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Interaction of Urea with Frequency and Amount of Distillers Grains Supplementation for Growing Steers on a High Forage Diet

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INTERACTION OF UREA WITH FREQUENCY AND AMOUNT OF DISTILLERS GRAINS SUPPLEMENTATION FOR GROWING STEERS ON A HIGH FORAGE DIET

by

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Two studies were conducted to determine interactions of urea inclusion to a dried distillers grains plus solubles (DDGS) supplement fed at two amounts and two frequencies to steers on a high forage diet. In Exp. 1, 120 steers were fed individually for 84 d. Steers received ad libitum grass hay and 1 of 8 treatments. Supplement was fed either every day (D) or 3x/week (ALT), amount of supplement fed was 6.36 kg/week (LO) or 12.73 kg/week (HI), and contain either no urea (-U) or 1.3% urea (+U). Hay DMI and steer BW were measured. In Exp. 2, 8 ruminally cannulated steers were used in a digestion trial for 6 periods. Treatment design was the same as Exp. 1, except that supplement was fed at a rate of 0.4% of BW (LO) or 0.8% of BW (HI). Hay DMI, rumen fluid, in situ NDF disappearance, and rumen pH were measured. In Exp. 1, ADG was only affected by amount of supplement with steers on HI gaining more than LO. Hay DMI was reduced by increased amount of supplement and by decreased frequency of supplementation. In Exp. 2, hay DMI was also reduced due to increase amount of supplement and decreased frequency of supplementation. Rumen pH was decreased on the day of feeding for steers on ALT and reduced for steers fed HI vs LO. There was an interaction of urea x amount for rumen ammonia-N concentration but no effect of

frequency. A reduction in in situ NDF disappearance was observed on the day ALT received supplement between HI and LO. There was no difference between NDF digestibility between D and ALT. Infrequent supplementation of DDGS results in no difference in ADG. No effect was seen of with the inclusion of urea and animal performance was only improved when increase the rate of DDGS supplementation. There is little change in rumen fermentation parameters between frequency of supplement feeding, indicating that forage digestion is not impacted by supplementation frequency. Thus, DDGS can be supplemented infrequently without reducing animal performance.

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CHAPTER I: LITERATURE REVIEW

Backgrounding Calves

The US beef production system is horizontally integrated with cattle changing production phases several times before slaughter**.** Cow-calf, backgrounding, and finishing operations vary in their goals and thus, their nutritional strategies to achieve these. Backgrounding operations are extremely diverse with no definite cattle age, weight, or length of growing program defining this phase. However, the primary goal of backgrounding is animal growth of frame and muscle without adding fat (Peel, 2003; Rasby et al., 1994). Backgrounding allows for producers to develop calves economically using inexpensive feed sources such as crop residues or forages (Rasby et al., 1994). Still, depending on forage quality and targeted rate of gain, supplementation may be necessary. Supplementation needs also vary depending on cattle age, weight, and frame. When adding supplementation to backgrounding diets though, it is important to note the cost of gain. In order for backgrounding operations to be an enterprise separate from other phases of production, they must be economically viable (Peel, 2003). Thus, the challenge to backgrounding producers is to determine a nutritional program that improves cattle performance in a cost-effective manner.

Limiting Nutrients in Forage Diets

Depending on the forage type and quality, limiting nutrients in the diet can vary. Lower quality forages have less crude protein (CP) and can limit intake, which can result in insufficient dietary protein and energy. The TDN:CP ratio of a forage impacts voluntary intake. If this ratio is greater than 7, such as in the case of low-quality forages,

protein is deficient and intake is decreased (Moore and Kunkle, 1998). In forages, majority of protein is rumen degradable (RDP). Deficiency of protein, in particular RDP, reduces forage utilization by the animal therefore, negatively impacting their performance. Due to the seasonality of backgrounding operations, as most backgrounding producers receive fall weaned calves, cattle in this phase are often grazing dormant forage or crop residues, which are considered to be low quality (Peel, 2003). Knowing forage quality, such as protein and fiber content, is crucial to developing beneficial and cost-effective supplementation program. To improve forage utilization and animal performance, determining type of supplementation (protein or energy) based off forage quality and targeted animal performance is key.

Growing Calf's Nutrient Requirements

Again, determining supplementation needs begins with knowing the nutritive value of the forage, but also, the physiological needs of the animal. Animal size and age play a role in determination of nutrient requirements as well as targeted rate of gain (NRC, 2016). Younger animals experience more muscle growth and therefore, protein needs are the greatest at this stage. For animals of the same age, increasing body size increases energy requirements for gain. Protein synthesis rate is first limiting as energy intake above maintenance increases excess energy is then stored as fat (NRC, 2016). Thus, the growing calf may need both supplemental energy and protein in order to increase growth without depositing excess fat.

Types of Supplementation

Energy Supplements

Energy supplements are fed to improve animal gain as growing calves have greater energy requirements than cattle at maintenance. This is because the amount of energy required for gain (NEg) is more than energy needed for maintenance, or maintaining a body weight (NEm; NRC, 2016). The rate of gain also impacts NEg requirements, with a greater ADG resulting in a greater amount of NEg per day. Management practices vary but typically, backgrounding operations target gains between 1 to 3 pounds per day (Peel, 2003; Rasby et al.).

Traditionally, energy supplements are thought of as cereal grains. These grains are typically high in starch content, low in crude protein, and have little fiber, thus, referred to as being high in non-structural carbohydrates (NSC). They are often selected for supplementation due to their high NE content and cost. In the rumen, the starch is rapidly digested by rumen microbes, which produce volatile fatty acids (VFA) as an end product. One of the VFA produced by starch fermentation is propionate. This VFA is critical to energy metabolism in ruminants because it is the only one that contributes to gluconeogenesis (Young, 1977). However, supplementation of a large amount of NSC, can have a negative associative effect on forage digestion and intake (Kunkle et al., 2000). This effect is due to a variety of factors including substitution of forage, ruminal pH, and rumen ammonia concentration.

Negative Associative Effects of Energy Supplementation

Energy supplements high in NSC have been shown to decrease intake of forage as well as forage utilization, which can result in gains less than expected from the amount of energy supplemented (Horn and McCollum, 1987; Bowman and Sanson, 1996; Moore et

al., 1999). The magnitude of these effects have been shown to be dependent on the amount of NSC fed and amount of protein in the diet (Kunkle et al., 2000).

Replacement of forage intake by supplement has been termed substitution (Caton and Dhuyvetter, 1997). The rate of substitution depends on a variety of factors including amount of supplement and supplemental TDN intake (Moore et al., 1999). Chase and Hibberd (1987) fed supplements containing 0, 1, 2 or 3 kg/d of corn to cows on low quality grass hay (4.2% CP). Increasing corn supplementation linearly decreased daily hay intake. From 1 kg/d to 3 kg/d of corn, daily hay intake decreased by 38%. Even 1 kg/d of corn decreased hay intake 7% from the control. Interestingly, in review of the literature the same year, Horn and McCollum (1987) concluded that concentrates could be fed up to 0.5% of body weight (BW) without causing large decreases in grazed forage intake. For the cattle on the Chase and Hibberd study (1987), 0.5% of BW would have been 1.8 kg/d. Therefore, supplementation amount is not the only determining factor in substitution rate. Moore et al. (1999) reviewed 66 publications to determine effects of supplementation on cattle consuming ab libitum forage. Their review found supplemental TDN intake greater than 0.7% of BW decreased forage intake. Forage quality can also play a role in the substitution effect, as forage intake was decreased by supplementation when forage TDN:CP ratio was less than 7 (sufficient N), but supplements increased forage intake when TDN:CP ratio was greater than 7 (deficient N) (Moore et al., 1999).

Energy supplementation with NSC can not only reduce forage intake, but also the utilization of forage, which could explain why large amounts of grain supplementation does not always yield the expected gain. Bacterial species in the rumen possess different enzymatic capabilities and thus, utilize different feed sources. Cellulolytic bacteria

possess the enzyme cellulase. This enzyme can hydrolyze the β 1,4 glyosidic bonds present in plant cell wall structure and free the repeating units of glucose. Amylolytic bacteria use amylase to free carbohydrates from plant polymers. Carbohydrates are further used to provide energy for the microbes. The different bacterial species yield different VFA due to their enzymatic capabilities, with cellulolytic bacteria producing acetate and butyrate, and amylolytic bacteria producing propionate as well as lactic acid. These byproducts can alter the rumen environment, with lactic acid and VFA's reducing ruminal pH (Moran, 2005). Environmental pH is crucial to the metabolic function of bacteria inhabiting the rumen. Cellulolytic bacteria are more sensitive to drops in pH and their ability to digest plant cell wall material is reduced when ruminal pH falls below 6.0. Reduction in pH to 5.5 not only impacts cellulolytic bacteria's digestion but their growth rate, only further reducing forage utilization (Hoover, 1986). Large amounts of NSC are rapidly fermented by amylolytic bacteria causing drops in ruminal pH and reducing forage digestion (Hoover, 1986). By reducing forage digestion, the animal no longer receives the full nutritional value from that feed, which in backgrounding operations is the majority of their diet. Thus, negatively impact their gains.

However, the drop in ruminal pH cannot be the sole factor impairing forage digestion seen in studies supplementing NSC (Caton and Dhuyvetter, 1997). In Caton and Dhuyvetter's review (1997) of 14 studies of energy supplementation's effects on ruminal pH, in cases where ruminal pH was reduced, it was not reportedly not below the threshold impacting fiber digestion. Furthermore, if ruminal pH is below the level for optimal forage digestion, the time at which it remains at this level plays a role. Increasing time spent at a pH of 5.5 was found to linearly decrease NDF and ADF digestion

(Cerrato-Sánchez et al., 2007). Sanson et al. (1990) reported a decrease in ruminal pH below 6 for 8 hours post feeding in cattle receiving a higher level of corn in their supplement. Still, the digestibility of hay was not significantly different between the lower and higher levels of corn but was reduced between the supplemented and non-supplemented groups, indicating pH was not solely responsible for impaired hay digestion.

Several studies have suggested rumen ammonia concentration could be a predominant factor in the negative associative effects of energy supplementation. Just as animals require energy and protein to grow and reproduce, as do microbes. However, bacteria possess the ability to synthesize their amino acids from available carbon skeletons and nitrogen in the rumen. Cellulolytic bacteria are heavily dependent on ammonia as their source of nitrogen for synthesis (Hoover, 1986). Increasing the energy in the diet increases microbial needs for N which may not be met by low quality forages. Furthermore, amylolytic bacteria can outcompete cellulolytic bacteria for this available N, only further reducing growth and synthesis of cellulolytic bacteria. The minimum level of rumen ammonia-N that is often cited is 2 mg/dL (Satter and Slyter, 1974). Concentrations below 2 mg/dL can impair microbial growth and alter microbial populations. The role of rumen ammonia concentration is supported by a decrease seen in the negative associative effects when rumen degradable protein is balanced with the TDN in the diet (J. S. Heldt et al., 1999; Bodine et al., 2001). Increasing the energy available in the diet without increasing rumen available nitrogen can reduce rumen fermentation thus, reducing forage digestibility. Feeding an energy supplement with a

low-quality forage may result in a deficiency in rumen available N, decreasing forage digestibility, and result in the negative associate effect observed.

Frequency of Energy Supplementation Feeding

Studies regarding infrequent energy supplementation feeding have had variable results, with some observing differences in performance and others not (Kartchner and Adams, 1982; Chase and Hibberd, 1989; Cooke et al., 2007; Loy et al., 2008; Drewnoski et al., 2011). Infrequent energy supplementation would result in cattle being fed a larger amount of concentrate on supplement days, which may exacerbate the negative associative effects observed with concentrate feeding. Kartchner and Adams (1982) reported that cows grazing dormant range receiving 7.3 kg of corn supplement daily gained twice as much weight over a 10-week period than their alterative day counterparts, 14.6 kg corn every other day, (65 vs 31 kg). In Chase and Hibberd (1989), corn supplementation was fed at two amounts (1.4 or 2 kg/d) on either daily or alternate days to cattle consuming low quality hay (5% CP). Supplements provide similar amounts of CP (255 g/d). However, supplements differed in TDN content (1087 vs 1713 g/d). In this study, hay OM digestibility was decreased by level of supplementation but not the frequency. Reducing frequency of supplementation reduced digestible OM intake by 0.23 kg/d, which may suggest the utilization of OM intake of alternate day cows is less than daily because frequency did not decrease total or hay OM intake. Feeding large amount of supplement less frequently reduces digestion due to negative associative effects of observed with energy supplementation. Therefore, negative effects of infrequent supplementation may be exacerbated by the amount of supplementation fed per feeding.

Cooke et al. (2007) offered growing steers on grass hay (54% TDN, 9.1% CP) a molasses-based supplement 3x/week or a citrus pulp-based supplement 3 or 7x/week. All supplements were formulated to provide the same energy and protein (75% TDN, 20% CP) and fed at 1% of BW/d. Cottonseed meal made up approximately 20% of both supplements. Forage DMI had a day x treatment interaction, with both 3x treatments decreasing forage intake on supplementation days. This is consistent with substitution effects seen in other supplementation studies. Mean BW change was greater for steers receive supplementation daily, but BW change was not significantly different for the 3x treatments. Daily supplementation resulted in gains of 0.3 kg/d while 3x gained 0.18 kg/d.

Loy et al. (2008) compared growing heifer performance of 3 supplement types (dry rolled corn, dry rolled corn + corn gluten mean, distillers grains), fed at 2 amounts (0.21% or 0.81% BW), and at 2 frequencies (7 or 3x/week). Supplements were formulated to provide meet MP requirements and provide the same amount of RUP. Supplement x concentration interactions were found for ADG. At a low amount, distillers grains (DDGS) had greater ADG than dry rolled corn + corn gluten meal (DRC + GCM) or dry rolled corn (DRC), 0.45 vs 0.34 kg/d. Though, at the high amount, both DDGS and DRC + GCM had greater gains than dry rolled corn DRC. There was a tendency for a supplement x frequency interaction. The DRC treatment did not result in a reduction of ADG when fed 3x. However, infrequent feeding of DDGS and DRC+CGM reduced ADG by 10.5% and 15%, respectively. These results are interesting as DRC has a greater amount of NSC than DDGS, and one would expect to see a greater impact of alternative day supplementation on performance due to increased negative associative effects on

forage utilization. While at first these results may not seem supportive of the negative effects of NSC on forage digestion, they do support the hypothesis of rumen ammonia-N concentration playing a key role in the negative associative effects of supplementation on forage utilization. In this study, urea was added to the treatments in case of RDP deficiencies unless the predicted MP supply was in excess to supply the rumen with N through urea recycling. Urea was added to DRC in both the high and low treatments because it offers little CP. However, DDGS and CGM are high in CP and a majority of that protein is unavailable to the rumen, so in these treatments urea was only added at the low levels. Relying on urea to be recycled from RUP to the rumen in time for forage digestion on alternative supplementation may have been an incorrect assumption. In the case of DRC, RDP was supplied at the time of supplement feeding and could contribute to the rumen ammonia-N pool. These results support the concept of nutrient synchrony, or suppling energy and protein source concurrently in the rumen to optimize microbial efficiency. Further studies of the addition of RDP to energy supplementation support the hypothesis of the impact of a supplement's RDP:TDN ratio on animal performance.

Drewnoski et al. (2011) looked at supplementation frequency of a soyhulls (SH) and corn gluten feed (CGF) blend on growing steer performance. These feeds were selected as they are low in NSC but high in energy and CGF provides RDP. Treatments included no supplement, 3x week supplementation frequency, or 7x supplementation. Steers received 19.1 kg of supplement per week, either 2.7 or 6.4 kg per feeding, and ad libitum medium quality fescue hay (8.8% CP, 67.1% NDF). Supplementation did reduce hay intake, with 3x reducing hay intake more than daily. Steers receiving no supplement had the lowest ADG (0.20 kg/d) but supplementation frequency did not change

performance (0.78 kg/d). As digestion study utilizing the same feeds was also conducted (Drewnoski and Poore, 2012). Hay intake was also reduced in this study between daily and alternate day supplementation (4.52 vs 3.88 kg/d). However, diet DM, NDF, ADF, cellulose, and CP digestibility did not differ between supplementation frequency. The amount of time that ruminal pH was below 6.5 also did not differ between supplementation frequency. These results suggest an energy supplement that is low in NSC may have less negative associative effects on forage digestion, as there would not be the drops in ruminal pH seen with starch digestion. Additionally, the addition of CGF allowed for there to be a source of RDP. This suggests again, rumen ammonia-N concentration may be the more significant factor in the negative associative effects seen on forage digestion with NSC feeding. By balancing the RDP:TDN ratio in the supplement, there is sufficient N for microbial usage to meet dietary energy availability.

Infrequent energy supplementation has yielded variable results, suggesting other dietary or metabolic factors have a role in the utilization of dietary available nutrients to improve animal performance. Degradable protein availability in the rumen may be one of these.

Protein Supplementation

Ensuring adequate protein in backgrounding diet is crucial. Undersupplying protein can reduce gains but also result in deposition of fat rather than muscle growth, especially if energy is oversupplied in the diet. Additionally, insufficient dietary protein can negatively effect rumen digestion, thereby decreasing the animal's ability to utilize forage. However, for ruminants, not all protein is created equal in terms of degradation in

the rumen. Where dietary protein is digested, either in the rumen or post-ruminally, and if the amount of that protein supply is sufficient can impact animal performance as well.

Protein Digestion and Utilization in the Ruminant

Protein in ruminant diets is commonly expressed as crude protein (CP), which is a measurement of nitrogen content of the feed. However, not all nitrogen containing compounds are protein. Crude protein content can be broken down into true protein and non-protein nitrogen (NPN). True protein can either be defined as rumen degradable protein (RDP) or rumen undegradable protein (RUP). Rumen Degradable Protein is the dietary protein the microbes in the rumen can digest for their own nitrogen requirements. Microbes can also use the NPN in feed to meet their needs for nitrogen for amino acid synthesis. Protein that is not digested in the rumen (RUP) by the microbes may be digested in the small intestine and utilized by the ruminant, digestible RUP (dRUP). However, the ruminant can also utilize microbes that are flushed from the rumen into the gastrointestinal tract (GIT) as protein. This source is referred to as microbial crude protein (MCP). Together, MCP and dRUP make up metabolizable protein (MP) or protein available for the animal to use. Metabolizable protein only considers the animal's protein requirements. Excess MP can contribute back to the microbial protein supply as excess nitrogen can be recycled back to the rumen via urea. Without adequate nitrogen for amino acid synthesis, microbial growth is reduced, decreasing the MCP supply as well as digestion in the rumen. Thus, when providing protein supplements to cattle, it is important to note what type of protein is supplied in the diet and in the supplement.

Protein Supplementation with Low Quality Forages

Low quality forages are often less than 8% CP, resulting in protein typically being the first-limiting nutrient for cattle grazing these forages (McCollum and Horn, 1990). This is especially true for growing cattle grazing low quality forages as their protein needs are greater than animals at maintenance. Offering protein supplementation to cattle on low quality forages provides nitrogen, which is a limiting nutrient in low quality forages. Nitrogen is crucial in the rumen environment for microbial growth and protein synthesis. Insufficient ruminally available nitrogen impairs microbial fermentation, therefore, decreasing forage digestibility. Reduction in forage digestibility can slow passage rate of digesta out of the rumen thus, reducing forage intake. Improvements in both forage digestibility and intake have been observed when supplementing cattle on a high forage diet with protein. Stokes et al. (1988) fed soybean meal (SBM), a protein supplement providing a majority of CP as RDP, to cannulated beef cows at 0, 0.12% or 0.25% of BW. Prairie hay (4.8% CP) was offered ad libitum. Hay intake increased linearly with SBM supplementation. Cows receiving 0.24% SBM consumed 7.82 kg/d of hay compared to 6.98 kg/d for 0.12% and 5.78 kg/d for control cows.

In DelCurto et al., (1990b) prairie hay (3% CP) and either a 0, 12%, 28%, or 41% CP supplement were fed to 242 kg steers. Supplements were a SBM and dry-rolled grain sorghum blend, formulated to be isocaloric and fed at 0.40% of BW. A 40% increase in forage intake was observed for steers receiving moderate and high crude protein compared to steers receiving low or no crude protein supplementation. There was a quadratic response to protein supplementation for NDF digestibility, with increasing digestibility from low to med protein but no change from med to high, suggesting that

once protein supplementation was provided at a certain amount, further feeding would not further improve forage intake or utilization.

A second study done by DelCurto et al. (1990b), compared low, moderate, and high protein supplementation to steers grazing dormant prairie grass. The SBM and dry-rolled grain sorghum blend supplements were fed at 0.5% of BW and provided 39.7% (LP), 79.3% (MedP), and 119.8% (HP) of the CP required by 318 kg yearling steers gaining 0.23 kg/d. Supplements were formulated be isocaloric. Forage intake, organic matter digestibility, and NDF digestibility, all responded in a quadratic matter, with MedP steers having the greatest values for all measurements. MedP steers consumed 50% and 32% more forage on a percent BW basis than LP and HP steers, respectively. NDF digestibility was 32.2% for LP, 34.2% for HP, but 44.0% for MedP. While MedP had the greatest forage intake and digestibility, HP steers still had greater values than LP steers, indicating that while oversupplementation of protein may not result in greater forage utilization, it still improves forage utilization relative to undersupplying protein. In these studies, SBM was provided as the protein supplementation, which is high in RDP. Low quality forages, which are typically low in CP, also do not provide enough RDP for microbial growth and synthesis. As with animals, microbes require amino acids for growth and reproduction. However, they can synthesize these from nitrogen and carbon skeletons, which are provided by catabolism of nutrients in the diet. Protein that is degraded in the rumen contributes to the microbes' nitrogen demands. When there is not enough nitrogen available to the microbes, their growth is reduced, limiting the microbial digestion of feed in the rumen. Thus, explaining why supplementation of a source in RDP increased forage utilization and intake. However, as seen in Del Curto et al. (1999a),

increasing amount of RDP supplementation does not linearly increase digestibility. The authors stated this result was unexpected. However, oversupplementation of protein may have caused the reduction of forage intake and digestibility as seen in this study due to insufficient energy available to rumen microbes. Since these supplements were formulated to be isocaloric to the animal and fed at the same amount, they varied in concentration of SBM and grain sorghum. In the case of these supplements, sorghum provided starch, or a source of energy directly available to the microbes. At the high CP level, more SBM was substituted for sorghum thus, reducing the starch content of the supplement. Microbial growth could have been impaired by limited availability of an energy source, resulting in decreased digestibility of forage, and then leading to a reduction in forage intake.

Since low quality forages offer little in terms of crude protein (<7%) and even less of that being rumen degradable, it was suspected that RDP was the limiting nutrient in these diets. Thus, to further understand what level of protein supplementation best improved forage utilization, Köster et al. (1996) evaluated digestibility of low-quality grass hay with incremental increasing amounts of RDP supplementation. Rumen degradable protein was intraruminal administered in the form of sodium caseinate. Amounts of RDP increased by 180 g/d, ranging from 0 to 720. Prairie grass hay (1.94% CP, 77% NDF) was offered ad libitum. Forage intake responded in a quadratic manner with 540 g/d of RDP having the greatest intake. The greatest increase in intake though was seen for the first incremental 180 g/d, with forage intake increasing 39% from control. True ruminal OM digestibility increased with increasing supplemental RDP, with digestibility as percent of intake increasing from 46.1% for control to 58.1% for 720 g/d.

Ruminal NDF digestibility also increased with supplemental RDP. The largest increase was for the first incremental level of supplementation, 47.2% to 55.6% digestibility. All total tract digestibility values increased with RDP supplementation, though were variable between levels. Greater NDF and OM digestibility as a result of RDP supplementation was likely due to increased availability of nitrogen for rumen microbes. However, due to the quadratic response of forage intake and digestibility, the authors set a recommended value that the digestible OM contain 11% RDP to maximize intake and digestibility.

Heldt et al. (1999) observed an interaction between of the amount and source of carbohydrate supplementation and amount of RDP on forage intake and utilization. Forage source was a prairie grass hay (5.9% CP, 74.9% NDF) and offered ad libitum. Treatments were arranged in a 2 x 2 x 3 factorial plus a negative control. Factors included level of RDP, sodium caseinate, (0.031 or 0.122% of BW), carbohydrate source (cornstarch, glucose, or oat fiber), and level of carbohydrate (0.15 or 0.30% of BW). When averaged across all carbohydrate levels and sources, supplemental RDP increased forage intake. Low levels of carbohydrate supplementation with supplemental RDP increased NDF digestibility compared to no supplementation. However, increasing the level of glucose or starch supplementation decreased NDF digestibility, regardless of RDP supplementation level. For the high level of fiber supplementation, NDF digestibility did decrease for low level but not at the high level of RDP supplementation. In this study, the greatest RDP supplementation level was set off the recommended level by Köster et al. (1996), 4 g RDP/kg BW. While the Köster et al. (1996) study utilized a similar forage, there was no additional carbohydrate supplemented in the diet. Thus, the RDP levels in this study still might have not been sufficient to maximize digestion,

explaining why NDF digestibility was decreased at higher levels of glucose or starch supplementation, even with additional RDP added.

Compling the results from various RDP supplementation to forage based diet studies, Cochran et al. (1998) recommended that 10-13% of the dietary TDN should be RDP to maximize forage intake and utilization. Rumen degradable protein supplementation is observed to have a quadratic response, with increasing supplementation over the 13% level not further improving forage response. The importance of balancing RDP and TDN is due to competition between amylolytic and fiberolytic bacteria for rumen available nitrogen. In the case of low RDP:TDN ratio, rapid fermentation of supplement by amylolytic would utilize majority of N present in both the supplement and forage, resulting in little for fiberolytic bacteria. Thus, impairing fiber digestion and reducing forage intake. However, providing enough RDP to meet the TDN content of the diet supports the demand of both the amylolytic and fiberolytic bacteria.

Bodine et al. (2001) fed starch, fiber, or protein-based supplements formulated to provide 1.1 g of RDP/kg of BW to determine effects on forage utilization. Ruminally cannulated steers were fed ad libitum prairie grass hay (5.5% CP, 72.6% NDF) in addition to either 0.5% of BW for the protein supplement or 1.0% of BW for the starch and the fiber supplements. Treatment supplements were: 1) MINCR, mineral/vitamin mix with cracked corn; 2) PROT, cottonseed meal based pelleted protein supplement; 3) HF, wheat middlings/soybean hull based high fiber supplement; or 4) HG, sorghum grain based energy supplement. Hay intake was greater for protein-based supplement animals than starch or fiber-based. However, those animals also received less supplement, which

resulted in similar total OM intake across all treatments. Supplement base type did not affect forage OM digestibility or in situ DM digestibility. In this study, all treatments except MINCR provided over 13% RDP:TDN, the value recommended by Cochran et al. (1998). In this study, negative associative effects of feeding large amounts of a starch-based supplement were not observed. However, suggested RDP requirements were met, indicating the importance of rumen available nitrogen to support both amylolytic and fiberolytic bacteria fermentation.

Performance data from Bodine and Purvis (2003) also supports the digestibility study's findings. Yearling steers grazed dormant prairie grass (6.8% CP, 69% NDF), and received one of four treatments: 1) corn and SBM, balanced for a RDP:TDN ratio of 7.5 (CSBM); 2) corn and soybean hulls, equal in supplemental TDN to CSBM (CORN); 3) soybean meal, equal in supplemental RDP to CSBM (SBM); or 4) cottonseed hull-based control supplement (CONT). The ratios of RDP:TDN for CORN, SBM, and CONL were 3.2, 51.9, and 4.7, respectively. Average daily gain and final BW was greatest for steers receiving the CSBM supplement. All treatments had greater ADG than CONL cattle. CSBM gained 0.73 kg/d while CORN and SBM steers gained 0.24 and 0.39 kg/d, respectively. CORN and SBM treatments were not significantly different. CONL cattle lost body weight during the trial and had an ADG of -0.17 kg/d. Animal performance was improved when energy or protein was supplemented yet, the greatest response was observed when energy and protein (RDP:TDN) of the total diet (supplement and forage) were adequately balanced per the 1996 NRC model.

Improvements in forage intake and forage utilization by supplemental RDP are also dependent on the initial forage quality. Low quality forages are considered to be less

than 7% CP and typically show the greatest response in forage intake and utilization with supplemental RDP. While forages over 7% CP still may not have adequate RDP values, response to RDP supplementation may not be observed due to nitrogen (N) recycling, or the mechanism in which N is conserved in the ruminant. Nitrogen that is not utilized by the animal can return to the rumen as urea and contribute to the N pool. In Mathis et al. (2000), three different forages with increasing levels of RDP supplementation were fed to steers (BW = 295±8 kg) to determine impacts on forage intake and utilization. Three independent experiments used either bermudagrass (8.2% CP, 70.8% NDF; Exp 1), bromegrass (5.9% CP, 65.4% NDF; Exp 2) or forage sorghum (4.3% CP, 59.4% NDF; Exp 3) hay and supplemented RDP (sodium caseinate) at 0.041, 0.082 or 0.124% of BW; control animals received no RDP supplementation. Values in terms of grams of RDP per day were 120, 240, and 365, respectively. Forages were selected as they were expected to respond to RDP supplementation, based off the 1996 NRC model. All forages had less than 13% RDP in the digestible OM, the value used as the default requirement in Level 1 of the model. Sodium caseinate was directly placed in the rumen, immediately prior to forage feeding. In Exp 1, there was no effect of supplemental RDP on forage intake or utilization. Across all treatments forage intake averaged 88.92 g/ kg BW and average total tract NDF digestion was 63.98% of intake. Rumen degradable protein supplementation also did not impact intake or digestibility of bromegrass hay in Exp 2. Forage intake was 112.1 g/ kg BW across treatments and NDF total tract digestibility was 53.68% of intake. However, in Exp 3, both forage intake and digestibility linearly increased with RDP supplementation level. Forage intake increased 28.1% from control to steers receiving 0.124% of BW in RDP supplementation. Neutral detergent fiber total

tract digestibility increased 35.2% from no RDP to the highest amount of RDP supplementation. While all these forages were low in their RDP as suggested by the 1996 NRC model, there was only a response to supplementation in forage intake and utilization for forage sorghum. Rumen degradable protein of these forages was estimated with an in situ technique. Forage sorghum had the lowest value, 2.5% of DM, followed by bromegrass, 2.9% of DM, then bermudagrass, 4.8% of DM. The varying CP of the forage may have resulted in RUP still contributing to the rumen ammonia pool through nitrogen recycling. This may explain why not all diets showed an effect of RDP supplementation, as microbial needs for nitrogen were met though dietary protein and recycled nitrogen.

Supplying supplemental protein to low quality forages can help improve forage intake and utilization, resulting in improved animal performance. However, due to the ruminant animal's ability to recycling nitrogen, not all protein supplementation will have large effects, especially if forage quality is greater or the animal requires less crude protein. Additionally, the amount of protein to supply can vary depending on the TDN of the diet, with an increased need for protein with greater TDN.

Nitrogen Recycling in the Ruminant

Ammonia Production

Nitrogen is required for tissue protein synthesis, nucleotide synthesis, and the production of nitrogenous compounds ranging in functions from hormones, neurotransmitters, and immune defenses (Tomé and Bos, 2000). Dietary protein provides the source of N for animals, but ruminants are less efficient than nonruminants in utilizing dietary proteins due to microbial conversion of protein to ammonia in the rumen

(Tan and Murphy, 2004). A majority of ruminants' dietary nitrogen is absorbed in the gastrointestinal tract as ammonia, and in some cases, more N is absorbed as ammonia (NH_3) than as α-amino N (Reynolds, 1992). Two main processes lead to the concentration of ammonia in the ruminant gut, one being the microbial catabolism of protein in the rumen, and the other from microbial hydrolysis of urea, which passes across the gut wall from blood and intestinal fluids (Parker et al., 1995). Endogenous sources, such as sloughed mucosal cells and salivary proteins, can also contribute to ammonia absorbed (Nolan, 1975). Ammonia that is absorbed across the rumen epithelium is a function of the rumen NH_3 concentration. The mechanism in which absorption occurs is a passive diffusion down a concentration gradient, thus, a higher ruminal concentration of ammonia increases the rate of NH_3 into the blood (Parker et al., 1995). Consequentially, the amount of NH_3-N absorption into the portal vein increases with increasing N intake (Firkins and Reynolds, 2005). Ammonia is a toxic compound to non-hepatic tissues and therefore, must be detoxified by the liver to prevent tetany and/or death (Symonds et al., 1981). Ammonia from the ruminant gastrointestinal tract is absorbed into the portal vein and extracted by the liver. The ruminant liver is extremely efficient in uptaking NH_3, even when portal NH_3 absorption varies threefold, arterial NH_3 concentrations remain constant (Parker et al., 1995). However, the liver can still only extract 70-95% of portal NH_3 (Parker et al., 1995). Thus, ruminants are susceptible to diet induced NH_3 toxicity when non-protein nitrogen is rapidly converted to ammonia in the rumen, absorbed into the portal vein, and overwhelms the liver's capacity to detoxify it to urea. Ammonia toxicity in ruminants is observed when circulating NH_3 concentrations

exceed 0.7 m, with normal arterial NH_3 concentrations in the 0.1 mM range (Parker et al., 1995).

Urea Synthesis

The liver converts ammonia to urea or glutamine in order to detoxify it. However, the conversion to urea is the major detoxification pathway, with 94% of portal ammonia converted to urea (Lobley et al., 1995). Rate of ureagenesis is dependent on several factors including the type of N metabolites in the liver and the productive state of the animal (Lapierre and Lobley, 2001). For growing or lactating animals, the demand for N for tissue synthesis is greater and thus, the preferred N source is in the form of amino acids (Lapierre and Lobley, 2001). In Lapierre et al. (1997), diets differing in percentage of rumen degradable protein (RDP) were fed to lactating dairy cattle. Diets were isonitrogenous but contained either 60 or 75% RDP. While the net portal drained viscera (PDV) supply of nitrogen was equal, the ratio between ammonia-N and amino acid-N in PDV was 46:54 and 54:46, respectively. A diet in more rumen undegradable protein resulted in a greater proportion of N absorbed as amino acids than ammonia, resulting in less urea synthesis by the animal. Bailey et al. (2012b) compared urea synthesis and recycling of steers weighing 208 vs. 391 kg and fed a diet deficient in RDP. The more mature steers had more urea synthesis and thus, more urea recycling than the younger animals. This is due to the greater amount of N deposition in tissue by the younger, growing animal. Still, even in ruminants offered high dietary N intakes, ureagenesis can exceed that digestible N (Archibeque et al., 2002). This excess N is a result of rumen microbes flowing into the small intestine. They can be utilized as a protein source, adding to the N available to the animal. Without a recycling system to recover nitrogen in cases

where dietary N intake is insufficient for both the animal and microbes, that animal would have a negative N balance. The recycling of nitrogen back to the rumen as urea helps to shift this balance by providing microbial synthesis, which in turn, supply N to the ruminant as amino acids (Lapierre and Lobley, 2001).

Fate of Urea Produced by the Liver

Urea produced by the liver is either excreted in urine or recycled back to the rumen. Between 40 and 80% of the urea-N synthesized by liver is returned to the rumen and provides a large contribution to the available N for the rumen microbes (Harmeyer and Martens, 1980). However, urea-N can also be cleared from the body through the kidney in urine. While there will always be some N loss though the urine pool, manipulation of the amount of these losses can done through reduction of dietary N or improvements in amount of urea recycled to the GIT (Tan and Murphy, 2004). Still, in either case, the amount of N demanded by the rumen plays an important role in determining the balance of urea recycling or excretion. When growing steers fed a diet of fescue hay with a ruminally protected methionine supplement, recycled urea-N increased the digestible N inflow up to 85%. However, differences between levels of supplement were detected in urinary urea-N excretion and percentage of urea-N returned to the ornithine cycle. Diets were fed to support adequate energy for 0.5 kg ADG and supplement was fed at levels to provide protein to support maintenance or 0.5 kg ADG. Steers receiving the higher amount of supplementation had a greater amount of urinary urea-N excretion, 34.6 g vs 24.8 g, but a lower percentage of urea-N produced that was returned to the ornithine cycle (Archibeque et al., 2002). Determination of the fate of urea, either to be utilized by the gut or excreted by the kidney is dependent on dietary

factors. Harmeyer and Martens reported a relationship between dietary crude protein content and renal excretion of urea, with increasing dietary crude protein content correlated with increasing renal urea clearance (1980). Plasma urea concentration is the main factor influencing the quantity of urea excreted by the kidney, with increasing plasma concentrations leading to greater amount of urea cleared. Within the kidney, glomerular filtration rates and tubular reabsorption of urea serves as other mechanisms to increase retention of urea when N supply is insufficient. However, in the case of these factors, they are an adaption when N intake is inadequate over a period of several months (Harmeyer and Martens, 1980). In times of limited dietary N, renal excretion of urea is reduced while when dietary N intake is sufficient for rumen function, renal excretion of urea is enhanced. Again, this mechanism improves the reutilization of N throughout the system to prevent a negative N balance and thus, supporting both animal and microbial needs.

Urea Recycling to the Rumen

Urea is recycled back to the gut through salvia and across the rumen wall (Houpt, 1959). However, the regulation of urea reentering the gut is still not well understood. Factors that have been suggested to play a role include plasma urea-N concentration, rumen NH_3 concentration, and fermentable carbohydrates in the GIT (Reynolds and Kristensen, 2008; Jin et al., 2018).

While plasma urea concentrations appear to have some role in urea transfer to the rumen, there is an upper limit on which increasing plasma urea concentration no longer has a linear effect on transfer. For cattle, this concentration is 4 mM (Harmeyer and Martens, 1980). Increasing plasma urea concentration above this value did not further

increase the amount of urea transferred to the rumen, which may indicate that plasma urea is not the only controlling factor. The concentration of ammonia in the rumen also appears to have a role in the transfer of urea. When urea was infused directly into the rumen, the amount of urea transferred to the rumen from plasma decreased as the rumen NH_3 concentrations increased. Intraruminal infusions were given over a 24 hour period with blood and rumen fluid samples collected at 45 minute intervals over the last 8 hours of the infusion period (Kennedy, 1980). Additionally, increased ammonia concentration could have a negative inhibitory effect on the microbial enzyme, urease, which could reduce diffusion of urea into the rumen from plasma (Abdoun et al., 2006; Jin et al., 2018). Hydrolysis of urea by urease is very rapid, occurring at an approximate rate of 0.2 mg ammonia-N per g of urea per hour (Pearson and Smith, 1943). However, utilization of ammonia by microorganism occurs at a slower rate than hydrolysis (Jin et al., 2018; Patra and Aschenbach, 2018). Therefore, due to the passive diffusion of ammonia from the rumen wall to the blood, this imbalance can result in decreased efficiency of urea-N utilization and in extreme cases, ammonia toxicity.

Finally, the amount of energy available to the rumen may have a role in urea transfer. Increasing energy available to rumen microbes results in an increase of fermentation end products, or volatile fatty acids (VFA). In Bodeker et al. (1992) the presence of VFA in a mucosal buffer solution stimulated the uptake of urea while lactic acid did not. Propionate increase not only the entry of urea to the rumen, but also the conversion to anabolic-N (Kim et al., 1999). Abdoun et al. (2010) also found that VFA and CO_2 stimulated transfer of urea across the rumen wall. Increased VFA presence or absorption may act as a signal that energy availability has increased in the rumen and

therefore, increased the demand for N. Though, not just fermentation end products stimulate urea transfer, but also the availability of a carbon source for the microbes. Increasing the amount of fermentable organic matter in the diet also increases the amount of urea transferred to the rumen (Kennedy and Milligan, 1980). Rémond et al. (1996) recorded that when feed was supplemented with a rapidly fermentable energy source, the passage of urea into the rumen could be doubled. Two studies regarding starch content in diets of growing steers found that higher starch diets also resulted in an increase of transfer of urea to the rumen (Huntington, 1989; Theurer et al., 2002). Increasing the amount of carbon available allows for increased fermentation by microbes. However, this also increases microbial N requirements. Thus, to match ruminal available nitrogen to fermentation needs, the amount of urea returned to the rumen needs to be increased.

Fate of Urea-N in the Rumen

Ureolytic bacteria in the rumen hydrolyze urea into ammonia and carbon dioxide through the enzyme, urease. This conversion of urea to ammonia is crucial as microbes only utilize N from ammonia for their synthesis of proteins (Jin et al., 2018). While rumen supply of nitrogen may appear to only effect the microbes, the ruminant does benefit from an increase in microbial protein synthesis as these can be digested by the animal in the small intestine and serve as a source of amino acids. However, the amount of urea-N that ultimately ends up being used in microbial protein synthesis is less than what is returned from the liver. In steers fed a diet of forage with 59% NDF and 16% CP, 50-60% of urea-N recycled went to anabolism in the rumen (Archibeque et al., 2001; 2002). Likewise, in Kim et al., the amount of microbial usage of urea-N ranged from 45-72% of recycled urea-N (1999). This inefficiency of microbial capture of urea-N may be

due to the fact that once urea is hydrolyzed into ammonia, it then can be absorbed across the rumen wall yet again. However, this does not mean this N is a total loss to the system, as it can once again be synthesized into urea and returned to the rumen. The return of N to the rumen multiple times only increases the likelihood that it ends up in microbial protein synthesis (Lapierre and Lobley, 2001). There is a 30% improvement of urea-N being used in anabolic processes when it has multiple entry to the rumen (Archibeque et al., 2001; 2002). Dietary N intake does impact the amount of urea-N utilized by microbes, as cattle fed diets higher in crude protein had less urea-N being used for anabolic purposes. Cattle fed diets that were less than 12% CP had up to 72% of urea entering the rumen used in microbial protein synthesis where higher protein diets only had 17-26% (Reynolds and Kristensen, 2008). Cattle that are on low protein diets (less than 7% CP) are much more dependent on recycled urea for a source of N and thus, are more efficient in recapturing urea-N.

Importance of N Recycling to the Ruminant

For cattle grazing low quality forage, nitrogen is the most limiting nutrient to the microbes (Köster et al., 1996). When these cattle do not receive protein supplementation, the N intake would be insufficient to meet the microbial needs. Thus, the animal needs to be efficient in salvaging N to meet microbial demands. Even in low protein diets, the total flow of N to the small intestine exceeds the animal's N intake due to microbial crude protein. This demonstrates how important of a role urea recycling is in the nitrogen metabolism in the rumen (Titgemeyer, 2012). In cattle fed prairie hay (2.8% CP), the microbial N reaching the duodenum was twice the rumen degradable protein intake, indicating that a majority of the N used in microbial synthesis was provided by urea-N

(Lintzenich et al., 1995). Protein deficient cattle are incredibly efficient in recycling urea to the rumen in order to supply N for microbial needs. Up to 99% of urea synthesized by the ruminant can be returned to the gut in the case of low protein intake, less than 7% CP (Wickersham et al., 2008a). Still, even in cases where rumen available nitrogen was sufficient for microbial fermentation, up to 95% of urea synthesized was returned to the gut, again highlighting the efficiency of N conservation in the ruminant (Wickersham et al., 2008a). Yet, even though this urea is returned to the gut, microbial usage is not guaranteed. When steers were provided with a supplemental RDP source, the amount of urea-N that was captured by the microbes decreased with increasing amount of supplementation (Wickersham et al., 2008b; Bailey et al., 2012b). In the case of providing protein supplementation as rumen undegradable protein, urea recycling plays a more critical role in meeting microbial demands for nitrogen. Since RDP is readily available to the microbes, they depend less on receiving N from recycled urea. However, with RUP before the microbes have access to the nitrogen, the protein first must be digested in the small intestine and the amino acid-N absorbed through the portal vein and synthesized into urea by the liver before it can be finally recycled to the rumen (Titgemeyer, 2012). Additionally, the amount of amino acid-N that enters the liver to be synthesized to urea varies. Metabolic status of the animal determines amino acid requirements. Growing or lactating animals have a greater demand for amino acids for anabolism and deposit more N into tissue synthesis thus, reducing the amount of amino acid-N entering the liver. Wickersham et al. (2008c) saw an increase in urea recycling when cattle on prairie hay were fed a RUP supplement. Rumen available nitrogen demands in this study were not met through dietary intake and therefore, to sufficiently

meet demands, more N was recycled back to the rumen. Additionally, the amount of MCP synthesized from urea-N increased with the RUP supplement. Thus, the microbes utilized more of recycled N since they did not have a source of N from dietary intake. The ability of the ruminant to recycle N also is key during periods of infrequent protein supplementation. When steers fed prairie hay received a RDP supplement either daily or every third day, animals receiving infrequent supplementation had more urea recycling and greater amount of MCP synthesized from urea-N (Wickersham et al., 2008b). Steers that received daily supplementation were less dependent on urea recycling to provide N for microbial needs but steers receiving infrequent supplementation were much more dependent on urea recycling to meet N demand. Overall, N recycling in the ruminant plays a crucial role in supporting rumen microbes. The efficiency of this process allows for even protein-deficient animals to meet microbial needs.

Dried Distillers Grains as a Supplement for Forage Fed Cattle

Distillers Grains Production

Nebraska is the third largest corn producer in the United States. While this corn can be used as a source of food for humans and animals, it can also be utilized as an energy source in the form of ethanol. Twenty-five plants across Nebraska use corn for ethanol production, resulting in over 2 million gallons of ethanol produced per year (Nebraska Ethanol Board). However, ethanol production process only uses the starch in corn grain, leaving the bran and germ. While nutrients such as protein and fat remain in these portions of corn grain, they are fibrous and not easily digested by monogastrics. However, ruminants can utilize this by-product, known as distillers grains, as a feed source (DiCostanzo, 2018). Due to amount of ethanol production in Nebraska, the

availability, cost, and nutrient content of distillers grains makes it an attractive feedstuffs for beef cattle producers.

Distillers grains is the by-product of the dry milling ethanol process. Ethanol is produced as a result of a yeast species *Saccharomyces cerevisiae* digesting simple sugars. The steps in the production process utilize the yeast's ability to digest starches to glucose and then ferment pyruvate, the product of glycolysis, to ethanol and carbon dioxide. Since the yeast just metabolizes the starch in corn, other components like the protein, fat, and fiber, are unchanged and concentrated. Since starch in corn makes up two-thirds the dry matter, all remaining nutrients are concentrated three-fold. After ethanol is distilled, the remaining product, referred to a stillage, is centrifuged. Centrifugation separates the grain from the liquid or solubles. The solids can either be sold as is, wet (WDG), or dried and the solubles added back (DDGS). Dried distillers grains are approximately 30% CP, 6-8% fat, and 36% NDF (Klopfenstein et al., 2008). Of the CP content of DDG, approximately 65% of the protein is rumen undegradable (RUP).

Distillers Grains Supplementation to Growing Cattle

In finishing-based diets, energy value of distillers grains was observed to be greater than corn (Larson et al., 1993). However, in forage-based diets, DDG was mainly fed as a protein supplement. But, due to the increased NDF and reduced starch, providing DDG as a supplement to cattle on forage-based diets, may reduce the negative associate effects seen with traditional starch-based supplements (Fieser and Vanzant, 2004). Therefore, Loy et al. (2008) compared the energy value of DDGS to dry rolled corn (DRC) to heifers on a high-forage diet. Three diets were fed at two different concentrations to produce either 0.45 or 0.80 kg/d of gain, respectively. Since the energy

value was not yet determined for DDGS, the energy value used was equal to corn for diet formulation. Diets were either DDGS, DRC, or a DRC + corn gluten meal blend (CGM). The DRC and DDGS were formulated to meet or exceed the metabolizable protein and RDP requirements per the 1996 NRC. The DRC + CGM supplement was formulated to provide a similar amount of RUP as the DDGS supplement. Diets were fed for 84 days in individual bunks and in addition, all heifers received ad libitum grass hay (8.7% CP, 54% NDF). Hay intake did not differ between the supplement type but did decrease with supplement concentration. Heifers receiving the low concentration consumed 4.99 kg/d of hay while high treatment consumed 4.47 kg/d. For ADG and gain to feed (G:F), there was a supplement x concentration interaction. For the low concentration, heifers receiving DDGS gained 0.49 kg/d, while those supplemented with DRC or DRC+CGM gained 0.36 kg/d. Those on the DDGS supplement also gained more efficiently. At the high concentration, heifers supplemented with DDGS or DRC+CGM gained 0.89 kg/d and DRC gained 0.71 kg/d. Both the DDGS and DRC+CGM heifers had greater G:F than DRC. The prediction of energy value of DDGS was estimated to be 130% of the value of DRC at the low concentration and 118% at the high concentration.

Morris et al. (2005) also reported increasing ADG for heifers supplemented with increasing levels of DDGS on forage diets. Heifers were supplemented with 0, 1.5, 3, 4.5, or 6 pounds of DM dried distillers grains on a high quality (65% TDN) or low quality (53% TDN) forage source. As DDG intake increased, forage intake decreased for both forage sources, but the rate of decrease was greater for the heifers consuming the high quality than low quality, 0.53 and 0.33 lbs forage per lb of DDG, respectively. Forage quality did impact ADG, with heifers on the high quality gaining more than heifers on

low, 1.41 and 0.42 lb/d, respectively. Average daily gain linearly increased with increasing DDG supplementation. However, between the forage qualities, the rate of increase in gain was greater for the low-quality forage (0.265 lbs per lb DDG) compared to the high quality (0.20 lbs per lb DDG).

The ability of distillers grains to serve as both a protein and energy supplement to cattle on forage diet was not well understood. It was hypothesized that due to the higher RDP content in forages, supplementation of RUP from DDG helped to balance a potential metabolizable protein deficiency for cattle on high forage diets (Klopfenstein, 1996). Additionally, the fat content of DDG could also contribute to additional energy. Therefore, MacDonald et al. (2007) looked at the effects of DDG or the equivalent RUP or fat on ADG and forage intake for heifers (BW= 368 ± 39 kg) grazing bromegrass. Treatments were arranged in a 3 x 3 + 1 factorial design, with factors being source and level of supplementation. Supplement sources were DDG, corn gluten meal (CGM), or corn oil (OIL). Level of DDG supplementation was 750, 1,500, or 2,250 g/d. The corn gluten meal and corn oil supplements were fed in amounts to provide equivalent RUP or ether extract (fat) as DDG. Control heifers received a corn bran and molasses supplement. For average daily gain, increasing DDG linearly increased ADG, while CGM tended to increase ADG. However, the rate of ADG was 39% less than that for DDG. Supplementation of OIL did not affect ADG and tended to be less than DDG. While suppling RUP equivalent to DDG supplementation did improve ADG, gains were not equal to what was observed for DDG. Additionally, supplementation of fat equal to that in DDG did not improve gains. Therefore, neither single component of RUP nor fat could independently explain the increase in ADG seen with DDG supplementation.

However, the increase in ADG with RUP but not with fat supplementation suggests that meeting a MP deficiency may explain some of the responses to DDG supplementation. Feeding levels of DDG in excess of MP requirements leads to deamination of protein and subsequent carbon skeletons to be metabolized as an energy source.

A meta-analysis of 20 forage-based growing studies evaluated the effects of different supplementation levels of DDGS on final BW, ADG, and forage intake (Griffin et al., 2012). The analysis utilized both studies in which cattle were grazing pasture or fed forage in confinement. In the pasture-based studies, grass was managed so forage intake was not limited. In these studies, cattle grazed from late spring to early fall, ranging from 60 to 196 days on pasture. Dry distillers grains were supplemented daily with amounts from 0 to 1.03% of body weight. Supplementing DDGS to cattle on pasture linearly increased ending BW and ADG with increasing amount of supplementation. For confinement-based studies, forage source was either grass or alfalfa hay, or a blend of hay with silage. Cattle were on study for an average of 86 days and fed levels of DDGS from 0 to 1.27% of BW. In these studies, increasing DDGS supplementation quadratically increased ADG and final BW. However, increasing DDGS supplementation had the opposite effect on forage DMI, quadratically decreasing forage intake. On average in the pasture-based studies, supplemented cattle gained 37 kg more than non-supplemented cattle. Additionally, forage intake was replaced at rates ranging from 0.50 to 0.79 kg of forage per 1 kg of DDGS intake. Overall, results from all studies support increasing levels of DDGS supplementation increases ADG and final BW, while replacing some of the forage intake for cattle on forage-based diets.

Daily supplementation of DDG increases ADG for growing cattle. However, daily supplementation can increase costs to producers. Therefore, effects of DDG supplementation frequency on gains was studied. In the previously mentioned Loy et al. (2008) study comparing the energy value of DRC to DDG, supplementation frequency was another treatment factor. All supplements of DDGS, DRC, and DRC+CGM were fed at the low or high amount either daily or three times weekly. Decreasing supplementation frequency decreased hay dry matter intake across all supplementation types and amounts, with daily heifers consuming 5.03 kg/d compared to alternate day consuming 4.44 kg/d. This leads to a total reduction in DMI, 12% between daily and alternate day frequency. Across all supplement types, there was a 10% reduction in ADG for daily to alternate day supplementation, 0.62 to 0.56 kg, respectively. A supplement x frequency interaction was not detected (P = 0.13). However, the authors report that for DDGS supplementation specifically, there was a 10.5% reduction in ADG for infrequent supplementation compared to daily supplementation.

Further performance study data by Stalker et al. (2009) also reported a decrease in gain for heifers and steers supplemented DDGS 3x/week compared to 6x/week. In the heifer performance trial, animals (BW = 193±20 kg) received ad libitum grass hay (6.6% CP, 67.2% NDF) and the equivalent of 1.3 kg/d of DDGS supplement. Heifers were fed either 6x/week (Monday- Saturday) or 3x/week (Monday, Wednesday, Friday) for 84d. Infrequent supplementation reduced heifer ADG by 0.07 kg/d, final BW of the heifers differed by 6 kg. In the steer performance trial, steers (BW = 213±22 kg) received one of four supplementation treatments. The control treatment was ad libitum grass hay (6.6% CP, 67.2% NDF) and a corn/SBM blend supplement fed at the equivalent of 2.0

kg/head/d 6x/week. Steers in the other three treatments grazed dormant winter range and were separated into pens 6x/week to receive their supplements. Supplements included a corn/SBM blend fed at the equivalent 2.7 kg/d, 6x/week, or DDGS fed at the equivalent of 1.9 kg/d, either 6x or 3x/week. All supplements were formulated to supply similar amounts of energy and meet RDP and MP requirements in the 1996 NRC. While the DDGS 3x animals were offered the full supplementation amount, they did not consume all of it in the allotted time and therefore, consumed the equivalent of 1.75 kg/d. Average daily gain was similar for steers in the control, CSM, and DDGS6 treatments, but reduced in the DDGS3 treatment, 0.88 vs 0.65 kg, respectively. While the DDG3 did not consume to equivalent amount of supplement as the DDGS6, the difference in amount should have only resulted in a 0.06 kg/d difference of ADG.

As animals in these studies received equivalent amounts of supplementation, their reduction in ADG may be due to changes in the forage component of their diet. In foraged-based diets, voluntary dietary intake can be controlled by two factors, physical or physiological factors (Van Soest, 1994). Physical factors include those relating to feed effects, such as fiber content and digestibility, and rate of fiber degradation in the rumen, all of which affect rumen distention and fill (Roche et al., 2008). Physiological factors impacting voluntary forage intake including chemostatic feedback, nutrient intake, and metabolic state sensing by the animal (Roche et al., 2008). When considering changes in forage intake with supplementation, particularly in terms of reduction with alternate day supplementation, both of these factors should be considered. Receiving a larger amount of supplement, the previous day would signal a greater nutrient balance thus, reducing nutrient needs be met by forage intake the subsequent day. However, infrequent

supplementation may impact rumen fermentation, reducing digestibility of the forage component in the diet, and therefore, decrease forage intake. Changes in forage intake and/or forage digestion seems to be the likely causes of decreased animal performance between daily and infrequently supplemented animal as supplementation amounts were consistent amongst treatments.

Therefore, Loy et al. (2007) used 10 ruminally cannulated heifers (416±24 kg) to compare the effects of the form of energy supplementation as well as the frequency on forage intake and digestibility. Dry rolled corn or DDGS were fed as supplements. Supplementation amount was 0.4% of BW (1.66 kg) and fed daily or on alternate days. Heifers also received ad libitum grass hay (8.2% CP). Urea was added to both supplement types to balance for RDP, based on the 1996 NRC model. Supplementation did reduce hay DMI (7.82 kg to 6.91 kg), but supplementation frequency only tended to decrease hay DMI (7.03 kg vs 6.73 kg). There was no difference in hay DMI between supplementation types. There was no difference in rumen pH for supplementation type or frequency, though supplemented heifers did have a lower pH than control. The rate of in situ hay NDF disappearance was greater for control animals than supplemented, 4.3 verses 3.8%/hour, respectively. However, supplementation frequency did not affect NDF disappearance rate, but supplementation type did. Despite there not being a difference in ruminal pH or forage intake, DDGS had a greater NDF disappearance rate than corn (4.05 to 3.54%/h). Infrequent supplementation did reduce hay intake but did not appear to negatively impact digestibility, implying that chemostatic feedback may have been the more important factor in limiting voluntary forage intake than physical factors of the forage.

In Stalker et al. (2009), six steers (371±30 kg) were used in a 3 x 3 Latin square design with 3 periods to study the effects of daily, alternate day, or every third day supplementation of DDGS on digestibility and intake. Dry distillers grains plus solubles were fed to consist of 16.7% of the diet DM, approximately 1.15 kg. Grass hay (6.7% CP) was fed ab libitum on the first 9 days of the period. Hay DMI decreased with infrequent supplementation, 8.75 vs 8.23 kg/d. Apparent total tract DM, OM, and NDF digestibility linearly decreased with infrequent supplementation. When only the NDF disappearance from hay was analyzed, there was also a linear decrease in digestibility. Thus, suggesting infrequent DDG supplementation causes a decrease in forage digestion, leading to reduction in animal performance. This result contrasted with Loy et al. (2007) results. However, forage quality differed between the two studies, with Stalker et al. (2009) utilizing a lower quality hay. Infrequent supplementation's impact on rumen fermentation may be exacerbated by low-quality forage, and thus, further reducing forage utilization and animal performance.

The decrease in animal performance seen with infrequent DDG supplementation may be contributed to the decrease in forage digestion. One possible reason for the decrease in forage digestion is inadequate nitrogen for the rumen microbes on alternative day feeding. Due to the high RUP content of DDG and the low CP in forages, supplementation of DDG for cattle on low quality forages may not balance a potential RDP deficiency. Thus, leading to a reduction in microbial digestion of forage, leading to decreased animal performance. Therefore, Stalker et al. (2007) included urea, a RDP source, to DDG supplements for cattle consuming low quality forages to determine effects on animal performance. Heifers were fed meadow hay (7.4% CP) and

supplemented with either 1.4 kg/d DDG or 1.4 kg/d plus 45 g of urea. This amount of urea was added to meet RDP requirements as predicted by the 1996 NRC. Heifer ADG, DMI, or G:F did not differ between treatments. These results suggest that RDP was not deficient in these diets or nitrogen recycling was adequate to meet microbial needs when DDG was fed daily. Excess MP can be not only be beneficial in terms of the animal receiving energy from catabolized carbon skeletons from protein degradation, but through nitrogen recycling, rumen microbes can receive cleaved nitrogen. In the liver, amino acids that are not used for tissue synthesis are deaminated, with the amino acid's carbon skeleton utilized for energy. However, the cleaved amine group must be synthesized into urea to prevent ammonia toxicity in the body. In the case of ruminants, urea synthesized by the liver can return to the rumen to contribute to the nitrogen pool available for microbes. This recycling system can help to overcome any RDP deficiency even when the DDG supplement itself does not directly contain RDP. Though, this was observed when cattle were supplemented daily with DDG. Infrequent supplementation may not allow for enough nitrogen to recycled back to the rumen on day when supplement is not provided, leading to a deficient rumen N supply at the time of feeding. Thus, impacting the cellulolytic bacteria who are highly dependent on rumen nitrogen availability.

Conclusion

Backgrounding producers utilize supplements to increase growing calf performance. However, some supplements can cause negative associate effects on forage digestion and utilization, particularly supplements high in NSC. Dried distillers grains is a popular supplement for growing calves on a high forage diet as it is low in NSC due the

energy content being in the form of highly digestibility fiber and rumen undegradable protein. However, when DDGS is fed infrequently, there is a 10% reduction in ADG. This reduction in gain in not observed across all infrequent supplementation, particularly those supplements which provide a source of RDP. While MP in excess of requirements can contribute to the rumen nitrogen pool through nitrogen recycling, this mechanism may be asynchronous to the demands of microbial fermentation. Supplying a form of RDP, such as urea, when feeding a DDGS supplement infrequently could allow for nutrient synchrony and thus, improve forage utilization and subsequent animal performance.

Therefore, the objectives for this research were to evaluate the effects of amount of supplement, frequency of supplementation, and inclusion of urea to a dried distillers grains supplement on growing steer performance, hay dry matter intake, NDF digestibility, ruminal pH, ruminal VFA and NH_3 concentration.

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CHAPTER II: INTERACTION OF UREA WITH FREQUENCY AND AMOUNT OF DISTILLERS GRAINS SUPPLEMENTATION FOR GROWING STEERS ON A HIGH FORAGE DIET

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Abstract

Two studies were conducted to determine interactions of urea inclusion to a dried distillers grains plus solubles (DDGS) supplement fed at two amounts and two frequencies to steers on a high forage diet. In Exp. 1, 120 (247 kg; SD = 20) steers were fed individually for 84 d. Steers received ad libitum grass hay (6.8% CP) and 1 of 8 treatments. Treatment design was a 2 x 2 x 2 factorial. Supplement was fed either every day (D) or 3x/week (ALT), amount of supplement fed was 6.36 kg/week (LO) or 12.73 kg/week (HI), and contain either no urea (-U) or 1.3% urea (+U). Steer BW was collected at the start and end of the trial and hay DMI was measured weekly. In Exp. 2, 8 ruminally cannulated steers (310 kg; SD = 25) were used in an 8 x 6 row-column design with eight steers and six 14 d periods. Treatment design was the same as Exp. 1, except that supplement was fed at a rate of 0.4% of BW (LO) or 0.8% of BW (HI). Hay DMI was collected all 4 d of the collection period. Rumen fluid was collected 2, 4, 8, 12, 16, and 24 hr post-feeding. In situ bags were inserted at the time of feeding then removed 2, 4, 8, 12, and 24 hr post-feeding. Rumen pH was collected every 10 min via an intraruminal pH bolus. In Exp. 1, ADG was only affected by amount of supplement with steers on HI gaining 0.30 kg/d more ($P < 0.01$) than LO. Hay DMI was reduced by increased amount of supplement (0.39 kg/d; $P < 0.01$) and by decreased frequency of supplementation (0.54 kg/d; $P < 0.01$). In Exp. 2, hay DMI was also reduced due to increase amount of supplement and decreased frequency of supplementation ($P < 0.01$). Rumen pH was decreased on the day of feeding for steers on ALT ($P <0.01$) and reduced for steers fed HI vs. LO. Total VFA concentration did not differ among any treatments ($P > 0.05$). There was an interaction of urea x amount for rumen ammonia-N concentration ($P <$

0.01) but no effect of frequency ($P > 0.05$). A reduction in in situ NDF disappearance was observed on the day ALT received supplement between HI and LO ($P < 0.01$). However, there was no difference between NDF digestibility between D and ALT ($P > 0.05$). Infrequent supplementation of DDGS results in no difference in ADG but decreased hay DMI from daily supplementation. No effect was seen of urea, suggesting RDP was not deficient. Animal ADG was only improved when increase the rate of DDGS supplementation. There is little change in rumen fermentation parameters between frequency of supplement feeding, indicating that forage digestion is not impacted by supplementation frequency.

Key words: beef cattle, distillers grains plus solubles, supplementation frequency, urea

Introduction

Reducing supplementation frequency is a strategy to reduce labor costs in backgrounding cattle operations. In the state of Nebraska, a popular supplement for growing cattle consuming forage-based diets is dried distillers grains **(DDGS)**, due to its cost and nutrient content. Unlike traditional energy supplements that provide energy in the form of non-structural carbohydrates **(NSC)**, distillers grains provides energy in the form of highly digestible fiber and rumen undegradable protein **(RUP)**, which may reduce negative associative effects seen with NSC supplementation, such as decreased digestibility of the forage component of the diet (Caton and Dhuyvetter, 1997). However, reducing supplementation frequency of DDGS from daily to alternate day may reduce average daily gain by 10% (Loy et al., 2008; Stalker et al., 2009). However, not all infrequent supplementation strategies on forage-based diets has been reported to cause

decreases in animal performance. When Drewnoski et al. (2011), fed a supplement of corn gluten feed **(CGF)** and soyhulls **(SH)**, there was no reduction in growing steer performance from daily to 2x/week supplementation frequency. This supplement was similar to DDGS in terms of being highly digestible, but low in NSC; however, a key difference was that much of the crude protein **(CP)** content of CGF is rumen degradable protein **(RDP)**, thus being readily available to contribute to the rumen available nitrogen **(RAN)** pool and microbial needs.

In the case of DDGS, the protein content is high in RUP but low in RDP. For cattle consuming a low-quality forage (<7% CP), RDP is often the first limiting nutrient (McCollum and Horn, 1990). Inadequate amounts of RDP can impair rumen microbial fermentation as fibrolytic bacteria are most sensitive to RAN (Köster et al., 1996). With a large amount of protein bypassing the rumen before microbes can access the nitrogen, forage digestion may be reduced and animal performance may be negatively impacted since the digestion of the forage, which makes up the largest portion of their diet, is not maximized. However, the ruminant animal is efficient in salvaging nitrogen to balance a RAN deficiency, especially in times of low dietary protein intake (Wickersham et al., 2008). When protein is fed above the animal's metabolizable protein requirement, N can be cleaved in the liver and recycled to the rumen in the form of urea thus, contributing to the RAN supply. In backgrounding operations, DDGS is often fed in excess of MP requirements as excess protein can be used for energy. Stalker et al., (2007) determined that performance was not improved when a RDP source, urea, was added to the DDGS supplement fed to growing calves consuming meadow hay, and concluded feeding a DDGS supplement daily that provided excess MP, predicted by the 1996 NRC model,

would provide enough N through recycling to meet microbial demands. Therefore, it has been assumed there is not an RDP deficiency when growing calves receive DDGS supplement in excess of MP requirements. However, while the inclusion of urea to a DDGS supplement did not impact animal performance when fed daily, providing DDGS infrequently may not allow for recycled nitrogen to contribute to the RAN supply at the time of peak microbial fermentation. Consequentially, RDP may be deficient and forage digestibility reduced. Additionally, the amount or rate of supplementation could contribute to impacts of supplementation frequency on ruminal digestion. The RDP-to-TDN ratio of a diet has been identified as an important factor in forage digestion (Cochran et al., 1998). As the amount of TDN increases, microbial needs for N also increase. Therefore,inadeqauate RDP relative to TDN could result in reduced forage utilization (Bodine et al., 2001). Increasing the TDN of a diet through increased amount or rate of supplementation could further exacerbate the deficiency of recycled nitrogen during infrequent supplementation of DDGS.

Therefore, we hypothesized that the addition of urea to a DDGS supplement would immediately contribute to RAN if the animals' nitrogen recycling system could not match microbial demands due to an infrequent supplementation pattern. Supplying urea at the time of supplementation would reduce a potential RDP deficiency and thus, improve forage digestibility and subsequent animal performance. The objective of these studies were to determine the interaction of the inclusion of urea with a dried distillers grains supplement fed at either a low or high amount, and either daily or on alternative days.

Materials and Methods

All animal-use procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Experiment 1: Performance Trial

One hundred and twenty crossbred steers (247 kg; SD = 20) were fed one of eight treatment for 84 days to determine the effects of the inclusion of urea with the frequency and amount of distillers grains supplementation on growing steer performance. There were two turns, or replication, of 60 steers through the same barn, turn one was conducted November through February, and turn two was March through June. Animals were blocked by turn then stratified by body weight within turn, and randomly assigned to treatment. Treatments were arranged in a 2 x 2 x 2 factorial design, with factors including frequency of supplement feeding, amount of supplement, and addition of urea to supplement. There was a total of 15 animals per treatment. To try and balance the treatments across the whole experiment, if there were 7 animals assigned to treatment in turn one, then 8 animals were assigned to that treatment in turn two, and vice versa.

Steers were individually fed in a Calan gate system. All steers received ad libitum grass hay and free choice mineral blocks (American Stockman Big 6; Compass Minerals; Overland Park, KS) containing 96% NaCl; 2,400 ppm Mn; 2,400 ppm Fe; 260 ppm Cu; 320 ppm Zn; 70 ppm I; and 40 ppm Cu. Supplement was dried distillers grains with solubles **(DDGS)** with limestone and molasses. Supplement was fed either every day **(D)** or Monday, Wednesday, Friday **(ALT)**. Amount of supplement fed was 6.36 kg/week **(LO)** or 12.73 kg/week **(HI)**, split equally between feedings. Steers on the D LO and D

HI treatments received 0.91 kg/d and 1.82 kg/d, respectively. Steers on the ALT LO and ALT HI received 2.12 kg and 4.24 kg, respectively, on each Monday, Wednesday, and Friday. Supplement contained either no urea **(-U)** or 1.3% urea **(+U)**. To ensure total consumption of supplement and ad libitum hay intake, hay was not fed until 5 hours post-supplement feeding. Supplement was fed at 0600 h.

To adjust for gut fill, steers were fed a common diet of 50% Sweet Bran (Cargill Corn Milling; Blair, NE) and 50% alfalfa hay at 2% of BW for five d at the beginning and end of the trial (Watson et al., 2013). Weights were recorded for the last three consecutive days of the limit-feeding period using a hydraulic squeeze chute with mounted load cells (Silencer, Moly Manufacturing Inc.; Lorraine, KS: scale readability ± 0.90 kg). On the last day of the starting limited-feeding period, steers were implanted with 36 mg zeranol (Ralgro; Merck Animal Health; Madison, NJ). Amount of hay offered was recorded daily and refusals were collected weekly. Weekly orts were dried with forced air at 60ºC for 48 h to measure dry matter.

Data were analyzed using the MIXED Procedure of SAS (SAS Inst. Inc., Cary, NC). Four animals were removed from the analysis, 2 due to death, 1 due to chronic illness, and the other was an uncastrated bull. Animal served as the experimental unit. The model was first analyzed with an interaction of turn and treatment. However, this interaction was not significant. Therefore, the interaction of turn and treatments were removed from the model and only the main effects of treatment were analyzed. The model effects also included amount of supplementation, frequency of supplementation,

inclusion of urea, and all factorial interactions. There were no significant ($P < 0.05$) factorial interactions so only the main effects are reported.

Experiment 2: Digestion Trial

Eight ruminally cannulated crossbred steers (310 kg; SD = 25) were used in a 8 x 6 row-column design with 8 steers and 6 periods to determine effects of inclusion of urea with the frequency and amount of distillers grain supplementation on rumen digestion parameters. Treatment design was a 2 x 2 x 2 factorial, with factors including amount of supplementation, frequency of supplementation, and inclusion of urea. Steers received supplement at 2.8% **(LO)** or 5.6% **(HI)** of BW per week. Supplement amount was split into feedings, either every day **(D)** or every other day **(ALT)**. For reference, the steers on D LO received 0.4% of BW/d and the steers on the D HI received 0.8% of BW/d of supplement. Urea was included at 0% **(-U)** or 1.3% **(+U)** of the supplement's dry matter.

Steers were housed in individual pens (2.45 x 1.85 m) with feed bunks and water cups in a temperature-controlled room. Each pen had two separate feed bunks, one for supplement and one for hay. Mineral lick blocks, same as those utilized in Experiment 1, were also available in every pen. Supplement was fed at 0700 h immediately followed by hay. Supplement was the same as in Experiment 1. Brome grass hay (11.5% CP), chopped to a particle length of 8 cm, was fed to attain ad libitum intake. To ensure hay intake was not limited, hay orts were removed and weighed daily. Adjustments to the amount of hay offered were made depending on refusal amount. Periods were 14 d, with 7 d for adaptation and 7 d for collections. Steers on the ALT treatment received supplement for a total of 7 d during the period (d 2, 4, 6, 8, 10, 12, 14).

Hay orts during the collection period were subsampled and dried in a forced air oven at 60ºC for 48 h to measure dry matter intake **(DMI)**. All animals consumed all supplement offered within 6 h so no supplement orts were collected. The same hay that was fed during the trial was also utilized for in situ incubations. Hay was ground through a 2mm screen using a Wiley mill (No. 4, Thomas Scientific, Swedesboro, New Jersey) and 1.25 g was placed in 5 x 10 cm, 50 µm pore size in situ bags (Ankom Technologies; Macedon, NY). Three in situ bags per time point were placed in a mesh laundry bag with a weight. Bags were inserted in the rumen through cannula at 0700 h then incubated for 4, 8, 12, 24 and 96 h. To determine if there were potential differences in rumen fermentation between days steers received supplement and days they did not, animals on the ALT treatment had two sets of in situ incubations; one on the day of feeding (d 10, 11), and a second on the subsequent non-supplemented day (d 11, 12). However, only one 96 h in situ incubation was conducted, removed on d 14. Animals on the D treatment had one set of in situ incubations, the same day the ALT animals had their supplemented day collections (d 10, 11). Following all incubation, bags were washed in a standard washing machine with 5, 1-minute agitation, 1-minute spin cycles. To account for washout, 3 unincubated in situ bags were included. Bags were then rinsed with distilled water and frozen. Thawed bags were placed into the Ankom Fiber Analyzer (A2000; Ankom Technologies, Macedon, NY) to determine NDF content.

Rumen fluid was collected at 2, 4, 8, 12, 16, and 24 h post-feeding to analyze rumen ammonia-N and VFA concentration. Like with in situ incubations, animals on the ALT treatment had two sets of collections, one on supplemented day (d 12) and not supplemented (d 13). Daily animals had rumen fluid collected on d 12. Approximately

100 mL of rumen fluid was collected via a vacuum hand pump into two separate 50 mL conical tubes then frozen until analysis.

For VFA analysis, samples were thawed and prepared in duplicate according to Erwin et al. (1961). Crotonic acid (Catalog # 107- 93-7, Sigma Aldrich, St. Louis, MO) was used as the internal standard for all samples. A Trace 1300 (Thermo Fisher Scientific, Inc., Omaha, NE) gas chromatograph fitted with a Zebron capillary column (Phenomenex, Torance, CA, Catalog # 7HM-G009-22,) was used. The column was 30 meters in length with an inside diameter of 0.32 mm, and a film thickness of 1 µm. Run time was 9.75 minutes; and inlet and flame ionization detector temperatures were held constant at 280°C. Oven temperature started at 160º C and increased at a rate of 8ºC per minute until it reached 200º. Column carrier flow was set at 2.4 mL/min and helium (Catalog #SGSPPULW800P, Matheson Tri-Gas, Lincoln NE) was used as the carrier gas. Flow rates of compressed air (Catalog # SGSPPULW700, Matheson Tri-Gas, Lincoln NE) and hydrogen (Catalog # SGSPPULW500P, Matheson Tri-Gas, Lincoln NE) were set at 350 mL/min and 30 mL/min, respectively.

Ruminal ammonia-N concentration was determined using the alkaline hypochlorite phenol coloromet ic procedure (Broderick and Kang, 1980) using a Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA). Samples were prepared in duplicate.

Rumen pH was measured using intraruminal pH probes (smaXtec Classic Bolus; Graz, Austria). Probes were first calibrated then inserted through the rumen cannula, into the reticulum, prior to the start of the trial and remained through the duration of the trial,

a total of 84 d. Readings were collected every 10 minutes. Recorded data was transmitted wirelessly in real-time to a smaXtec base station, then transmitted to smaXtec software on a PC.

For the digestion trial to best understand the impacts of frequency, two different data sets were analyzed. One set compared D to ALT, in which values for each measurement for ALT treatments were averaged across all collection days. The other set compared alternate fed **(ALT-F)** to alternate not fed **(ALT-NF)**, in which only the ALT treatments were analyzed but values were averaged for the collection days steers received supplement, and the collection days they did not.

The model for the D vs ALT data set included amount of supplementation, frequency of supplementation, inclusion of urea, and all factorial interactions. The ALT-F vs ALT-NF model included amount of supplementation, feeding of supplementation, inclusion of urea, and all factorial interactions. Time post feeding was also included in both models for those variables analyzed as repeated measures. Interactions that were not significant ($P < 0.05$) were removed from the models. Rumen ammonia-N, VFA, and pH data were analyzed using repeated measures over time. Covariance structure was based on lowest Akaike's Information Criteria. Rumen ammonia-N and pH both had unstructured compound symmetry covariance structure, and pH had AR(1) covariance structure. For DMI and in situ NDF disappearance rate, data were analyzed using the MIXED Procedure of SAS (SAS Inst. Inc., Cary, NC). To determine the in situ degradation ratio, the NCIN Procedure of SAS with the Marquardt degradation model was used.

Results

Experiment 1: Performance Trial

Final body weight did not differ between D and ALT treatments, nor +U and -U ($P > 0.56$; Table 2.3). However, final BW was greater for HI compared to LO steers, 319 kg and 293 kg, respectively ($P < 0.01$) Average daily gain was 0.30 kg/d greater for steers receiving a HI amount of supplement than LO, ($P < 0.01$; Table 3). Frequency and Urea Inclusion had no effects on steer ADG ($P > 0.86$). Hay dry matter intake was reduced by 0.39 kg/d for the steers on the HI treatment compared to the LO ($P < 0.01$; Table 2.3). Additionally, frequency of supplementation reduced hay dry matter intake. Steers receiving ALT supplementation consumed 5.52 kg/d of hay while D steers consumed 6.06 kg/d ($P < 0.01$). Urea inclusion had no effect on hay DMI ($P = 0.25$)

Experiment 2: Digestion Trial

Hay Intake Like the performance trial, both amount and frequency of supplementation impacted hay DMI ($P < 0.01$) of steers during the digestion trial (Table 2.4). High amount of supplement reduced hay DMI by 0.99 kg/d compared to LO, and ALT reduced hay DMI by 0.47 kg/d compared to D. Urea inclusion had no significant effect on hay DMI ($P = 0.21$)

In Situ NDF Disappearance There were no significant three-way interactions for neither D vs ALT treatments nor ALT vs ALT treatments. There were also no significant differences in the washout fraction, nor the potentially digestible fraction in either data set. For the D vs ALT comparison, there was an interaction of frequency x amount ($P = 0.05$) for rate of NDF disappearance. D LO had a faster rate of NDF disappearance

(5.22%/h) than D HI, ALT HI, and ALT LO (4.19%/h, 4.19%/h and 4.20%/h, respectively; Table 2.5). There were no other significant treatment effects for the washout fraction, potentially digestible fraction, or rate of NDF disappearance in the D vs ALT data set. For the ALT-F vs ALT-NF comparison, there was an interaction of feeding x amount ($P < 0.01$). Rate of NDF disappearance was greater for ALT-F LO and ALT-NF HIGH than ALT-F HI and ALT-NF LO ($P < 0.01$; Table 2.6). No other interactions or treatment effects were observed for washout fraction, potentially digestible fraction, or rate of NDF disappearance for the ALT-F vs ALT-NF data set.

Ruminal Ammonia-N Concentration In the D vs ALT data set, there was a significant interaction of amount x urea ($P < 0.01$). HI +U had the greatest average ruminal ammonia concentration, 8.05 mg/dL while HI -U and LO +U had an average of 5.00 mg/DL, and LO -U had the lowest, 3.60 mg/dL (Table 2.7). There was also a significant amount x urea x time interaction ($P < 0.01$). For all treatments, ruminal ammonia-N concentration was greatest 2 h post-feeding and decreased from 4 h post-feeding to 16 h post-feeding. Ammonia-N concentrations reached their lowest at 16 h post feeding for all treatments. None of these treatments reached a ruminal ammonia-N concentration below 2 mg/dL. Concentrations were then increased at 24 h post-feeding for all treatments. In the ALT-F vs ALT-NF data set, there was a significant interaction of feeding x amount x urea ($P < 0.01$). Steers on the HIGH +U treatment on the day they were fed, had the greatest ruminal ammonia-N concentration. Regardless of amount or whatever they received supplement that day, steers on the -U treatments had the lowest ruminal ammonia-N concentration (Table 2.8).

Ruminal VFA Concentration For the D vs ALT comparison, there were no significant three-way interactions. For both acetate and butyrate, the main effects of frequency and amount were significant ($P \leq 0.02$). However, only the main effect of amount was significant for propionate ($P < 0.01$). Alternate day supplementation animals had greater concentration of acetate compared to D, but lesser concentrates of butyrate (Table 2.9). Steers supplemented a HI amount of supplement had increased concentrations of propionate and butyrate, but decreased concentration of acetate compared to the LO supplemented steers. This resulted in HI steers having a lower A:P ratio than LO steers ($P < 0.01$). A frequency x urea interaction was significant for the A:P ratio ($P < 0.05$; Table 2.9). In the ALT-F vs ALT-NF data set, a feeding x amount interaction ($P < 0.01$) and feeding x urea interaction ($P < 0.05$) were observed (Table 2.10). Acetate and propionate concentration were affected by both feeding and amount. On the day not supplemented, steers had increased concentration of acetate, but decreased concentration of propionate and butyrate ($P < 0.01$). However, on the day steers were supplemented, concentrations of propionate and butyrate increase, but acetate concentration decreased ($P < 0.01$). HI steers also had greater concentration of propionate compared to the LO steers, but lesser concentration of acetate ($P < 0.01$).

Rumen pH In the DAILY vs ALT data set, there was an interaction of supplement amount x time on rumen pH ($P < 0.01$). Steers receiving a HIGH amount had a greater drop in their rumen pH post-feeding than steer receiving a LOW amount (Figure 2.1). For the ALT-F vs ALT-NF comparison, there was a significant interaction of feeding x amount x time. On the day they were supplemented, steers had a lower pH than on the day they were not supplemented. Additionally, while on supplemented days, steers

that received a HI amount had a greater drop in their ruminal pH than steers that received a LO amount. However, on non-supplemented days, there was no significant differences between pH of LO and HI fed steers (Figure 2.2).

Discussion

Growing steer ADG was only impacted by increasing the rate of DDGS supplement. This result was expected as other supplementation studies have reported increases in gain with increasing rate or amount of DDGS supplementation (Loy et al., 2008). An increased rate of supplementation also led to a reduction in hay DMI. Again, this effect has been observed as there is a substitution of forage intake with increasing supplement intake. Horn and McCollum (1987) observed a forage replacement effect when the supplementation rate was greater than 0.50% of BW daily. Furthermore, Loy et al., (2008) reported a 0.78 kg/d difference in hay intake between steers supplemented with DDGS at 0.21% or 0.81% of BW. In the current study, the steers on the D LO treatment received approximately 0.4% of their BW while steers on D HI received 0.8% of their BW. Supplementation frequency also reduced hay DMI, despite receiving the same amount of supplementation weekly, ALT steers did not consume as much hay as those on the DAILY treatment. This effect was also observed by Loy et al., (2007, 2008) and Drewnoski et al., (2011, 2012). Forage intake in ruminants can be influenced by two factors, physical or feed factors, or physiological or animal factors (Van Soest, 1994). In the case of physical factors, fill of the rumen limits intake while in physiological factors, metabolic feedback is the limiter. Since forage intake is correlated with NDF content of the forage, decreasing the NDF digestibility in the rumen could lead to decrease intake.

However, in situ NDF disappearance was not impacted solely by frequency in the digestion trial. Metabolic feedback may vary from day-to-day in infrequently supplemented animals depending on fed status. Receiving supplement the day prior may result in the animal receive feedback of an elevated nutritional status, and thus, limit their intake of forage that day. Drewnoski and Poore, (2012) did report an increase in ruminal VFA concentration on the day steers on the alternative supplementation treatments were fed, suggesting chemostatic feedback was a regulator of intake in the infrequent supplemented animals. However, in this study, there was no difference in total VFA concentration for ALT-F vs ALT-NF. While there was a decrease in the A:P ratio for ALT-F compared to ALT-NF, this result would be expected as on the day fed supplement steers consumed less forage. There was also no difference in the propionate concentration or A:P ratio for D vs ALT steers as one would expect if metabolic feedback were the main limiter in intake for animals on an infrequent supplementation pattern. The VFA data in Exp. 2 offers little explanation as to why the ALT steers consumed less total DMI than D but gained the same in Exp. 1.

Rumen pH is often cited as a factor that impairs fiber digestion as fibrolytic bacteria are most sensitive to a pH below 6.2 (Grant and Mertens, 1992; Russell and Wilson, 1996). Some supplements when fed infrequent have been observed to reduce rumen pH below 6.2 but these supplements were high in NSC and often did not meet RDP requirements (Caton and Dhuyvetter, 1997). However, since DDGS contains little NSC, no treatment reduced rumen pH below 6.2. Additionally, frequency of supplementation had no significant impact on ruminal pH. While there was an interaction between feeding x amount for ALT animals, the average of the ALT-F and ALT-NF days

was equal to that of the D treatments. Though the pH for the ALT-F HI animals never dropped below 6.2, there was a reduction in in situ NDF digestibility for this treatment compared to the ALT-F LO. It is possible the time near a pH of 6.2 may have reduced in situ NDF digestibility, but it was not enough to impact on animal performance.

Unlike previous studies, infrequent supplementation with DDGS did not reduce steer ADG. Loy et al. (2008) observed a 10% reduction in ADG when DDG was fed 3x/weekly compared to daily. Likewise, Stalker et al. (2009) reported a 10% decreased in ADG from 6x/weekly DDGS supplementation to 3x/weekly. The results of the current study agree with those of Drewnoski et al. (2011), which reported no difference in ADG of steers supplemented 7x, 3x, or 2x/weekly with a CGF and SH blend. However, the differences in the supplement types may have resulted in the difference in animal performance. The supplement in both Loy et al. (2008) and Stalker et al. (2009) contain a greater amount of fat, but less RDP than the supplement utilized by Drewnoski et al. (2011) and the current experiments. Both dietary fat and RDP can impact rumen fibrolytic bacteria, reducing forage digestion and thus, animal performance.

However, inclusion of urea, a RDP source, had no impact on animal performance or hay intake. Satter and Slyter (1974) reported that fibrolytic bacteria growth is inhibited when rumen ammonia concentration was below 2 mg/dL. While in the digestion trial there was a significant amount x urea x time interaction, none of the treatments had rumen ammonia-N concentrations drop below 2 mg/dL. Additionally, urea had no significant effect on in situ NDF disappearance, also suggesting that the RAN pool was not limiting for fiber digestion. Our hypothesis that infrequent supplementation resulted

in asynchrony between rumen available energy and rumen available nitrogen, leading to a reduction in forage digestibility was not support by data in either the performance or digestion trial.

One other key difference between the DDGS supplement fed in Loy et al., (2007, 2008) and Stalker et al., (2009), and these studies was the fat content. Distillers grains processing methods have changed since the early 2000's with processing plants extracting more of the fat. Currently, fat is centrifuged from the solubles stream. The fat removed through this process is more reactive in the rumen then the remaining fat in DDGS, which is held in the corn germ and likely bypasses. Since this centrifugation process was not commonly used during the time of Loy et al. (2007, 2008) and Stalker et al. (2009), the fat in the DDGS supplement in these studies was more reactive in the rumen environment. The ether extract **(EE)** content of DDGS in the previous studies was approximately 10-11% whereas the EE of the DDGS utilized in these studies was ~5%. The supplement in Drewnoski et al. (2011, 2012) also contained little EE, the value was not reported for the supplement itself but CGF has ~3.5 % EE and SH ~2.2% EE (NRC, 2016). Fat can reduce fiber digestion in the rumen as it is toxic to fibrolytic bacteria and can reduce the time for bacterial attachment to forage particles (Jenkins, 1993). In Loy et al., (2008) the total fat content of the diet was 5.2% and in Stalker et al. (2009) feeding their supplement 3x/weekly resulted in an additional 5.4% of fat to the diet. Feeding supplement less frequently would require a larger amount to be fed per feeding, resulting in a greater percentage of the diet as fat. It is recommended dietary fat content does not exceed 5% in forage-based diets, but in both Loy et al. (2008) and Stalker et al. (2009), dietary fat content exceeded that for animals receiving infrequent supplementation.

Again, this could have had negative effects on fiber digestion in the rumen, reducing forage utilization and subsequent animal performance. Interestingly though, a digestion study done by Loy et al. (2007) with DDGS containing 9.67% EE saw no reduction in in situ NDF disappearance rate. Likewise, there was no significant difference between D and ALT in situ NDF disappearance rate in the current digestion trial. However, Stalker et al. (2009) observed a linear decrease in total tract NDF digestibility as supplementation frequency decreased. Drewnoski and Poore (2012) also used total tract digestibility but did not see a difference in the potentially digestible NDF digestibility between frequency of supplementation. If an impact of fat on NDF digestibility could also be post-ruminal, this would suggest why a difference was observed between Drewnoski and Poore (2012) and Stalker et al. (2009), but not Loy et al. (2008) and these studies. Furthermore, none of the previously mentioned studies with DDGS measured passage rate. Drewnoski and Poore, (2012) did measure total tract passage rate through a rare earth marker tagged to the SH in the supplement, but did not report a difference in total tract passage rate between frequent and infrequently supplemented animals. However, this was also total tract passage rate, not just rumen passage rate. Rumen digestibility is impacted by both digestibility rate and passage rate. These factors have an inverse relationship, as increasing passage rate will decrease digestibility rate. Conversely, decreasing ruminal passage rate allows for an increase in digestibility rate but can reduce animal performance. Increasing the amount of supplementation would increase rumen passage rate but decrease digestibility rate. However, without measuring rumen passage rate, it is difficult to determine if supplementation frequency impacts rumen digestibility by altering passage rate

Implications

The results of these studies suggest that a DDGS supplement with a lower fat content can be fed infrequently to growing steers on a high forage diet with no reduction in performance. Additionally, decreasing supplementation frequency can reduce hay dry matter intake. Including urea had no impact on animal performance or hay DMI nor any ruminal digestion parameters, suggesting there is no deficiency in the RAN pool leading to a reduction in forage utilization.

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Table 2.1. Composition of supplements fed to steers in both the performance and digestion trial

	Composition, % of DM	
Ingredient	DDGS	DDGS+U
Dried distillers grains plus solubles	95.25	93.95
Molasses	2.50	2.50
Limestone	2.25	2.25
Urea	-	1.30

Table 2.2. Feedstuff nutrient content in both the performance and digestion trial

Item	DDGS	DDGS+U	Grass hay, performance trial	Grass hay, digestion trial
DM,%	92.1	92.3	92.9	89.9
OM, % DM	92.7	93.0	93.0	91.0
NDF, % DM	-	-	68.9	64.1
CP, % DM	29.4	31.1	6.8	11.5
Ether Extract, % DM	5.48	5.51	-	-

Table 2.3. Performance of steers fed steers fed distillers grains supplement either daily (D) or alternate days (ALT), at a high (HI) or low (LO) amount, and with (+U) or without (-U) the inclusion of urea

	Treatment									
	Freq[1]		Amt[2]		Urea[3]			*P*-value		
	D	ALT	LO	HI	-U	+U	SEM	Freq	Amt	Urea
Initial BW, kg	247	247	247	247	247	247	1.80	0.86	0.72	0.87
Final BW, kg	307	305	293	319	306	306	2.30	0.56	<0.01	0.99
ADG, kg/d	0.72	0.69	0.85	0.55	0.70	0.70	0.01	0.20	<0.01	0.82
Hay DMI, kg/d	6.06	5.52	5.99	5.60	5.89	5.70	0.12	<0.01	<0.01	0.25

[1] D = daily, ALT = every other day
[2] LO = 0.4% of body weight, HI = 0.8% of body weight
[3] +U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

Table 2.4. Hay intake of steers fed distillers grains supplement either daily (D) or alternate days (ALT), at a high (HI) or low (LO) amount, and with (+U) or without (-U) the inclusion of urea during digestion trial

	Treatment									
	Freq[1]		Amt[2]		Urea[3]			*P*-value		
	D	ALT	LO	HI	-U	+U	SEM	Freq	Amt	Urea
Hay DMI, kg/d	6.34	5.87	6.60	5.61	5.98	6.22	0.58	<0.01	<0.01	0.21

[1] D = daily, ALT = every other day
[2] LO = 0.4% of body weight, HI = 0.8% of body weight
[3] +U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

Table 2.5. In Situ NDF Disappearance for steers fed distillers grains supplement either daily (D) or alternate days (ALT), and at a high (HI) or low (LO) amount

	Treatment					P-Value		
	D		ALT					
	HI	LO	HI	LO	SEM	Freq[1]	Amt[2]	Interaction
Washout Fraction	0.25	-0.05	-0.08	0.12	0.12	0.82	0.90	0.51
Potentially Digestible Fraction, %	49.6	51.5	49.1	50.2	0.90	0.12	0.36	0.66
Rate, %/h	4.19[b]	5.22[a]	4.19[b]	4.23[b]	0.24	0.06	0.03	0.05

[a,b] Within a row, common superscripts indicate no significant difference between means, P > 0.05

[1] D = daily, ALT = every other day

[2] LO = 0.4% of body weight, HI = 0.8% of body weight

Table 2.6. In Situ NDF Disappearance for steers fed distillers grains supplement on alternative days comparing day fed (ALT-F) to day not fed (ALT-NF), and at a high (HI) or low amount (LO)

	Treatment					P-Value		
	ALT-F		ALT-NF					
	HI	LO	HI	LO	SEM	Day Fed[1]	Amt[2]	Interaction
Washout Fraction	-0.5	-0.2	0.4	0.5	0.7	0.44	0.63	0.91
Potentially Digestible Fraction, %	51.2	49.4	51.8	51.0	1.2	0.31	0.34	0.62
Rate of NDF Digestibility, %/h	3.76[b]	4.72[a]	4.63[b]	3.75[b]	0.43	0.89	0.92	<0.01

[a,b] Within a row, common superscripts indicate no significant difference between means, P > 0.05

[1] ALT-F = fed, ALT-NF = not fed

[2] LO = 0.4% of body weight, HI = 0.8% of body weight

Table 2.7. Ruminal Ammonia-N concentration for steers fed distillers grains supplement either daily (D) or alternate days (ALT), at a high (HI) or low (LO) amount, and with (+U) or without (-U) the inclusion of urea

	Treatment					P-Value		
	HI		LO					
	+U	-U	+U	-U	SEM	Amt[1]	Urea[2]	Interaction
Ammonia-N, mg/dL	8.05[a]	5.00[b]	5.01[b]	3.60[c]	0.325	<0.01	<0.01	<0.01

[a,b] Within a row, common superscripts indicate no significant difference between means, P > 0.05

By time interaction (*P* < 0.01*)*, data not shown

[1] LO = 0.4% of body weight, HI = 0.8% of body weight

[2]+U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

Table 2.8. Ruminal Ammonia-N concentration for steers fed distillers grains supplement on alternative days comparing day fed (ALT-F) to day not fed (ALT-NF), at a high (HI) or low amount (LO), and with (+U) or without (-U) the inclusion of urea

	Treatment									P-Value			
	ALT-F				ALT-NF								
	HI		LO		HI		LO						
	+U	-U	+U	-U	+U	-U	+U	-U	SEM	Day Fed	Amt	Urea	Interaction
Ammonia-N, mg/dL	10.56[a]	4.89[c]	5.63[b]	3.58[c]	5.13[b,c]	4.49[b,c]	4.17[c]	3.78[c]	0.489	<0.01	<0.01	<0.01	<0.01

[a,b] Within a row, common superscripts indicate no significant difference between means, P > 0.05

[1] ALT-F = fed, ALT-NF = not fed

[2] LO = 0.4% of body weight, HI = 0.8% of body weight

[3] +U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

Table 2.9. Ruminal VFA concentration for steers fed distillers grains supplement either daily (D) or alternate days (ALT), at a high (HI) or low (LO) amount, and with (+U) or without (-U) the inclusion of urea

	Treatment									P-Value			
	D				ALT								
	HI		LO		HI		LO						3-way
	+U	-U	+U	-U	+U	-U	+U	-U	SEM	Freq[1]	Amt[2]	Urea[3]	Interaction
Acetate, %	64.2	64.7	65.7	66.9	67.5	65.3	69.2	68.1	0.09	<0.01	<0.01	0.52	0.89
Butyrate, %	11.1	11.0	9.73	10.0	8.98	9.87	8.80	9.34	0.04	<0.01	0.02	0.46	0.94
Propionate, %	22.4	21.2	21.2	20.1	21.4	21.8	20.2	20.1	0.05	0.28	<0.01	0.22	0.58
A:P ratio[1]	2.94	3.12	3.19	3.37	3.24	3.07	3.51	3.47	0.10	0.02	<0.01	0.64	0.65

Freq x Urea interaction ($P < 0.05$). Urea did not affect A:P for alt, but tended to reduce A:P for alt $P < 0.08$

[1] D = daily, ALT = every other day

[2] LO = 0.4% of body weight, HI = 0.8% of body weight

[3] +U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

Table 2.10. Ruminal VFA concentration for steers fed distillers grains supplement on alternative days comparing day fed (ALT-F) to day not fed (ALT-NF), at a high (HI) or low amount (LO), and with (+U) or without (-U) the inclusion of urea

	Treatment									P-Value			
	ALT-F				ALT-NF								
	HI		LO		HI		LO						3-way
	+U	-U	+U	-U	+U	-U	+U	-U	SEM	Freq[1]	Amt[2]	Urea[3]	Interaction
Acetate, %	65.1	62.4	67.1	65.1	70.0	68.1	71.0	71.0	0.08	<0.01	<0.01	0.02	0.59
Butyrate, %	10.3	11.7	9.91	10.6	7.62	7.91	8.21	7.71	0.03	<0.01	0.26	<0.01	0.07
Propionate, %	23.2	23.1	21.4	22.1	19.5	20.0	19.0	18.4	0.05	<0.01	<0.01	0.68	0.03
A:P ratio	2.88	2.73	3.18	3.08	3.60	3.46	3.84	3.90	0.10	<0.01	<0.01	0.32	0.41

Freq x Amt interaction (P <0.01). Butyrate concentrations were not affected by amount of supplement on days when supplement was not fed (P > 0.47), but HI supplement resulted in greater butyrate concentration than LO on days when supplement was fed (P < 0.01).

Freq x Urea interaction (P <0.05). Butyrate concentrations were not affected by urea on days when supplement was not fed (P > 0.14), but urea decreased butyrate concentration on the day supplement was fed (P < 0.01).

[1] ALT-F = fed, ALT-NF = not fed

[2] LO = 0.4% of body weight, HI = 0.8% of body weight

[3] +U = inclusion of urea at 1.3% of supplement DM, -U = no inclusion of urea

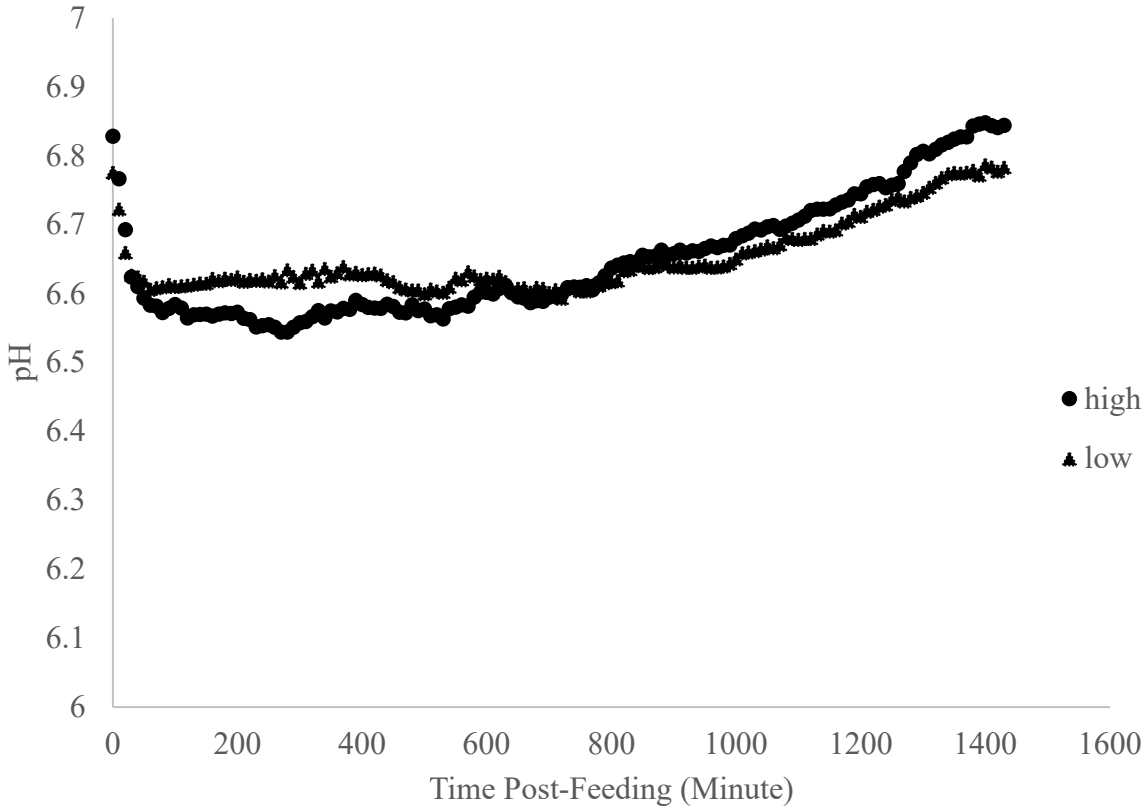

Figure 2.1. The ruminal fluid pH of steers fed distillers grains supplement at a high (0.8% of BW) or low (0.4% of BW) amount. Amount x time effect ($P < 0.01$, SEM = 0.0062)

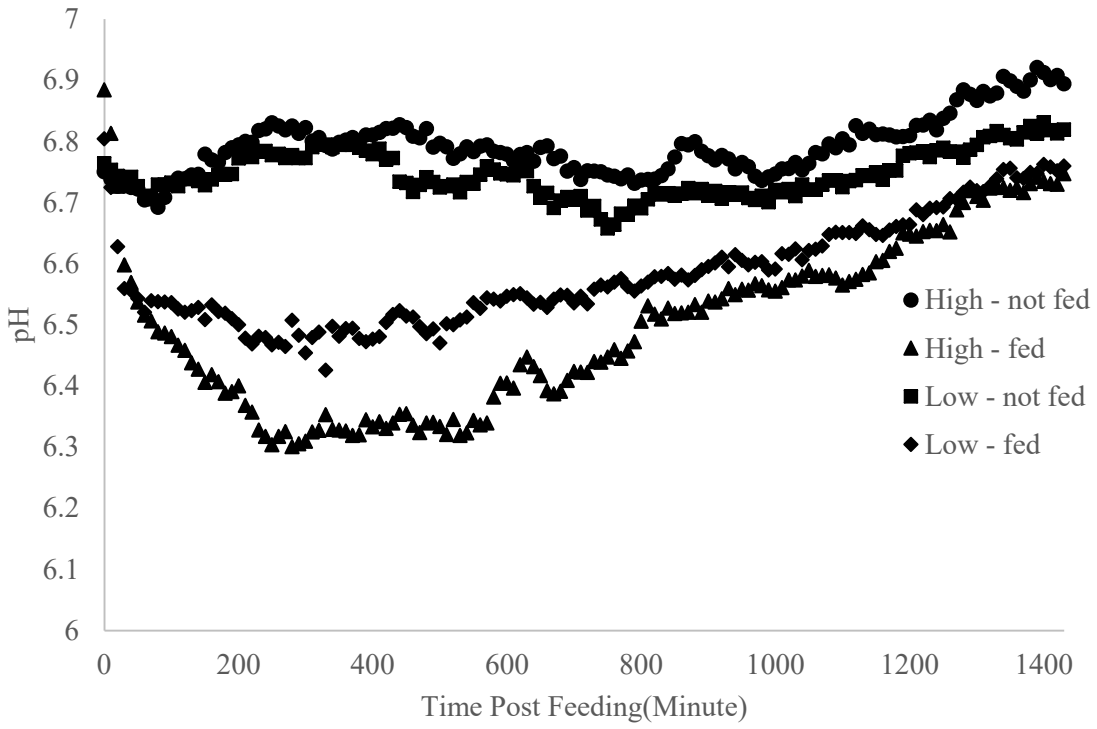

Figure 2.2. The ruminal fluid pH of steers fed distillers grains supplement on alternate days, comparing day fed to day not fed, at a high (0.8% of BW) or low (0.4% of BW) amount. Amount x time effect ($P < 0.01$, SEM = 0.0062). Feeding x amount x minute effect ($P < 0.01$, SEM = 0.07).