

## **ABSTRACT**

DREWNOSKI, MARY ELIZABETH. Understanding the Effect of Reduced Supplementation Frequency on Performance, Digestion and Metabolism of Stocker Cattle. (Under the direction of Matthew H. Poore).

Supplements are often fed to stocker cattle on forage-based diets to improve animal performance. Delivery costs can make up a substantial portion of the cost of supplementation, particularly for small producers and those that travel long distances to feed cattle. Reducing supplementation frequency can reduce labor and equipment operation costs and therefore has the potential to increase profit. However, less frequent feeding requires feeding larger quantities of supplement at once and can increase the likelihood of negative associative effects of supplementation on digestion and forage intake and can therefore decrease gains. Additionally, little is understood about the metabolic response of ruminants to large fluctuation in nutrient intake. Therefore, even if microbial digestion is not affected by less frequent supplementation, performance may still be altered. A 50:50 blend of soyhulls and corn gluten feed is widely used by producers to supplement growing cattle. This blend is high in energy but low in non-structural carbohydrates. It also contains a moderate amount of protein, much of which is ruminally degradable. Therefore, reducing the frequency of supplementation of a 50:50 blend of soyhulls and corn gluten feed may not cause negative effects on fiber digestion. The purpose of this research was to determine the effect of reducing supplementation frequency of a soybean hull and corn gluten feed blend on performance, digestion, and concentrations of metabolites and hormonal growth regulators in blood of growing cattle. In Experiment 1, growing steers consuming medium quality tall fescue hay were supplemented either daily (~1% BW), 3 times a week (~2.3% BW), or 2

times a week (~3.6% BW). Hay intake was decreased by reducing supplementation frequency but gains were not affected. As a result, the feed to gain ratio increased slightly with less frequent supplementation. In Experiment 2, six ruminally cannulated beef steers consuming medium quality fescue hay were used in a replicated 3 x 3 Latin square design to determine the effect of supplement frequency (daily at 1% BW or on alternate days at 2% BW) on digestion and ruminal parameters. Reducing supplementation frequency decreased hay intake but did not affect digestibility of the diet. On the day of supplementation molar proportions both of propionate and butyrate in the rumen of steers supplemented on alternate days was increased compared to those supplemented daily. In Experiment 3, growing steers were individually fed medium quality hay and supplemented daily (1% BW) or on alternate days (2% BW). Gains did not differ due to supplementation frequency. However, plasma IGF-1 was greater and insulin tended to be greater in steers supplemented less frequently. The effect of less frequent supplementation on insulin and IGF-1 deserves further examination as it may explain why the steers supplemented less frequently with a soybean hull and corn gluten feed blend appear to be more efficient. When supplementing medium quality hay with a blend of soybean hulls and corn gluten feed, producers can reduce supplementation costs by decreasing supplementation frequency to as little as 2 times a week without negatively affecting gains. Future research should focus on determining the main factors that influence the performance responses to less frequent supplementation so that the response can be predicted when utilizing different supplement and forage combinations.

Understanding the Effect of Reduced Supplementation Frequency on Performance, Digestion  
and Metabolism of Stocker Cattle

by  
Mary Elizabeth Drewnoski

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APPROVED BY:

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Geoff Benson

---

Vivek Fellner

---

Gerald Huntington  
Co-Chair of Advisory Committee

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Matthew Poore  
Co-Chair of Advisory Committee

## **BIOGRAPHY**

Mary Drewnoski grew up in a rural community in mountains of western North Carolina. After high school she moved to Kentucky and earned her B.S. Degree in Agriculture and Natural Resources from Berea College. While at Berea, Mary was employed at the college farms, working with a variety of animals such as goats, sheep, hogs, and beef cattle as well as growing and harvesting an assortment of crops, both forage and grain. Her focus while at Berea, however, was managing the beef operation. As a manager she learned that forage production and grazing management can have dramatic effects on profitability for beef producers. After completing her Bachelors, she decided to come to North Carolina State University to pursue a M.S. in Animal Science under guidance of Dr. Matthew Poore. Her research focused on the effects of endophyte status in tall fescue on agronomic and animal performance when stockpiled and strip-grazed. For her Ph.D., she has continued to work under Dr. Poore and is researching cost effective methods of supplementing stocker cattle on forage based diets.

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## INTRODUCTION

Stocker calves are weaned calves that are generally developed on forage-based diets until being placed in a feedlot. Supplements are often fed to stocker cattle to improve animal performance (rate and/or efficiency of weight gain) with the ultimate goal of increasing economic return. Many factors must be considered when determining the optimal supplementation program. Although the cost of the supplement itself is often weighed against the value of the added gain, the cost of the labor and equipment it takes to feed the supplement is sometimes overlooked. With the rising cost of fuel, the cost of the act of feeding a supplement is becoming more and more expensive and should be considered.

Supplement delivery methods can be classified into two main categories: self-fed or hand-fed. Hand-feeding implies that the supplement is regularly fed (often daily) to the animals in a form and amount that is readily consumed. Self-fed supplements are made available in bulk amounts, with the expectation of continuous, low-level consumption. Intake may be limited by the supplement's physical form, intake limiting compounds (such as salt), or a combination of these methods (Knukle et al., 2000).

Using self-fed supplements can reduce labor costs, since delivery times are designed to be less frequent than with hand-feeding. However, growing cattle are often checked several times a week to assess the health of individuals and if cattle are checked at times other than feeding, this labor and associated costs should be taken into account when calculating the cost of a self-feeding program. The amount of supplement consumption

expected, feed bunk volume and the desire/need to keep the supplement fresh will determine how frequently self-fed supplements will need to be provided. If supplements are to be consumed at low amounts, then self-feeding may be very cost effective. However, if larger amounts of supplement intake are needed extra feeder capacity or more frequent delivery may be necessary and thus may increase the cost of delivery. Additionally, use of an intake limiter may increase the cost of the self-fed supplement and offset savings in labor and transportation.

Although hand-feeding requires more labor than self-feeding it has several advantages, particularly when stockering cattle. One advantage of hand feeding is that animals are often easier to gather and/or check. They are also more accustomed to people and therefore are less stressed when worked and may perform better in the feedlot. Additionally, sick individuals can often be detected by their unwillingness to approach the bunk to eat.

Reducing the frequency of hand supplementation from daily to several times a week can decrease the labor and transportation costs associated with the hand-feeding while still providing the previously mentioned benefits of hand feeding. When supplementation frequency is reduced the amount of supplement offered per week is maintained at the same level but the amount offered per supplementation event is increased. However, the effect of less frequent supplementation on digestion and performance must also be considered. The cost advantages of less frequent supplementation could be off-set if performance is negatively affected.

In many cases ruminant responses to supplements are either greater or lesser than expected due to associative effects of the supplement on forage intake and digestion. Protein

supplementation generally does not result in negative associative effects and less frequent protein supplementation has been shown to be as effective as daily supplementation (Krehbeil et al., 1998; Huston et al., 1999; Bohnert et al., 2002). Supplementation of large amounts of energy particularly when the supplement contains high levels of non-structural carbohydrates (NSC), such as starch, can cause negative associative effects (Kunkle et al., 2000). Additionally, feeding large amounts of energy based supplements can potentially result in asynchrony of available energy and nitrogen in the rumen thus making microbial growth and digestion less efficient.

However, response to less frequent supplementation of energy has varied. In general when the degradable intake protein to total digestible nutrient ratio (DIP:TDN) of the diet was high, less frequent supplementation of grain based mixes has not greatly reduced performance (La Manna, 2002; Loy et al., 2008). However, when low protein grain based mixes were fed infrequently to cattle consuming low quality forage performance was greatly reduced (Kartchner and Adams, 1982). Therefore, it appears that response to less frequent supplementation may depend on the characteristics of the supplement, the forage, or the forage-supplement interaction.

A 50:50 blend of soybean hulls and corn gluten feed is widely used by producers to supplement growing cattle. This blend is high in energy but low in non-structural carbohydrates. It also contains a moderate amount of protein, much of which is ruminally degradable. We hypothesized that reducing the frequency of supplementation of a blend of soyhulls and corn gluten feed would not have large negative effects on fiber digestion and thus would not dramatically reduce animal performance. The following research was focused

on the impact of less frequent supplementation of 50:50 blend of soybean hulls and corn gluten feed on the performance of stocker cattle, the potential economic benefits for producers and the digestive and metabolic effects.

## **LITERATURE REVIEW**

### **Supplementation of forage based diets**

Generally, the substitution rate of forage for supplement depends on forage quality, level of protein in the supplement, energy source, and feeding rate. Supplements often decrease forage intake when forage TDN:CP ratio is less than 7, when supplemental TDN is greater than 0.7 % BW, or when forage intake when fed alone is greater than 1.75% of BW. However, supplements increase forage intake when forage TDN:CP ratio is greater than 7 (N deficit) and when forage intake when fed alone is low (Moore et al., 1999).

On medium and high quality forages substitution rate increases as forage quality increases (Horn and McCollum, 1987; Fieser and Vanzant, 2004). This indicates that the influence of energy-satiety intake control mechanisms may become increasingly important as forage quality increases. Often the rate of substitution decreases as the level of protein in the supplement increases. This is most likely due to a reduced competition for ruminally degradable N. The rate of substitution also tends to increase as supplement intake increases (Moore et al., 1999).

One reason for negative associative effects of supplementation is that consuming supplements, particularly those high in NSC, can cause a decrease in ruminal pH. Fiber

digestion is negatively affected below a pH of 6.2 (Grant and Merts, 1992). Microbial activity of fibrolytic bacteria is reduced at low pHs due to increases in their energy requirement for maintenance. These bacteria have to utilize more energy to maintain their intercellular pH which results in slower growth and can eventually lead to the wash out of specific microbial populations from the rumen (Strobel and Russell, 1986; Russell and Wilson, 1996). Based on this, Cerrato et al. (2007) suggested that the extent to which fiber digestion is decreased is correlated to the duration of suboptimal ruminal pH.

Another potential mechanism for negative associative effects involves the competition for available nutrients. Ruminally available N is often cited as a potentially limiting nutrient. When energy supplements are fed in situations where ruminal N is low, availability of N could potentially limit microbial growth and/or digestion. A drop in ruminal ammonia is often observed when energy supplements are fed. Ammonia is an important N source for fibrolytic bacteria (Hoover, 1986).

The classic and often cited in-vitro study by Satter and Slyter (1974), reported that the minimum level of ammonia-N required for maximal microbial growth was 2 mg/dL. The authors suggested that 5 mg/dL should be targeted to provide a margin of safety. Similarly, Schaefer et al. (1980) suggested that based on the ammonia saturation constant, the growth rate of ruminal microbes would be 95% of maximal when ammonia concentration was 1.4 mg/dL. However, there has been a wide range of ruminal ammonia-N values reported to be optimum for microbial growth (Hume et al., 1970; Slyter et al., 1979; Kang-Meznarich and Broderick, 1981) and for maximum rate of ruminal digestion of feed (Hume et al., 1970; Mehrez et al., 1977; Kang-Meznarich and Broderick, 1981; Erdman et al., 1986; Odle and

Schaefer, 1987). Reported ruminal ammonia-N concentration considered optimum to support maximum synthesis of microbial protein has ranged from 2 to 13 mg/dL, the optimum ammonia N concentration for maximal rate of ruminal fermentation has ranged from 2.5 to 25 mg/dL.

Differences in experimental design (in-vitro or in-vivo, diets, and method for increasing ammonia-N concentration) could explain some of these discrepancies. Relative to in-vitro data, Allison (1969) suggested that ammonia-N concentrations may need to be greater in-vivo to penetrate microenvironments within the rumen. It has also been shown that the minimum ruminal ammonia-N concentration required for optimum digestion increases with increasing fermentability of the feed (Erdman et al., 1986). The ammonia-N needed for maximal rate of digestion of low quality black spear grass hay (*Heteropogon contortus*) in-vitro was 2.5 mg/dl (Morrison et al., 1988) and 4.5 mg/dL under in-situ conditions (Boniface et al., 1986). The concentration of ammonia-N needed for maximal rate of digestion of in-situ digestion corn and barley grain were 6.1 mg/dL and 12.5 mg/dL, respectively (Odle and Schaeffer, 1987).

When formulating diets, sufficiency of ruminally available N is often evaluated by the balance of digestible intake protein (DIP) to total digestible nutrients (TDN) or DIP to digestible organic matter intake (DOMI) of the diet. Mathis et al. (2000) found that intake and digestibility of forages was maximized when dietary DIP was 8 to 13% of the DOMI. The NRC (1996) suggests that 13% of TDN as DIP is needed to maximize gain of cattle on forage based diets.

While negative associative effects of supplementation can occur the reverse is also true. In situations where there is a deficiency of N in the diet, positive associative effects of N supplementation on intake and digestibility are often observed (Moore et al., 1999). Other mechanisms where supplements can increase forage digestion and intake include a decrease in the lag time for microbial colonization of fiber (Hiltner and Dehority, 1983) or by supplying other deficient nutrients.

***Grain based supplements.*** High dietary levels of the most common type of energy supplement, cereal grains, which are high in NSC, can decrease forage digestibility (Hoover, 1986). Supplementation with large amounts of NSC has been suggested to cause decreases in fiber digestion by decreasing ruminal pH, decreasing cellulolytic enzyme production and activity (Martin et al., 2001; Bowman et al., 2004), impairing bacterial attachment to fibrous feedstuffs (Hiltner and Dehority, 1983), and increasing lag time for fiber digestion (Mertens and Loftin, 1980).

In addition to the DIP:TDN ratio of the base forage, the DIP:TDN ratio of the supplement may greatly influence responses to supplementation of forage diets (Bowman et al., 1997). The negative associative effects of corn based supplements have been shown to be partially alleviated by formulating supplements to meet DIP requirements (Bodine and Purvis, 2003). Klevesahl et al. (2003) provided steers consuming low-quality grass hay with supplements that varied in the ratio of DIP to starch. When supplement DIP was low, starch decreased forage intake and digestion, but when supplement DIP was high, starch had little effect on fiber digestion.

It also appears that the effect of supplements high in NSC on forage intake and digestibility depends on the level of supplementation. Garces-Yopez et al. (1997) studied the effect of supplemental energy source and amount on forage intake, performance, and digestibility. The authors reported that when growing cattle consuming bermudagrass were supplemented with high starch or high fiber concentrates at levels less than 0.5% BW, forage intake and digestibility were not affected. However, when supplementation was increased to 1%, forage intake was decreased by both supplement types, but cattle supplemented with high fiber concentrates had greater performance. It has been suggested that when NSC provided by supplements exceeds 0.4% BW, the forage digestible OM intake is decreased and that using a high fiber concentrate in these instances would result in 10 to 30% more gain per unit of supplemental TDN (Kunkle et al., 2000).

While a large amount of starch can be detrimental to fiber digestion, small amounts of starch would not be expected to have substantial adverse effects on fiber digestion and may even increase digestion. Galloway et al. (1993) showed a positive associative effect of mixing corn and SH on digestible OM intake. Supplementing a mixture of corn and SH increased digestible OM intake relative to the mean of corn and SH, on both orchardgrass and bermudagrass hay-based diets.

***Soybean hulls.*** Soybean hulls (SH), also referred to as soyhulls, are a byproduct of the soybean milling industry. Soybean hulls are actually the seed coat of the soybean which comes off during processing and are small in size and not very dense. Therefore, SH are often pelleted to increase ease of handling and bulk density. They are a starch-free (Garleb et al., 1988), low lignin (1.8 to 2.0% ADL) and high fiber feedstuff (57-71 % NDF; DePeters et

al., 2000). Nitrogen concentrations of SH vary and can range from 11 to 14% CP (DM basis; DePeters et al., 2000).

Supplementation with SH has a less dramatic effect on ruminal pH than supplementation with cereal grains (Klopfenstein and Owen, 1987). Martin and Hibberd (1990) showed that when supplementing 3 kg/d of SH to cows consuming low-quality native grass hay ruminal pH did not decrease below 6.2. Soyhulls are less rapidly degraded and have a higher N content than corn therefore they appear to decrease ruminal ammonia concentrations to a lesser degree than corn supplementation. When low-quality bromegrass hay (5.6% CP) was substituted with 0, 15, 30, 45, or 60% SH ruminal pH and ammonia concentration of steers decreased more rapidly, and to a greater extent and duration, as level of SH increased; however, neither was decreased to levels considered detrimental to fiber digestion (Grisby et al., 1992).

Furthermore, the absence of starch in SH may avert decreased fibrolytic activity that results from preferential starch utilization by fiber-digesting microbes (Hoover, 1986). Despite a difference in energy content (4.19 and 2.82 Mcal/kg for corn and SH, respectively; NRC, 1996), SH appear to be equivalent to corn as a source of supplemental energy in forage based diets (Anderson et al., 1988a). Additionally, Merrill and Klopfenstein (1985) reported that feeding 51% corn grain diets to lambs grazing summer bromegrass or cornstalks reduced fiber digestion, whereas soybean hulls had no detrimental effect on either forage intake or NDF digestibility.

Highfill et al. (1987) observed a negative associative effect on digestibility when cows consuming mature fescue hay were supplemented with a corn/soybean meal mix, but

not when SH was supplemented. In this study, microbial N flow at the abomasum tended to be greater for supplementation with SH than with corn/soybean meal and efficiency of microbial growth was slightly greater for the diet with SH.

***Corn gluten feed.*** Corn gluten feed (CGF) is a byproduct of the wet milling process. Wet milling separates the corn kernel into starch, oil, protein, and bran. First, the corn is soaked is steeped in a dilute sulfurous dioxide solution (Stock et al., 2000). The resulting steep liquor contains soluble protein, carbohydrates, minerals, and vitamins. The endosperm (starch) and germ (oil) are extracted from the swollen kernel and the remaining bran (fiber) is mixed with the steep liquor to produce CGF. Processors vary in how they handle the bran and steep liquor, which results in differing amounts of these two components in CGF. Therefore the nutrient profile can vary widely from plant to plant (Stack et al., 2000). Corn gluten feed is sold in both the wet (40 to 60% DM) and dry (90% DM) forms. Drying of CGF reduces its energy value (Ham et al., 1995) but increases its ability to be transported longer distances and simplifies storage. The cause of the reduced energy value is unknown but Stock et al., 2000 suggested that loss of volatile compounds during drying may be the cause. The published nutrient profile of CGF is 23% CP and 80% TDN (NRC, 1996). However, to get an acute estimate the nutrient content should be evaluated on a plant to plant basis rather than using the book value (Stock et al., 2000).

The CP from CGF is extensively degraded in the rumen due to the addition of corn steep liquor. The CP fraction of CGF consists of 75% DIP (NRC, 1996) and is degraded rapidly in the rumen (9.46%/h), having a rate of N disappearance similar to that of soybean meal (Firkins et al., 1984). Due to the highly digestible fiber and moderate CP content of

CGF, it can be used as a source of both energy and protein. Corn gluten feed is low in calcium (Ca; 0.07%) but has significant amounts of phosphorus (P; 0.95%). The Ca:P ratio is about 1:10. Therefore, when feeding high levels of CGF, additional dietary Ca should be provided. This can be accomplished by either mixing Ca carbonate (limestone) in the CGF supplement or providing a high Ca/low P mineral free-choice (Poore et al., 2002). Due to the corn being soaked in a dilute sulfur dioxide solution, the sulfur (S) content of CGF can be high (0.47% S; NRC, 1996) and potentially limit the level at which it can be fed. Feeding high levels of CGF can result in S intake above the suggested maximum tolerable level (0.4%; NRC, 1996) and can reduce feed intake, induce copper deficiency or cause polioencephalomalacia (Poore et al., 2002).

Corn gluten feed has less of a negative associative effect on digestion than corn. The 48 h in-situ dry matter digestibility of fescue hay was greater when supplemented (27% of the diet) with SH than corn/soybean meal and was intermediate for CGF (43, 39, and 41% for SH, corn/soybean meal and CGF, respectively; Highfill et al., 1987). When compared to a N equivalent corn/urea supplement, CGF has been shown to increase digestibility of hay and increase hay intake when supplemented at  $3 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$  (Cordes et al., 1988). However, at moderate feeding levels the feed value of CGF appears to be equal to or lower than corn when the CP content of the forage is not limiting gains. Performance of steers consuming an alfalfa haylage-based diet and supplemented with corn or CGF did not differ when supplemented at 20 or 60% of the diet (Hannah et al., 1990). Elizalde et al (1998) compared cracked corn and corn gluten feed as supplements for cattle grazing spring growth of endophyte-infected tall fescue. When supplemented at  $1.4 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$  gains were lower for

CGF than cracked corn (0.69 and 0.77 kg ·hd<sup>-1</sup>·d<sup>-1</sup>, respectively). However, when supplemented at 2.8 kg/d, gains were greater for CGF than cracked corn (0.83 and 0.71 kg ·hd<sup>-1</sup>·d<sup>-1</sup>, respectively). When CGF was substituted at increasing levels for corn/soybean meal in an orchardgrass hay-based diet performance of calves linearly decreased (Poore and Mueller, 1996). Nevertheless, compared to corn/soybean meal, the lower price of CGF usually causes it to be a more economical feed.

*Distillers grains* are a byproduct of dry milling and fermentation of grain (mainly corn) to produce ethanol. The production system can vary widely and therefore there are many different types of distillers byproducts available. The coarse particles remaining after fermentation or distillation, called distillers grains, can be removed and are sold in a wet (WDS) or dry (DDG) form. The liquid fraction (5 to 10% DM) remaining after separation of the coarse particles is called thin stillage and can be evaporated to produce condensed distillers solubles (CDS) which is a syrup like product that is 20 to 35% DM. The CDS may be dried with DDG to produce dried distillers grains plus solubles (DDGS), may be added to WDG to produce wet distillers grains plus solubles (WDGS), or may be sold as a feed ingredient (Stock et al., 2000).

Like corn gluten feed drying decreases the energy value of distillers products but increases its transportability (Stock et al., 2000). Dried distillers grains plus solubles is commonly produced and is becoming more and more readily available across the United States with expanding ethanol production. Corn is approximately 2/3 starch and during the production of ethanol the starch in the grain is fermented. Therefore, DDGS are low in starch and the protein, fat, and fiber concentrations are increased when compared with corn

(Klopfenstein et al., 2008). Dried distillers grains plus solubles contain 30% CP, 11% fat and 46% NDF (DM basis; NRC, 1996). Of the CP in DDGS, a little over half (52%) is ruminally undegradable (UIP; NRC, 1996). During ethanol production sulfuric acid is used to control pH in fermentation and for cleaning. The S levels vary significantly from plant to plant as well as from batch to batch within plant (Buckner et al., 2008) and can range from 0.6 to 1.0% (Batal and Dale, 2003). Both fat and S content of DDGS can limit the level at which it can be fed without negatively effecting performance.

Although supplementing fat to cattle on high-forage diets can increase the energy density of the diet, inclusion of fat in the diet can negatively affect fiber digestion (Van Soest, 1994). In general, limiting supplemental fat to 2% of dietary DM will prevent negative associative effects for ruminants fed high-forage diets. However, the energy density of high-forage diets will continue to increase until supplemental fat exceeds 4 to 5% of DM (Hess et al., 2008). Therefore, inclusion of supplemental fat at less than 3% of DM is recommended to obtain the most benefit from the energy contained within the fat and other dietary components in high-forage diets (Hess et al., 2008). However, differences in degree of saturation among fat sources is a primary property of fats that influences the degree to which they affect ruminal fermentation. Unsaturated fats are more inhibitory to ruminal fiber digestion than saturated fats (Eastridge and Firkins, 1991). However, unsaturated fatty acids have greater intestinal digestibility than saturated fatty acids (Zinn et al., 2000). Interestingly, the fatty acids in WDS are not hydrogenated in the rumen to the same extent as fatty acids in corn oil thus allowing more unsaturated fatty acids to reach the intestine.

Therefore the fat from wet distillers grains may have less of a negative effect in the rumen and be more digestible in the intestine than that of corn oil (Vander Pol et al., 2009).

A great deal of research has been conducted to determine the effects of including distillers grains in finishing rations (Klopfenstein et al., 2008). However, less work has been published on the effects of supplementing forage-based diets with distillers grains. One study showed that DDGS (25.4% CP, 9.8% fat) could be supplemented at levels up to 0.9% BW to cattle consuming medium quality (10% CP) grass hay without negatively affecting digestion (Leupp et al., 2007).

Loy et al. (2007) supplemented heifers consuming chopped grass hay (8.2% CP) with either dry rolled corn or DDGS at 0.4% BW. There was no difference in hay intake due to type of supplement but rate of hay NDF disappearance was higher for DDGS than dry rolled corn. Therefore, compared with corn, DDGS supplementation of forage-based diets appears to have less negative associative effects on fiber digestion. In addition, the greater content of UIP and greater energy density of DDGS contribute to the greater gains that are often observed when cattle are supplemented with DDGS (Macdonald et al., 2007). When supplemented at 0.21% BW or 0.81% BW, gains of growing heifers consuming medium quality hay were greater when supplemented with DDGS than dry rolled corn plus urea. Heifers supplemented with DDGS also gained more than those supplemented with dry rolled corn plus corn gluten meal (high UIP) when supplemented at 0.21% BW (0.49 and 0.37 kg/d, respectively). However, when supplemented at 0.81% BW, the ADG of heifers supplemented with DDGS (0.89 kg/d) or dry rolled corn plus corn gluten meal (0.90 kg/d) did not differ (Loy et al., 2008).

Dry distillers grains contain approximately 52% UIP (% of CP; NRC, 1996), consequently forage based diets that include dried distillers grains as an energy source are commonly deficient in DIP but contain excess metabolizable protein. However, when heifers consuming a forage based diet (58% ground corn cobs and 12% sorghum silage) and were supplemented with DDGS, gains were not increased by inclusion of extra DIP (urea) in the diet, despite the diet being predicted (based NRC, 1996) to have a DIP deficiency (Stalker et al., 2004). It was speculated that the cattle converted the excess metabolizable protein from DDGS to urea which was then recycled to the rumen and used as a source of ruminal N.

***Wheat middlings.*** A by-product of the wheat milling industry, wheat middlings (WM) consist of fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and some of the offal from the “tail of the mill” (Poore et al., 2002). Typical content of WM is 16-20% protein and 69% TDN (Poore et al., 2002) but like most by-products the nutrient content can vary from source to source and batch to batch. Wheat middlings contain between 40 and 59% wheat bran (Blasi et al., 1998) and the bran has an in-vitro NDF digestibility of 51% (Miron et al., 2001). While WM are considered a high fiber source of energy they also contain a moderate amount of starch (20 to 30%; Poore et al., 2002). Although WM are lower in starch content than corn, the starch in WM is much more rapidly digested than that the starch in corn (Poore et al., 2002). The protein in WM is considered to be fairly high in rumen degradability (21% UIP; NRC, 1996). Like CGF, WM also contain a high level of P (0.99%) and a low level of Ca (0.15%) and therefore may require supplemental Ca to balance Ca:P ratio if it is supplemented in large quantities.

The feed value of WM appears to vary and may depend on the by-product batch, forage type, and level of supplementation. When compared to corn/soybean meal, WM tended to be inferior at increasing weight of brood cows on winter range (Heldt et al., 1998). However, Lusby and Gill (1993) reported that when spring-calving cows were fed to provide the recommended level of supplemental CP, WM were equivalent to corn/soybean meal. Garces-Yepe et al. (1997) reported that WM and corn/soybean meal supplements gave similar performance when fed at 0.5% BW/d to growing cattle consuming bermudagrass hay, but when fed at 1% BW/d cattle supplemented with WM had 18% greater gains.

### **Research on supplementation frequency**

*Protein supplements.* The majority of research on frequency of supplementation has focused on ruminants consuming low-quality forage. When ruminants are fed low-quality forages, availability of N for microbial growth and fiber digestion is often limiting. Therefore, protein supplementation can increase digestion and has been shown to increase intake of low-quality forages (DelCurto et al., 1990; Köster et al., 1996). Many authors have reported no negative effect of reducing protein supplementation frequency on forage DMI or performance (Krehbiel et al., 1998; Huston et al., 1999; Bohnert et al., 2002b; Schaeur et al., 2005). It appears that supplementing cattle on low-quality forages with protein supplements such as cottonseed meal three times or once each week usually gives similar performance compared to daily feeding.

The lack of a negative effect of infrequent protein supplementation on N utilization has been attributed to the recycling of urea to the rumen, which provides N for microbes on days when protein is not supplemented and dietary protein concentration is low (Krehbiel et

al., 1998; Bohnert et al., 2002a; Archibeque et al., 2007). Krehbiel et al. (1998) demonstrated that mature ewes receiving supplemental protein every 3 d had greater transfer of urea to the rumen than those supplemented daily.

Collins and Pritchard (1992) studied the effect of frequency of supplementation when feeding a protein source high in UIP (corn gluten meal; CGM) to one high in DIP (soybean meal). An un-supplemented control was not used in this study. There was an interaction of source of protein and interval of feeding (daily or alternate days) for dry matter digestibility and digestible dry matter intake. Digestible dry matter intake and dry matter digestibility was slightly greater for daily supplementation of SBM than for alternate day supplementation whereas the reverse occurred for CGM. However, regardless of source, urinary N excretion was decreased by 30% when supplementation frequency was reduced and frequency of supplementation did not affect ADG of steer calves. Similarly, Cole (1999) found that oscillating the CP content of high-concentrate diets over a 48 h interval improved N retention in lambs and the response was greater when the protein source was higher in UIP. It has been suggested that the capacity of the urea cycle may be reduced by infrequent protein supplementation and therefore absorbed amino acids may be spared for anabolic uses on days when the supplement is fed (Wickersham et al., 2008).

Whole-body protein metabolism may be affected by less frequent protein supplementation. Liu et al. (1995) observed that, when lambs were switched from an adequate CP intake to a deficient CP intake, total-body protein flux, protein synthesis, and protein degradation all decreased immediately. On the first day of reduced CP intake, urinary N excretion was decreased by 20% and was further reduced (29%) on the second day.

***Grain based supplements.*** Supplementing grain based energy mixes less frequently requires the feeding of more grain per offering, thereby potentially exacerbating the negative effects of NCS on forage digestion. Kartchner and Adams (1982), fed cracked corn at a rate of 0.3% of BW daily or twice that amount every other day to pregnant cows grazing winter range. Over the 70 d winter feeding period cows supplemented daily gained 64 kg while those supplemented every other day only gained 31 kg.

Chase and Hibberd (1989) examined the effects of level of corn and frequency of supplementation on the digestion and intake of low-quality grass hay (5.0% CP and 46% ADF). The two levels of supplementation were designed to supplement 1 or 2 kg ·hd<sup>-1</sup>·d<sup>-1</sup> of corn for the low and high level of supplementation, respectively. The supplement for the low level of supplementation (1.39 kg/d) was 18.5% CP and contained 62% corn and 29% cottonseed meal. The supplement for the high level of supplementation (2.05 kg/d) was 12.3% CP and contained 85% corn and 9.6% cottonseed meal. Supplements were fed daily or twice the amount was fed on alternate days. Level of supplementation appeared to have more of an effect on hay intake and digestion than frequency. Increasing the level of supplementation decreased hay intake by 0.75 kg/d and decreased hay digestibility by 4%. However, frequency of supplementation did not affect hay intake and numerically reduced hay OM digestibility by only 1% at both levels of supplementation. Total diet digestibility was not affected by level of supplementation and was only numerically ( $P = 0.20$ ) reduced by less frequent supplementation (54.6 and 53.2% for daily and alternate day, respectively). Overall, reducing supplementation frequency caused a slight but significant decrease in digestible OM intake (5.5 and 5.3 kg/d for daily and alternate day).

Beaty et al. (1994) fed four supplements containing various levels of CP (12, 20, 30 or 39% CP) either daily (7X) at a rate of 0.4% BW/d or the equivalent amount three times a week (3X) to steers consuming wheat straw. The level of CP was varied by changing the ratio of rolled sorghum grain to soybean meal in the supplement. No frequency by level of CP interaction was detected. On the day that 3X steers were not supplemented, ruminal pH was higher than 7X. On the day that both 7X and 3X were supplemented, ruminal pH of 3X was less than 7X but remained at or above the level considered to be detrimental to fiber digestion (pH 6.2). When compared to 7X supplementation, supplementation 3X reduced straw intake (1.42 and 1.18% BW, respectively) but increased DM (50 and 54%) and NDF digestion (51 and 54%). The increase in diet digestibility was at least partially due to the supplement being a higher proportion of the diet for 3X.

In a second experiment, cows on native range during the winter were given the same supplements as previously described, either 7X or 3X at a rate of 0.42% BW/d over a 105 d period. Again, no interaction between frequency and CP was detected. All cows lost weight but cows on 3X lost more weight than 7X (-75.3 and -87.6 kg for 7X and 3X, respectively). However, at weaning no carryover effect on cow body weight or calf ADG was observed. In

a third experiment, cows on native range during the winter were fed a grain based supplement (21% CP) containing 74% grain (either corn or sorghum), 23% soybean meal and 3% molasses at a rate of 0.48% of BW/d. No effect of grain type was observed. Again, all cows lost weight and cows on 3X lost more weight than 7X (-79.2 and -88.7 kg for 7X and 3X, respectively). Overall, it appeared that feeding grain based supplements containing 12%

CP or greater to cows on low quality forage 3 times a week caused a small decrease in performance.

La Manna (2002) studied the effects of frequency of supplementing cracked corn to steers consuming chopped alfalfa hay (21% CP, 36% ADF). Steers were supplemented daily (0.5% BW), every other day (1% BW) or every three days (1.5% BW). Minimum ruminal pH of supplemented steers was 6.2, 6.0 and 5.7 when supplementation daily, on alternate days, or every third day, respectively. Hay intake decreased as supplementation frequency decreased. Steers consumed 11.5, 10.1, and 9.5 kg ·hd<sup>-1</sup>·d<sup>-1</sup> of hay when supplemented daily, alternate day and every third day, respectively. The ADF and NDF digestibility of the diet increased with reduced supplementation frequency. This was either due to decreased passage rate or corn making up a higher percentage of the diet. In a performance trial, average daily gain of steers did not differ between the daily and alternate day supplementation (0.77 and 0.75 kg/d, respectively) but was lower for steers supplemented every third day (0.62 kg/d; La Manna, 2002).

Loy et al. (2008) supplemented heifers consuming medium quality grass hay either 7X or 3X with two levels (0.21 or 0.81% BW/d) of a dry rolled corn based supplement that had either urea or CGM as a protein source. Regardless of supplement type or level, hay intake was decreased when supplemented less frequently. However, there was a tendency ( $P = 0.13$ ) for a frequency by supplement interaction on ADG. At both levels of supplementation, (0.21 and 0.81% of BW) the ADG of heifers supplemented with dry rolled corn plus urea did not differ due to frequency. Gains at the low level of supplementation were 0.36 vs. 0.35 kg/d for 7X and 3X, respectively. At the high level of supplementation

gains were 0.71 kg/d for both 7X and 3X. However at both levels of supplementation, gains of heifers supplemented 3X with the dry rolled corn plus CGM were lower than 7X (0.37 vs 0.28 kg/d and 0.90 vs. 0.79 kg/d for 7X and 3X with the low and high level of supplementation, respectively). The majority of the protein in CGM is ruminally undegradable while urea is rapidly and completely degraded in the rumen. Availability of ammonia for microbial growth and digestion may have been limiting when the dry rolled corn plus CGM was supplemented less frequently. Therefore, when supplementation frequency is reduced it may be important to provide ruminally degradable N with the supplemental energy in order to maintain forage digestion.

***Citrus pulp based supplements.*** Citrus pulp is a by product feed obtained during the manufacture of orange juice and processing of other citrus fruits (Grasser et al., 1995), therefore its availability is often limited to areas where citrus is grown and processed. Citrus pulp is high energy (82% TDN), low starch (< 10%; Hall, 2000) and low in crude protein (6 to 7%). Citrus pulp is 25% pectin (Keller et al., 1984) and 23% NDF (NRC, 1996) both of which are highly digestible in the rumen.

Cooke et al. (2007) examined the effect of feeding a supplement containing 75% citrus pulp and 25% cottonseed meal (70% TDN; 19% CP) either daily (7X) or three times a week (3X) at a rate of 1% BW/d to growing steers consuming low quality warm season grass hay. Mean insulin like growth factor (IGF-1) concentration in plasma did not differ due to supplementation frequency whereas plasma insulin and glucose concentrations were increased by less frequent supplementation. Despite the increase in insulin and glucose, 3X steers tended to have lower gains (0.18 kg/d) than 7X (0.30 kg/d); however, neither 7X nor

3X had substantial gain. In this study, plasma urea-N (PUN) concentrations of both treatments were low (3.55 and 2.92 mg/dL for 7X and 3X, respectively) and protein would probably be limiting growth. Cottonseed meal has relatively low rumen degradability and low PUN would not have provided much urea for recycling back to the rumen to aid in digestion. Assuming 60% of the CP in the hay was DIP the TDN:DIP ratio of the hay would have been 9. Based on the NRC (1996), the CP of the supplement would have been around 57% DIP and the TDN:DIP ratio would have been 7. Therefore, low rumen ammonia may have limited fiber digestion (of the hay and the supplement) when a large amount of this supplement was fed at one time.

***Wheat middling based supplements.*** Cooke et al. (2008) studied the effect of supplementation frequency (7X or 3X) of a WM-based supplement (66.7% WM, 26.9% SH, 3.8% molasses and 2.7% cottonseed meal) to growing heifers grazing bahiagrass pastures. During weeks 1, 3, 5 and 6, blood samples were taken from 2 heifers per pen (7 pens per treatment) on Monday, Tuesday, Wednesday, and Thursday starting 4 h after supplementation. On the day that 3X received supplement (Mondays and Wednesdays), insulin and glucose concentrations of heifers did not differ due to supplementation frequency, but on the day after supplementation (Tuesdays and Thursdays; 28 h after supplementation for 3X) glucose and insulin concentrations of 3X were greater than 7X. The IGF-1 concentration of 3X was lower than 7X on the day of supplementation but did not differ due to supplementation frequency on the day after supplementation (Tuesdays and Thursdays). The BUN concentration of 3X was lower than 7X on the day of supplementation but was greater on the day after supplementation.

Liver biopsies were taken 4 h after supplementation on d 35 and 36 (Monday and Tuesday) of the trial and hepatic mRNA expression of pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) was measured. On the day of supplementation, the hepatic mRNA expression for PC and PEPCK-C of 3X were greater than 7X. On the day after supplementation, mRNA expression of PC in the 3X decreased and did not differ from 7X but mRNA expression of PEPCK-C decreased and was lower than 7X.

Pyruvate carboxylase and PEPCK are potential rate-limiting enzymes for hepatic gluconeogenesis from precursors that enter the gluconeogenic path prior to the triose phosphates. Recent information indicates that propionate and other short chain fatty acids (VFA) stimulate the expression of PEPCK mRNA in rat liver cells (Massillon et al., 2003).

Therefore the changes in PEPCK were probably in response to the VFA absorption (particularly propionate) of 3X being greater on the day of supplementation and lesser on the day after supplementation. For lactate and many gluconeogenic amino acids to be utilized for gluconeogenesis they must be converted to pyruvate which then must be converted to oxaloacetic acid (OAA) by PC. While it is possible that there was an increase in the utilization of amino acids for glucose production on the day that 3X was supplemented, it is more likely that the increase in PC was due to an increase in the absorption of lactate and its subsequent utilization for glucose production.

Interestingly, insulin-like growth factor binding protein 3 (IGFBP-3) mRNA expression of 3X was greater than 7X on the day of supplementation but did not differ on the day after supplementation. Insulin-like growth factor binding protein 3 is the main carrier of

IGF-1 and greater serum concentrations of IGFBP-3 have been associated with faster growing animals (Vestergaard et al., 1995; Rausch et al., 2002).

The authors reported that gain was decreased by less frequent supplementation (0.41 vs 0.33 kg/d for 7X and 3X). However, gains were calculated from weights taken on d1 and d 108 but heifers were grouped for breeding (only one group per treatment) and not rotated among pastures from d 46-108. Forage availability, intake and quality of individual pastures were not reported. Unfortunately, the effect of supplementation frequency on forage intake and digestion was not measured. These results are intriguing and more frequent blood sampling would be useful to further elucidate the hormonal and metabolite status of animals supplemented less frequently and its effects on performance.

***Dry distillers grains plus solubles.*** Although, DDGS has a high fiber and low NSC content it appears that compared to daily supplementation less frequent supplementation of DDGS negatively affects performance.

Loy et al. (2008) supplemented heifers (n = 10/treatment) consuming medium quality grass hay either 7X or 3X with two levels (0.21 or 0.81% BW/d) of DDGS. Hay intake and ADG was decreased by less frequent supplementation. The decrease in hay intake and ADG was greater at the lower level of supplementation than the higher level. Hay intake was 5.30 and 4.78 kg ·hd<sup>-1</sup>·d<sup>-1</sup> at the low level of supplementation and 4.52 and 4.15 kg ·hd<sup>-1</sup>·d<sup>-1</sup> at the high level of supplementation for 7X and 3X, respectively. Gains were 0.49 and 0.41 kg ·hd<sup>-1</sup>·d<sup>-1</sup> at the low level of supplementation and 0.89 and 0.84 kg ·hd<sup>-1</sup>·d<sup>-1</sup> at the high level of supplementation for 7X and 3X, respectively.

In another study, supplementation of DDGS (~ 0.04% BW/d) every other day or every third day reduced diet apparent total tract DM disappearance compared to daily (Stalker et. al., 2009). Apparent DM disappearance was 58, 56 and 55% for 7X, every other day and every third day, respectively. On the day of supplementation, the DDGS would have added 3 and 5% fat to the diet of the steers supplemented every other and every third day, respectively.

When growing heifers (BW =193 kg) were fed the equivalent of  $1.3 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$  (0.07% BW/hd) of DDGS either 6 times a week or 3X, ADG was reduced by less frequent supplementation (0.79 and 0.72,  $\text{kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ , respectively; Stalker et al., 2009). On the day of supplementation, the diet of heifers fed 3X would have contained 5.4% fat. The high fat content of DDGS may be partially responsible for the reduced performance and digestion that has been observed when DDGS are supplemented less frequently. The imbalance of DIP: DOMI may also contribute to the reduced response to less frequent supplementation.

***Soybean hulls.*** Little data is available on the effect of less frequent supplementation of SH. When steers grazing cool season pasture with clover were supplemented with SH either daily at 0.5% of BW, every other day at 1% of BW or every third day at 1.5% BW, gains were reduced with less frequent supplementation. However, ADG was 14.3% greater for supplemented heifers compared with unsupplemented heifers. Frequency of supplementation resulted in a linear response with heifers supplemented daily having the greatest ADG (0.90 kg/d) and ADG of heifers supplemented every second and every third day being reduced by 6.6 and 2.0%, respectively (Mills et al. 2006). The nutrient content of the pasture and forage intake were not reported.

*Corn gluten feed.* No research is currently published related to less frequent supplementation of CGF. However, given that CGF has a low NSC content and a moderate protein content that is highly ruminally degradable it would appear to be the ideal candidate for programs utilizing less frequent supplementation.

## **Summary**

Response to less frequent supplementation of energy may depend on the characteristics of the supplement and the forage or the forage-supplement interaction. Feeding large amounts of energy-based supplements can potentially result in asynchrony of available energy and N in the rumen thus making microbial digestion less efficient. Using a supplement that has a balanced energy and protein profile and that is low in total NSC may decrease the negative effects associated with supplementing energy less frequently.

By-product commodities such as soybean hulls (SH), wheat middlings (WM), dry distillers grains plus solubles (DDGS), and corn gluten feed (CGF) are often a lower cost alternative to traditional grain and oil seed supplements and are commonly supplemented to growing cattle on forage based diets. These feedstuffs have unique physical and nutritional characteristics which may or may not lend themselves to production systems where supplementation frequency is reduced.

Based on recent studies (Loy et al., 2008; Stalker et al., 2009), it appears that compared to daily supplementation, less frequent supplementation of DDGS negatively affects performance of growing cattle on forage based diets despite DDGS being high in digestible fiber, low in NSC and high in CP. The high fat content (10.7%; NRC, 1996) or

imbalance of ruminally degradable protein to digestible organic matter of DDGS may be responsible for the reduced performance and digestion that has been observed when DDGS was supplemented less frequently.

Wheat middlings are higher in protein (16-20% CP) and lower in starch (20 to 30% starch) than corn (Poore et al., 2002). However, the starch in WM is much more rapidly digested than in corn (Poore et al., 2002). Therefore, WM may not be well suited for less frequent supplementation, however, little research is available.

Soybean hulls and CGF may be compatible with less frequent supplementation because they have moderate levels of CP (12 and 23%, CP, respectively), much of which is ruminally degradable and are high in degradable fiber and low in starch (NRC, 1996). However, little research has examined less frequent supplementation of SH or CGF.

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## **CHAPTER 2**

### **EFFECT OF FREQUENCY OF SUPPLEMENTATION OF A SOYHULLS AND CORN GLUTEN FEED BLEND ON HAY INTAKE AND OF PERFORMANCE GROWING STEERS**

## **Abstract**

Feeding supplements less frequently can reduce labor costs and could increase profit. However, reducing the frequency of delivery of grain-based supplements can negatively affect fiber digestion. Using supplements that have a moderate protein level and that are low in total nonstructural carbohydrates might alleviate the negative effects on fiber digestion and therefore decrease negative effects associated with supplementing energy less frequently. Corn gluten feed and soybean hulls are high in energy but low in non-structural carbohydrates. Additionally, corn gluten feed is also a good source of ruminally degradable protein. The objective of this study was to determine the effect of reducing supplementation frequency on steer performance when supplementing medium quality hay with a blend of soybean hulls and corn gluten feed (SH/CGF). The 86-d feeding trial was replicated over 4 years. Each yr, 40 steers (BW = 263, 281, 271, and 229 kg for yr 1 through 4, respectively) were stratified by weight and assigned to 8 groups which were randomly assigned to treatment. During yr 1 and 2, treatments consisted of ad-libitum medium quality fescue hay (7-10 % CP and 34-41% ADF) that was either not supplemented (HAY), supplemented daily (7X) with 2.73 kg/hd, or supplemented on Monday, Wednesday and Friday (3X) with 6.36 kg/hd. During yr 3 and 4, an additional treatment was added in which steers were supplemented on Monday and Thursday with 9.55 kg/hd of SH/CGF (2X). Average daily gain was greater ( $P < 0.01$ ) in supplemented steers compared to non-supplemented steers, but did not differ ( $P \geq 0.40$ ) due to supplementation frequency. Hay intake was reduced ( $P < 0.01$ ) by supplementation and was greater ( $P < 0.05$ ) for 7X compared to both 3X and 2X.

Hay intake did not differ ( $P = 0.37$ ) between 3X and 2X. The gain to feed ratio was increased by supplementation ( $P < 0.01$ ) and was further increased by less frequent supplementation ( $P \leq 0.02$ ). These results suggest that when supplementing hay with a blend of soyhulls and corn gluten feed, steers can be supplemented as little as two times a week without reducing performance.

## **Introduction**

There has been a considerable amount of research studying the effects of reduced frequency of protein supplementation in ruminants consuming low quality forage. Many of these studies have shown little to no effect of reduced protein supplementation frequency (Krehbiel et al., 1998; Huston et al., 1999). However, when energy supplements were delivered less frequently to cattle consuming low quality forages, performance was reduced (Kartchner and Adams, 1982; Beaty et al., 1994; Cooke et al., 2007). Results of less frequent supplementation of growing cattle consuming medium or high quality hay have been variable (La Manna, 2002; Loy et al., 2008). Response to less frequent supplementation of energy may depend on the characteristics of the supplement and the forage or the forage-supplement interaction.

Feeding large amounts of energy based supplements can potentially result in asynchrony of available energy and nitrogen in the rumen thus making microbial digestion less efficient. Feeding the total quantity of a gain based energy supplement less frequently requires the feeding of more grain per offering, thereby potentially exacerbating negative effects of non-structural carbohydrates on forage digestion (Hoover, 1986). Using a

supplement that has a balanced energy and protein profile and that is low in total non-structural carbohydrates may decrease the negative effects associated with supplementing energy less frequently.

Corn gluten feed and soyhulls are two byproduct feeds that are highly digestible (2.89 Mcal/kg of ME) but are low (< 30%) in non-structural carbohydrates (NRC, 1996).

Additionally, corn gluten feed is also a good source of protein (23% CP) much of which is ruminally degradable (75%; NRC, 1996). These byproducts are widely available and are an affordable source of supplemental energy and protein. However, the labor and equipment cost of hand feeding supplements can be very high; therefore, feeding a supplement less frequently could potentially increase profit, provided animal performance is not greatly affected. The objective of this study was to determine the effect of reducing supplementation frequency on steer performance when supplementing medium quality hay with a SH/CGF blend.

## **Materials and Methods**

Winter feeding trials were conducted over four years to determine the effect of supplementing hay with a mixture of SH/CGF daily, 3 times a week, or 2 times a week on steer performance. The protocol for this study was approved by the Institutional Animal Care and Use Committee at North Carolina State University.

*Animal care.* Prior to the initiation of the study, steers were treated for internal and external parasites using Dectomax pour-on (Pfizer Inc; New York, NY) and implanted with Ralgro (Intervet; Millsboro, DE). At the start of each year of the trial, Angus-cross steers

were weighed in the morning (prior to the addition of fresh hay and feeding of supplement) on two consecutive days to determine initial full weights. Access to hay and water was then removed and steers were reweighed after 24 h to determine initial shrunk weights. Each yr, 40 steers (BW = 263, 281, 271, and 229 kg for yr 1 through 4, respectively; SEM $\pm$  2.7) were stratified by weight into 8 groups (5 steers per group) and randomly assigned to treatment. During the first two yr, dietary treatments consisted of ad-libitum fescue hay that was supplemented daily (7X) with 2.73 kg/head (as-fed), supplemented 3 days a week (3X) with 6.36 kg/head (as-fed) or not supplemented (HAY). In yr 1 and 2, 3 groups were assigned to each of the 2 supplemented treatments and 2 groups to HAY. During the last two yr, an additional treatment was added in which steers were supplemented 2 times a week (2X) with 9.54 kg/head (as-fed). In yr 3 and 4, there were 2 groups for each of the 4 treatments. Each week, 3X steers were supplemented on Monday, Wednesday and Friday, and 2X steers were supplemented on Monday and Thursday. The supplement contained 47% soyhull pellets, 47% corn gluten feed pellets, 2 % feed grade limestone, and 4% liquid yeast. Interim weights were taken every 28 d. Final barn weights and shrunk weights were determined using the same protocol as initial barn and shrunk weights. Prior to the initiation of, and after the conclusion of the trial, an evaluator that was blind to treatment assigned body condition scores. Body condition score was based on a 9 point scale (1 = emaciated; 9 = extremely obese; NRC, 1996).

Each group was housed in a 92 m<sup>2</sup> pen that was partially covered by an open sided barn. Steers had access to a covered concrete bunk (61 cm bunk space per steer), a plastic

feed bunk (97.5 cm of bunk space per hd) and 1 automatic water bowl per pen. Each week the groups were rotated among pens to balance pen effect.

**Feeding.** A weighed amount of square baled fescue hay was offered in the concrete bunk each morning in quantities sufficient to ensure ad-libitum access (10% refusal). Prior to feeding, hay remaining from the previous day was collected and weighed. Hay intake per group was estimated as hay offered minus hay remaining. Supplement was fed in the plastic feed bunk under the cover of the barn. For the first 10 d, steers on the supplemented treatments were adapted to diets by gradually increasing the amount of supplement being fed (Table 1). Throughout the trial, the supplement offered was completely consumed by the steers. Therefore, supplemented groups consumed the same amount of supplement weekly (19.1 kg/hd each week). All groups were given free choice access to a mineral supplement (Beef Cow Special Mineral, Southern States Cooperative, Inc., Richmond VA) containing 25.5 % Ca; 5.0 % P; 24.0% Salt; 4,000 ppm zinc; 1,250 ppm copper (from sulfate); 3,700 ppm Mn; and 52 ppm Se. The mineral supplement was labeled to contain 75,000 IU/kg, 8,636 IU/kg and 68 IU/kg of Vitamin A, D, and E, respectively.

**Laboratory Analyses.** Samples of hay offered were taken weekly and samples of supplement offered were taken every two weeks throughout the trial. Samples were ground in a Model 4 Wiley mill (Arthur A. Thomas Co., Philadelphia, PA) to pass through a 5-mm screen, subsampled and then ground to pass through a 1-mm screen. Dry matter (105°C), ash, and Kjeldahl N were determined according to AOAC (1999) procedures. Neutral detergent fiber (with  $\alpha$ -amylase) and ADF were sequentially determined using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology, Fairport, NY). Hay and supplement

were analyzed for mineral content by a commercial laboratory (Cumberland Valley Analytical Services; Maugansville, MD). The nutrient content of the hay and supplement is presented in Table 2.

**Statistical Analyses.** Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Two models were used for analysis. The first model contained data for the HAY, 7X, and 3X treatments during all four years. The second model contained data from the last two years where 4 treatments (HAY, 7X, 3X, and 2X) were examined. In both models, group was considered the experimental unit, and the effect of year was considered random. Hay intake was analyzed using repeated measures (week) and the models included the fixed effects of treatment, day of the week, and their interaction. Significant differences were defined as  $P \leq 0.05$ .

## **Results**

The comparisons among HAY, 7X, and 3X are discussed using the model containing data from all four yr. Comparisons of the 2X with other treatments are discussed using the model containing data from yr 3 and 4. Hay intake was reduced by daily supplementation ( $P < 0.01$ ) and was further reduced ( $P < 0.05$ ) by supplementing 3X and 2X (Table 3). However, hay intake for 3X and 2X did not differ ( $P = 0.37$ ). There was a treatment by day of the week interaction ( $P < 0.01$ ) for hay intake (Figure 1). On the days 3X and 2X received supplement, hay intake was decreased and this substitution effect appeared to carry over to the day after supplementation but two days after supplementation hay intake was not different from the HAY. Despite the reduction in hay intake, supplementation increased ( $P <$

0.01) ADG and gain did not differ ( $P \geq 0.40$ ) due to frequency of supplementation (Table 3). Efficiency, as measured by the gain to feed ratio, was improved by supplementation (Table 3;  $P < 0.01$ ). Compared to supplementing daily, feed to gain was increased by less frequent supplementation ( $P = 0.02$  and  $P < 0.01$  for 3X and 2X, respectively). The gain to feed ratio of 2X did not differ from 3X ( $P = 0.14$ ). The coefficient of variation of the final weight of steers in each group did not differ ( $P = 0.18$ ) and was 6.41, 7.71 and 8.16% for HAY, 7X and 3X, respectively.

## **Discussion**

Decreasing the frequency of supplementation is a management practice that can decrease labor and equipment operation costs. However, animal performance response to less frequent supplementation has been variable and may depend on the characteristics of the supplement and/or the forage-supplement interaction.

Energy supplements often decrease forage intake when the forage is high quality (TDN: CP ratio is less than 7 and forage intake when fed alone is greater than 1.75% of BW) or when a large amount of supplemental energy (supplemental TDN is greater than 0.7 % BW) is provided (Moore et al., 1999). In our study, the quality of the hay varied from year to year and the TDN:CP ratio ranged from 6.3 to 8.8. However, in all years the intake of hay when fed alone was above 2% of BW and supplemental TDN provided was a minimum of 0.7% of BW/d. Therefore, the substitution effect observed in this study was not unexpected. The reduction of hay intake due to less frequent supplementation is also consistent with the literature (Beaty et al., 1994; La Manna, 2002; Loy et al., 2007; Loy et al., 2008).

Loy et al. (2008) supplemented heifers consuming medium quality grass hay either daily or three times a week with two levels (0.21 or 0.81% BW/d) of dry distiller's grains with solubles or a dry rolled corn based supplement that had either urea or corn gluten meal as the protein source. Regardless of supplement type or level, hay intake was decreased when supplemented less frequently. In the present study, hay intake was not only decreased on the day of supplementation but also on the day after supplementation. In ruminants, intake is regulated by both physical and metabolic controls (Forbes, 1996). On the day of supplementation steers would have consumed a great volume of feed (5.3 and 5.5 kg of NDF for 3X and 2X, respectively) much of which would have been highly digestible. It is unlikely that the increased energy supply and gut fill that steers experienced on the day of supplementation would have fully dissipated by the next day. Steers may have continued to respond to these signals on the day after supplementation thus causing the observed reduction in intake.

One factor that may play a role in the response to less frequent energy supplementation is the availability of sufficient dietary DIP for ruminal digestion of the carbohydrate from the hay and the energy-supplying supplement. The SH/CGF supplement used in this study was high energy and intermediate in CP (16-18%, DM basis). The DIP as a % of CP is 58% and 75% for soybean hulls and corn gluten feed, respectively (NRC, 1996). Therefore the majority of the protein in the supplement would have been ruminally degradable. The large amount of DIP provided by the supplement combined with the low nonstructural carbohydrate content of the supplement may have helped to maintain a favorable ruminal environment for microbial digestion. Cooke et al. (2007) fed ~1% of

BW/d of a supplement containing 75% citrus pulp and 25% cottonseed meal (70% TDN; 19% CP) either 7X or 3X to growing steers consuming Limpograss hay (54% TDN and 9% CP). Although, citrus pulp contains little starch the 3X steers tended ( $P = 0.13$ ) to have lower gains (0.18 kg/d) than 7X (0.30 kg/d). In this study, the plasma urea N concentration of both treatments were low (3.6 and 2.9 mg/dL for 7X and 3X, respectively). The CP in the supplement would have been 57% DIP (NRC, 2007). It seems likely that the rumen ammonia levels may have been low enough to negatively affected fiber digestion when a large amount of this supplement was fed at once.

Loy et al. (2008) reported a tendency ( $P = 0.13$ ) for a frequency by supplement interaction on performance. At both levels of supplementation, (0.21 and 0.81% of BW/d) the ADG of heifers supplemented with dry rolled corn plus urea did not differ due to frequency and was 0.36 vs 0.35 kg/d for daily and 3 times a week at the low level and 0.71 kg/d for both daily and 3 times a week at the high level of supplementation. However, gain of heifers supplemented with the dry rolled corn plus corn gluten meal (CP of corn gluten meal is 38% DIP) or the dry distiller's grains with solubles (36% of CP is DIP) was lower for the heifers supplemented 3 times a week than those supplemented daily at both levels of supplementation.

In another study, ADG of steers fed chopped alfalfa hay (21% CP and 36% ADF) and supplemented with corn (0.5% of BW/d) did not differ between daily and every other day supplementation (0.77 and 0.75 kg/d, respectively), but was lower for steers supplemented every third day (0.62 kg/d; La Manna, 2002). The CP in alfalfa hay is between 77 and 84% DIP (NRC, 1996) and for all treatments the rumen ammonia concentration was maintained

above 5 mg/dL throughout the feeding cycle. Taken together these data suggest that to successfully feed energy supplements less frequently the DIP available in the diet, either through the supplement or the hay, may need to be elevated.

The reason for the increase in efficiency that occurred in the present study when steers were supplemented less frequently is unclear. Alterations in ruminal microbial population or metabolism as well as changes in behavior or metabolic status of the animal itself may explain the increase in efficiency. Modification of eating behavior of steers supplemented less frequently may be one possible explanation. Energy expended during eating and rumination is not necessarily tied directly to the amount of DM consumed but to the amount of time spent eating (Adam et al., 1984). If cattle supplemented less frequently eat larger, less frequent meals then the reduction in energy expended by eating may compensate for the reduction in hay intake and thus make them more efficient. Loy et al. (2007) reported that reducing supplementation frequency of dry-rolled corn or dry distillers grains from daily to every other day, reduced the number of meals consumed per day and the amount of time spent eating. Tellier et al. (2004) reported that voluntary intake and apparent digestibility of gross energy were not affected by frequency of concentrate feeding when steers were fed barley straw and were supplemented with a barley grain-based concentrate. Despite the similarities in intake and digestion, they observed a 4% reduction in heat production when steers were supplemented every other day compared to daily supplementation.

Supplementation increased labor and equipment operation costs but also increased gain and resulted in a decrease in the cost of gain (Table 4). Based on the results of this

study reducing supplementation frequency would not have affected income (final body weights were similar) but would have an economic advantage in that labor and travel associated with the act of feeding was reduced and hay intake was reduced. In this study, hay intake was reduced by 0.8 kg/hd/d when supplementing 3X compared to 7X. The savings in hay cost would be \$5.91 per hd (hay cost estimated to be \$0.088/kg of DM) during a 84 d feeding period. If a producer was feeding 20 calves they would save \$118 over the 84 d period (Table 4). The majority of the economic benefit related to less frequent supplementation would be due to the reduced labor and cost of equipment operation associated with the travel to and from the area where the calves are being fed. If it took 15 minutes of travel time and 3 minutes to actually feed the supplement each time the calves were supplemented, during an 84 d period the cost of supplementing 7X would be \$230 in labor cost and \$242 for the cost of operating a ½ ton truck (Table 4). During an 84 d period, reducing supplementation frequency to 3X vs 7X would save \$110 in labor and \$138 in the cost of operating a ½ ton truck to get to the site of feeding (Table 4).

The reduction in costs associated with labor and travel would vary greatly among individual farms. Producers that have calves at several locations or have calves located further away from the main area of operation would realize more of a benefit than producers for whom travel time to feed calves is minimal. For a operation with 20 head, the cost of gain for 3X would be \$0.29/kg less than 7X, if travel time for each supplementation event was 15 minute but if travel time was 30 minutes per supplementation event then the cost of gain for 3X would be \$0.49/kg less than 7X (Table 5).

The size of the operation would also affect the extent to which reducing supplementation frequency would change the cost of gain. The cost reduction would be proportionally greater for smaller operations because the costs associated with each supplementation trip would be distributed over a smaller number of cattle and would constitute a larger percentage of the overall cost of supplementation (Table 5). Since hay intake did not appear to be further reduced by supplementing 2X the added benefit of 2X compared to 3X would only be associated with one less trip to supplement the calves per week.

Supplementation programs rely on the assumption that all animals consume the target amount of supplement. The effectiveness of supplementation programs is affected by the ability to reduce intake variation and to achieve the intended consumption of supplement. This is particularly important when backgrounding or stockering cattle because there are marketing advantages to having a uniform group of calves to sell. Therefore, the effect of supplementation frequency on the variability of supplement intake of individuals within a group and its subsequent effect on the individual's performance should be considered.

Offering a larger quantity of supplement per animal has been shown to reduce the variation in individual animal supplement consumption (Bowman and Sowell, 1997). Infrequent supplementation increases the amount of supplement offered per supplementation event, potentially allowing individual animals an increased opportunity to consume supplement. Huston et al. (1999) observed that cows supplemented infrequently (three times weekly or once weekly) exhibited approximately 33% less variation in supplement intake than cows supplemented daily.

In our study, the coefficient of variation of the final weight of steers in each group did not differ. However, each group consisted of only 5 steers and thus our ability to determine the effect of supplementation frequency on variability of performance within a group was limited. More research on the effect of supplementation frequency on variability of supplement intake and performance of growing cattle within a group would be beneficial.

## **Conclusions**

The response to less frequent supplementation depends on the characteristics of the supplement and the forage being fed. The results of this study suggest that when supplementing growing steers consuming medium quality hay with a soybean hull and corn gluten feed blend, frequency of supplementation can be reduced to as little as two times a week, thereby reducing costs without reducing gains. However, more research needs to be conducted to establish the mechanisms behind our observations and to be able to predict effects of supplementation frequency on animal performance when utilizing different supplement and forage combinations. The economic benefits of supplementing less frequently would vary greatly among individual farms. Small producers and producers that travel longer distances to feed cattle may benefit the most from reducing supplementation frequency.

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**Table 1.** Supplementation (kg/hd on as-fed basis)

of steers during the adaptation period<sup>1</sup>.

Day of week	Treatment		
	7X	3X	2X
Wednesday	0.91	1.82	1.82
Thursday	0.91	-	-
Friday	0.91	2.73	2.73
Saturday	0.91	-	-
Sunday	0.91	-	-
Monday	1.82	3.64	3.64
Tuesday	1.82	-	-
Wednesday	2.27	4.55	4.55
Thursday	2.27	-	-
Friday	2.73	5.45	5.45

<sup>1</sup>After first 10 days the steers on 7X were supplemented (as-fed) with 2.73kg/hd daily, 3X with 6.54 kg/hd on Mondays, Wednesdays and Fridays, and 2X with 9.54 kg/head on Mondays and Thursdays.

**Table 2.** Chemical composition of tall fescue hay and supplement<sup>1</sup> fed to steers during years 1 through 4

Item	DM, %	% of DM					
		CP	NDF <sup>2</sup>	ADF	Ca	P	S
Hay							
year 1	89.8	9.5	68.0	40.9	0.68	0.20	0.23
year 2	92.3	6.9	67.9	39.5	0.36	0.25	0.18
year 3	93.8	9.9	66.4	34.3	0.38	0.28	0.23
year 4	90.4	7.4	71.5	39.5	0.29	0.24	0.16
Supplement							
year 1	88.4	15.9	46.4	26.9	0.91	0.56	0.24
year 2	89.7	16.6	44.8	25.3	1.92	0.63	0.31
year 3	90.1	18.0	39.6	21.2	1.03	0.62	0.29
year 4	88.8	17.5	49.1	27.0	0.82	0.71	0.30

<sup>1</sup>The supplement was formulated to contain 47% soybean hull pellets, 47% corn gluten feed pellets, 2 % feed grade limestone and 4% liquid yeast

<sup>2</sup>Neutral detergent fiber determined using  $\alpha$ -amylase

**Table 3.** Effect of supplementation<sup>1</sup> frequency on the performance and intake of steers fed ad-libitum medium quality fescue hay

Item	Treatment <sup>2</sup>				SEM
	Hay	7X	3X	2X	
Years 1 through 4					
ADG, kg ·hd <sup>-1</sup> ·d <sup>-1</sup>	0.24 <sup>b</sup>	0.75 <sup>a</sup>	0.73 <sup>a</sup>	–	0.01
Hay intake, kg ·hd <sup>-1</sup> ·d <sup>-1</sup>	6.0 <sup>a</sup>	5.4 <sup>b</sup>	4.6 <sup>c</sup>	–	0.17
Gain to feed	0.040 <sup>c</sup>	0.098 <sup>b</sup>	0.106 <sup>a</sup>	–	0.003
Years 3 and 4					
ADG, kg ·hd <sup>-1</sup> ·d <sup>-1</sup>	0.32 <sup>b</sup>	0.85 <sup>a</sup>	0.84 <sup>a</sup>	0.85 <sup>a</sup>	0.02
Hay intake, kg ·hd <sup>-1</sup> ·d <sup>-1</sup>	5.8 <sup>a</sup>	5.0 <sup>b</sup>	4.4 <sup>c</sup>	4.2 <sup>c</sup>	0.24
Gain to feed	0.054 <sup>a</sup>	0.115 <sup>b</sup>	0.123 <sup>bc</sup>	0.130 <sup>c</sup>	0.003

<sup>1</sup> Supplement contained 47% soyhull pellets, 47% corn gluten feed pellets, 2 % feed grade limestone and 4% liquid yeast

<sup>2</sup> Treatment: Hay = hay only; 7X = hay plus 2.73 kg/hd of supplement daily; 3X = hay plus 6.36 kg/hd of supplement 3 times a week; 2X = hay plus 9.55kg/hd of supplement 2 times a week. Treatment effect for all measurements ( $P < 0.01$ ).

<sup>a-c</sup> Means within the same row not sharing a common superscript differ ( $P < 0.05$ ).

**Table 4.** Estimated operating, labor costs, and cost of gain for stockering 20 beef steers for 84 days when feeding medium quality hay alone (H) or supplementing hay daily (7X) or three times a week (3X) with mix of corn gluten feed and soybean hull pellets<sup>1</sup>

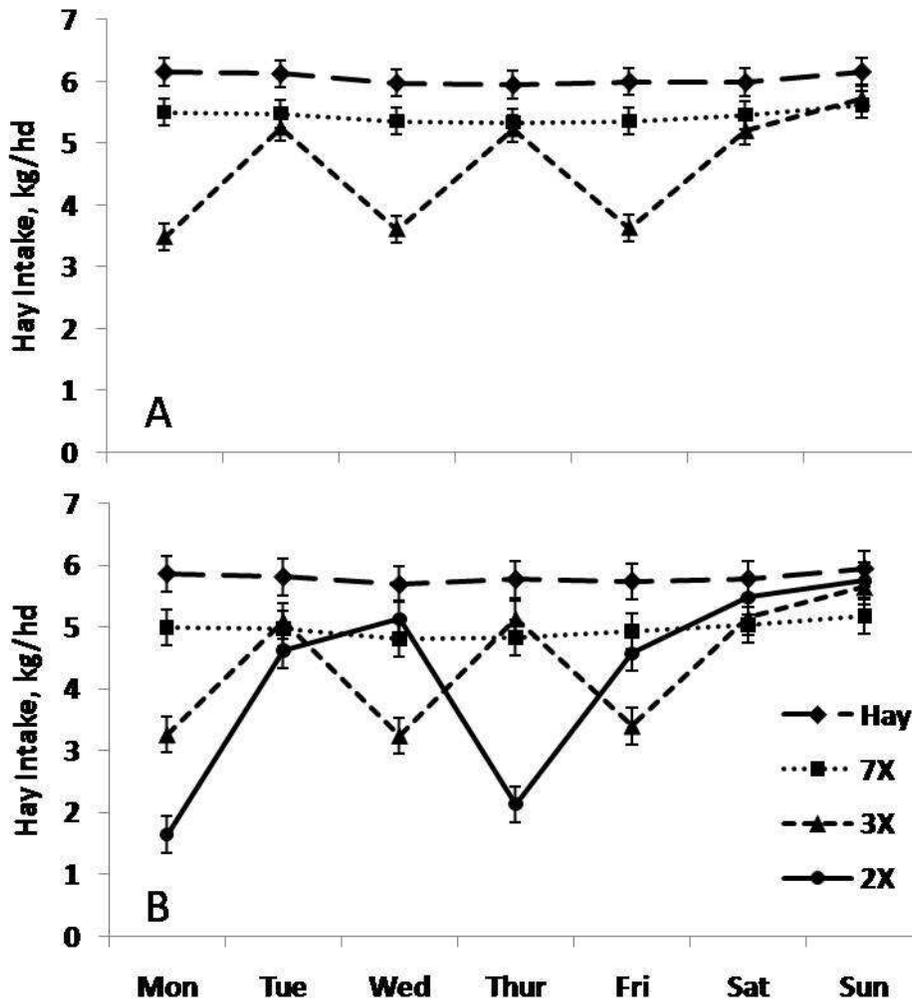
Item	Treatment		
	H	7X	3X
Hay, \$	887	798	680
Supplement, \$	-	590	590
Mineral, \$	84	84	84
Hay tractor, \$	67	67	67
Feed truck, \$	-	242	104
Labor for hay feeding, \$	55	55	55
Labor for travel to feed supplement, \$	-	192	82
Labor for supplement feeding, \$	-	38	38
Total operating & labor costs, \$	1,093	2,066	1,700
Cost, \$/hd	55	103	85
Cost, \$/kg of gain	2.71	1.66	1.37

<sup>1</sup>Hay, supplement and mineral costs were estimated at 0.088, 0.143, and 0.440 \$/kg of DM, respectively. Hay feeding estimated to take 30 minutes per week. Travel to feed supplement was estimated at 15 minutes per feeding event. Equipment operating costs (\$11.15/h for a 55 hp tractor and \$11.50/h for a ½ ton truck) are from Benson and Poore (2006) and labor was valued at \$9.15/h. Fixed costs were not considered in this scenerio. Hay intake and gain based on 4 years of data.

**Table 5.** Effect of travel time required to feed supplement and size of operation on estimated operating, labor costs for stockering beef steers for 84 days when feeding medium quality hay alone (H) or supplementing hay daily (7X) or three times a week (3X) with mix of corn gluten feed and soybean hull pellets<sup>1</sup>

Cost	15 minute travel time			30 minute travel time		
	H	7X	3X	H	7X	3X
20 head						
Feed, \$	971	1,472	1,354	971	1,472	1,354
Equipment operation, \$	67	308	170	67	550	274
Labor, \$	55	285	176	55	478	258
Cost, \$/hd	55	103	85	55	125	94
Cost, \$/kg of gain	2.71	1.66	1.37	2.71	2.01	1.52
50 head						
Feed, \$	2,428	3,680	3,385	2,428	3,680	3,385
Equipment operation, \$	134	375	237	134	617	341
Labor, \$	110	379	269	110	571	351
Cost, \$/hd	53	89	78	53	97	82
Cost, \$/kg of gain	2.65	1.43	1.25	2.65	1.57	1.31

<sup>1</sup>Hay, supplement and mineral costs were estimated at 0.088, 0.143, and 0.440 \$/kg of DM, respectively. Hay feeding estimated to take 30 minutes per week for 20 hd and 1 h for 50 hd. Equipment operating costs (\$11.15/h for a 55 hp tractor and \$11.50/h for a ½ ton truck) are from Benson and Poore (2006) and labor was valued at \$9.15/h. Fixed costs were not considered. Hay intake and gain based on 4 years of data.



**Figure 1.** Hay intake (kg/hd) of steers fed ad-libitum medium quality fescue hay and either not supplemented (Hay), supplemented daily (7X) with 2.73 kg/hd, supplemented on Monday, Wednesday and Friday (3X) with 6.36 kg/hd or supplemented on Monday and Thursday with 9.55 kg/hd (2X) with a blend of soyhulls and corn gluten feed during all four years (A) and during the last two years (B). Treatment by day of week interaction ( $P > 0.01$ ).

## **CHAPTER 3**

### **EFFECT OF SUPPLEMENTATION FREQUENCY ON RUMINAL FERMENTATION AND DIGESTION OF STEERS FED MEDIUM QUALITY HAY AND SUPPLEMENTED WITH A SOYHULL AND CORN GLUTEN FEED BLEND**

## Abstract

Reducing frequency of supplementation would reduce labor and equipment costs and has potential to increase profit if performance is not greatly affected. The effect of reducing supplementation frequency on ruminal fermentation and digestion is most likely a major determinant of the gain. Six ruminally cannulated beef steers ( $362 \pm 18$  kg BW) were used in a replicated 3 x 3 Latin square design to determine the effect of supplementation frequency (daily or on alternate days) on digestion and ruminal fermentation when feeding medium quality hay and supplementing with a mixture of soybean hulls and corn gluten feed. Dietary treatments consisted of fescue hay (8.8% CP and 34.8% ADF) fed ad-libitum that was supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H). The supplement (14.6% CP and 29.8% ADF) contained 47% soybean hull pellets, 47% corn gluten feed pellets, 2% feed grade limestone, and 4% molasses (as-fed). Each period consisted of a 12 d adaptation phase followed by 6 d of total fecal, urine, and ort collection. All supplement offered was consumed within 2 h. Ruminal fluid was collected every 4 h for 2 d. Hay intake was reduced ( $P < 0.01$ ) for SD and further reduced ( $P < 0.01$ ) for SA. Hay intake was 1.54, 1.19 and 1.02% BW (SEM  $\pm$  0.036) for H, SD and SA, respectively. There was a significant ( $P < 0.01$ ) treatment by day interaction for mean ruminal pH. On the day of supplementation, ruminal pH for SA (6.13) was lower ( $P < 0.01$ ) than both SD (6.29) and H (6.52). However, on the day SA did not receive supplement ruminal pH of SA (6.53) did not differ ( $P = 0.87$ ) from H and was greater ( $P < 0.01$ ) than SD. Ruminal pH of SD was lower ( $P < 0.01$ ) than H. Diet DM digestibility was increased ( $P < 0.01$ ) by supplementation but did not differ ( $P = 0.58$ ) due to frequency. Dry matter

digestibility was 57.9, 64.1, and 64.6% (SEM  $\pm$  0.65) for H, SD, and SA, respectively. The amount of N retained was greater ( $P < 0.01$ ; SEM  $\pm$  2.5) for both supplemented treatments (24.9 and 22.0 g/d for SD and SA, respectively) than H (4.2 g/d) but did not differ ( $P = 0.47$ ) due to frequency. When supplementing a blend of soybean hulls and corn gluten feed producers can reduce frequency of supplementation to every other day without reducing digestibility or N retention.

## **Introduction**

The labor cost associated with hand feeding supplements can contribute significantly to the overall cost of supplementation. Reducing the frequency of supplementation would reduce labor and equipment costs and has the potential to increase profit. The responses to less frequent supplementation have been variable and may be determined by the effects of this feeding strategy on ruminal fermentation. Reducing the frequency of protein supplementation to beef cows consuming low quality forage has been shown to have little to no effect on performance or digestion (Kunkle et al., 2000) while less frequent energy supplementation in the form of cereal grains has been reported to negatively effect performance (Kartchner and Adams, 1982). Grains are high in non-structural carbohydrates (NSC) and feeding large amounts can reduce ruminal pH and decrease fiber digestion (Hoover, 1986). Regardless of NSC content, energy supplementation can reduce the degradable intake protein to total digestible nutrient (DIP:TDN) ratio of the diet and cause negative associative affects on fiber digestion (Bodine et al., 2001). At moderate feeding rates ( $< 0.5\%$  BW/d), reducing supplementation frequency to 3 times a week when using a

grain-based supplement with intermediate protein levels has caused little or no reduction in digestion of low (Tellier et al., 2004) or medium quality forage (Loy et al. 2007).

However, growing cattle consuming hay based diets are often supplemented at 1% BW/d or higher. At high levels of supplementation (> 0.5% BW/d), feeding fibrous byproducts such as corn gluten feed and soybean hulls have been shown to have a less negative impact on forage intake and digestibility than grain based supplements (Bowman and Sanson, 1996). These fibrous byproducts are high energy, contain moderate amounts of ruminally degradable protein, and have a less negative effect on ruminal pH than grains. Therefore, they appear to be good candidates for use in systems where cattle are supplemented with larger quantities less frequently. The objective of this study was to examine the effect of frequency of supplementing a mixture of soybean hulls and corn gluten feed on ruminal parameters and digestibility when steers are fed medium quality hay and supplemented daily at 1% BW or on alternate days at 2% BW.

## **Materials and Methods**

Six cannulated steers ( $362 \pm 18$  kg) were used in a replicated, 3x3 Latin square design. Steers were housed in individual tie stalls (11.5 x 17.8 m) with individual feeders and water cups. At the beginning of the trial, steers were dewormed with Safeguard drench (Intervet, Millsboro, DE) and Cydectin (Fort Dodge Animal Health, Overland Park, KS). Dietary treatments consisted of ad-libitum hay that was supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a blend of soybean hulls and corn gluten feed. Steers were weighed in the morning on the first day of

each adaptation period and the amount of supplement offered during each period was based on this initial weight. The steers were allowed to exercise in an outdoor pen on a regular basis (2 times a week for at least 1 h in the morning) between collection periods. Steers had access to a trace mineral block (Buckeye Feed Mills, Inc., Dalton, OH; 96- 98% NaCl, 0.5% Zn, 0.4% Mn, 0.25% Cu and 0.004% Se) throughout the trial. To prevent heat stress, the temperature in the room was maintained below 27°C by use of air conditioning units located at the rear of the barn. Lights in the barn were set to provide 14 h of light each day.

The supplement was offered at 0800. The supplement contained 47% corn gluten pellets, 47% soybean hull pellets, 2% feed grade limestone, and 4% molasses (as-fed). Chopped medium quality tall fescue mix hay [*Lolium arundinaceum* (Schreb.) Darbysh] was offered in two equal portions (morning and afternoon). The morning hay was offered to H and SA steers at 0800 on the days they did not receive supplement. Hay was offered to SD and to SA steers at 1000 on the days they were supplemented. All supplement was consumed before the morning hay feeding. The afternoon hay portion was offered to all steers at 1500. Hay was offered at 115% of the previous day's intake for H and SD steers. The hay offered to SA steers was 115% of the intake from 2 d prior, therefore the amount fed was based on intake from the previous time that the steers were either supplemented or not supplemented. The experimental periods were 18 d, with 12 d of adaptation and a 6 d collection period. All steers were given supplement at 1% BW for 3 d prior to the start of each adaptation period. The nutrient content of the hay and the supplement is shown in Table 1.

***Digestibility and nitrogen balance.*** Prior to the collection phase pens were thoroughly scrubbed and washed. Separation boards, designed to allow visual contact among

steers, were attached to the pens to minimize cross-contamination of feces between steers. Hair on the underline and hind legs of each steer was clipped to minimize matting of fecal material and to allow for secure attachment of the urine harness. Total collection of urine, feces, and orts was conducted during the 6 d collection phase. Daily grab samples of hay and supplement were collected and pooled on an equal weight bases.

Urine was collected by aspiration into polypropylene jugs from a urine collection harness as described by Archibeque et al. (2001). Urine was acidified (pH < 5) with 6 N acetic acid to prevent N volatilization and 1 L of deionized water was added to prevent a crystal residue from forming. Urine was collected daily and a representative aliquot was retained. All aliquots were pooled for each steer in a given period. Urine samples were analyzed for Kjeldahl N using AOAC (1999) procedures and for urea content using the diacetyl monoxime method of Marsh et al. (1957), using a Technicon Auto Analyzer (Technicon Instruments Corporation, Tarrytown, NY).

Feces were also collected daily, weighed, and a representative sample (5% of total wt) was retained. After the sixth day of collection feces attached to the steers and harnesses were removed and collected, and steers were removed from the pens and the pens were thoroughly scraped to ensure total collections. Scrapings were included in the final day's output of feces. Fecal samples were dried with forced air at 55°C for 48 h. Due to a sampling error, one steer receiving SD in the third period was removed from digestibility and N balance measurements.

***Rate of passage.*** Marked feeds were prepared by soaking 75 g/L of hay or soybean hulls in distilled water which contained 2.5 g/liter of hydrated rare earth chloride for 24 h.

Hay was marked with dysprosium (Dy) and soybean hulls with ytterbium (Yb). After 24 h, the solution was decanted and replaced with deionized water 5 times at hourly intervals. Following the last rinse the feeds were dried at 60°C. Approximately 15 min prior to the morning feeding on d 2 of each collection period, marked feeds (approximately 500 mg of rare earth per ingredient) was mixed into a small portion (150 g) of unmarked supplement and was offered prior to morning feeding. Steers consumed all of the marked feed within 15 min. To determine liquid passage rate 10 g of Cobalt-EDTA was mixed with 200 mL of water and dosed directly into the rumen via the cannula.

Total feces was collected from the floor just prior to dosing and at intervals of 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96 and 120 h after dosing. Feces collected during each interval were thoroughly mixed and 100 g was retained for marker analysis. These samples were dried for at least 48 h at 60°C. The remaining feces were added to the total collection to be subsampled for digestibility. The grams of wet feces removed for marker analysis was recorded to adjust digestibility measures. Fecal samples (3 g) were ashed overnight at 600°C, solubilized by boiling in a 20 mL 50:50 mixture of 3N HCL and 3N HNO<sub>3</sub> and were allowed to stand overnight (Ellis et al., 1982). Then potassium chloride was added to control for ionization (final concentration was 150 ppm) and samples were brought up to 25 mL with deionized water and stored at room temperature until analysis. Prior to analysis samples were diluted 10X and analyzed on an inductively coupled plasma-optical emission spectrometer (Perkin Elmer Optima 2000 DV; Perkin Elmer, Wellesey, MA) at the wavelengths 228.616, 240.780 and 297.056 for Co, Dy, and Yb, respectively.

Excretion curves for were fit to a two compartment, time independent model as described by Grovum and Williams (1973) using linear regression (curve peeling).

***Rumen measurements.*** On d 2 and d 3 of the collection period, ruminal fluid was collected using a suction strainer from various locations in the rumen just prior to the morning feeding and at 4, 8, 12, 16, 20, and 24 h post morning feeding to determine pH, ammonia-N, and short chain fatty acids (VFA). Immediately after sampling, ruminal fluid pH was determined (Fisher Scientific Accumet AP63 portable pH meter, Cole-Parmer Instruments). Approximately 30 mL of ruminal fluid was retained and placed on crushed ice to stop fermentation and then frozen until preparation for analysis of ammonia and VFA concentration. This allowed samples to be taken on a day that both SD and SA treatments were supplemented (d 2) and on a day when only SD was supplemented (d 3). Area under the curve was calculated for ruminal fluid pH and VFA concentrations during the 48 h period using the trapezoidal method between each set of time points and summing areas. Grab samples of the rumen mat in the dorsal and ventral portions of the rumen were also collected every 4 h over the 48 h period. The fluid from these samples was extruded by hand (pooled within animal) and pH was immediately determined.

***Chemical analyses.*** Hay, ort, supplement, and fecal samples were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen and stored in sealed plastic containers at room temperature until analyzed. Hay, ort, supplement, and feces were analyzed for DM, ash, and Kjeldahl N using AOAC (1999) procedures. Neutral detergent fiber, ADF, and cellulose were sequentially determined using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology, Fairport, NY). Urine was kept at -20°C until analyzed

for Kjeldahl N using the AOAC (1999) procedure and for urea content using the diacetyl monoxime method of Marsh et al. (1957), adapted for use in a Technicon autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Concentrations of VFA were determined in the ruminal fluid using a Varion 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) with a Nikol fused-silica capillary column (15 m; 0.53 mm i.d.; 0.5- $\mu$ m film thickness; Supelco, Bellefonte, PA). Ruminal ammonia was determined by the colorimetric procedure used for Kjeldahl N (AOAC, 1999).

To determine the potentially digestible NDF (PDNDF) content of the hay, supplement, and orts, 2 ruminally cannulated steers (~525 kg BW) were fed ad-libitum novel endophyte infected fescue hay (9.5% CP and 38% ADF) and 3.63 kg·hd<sup>-1</sup>·d<sup>-1</sup> of the soybean hull and corn gluten feed blend (SH/CGF) for 10 d. Following the 10 d adaption, triplicate Dacron bags (4 x 5 cm; 25  $\mu$ m pore size) containing 0.39 g of sample were placed inside a weighted laundry bag and placed in the rumen of each steer (total of 90 bags with sample and 3 blank bags per steer) and incubated for 120 h. Immediately after the bags were removed from the rumen they were placed in ice water, transported back to the laboratory, and NDF content was determined. The NDF remaining after incubation was considered to be the indigestible NDF (INDF) fraction. The PDNDF was calculated as the difference between NDF and INDF.

***Statistical analysis.*** All data were analyzed using the Mixed procedure of SAS and the effects of period, animal, and square were considered random. The models for measures of digestibility, N balance, and area under the curve included the fixed effect of treatment. Analysis of hay intake included the effects of treatment, day of collection, and their

interaction. When treatment was significant ( $P \leq 0.05$ ), differences among means were separated using single df contrasts. The comparisons made were 1) hay only vs supplementation (H vs average of SD and SA) and 2) supplementation frequency (SD vs SA). For total VFA concentration, VFA molar proportions, pH, and ammonia-N concentration in the ruminal fluid, the model included the fixed effects of treatment, day, time, and their interactions.

## Results

**Hay intake.** Hay intake was reduced by supplementation ( $P < 0.01$ ) and hay intake of SA was lower ( $P < 0.01$ ) than SD (Table 2). On the day of supplementation, hay intake of SA was less ( $P < 0.01$ ) than both H and SD. On the days that SA did not receive supplement, hay intake was still reduced ( $P < 0.01$ ) compared to those H, but was greater ( $P < 0.01$ ) than SD (Figure 1). The intake of supplement ( $3.03$  and  $3.06 \text{ kg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ ) of SD and SA did not differ ( $P = 0.74$ ;  $\text{SEM} \pm 0.10$ ). Although hay intake was reduced for SA, the proportion of the diet that was supplement (44%) did not differ ( $P = 0.20$ ) from SD (40%;  $\text{SEM} \pm 1.07$ ).

**Passage parameters.** The passage parameters of hay did not differ due to treatment ( $P = 0.91$ ; Table 3). Whereas, ruminal passage rate of soybean hulls was increased ( $P = 0.02$ ) by supplementation, but did not differ due to frequency ( $P = 0.67$ ). Despite the increase in ruminal passage, the mean retention time of soybean hulls in the digestive tract did not differ ( $P = 0.15$ ) due to treatment. The passage parameters of liquid in the digestive tract of H and SA did not differ ( $P = 0.45$ ). Ruminal liquid passage rate was increased ( $P \leq 0.02$ ) for SD. Mean retention time of liquid in the digestive tract tended to be ( $P = 0.08$ ) and was ( $P = 0.05$ ) decreased for SD compared to H and SA, respectively.

**Diet digestibility.** Total diet DM, NDF, ADF and cellulose digestibility was increased ( $P < 0.01$ ) and CP digestibility was decreased ( $P < 0.01$ ) by supplementation (Table 4). Diet DM, NDF, ADF, cellulose and CP digestibility did not differ ( $P \geq 0.14$ ) due to supplementation frequency (Table 3). The percent of PDNDF that was digested was decreased ( $P = 0.01$ ) by supplementation but did not differ due to supplementation frequency ( $P = 0.43$ ; Table 4).

**Ruminal fluid pH, ammonia-N, and VFA.** For all ruminal fluid parameters (pH, ammonia-N, and VFA) there was a significant ( $P < 0.01$ ) treatment by day by time interaction. The pH of the fluid from the rumen mat was consistently lower than the pH of ruminal fluid collected from the liquid phase using the suction strainer method. The pH of the fluid from the rumen mat was highly correlated ( $R = 0.84$ ;  $P < 0.01$ ) with the pH of ruminal fluid collected from the liquid phase. Regardless of collection method, the differences among treatments and patterns throughout the day were similar (Figure 2). The pH of the ruminal fluid collected via the suction strainer was used for comparison of treatments as this method is more commonly employed. Ruminal pH decreased after supplementation and the minimum pH of SA (5.85) was lower ( $P < 0.01$ ) than SD (6.05). On the day of supplementation (d 2), SA had a lower ( $P < 0.01$ ) mean ruminal fluid pH than SD (6.12 and 6.29 for SA and SD, respectively; SEM  $\pm 0.05$ ). However, on the day SA was not supplemented (d 3), ruminal fluid pH (6.53) did not differ ( $P = 0.53$ ) from H (6.52) and was greater ( $P < 0.01$ ) than SD (6.29). It has been suggested that the impact of low pH on ruminal fermentation is dependent not only on mean pH but also on the amount of time that ruminal pH is suboptimal (Cerrato et al., 2007). When using the mean ruminal fluid pH of H (6.5) as

a baseline to determine the extent of pH depression over the 48 h period, the area under the curve for SA and SD did not differ ( $P = 0.94$ ; 11.20 vs 11.27, respectively).

During the 48 h period the ammonia-N concentration of H ranged from a high of 4.2 mg/dL at 4 h after feeding (1200) to a low of 2.2 mg/dL at 16 h after feeding (0000) with a mean of 3.0 mg/dL (SEM  $\pm$  0.64; Figure 3). For both supplemented groups, ammonia-N concentrations peaked 4 h after supplementation (1200; Figure 3). The peak ammonia-N concentration of SA (14.1 mg/dL) was greater ( $P < 0.01$ ) than SD (8.1 mg/dL; SEM  $\pm$  0.64). The initial rise in ammonia-N of the supplemented treatments was followed by a depression in ammonia-N concentrations. The nadir was lower ( $P = 0.04$ ) for SA (1.3 mg/dL at 0000 on d 2) than SD (2.6 mg/dl at 1600).

The total VFA concentration in the ruminal fluid sampled at 4 h intervals over the two day period is shown in Figure 4. Total VFA concentrations of SA peaked (114 mM) 8 h after supplementation (16:00 on d 2) and remained greater ( $P < 0.05$ ) than H until (32 h after supplementation (1600 on d 3). Total VFA concentrations of SD peaked (112 mM) at 4 h after supplementation (1200) and then began to decrease until the next supplementation event. Just prior to supplementation (24 h after previous supplementation event; 0800), total VFA concentration of SD did not differ ( $P \geq 0.19$ ) from H. Total ruminal VFA concentration (SEM  $\pm$  2.3) of SA and SD did not differ ( $P = 0.33$ ) on day of supplementation (100 and 102 mM for SD and SA, respectively) and were greater ( $P < 0.01$ ) than H (83 mM). Total VFA concentration of SA (87 mM) on the day after supplementation (d 3) was lower ( $P < 0.01$ ) than SD (100 mM) and did not differ ( $P = 0.18$ ) from H (84 mM).

The molar proportion of acetate was less ( $P < 0.01$ ) and propionate was greater ( $P < 0.01$ ) for SD and SA by four hours after supplementation than those H (Figure 5). Although decreasing in magnitude, the molar proportion of acetate for SA remained lower ( $P < 0.01$ ) than H until 20 h after supplementation (0400 on d 2) and the molar proportion of propionate remained greater ( $P < 0.01$ ) than H until 24 h after supplementation (0800 on d 3). However, by 8 h after supplementation (1600 on both d 2 and d 3) the molar proportion of acetate of SD did not differ ( $P \geq 0.51$ ) from H and by 12 and 8 h after supplementation on d 2 and 3, respectively (0000 on d 2 and 1600 on d 3) the proportion of propionate did not differ ( $P = 0.09$ ;  $P = 0.68$ ) from H. The proportion of butyrate for SA was greater ( $P < 0.02$ ) than those SD and H at 4 through 12 h after supplementation (1200, 1600, and 0000 on d 2; Figure 5). The area under the VFA concentration-time curve for total VFA, acetate, propionate, and butyrate was increased ( $P < 0.01$ ) by supplementation, but did not differ due to frequency ( $P \geq 0.32$ ; Table 5).

***N balance.*** Intake of N, fecal excretion of N, and the amount of N retained was increased by supplementation ( $P < 0.01$ ; Table 6). Due to the reduction in hay intake, the N intake and fecal excretion of SA were reduced ( $P = 0.02$ ) when compared to SD (Table 6). However, the amount of urinary N excreted and the proportion of urinary N that was urea did not differ ( $P = 0.58$ ;  $P = 0.57$ ) due to supplementation frequency (Table 6). Frequency of supplementation did not affect the amount of N retained ( $P = 0.47$ ; Table 6).

## Discussion

In this study, daily supplementation of a SH/CGF blend reduced hay intake and reducing supplementation frequency further decreased hay intake. Several studies have also shown an increased substitution rate when cattle are supplemented less frequently (Beaty et al., 1994; Loy et al. 2007; Loy et al, 2008). Intake of ruminants is regulated by metabolic feedback as well as physical constraints. Forbes (1996) proposed that these mechanisms are not mutually exclusive and can have an additive effect. On the day of supplementation, steers SA had elevated ruminal VFA concentrations, decreased ruminal fluid pH, and consumed 4.69 kg of NDF per steer. Most likely the combination of fill, ruminal acidity, and metabolic feedback from absorption of VFA caused the large decrease in hay intake that was observed when steers SA were supplemented. On the day after supplementation, VFA concentrations of steers SA remained elevated for the first half of the day. It is also likely that the increased gut fill that steers experienced on the day of supplementation would have not fully dissipated by the next day. Steers may have continued to respond to these signals on the day after supplementation causing their intake to remain lower than H.

Supplementation with soybean hulls and corn gluten feed increased the intake of digestible fiber and as would be expected, supplementation with these feeds increased total diet DM and fiber digestibility. However, the digestibility of PDNDF was decreased due to supplementation. The amount of fiber digested in the rumen is a function of the rate of digestion and the rate of passage out of the rumen. Reported estimates of the rate of NDF digestion of soybean hulls (0.01- 0.07/h) and corn gluten feed (0.02-0.048/h) has varied

(Firkins, 1997) but are within the range of estimates reported for tall fescue hay (0.062/h; Miller and Muntifering, 1985).

Therefore, the higher ruminal passage rate of the supplement may be a major factor explaining the lower digestibility of potentially digestible NDF. In this study, soybean hulls had a greater ruminal passage rate than the fescue hay and passage rate of soybean hulls was further increased in the supplemented treatments. The increased passage rate of soybean hulls caused by supplementation is most likely due to the replacement of hay by supplement in the diet which may have reduced the consistency of the ruminal mat. Both Weidner and Grant (1994) and Mulligan et al. (2002) observed lower ruminal mat consistency of diets with high amounts of soybean hulls when compared with high forage diets. Therefore, the replacement of forage with SH/CGF blend may have increased the amount of potentially fermentable fiber that escaped ruminal fermentation.

Despite lower nadirs in ruminal pH and ammonia-N, the total diet digestibility of steers SA did not differ from those SD. This verifies the need to consider the total amount of time in which ruminal conditions may limit rate of digestion, not just minimum concentrations of ruminal pH or ammonia-N. Additionally, the passage rate of hay and soybean hulls did not differ due to supplementation frequency. Although the proportion of the diet that was supplement was similar for steers SD and those SA over a 48 h period, on the day of supplementation dietary intake of steers SA consisted of a higher proportion of supplement (71%) than steers SD (40%). Therefore, it might be expected that passage rate of soybean hulls would be increased by SA. However, dietary intake of steers SA consisted solely of hay during the day prior to supplementation and the presence of a dense ruminal

mat at the time of supplement consumption may have helped to slow passage rate despite the high level of supplement intake.

The concentration of ammonia-N needed to maximize digestion appears to depend on the fermentability of the feedstuff (Erdman et al., 1986). In-vivo estimates of ammonia-N needed for maximal rate of digestion have ranged from 4.5 mg/dL for low quality grass hay (*Heteropogon contortus*; Boniface et al., 1986) to 12.5 mg/dL for barley grain (Odle and Schaeffer, 1987). While fiber digestion was not affected by less frequent supplementation in this study, the dramatic drop in ammonia-N that occurred in steers SA should be taken into consideration. The concentration of ammonia-N in the rumen was below 2 mg/dL for an 8 h period and was below 5 mg/dL for a period of 16 h during the 48 h period. The intake and digestion of a large amount of fermentable substrate (supplement) most likely caused this trough in ammonia-N concentration.

On the day of supplementation the diet of the steers SA consisted of mainly supplement therefore it may be important for the DIP:TDN of the supplement to be balanced to ensure adequacy of available N for microbial growth and digestion. In this study the supplement had a moderate CP content (15%) and the protein was highly ruminally degradable (75 and 78% DIP for soybean hulls and corn gluten feed, respectively; NRC, 1996). The DIP:TDN ratio of the supplement was calculated to be 0.14 when using NRC (1996) values and would have been considered to be adequate. However, when a supplement has a low level of ruminally degradable protein, the drop in ammonia-N may be more prolonged and could result in decreased digestion when supplemented less frequently.

Kartchner and Adams (1982) reported negative effects of less frequent energy supplementation on performance of beef cows when a low-protein energy supplement was used in a situation where digestion would have already been limited by ruminally available N (low quality forage). Research that used grain-based supplements with intermediate protein levels observed little or no reduction in digestion (Chase and Hibberd, 1987; Tellier et al., 2004; Loy et al. 2007). The results of this current study taken together with these previous studies suggest that when supplementation frequency is reduced the balance of ruminally degradable N to supplemental energy may be vital to the maintenance of digestion.

The VFA concentrations in the rumen are affected by pool size of the ruminal fluid and the rate of VFA absorption, which are in turn affected by ruminal fluid pH, osmolarity, and passage rate. The molar proportions of VFA in ruminal fluid do not necessarily represent the proportions that are formed or absorbed (Dijkstra, 1994; Lopaz et al., 2003). It has been shown that as ruminal pH decreases, the rate of VFA absorption increases (Annison, 1965), and often the rate of propionate and butyrate absorption relative to acetate increases (Danielli et al., 1945; Lopaz et al., 2003). Therefore, caution should be used when making extrapolations from measurement of VFA concentrations in the rumen.

On the day of supplementation VFA concentrations of steers SA was not greater than that of steers SD. Given that steers SA consumed twice as much supplement and that the ruminal liquid passage rate was lower, it would be expected that VFA concentrations of steers SA would have been greater than those SD. It is possible that on the day of supplementation steers SA had an increased rate of absorption due to a lower ruminal pH or

that the liquid pool of steers SA was greater than those SD although these factors were not measured.

It is also interesting to note that despite the lower pH on the day of supplementation there was a small increase in the proportion of propionate and butyrate in the ruminal fluid for steers SA compared to those SD. We have previously found that ADG of growing steers supplemented with a SH/CGF blend, 3 times a week, did not differ from those supplemented daily, despite having lower hay intake (Drewnoski et al., 2008). The differences in the VFA profile deserves further examination as it may help explain the greater gain to feed ratio that has been previously observed with less frequent supplementation of a SH/CGF supplement.

## **Conclusions**

Supplementing a blend of soybean hulls and corn gluten feed at 2% of BW on alternate days decreased hay intake but did not affect the digestibility of the diet or N balance of steers compared to daily supplementation at 1% of BW. Therefore, when supplementing medium quality hay with a blend of soybean hulls and corn gluten feed, producers can reduce supplementation costs by decreasing supplementation frequency without negatively affecting digestion or N retention. The DIP:TDN ratio of the supplement may be central to the maintenance of digestion when supplementation frequency is reduced.

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**Table 1.** Nutrient composition of hay and supplement fed to steers during collection period

	% of DM								
	DM,%	CP	NDF	ADF	Cellulose	INNDF <sup>1</sup>	Ca	P	S
Hay	91.0	8.8	67.1	34.8	31.0	21.7	0.31	0.23	0.17
Supplement <sup>2</sup>	90.5	14.6	50.2	29.8	27.8	3.9	0.81	0.45	0.16

<sup>1</sup>INNDF= indigestible NDF as determined by NDF still present after by 120 h in-situ incubation in rumen.

<sup>2</sup>The supplement contained 47% corn gluten pellets, 47% soybean hull pellets, 2% feed grade limestone, and 4% molasses.

**Table 2.** Intake of steers when fed ad-libitum medium quality fescue hay and either not supplemented (H), supplemented daily at 1% of BW (SD), or supplemented every other day at 2% of BW (SA) with a soybean hulls and corn gluten feed blend

Item	Treatment			SEM	<i>P</i> -value <sup>1</sup>	
	H	SD	SA		Supp	Freq
Hay DMI, % BW	1.54	1.20	1.02	0.04	< 0.01	< 0.01
Total DMI, % BW	1.54	2.00	1.83	0.04	< 0.01	< 0.01

<sup>1</sup>Contrast: Supp = Hay only (H) vs. supplementation (SD and SA) ; Freq = SD vs. SA.

**Table 3.** Passage parameters<sup>1</sup> of hay, soybean hulls and liquid when steers were fed ad-libitum medium quality fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soybean hulls and corn gluten feed blend

	Treatment			SEM	P-value <sup>2</sup>		
	H	SD	SA		Trt	Supp	Freq
Tall fescue hay							
k1	0.028	0.027	0.028	0.003	0.91	0.94	0.68
k2	0.28	0.33	0.25	0.03	0.15	0.58	0.06
Tau	4.4	5.3	6.1	1.15	0.46	0.29	0.54
MRT	50.1	47.1	49.8	4.07	0.53	0.51	0.37
Soybean hulls							
k1	0.034	0.038	0.039	0.003	0.06	0.02	0.67
k2	0.25	0.30	0.27	0.03	0.35	0.30	0.31
Tau	3.9	5.1	4.8	1.59	0.64	0.38	0.84
MRT	38.9	35.5	34.9	1.97	0.15	0.06	0.77
Liquid							
k1	0.047	0.054	0.048	0.002	0.02	0.04	0.02
k2	0.66	0.59	0.68	0.09	0.64	0.77	0.39
Tau	3.1	2.3	4.2	1.19	0.42	0.92	0.21
MRT	26.2	22.9	26.8	1.13	0.10	0.36	0.05

<sup>1</sup>k1 = passage rate (/h) of slow (second) compartment; k2 = passage rate (/h) of fast (first) compartment; Tau = time delay (h); MRT = mean retention time [(1/k1)+ (1/k2) + Tau]

<sup>2</sup>Trt = treatment effect; Supp = contrast of hay only (H) vs. supplementation (SD and SA); Freq = contrast of SD vs. SA.

**Table 4.** Digestibility (on DM basis) of the diet when steers were fed ad-libitum medium quality fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soybean hulls and corn gluten feed blend

Item	Treatment			SEM	<i>P</i> -value <sup>1</sup>	
	H	SD	SA		Supp	Freq
DM,%	57.9	64.1	64.6	0.65	< 0.01	0.58
NDF,%	58.9	63.0	63.1	0.88	< 0.01	0.93
ADF,%	56.3	63.0	62.1	0.73	< 0.01	0.28
Cellulose,%	63.3	68.3	67.6	0.82	< 0.01	0.54
CP,%	53.1	58.1	59.7	1.37	< 0.01	0.09
PDNDF <sup>2</sup> ,%	86.7	82.6	81.4	1.06	0.01	0.43

<sup>1</sup>Contrast: Supp = Hay only (H) vs. supplementation (SD and SA); Freq = SD vs. SA.

<sup>2</sup>Potentially digestible NDF.

**Table 5.** Area under the VFA concentration-time curve (mmol/ h) during a 48h period for steers fed ad-libitum medium quality fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soybean hulls and corn gluten feed blend

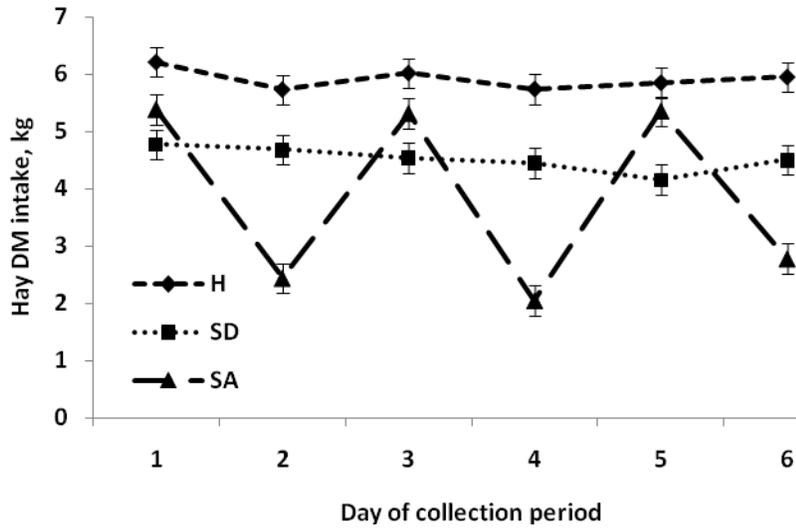
Item	Treatment			SEM	<i>P</i> -value <sup>1</sup>	
	H	SD	SA		Supp	Freq
Total VFA	412	510	491	15.3	< 0.01	0.32
Acetate	352	438	429	13.4	< 0.01	0.57
Propionate	240	301	307	10.2	< 0.01	0.57
Butyrate	224	281	290	9.8	< 0.01	0.37

<sup>1</sup>Contrast: Supp = H vs. supplementation (SD and SA) ; Freq = SD vs. SA.

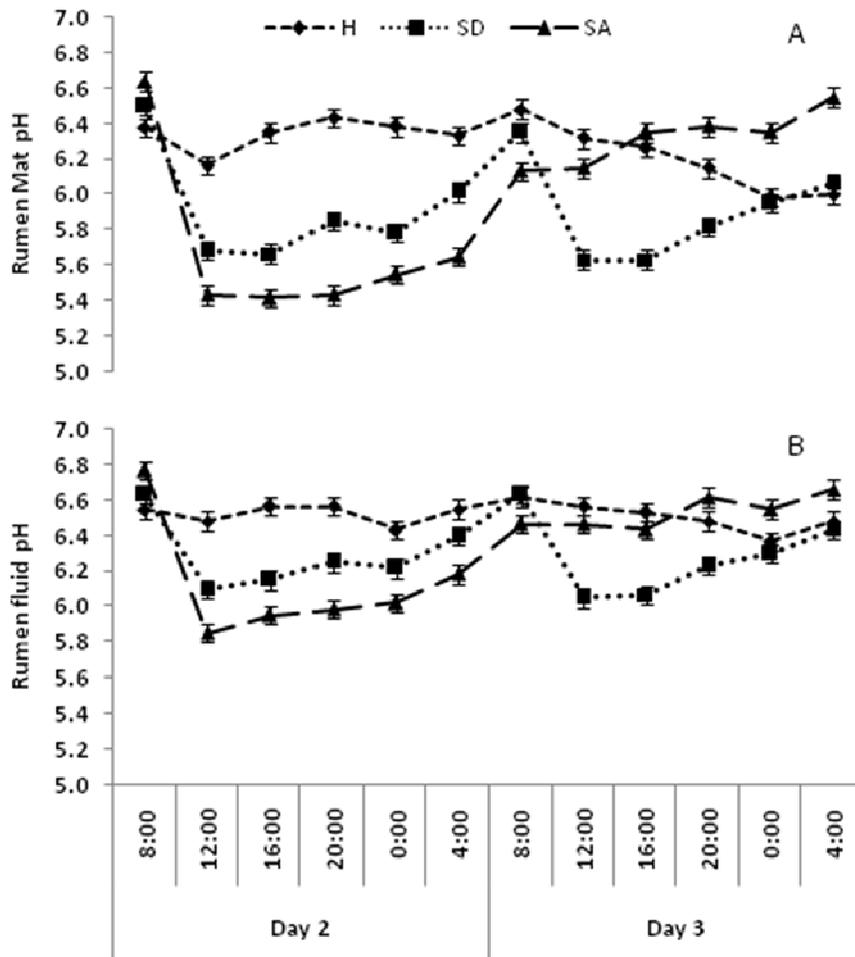
**Table 6.** Nitrogen balance and urinary urea N of steers fed ad-libitum medium quality fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soybean hulls and corn gluten feed blend

Item	Treatment			SEM	<i>P</i> -value <sup>1</sup>	
	H	SD	SA		Supp	Freq
N intake, g/d	85	138	126	5.9	< 0.01	0.02
Fecal N, g/d	39.5	57.3	51.0	2.5	< 0.01	0.02
Urinary N, g/d	41.2	55.1	53.4	2.6	< 0.01	0.58
N retained, g/d	4.2	24.9	22.0	4.9	< 0.01	0.47
N retained as a % of N intake	3.8	17.6	17.3	3.9	< 0.01	0.92
N retained as a % of N digested	7.1	29.9	28.5	6.6	< 0.01	0.82
Urinary urea N, g/d	9.8	12.1	12.1	1.3	0.14	0.98
Urine urea N, % of urinary N	18.2	24.0	25.9	2.2	0.04	0.57

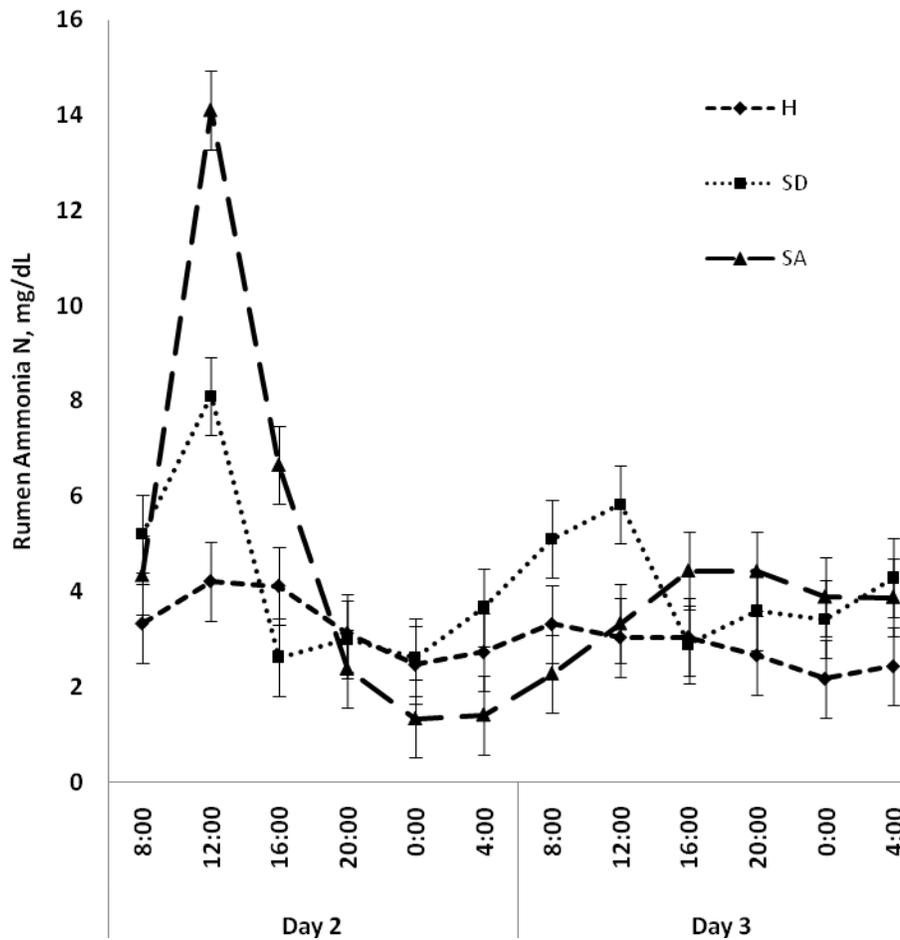
<sup>1</sup>Contrast: Supp = H vs. supplementation (SD and SA) ; Freq = SD vs. SA.



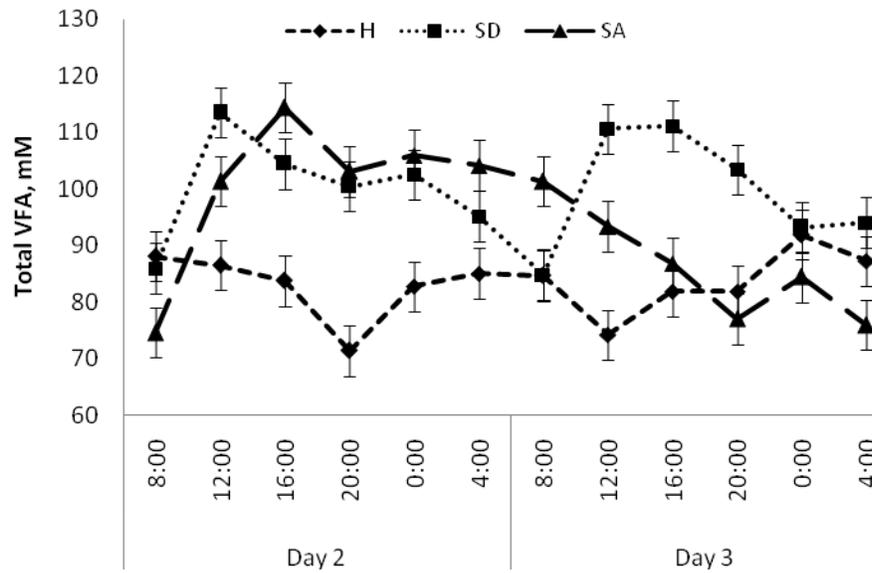
**Figure 1.** Hay intake of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend. Steers supplemented on alternate day received supplement on d 2, 4, and 6 of the collection period. Treatment by day effect ( $P < 0.01$ ).



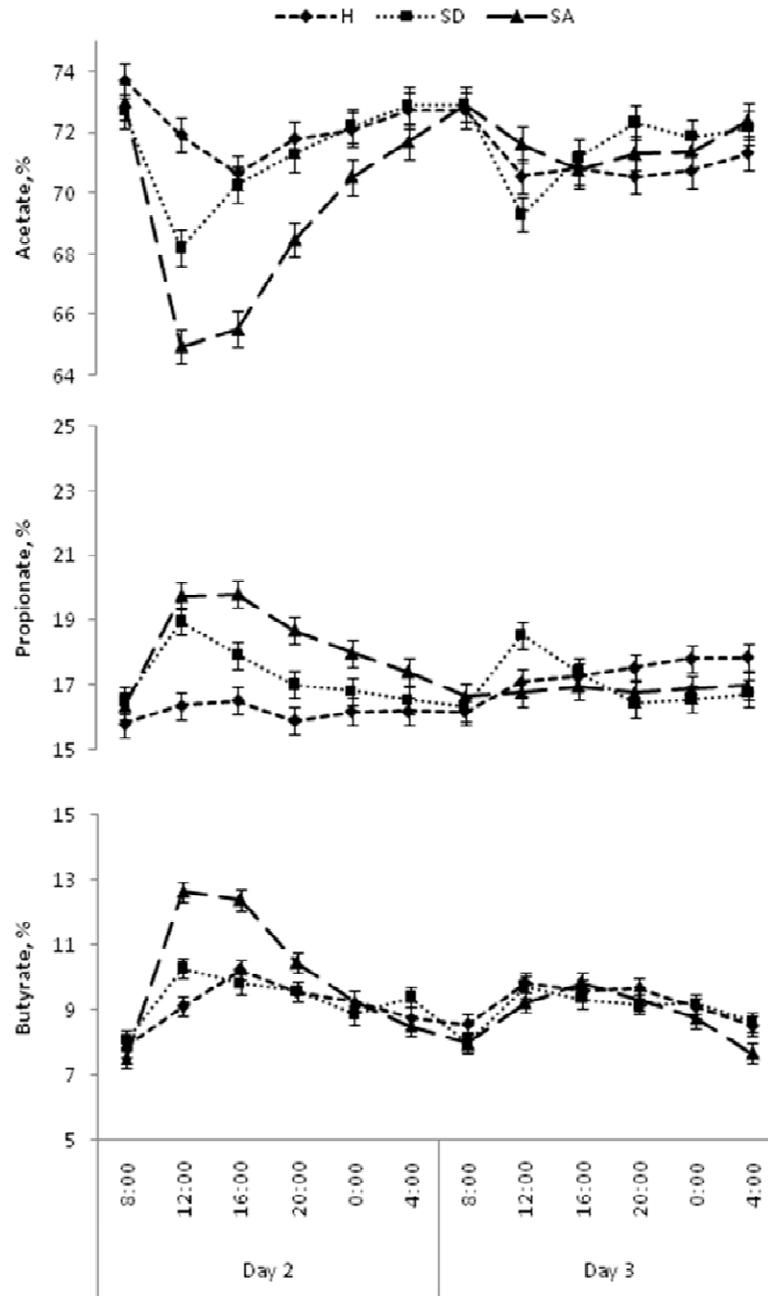
**Figure 2.** The pH of ruminal fluid from the rumen mat (B) and ruminal fluid collected via suction strainer (A) of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend. Steers were supplemented at 0800. Steers on SA only received supplement on d 2. Treatment by day by time effect ( $P < 0.01$ ).



**Figure 3.** The ammonia-N concentration of ruminal fluid collected via suction strainer of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend. Steers were supplemented at 0800. Steers on SA only received supplement on d 2. Treatment by day by time effect ( $P < 0.01$ ).



**Figure 4.** The VFA concentration (mM) in ruminal fluid of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend. Steers were supplemented at 0800. Steers on SA only received supplement on d 2. Treatment by day by time effect ( $P < 0.01$ ).



**Figure 5.** The molar proportion of acetate, propionate and butyrate in ruminal fluid of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend. Steers were supplemented at 0800. Steers on SA only received supplement on d 2. Treatment by day by time effect ( $P < 0.01$ ).

## **CHAPTER 4**

**REDUCED SUPPLEMENTATION FREQUENCY INCREASED INSULIN-LIKE  
GROWTH FACTOR-1 IN BEEF STEERS FED MEDIUM QUALITY HAY AND  
SUPPLEMENTED WITH A SOYBEAN HULL AND CORN GLUTEN FEED BLEND**

## Abstract

Reducing supplementation frequency in calf growing programs can reduce labor and equipment costs. However, little is understood about the metabolic response of ruminants to large fluctuations in nutrient intake. Eighteen Angus or Angus X Simmental cross steers ( $287 \pm 20$  kg and  $310 \pm 3.6$  d) were individually fed 1 of 3 dietary treatments using Calan gates. Dietary treatments consisted of ad-libitum hay and no supplement (H), ad-libitum hay and 1% BW of supplement daily (SD), or ad-libitum hay and 2% BW of supplement every other day (SA). The supplement contained (as-fed) 47% corn gluten feed, 47% soybean hulls, 2% feed grade limestone, and 4% molasses. Hay intake and ADG was measured over a 52-d period. Steers were then moved to individual tie stalls and blood samples were collected every hour from 0600 to 1400 and at 1800, 2200, and 0200 over a 2 d period. Gains were increased ( $P < 0.01$ ) by supplementation, but did not differ ( $P = 0.68$ ) due to supplementation frequency. Average daily gain was  $0.45$ ,  $0.90$ , and  $0.87$  kg  $\cdot$ hd<sup>-1</sup>  $\cdot$ d<sup>-1</sup> (SEM  $\pm 0.05$ ) for steers H, SD, and SA, respectively. Area under the concentration time curve (AUC) for plasma glucose was increased ( $P < 0.01$ ) by supplementation, but did not differ ( $P = 0.41$ ) due to supplementation frequency. The AUC for plasma insulin was increased by supplementation ( $P < 0.01$ ) and tended ( $P = 0.10$ ) to be increased by less frequent supplementation. Plasma IGF-1 was increased ( $P = 0.01$ ) by supplementation and further increased ( $P = 0.04$ ) by less frequent supplementation. Plasma urea-N concentrations of steers SD and SA fluctuated more than those H, but AUC did not differ due to treatment ( $P = 0.77$ ). Gains of steers supplemented with a soybean hull corn gluten feed blend on alternate days did not differ from those supplemented daily suggesting the steers were able to efficiently utilize large

quantity of nutrients fed every other day. The effect of less frequent supplementation on insulin and IGF-1 deserves further examination as these hormones have been shown to decrease protein degradation and increase protein synthesis.

## **Introduction**

Supplements are often fed to growing cattle to improve animal performance with the ultimate goal of increasing economic returns. Feeding supplements less frequently would reduce labor and equipment costs and has the potential to increase profit if performance is not negatively affected. When supplementation frequency is reduced the amount of supplement fed each week remains the same but the amount fed during each supplementation event is increased. This can exacerbate any negative effects of supplementation on forage intake and digestion (Knukle et al., 2000). A supplement containing a mixture of soybean hulls and corn gluten feed has many favorable characteristics (high in digestible fiber, low in non-structural carbohydrates and high in ruminally degradable protein) that allow it to be fed less frequently to cattle consuming forage without negatively affecting digestion (Drewnoski, 2009).

Compared to daily supplementation, less frequent supplementation (2 or 3 times a week) with a soybean hull and corn gluten feed mix did not appear to affect gains of steers consuming medium quality grass hay (7-10% CP and 34-41% ADF) but did consistently reduce hay intake (Drewnoski, 2009). Therefore, it appears that steers fed less frequently with a soybean hull and corn gluten feed blend have a greater gain to feed ratio (more efficient) than those supplemented daily.

Reduction of supplementation frequency changes the pattern of incoming nutrients and could therefore alter blood concentrations of hormones. Increased rates of gain in cattle have been associated with increased concentrations of insulin and IGF-1 (Bishop et al., 1989; Vizcarra et al., 1998; Lapierre et al., 2000). Insulin and insulin-like growth factor 1 (IGF-1) are anabolic hormones that respond to nutritional status of the animal (Thissen et al., 1994). These hormones have been shown to decrease protein degradation and increase protein synthesis (Florini et al., 1996; Tesseraud, 2006). Therefore increases in insulin or IGF-1 could explain the greater efficiency of steers supplemented less frequently. The objectives of this study were to compare the effect of daily vs. alternate day supplementation of a soybean hull and corn gluten feed mix on growth and hay intake of individually fed steers and to determine the effect of supplementation frequency on concentrations of metabolites and hormonal growth regulators in blood.

## **Materials and Methods**

Eighteen Angus or Angus X Simmental cross steers ( $287 \pm 20$  kg and  $310 \pm 3.6$  d) were blocked by BW and breed and randomly assigned to 1 of 3 treatments. Prior to the beginning of the trial steers were dewormed (Cydectin; Fort Dodge Animal Health, Overland Park, KS). Steers were housed in groups of 6 in a  $13 \text{ m}^2$  pen with slotted floors and an automatic waterer. Steers were individually fed using Calan gate electronic feeders (American Calan, Northwood, NH). Dietary treatments consisted of ad-libitum hay and no supplement (H), ad-libitum hay and 1% BW of supplement daily (SD), or ad-libitum hay and 2% BW of supplement every other day (SA). The supplement contained (as-fed) 47% corn

gluten feed, 47% soybean hulls, 2% feed grade limestone, and 4% molasses. Steers receiving supplement were fed at 0800 and hay was provided 30 min later. Steers not receiving supplement were fed hay at 0800. Hay was offered to all steers in two portions (1/2 in morning and 1/2 in afternoon at 1530). Hay was offered at 110% of the previous day's intake for H and SD steers. The hay offered to the SA steers on days they received supplement was 110% of the previous day they received supplement and hay offered on days they did not receive supplement was 110% of the intake from the previous day they did not receive supplement. Steers had access to a trace mineral block (Buckeye Feed Mills, Inc., Dalton, OH; 96- 98% NaCl, 0.5% Zn, 0.4% Mn, 0.25% Cu and 0.004% Se) throughout the trial.

Hay and supplement was sampled weekly for determination of nutrient content throughout the trial. Prior to feeding, the square baled tall fescue hay (*Lolium arundinaceum* (Schreb.) Darbysh) was sliced using a S600 VanDale Bale Processor 197 (J-Star Industries, Fort Atkinson, WI) with the blades spaced 12.5 cm apart. Due to a limited supply of a single lot of hay, three different hay lots were fed. During the first 20 d of the trial all steers received endophyte infected Kentucky-31 fescue hay. On d 21, 3 blocks of steers (one heavy, one medium and one light weight block) began receiving novel endophyte infected HiMag fescue hay (Ark+) and 3 blocks of steers (one heavy, one medium and one light weight block) began receiving novel endophyte infected Jessup fescue hay (MaxQ).

The two novel endophyte infected hays were cut from adjacent fields at the same time and thus were similar in nutrient content (Table 1). Feed was dried in a forced-air oven at 55°C to a constant weight and then air-equilibrated for 48 h to determine air-equilibrated DM. Samples were then ground in a Model 4 Wiley mill (Arthur A. Thomas Co., Philadelphia,

PA) to pass through a 1-mm screen. Dry matter (105°C), ash, and Kjeldahl N were determined according to AOAC (1999) procedures. Concentrations of NDF and ADF were sequentially determined as described by Van Soest et al. (1991) but modified for use with an Ankom apparatus (Ankom Technology, Macedon, NY). The DM, CP, NDF, and ADF content of the hay and supplement is shown in Table 1. The supplement was analyzed for mineral content by a commercial laboratory (Cumberland Valley Analytical Services; Maugansville, MD) and contained 0.76% Ca, 0.68% P and 0.30 % S on a DM basis.

### ***Performance and intake***

Prior to the beginning of the trial steers were weighed on two consecutive days (d -2 and -1) before the morning feeding. Then at 1500 on d -1 feed and water was removed and steers were weighted 16 h later (d 0). Steers were weighed prior to morning feeding on d 25 and 26 and the amount of supplement fed was adjusted based on these weights. Steer weights were taken in the morning prior to feeding on d 52 and 53 and in the afternoon (1500) on d 53 feed and water was removed and weights were taken the following morning (d 54). Hay intake from d 1 and 2 was not included in the analysis because intake was abnormally high due to steers being previously fasted for determination of shrunk weights.

### ***Serial blood sampling***

From d 63 to 80, serial blood samples for hormone and metabolites were collected. Prior to the start of the serial blood sampling phase the amount of supplement being fed was adjusted based on BW taken on d 52 and 53. On d 54, the heaviest 2 blocks of steers (one being fed MaxQ and the other Ark+) were placed in individual metal tie stalls (1.5 X 3 m) with a feeder and water cup. Steers were adapted to tie stalls for 8 d and on d 62 these steers

were fitted with indwelling jugular catheters to measure circulating hormone and metabolite levels. On d 63 and 64, intensive blood samples were collected every hour from 0600 to 1400 and additional blood samples were taken at 1800, 2200, and 0200 on both days.

Blood samples were collected into 10 mL Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) with heparin as the anticoagulant. During the blood sampling procedure 5 mL of blood and anticoagulant was drawn and discarded, then a blood 10mL sample was saved, and 4 mL of heparin was used to flush the catheter. Blood samples were placed on ice until plasma was harvested and frozen at -20°C. On d 59, the two medium weight blocks of steers began adaptation in wooden tie stalls (1.2 X 3 m) and were moved to metal tie stalls on d 65d. On d 70 they were catheterized followed by blood sampling on d 71 and 72. The lightest two blocks of steers began adaptation in the wooden stalls on d 67, were moved to tie stalls on d 73, were catheterized on d 77, and blood samples were taken on d 78 and 79.

### ***Blood analysis***

Insulin, glucose and ghrelin were measured on all blood samples taken during the two day period. Plasma insulin was measured using a commercial solid-phase radioimmunoassay (Coat-A-Count insulin kits; Diagnostic Products Corporation, Los Angeles, CA) using bovine insulin as a standard. Plasma was analyzed for glucose according to Bran and Luebbe's colorimetric method G-142-95, Rev. 1 (Bran and Luebbe Auto Analyzer Methods). Plasma urea-N (PUN) was analyzed by the diacetyl monoxime method of Marsh et al. (1957) on samples taken every 4 h over the two day period (0600, 1000, 1400, 1800, 2200 and 0200), using a Technicon Auto Analyzer (Technicon Instruments Corporation,

Tarrytown, NY). For determination of IGF-1 concentration, 200  $\mu$ l of plasma from samples taken every 4 h were pooled within day and quantified by radioimmunoassay using a commercial kit (DSL-2800; Linco Research, St. Charles, Missouri).

### ***Statistical analysis***

All data were analyzed using the Mixed procedure of SAS. Block, breed, and steer (treatment by hay) were considered random effects. The model for insulin, glucose, and PUN concentrations included the fixed effect of treatment, hay type, day, time and their interactions. Analysis of hay intake and IGF-1 concentration included the effects of treatment, day, hay type, and their interaction. The analysis of ADG and area under the concentration time curve for insulin, glucose, and PUN included the effects of treatment, hay type, and their interaction. Non-significant ( $P > 0.20$ ) interactions were removed from the model. When treatment was significant ( $P \leq 0.05$ ), differences among means were separated using single df contrasts. The comparisons made were 1) hay only vs. supplementation (H vs. average of SD and SA), and 2) supplementation frequency (SD vs. SA).

## **Results**

### ***Hay intake and average daily gain***

There was a treatment by hay type by day interaction for hay intake ( $P < 0.01$ ; Figure 1). For all hay types the hay intake of H was greater ( $P < 0.01$ ) than SD, and the hay intake of SA on the day they received supplement was lower ( $P < 0.01$ ) than both H and SD. Across all hay types, hay intake of steers SA increased on the day they did not receive supplement, but the extent of the increase varied among hay types. Hay intake of SA on unsupplemented

days was intermediate between H and SD when steers were fed Kentucky-31 during the first part of the trial. During the second half of the trial hay intake of SA on unsupplemented days was greater ( $P < 0.01$ ) than SD and did not differ ( $P = 0.54$ ) from H when steers were fed Ark+. Intake of SA only tended to be greater ( $P = 0.07$ ) than SD and was lower ( $P < 0.01$ ) than H and when steers were fed MaxQ. For all hay types, the mean hay intake of SD and SA did not differ ( $P \geq 0.66$ ), but were lower ( $P < 0.01$ ) than H. The mean hay intake across all hay types for each treatment is shown in Table 2.

The ADG of steers was increased ( $P < 0.01$ ) by supplementation but did not differ ( $P = 0.68$ ) due to supplementation frequency (Table 2). There was a tendency for hay type to affect ( $P = 0.10$ ) ADG with steers being fed MaxQ gaining more than those being fed Ark+ (0.69 and 0.78 kg  $\cdot$ hd<sup>-1</sup>  $\cdot$ d<sup>-1</sup>, respectively; SEM  $\pm$  0.04). The gain to feed ratio was increased ( $P < 0.01$ ) by supplementation but did not differ ( $P = 0.89$ ) due to supplementation frequency (Table 2).

### ***Blood metabolites and hormones***

There was no effect ( $P > 0.20$ ) of hay type on plasma concentrations of metabolites or hormones. There were significant ( $P < 0.01$ ) treatment by day by time interactions for plasma insulin and glucose. At all time points during the 48 h period plasma glucose concentrations were increased ( $P < 0.05$ ) by supplementation (Figure 2A). Mean glucose concentrations of SA and SD did not differ ( $P = 0.45$ ). For both supplemented treatments there was a short period (~ 3 h) of decreased glucose concentration around supplementation. A short period (~ 2 h) of decreased glucose concentration was also observed for H around the morning hay feeding. However, the glucose of SA on the day supplement was not fed (d 2) did not

decrease due to the morning hay feeding. This caused the glucose concentration of SA on the day they did not receive supplement (d 2) to be greater ( $P < 0.02$ ) than SD for a 3 h period on d 2 around supplementation. Glucose did not differ ( $P > 0.10$ ) during the rest of the day.

Insulin concentrations (Figure 2B) of H remained steady with a mean concentration of 11.5  $\mu\text{IU/mL}$  over the 48 h period. Insulin concentration of SD also remained relatively steady over the 48 h period. Insulin concentration of SD was greater ( $P < 0.05$ ) than H, except during the 2 h period immediately prior to supplementation during which time insulin decreased and did not differ ( $P > 0.20$ ) from H. Insulin concentrations of SA had a greater range in concentration over the 48 h period. Insulin concentration of SA prior to supplementation (0200 through 0800 on d 1) did not differ ( $P > 0.20$ ) from H but increased following supplementation and remained greater ( $P < 0.05$ ) than H until 2200 on d 2 (a 34 h period). By 2 h after supplementation (d 1) insulin concentration of SA was greater ( $P \leq 0.05$ ) than SD and remained greater ( $P < 0.05$ ) than SD throughout the 22 h period after supplementation. On d 2, insulin concentrations of SA began to decrease and by 2200 insulin concentrations of SA were lower ( $P < 0.01$ ) than SD and remained lower ( $P < 0.05$ ) until the next supplementation event.

Plasma IGF-1 did not differ due to day ( $P = 0.72$ ), but was affected by treatment ( $P < 0.01$ ; Figure 3). Plasma IGF-1 was increased ( $P < 0.01$ ) due to supplementation and was greater ( $P = 0.04$ ) for SA compared with SD.

There was significant ( $P < 0.01$ ) treatment by day by time interaction for PUN. Peak concentrations of both SD (13.2 mg/dL) and SA (13.4 mg/dL) were greater ( $P < 0.01$ ) than H (11.50 mg/dL). Despite the intake of a larger bolus of N on the day SA were supplemented (d

1), the peak PUN concentration of SA did not differ ( $P = 0.82$ ) from SD (Figure 4). The PUN nadir of SA (6.19 mg/dL) did not differ ( $P = 0.25$ ) from SD (7.12 mg/dL) but was lower ( $P < 0.01$ ) than H (8.15 mg/dL). Although SD and SA had more fluctuation in PUN concentrations than H, the mean PUN concentration (9.7 mg/dL) did not differ due to treatment ( $P = 0.77$ ).

The area under the glucose, insulin and plasma urea-N concentration-time curves are shown in Table 3. The area under the glucose concentration-time curve was increased ( $P < 0.01$ ) by supplementation but did not differ ( $P = 0.41$ ) due to supplementation frequency. The area under the insulin concentration-time curve was increased ( $P < 0.01$ ) by supplementation and tended ( $P = 0.10$ ) to be greater for SA than SD. The area under the PUN concentration-time curve did not differ ( $P = 0.77$ ) due to treatment.

## **Discussion**

Growing cattle consuming hay based diets are given high energy supplements containing moderate amounts of protein to increase performance. We have previously observed that less frequent supplementation (2 or 3 times a week) of growing steers with a soybean hull and corn gluten feed blend (similar to the one used in the current study) reduced hay intake compared to those supplemented daily, but did not affect gain (Drewnoski, 2009). A blend of soybean hulls and corn gluten feed is high in energy but low in non-structural carbohydrates (NRC, 1996). It also contains a moderate amount of protein (17%; Table 1), much (77%) of which is ruminally degradable (NRC, 1996). Therefore, it can be fed less frequently without negatively affecting digestibility (Drewnoski, 2009).

In agreement with our previous study, gains in the current study did not differ due to supplementation frequency. However, in the current study reduced hay intake was not observed when cattle were supplemented on alternate days. For steers supplemented three times a week, hay intake on the day following supplementation was equal to those that were supplemented daily (Drewnoski, 2009). In the current study, the hay intake of SA steers on the day they did not receive supplement was usually greater than those supplemented daily thus causing hay intake over the 48 h period to be similar for SA and SD.

Success of less frequent supplementation of energy based supplements may at least partially depend on availability of ruminal N for efficient microbial growth and digestion. Loy et al. (2008) showed that the effects of supplementation frequency on gains of heifers consuming medium quality hay tended to differ depending on the source of protein used in the energy supplement. Hay intake was decreased but gains were not negatively affected by reducing supplementation frequency to 3 times a week (3X) when a supplement high in DIP (dry rolled corn plus urea) was fed. Thus the heifers supplemented with dry rolled corn and urea 3X, had a greater gain to feed ratio than those supplemented daily. Interestingly, the purine derivative to creatinine ratio in the urine was greater for heifers supplemented with dry rolled corn plus urea 3X than those supplemented daily suggesting that heifers supplemented 3X absorbed more microbial protein.

We have previously observed that steers SA had a greater peak in rumen ammonia-N following supplementation than those supplemented daily (14 vs. 8 mg/dL) and also had a lower ruminal pH (5.8 vs. 6.1) than SD following supplementation (Drewnoski, 2009). In the present study, the peak concentration of PUN following supplementation did not differ

between SD and those SA despite SA consuming twice as much supplement at once. The lower pH in the rumen of SA may have decreased the rate absorption of ammonia into the blood stream as the ionized molecule ( $\text{NH}_4^+$ ) is less permeable than its unionized ( $\text{NH}_3$ ) counterpart (Smith, 1979). It is possible that reduced rate of absorption may have prolonged the supply of ruminal-N and allowed for increased microbial growth.

Compared to SD the insulin concentration of SA was greater for 24 h, equal for 14 h and lower for 10 h during the 48 h period. Therefore, the area under the insulin concentration time curve tended to be greater for SA than SD. The increase in insulin concentration may have been due to increased absorption of VFA in to the portal blood or changes in the profile of VFA that were absorbed. In ruminants, insulin increases in response to increased absorption of propionate and butyrate (Manns and Boda, 1967). We have previously observed that on the day of supplementation molar proportions of both propionate and butyrate in the rumen of SA were increased compared to SD (Drewnoski, 2009). The major role of insulin is the promotion of metabolite storage in peripheral tissues. Insulin is the major regulator of glucose metabolism, and is also known to regulate protein metabolism. Insulin induces protein accretion by stimulating protein synthesis and inhibiting proteolysis (Lobley, 1998). Increased concentrations of insulin have been shown to decrease the utilization of amino acids for glucose production in ruminants and increase the incorporation of amino acids into muscle protein (Tesseraud, 2007). Increased concentrations of insulin can decrease the utilization glucogenic amino acids for glucose production (Huntington et al., 2006). Therefore, increased insulin of SA may have spared some glucogenic amino acids and allowed them to be utilized for growth.

Insulin like growth factor 1 is an endocrine regulator of muscle growth in cattle and forms a vital link between growth hormone and the metabolic process of growth. Like insulin, IGF-1 has been shown to increase protein synthesis in skeletal muscle and reduce the rate of protein degradation (Florini et al., 1996). The IGF-1 concentration of SA was greater than SD. Concentrations of IGF-1 are responsive to both plane of nutrition as well as the composition of the diet with level of protein appearing to be more important than energy content (Pell and Bates, 1990). Increased availability of amino acids for anabolism may be responsible for greater concentration of IGF-1 observed in SA. Steers on SA may have had increased absorption of microbial protein as was observed by Loy et al. (2008) or decreased utilization of amino acids for gluconeogenesis due to increased concentrations of propionate and insulin on the day of supplementation. More work is needed to understand why SA steers had greater IGF-1 than SD steers despite both treatments essentially consuming the same amount of nutrients over a 48 h period.

Little research has examined the effects of supplementation frequency on metabolic regulators. Studies from the University of Florida have examined the effects of supplementation frequency when growing cattle consuming low quality forage were supplemented with a citrus pulp/cottonseed meal supplement (Cooke et al., 2007) or a wheat middling-based supplement (Cooke et al, 2008) daily (1% BW) or 3X (2.3% BW). Unlike the present study, gains of supplemented cattle were low ( $< 0.45 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ ) and were reduced by less frequent supplementation in both studies. Insulin like growth factor-1 was not increased by less frequent supplementation. However, no unsupplemented control was used to determine if supplementation had an effect on IGF-1. In both studies, the nutrient

content of the forage alone would probably have been below maintenance. The nutrient intake of SA in our studies would have been above maintenance even on unsupplemented days. Therefore, plane of nutrition may explain the difference observed between our studies and those of Cooke et al., (2007 and 2008).

The effect of less frequent supplementation of a soybean hull and corn gluten feed blend on insulin and IGF-1 deserves further examination as it may explain why the steers supplemented three or two times a week with a soybean hull and corn gluten feed blend appear to be more efficient in terms of gain to feed ratio.

## **Conclusions**

Steers fed medium quality hay and supplemented with a soybean hull and corn gluten feed blend on alternate days were able to as efficiently utilize the supplement as those supplemented daily despite a having larger fluctuations in incoming nutrients. In this study the metabolic response of growing cattle to less frequent supplementation appeared to be positive as steers supplemented on alternate days had a greater concentration of IGF-1 and tended to have a greater area under the insulin concentration time curve. Further research is needed to elucidate the factors that affect the metabolic responses to less frequent supplementation.

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**Table 1.** Chemical composition of tall fescue hay<sup>1</sup> and supplement<sup>2</sup> fed to steers

Item	DM, %	% of DM		
		CP	NDF	ADF
Kentucky-31	92.8	11.6	64.8	35.6
MaxQ	93.8	9.1	67.8	36.6
Ark +	94.9	8.8	67.7	36.6
Supplement	89.6	17.2	48.0	26.6

<sup>1</sup>Kentucky-31 endophyte-infected fescue hay fed to all steers from d 1-20. Starting on d 21, steers began receiving MaxQ or Ark+ novel endophyte-infected tall fescue hay.

<sup>2</sup>The supplement was formulated to contain 47% soybean hulls, 47% corn gluten feed, 2% feed grade limestone and 4% molasses.

**Table 2.** Average daily gain, mean hay intake and gain to feed ratio of steers fed ad-libitum medium quality tall fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend

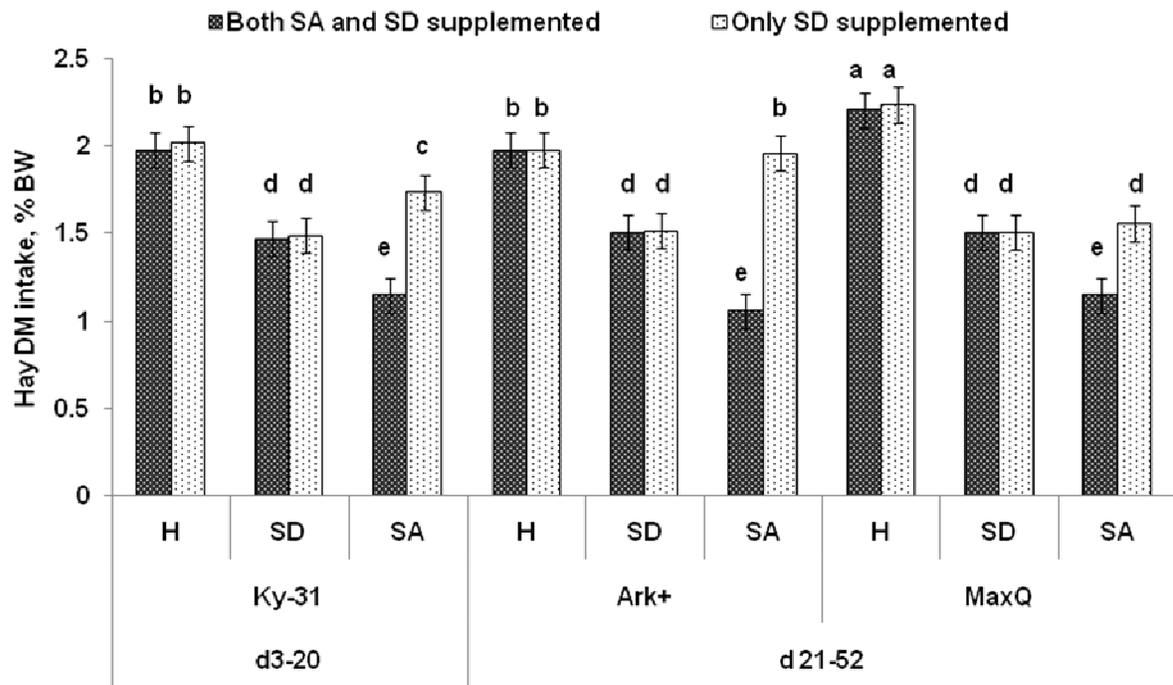
Item	Treatment			SEM	Supp	Freq
	H	SD	SA			
ADG, kg ·hd <sup>-1</sup>	0.45	0.90	0.87	0.05	< 0.01	0.68
Hay intake, kg ·hd <sup>-1</sup> ·d <sup>-1</sup>	6.06	4.50	4.31	0.33	< 0.01	0.41
Gain to feed ratio	0.070	0.123	0.122	0.008	< 0.01	0.89

**Table 3.** Area under the glucose, insulin and plasma urea-N concentration-time curves of steers fed ad-libitum medium quality tall fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend

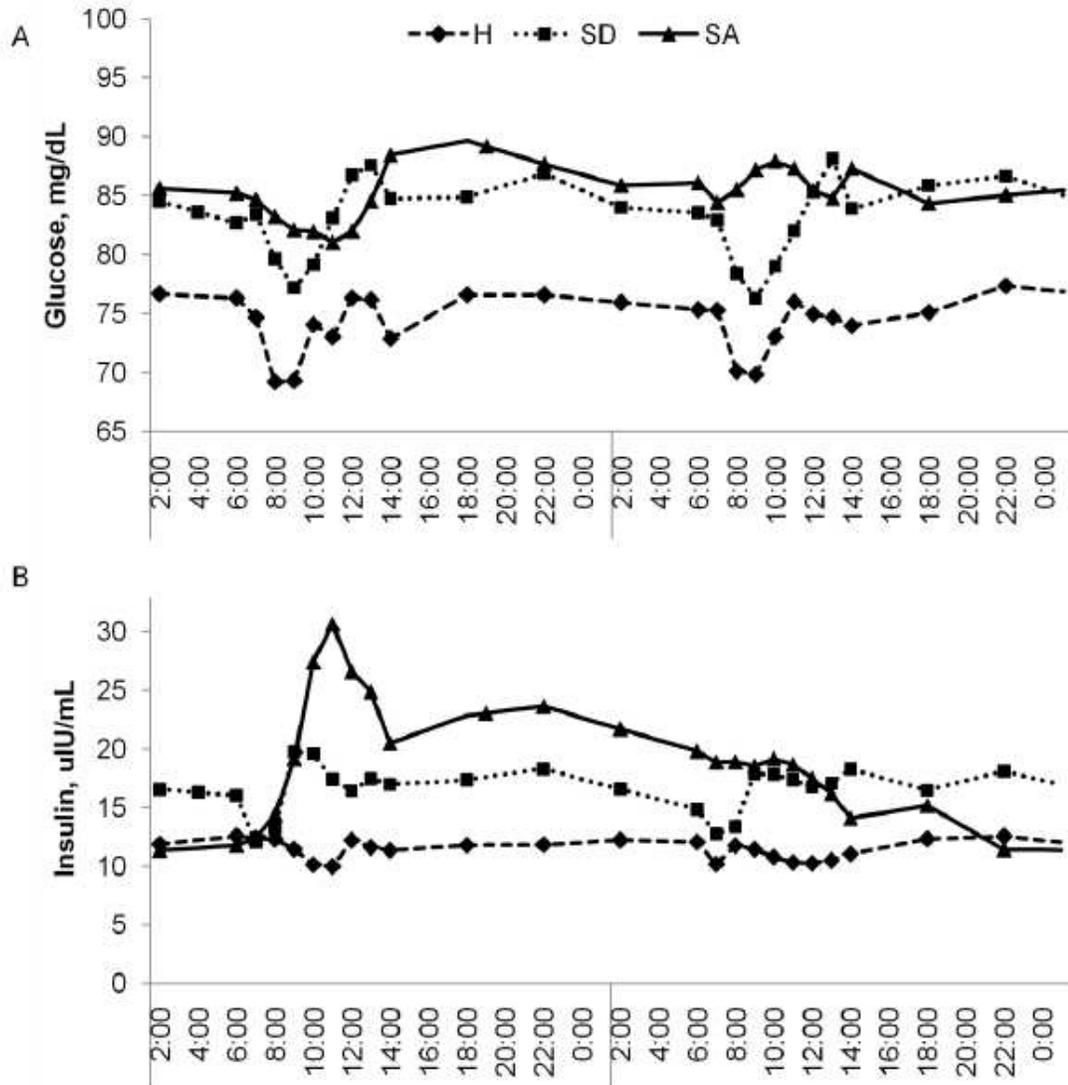
Area under the concentration time curve <sup>1</sup>	Treatment			SEM	P-value <sup>2</sup>	
	H	SD	SA		Supp	Freq
Glucose, mg·dL <sup>-1</sup> ·h	1707	1911	1962	42.2	< 0.01	0.41
Insulin, μIU·mL <sup>-1</sup> ·h	263	382	445	40.8	< 0.01	0.10
Plasma urea-N, mg·dL <sup>-1</sup> ·h	218	216	207	11.3	0.67	0.59

<sup>1</sup>Area under the curve determined by trapezoidal method

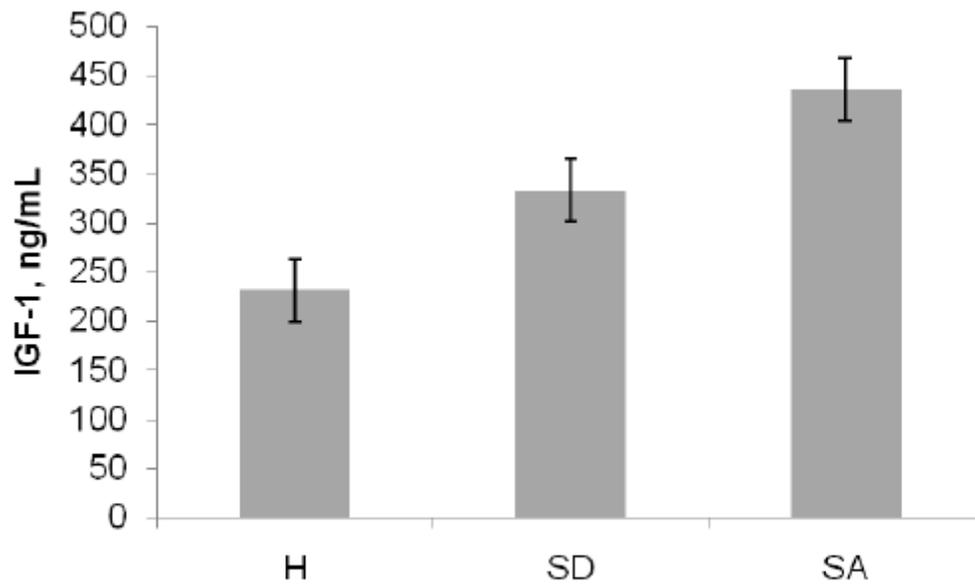
<sup>2</sup>Contrasts: Supp = H vs. supplementation (SD and SA); Freq = SD vs. SA.



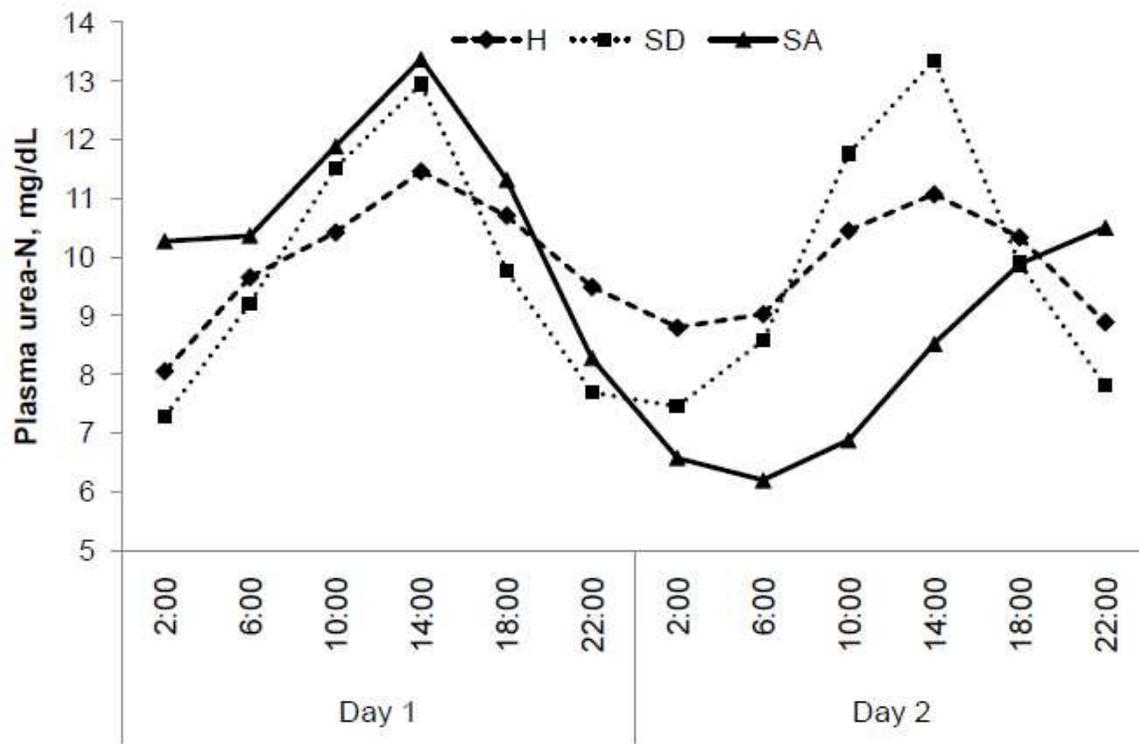
**Figure 1.** Hay intake of steers given ad-libitum access to medium quality Kentucky-31 (Ky-31) endophyte infected tall fescue hay from d 3-20 and novel endophyte infected Hi Mag (Ark+) or Jessup (MaxQ) tall fescue hay from d 21-52 and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend. Bars without common letters differ ( $P < 0.05$ ).



**Figure 2.** Plasma glucose (SEM  $\pm$  2.1) and insulin (SEM  $\pm$  2.0) concentration of steers fed ad-libitum medium quality tall fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend.



**Figure 3.** Plasma insulin like growth factor-1 (IGF-1) concentration of steers fed ad-libitum medium quality tall fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend. Treatment effect ( $P < 0.01$ ; SEM  $\pm 31$ ).



**Figure 4.** Plasma urea-N (SEM  $\pm$ 0.54) concentration of steers fed ad-libitum medium quality tall fescue hay and either not supplemented (H), supplemented daily (SD) at 1% of BW, or supplemented every other day (SA) at 2% of BW with a soyhulls and corn gluten feed blend.

## SUMMARY

Reducing supplementation frequency can reduce the cost of supplementation. The economic benefits of supplementing less frequently will vary greatly among individual farms. Small producers and producers that travel longer distances to feed cattle would benefit proportionally more from reducing supplementation frequency. A review of the literature indicates that the animal response to less frequent supplementation may depend on the characteristics of the supplement and the forage being fed. Protein supplementation generally does not result in negative associative effects and less frequent protein supplementation has been shown to be as effective as daily supplementation. Based on the current body of knowledge, the performance of cattle fed energy-based supplements less frequently cannot be predicted. However, it appears that the degradable intake protein to total digestible nutrient ratio of the diet may be important in determining response.

Soybean hulls and corn gluten feed are readily available, are an affordable source of supplemental energy and protein, and are widely used by producers to supplement growing cattle. The addition of a small amount calcium (limestone) to a blend of equal parts soybean hulls and corn gluten feed produces a well rounded supplement that is high energy, has a moderate CP content (15%) that is highly ruminally degradable, and has a balanced Ca:P ratio.

The goal of this research was to study the impact of less frequent supplementation of a blend of soybean hulls and corn gluten feed on performance of stocker cattle, the potential economic benefits for producers and determine the digestive and metabolic effects of this

feeding strategy. A series of performance trials were conducted to determine the effects of supplementing growing cattle consuming medium quality hay with a soybean hull and corn gluten feed blend 2 or 3 times a week. These studies showed that hay intake was reduced by less frequent supplementation but gains were not affected. As a result, the feed to gain ratio increased slightly with supplementing a blend of soybean hulls and corn gluten feed 2 or 3 times a week (compared to daily supplementation).

In a second experiment the effect of alternate day supplementation on digestion on ruminal fermentation was evaluated. Supplementation with a blend of soybean hulls and corn gluten feed increased digestibility of the diet and N balance but decreased hay intake. Hay intake was further decreased by reducing supplementation frequency to every other day but digestion and N balance were similar to those supplemented daily. Fluctuations in ruminal ammonia-N suggest that ruminally degradable N content of the supplement may be important for maintenance of digestion when supplementation frequency is reduced.

In a third experiment the metabolic response of growing steers supplemented on alternate days was assessed. Steers supplemented on alternate days had a greater concentration of IGF-1 and tended to have a greater area under the insulin concentration time curve than steers supplemented daily. These results may explain why the steers supplemented three or two times a week with a soybean hull and corn gluten feed blend appear to be more efficient than those supplemented daily.

The overall results of these suggest that when supplementing medium quality hay with a blend of soybean hulls and corn gluten feed, producers can reduce supplementation costs by decreasing supplementation frequency to as little as 2 times a week without

negatively affecting gains. However, responses to other forage and supplement combinations cannot be predicted and further research is needed. Success of less frequent supplementation may depend on the quality of forage being supplemented as well as the characteristics of the supplement. Future research should focus on determining the main factors that influence the performance responses to less frequent supplementation.

## APPENDIX

**Table 1a.** Mean body weight of steers (BW), amount of total digestible nutrients (TDN) supplemented to steers (% of BW) and quality of hay as determined by intake when fed alone (% of BW), TDN content (% of DM) and TDN to CP ratio of hay in years 1 through 4 of performance trial

	BW, kg	Supplemental TDN <sup>1</sup>	Hay		
			Intake	TDN <sup>2</sup>	TDN:CP
Year 1	263	0.76	2.62	59.9	6.31
Year 2	281	0.71	2.15	61.0	8.84
Year 3	271	0.74	2.46	65.2	6.58
Year 4	229	0.88	2.23	59.9	8.10

<sup>1</sup>Total digestible nutrient content of supplement was calculated to be 73.8% as determined by TDN values for soy hulls (77%) and corn gluten feed (80%) from the NRC,1996 and assuming that liquid yeast and limestone had no energy value

<sup>2</sup>Total digestible nutrient content was calculated using the equation: %TDN = 92.5135-(0.7965 x %ADF)

**Table 2a.** Treatment by day effect on rumen parameters<sup>1</sup> of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (SD), supplemented 2% BW on alternate days (SA), or not supplemented (H) with a soybean hull and corn gluten feed blend.

Item	Day 2			Day 3			SE
	H	SD	SA	H	SD	SA	
pH <sup>2</sup>	6.53 <sup>a</sup>	6.29 <sup>b</sup>	6.13 <sup>c</sup>	6.51 <sup>a</sup>	6.29 <sup>b</sup>	6.53 <sup>a</sup>	0.03
NH <sub>3</sub> , mg/dL	4.3 <sup>d</sup>	5.4 <sup>b</sup>	6.5 <sup>a</sup>	3.6 <sup>e</sup>	5.4 <sup>bc</sup>	4.7 <sup>cd</sup>	0.34
Total VFA, mM	82.9 <sup>b</sup>	100.3 <sup>a</sup>	102.4 <sup>a</sup>	83.6 <sup>b</sup>	99.6 <sup>a</sup>	86.5 <sup>b</sup>	2.13
	Molar %						
Acetate	72.2 <sup>a</sup>	71.3 <sup>b</sup>	69.0 <sup>c</sup>	71.1 <sup>b</sup>	71.6 <sup>ab</sup>	71.7 <sup>ab</sup>	0.24
Propionate	16.2 <sup>d</sup>	17.3 <sup>b</sup>	18.3 <sup>a</sup>	17.3 <sup>b</sup>	17.0 <sup>c</sup>	16.8 <sup>c</sup>	0.13
Butyrate	9.1 <sup>b</sup>	9.2 <sup>b</sup>	10.1 <sup>a</sup>	9.2 <sup>b</sup>	9.0 <sup>bc</sup>	8.8 <sup>c</sup>	0.15
Isobutyrate	0.72 <sup>b</sup>	0.61 <sup>d</sup>	0.60 <sup>d</sup>	0.66 <sup>c</sup>	0.64 <sup>cd</sup>	0.80 <sup>a</sup>	0.02
Isovalerate	0.98 <sup>b</sup>	0.83 <sup>d</sup>	0.92 <sup>bc</sup>	0.86 <sup>cd</sup>	0.87 <sup>cd</sup>	1.05 <sup>a</sup>	0.03
Valerate	0.83 <sup>b</sup>	0.84 <sup>b</sup>	0.98 <sup>a</sup>	0.85 <sup>b</sup>	0.84 <sup>b</sup>	0.80 <sup>b</sup>	0.03
A:P ratio <sup>3</sup>	4.47 <sup>a</sup>	4.14 <sup>c</sup>	3.81 <sup>d</sup>	4.13 <sup>c</sup>	4.23 <sup>b</sup>	4.27 <sup>b</sup>	0.04

<sup>1</sup> Both SD and SA steers were supplemented on day 2; Only SD steers were supplemented on day 3.

<sup>2</sup> pH of ruminal fluid collected via suction strainer method

<sup>3</sup> Acetate to propionate ratio

<sup>a-d</sup> Means within row without common superscripts differ

**Figure 1a.** Fecal excretion curves for hay marked with Yb (A) and soybean hulls marked with Dy (B) of steers fed ad-libitum fescue hay and supplemented at 1% BW daily (■), supplemented 2% BW on alternate days (▲), or not supplemented (◆) with a soybean hull and corn gluten feed blend.

