with a soybean meal:sorghum grain mixture either daily or 2 d/wk. Steers supplemented 2 d/wk maintained greater ruminal NH_3 concentrations 24 to 48 h post-supplementation. Steers supplemented daily had elevated NH_3 concentrations for 12 h post-supplementation. In addition, peptide- and amino acid-fermenting bacteria counts peaked at higher concentrations and remained in the rumen longer (up to 48 h) with infrequent supplementation. As a result, total absorbed N was similar to daily supplementation. Longer periods of elevated ruminal NH_3 and peptide-fermenting bacteria on the day of supplementation in infrequently supplemented steers could allow for more complete forage digestion and ultimately translate to greater efficiency of forage use. Additionally, urea N recycled to the rumen on non-supplementation days may continue to improve forage digestion.

Bohnert et al. (2002a) fed wethers a soybean meal-based diet or a blood meal:soybean meal mixture. In wethers supplemented as infrequently as once every 6 d and consuming low-quality forage (5% CP), there was no difference in how efficiently the rumen utilized dietary N when compared to wethers supplemented daily, as evidenced by similar retained N. Conversely, McGuire et al. (2013) observed greater retained N in wethers consuming fescue straw (4.7% CP) and supplemented with soybean meal every 2 days compared to wethers supplemented daily (57.5% and 42.5%, respectively). However, researchers also observed no effect of supplementation frequency on OMI, NDFD, and N digestibility in both wethers and steers

consuming low-quality hay and supplemented with soybean meal. This has been interpreted to indicate a more efficient use

Daily delivery of supplements increases fermentable substrate available to ruminal microbes which increases passage rate; whereas, ruminants supplemented infrequently would potentially consume solely a low-quality forage on non-supplementation days, slowing passage rate, increasing ruminal retention time, and possibly allowing for more complete forage digestion. Atkinson et al. (2010) observed that on non-supplementation days when the forage base (crested wheatgrass hay; 4.2% CP) was the sole dietary component for wethers, passage rate slowed resulting in greater retention time and apparent digestibility. Infrequent supplementation with either isolated soy-protein or corn gluten feed also improved ruminal OM, NDF, and ADF digestibility (as a proportion of intake) compared with daily supplementation. Additionally, indigestible ADF (IADF) was greater with daily supplementation due to increasing passage rate and reduced apparent digestibility of the forage (4.3% CP) compared to 3 d/wk supplementation (Beaty et al., 1994).

Cow performance improves with infrequent protein supplementation due to improved forage digestion (Farmer et al., 2001). Late gestation beef cows were supplemented with a pelleted mixture of sunflower and cottonseed meal either frequently (7 d/wk) or infrequently (2 d/wk) while grazing dormant tallgrass-prairie. Infrequently-supplemented cows compensated for BCS loss early in the last trimester of gestation by improving BCS status during the 30 d preceding calving, ultimately resulting in similar overall BCS loss during the last trimester of gestation compared to frequently supplemented cows. Cows supplemented 7 d/wk still experienced less total BCS loss than cows supplemented 2 d/wk; however, the productive responses resulting from infrequent supplementation were not drastically different compared to those resulting from daily supplementation. In addition, supplementing late gestation beef cows with cottonseed meal daily, 3 d/wk, or 1 d/wk during late gestation did not affect ending BW or BCS (Huston et al., 1999).

Hunt et al. (1989) supplemented growing steers fed harvested fescue hay (6.6% CP) with cottonseed meal at 12 h, 24 h, or 48 h intervals. Steers supplemented on alternate days had greater digestible DMI than non-supplemented steers and greater ADG compared to steers supplemented daily. Additionally, total-tract DMD was greater in steers supplemented 3 d/wk versus steers supplemented 7 d/wk when consuming wheat straw (Beaty, et al., 1994).

Dried Distiller's Grains with Solubles

The use of dried distiller's grains with solubles (DDGS) in livestock diets has dramatically increased with the rapid expansion of ethanol production in the U.S. This byproduct of ethanol production is readily available at a relatively inexpensive cost to the producer, compared to traditional supplemental protein feedstuffs. Since DDGS are derived from corn and sorghum (which are inherently lower in CP) and Maillard reactions denature existing protein during the heating process, it is lower in CP when compared to traditional oilseed meals (28-32% CP; NRC, 1996; Shurson and Noll, 2005). Soybean meal is approximately 60-75% RDP (NRC, 2000; Anderson et al., 2001); whereas DDGS contain more moderate amounts of RDP (40-50%; NRC, 1996; Shurson and Noll, 2005; Winterholler et al., 2009).

Morris et al. (2005) supplemented DDGS (from 0 – 2.72 kg/d in 0.68 kg increments) in both summer and winter feeding scenarios to growing heifers. Brome hay (53% TDN) simulated low-quality forage or hay feeding. An alfalfa:sorghum silage mixture (60:40) represented high-quality summer forage (65% TDN). Average daily gain was greater under simulated winter feeding compared to summer supplementation (0.12 kg/kg DDGS and 0.09 kg/kg DDGS, respectively). For every kilogram of DDGS supplied, a 0.15 kg decrease in forage intake on the day of supplementation was observed. The observed decrease in forage intake was attributed to ruminal fill and did not alter performance. The ability for DDGS to correct a protein deficiency while augmenting carbohydrate intake makes it a versatile product that can potentially allow for improved utilization of standing forage at the end of winter grazing or allow for conservation of hay reserves.

Stalker et al. (2009) observed a decrease in apparent total-tract DM, OM, and NDF disappearance when DDGS was supplemented every 3 d compared to daily supplementation. These observations may be in part attributable to the additional fat intake on the day of supplementation which may have elevated dietary fat levels above 7%. These levels may depress OM digestibility and account for lower total-tract OM disappearance (Shurson and Noll, 2005).

When compared to a RDP-based protein supplement such as soybean hulls, heifers in the last trimester of gestation and supplemented with DDGS maintained similar performance (Engel et al., 2008). The soybean hulls utilized in this experiment were calculated to be approximately 80% RDP, while the DDGS were below 60% RDP. Over the 2 year experiment, BW and BCS change were not different between treatments. Subsequent calf ADG and cow pregnancy rate

followed the same trend. Other researchers (Rusche et al., 1993; Mulliniks et al., 2012) observed similar trends in cow pregnancy rate when comparing RDP- and RUP-based supplements.

Winterholler and colleagues (2012) compared DDGS to a wheat middlings/cottonseed meal mixture when fed to spring-calving beef cows consuming low-quality hay (5.6% CP). An intermediate feeding level (1.54 kg/d) of DDGS and a wheat middling/cottonseed meal mixture provided equal dietary CP and approximate RDP balance and excess, respectively. Changes in BW and BCS preceding parturition were similar, regardless of whether the RDP requirement was met, or in excess (DDGS vs. wheat middlings/cottonseed meal mix). Cows fed the wheat middlings/cottonseed meal mix demonstrated greater serum urea nitrogen (SUN) concentrations compared to DDGS-supplemented cows, indicating inefficient N use (Hammond, 1997) and increased urinary N excretion (Kohn et al. 2005).

Based on the previous research, use of DDGS as a protein supplement when livestock are consuming low-quality forages can elicit results similar to the use of traditional oilseed meal supplements. This was interpreted to indicate that minimal RDP supplementation and a shift to a protein supplement that will be utilized post-rationally will not adversely affect performance. The additional dietary RUP from DDGS may be effectively converted to urea and recycled to the rumen for utilization by ruminal microorganisms.

Summary

Forage availability and quality affect the type and source of supplement required to maintain cow performance. Determining the appropriate supplement required and the optimum frequency of delivery allows nutrient requirements to be met while minimizing the operational costs associated with supplement delivery.

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Chapter 2 - Effect of Supplementation Frequency on Performance of Spring-Calving Cows Supplemented with Dried Distiller's Grains with Solubles

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Abstract

Pregnant Angus-cross cows (Year 1: $n = 79$, age = 5.3 ± 3.0 yr, BW = 578 ± 76 kg, BCS = 5.1 ± 0.5 ; Year 2: $n = 148$, age = 4.9 ± 2.5 yr, BW = 578 ± 75 kg, BCS = 5.6 ± 0.5) were stratified by age, BW, and BCS and assigned randomly to one of three dried distiller's grains with solubles (DDGS) supplementation frequency treatments in a completely randomized design: 1) daily supplementation (DDGD; control); 2) supplementation every 3 d (DDG3); 3) supplementation every 6 d (DDG6). Cows in Year 2 were assigned randomly to treatments irrespective of previous treatment. Cows were maintained on common native range. Treatments were initiated 84 d prior to expected onset of calving. Supplement was prorated to supply 0.27 kg CP·head⁻¹·d⁻¹. Cows were sorted daily before supplementation at 0830. Forage sorghum hay was supplied at 50% of expected DMI during Year 1 due to drought and to ensure ample forage supply (annual precipitation was 81.5% of the National Weather Service 30-yr average). Forage sorghum hay was only supplied in Year 2 when snow cover prohibited grazing. Cow BW and BCS were measured every 28 d prior to supplementation and within 12 h of parturition. Progesterone (P4) concentrations were measured in paired serum samples collected before ovulation synchronization initiation to determine proportion of estrual cows during Year 1. Proportion of cows consuming hay 60 min post-feeding was observed during an 11 d period in Year 1. Supplementation frequency did not affect ending BW and BCS ($P = 0.69$ and 0.49 , respectively) or BW and BCS change ($P = 0.82$ and 0.70 , respectively). No differences ($P \geq 0.23$) were observed for calf BW at birth or weaning, or calf ADG in year 1. Supplementation frequency did not affect ($P \geq 0.62$) proportion of estrual cows (36%), first service conception rate (73%) or final pregnancy rate (95%) in Year 1. A greater proportion ($P = 0.01$) of cows assigned to DDGD, DDG3 or DDG6 (and not supplemented on the d of observation) were consuming hay 60 min post-feeding compared to cows assigned to DDG6 (and supplemented on the d of observation). Reducing supplementation frequency to once every 6 d did not adversely affect performance, reproductive success, or subsequent calf performance of spring-calving beef cows supplemented with DDGS.

Key words: cow performance, dried distillers grains, supplementation frequency

Introduction

During the winter cattle commonly graze standing dormant native range (< 7% CP). The lack of dietary protein supplied by this dormant forage may limit cow performance through decreased microbial efficiency and forage utilization. Thus it is vital that cattle grazing dormant native range be offered supplemental protein to sustain BW and BCS during the last trimester of gestation (Beatty et al., 1994; Huston et al., 1999; Mathis et al., 1999; Mathis and Sawyer, 2007; Olson and Harty, 2007). The production responses associated with protein supplementation occur not only from the additional protein provided by the supplement, but a more efficient utilization of the dormant forage base (Bohnert et al., 2002a).

Protein supplementation and delivery burden cattlemen with extra cost, but reducing supplementation frequency may be used to reduce delivery costs (Mathis and Sawyer, 2007). Supplementing a high protein feedstuff (> 30% CP) as infrequently as once per week has resulted in similar BW and BCS changes when compared to daily delivery of the same supplement (Wetteman and Lusby, 1994; Bohnert et al., 2002b; Schauer et al., 2005).

Dried distiller's grains with solubles (DDGS) have become an increasingly popular feedstuff in beef cattle production systems. The nutrient profile, availability, and cost of DDGS make it a suitable substitute for conventional protein feedstuffs. Morris and coworkers (2005) observed a 0.12 kg increase in ADG with every 0.45 kg of DDGS supplemented to cattle fed low-quality hay. More recently, pregnant cows fed tallgrass-prairie hay and supplemented with DDGS exhibited similar BW and BCS losses compared to a wheat middlings/cottonseed meal supplement (Winterholler et al., 2012).

Therefore the objective of this study was to evaluate the effects of DDGS supplementation frequency on cow performance, reproductive success, eating behavior, and subsequent calf performance of spring-calving cows grazing dormant native range during the last trimester of gestation.

Materials and Methods

All procedures involving the handling and care of animals used in our experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol no. 3175)

Animals and Experimental Design. Pregnant Angus-cross cows (Year 1: $n = 79$, age = 5.3 ± 3.0 yr, BW = 578 ± 76 kg, BCS = 5.1 ± 0.5 ; Year 2: $n = 148$, age = 4.9 ± 2.5 yr, BW = 578 ± 75 kg, BCS = 5.6 ± 0.5) were stratified by age, BW, and BCS and assigned randomly to one of three supplementation frequency treatments: 1) supplementation daily (DDGD; control); 2) supplementation every 3 d (DDG3); 3) supplementation every 6 d (DDG6). Cows in Year 2 were assigned randomly to treatments irrespective of previous treatment. Cows were maintained on common native range (Table 2.1) for 84 d and sorted daily into treatment groups. Composition of the range site was determined using a step point method (Evans and Love, 1957). The range site was comprised of the following species; Sideoats Grama (*Bouteloua curtipendula*; 31.8%), Western Wheatgrass (*Agropyron smithii*; 20.2%), Blue Grama (*Bouteloua gracilis*; 11.9%), Japanese Brome (*Bromus japonicus*; 11.4%), and Buffalograss (*Bouteloua dactyloides*; 3.0%) (K. Harmony, 2011, Kansas State University Agriculture Research Center – Hays, 1232 240th Ave, Hays, KS, personal communication). Dried distiller's grains with solubles (Table 2.1) originated from a single location each year, was delivered, and stored in bulk for use throughout the treatment period. Supplement was delivered at approximately 0830 into a bunk for consumption. Only one set of bunks was available, therefore on days when multiple supplement treatments were fed each group would be given ample time to finish the supplement before being moved out of the feeding area. Cows were allotted 71.1 cm of linear bunk space/head. Supplement was offered to supply $0.27 \text{ kg CP} \cdot \text{head}^{-1} \cdot \text{d}^{-1}$.

Forage sorghum hay (Table 2.1) was supplied at 50% of expected DMI during Year 1 to ensure ample forage supply, as pastures had been previously grazed by cow/calf pairs from April to October and due to the persistence of drought conditions (Table A.1). During Year 2, forage sorghum hay was provided ($n = 12$ d) when snow cover prohibited grazing. Mineral (Suther's Prairie Cow 4P; Suther's Feeds, Frankfort, KS) was available ad libitum before and during the experiment. At the end of 84 d, treatments were discontinued and cows were offered DDGS daily in a common pasture and fed ad libitum forage sorghum hay. Cows were maintained in this manner until turnout on summer pasture (d 132, Year 1; d 130, Year 2).

Data Collection. Range forage samples for nutrient analysis were obtained prior to trial initiation in both years. Samples were collected from multiple random 1 m^2 areas, clipped 2 cm above the surface, placed in plastic sealable bags, and immediately frozen. All samples were thawed and ground through a Wiley Mill (2 mm screen; Arthur H. Thomas, Philadelphia, PA),

composited, and frozen at -20°C until laboratory analysis for nutrient content. Core samples were collected each year from a random sample of forage sorghum bales (n = 20), composited, and frozen. Random samples of DDGS were collected each year at delivery, composited, and frozen. Feed samples were submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) and analyzed for DM, CP, NDF, ADF, Ca, P, and S.

Cow BW and BCS were measured every 28 d and within 12 h following parturition. Cows BW was measured at 0900 on each respective weigh day. Supplement and hay were withheld the morning of BW collection and fed immediately after all cows had been weighed. Two independent, qualified observers assigned BCS using a 9-point scale (1= extremely emaciated, 9=extremely obese; Wagner et al., 1988) on each weigh date, including the day of parturition. Calf BW was obtained during both years within 12 h of birth and at weaning (d 196, Year 1). Calves were weaned at 113 ± 17 d in Year 1 due to the persistence of drought conditions.

Backfat thickness (BF), longissimus dorsi muscle (LM) depth, and marbling score (MS) were measured at the 12th-rib ultrasonically on d 0 and 84 in Year 1 using an Aloka 500V (Aloka Co., Ltd., Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-12mm window). Ultrasound images were collected with Cattle Performance Enhancement Company (CPEC, Oakley, KS) software. Backfat thickness, LM, and MS were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC software.

Estrus Determination. Blood samples were collected via coccygeal venipuncture 10 d before and on the d ovulation synchronization was initiated during both years. Samples were collected into 10 mL serum vacutainer tubes (BD Vacutainer™; Becton, Dickinson, and Company, Franklin Lakes, NJ) then immediately placed on ice, allowed to clot for 24 h at 4°C and then centrifuged ($1,500 \times g$) for 10 min. Serum was decanted into 12 × 75 mm plastic tubes and immediately frozen (-20°C). Concentration of P4 in serum was subsequently quantified using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA; Stevenson, 2011). Intra- and inter-assay CV were 3.4 and 7.6% and assay sensitivity was 0.009 ng/mL for Year 1 and 4.2%, 7.5%, and 0.014 ng/ml, respectively for Year 2. Blood collected was used to verify the functional presence of a corpus luteum. If any 1 of the 2 samples contained $P4 \geq 1$ ng/mL (typical of cows in the luteal phase of the estrous cycle), cows were assumed to be cycling before the onset of ovulation synchronization

treatments. If concentrations in the 2 samples were <1 ng/mL, cows were considered to be noncycling.

Ovulation Synchronization and Breeding. During Year 1, ovulation was synchronized in equal proportions of the three supplementation frequency treatments by assigning cows from each treatment randomly to the 7 d Co-Synch + controlled internal drug release (CIDR; EAZI-Breed CIDR[®]; Zoetis, Madison, NJ) protocol (Larson et al., 2006) or a modification of the 7 d Co-Synch + CIDR protocol. Cows assigned to the 7 d Co-Synch+CIDR protocol received 100 µg of GnRH intramuscularly (2 mL of Cystorelin; Merial, Duluth, GA) and a CIDR (containing 1.38 g of P4) insert on d -10 followed by an injection of 25 mg prostaglandin F_{2α} (PGF_{2α}) intramuscularly (5 mL of Lutalyse; Zoetis, Madison, NJ) and the CIDR removal on d -3. This was followed in 62 h by fixed-time AI (FTAI) and a second 100 µg injection of GnRH (d 0). Cows assigned to the modified 7 d Co-Synch+CIDR protocol were treated similarly but also received an injection of 25 mg PGF_{2α} intramuscularly (d -20) and an injection of 100 µg GnRH intramuscularly (d -17).

Cows were exposed to 5 fertile bulls 10 d after FTAI for the remaining 35 d of a 45-d breeding season during both years. First service conception rate (FSCR) was determined by transrectal ultrasonography (Aloka 500V, 5MHz transrectal transducer; Wallingford, CT) 35 d after FTAI. A positive pregnancy outcome required the presence of uterine fluid and an embryo with a heartbeat. A final pregnancy rate (PR) was determined 35 d after the end of the breeding season via transrectal ultrasonography.

Eating Behavior. For an 11-d period in Year 1 (d 69-79), cow eating behavior was observed. This time frame was chosen to ensure a minimum of 2 supplementation events would coincide within the observation period for each treatment group. Cows were fed hay approximately at 0930 each day. The number of cows, within each treatment group, actively consuming hay was recorded 60 min post-feeding each day during the observation period.

Statistical Analysis. Cow was utilized as the experimental unit. The model included the main effects of supplementation frequency, year, and their interaction. Performance data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Cow BW and BCS at d 0, 28, 56, 84, and parturition, BW change, BCS change, calf BW at birth, calf BW at weaning, calf ADG from birth to weaning and average Julian calving date were used as the dependent variables. Number of cows consuming hay 60 min post-feeding was converted to proportion

within supplementation frequency treatment group. Data were transformed, due to non-normality, using the arcsine-square root (angular) transformation according to Martin and Bateson (1986). Behavior data were analyzed using the GLM procedure of SAS using supplementation frequency treatment group, supplementation status (supplemented or non-supplemented on the day of observation), and their interactions as independent variables. Behavior data are reported as proportion of supplementation frequency treatment group. Reproductive data was analyzed as a generalized linear mixed model with a 1-way treatment structure in completely-randomized design (PROC GLIMMIX), using the binary distribution with logit-link function. The model included the main effects of supplementation frequency, ovulation synchronization method, and their interaction. Reproductive success among supplementation frequency groups was not affected ($P > 0.50$) by ovulation synchronization method and it was removed from the model for final analysis. The class factor was treatment and the model statement included only the treatment fixed effect. Treatment means with SE and F-test p-value were reported. Least square means are presented and differences were considered significant at $P \leq 0.05$. Tendencies were considered to exist from $0.05 < P \leq 0.10$.

Results and Discussion

Initial cow BW and BCS were not different among treatments ($P \geq 0.94$; Table 2.2, 2.3). There was no treatment \times year effect ($P \geq 0.22$) for BW across weigh dates. Cow BW was greater across treatments at d 28 ($P = 0.01$; Table B.1) during Year 2 and at parturition ($P = 0.04$) during Year 1. A treatment \times year interaction was observed for BW change during Periods 2 and 3, but no treatment \times year effect for overall BW change (d 0-84). Change in BW across all periods was affected by year ($P \leq 0.002$). Cows supplemented daily had greater weight gain during Period 3 ($P < 0.001$) compared to cows supplemented every 3 or 6 d. Treatment (supplementation frequency) did not affect ending BW (d 84; $P = 0.69$) or overall BW change ($P = 0.82$).

A tendency for a treatment \times year effect was observed for calving BCS ($P = 0.06$). Cow BCS across all weigh dates was greater in Year 2 compared to that of Year 1 ($P \leq 0.008$). Change in BCS during Periods 1 and 2 were affected by treatment \times year and year ($P \leq 0.002$). Period 3 change in BCS was affected by year ($P = 0.02$). Cows supplemented once every 3 d lost BCS during Period 1 ($P = 0.02$) compared to cows supplemented daily or every 6 d; however,

the change in BCS is miniscule and may not play a role in typical production scenarios (0.04, -0.04, and 0.13 for DDGD, DDG3, and DDG6, respectively). Treatment (supplementation frequency) did not affect ending BCS (d 84; $P = 0.49$) or overall BCS change ($P = 0.70$).

Cow BF on d 84 was greater for cows supplemented daily when compared to cows supplemented every 6 d ($P = 0.06$; Table 2.4). However, cows supplemented every 6 d tended ($P = 0.07$) to have greater MS on d 84 compared to cows supplemented daily.

Average Julian calving date was influenced by year ($P = 0.005$; Table 2.2). Calf BW at birth, weaning, and ADG during Year 1 were not affected ($P \geq 0.23$; Table 2.5) by dam's supplementation frequency treatment. Beaty et al. (1994) also reported similar performance for calves from dams that were infrequently supplemented compared supplemented daily.

Infrequent supplementation has resulted in similar cow performance when compared to daily supplementation. Wetteman and Lusby (1994) observed similar changes in cow BW and BCS between cows supplemented 6 d/wk and 3 d/wk and grazing dormant native range. Farmer et al. (2001) observed similar BW and BCS at parturition in cows supplemented either 7 d/wk or 2 d/wk. Bohnert et al. (2002b) and Schauer et al. (2005) also observed no differences in ending cow BW and BCS after 84-d supplementation periods consuming low-quality meadow hay. These data agree with those observed in the current study and collectively, demonstrate the efficacy of infrequent supplementation as a mature cow winter feeding system.

The first limiting nutrient for cattle grazing dormant native range is typically RDP (Mathis et al. 1999; Bandyk et al., 2001; Bohnert et al., 2002b). Therefore, high-RDP (> 50% RDP) oilseed meal supplements (i.e. soybean and cottonseed meal) have become common in winter feeding systems. The RDP fraction of DDGS is lower than traditional supplements (40-50%; NRC, 2000; Shurson and Noll, 2005; MacDonald 2007). However, wethers supplemented with a 50:50 RDP/RUP mixture had improved total ruminal OM digestibility compared to wethers supplemented with high-RDP alone. This relative split between RDP and RUP potentially provides adequate nitrogen to the rumen to stimulate microbial efficiency, while increasing basal flow of AA to the small intestine compared to traditional high-RDP supplements (Atkinson et al., 2010). Swanson et al. (2004) observed greater retained N (as a % of intake) in wethers infused with casein both ruminally and post-ruminally (33:67 ratio) compared to 100% ruminal infusion alone. The split between ruminal and post-ruminal infusion is comparable to

differences in RDP and RUP fractions of DDGS; however, the effect of DDGS on forage digestibility and N efficiency warrants further study.

Bohnert et al. (2002b) theorized that high-RUP feedstuffs were more conducive to infrequent supplementation through increased urea N recycling. Excess N ingested on supplementation days would be maintained in the bloodstream as urea for use on non-supplementation days, when the concentration gradient in the rumen would allow for recycling via saliva, or by profusion of the ruminal wall. Cows receiving DDGS once every 3 or 6 d in the current study may have compensated for a lack of daily CP through this mechanism, resulting in similar performance of those cows supplemented daily.

There was a significant difference ($P = 0.01$) between supplementation frequency-supplementation status groups for the proportion of cows consuming hay 60 min post-feeding (Fig. 2.1). A lower proportion of cows delivered supplement once every 6 d (and supplemented on the d of observation; DDG6-S) were consuming hay 60 min post-feeding compared to daily-supplemented cows (DDGD-S) and cows not supplemented on the d of observation (DDG3-NS, DDG6-NS; $P = 0.01$). These data indicate that the amount of supplement provided may affect cow eating behavior. However, the decreased proportion of cows consuming hay 60 min post-feeding on the day of supplementation did not affect performance as measured by BW, BCS, or subsequent reproductive success and is similar to observations reported by Morris et al. (2005). Conversely, although a smaller proportion of DDG6-S cows were consuming hay 60 min post-feeding, they may have consumed more standing dormant range on the d of supplementation. Additionally, the limited observations for DDG6-S cows resulted in a large SE. Additional observations are necessary to fully elucidate the effect of infrequent supplementation on eating behavior.

During Year 1, the proportion of cows considered to be estrual at initiation of ovulation synchronization was not different ($P = 0.77$; Table 2.6) between supplementation frequency treatments and averaged 36.3%. Frequency of late gestation supplementation with DDGS did not affect first service conception rate (73.3%, overall average; $P = 0.62$) or final pregnancy rate (95.1%, overall average; $P = 0.76$). Our study agrees with others (DelCurto et al., 1990; Beaty et al., 1994) who also observed no effect of late gestation infrequent supplementation on subsequent pregnancy rate.

Under the conditions of our study, reducing the frequency of DDGS supplementation to as little as once every six days did not adversely affect cow and calf performance, or subsequent reproductive success.

Implications

The presumed economic benefits associated with reducing supplementation frequency of DDGS make this feeding system a viable option for cattle producers seeking to reduce production costs without adversely affecting performance. The reduction in labor and fuel costs may aid producers in maintaining the long-term sustainability of a cow-calf enterprise in a high cost environment.

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Figure 2.1 Proportion of supplementation frequency treatment group (dried distiller's grains with solubles daily = DDGD; dried distiller's grains with solubles every 3 d = DDG3; and dried distiller's grains with solubles every 6 d = DDG6) and supplementation status on day of observation (supplemented = S; not supplemented = NS) consuming hay 60 min post-feeding during an 11 d observation period in the course of an 84-d supplementation frequency experiment. ^{a,b} Bars without a common superscript differ (supplementation-frequency \times supplementation status interaction; $P = 0.01$).

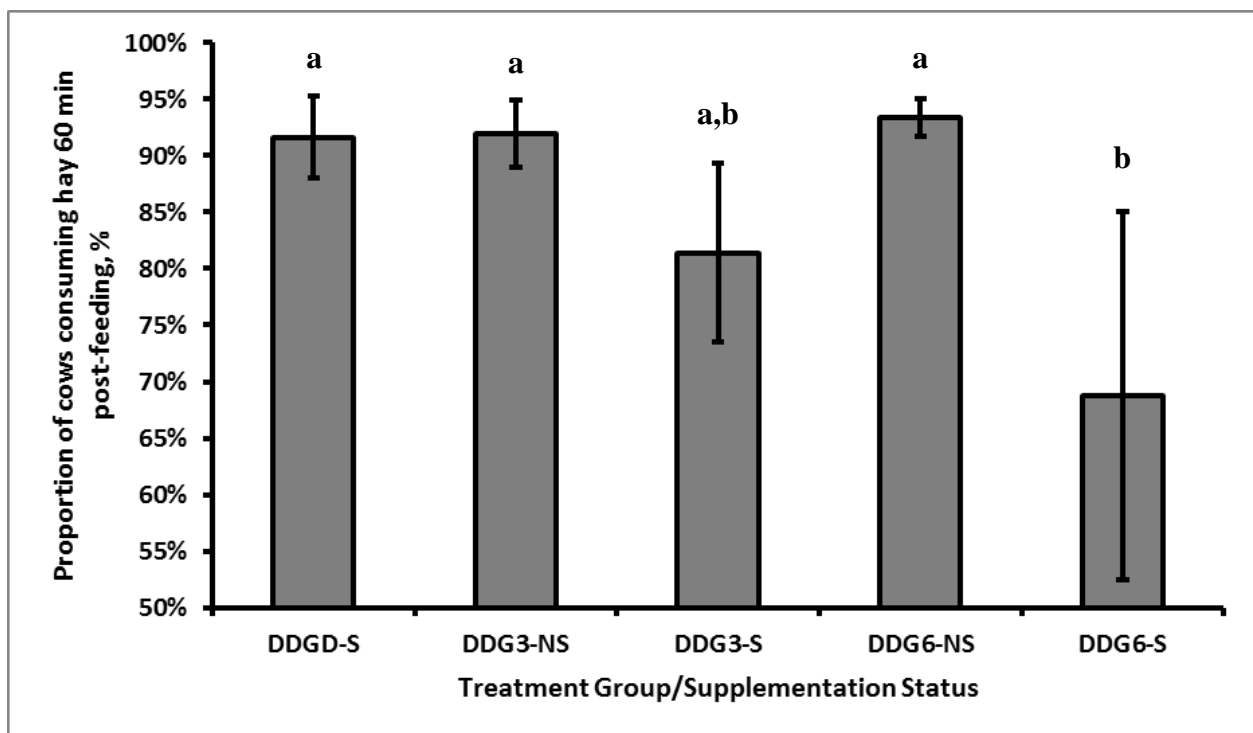


Table 2.1 Nutrient composition (DM basis) of native range, forage sorghum hay, and dried distiller's grains with solubles (DDGS).

Item	Nutrient Analysis, DM basis					
	Native Range		Sorghum Hay		DDGS	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
CP, %	7.49	8.35	6.93	10.84	29.53	31.00
NDF, %	59.18	-	59.30	-	30.39	25.97
ADF, %	44.43	47.17	36.89	33.46	17.08	20.00
Ca, %	0.58	0.45	0.49	0.65	0.08	0.11
P, %	0.11	0.06	0.15	0.13	0.80	0.78
S, %	0.09	-	0.10	-	0.43	-
ME ¹ , Mcal/kg	1.74	1.64	2.02	2.14	2.73	2.63
NE _m ² , Mcal/kg	0.87	0.80	1.17	1.28	1.81	1.72

¹ ME, Mcal/kg = $(4.103 - 0.0446 \times \% \text{ADF}) \times 0.82$ (Harlan et al., 1991).

² NE_m, Mcal/kg = $(1.37 \times \text{ME}) - (0.138 \times \text{ME}^2) + (0.0105 \times \text{ME}^3) - 1.12$ (NRC, 2000).

Table 2.2 Performance of cows, maintained on common native range, and supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3) or every 6 d (DDG6) for 84 d over a two year period.

Item	Treatment			SEM ¹	P-value ²		
	DDGD	DDG3	DDG6		Trt	Year	Trt × Year
n	77	69	75				
Avg. Julian calving date, d	83.3	82.7	82.2	0.69	0.79	0.005	0.67
Cow BW, kg							
d 0	577.3	581.4	577.2	5.08	0.94	0.92	0.35
d 28	607.7	610.0	606.8	4.53	0.96	0.01	0.38
d 56	618.8	624.1	619.2	4.45	0.88	0.32	0.38
d 84	643.8	641.7	634.3	4.61	0.69	0.46	0.28
Calving	601.4	602.5	597.2	4.37	0.88	0.04	0.22
Change, kg							
Period 1, d 0-28	30.4	28.6	29.6	2.41	0.96	<0.001	0.86
Period 2, d 29-56	11.1	14.0	12.4	0.85	0.24	<0.001	<0.001
Period 3, d 57-84	25.0	17.6	15.2	1.11	<0.001	<0.001	<0.001
Overall, d 0-84	24.1	21.1	20.0	2.71	0.82	0.002	0.99

¹ n = 221.

² P-value for treatment effect (Trt), year effect (Year), and treatment × year interaction (Trt × Year).

Table 2.3 Body condition score of cows, maintained on common native range pasture, and supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3), or every 6 d (DDG6) for 84 d over a two year period.

Item	Treatment			SEM ¹	P-value ²		
	DDGD	DDG3	DDG6		Trt	Year	Trt × Year
Cow BCS							
d 0	5.37	5.44	5.36	0.04	0.59	<0.0001	0.20
d 28	5.40	5.42	5.47	0.03	0.64	<0.0001	0.24
d 56	5.46	5.50	5.47	0.03	0.86	0.008	0.26
d 84	5.60	5.58	5.52	0.03	0.49	<0.0001	0.45
Calving	5.60	5.63	5.53	0.03	0.47	<0.0001	0.06
Change							
Period 1, d 0-28	0.04	-0.04	0.13	0.03	0.02	0.002	0.0007
Period 2, d 29-56	0.05	0.08	0.00	0.02	0.32	0.0005	0.0009
Period 3, d 57-84	0.14	0.08	0.04	0.02	0.22	0.02	0.78
Overall, d 0-84	0.23	0.18	0.17	0.03	0.70	0.75	0.88

¹ n = 221.

² P-value for treatment effect (Trt), year effect (Year), and treatment × year interaction (Trt × Year).

Table 2.4 Effect of supplementing cows, grazing dormant native range, with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3), or every 6 d (DDG6) on backfat thickness (BF), longissimus muscle depth (LM), and marbling score (MS) measured at the 12th rib in year 1.

Item	Treatment			SEM ¹	P-value ²
	DDGD	DDG3	DDG6		
Cow BF ³ , mm					
d 0	4.23	4.18	3.62	0.18	0.30
d 84	5.24 ^a	4.88 ^{ab}	4.09 ^b	0.21	0.06
Change	1.01	0.70	0.50	0.15	0.38
Cow LM ⁴ , mm					
d 0	44.11	41.83	42.18	0.53	0.16
d 84	46.74	44.70	46.26	0.55	0.33
Change	2.62	2.86	4.36	0.56	0.38
Cow MS ⁵					
d 0	5.56	5.86	6.11	0.11	0.11
d 84	4.86 ^a	5.22 ^{ab}	5.39 ^b	0.10	0.07
Change	-0.70	-0.64	-0.69	0.10	0.97

¹ n = 79.

² P-value for main effect of treatment.

³ BF = Backfat thickness.

⁴ LM = Longissimus dorsi muscle depth.

⁵ MS = Marbling score: 4.0 = low select (SE⁻), slight; 5.0 = choice (CH⁻), small; 8.0 = prime (PR⁻), slightly abundant.

^{a,b} Means with different superscripts are different ($P < 0.05$).

Table 2.5 Performance of early-weaned calves from dams supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3), or every 6 d (DDG6) during an 84-d period in year 1.

Item	Treatment			SEM ¹	<i>P</i> -value ²
	DDGD	DDG3	DDG6		
n	26	23	27		
Calf BW, kg					
Birth	40.4	39.9	38.3	0.49	0.19
Weaning ³	144.6	153.9	153.6	2.00	0.12
Calf ADG, kg/d	0.98	1.06	1.06	0.02	0.10

¹ n = 76.

² *P*-value for main effect of treatment.

³ Calves weaned at 113 ± 17 d of age.

Table 2.6 Reproductive performance of cows supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3) or every 6 d (DDG6) during an 84-d period in year 1.

Item	Treatment						SEM ¹	P-value ²
	DDGD		DDG3		DDG6			
	n	%	n	%	n	%		
n	28		22		29			
Estrual cows	9	32.1	8	36.4	12	41.4	0.04	0.77
FSCR ²	19	67.9	16	72.7	23	79.3	0.03	0.62
PR ³	28	100.0	21	95.5	26	89.7	0.01	0.76

¹ n = 79.

² *P*-value for the main effect of treatment.

³ FSCR = First service conception rate.

⁴ PR = Final pregnancy rate.

Chapter 3 - Review of Literature – Post-Breeding Administration of Flunixin Meglumine to Beef Cattle

Introduction

The capital investment (feed, labor, etc.) required to develop replacement females is often high, thus making the inherent cost of new genetics expensive. The cost of developing replacement females is also compounded by an immature reproductive system that may be unreceptive to timely breeding practices. Therefore, prompt and early conception is imperative for long-term herd success and maintaining a yearly calving interval. Despite improved, highly specific breeding technologies and a more complete understanding of proper heifer development, early embryonic mortality (before d 42 of pregnancy) is still the main cause of pregnancy loss (Thatcher et al., 1994). Processing naïve heifers through unfamiliar handling facilities or long distance transportation both increase animal stress and can lead to reduced pregnancy rates. The use of flunixin meglumine (FM; Banamine[®]; Merck Animal Health, Summit, NJ) between 12 and 16 d post-breeding has been demonstrated to reduce early embryonic mortality in situations where transportation post-breeding is necessary (Merrill et al., 2004; Purcell et al., 2005; Merrill et al., 2007). The possibility exists that even in cases where heifers do not require transportation administration of FM post-breeding may elicit similar improvements in conception rate.

Maternal Recognition

Early pregnancy maintenance relies on paracrine secretions of the conceptus onto the endometrial tissues of the uterus (Thatcher et al., 1995). The conceptus has this ability after multiple mitotic divisions that begin immediately following fertilization. These secretions begin on d 13 post-ovulation and are the primary determinant of successful maternal recognition (Senger, 2003).

In cases where maternal recognition does not occur, release of oxytocin by the corpus luteum (CL) signals production of PGF_{2α} by endometrial cells, and thus, the start of luteolysis (Senger, 2003). Regression of the CL, decreasing P4 concentrations, and pregnancy loss would follow; eventually, a new estrous cycle would be initiated.

Bovine interferon- τ (bIFN- τ) is the main paracrine secretion released by the conceptus onto the uterine epithelium (Senger, 2003). The action of bIFN- τ blocks oxytocin receptors that

stimulate $\text{PGF}_{2\alpha}$ release (Thatcher et al., 1994; Thatcher et al., 1995). When bIFN- τ is produced in adequate levels by the conceptus, P4 continues to be the primary reproductive tract hormonal secretion. Progesterone is the pregnancy maintenance hormone released by a functioning primary CL that attenuates uterine contractions, promotes involution of the uterus, and is important for embryo attachment to the epithelium. Concentration of P4 in plasma has been proven to be vital in early pregnancy establishment. Recently, Atkins et al. (2013) observed that P4 concentration on d 7 after ovulation of the dominant follicle is the highest correlating factor to pregnancy establishment ($r^2 = 0.231 \pm 0.049$).

Handling and Transportation Stress

Central processing facilities or limited availability of land (grazing) resources often necessitates the untimely handling and transportation of cattle immediately following artificial insemination (AI). Transportation elevates stress and cortisol concentration in the bloodstream (Crookshank et al., 1979). The combination of these factors has been linked to increased pregnancy loss (Merrill et al., 2007). Exogenous stressors such as unfamiliarity and noise, as well as activation of the sympathetic nervous system, activate the hypothalamic-pituitary-adrenocortical axis and promote release of cortisol (Yavas et al., 1996). These are considered the precursors to increased blood cortisol and may impede conceptus function and timely paracrine secretion.

Crookshank et al. (1979) evaluated the effect of transportation for on serum cortisol concentrations in newly-weaned steers. Serum cortisol concentrations significantly increased in steers transported for 12 h versus non-transported steers. A spike in cortisol concentration occurred 24 h after treatments were applied (12 h post-transport) and these elevated levels continued for 4-7 d. Considering conceptus development is rapid during this period, extrapolating this data to recently bred females could explain the detrimental effect that transportation has on recognition of pregnancy.

The stress of handling may inhibit maternal recognition, especially for naïve heifers that may be unfamiliar with facilities and human interaction. Echternkamp (1984) evaluated both cortisol and luteinizing hormone (LH) concentrations in cows that had been acclimated to handling and restraint or were not acclimated. Cows acclimated to handling and restraint exhibited lower serum cortisol concentrations and significantly higher serum LH concentrations

compared to non-acclimated cows. A correlation between cortisol and LH was also observed. Low cortisol concentrations resulted in normal LH concentrations and regular LH pulse intervals; however, at cortisol concentrations above 50 ng/ml, LH concentrations remained low and LH pulses were absent. It is possible that a decrease in LH secretion in stressed females is mediated by release of cortisol, ultimately leading to early embryonic loss (Echternkamp, 1984).

Serum cortisol concentration in heifers and cows bred 14 d prior and then subjected to transportation stress (for 5 h) were observed to be highly variable by Merrill et al (2004). For both heifers and cows, sampling during transportation (2.5 h) showed higher serum cortisol concentrations for transported females compared to non-transported females. However, transported females surprisingly exhibited significantly lower serum cortisol concentrations immediately following transportation compared to non-transported females. This contradicts earlier research (Crookshank et al. 1979) that demonstrated a prolonged effect on transported cattle and could be the result of livestock's ability to become comfortable and acquainted to a stressful situation over time. Also, the much shorter transport time used in this study (5 h vs. 12 h) may not have been long enough to sufficiently stress females to the point of pregnancy loss.

Similar trends in serum cortisol concentration were also observed in a follow-up study (Merrill et al., 2007). However, first service conception rates were not different between transported and non-transported groups (64% and 67%, respectively). Based on these results, the relative amount of cortisol in the blood stream, although directly correlated to stress, may not directly affect the conceptus and its ability to establish pregnancy.

Similar conception rates were also reported for heifers subjected to 1 h of transport either pre- or post-AI compared to non-transported females (Yavas et al., 1996). It has been postulated that cortisol interferes with the LH surge that occurs at the onset of standing estrus (Echternkamp, 1984). However, under proper AI protocol, insemination does not occur until approximately 12 h after standing heat. Thus, the effect of transportation stress and cortisol may be time-dependent with the onset of standing estrus, as opposed to the timing of AI.

Geary (2012) evaluated the effect of injection of adrenocorticotrophic hormone (ACTH) on serum cortisol concentration and pregnancy rate in mature cows. Cortisol concentrations were increased in ACTH-treated cows compared to that of untreated cows. However, increased serum cortisol concentrations did not affect pregnancy rates, as no ACTH-treated cows lost a

pregnancy. These data may show serum cortisol does not have a direct effect on pregnancy rate, possibly leaving the true cause of early embryonic death more uncertain.

Flunixin Meglumine

Oxytocin receptors that will eventually trigger $\text{PGF}_{2\alpha}$ release can be blocked via administration of flunixin meglumine (Thatcher et al., 2001), potentially overcoming the inability of the conceptus in a stressed female to produce sufficient concentrations of bIFN- τ . Inability to secrete bIFN- τ has been associated with unusually low serum P4 concentrations on the days just following fertilization (d 3-8; Beltman et al., 2009), resulting in early embryonic loss.

Flunixin meglumine possesses characteristics that may improve pregnancy establishment in times of unavoidable stress. Thatcher et al. (2001) hypothesized that early embryonic losses may be associated with the inability of the conceptus to secrete adequate bIFN- τ to block secretion of $\text{PGF}_{2\alpha}$ at the time of maternal recognition. Flunixin meglumine inhibits cyclooxygenase, which converts arachadonic acid to $\text{PGF}_{2\alpha}$ (Anderson et al., 1990; Odensvik, 1995; Merrill et al., 2007). Under these conditions, the ability for the conceptus to produce adequate bIFN- τ is not necessary due to the auxiliary action of FM. Administration of FM post-breeding, and immediately prior to maternal recognition, may overcome many endogenous problems (inadequate P4 or bIFN- τ production, or a small ovulatory follicle).

Merrill et al. (2007) examined the effect of FM administered intramuscularly on pregnancy establishment in cows transported for 5-6 h. Pregnancy rates were greater in transported cows administered FM compared to transported cows that did not receive FM and non-transported cows (74%, 64%, and 67%, respectively). Additionally, non-transported cows administered FM had higher AI pregnancy rates compared to untreated non-transported cows (73% and 64%, respectively). Administration of FM lowered $\text{PGF}_{2\alpha}$ metabolite concentration in the blood. This is correlated to a reduction in $\text{PGF}_{2\alpha}$ secretion, which may potentially create an environment where early embryo survival and maternal recognition of pregnancy are more likely to occur.

Geary et al. (2010) evaluated the efficacy of FM administration intramuscularly in non-transported heifers and cows under different ovulation synchronization strategies. In a series of three experiments, females were synchronized using either melengestrol acetate (MGA) + $\text{PGF}_{2\alpha}$,

CoSynch + CIDR or a SelectSynch + CIDR protocol. Pregnancy rates were reduced (66%) in heifers administered FM compared to non-handled heifers (72%) when synchronized using MGA. Conversely, utilizing the CoSynch + CIDR protocol elicited no difference in pregnancy rate in treated or untreated heifers, as did the SelectSynch + CIDR protocol utilized on mature cows.

Geary (2012) utilized non-lactating females and observed that administration of FM intramuscularly decreased serum PGF_{2α} metabolite concentration. Additionally, there was no pregnancy loss, regardless of FM administration. However, this may be a function of small sample size (n = 40) and, under typical production scenarios, multiparous females would be lactating and establishing estrous cycles at the same time. Without the nutrient demand of peak lactation, establishment of cyclicity and pregnancy maintenance may have been less taxing than under normal conditions.

Flunixin meglumine administration to recipient cows was also evaluated by Purcell et al. (2005). Administration of FM intramuscularly occurred 2-12 min prior to embryo transfer (on d 6-9 of the estrous cycle) at three locations. Females administered FM were 1.9 times more likely to conceive compared to those that were untreated. However, this effect was mainly due to a location interaction. The improvement in conception at one location was higher (32%) than for the other locations (5% increase and 3% decrease, respectively). Additionally, Rabaglino et al. (2010) evaluated the effect of FM administered intramuscularly 15.5 and 16 d post-FTAI in non-transported dairy heifers. First service conception rate and final pregnancy rate were not different between treatments.

Summary

The pharmacokinetic effect that FM has on the reproductive system has been intensively evaluated. Theoretically, administration of FM in a practical environment should yield favorable pregnancy results. However, the efficacy of FM administration cannot be completely vetted at this point, as the current reproductive responses to FM are highly variable. It is potentially possible that other hormonal, paracrine, and external factors also play an integral role in early pregnancy maintenance.

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Chapter 4 - Effect of Flunixin Meglumine on First Service Conception Rate in Beef Heifers

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Abstract

The objective of this study was to determine if post-breeding injection of flunixin meglumine would improve first service conception rate in non-transported beef replacement heifers. Following weaning, heifers ($n = 220$; $BW = 367 \pm 37$ kg; $BCS = 5.3 \pm 0.04$) originating from the Kansas State University Commercial Cow-Calf Unit (Manhattan, KS) and Agricultural Research Center (Hays, KS) were developed in the feedlot at Hays, KS and then returned to their respective origins for the breeding season. Heifers were stratified by origin, BW, and BCS and assigned randomly to one of two treatments: 1) injection with physiological saline (CON); or 2) injection with flunixin meglumine (BAN). Injections were administered intramuscularly at 1.1 mg/kg BW 14 d post fixed-time AI (FTAI). Serum progesterone (P4) concentrations were quantified from paired blood samples collected 10 d prior and on the d ovulation synchronization was initiated to determine proportion of pubertal heifers. Ovulation was synchronized using a 7 d CO-Synch + controlled internal drug release (CIDR) protocol. Heifers at each location were inseminated via FTAI 54 h after CIDR removal and exposed to 4 fertile bulls 10 d after FTAI for the remaining 35 d of a 45-d breeding season. Transrectal ultrasonography was used to determine conception to FTAI 35 d after insemination and final pregnancy rate was determined 35 d after the breeding season. The statistical model included the main effects of flunixin meglumine treatment, location, and their interaction. Reproductive performance among treatment groups were not affected ($P > 0.50$) by location and it was removed from the model for final analysis. Heifer BW and BCS at breeding were not different ($P > 0.40$) between treatments. Proportion of pubertal heifers (98.5%) did not differ between treatments ($P = 0.99$). First service conception rate of CON heifers (44.0%) did not differ ($P = 0.87$) from that of BAN heifers (43.0%). Final pregnancy rate was similar ($P = 0.40$) for CON and BAN heifers and averaged 81.8%. Under the conditions of our study, flunixin meglumine injection 14 d post-FTAI did not improve first service conception or final pregnancy rate in beef replacement heifers.

Key words: beef heifers, conception rate, flunixin meglumine

Introduction

The development of replacement heifers requires many resources (feed, labor, etc.). Therefore, the inherent cost to establish new genetics is often high. Timely conception and maintenance of the pregnancy are vital to the long-term success and retention of replacement females in the herd. Improvement of first service conception rate remains challenging for many operations, despite enhanced knowledge and use of heifer development strategies.

Facility limitations often dictate that cattle be processed and bred in a central location, and then transported to other locations for the remainder of the breeding season. The physiological stress associated with handling and transportation increases serum cortisol concentrations, which may be used as indication of animal stress (Crookshank et al., 1979). Physiological stress has been correlated with an inability for the embryo to suppress prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretions and failure of maternal recognition (Merrill et al., 2007; Geary et al., 2010). Early embryonic death accounts for the greatest proportion of reproductive failure (Thatcher et al., 1994). Injection of flunixin meglumine (Banamine[®]) at breeding or post-breeding has been demonstrated to inhibit production of $PGF_{2\alpha}$ mitigating the effect of transportation stress, and allowing for pregnancy maintenance (Merrill et al., 2004; Purcell et al., 2005; Merrill et al., 2007).

Even in cattle that do not require transportation after breeding, human interaction and handling often results in increased stress. This unavoidable stress may potentially contribute to reduced first service conception rates. Therefore, the objective of this study was to evaluate the effects of flunixin meglumine on first service conception rate in non-transported replacement heifers.

Materials and Methods

All procedures involving the handling and care of animals used in this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 3175).

Animals and Experimental Design. Angus-cross heifers ($n = 220$; $BW = 367 \pm 36.7$ kg; $BCS = 5.3 \pm 0.04$) originating from the Kansas State University commercial cow-calf herds in Manhattan and Hays, KS were developed in the feedlot at the Agricultural Research Center–Hays following weaning and were returned to their respective origins for the breeding season.

Prior to initiation of ovulation synchronization, heifers were stratified by origin, body weight, and body condition score and assigned randomly to one of two treatments: 1) injection with physiological saline (CON) or 2) injection with flunixin meglumine (BAN; Flu-Nix D; AgriLabs, St. Joseph, MO). Treatments were administered intramuscularly at 1.1 mg/kg BW, 14 d after fixed-time AI according to the methods used by Merrill et al. (2007).

Data Collection. Heifers were weighed and BCS was assessed by two independent, qualified observers using a 9-point scale (1=extremely emaciated, 9=extremely obese; Wagner et al., 1988) on the day of ovulation synchronization and day of breeding.

Puberty Determination. Blood samples were collected from all heifers via coccygeal venipuncture 10 d before and on the day ovulation synchronization was initiated. Samples were collected in to 10 ml serum vacutainer tubes (BD Vacutainer™, Becton, Dickinson, and Company, Franklin Lakes, NJ), immediately placed on ice, allowed to coagulate for 24 h at 4°C and then centrifuged ($1,500 \times g$) for 10 min. Serum was decanted into 12 × 75 mm plastic tubes and immediately frozen (-20°C). Concentration of progesterone (P4) in serum was subsequently quantified using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA; Stevenson, 2011). Intra- and inter-assay CV were 7.0 and 8.6%, respectively and assay sensitivity was 0.009 ng/ml. Blood collected on the two sampling dates was used to verify the functional presence of a corpus luteum (when serum concentrations of P4 ≥ 1 ng/mL) at the onset of ovulation synchronization. If any 1 of the 2 samples contained P4 ≥ 1 ng/mL (typical of heifers that have attained puberty and are in the luteal phase of the estrous cycle), heifers were assumed to be pubertal before the onset of ovulation synchronization treatment (d 10). If concentrations in the 2 samples were <1 ng/mL heifers were considered to be pre-pubertal.

Ovulation Synchronization and Breeding. Ovulation was synchronized using the 7 d Co-Synch + controlled internal drug release (CIDR; EAZI-Breed CIDR®; Zoetis, Madison, NJ) protocol (Larson et al., 2006). Heifers received an injection of 100 µg of GnRH intramuscularly (d -10; 2 mL Cystorelin; Merial, Duluth, GA) and a CIDR (containing 1.38 g of P4) insert followed in 7 d by an injection of 25 mg PGF_{2α} intramuscularly (d -3; 5 mL of Lutalyse; Zoetis) and the CIDR was removed followed in 54 h by fixed-time AI (FTAI) and a third 100 µg injection of GnRH (d 0). Heifers were exposed to 4 fertile bulls 10 d after FTAI for the remainder of the 45-d breeding season.

First service conception rate (FSCR) was determined 35 d after FTAI. Pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of uterine fluid and an embryo with a heartbeat. A final pregnancy diagnosis (PR) was determined 35 d after the end of the breeding season via transrectal ultrasonography.

Statistics. The statistical model included the main effects of flunixin meglumine treatment, location, and their interaction. Reproductive performance among treatment groups were not affected ($P > 0.50$) by location and it was removed from the model for final analysis. Body weight and BCS were analyzed using the GLM procedure of SAS (SAS Inc., Cary, NC). Reproductive data was analyzed as a generalized linear mixed model with a 1-way treatment structure in completely-randomized design (PROC GLIMMIX), using the binary distribution with logit-link function. Class factors included treatment and location. The model statement included only the treatment fixed effect. Treatment means with SE and F-test p-value were reported. Least square means are presented and differences were considered significant at $P \leq 0.05$. Tendencies were considered to exist from $0.05 < P \leq 0.10$.

Results and Discussion

Heifer BW and BCS at breeding were not different among treatments ($P = 0.41$ and 0.60 , respectively; Table 4.1). Proportion of heifers pubertal before onset of ovulation synchronization was not different between treatments ($P = 0.99$), was not affected by location, and averaged 98.5%. Treatment with BAN did not improve ($P \geq 0.40$) FSCR or PR compared to CON heifers and averaged 44.0 and 81.8%, respectively (Table 4.1).

Blocking $\text{PGF}_{2\alpha}$ secretions by bovine interferon-tau (bIFN- τ) 12-17 d post-breeding allows for maternal recognition of pregnancy (Bazer et al., 1991; Roberts et al., 1992). Flunixin meglumine injection post-FTAI is presumed to assist in maternal recognition of a pregnancy by inhibiting cyclooxygenase, thus preventing conversion of arachadonic acid to $\text{PGF}_{2\alpha}$ (Anderson et al., 1990; Odensvik, 1995). This could potentially decrease early embryonic loss. Our data agrees with Rabaglino et al. (2010) who found no difference in FSCR between non-transported dairy heifers injected with flunixin meglumine and control heifers (60.8% and 59.4%, respectively).

Conversely, Merrill et al. (2007) found that flunixin meglumine increased AI pregnancy rate (71%) compared to that of untreated females (61%). However, females were subject to transportation stress, unlike the current study. It is possible that the elevated serum cortisol concentrations associated with transportation can be mitigated by flunixin meglumine; whereas elevations in serum cortisol due to standard breeding procedures and handling may be less severe, thus negating the potential positive effects of flunixin meglumine treatment.

Under the conditions of our study, injection of flunixin meglumine did not improve FSCR or PR of non-transported beef heifers.

Implications

Previous research demonstrated that under certain conditions (i.e. where transportation of recently bred females is necessary) that flunixin meglumine may improve first service conception rate. However, in situations where transportation is not necessary the use of flunixin meglumine does not appear to improve FSCR.

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Table 4.1 Reproductive performance of non-transported beef heifers injected with flunixin meglumine (BAN) or physiological saline (CON) 14-d after fixed-time artificial insemination.

Item	Treatment		SEM ¹	<i>P</i> -value ²
	BAN	CON		
n	109	111		
BW, kg	365	369	2.49	0.41
BCS	5.3	5.3	0.04	0.60
Estrual, %	98.5	98.5	0.001	0.98
FSCR ³ , %	43.0	44.0	0.02	0.87
PR ⁴ , %	84.5	80.1	0.01	0.40

¹ n = 220.

² *P*-value for the main effect of treatment.

³ First service conception rate.

⁴ Final pregnancy rate.

Appendix A – Precipitation

Table A.1 Precipitation totals by month for 2011 and 2012

Month	Total Precipitation		Official Avg. ¹
	2011	2012	
Jan	0.35	0.03	0.50
Feb	0.57	1.27	0.70
Mar	0.67	1.40	1.81
Apr	1.03	2.87	2.13
May	2.41	1.59	3.26
Jun	2.41	0.85	2.84
Jul	1.95	0.22	3.86
Aug	4.09	3.37	3.04
Sep	0.86	1.07	2.05
Oct	1.57	0.94	1.60
Nov	1.20	0.00	0.93
Dec	2.01	0.78	0.72
Total	19.12	14.39	23.46
% of Normal	81.5%	61.3%	

¹ Official 30-yr National Weather Service Average

Appendix B – Cow Performance

Table B.1 Performance of cows, maintained on common native range, and supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3), or every 6 d (DDG6) for 84-d in consecutive years.

Item	Treatment						SEM ¹	<i>P</i> -value ²		
	DDGD		DDG3		DDG6			Trt	Year	Trt × Year
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2				
n	28	49	22	47	29	46				
Avg. Julian calving date, d	86	80	85	81	84	81	0.69	0.79	0.005	0.67
Cow BW, kg										
d 0	582.9	571.6	587.7	575.2	567.0	587.4	5.08	0.94	0.92	0.35
d 28	599.3	616.0	603.2	616.8	585.6	628.0	4.53	0.96	0.01	0.38
d 56	612.1	625.4	628.4	619.7	607.5	630.8	4.45	0.88	0.32	0.38
d 84	653.4	634.2	650.0	633.3	627.2	641.5	4.61	0.69	0.46	0.28
Calving	616.0	586.7	617.4	587.7	595.4	599.0	4.37	0.88	0.04	0.22
Change, kg										
Period 1, d 0-28	16.4	44.4	15.6	41.7	18.6	40.6	2.41	0.96	<0.001	0.86
Period 2, d 29-56	12.8	9.4	25.2	2.9	21.9	2.8	0.85	0.24	<0.001	<0.001
Period 3, d 57-84	41.3	8.8	21.6	13.6	19.6	10.7	1.11	<0.001	<0.001	<0.001
Overall, d 0-84	33.1	15.1	29.7	12.5	28.4	11.6	2.71	0.82	0.002	0.99

¹ n = 221.

² *P*-value for treatment effect (Trt), year effect (Year), and treatment × year interaction (Trt × Year).

Table B.2 Body condition score of cows, maintained on common native range, and supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3), or every 6 d (DDG6) for 84 d in consecutive years.

Item	Treatment						SEM ¹	<i>P</i> -value ²		
	DDGD		DDG3		DDG6			Trt	Year	Trt × Year
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2				
Cow BCS										
d 0	5.18	5.56	5.24	5.65	5.03	5.69	0.04	0.59	<0.001	0.20
d 28	5.30	5.51	5.19	5.65	5.32	5.63	0.03	0.64	<0.001	0.24
d 56	5.40	5.51	5.45	5.55	5.31	5.64	0.03	0.86	0.008	0.26
d 84	5.47	5.72	5.47	5.69	5.32	5.71	0.03	0.49	<0.001	0.45
Calving	5.43	5.77	5.44	5.81	5.19	5.87	0.03	0.47	<0.001	0.06
Change										
Period 1, d 0-28	0.13	-0.05	-0.09	0.00	0.33	-0.06	0.03	0.02	0.002	<0.001
Period 2, d 29-56	0.10	0.00	0.26	-0.10	-0.01	0.01	0.02	0.32	0.005	<0.001
Period 3, d 57-84	0.07	0.21	0.01	0.14	0.01	0.08	0.02	0.22	0.02	0.78
Overall, d 0-84	0.25	0.21	0.20	0.16	0.16	0.18	0.03	0.70	0.75	0.88

¹ n = 221.

² *P*-value for treatment effect (Trt), year effect (Year), and treatment × year interaction (Trt × Year).