EFFECT OF PROTEIN SUPPLEMENTATION AND FORAGE QUALITY ON INTAKE AND DIGESTION IN CATTLE

A Thesis

by

JAMIE LEE KUNKEL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Tryon A. Wickersham
Committee Members,	Jason E. Sawyer
	Jason P. Banta
Head of Department,	H. Russell Cross

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ABSTRACT

Effect of Protein Supplementation and Forage Quality on Intake and Digestion in Cattle.

(December 2011)

Jamie Lee Kunkel, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Tryon A. Wickersham

In many pasture and rangeland scenarios, low-quality forages (< 6-8% CP) are the primary energy source for some portion of the year. At these times, energy is typically the first limiting nutrient to the ruminant. Low-quality forages are generally not limited in availability; however, the high cell wall content and reduced levels of CP prevent ruminants from being able to extract the harvested energy. Without provision of supplemental nutrients, the available energy may be inadequate to meet performance expectations. Protein supplementation during periods of inadequate forage quality has been observed to alleviate ruminal nitrogen deficiencies and increase forage utilization. Increased forage utilization translates into greater energy extraction allowing for increased animal performance.

The first trial was conducted to evaluate the effects of utilizing non-protein nitrogen (a slow-release urea compared to urea) on intake and digestion of beef steers consuming low-quality bermudagrass hay. Steers were provided ad libitum access to a low-quality bermudagrass hay (7.3 % CP and were ruminally dosed once daily with either urea or slow-release urea (SRU) at levels to provide 0, 64, 128, or 192 mg of N/kg

of initial BW per day. Additionally, steers were supplemented with glycerol at levels of 0 or 0.1% of initial BW per day. Total OMI and forage OMI (**FOMI**) increased quadratically ($P < 0.01$) with NPN supplementation. However there was not a difference in total OMI or FOMI between urea and the SRU ($P = 0.24$ and 0.21, respectively). The largest increases in FOMI and total OMI occurred with the first level of supplementation (64 mg N/kg BW) for both urea and SRU and intake peaked when 128 mg N/kg BW was supplemented. Total OMD was not affected by N supplementation level however N from urea tended to elicit a greater response than from the SRU ($P = 0.01$). Ruminal OMD increased linearly ($P = 0.07$) and ruminal NDF digestion increased quadratically ($P = 0.09$) with N supplementation.

The second experiment was conducted to evaluate the effects of hay crude protein and protein supplementation on intake and digestion in beef steers. Steers received one of three bermudagrass (*Cynodon Dactylon*) hays of differing nutritive value (7.0, 8.4, or 13.4% CP) and either 0 or 156 mg N/kg BW supplemented as cottonseed meal once daily. No significant Hay × CSM interactions were observed for hay OMI, total OMI, TDOMI, or digestible NDF intake ($P \geq 0.67$). Hay OMI and total OMI increased linearly ($P < 0.01$) with hay nutritive value. A quadratic response ($P = 0.03$) was observed for TDOMI with increasing hay nutritive value. This response was largely driven by a quadratic increase ($P < 0.01$) in OM digestion with increasing nutritive value of hays. Supplementing CSM generally did not affect HOMI ($P = 0.63$) although TDOMI ($P = 0.03$) was increased. Similarly, OM digestion ($P = 0.61$) and NDF digestion ($P = 0.11$) were not impacted by CSM supplementation.

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction

Increasing population leads to increasing demand for food and fiber products. Additionally, as a greater number of people enter the middle class, the demand for animal products increases. Roughly a quarter of the world's land is in pasture and rangeland compared to the 10% which is arable (Darwin et al., 1995). With a large portion of the world's land resources in pasture and rangeland, efficient utilization of this resource will enable grazing to shoulder a larger portion of food and fiber production. Livestock, specifically ruminants, convert forages, which are largely unusable by humans, into valuable food and fiber products. Efficient utilization of forages is part of the solution to the problem of meeting the increasing demand for food and fiber products.

In many pasture and rangeland scenarios, low-quality forages (< 7% CP) are the primary energy source for some portion of the year. At these times, energy is typically the first limiting nutrient to the ruminant. Low-quality forages are generally not limited in availability; however, the high cell wall content and reduced levels of CP prevent ruminants from being able to harvest the available energy. Nitrogen (**N**) deficiencies often result in decreased utilization of forage because of decreased intake and digestibility which reduces the total digestible energy available to the animal.

This thesis follows the style of Journal of Animal Science.

Without provision of supplemental nutrients, the available energy may not be adequate to meet performance expectations (Moore et al., 1999). Protein supplementation during periods of inadequate forage quality has been observed to alleviate ruminal N deficiencies and increase forage utilization. Increased forage utilization translates into greater energy availability allowing for increased animal performance.

Protein supplementation is relatively expensive. Developing a cost effective supplementation plan is complicated by the forage, animal, and expectations of each producer. Forage quality is impacted by management, environment, and forage type. All of these factors complicate the efficient delivery of protein supplementation and need to be addressed.

Effects of Protein Supplementation on Intake, Digestion, and Rate of Passage

The main objective of supplementation is to improve or maintain animal performance by targeted delivery of nutrients in a cost effective manner. Direct supplementation of energy to improve the energy status of cattle consuming low-quality forage has not been effective (Kartchner, 1981; Moore et al., 1999). Sanson et al. (1990) observed that cattle grazing native winter range and supplemented with corn and a protein supplement (to supply 1.16 kg TDN and 290 g CP/d) lost 3.5% of their initial BW whereas cattle supplemented with a protein supplement (to supply 0.72 kg TDN and 290 kg CP/d) gained 1.1% of their initial BW. Data from another experiment in the same paper suggests that the BW loss was the result of reduced intake and digestion of native range in cattle fed corn and protein compared to the protein supplement alone. Protein supplementation of low-quality forages, on the other hand, increased total energy

availability to the animal by increasing intake and digestion of the available forage (McCollum, 1997).

Milford and Minson (1965) observed that voluntary intake of tropical grasses decreased sharply after CP concentrations of forages falls below 7%. Similarly, Moore and Kunkle (1995) compiled intake data from 30 trials utilizing 58 forages and conclude that intake decreases as forage CP declines below 6-8% CP. The greatest forage intake response to protein supplementation occurs below this forage CP range (NRC, 1996) and the response to protein supplementation tends to become less as forage CP increases.

Intake

Considerable research has been conducted determining the effects of N supplementation on a variety of low-quality forages using different levels and sources of N. Stokes et al. (1988) conducted a trial with cattle consuming prairie hay (4.8% CP) and receiving soybean meal **(SBM)** at 0, 0.12, or 0.24% of BW. Both forage DMI and total DMI **(TDMI)** increased linearly as supplementation level increased. Cows receiving the control, low level SBM, and high level SBM supplements had hay DMI of 1.5, 1.9, and 2.1% of BW, respectively. In their study, the intake response was greatest with the first increment of supplementation; however, additional SBM increased hay intake, though the magnitude of this increase was smaller. In a similar study, steers fed prairie hay (6.1% CP) were either supplemented with 0 or 800 g/d of cottonseed meal **(CSM;** McCollum and Galyean, 1985a). Supplementation with CSM increased forage intake by 27%. Guthrie and Wagner (1988) conducted two trials to quantify the effects of SBM and corn grain supplements on prairie hay (4.2% CP) utilization. The first trial

utilized two levels of SBM and one level of corn grain. Supplemental SBM was fed to provide 114 g/d of protein, low level, or 230 g/d, high level. Supplemental corn was provided to supply an intermediate amount of protein (189 g/d). Forage DMI was greater for all treatments versus control with the high SBM level resulting in the greatest forage DMI. There were no statistical differences in forage DMI between the low level SBM and corn grain supplement. Total dry matter intake followed the same trend, but differed in that TDMI of cattle receiving the corn grain supplement was intermediate between the two levels of SBM supplementation. The second trial utilized prairie hay (5.2% CP) supplemented with SBM at five levels: 0, 121, 241, 362, and 603 g/d. Both TDMI and forage DMI (**FDMI**) increased quadratically as SBM level increased. However, the greatest increase in intake was observed with the 241 g/d of SBM and not the first supplementation level as observed by Stokes et al. (1988). This observation is likely the result of a large response for supplemental protein to act on their forage resource, and the inability of the 121 g/d of SBM to satisfy ruminal N requirements. In contrast, 241 g/d of SBM provided sufficient ruminal N to maximize the effectiveness of each unit of supplemental N.

DelCurto et al. (1990a) conducted a trial supplementing tallgrass-prairie hay (2.9% CP) with a SBM-sorghum supplement. Four ratios of SBM:sorghum were used to create supplements of 0%, 12%, 28%, or 41% CP and were fed at 0.4% of BW. Sorghum was mixed with SBM to make the supplements isocaloric. Additionally, there was a control treatment that received no supplement. Both FDMI and TDMI increased quadratically as protein supplementation increased. Forage dry matter intake was

greatest when the 28% CP supplement was offered and decreased when the 41% CP supplement was supplied. However, FDMI for the highest level of CP was greater than both the control and low CP supplement. Mathis et al. (1999) observed similar results with SBM provided at five levels to quantify the response to protein supplementation. Forage organic matter intake **(FOMI)**, TOMI, and total digestible organic matter intake **(TDOMI)** increased cubically with increasing SBM. Supporting Stokes et al. (1988), the first increment of SBM resulted in the greatest percent increase of FOMI at 129%, TOMI at 133%, and TDOMI at 151%. Forage organic matter intake tended to plateau as SBM level increased past 0.16% BW. Mathis et al. (1999) also supplemented eight levels of SBM to cows grazing dormant tallgrass-prairie (2.7% CP) and observed changes in BW and body condition score. Both BW and BCS loss decreased quadratically as SBM supplementation increased and plateaued at the highest levels (0.3% of BW) of supplementation. Additionally, Church and Santos (1981) conducted a trial supplementing SBM to cattle consuming wheat straw (3.8% CP) to supply an additional 0, 160, 320, 480, and 640 mg N/kg $BW^{0.75}$. Both forage DMI and TDMI increased as more N was supplied, up to the 480 mg N/kg $BW^{0.75}$. As in DelCurto et al. (1990a), the highest level of CP supplementation resulted in a depression in DMI although intake was still statistically greater than the control and low CP supplemented groups. Furthermore, the greatest increase in DMI was at the lowest CP supplementation level as observed by both Mathis et al. (1999) and Stokes et al. (1988).

The aforementioned studies demonstrate that protein supplementation can be utilized to increase both forage and total intake of cattle consuming low-quality forage.

Most often the forage intake response follows a quadratic response with the largest increase in forage intake occurring generally at the first level of protein supplementation with additional supplement producing diminished increases in intake. Two studies observed a slight depression in intake at the highest levels of protein supplementation although intakes at these levels were still greater than controls. Trials discussed so far have utilized supplements that are high in degradable intake protein **(DIP)**, but these supplements also contain other nutrients such as carbohydrates and vitamins and minerals. The addition of several nutrients can all potentially contribute to observed intake responses and mask factors involved in intake responses to protein supplementation. In order to elucidate the intake response to protein supplementation, experiments utilizing supplements that are highly degradable and constitute only protein are needed to minimize the confounding effects of other nutrients.

Köster et al. (1996) conducted an experiment evaluating the effects of DIP on intake of low-quality, tallgrass-prairie hay (1.94% CP). Casein, a phosphoprotein found in milk, was used as the DIP source to eliminate effects of other nutrients associated with typical protein supplements. Casein was supplemented at 0, 180, 360, 540, and 720 g/d and dosed intraruminally twice/d. Forage organic matter intake, TOMI, TDOMI, and N intake increased quadratically with increased DIP supplementation. Similar to several trials already discussed, the first addition of casein resulted in the greatest increase (64%) in forage intake. Also, in accordance with several trials already discussed, FOMI and TOMI peaked at 540 g/d of supplemental DIP and the highest level of supplementation depressed intake. Olson et al. (1999) conducted a study to evaluate

the effects of five levels of DIP supplementation (0, 0.03, 0.06, 0.09, and 0.12% of initial BW) and three levels of starch (0, 0.15, and 0.3% of initial BW) on forage utilization of steers. Again casein was used as a DIP source to eliminate effects of nutrients associated with typical protein supplements and corn-starch grits were used to provide a readily fermentable source of starch. Forage OMI, TOMI, and TDOMI increased linearly as casein level increased with and without starch supplementation. Starch supplementation had a negative linear relationship with FOMI and TDOMI. Compared to previous trials, a plateau or depression of intake was not observed at the high DIP supplementation level. Klevesahl et al. (2003) also evaluated DIP and starch supplementation to low-quality forages; however, they used a wider range of DIP supplementation levels. Degradable intake protein from casein in this trial was provided at 0, 0.015, 0.051, 0.087, 0.123, 0.159, and 0.195% of initial BW with starch, from corn-starch grits, supplemented at 0 and 0.3% of initial BW. Observations from this study with the initial increments of DIP supplementation were similar to observations by Olson et al. (1999). However, the wider range in DIP levels allowed them to observe quadratic increases in both FOMI and TDOMI as DIP supplementation increased when no starch was supplemented. Both FOMI and TOMI peaked at supplementation rate of 0.123% of BW and further increases in DIP supplementation resulted in reduced intake. Results when starch was supplemented followed the same trends as when starch was not supplemented. Forage OMI was numerically lower for treatments with starch compared to no starch treatments. Mathis (2000) supplemented casein at 0, 0.041, 0.082, and 0.124% of BW to three forages: bermudagrass (8.2% CP), bromegrass (5.9% CP), and

forage sorghum (4.3% CP). Protein supplementation to cattle consuming bermudagrass did not increase intake. Protein supplementation of bromegrass did not affect FOMI, but tended to linearly increase TOMI and TDOMI. The forage sorghum was the lowest quality forage and supplementation resulted in linear increases in FOMI, TOMI, and TDOMI. Two additional trials utilizing casein to determine the effects of level of DIP supplementation on intake were also performed Wickersham et al., (2008a and 2008b). Similar to other trials, FOMI, TOMI, and TDOMI linearly increased with N supplementation in both trials.

By utilizing a purified protein source it is clear that degradable intake protein is the main driver of increased forage utilization of low-quality forages when supplemental protein is provided. Additionally, provision of starch (carbohydrate) with protein resulted in FOMI that was lower than when starch was not supplemented. These results plus results from trials utilizing commercially available protein supplements have shown that intake response to DIP supplementation is quadratic and plateaus or slightly decreases as protein supplementation increases, indicating that DIP requirements have been met or exceeded. Besides improving protein status of cattle, increases in intake of low-quality forage due to DIP supplementation can be attributed to increases in extent and rate of digestion and increased passage rate out of the rumen and through the gastro-intestinal tract.

Digestion

Protein supplementation of low-quality forage has been clearly demonstrated to increase forage, total, and total digestible OM intake. Many of these same studies also

demonstrate the ability of protein supplementation to increase ruminal digestion, total tract digestion, or both. Increasing the extent of diet digestion is one factor that contributes to the increased intake of energy observed with protein supplementation. Furthermore, increasing digestion ruminally or in the hindgut allows the animal to extract more nutrients from the diet resulting in greater availability of energy, protein, and other nutrients to the animal.

Guthrie and Wagner (1988) supplementing SBM to steers consuming prairie hay observed in one trial that dry matter digestion **(DMD)** and cellulose digestion increased as protein level increased over controls. Though intake was not different between the low SBM treatment and a corn grain supplement, cellulose digestion was reduced by supplementation with corn. In a second trial, utilizing five levels of SBM supplementation, Guthrie and Wagner (1988) observed similar results with DMD and cellulose digestion linearly increasing as supplemental protein was added. DelCurto et al. (1990a) observed greater DMD in steers supplemented with the lowest level of SBM-sorghum supplement compared to control steers. However, no further increases in DMD were observed above the first supplement level. Neutral detergent fiber digestion, however, was reported to increase quadratically as supplement level increased and plateaued at the moderate supplement level. Hannah et al. (1991) observed increases in ruminal organic matter apparent and true digestion, as well as, increases in total OM digestibility. Stokes et al. (1988) observed true and apparent ruminal OM digestion and total OM digestion increasing quadratically as SBM supplementation of prairie hay increased. The first increment of supplementation resulted in the largest increase in

digestion with apparent ruminal OM, true ruminal OM, and total OM digestion increasing 15.1, 11.7, and 15.2%, respectively, over control treatments. Protein supplements have been observed to increase the digestion of low-quality forage diets.

Trials utilizing casein as a protein source have also reported increases in diet digestibility supporting the observations using commercially available supplements. Köster et al. (1996) observed increases in ruminal true OM and NDF digestion with the first level of protein supplementation above controls. Further protein supplementation increased ruminal digestion only slightly. Similar trends were observed for total tract digestion as well. Mathis et al. (1999) observed quadratic increases in TDOM and total tract NDF digestion as SBM supplementation increased. The first increment of supplementation resulted in the largest increase in digestion and tended to plateau with higher supplementation rates. Similar to observed intakes, Mathis et al. (2000) observed no increase in TDOM or NDF digestion for bermudagrass or bromegrass hays. Forage sorghum, the lowest quality forage in their study, responded to DIP supplementation linearly with increases in both TDOM and NDF digestion. Klevesahl et al. (2003) observed a linear increase in TDOM as DIP supplementation increased. No statistical difference in TDOM was observed in their work when starch was supplemented at 0.3% of BW. However, NDF digestion was observed to increase with the first two levels of DIP supplementation and plateaued at higher levels. When starch was provided, NDF digestion was significantly lower compared to treatments without starch, until DIP supplementation levels were equal to or greater than 0.123% of BW. At these levels of DIP supplementation NDF digestion was not reduced when starch was provided.

Similarly, Olson et al. (1999) observed that TDMD, TDOM, and NDF digestion increased linearly in response to casein supplementation and tended to decrease linearly in response to starch supplementation. Additionally, Wickersham (2008a) observed linear increases in TDOM and NDF digestion as DIP supplementation increased. Ruminal NDF and OM digestion were not statistically significant, but tended to respond quadratically and plateau at the highest level of supplementation. These trials suggest that protein status of animals play a role in digestion with increased protein status generally allowing the animal to more extensively extract nutrients from the diet.

Rate of Passage

Typically, trials observing increased intake of low-quality forages due to protein supplementation also observe increases in particulate and liquid rate of passage. The increase in intake must be balanced with increased digestion and absorption, passage, ruminal fill, or a combination of all three. Theoretically, as passage rate increases and intake is held constant diet digestibility is decreased due to decreased time for microbial and enzymatic digestion. Conversely, as passage rate increases and digestion is held constant, intake increases due to a lessening of the 'fill' effect and a greater tolerance for distention. Increasing rates of passage can be useful in increasing nutrient flow such as microbial CP **(MCP)** and undegradable intake protein **(UIP)** to the small intestine for further enzymatic digestion and absorption by the animal (Köster et al., 1996).

In a study with steers consuming prairie hay, steers fed CSM had 55% faster ruminal passage rates and 27.5% shorter mean retention times than control steers (McCollum and Galyean, 1985a). In a subsequent study, Guthrie and Wagner (1988)

reported that increases in SBM supplementation linearly increased particulate passage rate. DelCurto et al. (1990a) observed increased (quadratic) passage of indigestible ADF with protein supplementation. Low level protein supplementation increased passage rate 45% over control animals. Olson et al. (1999) reported linear increases in acid detergent insoluble ash **(ADIA)** passage rate with increasing DIP supplementation. Starch supplementation did not statistically affect ADIA passage rate. Similar to DelCurto et al. (1990a), Klevesahl et al. (2003) observed ADIA passage rate to respond quadratically to protein supplementation. Passage rate was highest when DIP was provided at 0.123% BW and slightly declined at the higher supplementation rates. Increases in particulate passage rate have been associated with increased intakes. Increasing passage rate can alleviate distention and rumen fill allowing greater intake of feed that in turn can lead to increases in total nutrients moving through the gastro-intestinal tract and available for use by the animal.

In addition to protein supplementation increasing particulate passage rate, liquid passage rate has also been observed to increase. McCollum and Galyean (1985a) observed that dilution rate increased 19% and rumen fluid turnover time decreased 29% in CSM supplemented steers compared to controls. DelCurto et al. (1990a) observed increases in both dilution rate and liquid flow as protein supplementation increased. Köster et al. (1996) and Olson et al. (1999) observed linear increases in fluid dilution rate as DIP supplementation increased. Additionally, Olson et al. (1999) observed no interaction of starch on fluid dilution rate.

Ruminal Microbes

Addressing the nutritional requirements of ruminants can, for the most part, be addressed by meeting the requirements of ruminal microbes. Ruminal microbes produce a substantial proportion of the energy and protein utilized by the animal (McCollum, 1997). This is particularly true in animals maintained on low-quality forage. Microbial CP can supply 50% to nearly all MP required by the animal (NRC, 1996). To maximize the efficiency of ruminal digestion, the requirements of the ruminal microbes must be met to efficiently and economically address the requirements of the ruminant animal. Low-quality forage diets generally do not contain N in sufficient quantities to optimize microbial activity and thus limit ruminal digestion of forage (Guthrie and Wagner, 1988; Hannah et al., 1991; Stokes et al., 1988). Ultimately, this limits both energy (VFA) and protein (MCP) available to the host animal. Exacerbating the low N content of low-quality forage is the presence of degradable N sources present in bundle sheaths rather than mesophyll tissue, resulting in less ruminally available N to microbes (Coleman et al. 2004).

Nitrogen Requirements

Ruminal microbes have the ability to access and metabolize relatively low-quality nutrients into products that can meet the host animal's requirements. Optimizing the activity of ruminal microbes is most often the best means for a producer to improve animal performance in an economically sustainable manner. Rumen microbes require both adequate fermentable energy and N substrates to achieve maximum efficiency (Stern and Hoover, 1979; Satter and Slyter, 1974). Like their host animals, when either

energy or protein is limiting microbial efficiency (performance) is hindered. Low-quality forages do not always meet the N needs of rumen microbes made obvious by increases in ruminal digestion, a proxy for microbial activity, with protein supplementation. Microbes can extract N from a number of sources including feed, and endogenous proteins that are degraded into amino acids, hydrolysis of urea from N recycling to the rumen, or hydrolysis of urea, biuret, and other non-protein nitrogen **(NPN)** compounds in feed. Most N from these sources is converted to ammonia which is the most important N source for ruminal bacteria (Allison, 1969; Mathison and Milligan, 1971; Pilgrim et al., 1970). Results from an *in vitro* trial conducted by Satter and Slyter (1974) support suggest that 5 mg/100 ml ruminal ammonia N supports maximal microbial protein synthesis. This number is not a fixed point and can vary with fermentable energy content of the diet.

Maximizing Microbial Efficiency

Since low-quality forages are typically deficient in N and limit microbial efficiency, protein supplementation to supply additional N can be utilized to alleviate N deficiencies and in turn increase microbial efficiency. Microbial efficiency is an indicator of growth of the microbe population by measuring the ability of microbes to incorporate available N in the rumen into microbial products such as VFAs and MCP and is normally reported as g N/kg OM digested. Köster et al. (1996) and later Wickersham et al. (2008a) observed linear increases in microbial efficiency as protein supplementation increased. In both studies total VFA concentration and amount of microbial N and total N reaching the duodenum increased as protein supplementation

increased. Köster et al. (1996) utilizing a wider range of protein supplementation observed quadratic increases in VFA production and total N reaching the duodenum and a linear increase in microbial N. Wickersham et al. (2008a) utilizing a narrower protein supplementation range observed linear increases for VFA production and microbial and total N reaching the duodenum. When SBM supplementation was increased to cows consuming prairie hay, microbial efficiency was not altered, but VFA production and microbial and total N reaching the duodenum linearly increased (Stokes et al., 1988). Mathis et al. (2000) observed a quadratic response of total VFA production to DIP supplementation for cattle consuming a basal diet of bermudagrass (8.2% CP) or bromegrass hay (5.9% CP). The highest DIP supplementation level decreased VFA production for both forages. Volatile fatty acid production increased linearly with DIP supplementation for the lowest quality hay forage sorghum (4.3% CP). Trials utilizing SBM as protein supplement have also seen increases in VFA production (Hannah et al., 1991; Mathis et al., 1999). Olson et al. (1999) and Klevesahl et al. (2003) both observed increases in VFA production with DIP supplementation and a decreasing trend when starch was supplemented. Increases in microbial activity result in increased VFA concentrations and MCP reaching the duodenum.

Substituting Non-protein Nitrogen for True Protein in Protein Supplements

The primary goal of protein supplementation is to increase nutrient intake by the animal. Protein supplementation with traditional supplements (i.e. SBM, CSM, etc.) have been shown to alleviate N deficiencies in low-quality forage diets leading to more efficient utilization of the basal diet by rumen microbes. Other sources of N not in the

form of true proteins can be utilized by rumen microbes. This protein is termed NPN and is most commonly provided as urea or biuret.

Nonprotein N typically contains 5-7 times more N and is cheaper per unit of N than true protein. However, there are a several drawbacks to using NPN. One such drawback is that NPN supplements do not contain other nutrients such as carbohydrates, vitamins and minerals that plant based protein supplements contain and which can contribute to improved animal performance. Sulfur, one such mineral not contained in NPN sources, is needed by ruminal microbes for production of the sulfur containing amino acids methionine and cysteine. Deficiencies in sulfur lead to deficiencies of methionine and cysteine reducing MCP which in turn can limit protein available to the host animal and hinder performance. Probably most important is that high inclusion levels of NPN in the diet can cause toxicity. Additionally, supplements containing high percentages of NPN can be unpalatable to animals (Owens et al, 1980) or sublethal ammonia toxicity may result in a negative feedback (Kertz et al., 1980) reducing intake.

Intake

Supplementation of low-quality forages with NPN sources has produced similar responses as true protein supplementation. Campling et al. (1962) conducted several trials supplementing urea to cattle consuming a basal diet of oat straw. In the first trial, cattle supplemented with 150 g of urea had a 39% greater intake of oat straw than cattle not receiving urea. Similar results were observed in a second trial when urea was supplemented at 0, 25, 75, and 150 g/d. Intake of oat straw increased linearly up to the 75 g/d urea treatment and leveled off at the higher treatment. The largest increase in

intake was for the 25 g/d treatment with a 26% increase in intake over control animals. The 75 and 150 g/d treatments had intake increases of 40 and 44% over control animals respectively. A third trial was conducted similar to the first with urea supplemented at 0 and 150 g/d with *ad libitum* access to oat straw except digestibility was also measured. Again the supplemented cattle had a greater intake of roughage than the unsupplemented cattle. Fick et al. (1973) conducted two trials looking at the effects of increasing N from biuret and increasing levels of an energy supplement on utilization of pangolagrass hay (4.5% CP) in wethers. In the first trial, biuret was fed at 0 or 10 g/d in a vitamin/mineral mix with or without the energy supplement. The addition of biuret increased FOMI and TOMI over control animals. In contrast, the energy supplement tended to depress intakes in wethers not supplemented with biuret. The second trial was similar to the first except that biuret was fed at 0, 8, and 16 g/d. Again, supplementing with NPN increased FOMI and TOMI. Currier et al. (2004) also using wethers, conducted a trial comparing urea and biuret supplements fed daily or on alternate days to wethers not receiving a N supplement. All sheep were consuming hard fescue straw (4.3% CP). In contrast to Fick et al. (1973), FOMI did not respond to NPN supplementation; however, TDMI and TOMI increased with supplementation.

Several studies have also compared NPN supplementation to true protein supplementation. Raleigh and Wallace (1963) conducted a trial with growing steers consuming meadow hay (5.5% CP) and supplemented with urea, CSM, or urea + CSM to create diets of 5.5, 6.0, 9.0, or 12.0% CP. Total intake when hay was supplemented with urea was not different from intakes when hay was supplemented with the CSM or

urea + CSM supplements at each CP level. As supplementation level increased, total intake also increased and plateaued at the highest supplementation level. Later, Ammerman et al. (1972) conducted several trials with wethers consuming pangolagrass hay comparing urea and biuret supplementation to CSM supplementation. In the first trial, wethers received no supplement, 150 g CSM (39.6% CP), or 158 g CSM + urea supplement (37.7% CP). Forage intake did not differ between the protein supplemented sheep, but was lower for the sheep not receiving a protein supplement. The second trial also utilized wethers consuming pangolagrass hay and receiving one of four treatments: no supplement, a citrus pulp based supplement (24 mg N/kg BW), the same citrus pulp supplement with biuret (179 mg N/kg BW) or CSM (176 mg N/kg BW). There was no difference in forage or total intakes between the biuret and CSM treatments. Forage intake of wethers receiving the citrus pulp, citrus pulp with biuret, and CSM treatments were 90, 109, and 115% of the control treatment's intake. Total intake was greatest for the CSM and biuret treatments and least for the citrus pulp treatment. In the final trial, treatments consisted of no supplement, SBM (38.2% CP), SBM + urea (39.6% CP), and SBM + biuret (39.3% CP). All supplements were fed at the same rate of 150 g/d. Forage intake was the same for all protein supplements. Forage and total intakes for the protein supplemented treatments averaged 25 and 43% greater than the control treatment. Another study utilized low-quality pasture hay and three N supplements consisting of urea fed at 112 g/d, molasses fed at 250 g/d, and a protein meal fed at 800 g/d (Lee et al., 1987). Urea and protein meal supplements were isonitrogenous. Both the urea and protein meal treatments increased FOMI and DOMI similarly and had

greater intakes when compared to the molasses treatment. Köster et al. (1997 and 2002) conducted two trials looking at the utilization response of increasing levels of urea on tallgrass-prairie hay. The first trial treatments had urea supplemented to provide 0, 25, 50, 75, and 100% of supplemental DIP with remainder of DIP provided by casein. All casein-urea supplements were balanced with cornstarch to contain 40% CP. No differences in FOMI or TOMI were observed at any urea inclusion level. In contrast, TDOMI decreased linearly with higher inclusion rates of urea due to decreased digestion. In the second experiment, urea was included to provide 0, 20, and 40% of supplemental DIP from urea. The remainder of the supplemental DIP was provided by a SBM-sorghum grain based supplement. Similar to the previous trial, FOMI and TOMI were not different. In contrast to the previous trial, TDOMI was not different between treatments.

Digestion

As with intake, digestion has also been shown to respond to NPN supplementation. Campling et al. (1962) observed that total tract DM, OM, and crude fiber digestibility increased 20, 20, and 23% respectively when urea was supplemented at 150 g/d compared to 0 g/d. Furthermore, reticulo-rumen OM and crude fiber digestibility were observed to increase 54 and 52% respectively. Raleigh and Wallace (1963) observed increases in cellulose digestibility, TDMD, and TDOM when urea was supplemented alone to steers consuming meadow hay and were similar to increases in digestibility when CSM and CSM + urea were supplemented. The highest levels of supplementation of all three treatments tended to result in a plateau in digestibility.

Ammerman et al. (1972) conducted several experiments observing the response to supplemental urea and biuret and observed results similar to those Raleigh and Wallace (1963) had previously reported. In their first trial, Ammerman et al. (1972) observed increases in total OM digestibility when CSM or CSM + urea were supplemented compared to control animals. Organic matter digestibility in another two trials responded similarly to the first trial when urea and biuret was supplemented resulting in digestibilities comparable to when either CSM or SBM was supplemented and greater than control treatments. Hunter and Siebert (1980) conducted a trial supplementing spear grass (4.2% CP) with either urea and sulfur or CSM. Forage OM digestibility was similar between control and NPN treatments and slightly less for the CSM treatment although forage intake was higher for the N supplemented treatments than for controls. Total OM digestibility as a percent was similar for all three treatments although TOMD (kg/d) for the NPN and CSM supplements was 27 and 54% greater than control animals. Lee et al. (1987) observed that both urea and a protein meal (fed to be isonitrogenous) increased TOMD 41 and 31% compared to steers receiving a molasses supplement. Köster et al. (1997) observed that true ruminal OM digestion remained constant as N from urea was increased in the diet until the highest level of urea inclusion where ruminal OM digestion decreased. Similarly, ruminal NDF digestion remained constant until N from urea reached 75%. At the two highest urea inclusion levels ruminal NDF digestibility decreased. Total digestible OM and total NDF digestion followed trends similar to ruminal digestion. Supporting this study, Köster et al. (2002) observed no

differences in TDOM or total NDF digestibility as supplemental DIP from urea increased from 0 to 40%.

Effects of Forage Quality on Forage Utilization

Forage quality plays an extensive role in forage utilization. Forage quality combines both nutrient composition and digestibility into one value (Coleman et al., 2004) and is essentially TDN. Forage quality is typically at its apex when forages are immature and actively growing and declines as forages mature. The decrease in forage quality is attributed to a build up of cell wall constituents (fiber) that is less digestible and dilutes more readily fermentable nutrients such as soluble carbohydrates (Coleman, 2004). Lignin, a component of fiber found in both the NDF and ADF fractions, is an indigestible compound that associates with structural carbohydrates and decreases microbial access to structural carbohydrates reducing their fermentability. Forages high in NDF and ADF have been correlated to lower intakes and digestibility than forages with less NDF and ADF (Reid et al., 1988; Jung and Allen, 1995). Reductions in the concentration of readily fermentable cell contents of forages can cause microbes to be deficient in nutrients (i.e., N) that reduce microbial efficiency.

Effects of Nitrogen Fertilization

The primary factor affecting both forage yield and protein content is the availability of N. Deficiencies in N limit nucleic acid synthesis in plant cell nuclei disrupting mitosis and stopping cell division and therefore limit forage growth. Additionally, N is needed to produce plant proteins. Forages that are N deficient therefore have reduced protein content. Numerous trials have demonstrated the use of N

fertilizers to alleviate N deficiencies of forages increasing both CP content and yields of the forage. Burton et al. (1963) conducted a N fertilization trial with bermudagrass over a two year period. Nitrogen was applied at rates of 0, 112, 224, 336, 672, and 1008 kg/ha. Forage yields were observed to increase quadratically in response to N fertilization and tended to reach a plateau at the highest N fertilization levels. Forage CP levels increased cubically with CP not differing with the first N rate applied and reaching a plateau at the highest levels of N fertilization rates. Crude fiber content of the forages was not affected by N fertilization rate. An earlier study by Burton and DeVane (1952) observed similar results when applying increasing rates of N fertilizer (0, 56, 112, 224, and 448 kg/ha) to bermudagrass. Protein content of the forage increased linearly with N fertilization rate and forage yields increased quadratically. Fertilizing at a rate of 56 kg N/ha resulted in the largest percent increase in forage yields. Prine and Burton (1956) also observed quadratic responses to both forage yield and protein content of coastal bermudagrass when N fertilization rates increased. Treatments consisted of fertilization rates of 0, 112, 336, 672, and 1009 kg N/ha. Forage yield reached a plateau at 672 lbs kg N /ha while protein content of the forage was still increasing at 1009 kg N/ha. Burton and Jackson (1962) observed the response of coastal bermudagrass to six N sources applied at three rates (112, 224, and 448 kg/ha). Similar to previous studies, both forage yields and CP content increased linearly in response to N fertilization for each N source. Furthermore, Monson and Burton (1982) conducted trials on eight different bermudagrass cultivars over two years observing yield and protein content responses to N fertilization rates of 336 and 672 kg N/ha. As reported in previous

studies, N fertilization increased forage yields and protein content of each bermudagrass cultivar.

Voluntary intake of low-quality forages by cattle has been observed to be limited by rumen capacity, digestion, and passage rate (Allison, 1985; Collins and Fritz, 2004). Trials looking into effects of forage nutritive value on intake, digestion, and passage rate have reported variable results, but suggest that forage nutritive value is important for predicting animal productivity and response to supplementation. Reid and Jung (1965) conducted a trial fertilizing tall fescue at rates of 0, 56, 168, and 504 kg N/ha. Two hay cuttings were then made after treatment application. Forage yield responded quadratically with the largest increase in yield response occurring with the first increment of N fertilization (91% increase) and a plateau occurring at the highest fertilization rate. Similarly, CP increased quadratically for both hay cuttings again with the greatest increase in CP occurring with the first increment of N fertilization; 54 and 42% increase for each hay cutting. Cell wall constituents also tended to decrease with increases in N fertilization rate. In addition, no differences in intake, DMD, or cellulose digestibility were observed for the first hay cutting when fed to sheep. Intake was also not affected in the second hay cutting however, DMD increased quadratically and cellulose digestibility increased linearly. Reid et al. (1966) conducted a similar study with orchardgrass fed to sheep and fertilized at rates of 0, 56, 112, 224, and 448 kg N/ha over two hay cuttings. Similar to the previous study, CP increased with N fertilization for both hay cuttings. There were no significant differences in intake or DMD and cellulose digestibility in the first hay cutting. Intakes of the second hay cutting were not

affected significantly by fertilization rate but tended to follow a quadratic response with a peak at 112 kg N/ha and the lowest intake at 448 kg N/ha. Dry matter digestibility and cellulose digestibility were significantly affected by fertilization rate and responded quadratically. Both DMD and cellulose digestibility reached a plateau when N was applied at 224 kg/ha.

Monson and Burton (1982) observed in eight different varieties of bermudagrass that increases in N fertilization from 336 to 672 kg N/ha increased *in vitro* DMD over a two year period. In contrast, Webster et al. (1965) observed no differences in *in vitro* digestibility of bermudagrass receiving 0, 448, or 1568 kg N/ha.

Messman et al. (1991) conducted a trial feeding bromegrass at two maturity stages and fertilized with 0 or 89 kg N/ha to Holstein cows. Similar to previous studies, CP was increased when N fertilizer was applied. However, N fertilization was not observed to increase intake, DMD, NDF, or ADF digestibility. Similarly, VFA concentrations were not affected by N fertilization rate. Puoli et al. (1991) observed that when switchgrass and big bluestem were fertilized with 0 or 75 kg N/ha as urea, CP in the forages increased from 5.3% to 6.4% in switchgrass and 5.6% to 7.3% in big bluestem. The increase in CP due to N fertilization of the forages increased DMI 11 and 16% for switchgrass and big bluestem respectively. The increase in CP due to N fertilization did not significantly increase DMD or NDF digestibility of either forage although ruminal DM turnover was decreased 9.3% for switchgrass and 18.5% for big bluestem. Kelsey et al. (1973) observed the responses of intake and digestibility of sheep consuming blue grama range and fertilized with two levels of N (0 and 44.8 kg

N/ha). Crude protein of the forages was increased from 7.0 to 8.8% with N fertilization. Crude fiber was not affected by N fertilization although ADF content decreased. Dry matter intake, OM digestibility, and TDN increased 29, 11, and 11% respectively with N fertilization. Delagarde et al. (1997) conducted a trial with dairy cows grazing ryegrass fertilized at either 0 or 60 kg N/ha. Fertilization level did not affect total forage yields but increased forage above 5 cm in height. Crude protein of the ryegrass increased from 10.6% in the low fertilization rate to 17.3% in the high fertilization rate. Furthermore, TOMI, TDOMI and apparent total OM digestibility increased 20, 25, and 04%, respectively, with N fertilization. Ruminal OM digestion was not affected by fertilization. Another study fertilized three forages, *Chloris gayana, Digitaria decumbens,* and *Pennisetum clandestinum*, with either 125 or 500 kg N/ha (Minson, 1973). As with previous studies, forage yields were increased for all three forages with additional N. Crude protein of the C. *gayana, D. decumbens,* and *P. clandestinum* were increased from 10.7 to 14.1%, 8.1 to 13.3%, and 10.6 to 14.7% respectively. Neither DMD nor OMD was affected by N fertilization for any of the forages over the entire study however they were increased significantly during certain periods of the study. Likewise, DMI and OMI were not significantly altered over the entire trial.

Nitrogen fertilization has been observed to increase protein content of forages although digestibility has been variable. Additionally, forage yields and carrying capacity have also been observed to increase though animal performance is typically not improved due to reductions in soluble carbohydrates and TDN not being increased (Blaser, 1962). Many of the studies reported forage CP without fertilization in excess of

the 6-8% observed by Milford and Minson (1965) and Moore and Kunkle (1995) needed to not adversely affect intake and thus explains why increases in forage intake were not observed in many of the studies. The increase in intake observed by Delagarde et al. (1997) may not be due to increases in forage CP content but an increase in available forage due to N fertilization. However, it is feasible that ruminants consuming forages with CP below the 6-8% will increase intake if N fertilization is utilized to increase forage CP levels as seen by Puoli et al (1991) and Kelsey (1973). Lima et al. (1999) further supports this concept. Average daily gain of heifers grazing limpograss, fertilized with either 50 or 150 kg N/ha, were measured. Nitrogen fertilization increased CP of the forage from 5.6 to 7.3% and *in vitro* OM digestibility increased 6.4%. Average daily gains of the heifers consuming limpograss fertilized with high rate of N were six times greater than ADG of heifers on the lower N rate limpograss.

Effects of Forage Maturity

Forage maturity also plays a role in overall forage quality with forage quality being negatively related to maturity. Burns et al. (1997) observed as forage maturity of switchgrass increased NDF content of the diet increased and was associated with decreases in DMI and DMD of steers. Matejovsky and Sanson (1995) conducted a trial looking at differences in intake of three different quality forages; a low-quality (5.2% CP), medium-quality (10.2% CP), and a high-quality (14.2% CP). Dry matter intake increased linearly as quality of the forage increased. Dry matter digestibility and NDF and ADF digestibility increased from the low-quality forage to the medium-quality forage, but did not increase with the high-quality forage. McCollum and Galyean

(1985b) and McCollum et al. (1985) conducted trials using the same cattle and rangeland to determine affects of forage quality on rumen fermentation and forage utilization of cattle consuming blue grama rangeland. *In vitro* organic matter digestibility and CP of the forage declined and ADF and acid detergent lignin increased as the growing season progressed showing that forage quality declined. Cattle grazing blue grama rangeland decreased OMI as the growing season progressed; however, intake increased slightly near the end of the growing season. Fecal organic matter excretion was constant throughout the trial suggesting that forage quality and not forage availability was limiting intake. Particulate passage rate decreased and rumen retention time increased as grazing period progressed and forage quality declined. Fluid passage rate also decreased with the grazing period. Additionally, Adams et al. (1987) observed responses of intake, rumen fermentation, and fluid flow of steers to maturity level of native range. Both CP and in vitro OM digestibility increased during the beginning of the growing period and decreased afterward. Acid detergent fiber tended to increase through the entire study. In contrast to McCollum and Galyean (1985b), OMI was not affected by stage of maturity. Rumen dilution rate decreased as forages matured throughout the growing season, but increased at the end of the growing season. Fluid turnover was increased with forage maturity until the end of the growing season where it decreased slightly. Ruminal fluid flow rates increased during the first period of the trial and leveled out for the remainder of the trial. Total VFA concentrations tended to decline throughout with increasing forage maturity through the beginning of the study then level out to the end of the study. Messman et al. (1991) similarly observed that as bromegrass matured from a late boot to

full head stage that CP decreased and NDF and ADF increased. Cellulose content was unaffected by stage of maturity indicating that increases in NDF and ADF can largely be attributed to increased lignin content. Dry matter, NDF, and cellulose digestibility was also seen to decrease with maturity. However, intake was unaffected by stage of maturity.

Forage quality has been demonstrated to be a major factor in determining animal performance and developing supplementation protocols. Efficiently managing forage quality is another avenue that producers can utilize to increase animal performance. Managing and selecting for higher quality forages decreases the need for expensive supplements to meet animal production goals.

Conclusion

Forage quality is an important consideration in forage based production systems. Reduced forage quality leads to decreases in intake and digestion of the basal forage diet which in turn decreases energy available to the animal reducing performance. Protein supplementation either through plant protein sources such as SBM and CSM, NPN sources, or a combination there of both has been documented to alleviate N deficiencies and increase intake and digestion of low-quality forages (< 7% CP) leading to increases in both protein and energy status of the animal. This is accomplished primarily through relieving N deficiencies of microbes and increasing VFA and MCP production. Further work is needed into the relationship of forage quality and the response to protein supplementation for producers to more efficiently manage and utilize forage in there livestock systems to meet the increasing demands of food and fiber production.

CHAPTER II

EFFECT OF SLOW RELEASE UREA AND GLYCEROL ON INTAKE AND DIGESTION OF BERMUDAGRASS HAY IN BEEF STEERS

Overview

This trial was conducted to evaluate the effects of utilizing non-protein nitrogen (a slow-release urea compared to urea) on intake and digestion of beef steers consuming low-quality bermudagrass hay. Thirteen duodenally and ruminally fistulated steers (average initial BW 194 ± 21 kg) were used in a 13 × 4 incomplete Latin square and were provided ad libitum access to low-quality bermudagrass hay (7.3 % CP). Steers were then ruminally dosed once daily with either urea or slow-release urea (SRU) at levels to provide 0, 64, 128, or 192 mg of N/kg of initial BW per day. Additionally, steers were supplemented with glycerol at levels of 0 or 0.1% of initial BW per day. Total OMI and forage OMI (**FOMI**) increased quadratically ($P < 0.01$) with NPN supplementation. However there was not a difference in total OMI or FOMI between urea and the SRU ($P = 0.24$ and 0.21, respectively). The largest increases in FOMI and total OMI occurred with the first level of supplementation (64 mg N/kg BW) for both urea and SRU. Intake peaked when 128 mg N/kg BW was supplemented, and the highest supplementation level (192 mg N/kg BW) resulted in decreased intake. Total OMD was not affected by N supplementation level however N from urea tended to elicit a greater response than from the SRU ($P = 0.01$). Ruminal OMD increased linearly ($P =$

0.07) and ruminal NDF digestion increased quadratically ($P = 0.09$) with N supplementation.

Introduction

Previous research has clearly demonstrated that delivery of supplemental protein to cattle consuming low quality forage (< 7% CP) increases forage intake and digestion (Guthrie and Wagner, 1988; Stokes et al., 1988; Köster et al., 1996) while improving animal performance (DelCurto et al., 1990b; Mathis et al., 1999). Similarly, protein supplements containing NPN increase intake and digestibility of low-quality forages (Campling et al., 1962; Fick et al., 1973) in a similar fashion as supplements containing true protein (Raleigh and Wallace, 1963; Ammerman et al., 1972; and Köster et al., 1997 and 2002). A benefit of NPN inclusion is the reduced cost per unit of equivalent CP. However, sources of NPN are, typically, rapidly hydrolyzed to ammonia in the rumen, absorbed by the rumen epithelium and, subsequently, transferred to the blood limiting the efficacy of the supplement (Helmer and Bartley, 1971). Rapid hydrolysis of NPN to ammonia is in contrast to the slow digestion rate of low-quality forages, thus synchrony of the two constituents is not realized reducing the microbes' ability to convert NPN into microbial CP **(MCP)**. However, nitrogen recycling mechanisms in the ruminant have the potential to salvage significant quantities of absorbed ammonia in cattle fed low-quality forage (Wickersham et al., 2008a and 2008b).

Several areas of research have been studied to increase the efficiency of urea use by rumen microbes. Inhibiting of urease activity is one such area, but has not been observed to be overly effective (Clifford et al., 1968; Harbers et al., 1962). Another area

of interest has been to slow the release of urea through the use of different coatings and treatments. The objective of this study was to compare the effects of urea and a slow release urea **(SRU)** on intake and digestion of bermudagrass hay in beef steers with and without glycerol infusion.

Material and Methods

Thirteen duodenally and ruminally fistulated steers (average initial BW 194 ± 21 kg) were used in a 13 × 4 incomplete Latin square with 13 treatments and 4 periods (Cochran and Cox, 1957). Treatments were arranged as a 3 × 2 × 2 factorial plus a negative control, which received no supplement. The first factor consisted of 3 levels of supplemental DIP (64, 128, and 192 mg of N/kg of initial BW per d). These levels of supplemental protein were based on previous research (Köster et al., 1996, Klevesahl et al., 2003, and Wickersham et al., 2004) and were expected to increase total digestible OM intake (**TDOMI**) with a plateau occurring at the higher levels of supplementation. One of two sources of DIP, urea or Ruma Pro (AniPro, Greeley, CO), made up the second factor and the third factor was level of glycerol supplementation (none or 0.1% of initial BW/d).

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University and included the use of anesthesia when surgical procedures were performed.

Steers were housed in an enclosed, climate controlled barn in individual pens (2.1 m × 1.5 m) with continuous lighting and ad libitum access to water and a trace mineral salt block (composition: 98.0% NaCl, 1.0% S, 0.15% Fe, 0.25% Zn, 0.30% Mn,

0.01% I, 0.02% Cu, and 0.003% Co; United Salt Corp, Houston, TX). Low-quality bermudagrass hay (Table 2-1; all tables and figures found in Appendix A) was chopped through a 75 mm × 75 mm screen and fed at 130% of the previous 4-d average intake to insure that access to forage was not restricting intake. The four experimental periods were divided into three phases: (1) 10 d adaptation to treatments; (2) 7 d measurement of hay intake and digestion; (3) 1 d ruminal and duodenal sampling. Supplements were administered at 0630 each morning by placing the supplement directly in to the rumen through the rumen fistulae, and hay was fed at approximately 0700 h. Approximately 1 kg of hay was retained daily for subsequent analysis. Observations of intake and digestion were made on days 11 - 17 of each period. Hay and ort samples were collected on days 11 – 16, and total fecal output was collected on days 12 – 17. These observations were used to determine total tract digestion according to the guidelines of Cochran and Galyean (1994). Orts were removed at 0600 h and approximately 200 g per day were retained for analysis. Fecal bags were removed and contents weighed at 0600 h daily. Feces collected over each 24 h period were thoroughly mixed, and approximately 600 g per day was retained for analysis.

On the day following the completion of fecal collections ruminal fluid samples were collected by suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) just before dosing (0 h) and at 1, 2, 3, 4, 8, 12, 16 and 20 h after supplements were dosed. Immediately after sampling, ruminal pH was measure using a portable pH meter with a combination electrode (Thermo Scientific, Singapore). Eight ml of ruminal fluid was combined with 2 ml of 1 N HCl and frozen for VFA and NH_3 analysis.

Additionally, duodenal digesta samples were collected at 0, 6, 12, and 18 h after dosing to determine duodenal flows. Blood was collected from the jugular vein 0, 4, and 12 h after feeding on d 18 for later determination of plasma urea N concentration.

Laboratory Analysis

Partial DM of hay, orts, and fecal samples were performed by drying at 55°C in a forced-air oven for 96 h. Duodenal samples were frozen and lyophilized. All dried samples were then ground in a Wiley mill to pass through a 1 mm screen. Hay samples collected during the measurement period were pooled across days on an equal weight basis. Ort and fecal samples were composited in proportion to their daily refusal or output, respectively, by steer among days for each measurement period. Hay, supplement, ort, fecal, and duodenal samples were then dried for 24 h at 105°C in a forced-air oven to determine DM and then combusted for 8 h at 450°C in a muffle furnace to determine OM. Nitrogen content of samples was determined by total combustion (Rapid N Cube, Elementar Americas, Inc, Mt Laurel, NJ). Crude protein was then calculated by N × 6.25. The ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY) was used to determine NDF and ADF (Komarek et al., 1993) of hay, ort, fecal, supplement, and duodenal samples. The ANKOM bags containing ADF residues were combusted for 8 h at 450°C in a muffle furnace to determine ADIA of hay, orts, fecal and duodenal samples. Ruminal VFAs were determined by GLC as described by Vanzant and Cochran (1994). Colorimetric determination of ruminal ammonia (Broderick and Kang, 1980) and plasma urea (Marsh et al, 1965) were made using an UV/VIS (DU 730 UV/VIS Spectrometer, Beckman Coulter, Inc, Fullerton, CA). Total

tract digestion coefficients for DM, OM, and NDF were determined, using total collections, as described by Cochran and Galyean (1994). Duodenal flow was calculated by dividing fecal ADIA output (g/d) by the ADIA concentration of the duodenal digesta.

Statistical Analysis

Intake, digestion, and duodenal flows were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Terms in the model were treatment and period with steer included as a random effect. Fermentation profile variables and plasma urea N concentrations were analyzed using the MIXED procedure of SAS. Terms in the model were treatment, period, hour, and hour × treatment with steer and treatment × period × steer included as random terms. The repeated term was hour with treatment × steer serving as the subject. Compound symmetry was used for the covariance structure. The LSMEANS option was used to calculate treatment means. Orthogonal polynomial contrasts (linear, quadratic, and cubic) were used to partition treatment sums of squares.

Results

Total OMI and forage OMI (**FOMI**) increased quadratically ($P < 0.01$) with NPN supplementation (Table 2-2). Nitrogen source, however, did not impact either total OMI (P=0.24) or FOMI (P=0.21). The largest increase in FOMI and total OMI occurred with the first level of NPN supplementation (64 mg N/kg BW) and increased intake 20.3 and 23.0%, respectively. Intake was observed to peak when 128 mg N/kg BW was supplemented and to slightly decrease at the highest N supplementation level (192 mg N/kg BW). However intake was still greater at the 192 mg N/kg BW level compared to unsupplemented cattle. Total digestible OM intake increased quadratically ($P < 0.01$)

with N supplementation. This was mainly due to the aforementioned increases in intake. A N source effect ($P = 0.03$) was observed for TDOMI with N from urea generally resulting in a greater increase (specifically at the 128 mg N/kg BW supplementation level) in TDOMI than N from the SRU largely driven by greater digestibility when supplementing with urea. The greatest increase in TDOMI was observed with the first increment of N supplementation with increases of 28.3 and 29.9% for the urea and SRU. However, supplementing with 128 mg N/kg BW resulted in TDOMI increasing 15.5% for urea where as the SRU only increased 2.5%. As per the design, total N intake increased linearly ($P < 0.01$) with N supplementation from either source. The addition of the glycerol supplement did not significantly impact FOMI (P=0.17), total OMI (P=0.82), or TDOMI (P=075). However, supplementation with 0.1% glycerol lowered total digestible NDF intake (**TDNDI**) compared to when glycerol was not supplemented ($P = 0.02$). This effect was mainly caused by reductions in total tract NDF digestibility (Table 2-3; $P = 0.07$) when glycerol was supplemented.

Nitrogen supplementation level did not impact either total OMD or total NDF digestion. Both total OM digestion (**OMD**) ($P = 0.01$) and total NDF digestion ($P = 0.02$) were effected by N source with N from urea generally resulting in higher digestibilities. However, it is of interest that supplementing cattle with 64 mg N/kg BW resulted in a 2.7% increase in total OMD over unsupplemented cattle and supplementing at the 128 mg N/kg BW resulted in a 11.1% increase in total OMD than unsupplemented catte. Conversely, supplementing the SRU at 64 mg N/kg BW increased total OMD 5.3% over unsupplemented catle and supplementing at the 128 mg N/kg BW level only

increased 3.4% over unsupplemented cattle. Ruminal OMD increased linearly (P = 0.07) with N supplementation while ruminal NDF digestion was increased quadratically (P = 0.09) with N supplementation. Similar to total tract digestibilities, ruminal NDF digestion was effected by N source (P = 0.08) although ruminal OMD was not effected (P = 0.97).

Glycerol did not effect either total OMD (P = 0.71) nor ruminal OMD (P = 0.31) or NDF digestion (P = 1.00). However, total NDF digestion (P = 0.07) was decreased when 0.1% glycerol was supplemented either with urea or SRU.

Ruminal pH and most VFA were characterized by treatment × time (P < 0.01) interactions. However, the interactions were largely due to variation in magnitude of differences within each time period. Therefore, data were averaged across time. Ruminal pH was not effected by glycerol or N source and increased linearly (P = 0.01) with N supplementation (Table 2-4). However, the linear relationship was driven largely by the difference in pH between control animals and animals receiving the first N supplementation level. The pH increased from 6.62 to 6.72 with the first N supplementation treatment and then hovered between 6.72 and 6.74. Total VFA concentrations did not differ with N level, N source (P = 0.35), or glycerol treatment (P = 0.17). The molar proportion of acetate was significantly decreased when glycerol was supplemented (P < 0.01; Table 2-5). Acetate responded quadratically (P < 0.01) to N supplementation level; however, this is the result of the marked decrease in acetate with glycerol provision and the lack of a treatment that provided no supplemental N and did provide glycerol. In contrast to acetate, the proportion of propionate was increased with

glycerol supplementation ($P < 0.01$). Propionate was linearly affected by N supplementation level ($P = 0.02$), but, similar to acetate the effect is the result treatment combinations. Butyrate responded similar to propionate with glycerol increasing butyrate ($P < 0.01$) and increasing quadratically ($P < 0.01$) with N supplementation level. Significant differences were also noted for isobutyrate, valerate, and isovalerate, however, the difference in these proportions were extremely small and the biological significance is likely small.

Mean ruminal ammonia levels were not affected by N source ($P = 0.92$) and increased linearly ($P < 0.01$) with increases in N supplementation. The addition of glycerol did not affect mean ruminal ammonia ($P = 0.53$); however, the provision of glycerol supplements tended to decrease ruminal ammonia N more quickly when urea was supplemented than when glycerol was not provided (Figure 2-1). This is probably due to the synchrony of readily available glycerol and rapidly hydrolyzable urea and the ruminal microbes' ability to more efficiently utilize them for MCP production. The SRU tended to produce ruminal ammonia N concentrations similar to urea with peaks occurring at hours 1, 2, and 3 for 64, 128, and 192 mg N/kg BW treatments respectively. Additionally, higher N supplementation levels resulted in larger sustained ruminal ammonia levels over time. However, at hour 20, ruminal ammonia concentrations returned to similar levels for each N supplementation level, but were larger than controls.

A N source interactions ($P = 0.04$) was significant for mean plasma urea N concentrations (**PUN**) resulting in SRU supplementation having depressed mean PUN

compared to urea supplementation. Mean PUN also increased linearly ($P < 0.01$) with N supplementation (Figure 2-2). No glycerol interaction ($P = 0.43$) was observed for PUN.

Discussion

Forage OMI responded quadratically to NPN supplementation in our study with a peak occurring at the 128 mg N/kg BW and a plateau occurring at the higher NPN supplementation level. The increase in forage intake with N supplementation indicates that N was a factor limiting intake. Additionally, the N requirement was met when less than or equal to 128 mg N/kg BW was supplemented, as indicated by maximal intake being observed at this level and intake reaching a plateau at the higher level. Our results are in agreement with Campling et al. (1962) who also observed a quadratic forage intake response to urea supplementation which reached a plateau at the highest urea supplementation levels. Additionally, studies utilizing true protein sources have also observed forage intake to respond quadratically to N supplementation (DelCurto et al., 1990a and 1990b; Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2004). Increases in FOMI were also observed by Hunter and Siebert (1980) and Lee et al. (1987) when urea was supplemented to steers consuming a low-quality forage and Ammerman et al. (1972) when biuret was supplemented. Total OMI and TDOMI was also observed to increase quadratically in our study. Hunter and Siebert (1980) observed total OMI and Lee et al. (1987) observed TDOMI to increase with urea supplementation. Raleigh and Wallace (1963) observed an increase in total intake when urea was included in the diet of steers consuming a low-quality meadow roughage. The largest increase in intake observed in our study with the first increment of N supplementation is also in

agreement with Campling et al. (1962), Köster et al. (1996), and Wickersham et al. (2004). These studies also reported diminishing responses to N supplementation which eventually formed a plateau similar to our study. The diminishing response and eventual plateau is due to the ruminal N deficiency being met and eventually exceeded as N supplementation level increases.

There was no N source effect on FOMI or total OMI in our study although TDOMI was affected. The use of the SRU resulted in diminished intake response (approximately 98%) of TDOMI when compared to urea. A study with dairy cows consuming a TMR also observed decreases in DMI when a SRU was included in the diet (Golombeski et al., 2006). Taylor-Edwards et al. (2009) also observed a decrease in DMI of growing steers consuming a basal diet of corn silage and supplemented with a SRU compared to urea during the second period of a growing trial although DMI did not differ during the first period or over the entire trial. Other studies comparing SRU's to urea have not observed differences in intake with cattle consuming either forage based or concentrate diets (Galo et al., 2003; Tedeschi et al., 2002; Thompson et al., 1972). In contrast to our results, Owens et al. (1980) observed increases in digestible DM intake and roughage intake when a SRU was supplemented to steers consuming cottonseed hulls compared to urea.

Glycerol did not elicit any effects on FOMI, total OMI, or TDOMI in this study although TDNDFI was reduced when glycerol was supplemented. Heldt et al. (1999) similarly did not observe any differences in FOMI when steers were supplemented with several carbohydrate sources although total OMI and TDOMI were observed to

increases with supplemental sugar and starch. In contrast to our study, Olson et al. (1999) observed that supplementing starch to steers consuming low-quality forage decreased FOMI and TDOMI. Supplementing DIP to these steers helped to alleviate the negative effects of starch supplementation, but was unable to overcome them. Klevesahl et al. (2003), on the other hand, observed reductions in FOMI when starch was supplemented, but total OMI was not affected. Sanson et al. (1990) also observed decreases in forage DMI and total digestible DMI in steers consuming a low-quality hay and supplemented with increasing levels of corn. The relatively low supplementation level (0.1% BW) and the higher CP content of our forage compared to most other studies are probably the main drivers why we did not see reductions in intake with energy supplementation that other studies have observed.

Increases in OMD with NPN supplementation have been observed in several studies (Campling et al., 1962; Raleigh and Wallace, 1963; Hunter and Siebert, 1980; Lee et al., 1987) although total tract digestibility was not affected in our study. Additionally, Campling et al. (1962) and Raleigh and Wallace (1963) observed increases in fiber digestion although Fick et al. (1973) and Ammerman et al. (1972) did not observe any differences in cellulose digestibility with or without NPN supplementation. Furthermore, Mathis et al. (1999) and Stokes et al. (1988) also observed a quadratic response of OMD and NDF digestion when SBM was supplemented to cattle consuming low-quality prairie hay. The improvement in ruminal digestion observed in our study can be attributed to the increase in N status of ruminal microbes when N was supplemented allowing the ruminal microbial population to grow and increase

fermentation of the feed in the rumen. The low concentrations of ruminal ammonia N observed when no supplemental N was provided probably limited microbial population growth (Satter and Slyter, 1974) and therefore limited ruminal digestion. Supplementing N caused ruminal ammonia N levels to increase allowing microbial growth to increase and resulting in the increased ruminal OMD and NDF digestion observed. Campling et al. (1962) also observed an increase in ruminal fiber digestibility when urea was supplemented to cattle. In contrast to the effect of N source on digestion that was observed in our study, Galo et al. (2003) did not observe any differences in either OMD or NDF digestibility of a TMR with or without a SRU when fed to dairy cattle although DMD was increased with addition of the SRU. Additionally, Owens et al. (1980) and Forero et al. (1980) did not observe any differences in DMD between cattle consuming low-quality roughages and receiving either urea or a SRU supplement.

Although there was no effect of glycerol on total OM or ruminal digestion in this study, other studies have generally reported decreases in digestibility with CHO supplements. Similar to the decrease in NDF digestibility observed in our study, Olson et al. (1999) reported decreases of both OMD and NDF digestion with increased starch supplementation. Additionally, Klevesahl et al. (2003) and Heldt et al. (1999) observed a decrease in NDF digestion with addition of CHO supplements although OMD was not affected. Sanson et al. (1990) also observed decreases in NDF digestion although OMD was reported to increase with addition of starch from corn. The increase in OMD is probably due to the increased amounts of highly digestible corn in the diet. Sanson and Clanton (1989) did not observe any significant change in either OMD or NDF digestion

although NDF digestion tended to decrease with increased corn in the diet and OMD increase.

Rumen pH levels in our study tended to increase with N supplementation. In contrast, pH levels have been observed to decrease with N supplementation in many other studies (Stokes et al., 1988; Olson et al., 1999; Klevesahl et al., 2003; Köster et al., 1996). Decreases in pH levels have been attributed to increases in total VFA concentrations due to increased ruminal fermenatation. However, the increase in rumen pH with N supplementation in our study can be explained by a lack of change in total VFA concentrations with N supplementation and the high levels of ruminal ammonia possibly being converted to ammonium and acting as a H^+ sink. The lack of change in total VFA concentration with N supplementation has been observed previously (Wickersham et al., 2004; McCollum and Gaylean, 1985) although many studies show increases in total VFA concentration with N supplementation due to increases in ruminal fermentation (Stokes et al., 1988; Olson et al., 1999; Köster et al., 1996; Hannah et al., 1991). Supplementation of glycerol decreased acetate concentrations and increased propionate and butyrate concentrations. In agreement with our results, Rémond et al. (1993) utilizing both *in vivo* and *in vitro* techniques observed increases in propionate and butyrate concentrations and a decrease in acetate concentration when glycerol was administered. Additionally, Czerkawski and Breckenridge (1972) observed increases in propionate and butyrate *in vitro* when glycerol was added as a substrate. Other studies have observed similar results when supplementing various CHOs to cattle on low-quality

forage (Heldt et al., 1999; Sanson and Clanton, 1989; Olson et al., 1999; Klevesahl et al., 2003).

As stated earlier, cattle receiving no supplemental N had ruminal ammonia N concentrations below levels needed to optimize microbial efficiency (Satter and Slyter, 1974). However, the first level of N supplementation (64 mg N/kg BW) as well as subsequent supplementation levels alleviated this deficiency. When N was supplemented, resulting ruminal ammonia concentrations were quite larger than previous studies utilizing either NPN or true protein supplements (Lee et al., 1987; Köster et al., 1996 and 1997; Wickersham et al., 2004, Klevesahl et al., 2003). This may be due to our use of a liquid supplement compared to a dry supplement normally used and our forage quality being higher. No difference in ruminal ammonia N levels was observed between N source. In fact, it the SRU did not seem to release urea slowly with ruminal ammonia N trends over time similar between both urea and the SRU (Figure 1).

Implications

It seemed that the use of the SRU did not reduce the rate of urea release in the rumen as indicated by ruminal ammonia N levels. The utilization of the SRU in place of urea did not effect either FOMI or TOMI intake although TDOMI was generally greater with urea than the SRU. Additionally, the SRU reduced both total and ruminal fiber digestion as well as total OMD compared to urea. Furthermore, rumen fermentation characteristics were not observed to be different between the two N sources. Although intake and digestion were reduced with SRU, if animal performance is maintained the use of a SRU could still be beneficial with increased efficiency in feed:performance

ratios. However, animal performance has been observed to be similar in other studies between the two N sources (Thompson et al., 1972; Forero et al., 1980; Tedeschi et al., 2002; Taylor-Edwards et al., 2009). Overall, this study has shown that SRU can be utilized to alleviate N deficiencies, but results in reduced responses compared to urea.

CHAPTER III

EFFECT OF HAY CRUDE PROTEIN CONTENT AND PROTEIN SUPPLEMENTATION ON INTAKE AND DIGESTION IN BEEF STEERS

Overview

This experiment was conducted to evaluate the effects of hay crude protein and protein supplementation on intake and digestion in beef steers. Twelve duodenally and ruminally fistulated steers (average initial BW 212 ± 39 kg) were used in a 12 × 2 crossover design experiment. Steers had ad libitum access to one of three bermudagrass (*Cynodon dactylon*) hays of differing nutritive value (7.0, 8.4, or 13.4% CP) throughout the entire trial and were supplemented with either 0 or 156 mg N/kg BW supplemented as cottonseed meal once daily. No significant Hay × CSM interactions were observed for hay OMI, total OMI, TDOMI, or digestible NDF intake ($P \geq 0.67$). Hay OMI and total OMI increased linearly ($P < 0.01$) with increasing hay nutritive value. Despite linear increases in HOMI and TOMI, a quadratic response ($P = 0.03$) was observed for TDOMI with increasing hay nutritive value. This response was largely driven by a quadratic increase ($P < 0.01$) in OM digestion with increasing hay nutritive value. Supplementing CSM generally did not affect HOMI ($P = 0.63$) although TDOMI ($P = 0.03$) was increased. Similarly, OM digestion ($P = 0.61$) and NDF digestion ($P = 0.11$) were not impacted by CSM supplementation. Increases in intake and digestibility with hay nutritive quality in this trial suggests that the practice of N fertilization can be utilized in lieu of protein supplementation when it is cost effective.

Introduction

Targeted delivery of small amounts of supplemental protein is effective at stimulating forage utilization when a deficiency of ruminal available N is present (Wickersham et al., 2008a; Köster et al., 1996; Hannah et al., 1991) and maintaining cow performance under the same conditions (Delcurto et al., 1990b; Sanson et al. 1990). In contrast to the relatively predictable forage-quality observed in native range, bermudagrass (*Cynodon dactylon*) quality can be variable, subject to manipulation by management, namely N fertilization and maturity at harvest (Burton et al., 1963; Webster et al., 1965; Monson and Burton, 1982), and difficult to adequately describe. These factors coupled with the resistance of producers to quantify hay nutritive value creates a less than ideal scenario for making supplementation decisions and recommendations. To improve the efficiency of supplementation programs for cattle consuming a bermudagrass based diet additional information on supplementation responses in cattle consuming bermudagrass hays of divergent nutritive value are required. Therefore, our study was conducted to evaluate the interactions of protein supplementation and bermudagrass hay nutritive value on intake and digestion in beef cattle.

Materials and Methods

Twelve duodenally and ruminally fistulated steers (average initial BW 212 ± 39 kg) were used in a 12 × 2 crossover design with 6 treatments and 2 periods (Cochran and Cox, 1957). Treatments were arranged as a 3 × 2 factorial. Steers received one of three bermudagrass (*Cynodon Dactylon*) hays of differing nutritive value (Table 3-1)

throughout the entire trial and either 0 or 156 mg N/kg BW fed as cottonseed meal (**CSM**). All hays were produced from an established stand of bermudagrass in College Station, Texas and harvested on the same day. Level of supplemental protein was based on previous research (Köster et al., 1996, Klevesahl et al., 2003, and Wickersham et al., 2004) and was chosen to maximize total digestible OM intake (**TDOMI**). Cottonseed meal was used as the protein supplement due to its general use as a protein supplement and its high protein content (45%).

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University and included the use of anesthesia when surgical procedures were performed.

Steers were housed in an enclosed, climate controlled barn in individual pens (2.1 m × 1.5 m) with continuous lighting and ad libitum access to water and a trace mineral salt block (composition: 98.0% NaCl, 1.0% S, 0.15% Fe, 0.25% Zn, 0.30% Mn, 0.01% I, 0.02% Cu, and 0.003% Co; United Salt Corp, Houston, TX). Bermudagrass hay was chopped through a 75 mm × 75 mm screen and fed at 130% of the previous 4-d average intake to insure that access to hay was not restricting intake. The two experimental periods were divided into three phases: (i) 9 days adaptation to treatments; (ii) 7 days measurement of hay intake and digestion; (iii) 1 day ruminal sampling. The CSM supplement was fed at 0630 each morning and hay was fed at approximately 0700 h. Approximately 1 kg of hay was retained, daily, for subsequent analysis. During days 10 - 16 of each period, both voluntary hay intake and total fecal output were determined. Hay and ort samples collected on days 10 – 15, and total fecal output collected on days

11 - 16 were used to determine total tract digestion according to the guidelines of Cochran and Gaylean (1994). Orts were removed at 0600 h and approximately 200 g per day were retained for analysis. Fecal bags were removed and contents weighed at 0600 h daily. Feces collected over each 24 h period were thoroughly mixed, and approximately 600 g per day was retained for analysis.

On the day following the completion of fecal collections ruminal fluid samples were collected by suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) just prior to dosing (0 h) and at 2, 4, 8, 12, and 16 h after supplements were fed. Immediately after sampling, ruminal pH was measure using a portable pH meter with a combination electrode (Thermo Scientific, Singapore). Eight ml of ruminal fluid was combined with 2 ml of 1 N HCl and frozen for VFA and NH_3 analysis. Blood was collected from the jugular vein 0, 4, and 12 h after feeding on d 18 for later determination of plasma urea N concentration.

Laboratory Analysis

Hay, orts, and fecal samples were dried at 55°C in a forced-air oven for 96 h and then ground in a Wiley mill to pass through a 1 mm screen. Hay samples collected during the measurement period were pooled across days on an equal weight basis. Ort and fecal samples were composited in proportion to their daily refusal or output, respectively, by steer among days for each measurement period. Hay, supplement, ort, fecal, and duodenal samples were then dried for 24 h at 105°C in a forced-air oven to determine DM and then combusted for 8 h at 450°C in a muffle furnace to determine OM. Nitrogen content of samples was determined by total combustion (Rapid N Cube,

Elementar Americas, Inc, Mt Laurel, NJ). Crude protein was then calculated by N × 6.25. The ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY) was used to determine NDF and ADF (Komarek et al., 1993) of hay, ort, supplement, and duodenal samples. Ruminal VFAs were determined by GLC as described by Vanzant and Cochran (1994). Colorimetric determination of ruminal ammonia (Broderick and Kang, 1980) and plasma urea (Marsh et al, 1965) were made using an UV/VIS (DU 730 UV/VIS Spectrometer, Beckman Coulter, Inc, Fullerton, CA). Total tract digestion coefficients for DM, OM, and NDF were determined, using total collections, as described by Cochran and Galyean (1994).

Statistical Analysis

Intake and digestion were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Terms in the model were treatment and period with steer included as a random effect. Fermentation profile variables and plasma urea N concentrations were analyzed using the MIXED procedure of SAS. Terms in the model were treatment, period, hour, and hour × treatment with steer and treatment × period × steer included as random terms. The repeated term was hour with treatment × steer serving as the subject. Compound symmetry was used for the covariance structure. The LSMEANS option was used to calculate treatment means. Orthogonal polynomial contrasts (linear, quadratic, and cubic) were used to partition treatment sums of squares.

Results and Discussion

No significant Hay × CSM interactions were observed for hay OMI (**HOMI**), total OMI, TDOMI, or digestible NDF intake ($P \geq 0.67$; Table 3-2). Significant

increases in utilization (i.e., intake and digestion) of the 7% or 8.4% CP hays would have likely resulted in significant Hay × CSM interactions. Our expectation, based on the conclusions of Milford and Minson (1965) and Moore and Kunkle (1995), were that steers consuming the 7.0% CP would increase hay utilization in response to CSM supplementation: however, hay intake was not increased ($P = 0.57$) by CSM. Total OMI and TDOMI intake increased ($P = 0.04$ and 0.03, respectfully) within CSM provision though these increases can largely be attributed to the provision of CSM and not improvements in hay utilization. It is noteworthy, that there was a 10.4% increase in HOMI when CSM was provided to cattle consuming the 7.0% CP hay.

Hay OMI increased linearly ($P < 0.01$) with hay nutritive value. Hay OMI was 16% greater in unsupplemented cattle consuming the 13.2% CP than in unsupplemented cattle fed the 7.0% CP hay (61.64 vs. 71.77 g/kg $BW^{0.75}$, respectively). Similarly, TDOMI and total digestible NDF intake were 83 and 56% greater when the 13.2% CP hay was fed versus the 7.0% CP hay. Despite linear increases in HOMI and TOMI, a quadratic response ($P = 0.03$) was observed for TDOMI with increasing hay nutritive value. This response was largely driven by a quadratic increase ($P < 0.01$) in OM digestion (**OMD**) with increasing nutritive value of hays. When hay nutritive value increased from 7.0 to 8.4% CP there was a reduction in OMD from 55.56 to 49.24% for 7.0 and 8.4% CP, respectively followed by increase in OMD for the 13.2% CP hay (65.23% OMD). The reduction in OMD for the 8.4% CP hay was unexpected and can likely be attributed to the higher ADF content of (49.9 versus 47.2% ADF, 8.4 and 7.0% CP, respectively) and the greater HOMI for 8.4 than 7.0% CP, which may have

increased passage rate. There was a tendency ($P = 0.08$) for a Hay × CSM interaction for NDF digestibility (NDFD) this tendency was likely the result of greater NDFD in the unsupplemented steers receiving the 7.0% CP hay than supplemented steers followed by similar NDFD for supplemented and unsupplemented steers for both 8.4 and 13.2% CP hays.

In contrast to our results Mathis et al. (2000) reported HOMI, TDOMI, OMD and NDFD (5.24 kg/d, 3.32 kg/d, 63.2% and 65.9%, respectively) for an 8.2% CP bermudagrass hay, which were more similar to our observations with the 13.2% CP hay. The bermudagrass hay used in their project contained 71% NDF and 37% ADF, which was similar in content to the 13.2% CP hay used in our project. This comparison indicates that despite a degradable intake protein (DIP) deficiency which was observed in Mathis et al. (2000) and was likely in our project (as evidenced by the increases in intake observed) digestibility is likely a greater barrier to the utilization of bermudagrass than DIP deficiency specifically.

Also in contrast to our results, Reid and Jung (1965) did not observe any difference in dry matter digestibility **(DMD)** or cellulose digestibility in first cutting tall fescue hay but did see increased digestibilities in aftermath tall fescue hay with increasing N fertilization. Also, Puoli et al. (1991) did not observe any differences in digestibility in switchgrass or big bluestem with increases in N fertilization.

Ruminal ammonia concentrations increased quadratically ($P < 0.01$) as hay quality increased (Table 3-3). The largest increase in ruminal ammonia occurred between the low to medium-quality hays (263% increase) compared to between the

medium to high-quality hay (19% increase). Supplementation with CSM increased ruminal ammonia concentrations compared to when CSM was not fed ($P < 0.01$) and followed a similar trend across hay qualities as when CSM was not supplemented. Ruminal ammonia concentrations over time are illustrated in Figure 3-1. Ammonia levels tended to peak by hour 2 for all treatments except for animals consuming the 8.4% CP hay and not supplemented with CSM whose ammonia concentrations peaked at hour 4. The later peak is probably a consequence of the lower digestibility of the 8.4% CP hay compared to the other hays. Furthermore, higher hay quality tended to extend higher ammonia concentrations in the rumen regardless if CSM was or was not supplemented. However, addition of CSM to the diet did result in higher ruminal ammonia levels throughout the whole day.

The quadratic response we observed can be explained by the decreased digestibility of the 8.4% CP hay compared to the 7.0% CP hay resulting in a build up of ruminal NH_3. This was then followed by an increase in digestibility with the 13.2% CP hay. The increased protein content of the hay caused NH_3 levels to increase however the increased digestibility, on the other hand, allowed ruminal microbes to utilize more of the ruminal NH_3 therefore resulting in a smaller increase in ruminal NH_3. Similar to our results, Messman et al. (1991) observed higher average ruminal NH3 concentrations in cattle fed a 15.6% compared to a 10.7% CP bromegrass hay.

Plasma urea N (**PUN**; $P < 0.01$), ruminal pH ($P < 0.01$), and total VFA ($P = 0.06$) were characterized by treatment × time interactions. However, the interactions were largely due to variation in magnitude of differences within each time period.

Therefore, data were averaged across time. Hay × CSM interactions were not evident for PUN ($P = 0.72$), ruminal pH ($P = 0.69$), or total VFA ($P = 0.66$). Plasma-urea N followed similar trends as ruminal ammonia concentrations and increased quadratically ($P < 0.01$) with higher quality hay. Moving from the low-quality hay to the medium quality hay resulted in a larger increase (136% increase) in PUN than from the medium to high quality hay (17% increase). As would be expected, supplementing CSM increased PUN concentration for each hay quality ($P < 0.01$) and followed a similar trend to when CSM was not supplemented. Rumen pH levels were not affected by either hay quality ($P = 0.44$) or CSM supplementation ($P = 0.94$). Ruminal pH level averaged 6.50 over all treatments.

Total VFA concentration was not affected by Hay × CSM interactions ($P = 0.66$), hay quality ($P = 0.32$) or CSM supplementation ($P = 0.38$). However, when averaged across treatments, total VFA concentration was numerically greater for the high-quality hay compared to the other hays. Acetate concentrations decreased as hay quality increased ($P < 0.01$) but was not affected by CSM supplementation ($P = 0.30$). Butyrate concentration was also significantly affected by hay quality ($P < 0.01$) with the medium-quality hay suppressing butyrate concentrations compared to the low-quality hay and the high-quality hay having greater butyrate concentrations than the low-quality hay. Isobutyrate, valerate, and isovalerate were each significantly affected by both hay quality and CSM supplementation, but the changes in their concentrations were minute and their biological significance would also be small.

Wickersham et al. (2008a) observed linear increases in PUN with protein supplementation compared to the quadratic response observed in our study. The differences in responses observed is similar to differences in ruminal NH_3 levels observations dicussed above and are probably due to differences in forage digestibility. Additionally, our PUN values are larger than those reported by Wickersham et al. (2008a), however the CP concentration in our forage was higher than the forage they utilized. Similar to our study, Mathis et al. (2000) did not observe differences in ruminal pH levels when increasing levels of protein were supplemented to cattle consuming bermudagrass hay. The pH values that we observed are also similar to those observed by Mathis et al. (2000), however, Köster et al. (1996) observed a higher rumen pH levels when N supplements were not administered. However, as supplementation level increased pH in their study also decreased to levels similar to those that we observed in our study. The decrease in rumen pH in their study was attributed to increases in ruminal fermentation evident by increases in total VFA concentrations. Total VFA concentrations were not changed in our study therefore ruminal pH also did not change. Additionally total VFA concentrations observed by Köster et al. (1996) were lower when protein supplements were not administered, but then increased with supplementation to levels similar to those in our study. The reduction in acetate levels as hay quality increased in our study is similar to observations by Koster et al. (1996) and Mathis et al. (2000) and are probably due to the increases in minor VFA production. In contrast, Messman et al. (1991) did not observe differences in acetic, propionic, or butyric acid levels when feeding bromegrass of different nutritive values.

Implications

Supplementing CSM generally did not affect intake or digestibility in this trial mainly due to the two highest hay qualities being greater than 6-7% CP. However, intake of the lowest quality hay was increased with supplementation. Hay quality did have an impact on intake with higher quality hays having an increased intake. Similarly, total tract digestibilities tended to increase with hay quality. The results from this trial indicate that N fertilization of hays can have an impact on the energy status of cattle and that N fertilization should be utilized in lieu of protein supplementation when it is cost effective relative to protein supplements. Additionally, future trials conducted with lower hay qualities would give better data to when either protein supplements or N fertilization is the most cost effective.

CHAPTER IV

SUMMARY

In our first trial, it seemed that the use of the SRU did not reduce the rate of urea release in the rumen as indicated by ruminal ammonia N levels. The utilization of the SRU in place of urea did not effect either FOMI or TOMI intake although TDOMI was generally greater with urea than the SRU. Additionally, the SRU reduced both total and ruminal fiber digestion as well as total OMD compared to urea. Furthermore, rumen fermentation characteristics were not observed to be different between the two N sources. Although intake and digestion were reduced with SRU, if animal performance is maintained the use of a SRU could still be beneficial with increased efficiency in feed:performance ratios. However, animal performance has been observed to be similar in other studies between the two N sources (Thompson et al., 1972; Forero et al., 1980; Tedeschi et al., 2002; Taylor-Edwards et al., 2009). Overall, this study has shown that SRU can be utilized to alleviate N deficiencies, but results in reduced responses compared to urea.

In our second trial supplementing CSM generally did not affect intake or digestibility in this trial mainly due to the two highest hay qualities being greater than 6-7% CP. However, intake of the lowest quality hay was increased with supplementation. Hay quality did have an impact on intake with higher quality hays having an increased intake. Similarly, total tract digestibilities tended to increase with hay quality. The results from this trial indicate that N fertilization of hays can have an impact on the energy status of cattle and that N fertilization should be utilized in lieu of protein

supplementation when it is cost effective relative to protein supplements. Additionally, future trials conducted with lower hay qualities would give better data to when either protein supplements or N fertilization is the most cost effective.

LITERATURE CITED

Adams, D.C., R. C. Cochran, and P. O. Currie. 1987. Forage maturity effects on rumen fermentation, fluid flow, and intake in grazing steers. J. Range Mgmt. 40:404-408.

Allison, C. D. 1985. Factors affecting forage intake by range ruminants: A review. J. Range Manage. 38:305-311.

Allison, M. J. 1969. Biosynthesis of amino acids by ruminal microorganisms. J. Anim. Sci. 29:269-277.

Ammerman, C. B., G. J. Verde, J. E. Moore, W. C. Burns, and C. F. Chicco. 1972. Biuret, urea and natural proteins as nitrogen supplements for low quality roughage for sheep. J. Anim. Sci. 35:122-127.

Blaser, R. E. 1962. Symposium on forage utilization: Effects of fertility levels and stage of maturity on forage nutritive value. J. Anim. Sci. 23:246-253.

Broderick, G. A. and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.

Burns, J. C., K. R. Pond, D. S. Fisher, and J. M. Luginbuhl. 1997. Changes in forage quality, ingestive mastication, and digesta kinetics resulting from switchgrass maturity. J. Anim. Sci. 75:1368-1379.

Burton, G. W. and E. H. DeVane. 1952. Effect of rate and method of applying different sources of nitrogen upon the yield and chemical composition of bermudagrass. Agron. J. 44:128-132.

Burton, G. W. and J. E. Jackson. 1962. Effect of rate and frequency of applying six nitrogen sources on Coastal bermudagrass. Agron. J. 54:40-43.

Burton, G. W., J. E. Jackson, and R. H. Hart. 1963. Effects of cutting frequency and nitrogen on yield, *in vitro* digestibility, and protein, fiber, and carotene content of Coastal Bermudagrass. Agron. J. 55-500-502

Campling, R. C., M. Freer, and C. C. Balch. 1962. Factors affecting the voluntary intake of food by cows. 3. The effect of urea on the voluntary intake of oat straw. Brit. J. Nutr. 16:115-124.

Church, D. C. and A. Santos. 1981. Effect of graded levels of soybean meal and of a nonprotein nitrogen-molasses supplement on consumption and digestibility of wheat straw. J. Anim. Sci. 53:1609-1615.

Clifford, A. J., J. R. Bourdette, and A. D. Tillman. 1968. Effects of urease inhibitors on sheep fed diets containing urea. J. Anim. Sci. 27:1073-1080.

Cochran, R. C. and G. M. Cox. 1957. Experimental Design (2nd Ed.). John Wiley & Sons, New York.

Cochran, R. C. and M. L. Galyean. 1994. Measurement of in vivo forage digestion by ruminants. In: G. C. Fahey, Jr., M. C. Collins, D. R. Mertens, and L. E. Moser (Ed.) Forage Quality, Evaluation, and Utilization. Pp 613-643. ASA-CSSA-SSSA. Madison, WS.

Coleman, S. W., J. E. Moore, J. R. Wilson. 2004. Quality and Utilization. In: L.E. Moser, B.L. Burson, and L.E. Sollenberger. (Ed.) Warm-Season (C4) Grasses. Pp 267-308. ASA-CSSA-SSSA. Madison, WI.

Collins, M. and J. O. Fritz. 2004. Forage Quality. In: R. F. Barnes, C. J. Nelson, M. Collins, and K. J. Moore (Ed.) Forages: An introduction to grassland agriculture (6^{th} Ed.). Pp 363-390. Iowa State Press. Ames, IA.

Currier, T. A., D. W. Bohnert, S. J. Falck, and S. J. Bartle. 2004. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and the efficiency of nitrogen use in wethers. J. Anim. Sci. 82:1508-1517.

Czerkawski, J. W. and G. Breckenridge. 1972. Fermentation of various glycolytic intermediates and other compounds by rumen micro-organisms, with particular reference to methane production. Br. J. Nutr. 27:131-146.

Darwin, R., M. Tsigas, J. Lewandrowski, and A. Raneses. 1995. World agriculture and climate change: Economic adaptations. Agric. Economic Report No. 703. ERS-USDA, Washington, DC.

DelCurto, T., R. C. Cochran, D. L. Harmon, A. A. Beharka, K. A. Jacques, G. Towne and E. S. Vanzant. 1990a. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and (or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. 68:515-531.

DelCurto, T., R. C. Cochran, L. R. Corah, A. A. Beharka, E. S. Vanzant, and D. E. Johnson. 1990b. Supplementation of dormant tallgrass-prairie forage: II. Performance and forage utilization characteristics in grazing beef cattle receiving supplements of different protein concentrations. J. Anim. Sci. 68:532-542.

Delagarde, R., J. L. Peyraud, and L. Delaby. 1997. The effect of nitrogen fertilization level and protein supplementation on herbage intake, feeding behaviour and digestion in grazing dairy cows. Anim. Feed Sci. and Tech. 66:165-180.

Fick, K. R., C. B. Ammerman, C. H. McGowan, P. E. Loggins and J. A. Cornell. 1973. Influence of supplemental energy and biuret nitrogen on the utilization of low quality roughage by sheep. J. Anim. Sci. 36:137-143.

Forero, O., F. N. Owens, K. S. Lusby. 1980. Evaluation of slow-release urea for winter supplementation of lactating range cows. J. Anim. Sci. 50:532-538.

Galo, E., S. M. Emanuele, C. J. Sniffen, J. H. White, and J. R. Knapp. 2003. Effect of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. J. Dairy Sci. 86:2154-2162.

Golombeski, G. L., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2006. Slow-release urea and highly fermentable sugars in diets fed to lactating dairy cows. J. Dairy. Sci. 89:4395-4403.

Guthrie, M. J. and D. G. Wagner. 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization, and passage rate of prairie hay in beef steers and heifers. J. Anim. Sci. 66:1529-1537.

Hannah, S. M., R. C. Cochran, E. S. Vanzant, and D. L. Harmon. 1991. Influence of protein supplementation on site and extent of digestion, forage intake, and nutrient flow characteristics in steers consuming dormant bluestem-range forage. J. Anim. Sci. 69:2624-2633.

Harbers, L.H., A. D. Tillman, and W. J. Visek. 1962. Effect of barbituric acid on urea diets for ruminants. J. Anim. Sci. 21:754-756.

Heldt, J. S., R. C. Cochran, G. L. Stokka, C. G. Farmer, C. P. Mathis, E. C. Titgemeyer, and T. G. Nagaraja. 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by steers. J. Anim. Sci. 77:2793-2802.

Helmer, L. G. and E. E. Bartley. 1971. Progress in the utilization of urea as a protein replacer for ruminants. A review. J. Dairy Sci. 54:25-51.

Hunter, R. A. and B. D. Siebert. 1980. The utilization of spear grass (*Heteropogan contortus*). IV. The nature and flow of digesta in cattle fed on spear grass alone and with protein or nitrogen or sulfur. Aust. J. Agric. Res. 31:1037-1047.

Jung, H. G. and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774-2790.

Kartchner, R. J. 1981. Effect of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. J. Anim. Sci. 51:432-438.

Kelsey, R. J., A. B. Nelson, G. S. Smith and R. D. Pieper. 1973. Nutritive value of hay from nitrogen-fertilized blue grama rangeland. J. Range Manage. 26:292-294.

Kertz, A. F., M. K. Koepke, L. E. Davidson, N. L. Betz, J. R. Norris, L. V. Skoch, B. R. Cords, and D. T. Hopkins. 1980. Factors influencing intake of high urea-containing rations by lactating dairy cows. J. Dairy Sci. 65:587-604.

Klevesahl, E. A., R. C. Cochran, E. C. Titgemeyer, T. A. Wickersham, C. G. Farmer, J. I. Arroquy, D. E. Johnson. 2003. Effect of a wide range in the ration of supplemental rumen degradable protein to starch on utilization of low-quality, grass hay by beef steers. Anim. Feed Sci. and Tech. 105:5-20.

Komarek, A R., J. B. Robertson, and P. J. Van Soest. 1993. A comparison of methods for determining ADF using the filter bag technique versus conventional filtration. J. Dairy Sci. 77:24-26.

Köster, H. H., B. C. Woods, R. C. Cochran, E. S. Vanzant, E. C. Titgemeyer, D. M. Grieger, K. C. Olson, and G. Stokka. 2002. Effect of increasing proportion of supplemental N from urea in prepartum supplements on range beef cow performance and on forage intake and digestibility by steers fed low-quality forage. J. Anim. Sci. 80:1652-1662.

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

Köster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, T. G. Nagaraja, K. K. Kreikemeier, and G. St. Jean. 1997. Effect of increasing proportion of

supplemental N from urea on intake and utilization of low-quality tallgrass-prairie forage by beef steers. J. Anim. Sci. 75:1393-1399.

Lee, G. J., D. W. Hennessy, J. V. Nolan, and R. A. Leng. 1987. Responses to nitrogen and maize supplements by young cattle offered a low-quality pasture hay. Aust. J. Agric. Res. 38:195-207.

Lima, G.F.da C., L.E. Sollenberger, W. Kunkle, J.E. Moore, and A.C. Hammond. 1999. Nitrogen fertilization and supplementation effects on performance of beef heifers grazing limpograss. Crop Sci. 39:1853-158.

Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11:624-627.

Matejovsky, K. M. and D. W. Sanson. 1995. Intake and digestion of low-, medium-, and high-quality grass hays by lambs receiving increasing levels of corn supplementation. J. Anim. Sci. 73:2156-2163.

Mathis, C. P., R. C. Cochran, G. L. Stokka, J. S. Heldt, B. C. Woods, and K. C. Olson. 1999. Impact of increasing amounts of supplemental soybean meal on intake and digestion of beef steers and performance by beef cows consuming low-quality tallgrass-prairie forage. J. Anim. Sci. 77:3156-3162.

Mathis, C. P., R. C. Cochran, J. S. Heldt, B. C. Woods, I. E. O. Abdelgadir, K. C. Olson, E. C. Titgemeyer, and E. S. Vanzant. 2000. Effects of supplemental degradable intake protein on utilization of medium- to low-quality forages. J. Anim. Sci. 78:224-232.

Mathison, G. W. and L. P. Milligan. 1971. Nitrogen metabolism in sheep. Br. J. Nutr. 25:361-366.

McCollum III, T. 1997. Supplementation strategies for beef cattle. Bulletin No. B-6067. TAES-Texas A&M University System, College Station, TX.

McCollum, F. T. and M. L. Galyean. 1985a. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. 60:570-577.

McCollum, F. T. and M. L. Gaylean. 1985b. Cattle grazing blue grama rangeland II. Seasonal forage intake and digesta kinetics. J. Range Manage. 38:543-546.

McCollum, F. T., M. L. Galyean, L. J. Krysl, and J. D. Wallace. 1985. Cattle grazing blue grama rangeland I. Seasonal diets and rumen fermentation. J. Range Manage. 38:539-543.

Messman, M. A., W. P. Weiss, and D. O. Erickson. 1991. Effects of nitrogen fertilization and maturity of bromegrass on in situ ruminal digestion kinetics or fiber. J. Anim. Sci. 69:1151-1161.

Milford, R. and D. J. Minson. 1965. Intake of tropical pasture species. In: Proc. 9th Int. Grasslands Congress, São Paulo, Brazil.

Minson, D.J. 1973. Effect of fertilizer nitrogen on digestibility and voluntary intake of Chloris gayana, Digitaria decumbens and Pennisetum clandestinum. Australian J. of Experimental Agriculture and Animal Husbandry. 13:153-157.

Monson, W. G. and G. W. Burton. 1982. Harvest frequency and fertilizer effects on yield, quality, and persistence of eight bermudagrasses. Agron. J. 74:371-374.

Moore, J. E., M. H. Brant, W. E. Kunkle, and D. I. Hopkins. 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. J. Anim. Sci. 77 (2):122-135.

Moore, J. E. and W. E. Kunkle. 1995. Improving forage supplementation programs for beef cattle. In: 6th Florida Ruminant Nutrition Symposium, Gainsville, FL.

NRC. 1996. Nutrient Requirements of Beef Cattle (7th Ed.). National Academy Press, Washington, DC.

Olson, K. C., R. C. Cochran, T. J. Jones, E. S. Jones, E. S. Vanzant, E. C. Titgemeyer, and D. E. Johnson. 1999. Effect of ruminal administration of supplemental degradable intake protein and starch on utilization of low-quality warm-season grass hay by beef steers. J. Anim. Sci. 77:1016-1025.

Owens, F. N., K. S. Lusby, M. Mizwicki and O. Forero. 1980. Slow ammonia release from urea: Rumen and metabolism studies. J. Anim. Sci. 50:527-531.

Pilgrim, A. F., F. V. Gray, R. A. Weller, and C. B. Belling. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Br. J. Nutr. 24:589-598.

Prine, G. M. and G. W. Burton. 1956. The effect of nitrogen rate and clipping frequency upon the yield, protein content and certain morphological characteristics of Coastal bermudagrass (*Cynodon dactylon*, (L) Pers,). Agron. J.48:296-301.

Puoli, J. R., G. A. Jung, and R. l. Reid. 1991. Effects of nitrogen and sulfur on digestion and nutritive quality of warm-season grass hays for cattle and sheep.

J. Anim. Sci. 69:843-852.

Raleigh, R. J. and J. D. Wallace. 1963. Effect of urea at different nitrogen levels digestibility and on performance of growing steers fed low quality flood meadow hay. J. Anim. Sci. 22:330-334.

Raun, N. S. and W. Burroughs. 1962. Suction Strainer technique in obtaining rumen fluid samples from intact lambs. J. Anim. Sci. 21:454-457.

Reid, R. L., and G. A. Jung. 1965. Influence of fertilizer treatment on the intake, digestibility and palatability of tall fescue hay. J. Anim. Sci. 24:615-625.

Reid, R. L., G. A. Jung, and S. J. Murray. 1966. Nitrogen fertilization in relation to the palatability and nutritive value of orchardgrass. J. Anim. Sci. 25:636-645.

Reid, R. L., G. A. Jung, and W. V. Thayne. 1988. Relationships between nutritive quality and fiber components of cool season and warm season forages: A retrospective study. J. Anim. Sci. 66:1275-1291.

Rémond, B., E. Souday, and J. P. Jouany. 1993. In vitro and in vivo fermentation of glycerol by rumen microbes. Anim. Feed Sci. and Tech.y 41:121-132.

Sanson, D. W., and D. C. Clanton. 1989. Intake and digestibility of low-quality meadow hay by cattle receiving various levels of whole shelled corn. J. Anim. Sci. 67:2854-2862.

Sanson, D. W., D. C. Clanton, and I. G. Rush. 1990. Intake and digestion of low-quality meadow hay by steers and performance of cows on native range when fed protein supplements containing various levels of corn. J. Anim. Sci. 68:595-603.

Satter, L. D. and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199-208.

Stern, M. D. and W. H. Hoover. 1979. Methods for determining and factors affecting rumen microbial protein synthesis: A review. J. Anim. Sci. 49:1590-1603.

Stokes, S. R., A. L. Goetsch, A. L. Jones and K. M. Landis. 1988. Feed intake and digestion by beef cows fed prairie hay with different levels of soybean meal and receiving postruminal administration of antibiotics. J. Anim. Sci. 66:1778-1789.

Taylor-Edwards, C. C., G. Hibbard, S. E. Kitts, K. R. McLeod, D. E. Axe, E. S. Vanzant, N. B. Kristensen, and D. L. Harmon. 2009. Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. J. Anim. Sci. 87:200-208.

Tedeschi, L. O., M. J. Baker, D. J. Ketchen, and D. G. Fox. 2002. Performance of growing and finishing cattle supplemented with a slow-release urea product and urea. Canadian J. Anim. Sci. 82:567-573.

Thompson, L. H., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1972. Starea, urea, and sulfur in beef cattle rations. J. Anim. Sci. 35:474-480.

Vanzant, E. S. and R. C. Cochran. 1994. Performance and forage utilization by beef receiving increasing amounts of alfalfa hay as a supplement to low-quality, tallgrass-prairie forage. J. Anim. Sci. 72:1059-1067.

Webster, J. E., J. W. Hogan, and W. C. Elder. 1965. Effect of rate of ammonium nitrate fertilizer and time of cutting upon selected chemical components and the *in vitro* rumen digestion of bermudagrass forage. Agron. J. 57:323-325.

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3079-3088.

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and E. S. Moore. 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3089-3099.

Wickersham, T. A., R. C. Cochran, E. C. Titgemeyer, C. G. Farmer, E. A. Klevesahl, J. I. Arroquy, D. E. Johnson, D. P. Gnad. 2004. Effect of postruminal protein supply on the response to ruminal protein supplementation in beef steers fed a low-quality grass hay. Anim. Feed Sci. and Tech. 115:19-36.

APPENDIX A

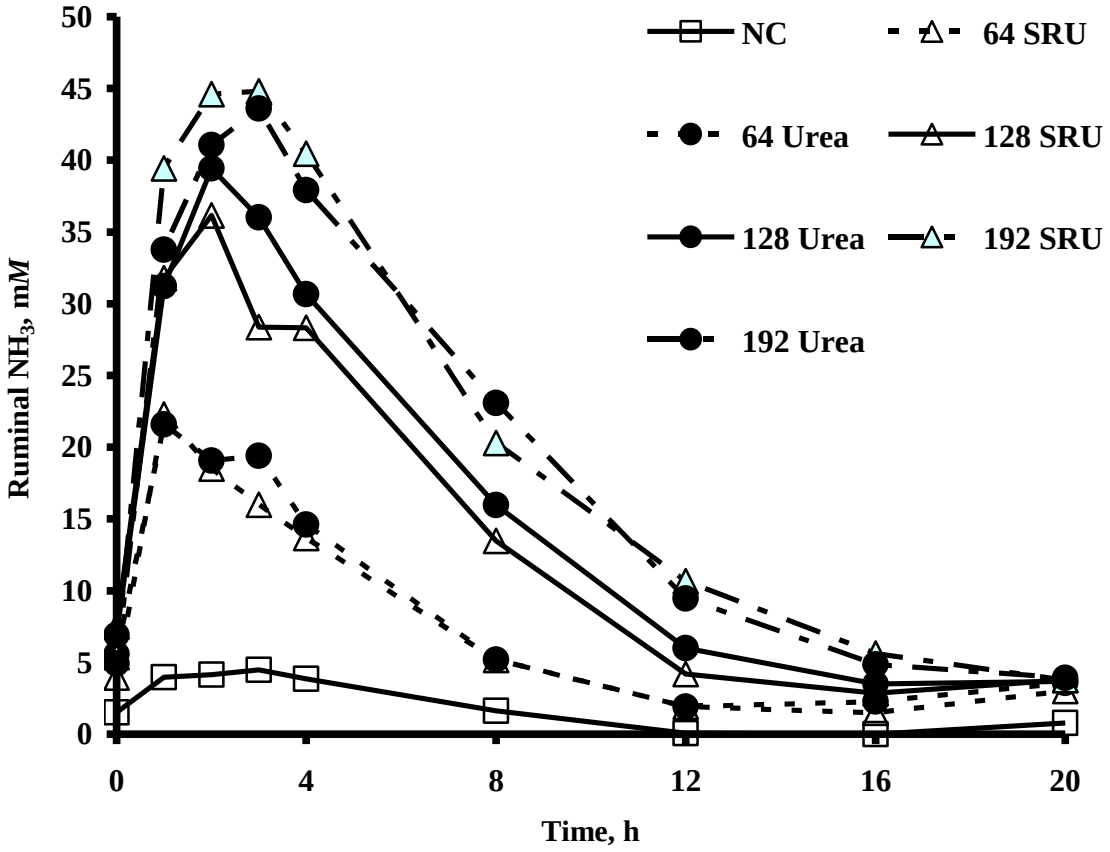

Figure 2-1. Influence of N level and source on ruminal ammonia concentrations.

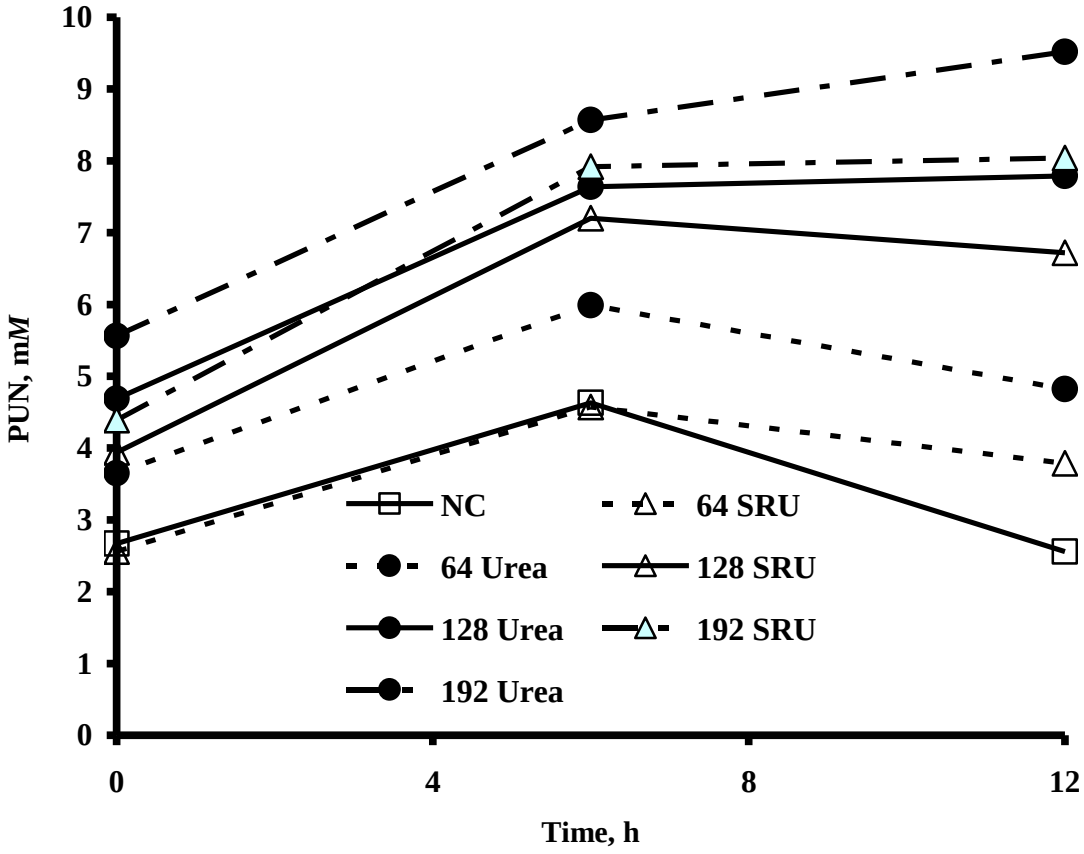

Figure 2-2. Influence of N level and source on plasma urea-N.

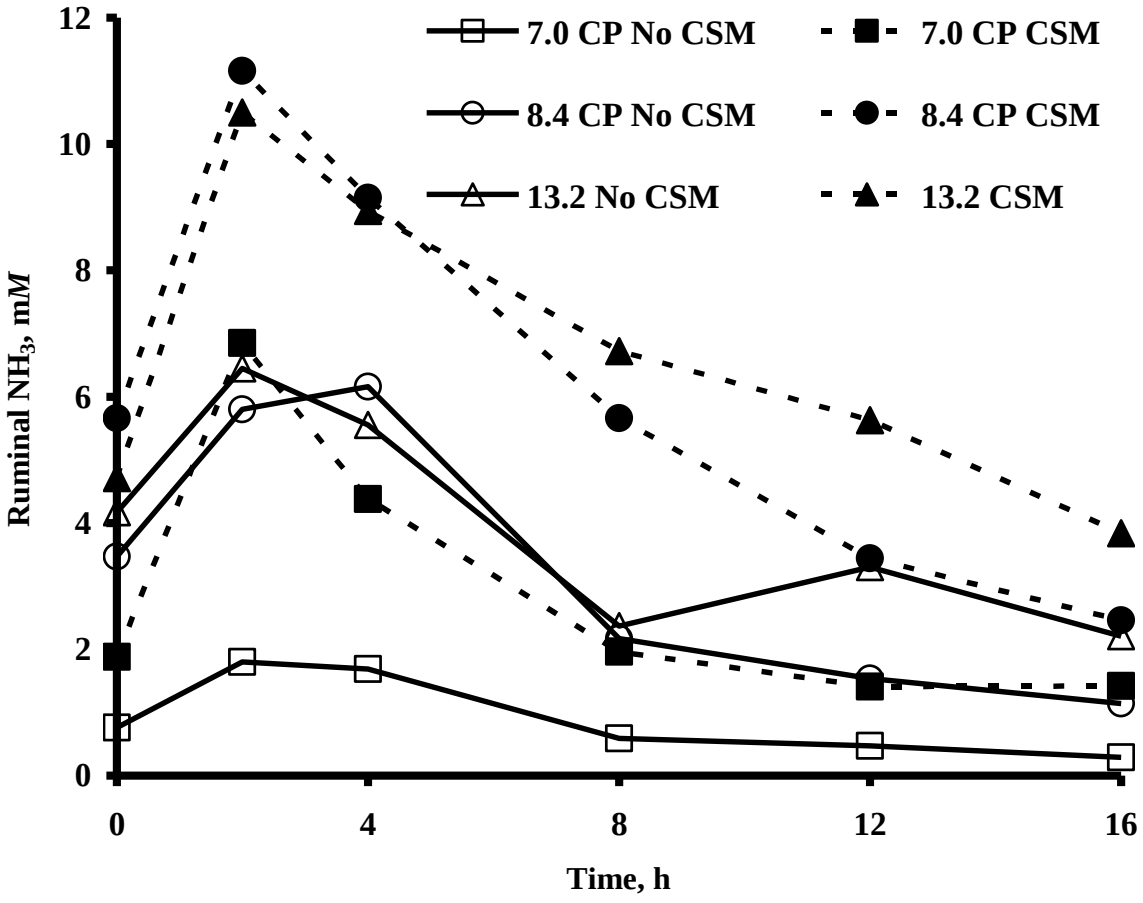

Figure 3-1. Effect of CSM supplementation and hay nutritive value on ruminal ammonia concentrations.

Table 2-1. Nutrient composition of forage and supplements

Item	Forage	Urea	SRU[a]	Glycerol
	% Dry matter			
DM	88.0	50.0	66.5	69.9
OM	93.1	98.9	70.4	96.6
CP	7.3	270.6	208.9	2.8
NDF	73.0	-	-	-
ADF	41.8	-	-	-

[a] SRU = Slow release urea

Table 2-2. Effect of level and source of NPN and glycerol supplementation on intake in beef steers consuming low-quality Bermudagrass hay

			Intake, g/kg $BW^{0.75}$					
Protein level[a]	Protein source[b]	Gly[c]	Forage OM	Total OM	Total NDF	N	Total DOM[d]	Total DNDF[e]
0	-	0.0	61.64	61.64	48.50	0.76	30.24	24.09
64	Urea	0.0	75.28	75.66	59.58	1.18	38.80	32.14
128	Urea	0.0	78.56	79.36	61.77	1.45	44.80	35.01
192	Urea	0.0	72.05	73.26	56.68	1.61	37.27	29.19
64	Urea	0.1	71.77	72.27	56.85	1.13	34.63	28.25
128	Urea	0.1	74.66	75.68	59.06	1.41	37.04	29.47
192	Urea	0.1	71.02	72.53	55.88	1.61	38.16	29.77
64	SRU	0.0	73.01	75.97	57.32	1.15	39.29	29.29
128	SRU	0.0	75.51	78.83	59.57	1.42	40.27	29.86
192	SRU	0.0	68.37	72.13	53.89	1.57	37.88	27.49
64	SRU	0.1	71.35	74.39	56.29	1.13	36.63	27.52
128	SRU	0.1	72.89	76.42	57.45	1.39	37.84	28.43
192	SRU	0.1	64.00	68.02	50.51	1.51	35.64	25.74
SEM			3.83	3.84	3.03	0.05	2.19	1.82
			Contrasts					
Treatment			0.20	0.18	0.20	<0.01	0.05	0.06
Glycerol			0.17	0.82	0.16	0.17	0.75	0.02
N Source			0.21	0.24	0.23	0.24	0.03	0.04
Contrast *P*-value								
N Level Linear			0.07	0.02	0.07	<0.01	<0.01	0.05
N Level Quadratic			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Protein level, mg N/kg BW
[b]Protein source, Urea = urea, SRU = slow release urea
[c]Gly = glycerol
[d]Total DOM = total digestible OM intake
[e]Total DNFD = total digestible NDF intake

Table 2-3. Effect of level and source of NPN and glycerol supplementation on digestion in beef steers consuming low-quality Bermudagrass hay

Protein level[a]	Protein source[b]	Gly[c]	Ruminal Digestion, %		Total Tract Digestion, %	
			OM	NDF	OM	NDF
0	-	0.0	19.82	37.13	49.40	50.00
64	Urea	0.0	23.68	41.79	50.72	53.03
128	Urea	0.0	24.22	40.45	54.88	55.56
192	Urea	0.0	26.53	43.33	50.49	50.95
64	Urea	0.1	20.61	42.05	47.28	48.75
128	Urea	0.1	23.26	46.24	49.41	50.54
192	Urea	0.1	23.27	39.60	51.20	51.22
64	SRU	0.0	21.95	38.09	52.01	51.36
128	SRU	0.0	24.45	41.32	51.10	50.12
192	SRU	0.0	26.58	38.80	52.36	50.68
64	SRU	0.1	24.51	42.00	49.18	48.80
128	SRU	0.1	26.81	50.00	49.27	48.77
192	SRU	0.1	28.51	43.20	51.96	50.74
SEM			3.06	3.12	1.15	1.47
			Contrasts			
Treatment			0.80	0.27	0.11	0.15
Glycerol			0.31	1.00	0.71	0.07
N Source			0.97	0.08	0.01	0.02
Contrast *P*-value						
N Level Linear			0.06	0.14	0.13	0.50
N Level Quadratic			0.75	0.09	0.97	0.67

[a]Protein level, mg N/kg BW
[b]Protein source, Urea = urea, SRU = slow release urea
[c]Gly = glycerol

Table 2-4. Effect of level and source of NPN and glycerol supplementation on ruminal pH, ruminal ammonia, plasma urea-N, and total VFA concentrations in beef steers consuming low-quality Bermudagrass hay

Protein level[a]	Protein source[b]	Gly[c]	pH	mM		
				Ammonia N	PUN	Total VFA
0	-	0.0	6.62	2.26	3.29	69.51
64	Urea	0.0	6.72	10.35	4.82	63.78
128	Urea	0.0	6.74	19.26	6.71	71.59
192	Urea	0.0	6.70	22.51	7.88	68.23
64	Urea	0.1	6.72	9.72	4.63	65.32
128	Urea	0.1	6.72	15.95	6.01	69.15
192	Urea	0.1	6.83	23.75	7.05	68.14
64	SRU	0.0	6.74	9.56	3.64	63.56
128	SRU	0.0	6.72	17.38	5.95	70.05
192	SRU	0.0	6.71	23.89	6.78	71.32
64	SRU	0.1	6.71	9.79	4.15	62.36
128	SRU	0.1	6.68	16.26	4.95	66.26
192	SRU	0.1	6.71	25.03	7.51	63.00
SEM			0.04	1.18	0.56	2.95
				Contrasts		
Treatment			0.26	<0.01	<0.01	0.30
Glycerol			0.89	0.53	0.43	0.17
N Source			0.25	0.92	0.04	0.35
Contrast *P*-value						
N Level Linear			0.01	<0.01	<0.01	1.00
N Level Quadratic			0.13	0.50	0.60	0.29

[a]Protein level, mg N/kg BW
[b]Protein source, Urea = urea, SRU = slow release urea
[c]Gly = glycerol

Table 2-5. Effect of level and source of NPN and glycerol supplementation on volatile fatty acid ratios in beef steers consuming low-quality Bermudagrass hay

Protein level[a]	Protein source[b]	Gly[c]	mol/100mol					
			Ace[e]	Pro[f]	But[g]	Isobut[h]	Val[i]	Isoval[j]
0	-	0.0	71.36	15.99	9.91	0.79	0.98	0.92
64	Urea	0.0	71.58	15.86	9.90	0.79	1.05	0.97
128	Urea	0.0	69.76	16.71	10.51	0.83	1.07	1.00
192	Urea	0.0	71.62	16.26	9.13	0.72	1.08	0.96
64	Urea	0.1	65.87	17.81	13.35	0.81	1.01	1.19
128	Urea	0.1	65.29	17.96	13.99	0.73	0.99	1.20
192	Urea	0.1	66.61	18.02	12.80	0.69	0.92	1.04
64	SRU	0.0	71.09	16.15	9.83	0.76	1.01	0.93
128	SRU	0.0	72.03	15.68	9.33	0.73	1.01	0.91
192	SRU	0.0	71.90	16.04	9.49	0.72	1.00	0.98
64	SRU	0.1	66.56	17.84	12.74	0.76	1.01	1.13
128	SRU	0.1	66.88	17.60	12.81	0.71	1.10	1.05
192	SRU	0.1	67.32	18.36	11.77	0.66	1.07	0.98
SEM			0.70	0.44	0.58	0.03	0.03	0.08
			Contrasts					
Treatment			<0.01	<0.01	<0.01	<0.01	0.04	0.15
Glycerol			<0.01	<0.01	<0.01	0.06	0.42	<0.01
N Source			0.04	0.51	0.07	0.03	0.49	0.15
Contrast *P*-value								
N Level Linear			0.01	0.02	0.16	<0.01	0.28	0.48
N Level Quadratic			<0.01	0.19	<0.01	0.27	0.16	0.09

[a]Protein level, mg N/kg BW
[b]Protein source, Urea = urea, SRU = slow release urea
[c]Gly = glycerol
[e]Ace = acetate
[f]Pro = propionate
[g]But = butyrate
[h]Isobut = isobutyrate
[i]Val = valerate
[j]Isoval = isovalerate

Table 3-1. Chemical composition of hays and supplement

	Hay			
	7.0% CP	8.4% CP	13.2% CP	Cottonseed meal
Item				
	----------% of DM----------			
CP	7.0	8.4	13.2	45.0
OM	93.8	92.6	92.8	94.2
NDF	77.7	77.7	69.8	28.9
ADF	47.2	49.9	35.3	19.9

Table 3-2. Effect of cottonseed meal supplementation on intake and digestion of steers fed Bermudagrass hays of divergent nutritive values

		Intake, g/kg $BW^{0.75}$					Total tract Digestion, %	
Hay, %CP	CSM[a]	Hay OM	Total OM	Total NDF	Total DOM[b]	Total DNDF[c]	OM	NDF
7.0	0	70.33	70.33	58.62	38.94	34.81	55.56	59.44
7.0	156	77.61	84.76	66.94	45.46	36.18	53.70	54.08
8.4	0	81.92	81.92	69.19	40.22	36.00	49.24	52.13
8.4	156	81.30	88.26	70.56	44.91	35.85	50.90	50.77
13.2	0	109.33	109.33	82.72	71.28	54.38	65.23	65.72
13.2	156	109.96	116.78	85.23	78.24	56.98	67.04	66.84
SEM		5.10	5.08	4.09	3.14	2.65	1.26	1.34
				Contrasts				
P-values								
Hay		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CSM		0.57	0.04	0.24	0.03	0.57	0.61	0.11
Contrast *P*-value								
Hay linear		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Hay quadratic		0.96	0.95	0.52	0.03	0.11	<0.01	<0.01

No significant Hay × CSM interactions were observed for hay OMI, total OMI, TDOMI, or digestible NDF intake ($P \geq 0.67$)

[a]CSM = cottonseed meal supplemented at 0 or 156 mg N/kg BW

[b]Total DOM = total digestible OM intake

[c]Total DNDF = total digestible NDF intake

Table 3-3. Effect of cottonseed meal supplementation on plasma urea-N concentration and ruminal fermentation characteristics in steers fed Bermudagrass hays of divergent nutritive values

	Hay										
	7.0 % CP		8.4 % CP		13.2 % CP			*P*-values[b]		Contrast *P*-values	
Item	No CSM[a]	CSM	No CSM	CSM	No CSM	CSM	SEM	Hay	CSM	Hay linear	Hay quadratic
Plasma urea-N, m*M*											
h 0	1.25	2.19	2.75	4.23	3.83	3.82	0.31	< 0.01	0.02	<0.01	<0.01
h 4	1.63	3.26	4.06	4.56	4.16	5.24	0.31	< 0.01	0.02	<0.01	<0.01
Ruminal Ph	6.58	6.49	6.55	6.51	6.37	6.48	0.10	0.44	0.94	0.22	0.81
Ruminal NH_3, m*M*	0.93	2.98	3.38	6.25	4.01	6.72	0.50	< 0.01	< 0.01	<0.01	<0.01
Total VFA, m*M*	70.67	70.69	65.77	73.05	74.66	76.47	4.14	0.32	0.38	0.17	0.54
Molar percentages											
Acetate	72.29	72.29	72.54	71.58	70.33	69.67	0.62	< 0.01	0.30	<0.01	0.63
Propionate	15.73	15.80	16.04	16.64	16.01	16.29	0.39	0.35	0.34	0.57	0.19
Butyrate	9.61	9.20	8.62	8.67	10.74	10.57	0.45	< 0.01	0.64	<0.01	0.02
Isobutyrate	0.66	0.67	0.74	0.79	0.64	0.81	0.03	0.03	0.01	0.39	0.01
Isovalerate	0.78	0.97	0.92	0.94	1.34	1.46	0.07	< 0.01	0.09	0.84	<0.01
Valerate	0.93	1.07	1.15	1.37	0.94	1.20	0.08	0.01	< 0.01	<0.01	0.36

[a] CSM = cottonseed meal supplemented at 0 or 156 mg N/kg BW

[b] No significant Hay × CSM interactions were observed ($P > 0.50$) with the exception of Isobutyrate ($P = 0.07$). Significant Hay × CSM × Time interactions ($P < 0.01$) were observed for plasma urea-N and ruminal pH.

VITA

Name: Jamie Lee Kunkel

Address: Texas A&M University, Dept. Animal Science
c/o Dr. Tryon Wickersham
133 Kleberg
2471 TAMU
College Station, TX 77843-2471

Email Address: jtexk@yahoo,com

Education: B.S., Animal Science, Texas A&M University, 2007
M.S., Animal Science, Texas A&M University, 2011