ABSTRACT

MAGEE, KELLY JEAN. Nitrogen Metabolism of Beef Steers Fed Either Gamagrass or Orchardgrass Hay With or Without A Supplement. (Under the direction of Gerald B. Huntington)

This experiment evaluated rumen protein:energy synchronization in steers fed either gamagrass (*Tripsacum dactyloides*) or orchardgrass (*Dactylis glomerata*) hays with or without a soybean hull/corn supplement. A N balance trial was conducted to compare the effects of the supplement and hay on N intake, N digestion, and N metabolism. The supplement consisted of 50:50 corn: soybean hulls mix with 50 g molasses per kg of corn: soybean hulls mixture. The supplement contained 11.3% CP, 34.2% NDF, 22.9% ADF, and 0.87 kg was fed at 0830 and 1600 followed by hay offered in two equal portions. Steers had ad libitum access to water and a trace mineralized salt block throughout the study. Hay compositions were 8.3% CP, 71.8% NDF, and 37.8% ADF for gamagrass and were 11.9% CP, 70.5% NDF, and 36.6% ADF for orchardgrass. Steers were individually fed, blocked into two weight groups, and randomly assigned to either gamagrass or orchardgrass hay (4 steers per hay). Within hays, steers were assigned to receive supplement or no supplement in a crossover design. Ad libitum DM intake for each steer was equal to their intake during the last 8d of the 21-d ad libitum period. Steers were then placed in metabolism crates for an 8-d adjustment followed by a 5-d balance trial. During the balance trial, steers were fed 90% of ad libitum intake. Compared with gamagrass, orchardgrass had similar true IVDMD (64.2 vs 62.2%), similar leaf true IVDMD (58.2 vs 63.2%), and similar stem true IVDMD (46.7 vs 51.7%). Effects were significant at $P < 0.10$. Compared with gamagrass, orchardgrass had a lower ad libitum

DM intake (4.62 vs 5.37 kg/d), higher N intake (96.6 vs 81.1 g/d), similar fecal N (36.1 vs 34.5 g/d), higher N digestibility (62.4 vs 57.4 %), similar N retained (27.2 vs 27.7 g/d), and lower digestible DMI (3.20 vs 3.53 kg/d). Compared with no supplement, supplement increased digestible DM intake (3.70 vs 3.03 kg/d), total DM intake (5.87 vs 5.07 kg/d), N intake (96.9 vs 80.8 g/d), fecal N (39.4 vs 31.1 g/d) and N retained (31.7 vs 23.2 g/d). Supplement (1.74 kg/d) decreased hay ad libitum intake by 0.73 kg/d. Supplement decreased, blood urea N, urine urea N and urine urea N as a percentage of urine N more for orchardgrass than for gamagrass. Supplement tended ($P < 0.11$) to improve N retained as a percentage of N intake or percentage of N digested more for orchardgrass than for gamagrass hay. Blood urea N was reduced (2.21 vs 0.14 mM)more for steers fed orchardgrass hay than for steers fed gamagrass. We believe that the increase in digestible OM intake is due in part to the presence of soybean hulls in the supplement.

Key Words: Beef Steers, Soybean Hulls, N Metabolism, Tripsacum dactyloides, Dactylis glomerata

NITROGEN METABOLISM OF BEEF STEERS FED EITHER GAMAGRASS OR ORCHARDGRASS HAY WITH OR WITHOUT A SUPPLEMENT

by
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BIOGRAPHY

Kelly Jean Magee was born February 15, 1979 in Cedar Rapids, Iowa to Craig and Barb Magee. In 1988, her family moved to Nashville, North Carolina where she lived with her parents, older sister, Erinn, and younger brother, Mike until she left home for college. She graduated from Northern Nash Senior High and entered North Carolina State University in the fall of 1997. Kelly received a B. S. degree in Animal Science in May of 2001. She entered the North Carolina State University animal science program in the fall of 2001 in pursuit of a M.S. degree in nutrition and animal science under the direction of Dr. Gerald Huntington.

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LITERATURE REVIEW

Uptake and Transfer of Nitrogen

Nitrogen can enter the rumen in three basic ways, as crude protein (CP) from the diet, salivary nitrogen (N) input, and influx across the rumen wall (Waldo, 1967). The primary source of N entry into the rumen is from the diet. Crude protein in hay and other dietary ingredients can be divided into two components, true protein and non protein nitrogen (NPN). True protein is defined as the nitrogenous compound which will hydrolyze completely into amino acids (Ensminger, 1990). Non protein nitrogen is the N that does not come from protein, such as peptides, nitrates, ammonia, urea and nonessential amino acids, but may be used for the building of protein. Dietary proteins can be hydrolyzed in the rumen to various degrees depending on solubility (Tillman and Sidhu, 1968), and at least part of the amino acids can be deaminated to yield ammonia (Armstrong and Weeks, 1983). Ammonia is produced in the rumen by metabolism of proteins, peptides, amino acids, amides, urea, and NPN compounds (Tillman and Sidhu, 1968). Non-protein nitrogen sources include urea and ammonia. Fresh forages contain from 10 to 30% NPN and drying these forages increases the NPN to 25 to 50% of total N (Brady, 1960). Sniffen et al. (1992) summarized the NPN content of a variety of common feedstuffs. The NPN concentration of grass forages in the pasture state ranged from 2.3 to 4.8 % and increased to 96 % of the soluble protein when in hay form. Soybean meal contains 55 % and soybean hulls contain 63% of soluble protein as NPN.

Entrance of N into the rumen also occurs through saliva. Saliva contains N and in cattle, 77% of the total N is in the form of urea (Waldo, 1967). McDonald (1948) found that small quantities of blood urea have been found to enter the rumen with saliva. In 1959, Houpt measured the movement of blood urea with saliva into the rumen. In that experiment,

ingesta of the rumenoreticular cavity in four mutton type ewe sheep and one Nubian doe goat was replaced with saline. The accumulation of ammonia, representing the hydrolytic products of urea, was measured along with salivary-urea N. Salivary secretion rates averaged 0.3 mmole urea-N/h in both the sheep and the goat. Total urea transfer from blood to rumen with salvia averaged 5.3 mmole urea-N/h for the sheep and 3.4 mmole urea-N/h for the goat. In this experiment, 16 times more urea passed directly from blood to the rumen as moved with saliva (Houpt, 1959). Norton et al. (1978, 1979), however, demonstrated that with very low N intake, daily salivary secretion will account for all the urea transferred into the rumen in both sheep and cattle. This is assuming that the concentration of urea in saliva is 60 to 67% of plasma urea concentration and that daily salivary secretion is ten liters (Norton et al., 1978, 1979).

Neutze and Kellaway (1986) fed merino wethers a diet of alkali treated wheat straw supplemented with three different levels of urea consisting of 3.5, 5.9, and 11.6 g N/kg DM. The wethers were infused with ^{15}N urea into the jugular vein. Rumen and blood samples were taken at 0.5, 1.5, and 2.5 h after feeding. Salivary urea N contributions did not account for all of the urea N transferred from the rumen. They concluded that the quantity of salivary urea N entering the rumen is influenced by total salivary output and blood urea N concentration, both of which are dependent on diet (Neutze and Kellaway, 1986).

The third source of rumen N is urea entry from the blood across the rumen wall (Waldo, 1967). Houpt (1959) hypothesized that if blood urea moved readily to the rumen it would be a source of N for protein synthesis by rumen microflora and as a result, the least amount of N intake necessary to maintain life would be considerably lower than that of most nonruminants. Houpt (1959) fed two young mature ewes poor quality chopped timothy hay,

and a supplement of cornstarch and sucrose. The author did not provide nutrient information concerning the timothy hay and I assumed that the hay was low in CP and high in cellulose. The sheep were injected with a 0.5% NaCl solution containing 10% urea into the external jugular vein. When sheep were fed added carbohydrate to the hay and injected with urea, the rate of urea utilization averaged 53% of injected urea. Urea utilization was calculated as the amount of urea that disappeared from body fluids minus the amount excreted in the urine. This amount presumably moved into the rumen and was used by microorganisms (Houpt, 1959). The higher percentage of utilization of urea in the sheep fed poor quality hay with added energy indicates that the microorganisms were better able to capture urea in the presence of available energy. When sheep were fed low protein timothy hay alone, the utilization of injected urea was lower indicating lower capture of available urea by microorganisms. There was little or no net utilization of blood urea in protein synthesis when ruminants were consuming a balanced high protein ration. When ruminants consumed a low protein ration with considerable cellulose, some of the blood urea is utilized (Houpt, 1959). Houpt showed that diet played a large role in the transfer of urea from the blood with higher utilization of blood urea occurring in animals fed low N diets.

Kennedy and Milligan (1978) also conducted an experiment tracing the movement of urea from the blood to the rumen and further examined the effects of infusing urea into either the rumen or the abomasum. Sheep were fed either bromegrass or lucerne pellets at varying amounts. Sheep fed bromegrass had 4.9 to 7.5-fold greater transfer of plasma urea-N to rumen ammonia than sheep fed lucerne. When the infusion of urea was changed from the abomasum to the rumen, there was a substantial increase in the rate of irreversible loss of rumen ammonia and the increase in the rate of irreversible loss of rumen ammonia-N was

equal to the quantity of infused urea-N. Kennedy and Milligan (1978) provide evidence that urea transport is dependent on rumen ammonia concentrations and that there is an inverse relationship between transfer from plasma urea to the rumen and concentrations of ammonia in the rumen. Once protein enters the rumen either by diet or endogenously, rumen microorganisms are then able to utilize the N for microbial growth and microbial protein synthesis.

Metabolism of Ammonia and Urea

Ammonia and urea and the movement of these metabolites through the ruminant system are two major components of N metabolism. As previously mentioned, dietary protein can be deaminated to yield ammonia and ammonia can then be used for microbial growth, absorbed through the rumen wall, or passed down the gastrointestinal tract in rumen fluid. Once ammonia is absorbed through the rumen wall and taken to the liver it is quickly converted to urea. Urea can then be excreted in the urine or recycled back to the gastrointestinal tract. The absorption and conversion of ammonia to urea and urea recycling is a complex system that is affected by many factors such as diet, microorganisms, and the status of the animal.

Ammonia

Ammonia is an important rumen fermentation product and is an essential nutrient for the growth of rumen microbes, for the synthesis of microbial protein and is important for acid-base metabolism and for the movement of molecules across the membranes (Huntington and Archibeque, 1999). Ammonia is the preferred substrate for protein synthesis by methanogenic and cellulolytic bacteria and it is an essential nutrient for growth specifically

in *Bacteriodes succinogenes, Ruminoccocus flavefacienes, Ruminoccocus albus, Bacteriodes amylophilus, Methanobacterium ruminantium and Eubacterium ruminantium* (Bryant, 1963).

Ammonia production rates and amount in the rumen are affected by the solubility and fermentability of the dietary and endogenous sources of N (Huntington and Archibeque, 1999). Ammonia N concentrations and rates of N metabolism are determined by various interactions among a variety of components such as: 1) dietary N conversion to amino acids and ammonia; 2) ammonia assimilation by rumen microorganisms; 3) rumen microbial lysis and degradation of cell constituents in the rumen; 4) ammonia absorption through the rumen wall; 5) urea N secretion into the rumen; and 6) ammonia passage to the lower gut (Al-Rabbat et al., 1970). Rumen concentrations of ammonia rise after the consumption of a meal and the net absorption of ammonia is positively correlated with rumen ammonia concentrations (Huntington, 1986). Huntington and Archibeque (1999) reported that the amount of N absorbed as ammonia is equivalent to 16 to 73% of N intake. Huntington and Prior (1983) reported that when heifers were fed a high concentrate diet, the amount of ammonia-N absorbed increased linearly but decreased as a percentage of total N intake.

Ammonia N is lost irreversibly from rumen fluid by incorporation into microbial cells, by absorption of ammonia through the rumen wall or by fluid passing out of the rumen (Leng and Nolan, 1984). Ammonia diffuses across the rumen wall independently of rumen fluid concentrations of Na, K, lactate, chloride, CO_2, or water flux across the rumen epithelium (Hogan, 1961). Ammonia absorption increases as rumen ammonia concentration increases. Once ammonia is diffused from the rumen, it is carried by the portal vein to the liver where it is converted to urea (Tillman and Sidhu, 1968).

Ruminants depend on the liver to detoxify portal blood that contains ammonia absorbed from the gut. Liver ammonia removal is especially important for ruminants consuming legumes or immature grasses which tend to have greater concentrations of rapidly degradable N (Huntington and Archibeque, 1999). Normal blood levels of ammonia remain low because of the rapid conversion of ammonia to urea maintained by the liver. Blood ammonia concentrations rise when excess dietary NPN is fed. Lewis et al. (1957) reported that the liver was able to convert all of the absorbed ammonia into urea until the concentration of ammonia in the rumen fluid reached 55 to 60 mM.

Urea

Urea produced in the liver can either be excreted in the urine or recycled to the gastrointestinal tract by direct transfer across the gut wall or through saliva. Urea production, recycling, and excretion are linked to diet composition, intake, and the production priorities of the animal (Huntington and Archibeque, 1999; Kennedy and Milligan, 1980). Urea recycling and N conservation is based on the assumption that endogenous urea not excreted in the urine is recycled to the rumen. Then it is degraded by microbes in the digestive tract where the ammonia produced from urea is then converted to amino acids for absorption and utilization by the animal (Norton et al., 1979). When dietary N intake is low, recycling becomes very efficient with the majority of urea coming from endogenous metabolism of tissue and absorbed amino acids (Van Soest, 1994). The production of endogenous urea that may be recycled to the gut may be from 19 to 96% (Kennedy and Milligan, 1980). Of this, 15 to 94% may transfer in saliva, and 25 to 90% of the urea degraded in the gut may be degraded postruminally (Norton et al., 1979). Houpt (1959) suggested that the formation of urea in the liver and its movement via blood into the rumen, the utilization of urea in the

rumen for protein synthesis, and subsequent digestion and the absorption of protein in the lower tract, constitutes the 'protein regeneration cycle.' Ammonia produced from the diet is absorbed and converted to urea in the liver. Nitrogen from urea may be recycled from the blood back to the rumen if there were a N shortage (Henning et al., 1993) or excreted in the urine during times of N excess.

In early studies done by Houpt and Houpt (1968), it was reported that urea diffused into the rumen from the blood and was almost completely hydrolyzed before it entered the rumen fluid. These workers suggest that the rumen urease penetrated the rumen epithelial layers and that the ammonia produced in the epithelium diffused more rapidly than urea through the remaining layers of epithelium. Evidence of another form of passage through the rumen wall was obtained in the 1970's in which urea transport in human red blood cells could be inhibited without significantly altering water transport (Wieth et al., 1974).

Around the 1980's, urea transporters were found in human cells. Mayrand and Levitt (1983) confirmed the presence of urea transporters in a study that showed that urea transport across human red blood cell membranes was a saturable facilitated diffusion process, and this process could be inhibited by a large number of urea analogs. Further discoveries were made concerning human urea transporters, but it was not until 1997 that Ritzhaup et al. (1997) reported a facilitated transporter of urea from the mucosal to the serosal side of the rumen epithelium confirming the presence of urea transporters in the rumen. In a study conducted by Marini and Van Amburgh (2003), urea transporters were shown to be responsible for redirecting urea destined for excretion instead to the gastrointestinal tract for use by rumen microorganisms to derive more N at low N intakes. When dietary N increases, the proportion of total urea degraded decreases with the balance excreted in urine. Marini and

Van Amburgh (2003) hypothesized that there would be an increase in the transfer of urea into the gastrointestinal tract by the urea transporters when low-N diets are fed. Results from their study showed, however, that the urea transporters were expressed more when the animals were fed high-N diets. A suggested explanation for the contrasting data may be the possibility of urea diffusing through the gastrointestinal tract and then returning to the blood through the urea transporters (Marini and Van Amburgh, 2003).

Energy and Protein Synchronization in the Rumen

Synchronization of carbohydrate and protein fermentation in the rumen is another way in which scientists have tried to better capture N. Fresh forages and some hays high in non-protein nitrogen and soluble components are rapidly degradable and produce high amounts of ammonia. When production of ammonia exceeds capture by rumen microbes, absorption of ammonia occurs and thus loss of ammonia from the rumen to the blood. Once ammonia is absorbed into the blood, it is converted to urea in the liver and excreted in the urine. Diets low in soluble carbohydrates and high in mature plant cell wall are slowly digested in the rumen. In this situation, there is not enough available energy to capture the ammonia causing unsynchronized energy and protein release in the rumen.

The CP in high quality forages consumed by beef cattle can be rapidly hydrolyzed in the rumen into amino acids and non-protein N. Rumen microbes are often not able to utilize all of the amino acids or ammonia that is rapidly produced and subsequent inefficiency can then limit the efficiency of N utilization by the animal. Synchronization of readily fermentable carbohydrate and N sources in the rumen may increase N capture as microbial protein and decrease the amount of N absorbed as ammonia. Increased capture of N through

enhanced synthesis of microbial protein and supply of amino acids may result in production responses such as more efficient growth through increased N retention.

In diets low in protein and high in cellulose, urea can be recycled from the blood back to the rumen and captured as microbial protein and then used to enhance the total amount and quality of protein fed to the animal. In high protein diets, excessive protein is hydrolyzed to ammonia. Increased appearance of ammonia in the rumen without an increase in ammonia utilization results in increased ammonia absorption to the blood, increased plasma urea concentrations, and increased urinary urea excretion (Cocimano and Leng, 1967). This increase in urinary N excretion reflects inefficient capture of ammonia N (Henning et al., 1993). Inefficient capture of N results in high feed to gain ratios and wastage of N from high quality feeds for the producer.

Forage based diets fed to ruminants have been shown to be beneficial for rumen function and animal health (Jung and Allen, 1995), thus it is important to include forage as the bulk of the diet. Plant manipulation and rumen protein and carbohydrate synchronization are a couple of ways in which scientists have tried to improve N capture while keeping the feed ration based on forage.

Genetic alteration of forages has been one approach scientists have used to improve forage digestibility. Genetic altering of forages can be advantageous through improved animal performance but the complexity of identifying specific genes to alter the plant and obtain increased production in the ruminant is a very complex process requiring a great amount of time. Anderson et al. (1988a) grazed yearling cattle on genetically modified switchgrass. The switchgrass lines were modified to have high in-vitro DM digestibility (IVDMD) or low IVDMD. Steers grazing high IVDMD line switchgrass had a 14% greater

average daily gain and a 17% improvement in gain to feed compared to steers grazing low IVDMD line switchgrass.

Ghesquire et al. (1993) fed a line of alfalfa pasture that was selected for low crude fiber concentration and found reduced in vivo crude fiber digestibility by lactating dairy cows when compared with the control. They also found increased DMI and milk production and concluded that the reduction in fiber digestibility was offset by the reduction in fiber concentration and resulted in increased intake.

Synchronization of carbohydrates and proteins within the rumen is also a difficult task because of complicated factors such as rate of passage of the liquid and DM from the rumen, voluntary intake, the transfer of metabolites and the transfer of fermentation products across the rumen wall, adaptation of rumen microorganisms, and interactions that can affect the efficiency of microbial growth (Dijsktra et al., 1998).

Kolver et al. (1998) showed that a synchronized release of carbohydrate and pasture N to the rumen increased the amount of pasture N retained by lactating dairy cows, which enhanced growth and milk production. Cows were fed fresh-cut pasture with a concentrate based supplement at the same time as the pasture feeding or four hours later. The supplement fed with the pasture feedings represented a synchronous diet and the supplement fed four hours later represented an asynchronous diet. The synchronized diet resulted in a significant decrease in concentration of rumen ammonia at 3 h after feeding when compared to the unsynchronized diet. Blood urea nitrogen (BUN) mirrored ammonia concentration by having lower concentrations in the blood 2 h after feeding. These differences, however, were not significant when averaged over 24 h indicating that there was improved capture of rumen N but the changes were temporary and did not change the N status of the dairy cow overall.

Furthermore, while blood urea nitrogen (BUN) levels differed at 2h post-feeding, the recycling of BUN between the last feeding and the morning feeding may have reduced dietary differences in BUN concentration and thus urinary N. The diet was also adequate in dietary protein and the cows did not need extra protein, thus explaining the lack of differences in the amount of N retained for production (Kolver et al., 1998). Kolver et al. (1998) concluded that to obtain metabolic advantage for tissue mobilization that a more even pattern of feed intake throughout the day should be provided and that the match of rapid rumen release of pasture N with carbohydrate did not show an advantage in N use. The cows on this study were provided adequate N for production in the 22% CP grass and treatments did not change (Kolver et al., 1998). This may not be the case in growing beef steers fed hay or grazing fresh grass moderate in CP. Growing steers require protein adequate for growth and retention and moderate levels of CP found in some hays and pastures may not meet the needs of their higher requirements. Providing a supplement that closely matches the degradation rate of the grass or hay should be beneficial because of improved capture of ammonia. The ammonia can then be used for microbial growth and rumen function and thereby decrease the amount of ammonia absorbed into the blood and eventually excreted.

Another study concerning protein and energy synchronization was conducted by Herrera-Saldana et al. (1990). The purpose of the experiment was to examine the effects that protein and starch degradation in the rumen had on nutrient digestion and microbial protein synthesis. Four multiparious dairy cows in mid-lactation fitted with duodenal cannulae were fed diets formulated to four different treatments. Protein was sufficient in both diets to meet the requirements of the cows. Cows were fed barley and cottonseed meal to synchronize rapid fermentation, and milo was mixed with brewers dried grains to synchronize slow

fermentation. Two unsynchronized diets were also formulated. Barley with brewers dried grain was used to formulate a rapidly fermenting unsynchronized diet and milo with cottonseed meal was used to formulate a slowly fermenting unsynchronized diet. The four supplements were fed with 35% alfalfa hay. Protein degradability was higher for cottonseed meal than brewers dried grain and barley was higher in starch degradability than the milo. Rumen ammonia concentrations tended to be lower for the barley, cottonseed meal and barley brewers dried grain diets than for the milo, cottonseed meal and milo brewers dried grain diets reflecting faster and more extensive starch degradation in the rumen. Protein degradability affected utilization of nutrients in the rumen less than starch degradability and the synchronization for the rapid fermentation with the more degradable starch and protein stimulated greater microbial protein passage than the unsynchronized or less degradable synchronized diets (Herrera-Saldana et al., 1990).

Chemical Composition

Protein and energy synchronization can be affected by ingredient compositions in addition to the above mentioned factors. Using different ingredients with differing fermentation rates can cause disparity in treatments in respect to the ratio and total amounts of energy and N released into the rumen (Henning et al., 1993) and specific dietary compositions may be a confounding factor in some of the protein energy synchronization studies (Henning et al., 1993; Kolver et al., 1998). The chemical compositions that result from morphological differences among hays and supplement can affect N digestion and metabolism. Ruminants pick and choose particular parts of the plant, preferring the leaf over the stem (Burns et al., 1991; Cherney et al., 1990). The leaf portion of the plant is lower in cellulose and higher in CP making it more digestible than the stem portion of the plant

(Bourquin and Fahey, 1994). Because the leaf of the plant contains more protein than the stem, ruminants can actually consume more protein than what the whole plant appears to provide.

Bourquin and Fahey (1994) hypothesized that digestion of cell wall components of the plant morphological fractions were needed to fully understand the kinetics of plant cell wall digestion. Bourquin and Fahey (1994) wanted to determine the cell wall composition and rumen degradability of cell wall components of leaf and stem fractions of alfalfa, orchardgrass, and wheat straw. Leaf and stem fractions of the forages were hand-separated with seed heads, buds, and flowers discarded. Orchardgrass had a leaf:stem ratio of 61% to 38%, alfalfa 35% to 64%, and wheat straw 39% to 60%. The leaf and stem fractions were also analyzed for digestibility using in situ procedures. For all forages, leaves contained lower concentrations of all fiber fractions relative to the stems and the extent of DM digestion was greater for the leaf than the stem. Further, differences in extent of digestion of leaf vs stem fraction were due to the smaller quantities of cell wall polysaccharides in leaves vs the stems and to the greater extent of digestion of leaf than stem cell walls. Bourquin and Fahey (1994) clearly showed that the leaf of the plant was higher in CP concentrations and lower in cell wall components than stems of the plant and differences in cell wall digestibility between leaf and stem fractions were greater in orchardgrass and alfalfa than in wheat straw. After prolonged fermentation, undegraded residues were more similar than different in composition across the three forages. The results from this study indicate that the ratio of leaf to stem and the rate of fermentation of each plant differ among species and that this contributes to observed differences in the digestibility of cell walls in ruminants (Bourquin and Fahey, 1994).

Rafiq et al. (2002) hypothesized that the leaf stem ratio of low quality forage with various types of supplements would have an effect on digestibility and productivity. Ewe lambs were fed two types of straw with four supplement regimes. The straw was either stem-rich or leaf-rich fractions of chopped barley straw. Supplements were urea and sodium sulfate, barley grain mixed with urea and sodium sulfate, fish meal, or no supplement. Adding supplement to both straw diets increase the digestibilities and all supplements increased N retention in the lambs (Rafiq et al., 2002). Both the stem-rich and leaf-rich straw were deficient in N according to sheep requirements. Feeding the straws alone put the sheep into a negative N balance, and by providing a supplement, digestibility and intake were both increased for the leaf-rich straw and the stem-rich straw. The supplemented straws caused an increase in live-weight, N retention and wool growth but the straws responded differently to supplements. The leaf-rich straw responded more to the urea/sodium sulfate and fishmeal supplement than the stem-rich straw. The authors concluded that the morphological compositions of straw had an affect on the response of the sheep to supplement. The responses to supplement may be in conjunction to the protein fractions of both the straw and the supplement. Bourquin and Fahey (1994) examined the leaf and stem fractions of wheat straw and found that the differences in extent of fermentation for leaf vs stem was the smallest for the wheat straw when compared to alfalfa and orchardgrass. Bourquin and Fahey (1994) related the small differences to the similarities in ADF in wheat straw for both the leaf and stem fractions. The slightly higher amount of CP found in the leaf-rich barley straw may have lead to the increase in digestibility when sodium sulfate and urea was supplemented. The readily digested non-protein-fraction of urea in conjunction with the

available true protein in the leaf-rich straw may have resulted in the greater response of sheep through better protein: carbohyrdrate synchronization in the rumen.

Along with analyzing the portions of the whole plant into proportions of leaf and stem, the fractions of protein that are digested at different rates are also very important. As mentioned before, fractions of the plant can be divided into categories based on the degree to which it is degraded or not degraded.

Van Soest (1994) divides protein into five basic categories; fraction A, B, B_1, B_2, B_3 and C. Fraction A consists of non-protein nitrogen and peptides. Fraction B_1, is the true soluble protein of the feedstuff and is precipitable and soluble in buffer. Neutral detergent soluble protein is considered fraction B_2 and is defined by the difference between crude-protein and fraction A and B_1 and protein in neutral detergent. Fraction B_3 is the fraction of protein insoluble in neutral detergent but soluble in acid detergent. Fraction C is indigestible and insoluble in acid detergent. Protein fraction C includes heat damaged protein and N associated with lignin (Van Soest, 1994). The difference in rate of degradation among fractions will lead to differences in rates of metabolism according to the percentage of fractions comprising the plant.

Elizalde et al. (1999a) analyzed protein fractions from four different fresh forages to determine whether or not maturity had an effect on chemical composition. Endophyte-infected tall fescue, endophyte-free fescue, bromegrass, and alfalfa were sampled in mid-to-late spring and analyzed for NDF, ADF, CP, and CP fractions. They found that soluble CP was a constant fraction for both the alfalfa and the grasses even when the CP decreased with later cuttings. The B_2 fraction was the largest CP fraction of all the forages and showed a decrease with days after first cutting but only in alfalfa. Fraction C showed an increase for

days after first cutting but was not affected by forage species. The overall implication from this study was that the species of forage rather than the stage of maturity had a greater impact on the CP composition (Elizalde et al., 1999a).

Johnson et al. (2001) examined the effects of fertilization and harvest date on protein fractions in Bermudagrass, stargrass, and bahiagrass. They determined that increasing N fertilization linearly decreased NDF concentration in all three forages. The overall effect of increased N fertilization on protein fractions was a linear increase in fraction A, B_1, and B_2 for all grasses, a linear effect for bahiagrass and bermudagrass with a cubic response in stargrass. For fraction C, there was a linear response in bermudagrass with a quadratic response in bahiagrass and stargrass. Across all species there was not a consistent effect of harvest date on NDF concentration. As for CP fraction response to harvest dates, the results varied for each forage, particularly in the B fractions. Across all species, fraction A responded quadratically to the harvest date by decreasing. The B_1, B_2, and B_3 protein fractions all responded differently to the harvest date. The C fraction, however, had a quadratic response across all species (Johnson et al., 2001). This appears to be in agreement with Elizalde et al. (1999a) in that harvest date produced varying results depending on the forage species. Even within warm-season grasses, the changes in chemical composition of protein fractions vary with time. Nitrogen fertilization, however, proved to increase CP across all species (Johnson et al., 2001).

The question now arises as to how does making more N available in the feed affect the animal? In an experiment conducted by Archibeque et al. (2001), gamagrass and switchgrass, two species of warm-season native grasses were fertilized at low and high N levels, harvested, and fed to steers. The switchgrass responded to increased N with a

decrease in hemicellulose, a decrease in cellulose, and similar lignin levels. Gamagrass had a very small increase in hemicellulose, an increase in cellulose and lignin. The IVTDMD reflected the changes in cellulose with levels decreasing in gamagrass and increasing with switchgrass. Both grasses showed an increase in CP with an increase in N fertilization (Archibeque et al., 2001). The response of the steer to increased levels of N varied depending on the grass. Retained N increased with increasing N levels in steers fed gamagrass but decreased in steers fed switchgrass with increased N levels. Archibeque et al. (2001) concluded that increasing N concentration in hays may increase N digestibility in steers but the efficiency of N use or N recycling may not be improved by making more N available and that once the N intake exceeds the needs of the steer the excess will be excreted in the urine.

While making more N available to the steer may not result in increased efficiency or recycling, the capture of the available N may be improved through supplementation. Archibeque et al. (2001) noted that the greatest change in chemical composition of the plant through increased N fertilization was in the readily soluble portion of CP, fraction A. The soluble portion of protein is degraded quickly and will not be captured by the microbes if there is not enough fermentable energy available, which will result in increased absorption of ammonia and subsequent excretion of urea in the urine.

Supplements

One approach to improving the capture of N is by providing additional fermentable energy in the form of a supplement. Providing additional energy in synchronization with forage consumption has the possibility of enhancing the use of forage N by reducing loss of N as NH_4 and improving capture by microbes. In an experiment conducted by Elizalde et al.

(1999c), steers were fed fresh alfalfa ad libitum and increasing levels of a corn supplement. Fresh alfalfa is usually high in CP with a substantial percentage of the CP in the A fraction of the plant making it a highly degradable forage. Inefficient capture of this available N by the microbes can result in N being excreted. Elizalde et al. (1999c) hypothesized that feeding cracked corn may improve N capture by adding a fermentable energy source in combination with a highly fermentable forage. While energy supplements can improve animal performance there is the risk of a decrease in forage intake and some decrease in utilization. In this experiment, they found that the total N intake was affected by the amount of forage consumed which was influenced by the amount of corn fed. Elizalde et al. (1999b) also specifically examined different levels of energy supplementation to try and decrease the negative effects associated with a high level of energy supplement. They found that forage and total N intakes were influenced by the level of corn fed. Supplementation with cracked corn decreased the N content of the total diet organic matter and rumen CP in comparison to unsupplemented steers. Adding corn supplement to fresh alfalfa resulted in reducing rumen N losses and increased N disappearance in the small intestine. The authors concluded that supplementation of corn to steers fed fresh alfalfa had positive effects on N digestion and utilization and that on average, supplemented steers had greater escape CP and higher small intestinal digestibilities of N and amino acids.

Substitution effects are a problem that can be associated with feeding concentrate supplements. When feeding a high energy supplement to animals fed mainly forage diets, a reduction in forage intake may occur and is due to preference for the supplement. The term substitution is commonly used to describe the event and is a challenge to producers trying to maintain a high forage intake and provide a high energy supplement. Substitution rates can

be calculated by measuring the reduction in voluntary forage DM intake per kg DM of added concentrate (Jarrige et al., 1986; Elizalde et al., 1999b). Substitution rates of concentrates are positively related to the forage ad libitum intake and negatively related to the forage fill value. Concentrates interact with forages to a variable extent in the rumen and small intestine. In the rumen concentrates can influence the breakdown and disappearance rates of forage cell wall mass. Substitution rates reported by Elizalde et al. (1999c) were higher when grain supplement was fed with low quality forage but lower when high quality fresh ryegrass was fed with grain supplement. There were also substitution effects when corn supplement was fed with alfalfa fed ad libitum (Elizalde et al., 1999c). As the amount of corn supplement consumption increased, the consumption of alfalfa decreased. The rate at which corn was substituted for alfalfa was about 0.69 g/g of supplemental corn (Elizalde et al., 1999b). In a review, Moore et al. (1999) reported that supplements decreased voluntary forage intake when supplemental TDN intake was >0.7 % BW, when voluntary forage intake was > 1.75 % BW, or when forage TDN:CP ratio was < 7. When the amount and source of protein in the concentrate adequately supplements the forage, substitution rates are at a minimum (Jarrige et al., 1986).

Cereal grains as a supplement provide energy and are high in starch with a lower filling effect than that of forages (Joanning et al., 1981). Negative effects of concentrate supplementation can ensue when rumen fermentation is disrupted and organic matter digestibility is decreased by increasing the rate of passage from the rumen (Elizalde et al., 1999b). In an experiment conducted by Chase and Hibberd (1987), mature beef cattle were supplemented with increasing quantities of ground corn to evaluate the effects of grain supplementation on nutrient digestion, rumen function, and intake of low protein native grass

hay. Corn supplement was fed at increasing levels of 0, 1, 2, or 3 kg/d. Feeding 3 kg/d of corn with low quality, native grass hay (4.2% CP) resulted in a 56 % reduction in hemicellulose digestibility in comparison to the control of no corn supplement. Hay and fiber digestibility decreased as the amount of supplemental corn increases and total DM and organic matter increased as supplement increase due to the increase in highly digestible corn. Substitution rates increased with increasing rates of supplementation (Chase and Hibberd, 1987). Intake of low quality native grass hay decreased linearly with increased corn supplementation. When cattle were fed 1 kg corn supplement there was a substitution rate of 0.83 g of hay per g of supplement. The substitution became more unequal as the supplement increased from 1 kg to 2 and 3 kg. The change in intake from 1 kg of supplement to 2 kg supplement produced the greatest jump in substitution rate with an increase from 0.83 to 2.68. Rumen ammonia concentration was also changed by the increase in corn supplement. As supplement increased rumen ammonia decreased and remained below 1 mg/dl when 3 kg of corn/d was fed. Low ammonia concentrations can be detrimental in the rumen if the concentration drops below the minimum amount needed for maximal microbial growth thus limiting microbial activity and rate and extent of fiber digestion (Chase and Hibberd, 1987). Conclusions from the study were that corn supplements formulated with inadequate rumen available protein appear to depress hay digestibility and intake. The results also suggest that greater amounts of a corn based supplement may reduce microbial activity, cause high substitution rates and decrease forage utilization overall.

One possible way to avoid these negative associative effects when feeding an energy supplement may be to offer a high fiber, low starch, high energy supplement such as soybean hulls. Soybean hulls are a by-product of soybean processing and consist of the seed coat

from the soybean seed. They are highly digestible with about 95% of the hulls digested in vitro after 72 h. While the hulls will most likely not stay in the rumen 72 h, a digestion rate was determined to be around 7.0% per hour (Klopfenstein and Owen, 1987). The CP of the hulls can range in value depending on how much of the soybean meal gets into the mix. The clean soybean hull has about 9.4% CP but can range from 9 to 14% CP (Klopfenstein and Owen, 1987). Soybean hulls are also a high fiber supplement with 60.3% neutral detergent fiber, 44.6% acid detergent fiber, and 2.5 % lignin (National Research Council, 2001). Soybean hulls are very low in nonstructural carbohydrates, high in TDN and are palatable with low risk of acidosis (Kunkle et al., 1999). Many trials have been conducted comparing corn supplement to soybean hull supplements and have resulted in the favor of soybean hulls. Soybean hulls contain less starch and more cell wall carbohydrates than cereal-based concentrates and with increasing concentrate proportions of soybean hulls, the substitution rate appears to increase at a much slower rate when compared to a high energy concentrate such as barley (Jarrige et al., 1986).

Many comparison studies were conducted to answer the question of how soybean hulls compare to popular concentrate supplements like corn. Anderson et al. (1988b) evaluated supplementation of soybean hulls as an alternative to corn when steers were grazing fresh grass. Steers were allotted to three treatments; no supplement, rolled corn, or whole untoasted soybean hulls while grazing smooth bromegrass pastures. Steers were supplemented at the rate of 1.36 kg DM $\cdot hd^{-1} \cdot d^{-1}$. There were no significant differences found between corn and soybean hulls supplementation, and soybean hulls supported daily gains equal to those of corn. They explained that the lack of differences between the two supplements was due to high forage quality and availability and that supplementation may

have reduced forages consumption on an energy-equivalent basis. In another trial, spayed heifers were grazed on cornstalks and supplemented at three different levels; no supplement, 1.36 kg DM ·hd $^{-1}$ · d $^{-1}$ of corn, or 1.36 kg DM ·hd $^{-1}$ · d $^{-1}$ of soybean hulls. A CP supplement was also fed. The heifers supplemented with either corn or soybean hulls gained faster than those not supplemented. Furthermore, soybean hulls were shown to support daily gains equal to those supported by corn. Overall, soybean hulls proved to be at least equal to corn as an energy supplement to grazing cattle.

Anderson et al. (1988c) conducted a second experiment to determine the effect of mechanical processing on digestibility and utilization of soybean hulls and compared soybean hulls as an energy supplement to corn. Steers were fed 2.3 and 4.6 kg DM·hd^{-1}·d^{-1} with cracked corn, ground soybean hulls or whole soybean hulls as energy supplements. The basal diet consisted of 1:1:1 ratio of mature Indiangrass hay, NH_3-treated oat straw, and alfalfa hay. There was no real benefit in feeding whole or pelleted soybean hulls over ground hulls and that soybean hulls appeared to be equal in energy to corn in supporting the growth of steers. While corn had a greater DM digestibility than soybean hulls, corn supplementation caused a more rapidly depressed pH than that of soybean hulls. Because of the negative associative effects on forage when corn is fed, the net energy values of soybean hulls and corn were equal (Anderson et al., 1988c). Soybean hulls are a high fiber, palatable by-product supplement that performed equally in this study without any negative effects making it a very attractive supplement alternative to producers.

Soybean hulls have been shown to increase daily gains at least comparable to that of corn without negative rumen changes. Corn is a high energy concentrate that is widely available. What about a combination of the two as a supplement to growing steers?

Galloway et al. (1993) conducted an experiment investigating the effects of supplementing steers consuming moderate quality, tropical or temperate grass hays with corn, soybean hulls, or a mixture of the two. Supplements were fed at equal amounts at 0800 and at 1600 h. The treatments were bermudagrass or orchardgrass with no supplement, 0.5% of BW ground corn, 0.7% BW of soybean hulls, or 0.25% of BW of corn plus 0.35% of BW of soybean hulls. Galloway et al. (1993) hypothesized that feed intake and digestibility by steers consuming these hays would be affected by supplementation with corn, soybean hulls, or a mixture of the two. Mixing corn and soybean hulls resulted in significantly increased digestible organic matter intake compared to the mean of corn and soybean hulls alone. The lower level of starch present in the soybean hull:corn mix compared to corn alone may be one factor responsible for the positive associative effect on digestible organic matter intake. With the moderate level of corn mixed with soybean hulls, adverse effects on fiber digestibility were not seen in their experiment (Galloway et al., 1993). Corn alone improved capture of N in the rumen but increased the risk of rumen acidosis and decreasing fiber digestion at high levels. Soybean hulls have performed comparably to corn in numerous studies without adverse affects associated with the risk of acidosis or lowering fiber digestion. A corn soybean hull mixture may actually improve digestibility and N capture at adequate levels without adverse effects.

Nitrogen in the Environment

Because of increased concerns for environmental damage caused by animal waste over the years, there is a great need for producers to decrease the amount of N excreted in urine and feces and increase the amount of N recycled back to the animal. Fecal N loss results from excretion of undigested feed N, undigested microbial N, and endogenous N.

Losses of urinary N can originate from rumen losses, metabolic losses in the gut, and maintenance losses (Tamminga, 1992).

Nitrogen can contribute to environmental pollution either as ammonia in the air or as nitrate in the soil and ground water (Tamminga, 1992). Ammonia can come from manure that has been broken down by urease present in the environment (James et al., 1999). Although ammonia is a highly soluble gas with a short atmospheric time, the high reactivity of ammonia gas causes significant enrichment of ammonia in the atmosphere. Gaseous ammonia is the most abundant cationic chemical species in the air and it neutralizes atmospheric acids produced by the oxidation of S and N to form aerosols (James et al., 1999). The aerosols have a much longer atmosphere residence time than the constituents of the gas phase (James et al., 1999).

Ammonium is a very water soluble compound and has a very mobile nature. Concerns for water contamination in areas of concentrated animal productivity are of major concern.

Nitrogen captured in beef steers is captured in the muscle and carried over into a salable profit in the meat. Nitrogen that is not captured is excreted in feces and urine which results in reduced profit and damage from excess N in the environment. Inefficient capture of N can result in high feed to gain ratios and wastage of high quality feeds for the producer. Diet manipulation through synchronization of rumen energy and protein release, forage composition, and supplementation as previously discussed are just a few ways in which fecal and urinary N can decrease and N capture can increase. When diets are low in soluble carbohydrates and high in mature plant cell walls the hay is slowly digested and are usually

energy limited. Providing a highly fermentable energy supplement to moderately high protein hay can result in better capture of available N.

Summary

Based on the literature, N from forage can be better captured by microorganisms when the protein and energy sources are well coordinated. Synchronization of energy and protein released from feeds can be affected by morphology, and chemical composition of the plant, and/or the presence of a supplement.

For my research, the portions of the plant that are of the greatest importance are that of the leaf and stem. The ratio of the leaf to stem has an effect on digestibility and may also alter N fermentation in the rumen. The leaf portion of the plant usually contains more protein and less fiber relative to the stem portion. Gamagrass hay is relatively high in leaf and lower in stem when compared to other warm-season grasses. Orchardgrass hay, a cool-season grass, has a lower leaf to stem ratio compared to gamagrass. We chose gamagrass for its high leaf to stem ratio and orchardgrass for its low leaf to stem ratio and expected these morphological differences to affect N metabolism in growing steers when fed an energy supplement.

Chemical composition also affects the digestibility of the forage in the rumen. Cool-season grasses are higher in B_2 protein and B_3 fractions than are warm-season grasses. The B_2 fraction of protein is degraded in the rumen and at a faster rate than the B_3 protein fraction. The differences in protein fractions between the two hays will affect interaction with a highly degradable supplement and therefore affect N metabolism.

Providing steers with an energy supplement to a forage-based diet can enhance the capture of N by reducing the loss of N as ammonia. Soybean hulls combined with corn provide a

supplement that is energy dense yet high in fiber. Supplementing growing steers at a low percentage of BW will result in less substitution of supplement for forage. The readily available energy of corn combined with the digestible fiber in soybean hulls in coordination with a moderate quality forage reduces the amount of N absorbed as ammonia.

The hypothesis of my experiment is that the synchronization of protein and energy released within the rumen will affect the proportions of nitrogen absorbed as ammonia and affect urea production and recycling by beef steers.

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NITROGEN METABOLISM OF BEEF STEERS FED GAMAGRASS OR ORCHARDGRASS HAY WITH OR WITHOUT A SUPPLEMENT

Abstract

This experiment evaluated rumen protein:energy synchronization in steers fed either gamagrass (*Tripsacum dactyloides* (L.) L.) or orchardgrass (*Dactylis glomerata* L.) hays with or without a soybean hull/corn supplement. A nitrogen (N) balance trial was conducted to compare the effects of the supplement and hay on N intake, N digestion, and N metabolism. The supplement consisted of 50:50 corn: soybean hulls mix with 50 g molasses per kg of corn: soybean hulls mixture. The supplement contained 11.3% CP, 34.2% NDF, 22.9% ADF, and 0.87 kg was fed at 0830 and 1600 followed by hay offered in two equal portions. Steers had ad libitum access to water and a trace-mineralized salt block throughout the study. Hay compositions were 8.3% CP, 70.6% NDF, and 36.2% ADF for gamagrass and 11.9% CP, 71.9% NDF, and 37.9% ADF for orchardgrass. Steers were individually fed, blocked into two weight groups, and randomly assigned to either gamagrass or orchardgrass hay (4 steers per hay). Within hays, steers were assigned to receive supplement or no supplement in a crossover design. Ad libitum DM intake for each steer was equal to their intake during the last 8d of the 21-d ad libitum phase. Steers were then placed in metabolism crates for an 8-d adjustment followed by a 5-d balance trial. During the balance trial, steers were fed 90% of ad libitum intake. Compared with gamagrass, orchardgrass had similar in vitro cell wall disappearance (IVCWD) (64.2 vs 62.2%), similar leaf IVCWD (58.2 vs 63.2%), and similar stem IVCWD (46.7 vs 51.7%). Effects were significant at $P < 0.10$. Compared with gamagrass, orchardgrass had decreased ad libitum DM intake (4.62 vs 5.37 kg/d), decreased digestible DM intake (3.20 vs 3.53 kg/d), increased N intake (96.6 vs 81.1 g/d), similar fecal N (36.1 vs 34.5 g/d), increased N digestibility (62.4 vs 57.4 %), and similar N retained (27.2 vs 27.7 g/d). Supplementation increased digestible DM intake (3.70 vs 3.03 kg/d), total DM

intake (5.87 vs 5.07 kg/d), N intake (96.9 vs 80.8 g/d), fecal N (39.4 vs 31.1 g/d) and N retained (31.7 vs 23.2 g/d). Supplementation (1.74 kg/d) decreased hay ad libitum intake by 0.73 kg/d. Supplementation decreased blood urea N, urine urea N and urine urea N as a percentage of urine N more for orchardgrass than for gamagrass. Supplementation tended ($P < 0.11$) to increase N retained as a percentage of N intake and increased N retained as a percentage of N digested more for steers fed orchardgrass than for steers fed gamagrass. Blood urea N was reduced (2.21 vs 0.14 mM) more with supplement for steers fed orchardgrass than for steers fed gamagrass. We believe that the differences in CP between the hays explain the interactions between hay and supplement.

Key Words: Beef Steers, Soybean Hulls, N Metabolism, Tripsacum dactyloides, Dactylis glomerata

Introduction

As the concerns for environmental damage caused by agricultural waste increase, so does the need for better management of waste by producers. Nitrogen is one waste product that has come into concern in the past years. Diet management by producers is one practical way by which excretion of N can be reduced and accretion of N in the form of protein may be increased.

Ammonia absorbed from the rumen or produced from the catabolism of amino acids is converted to urea in the liver (Tillman and Sidhu, 1968). Urea can be excreted in the urine or recycled to the gastrointestinal tract across the gut wall or in the saliva. The amount of urea N that enters the gastrointestinal tract ranges from 30 to 77% of dietary N (Huntington, 1988) and the quantity of endogenous urea that may be recycled to the gut ranges from 19 to 96% of the urea produced (Kennedy and Milligan, 1980). The synchronization of a N source

and a readily degradable energy source through supplementation can increase capture of ammonia by microorganisms (Herrera-Saldana et al., 1990), reduce the amount converted to urea in the liver, thereby making more N available for protein production. Soybean hulls are a highly degradable energy supplement that is high in fiber but low in lignin (Klopfenstein and Owen, 1987) and when fed with forage may degrade in the rumen at a comparable rate to the forage fiber. The chemical composition and degradability of forage and supplement influences the synchronization of protein and energy in the rumen. Digestibility differences among forages are influenced by morphological variation (Cherney et al., 1990). The purpose of this study was to determine the effects of N metabolism in growing steers fed a cool-season grass hay and a warm-season grass hay either with or without a highly fermentable energy supplement.

Materials and Methods

Animal and Experimental Procedures

The experimental design was a crossover design with a 2 X 2 factorial arrangement of treatments. The experiment began in June and ended in August, 2002. Treatments consisted of feeding gamagrass (*Tripsacum dactyloides* (*L.*) *L.*) hay or orchardgrass (*Dactylis glomerata L.*) hay with or without a soybean hull/corn supplement mix (Table 1). The supplement mix contained equal portions of ground soybean hulls and ground corn with 50 g molasses per kg supplement added for palatability.

Nine Hereford steers (initial BW 275.5 ± 31.3 kg) from the Beef Educational Unit of North Carolina State University were selected for the study. Eight steers were selected on the basis of BW and demeanor. Handling, sampling, and care of these steers were approved by the North Carolina State University Animal Care and Use Committee (IACUC 01-029-A).

The steers were trained to be lead by halter and accustomed to close human contact, passage through the animal handling facilities, and familiarization with equipment necessary for blood, urine, and fecal collections.

Steers were transported from the Beef Educational Unit to the Metabolism Educational Unit of North Carolina State University. Steers were divided according to weight into a heavy group (average BW 296.5 ± 29.2 kg) and a light group (average BW 254.5 ± 16.3 kg) and then randomly assigned orchardgrass or gamagrass (4 steers per grass). This process evenly distributed the steers according to weight. Gamagrass steers averaged an initial BW of 288.9 ± 34.4 kg and orchardgrass steers averaged 262.2 ± 25.2 kg. During period one of the crossover, steers were then randomly assigned to receive either no supplement or supplement. Four heavy steers shared a pen with individual Calan gates (American Calan Inc., Northwood, NH) and the four light steers were similarly housed and fed in an adjacent pen. All steers were implanted with eight Synovex-S pellets (200 mg progesterone and 20 mg estradiol benzoate) in the middle of the left ear between the skin and cartilage on July 1, 2002. Steers were adapted to the Calan gate feeding system for approximately one week and fed wheat hay ad libitum during this phase. Once the steers were adapted to the Calan gates, a 21-d ad libitum intake phase began. The last 8 d of the 21-d ad libitum intake phase measured the ad libitum DM intake for each steer. Following the ad libitum phase, steers were housed in metabolism crates with a 7-d adjustment phase followed by a 5-d collection phase. Natural daylight during the experiment extended approximately 14.5 h which determined the daily light and dark time in the barn. Steers had ad libitum access to water and a trace-mineralized salt block throughout the study. While

steers were housed in metabolism crates, barn temperatures were monitored and box fans were used to circulate air. Barn doors remained open to aid in airflow.

Hay was initially fed at 2% of BW and subsequently offered at 110% of previous day intake during the ad libitum phase. At DM intake equal to 2% BW, unsupplemented diets contained CP and TDN to support maintenance and 0.454 kg average daily gain (NRC, 1996). Hay samples were taken periodically throughout the study prior to passing the bales through the S600 Bale Processor (Van Dale Inc., Petersburg, VA). These samples were composited and a sub sample was later separated into leaf and stem fractions to be used for analysis. Plants were hand-separated into leaf, stem, and flower heads. The heads were severed, in the case of orchardgrass, directly below the bottom ray of the panicle with the remaining peduncle left with the stem fraction. In the case of gamagrass, the cut was made directly below the bottom ovule with the seed stalk combined with the stem fraction. A final fraction consisted of unidentifiable material. Supplement was formulated at 0.75% average initial BW and fed at a constant rate of 1.76 kg DM per day. Supplement was fed at 830 and 1600 in two equal portions. Steers were given 30 min to consume supplement. Hay was offered at 0900 and 1630 in two equal portions. During the balance trial, hay was fed at 90% of the previous 5-d average of ad libitum intake to simulate the 10% feed reduction normally found when animals are confined to metabolism crates. Supplement was fed at 90% as well. Prior to the balance trial collection, all crates were thoroughly scrubbed and washed. Plastic tarps were placed directly behind the crates and urinal collection containers were placed under the metal crate urine pans.

Total collections of orts, urine, and feces were conducted for 5 d during the collection phase. Hay was sampled daily and composited to represent hay composition. Grab samples

of the supplement were also taken. Urine was collected via urine pans funneled into collection jugs. The urine jugs contained a daily allotment of H_2O and 6N HCL for acidification to ensure that the collected urine was maintained at a pH < 4. This was verified using pH-sensitive paper prior to collection of the aliquot. Feces and urine were collected daily, weighed, and a 5% daily aliquot was retained. The urine aliquots were pooled by steer and kept frozen at < -4°C. Fecal aliquots were dried at 60°C and pooled by steer. At the end of collection, each steer was removed from the crate and the crate was thoroughly scraped. Feces recovered from the crates were added to the fecal collection for that day. Daily rectal temperatures were taken for all steers during the 5-d collection period. Temperatures for each steer were averaged together for period one and period two of the experiment. After completion of the balance trial in period one, steers returned to group housing, and were switched from either no supplement to supplement or supplement to no supplement treatments and procedures were then repeated.

Chemical Analysis

All feed, feces, and orts were ground in a Wiley Mill (Thomas Specific, Swedesboro, NJ) to pass through a 1 mm screen. Samples were sealed in whirl pack bags and stored at room temperature until analyzed. Urine samples were stored in 125-mL plastic bottles and frozen at < -4°C until analyzed. Feed, feces, and orts were analyzed for DM, organic matter (OM), and Kjeldahl N using (AOAC, 1990) procedures. Duplicate samples of feed and orts were analyzed sequentially for NDF and ADF using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp, Fairport, NY). Whole hay, leaf, stem, brown, flower heads, and other samples were analyzed for NDF, ADF, and Kjeldahl N. In vitro cell wall disappearance (IVCWD) was determined by in vitro fermentation using the Ankom II

Daisy batch fermenter (Ankom Technologies, Fairport, NY). Each fermenter received 2,000 mL of inoculate consisting of 1,600 mL of McDougal's buffer (Tilley and Terry, 1963) and 400 mL strained rumen fluid. Samples (0.25 g) were weighed in quadruplicate in Ankom fiber bags (Ankom Technologies, Fairport, NY) and incubated for 0, 6, 12, 18, 24, 48, and 72-h time intervals. Fermentation was terminated by NDF procedure in the Ankom 200 fiber analyzer to remove residual microbial fractions. The equation $y = ae^x$ was used to determine the rate of NDF and neutral detergent fiber N (NDFN) disappearance and the data points were plotted on a graph after the removal of data from 0 h and 6 h which represented the lag phase of disappearance (Gill et al., 1969). Sample fiber bags were then dried overnight at 60°C in a forced air oven. Residue from the Ankom bags was scraped into Kjeldahl tubes and analyzed for Kjeldahl N. Neutral detergent fiber nitrogen was calculated using the same equation as IVCWD. Urea content of plasma and urine was determined using the diacetyl monoxime method of Marsh et al. (1957) using a Technicon Auto Analyzer (Technicon Instrument Corp, Tarrytown, NY, USA). Protein fractions of the hay, leaf and stem were determined as described by Licitra et al. (1996). Protein fraction A was determined using borate phosphate buffer. Protein fractions B_1, B_2, B_3, and C were determined using NDF and ADF procedures followed by Kjeldahl N determination.

Statistical Analysis

Statistical analysis of data was performed using analysis of variance for a crossover 2 x 2 factorial design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model for the balance trial data included independent variables hay, steer(hay), supplement, hay x supplement, periods(hay), and residual as a source of variation. Hay was tested by the test term steer(hay) while the remaining variables were tested against the residual. Statistical

analysis for substitution rates was determined using analysis of variance for GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with the model including independent variables hay, supplement, steer, and period. Statistical significance was declared at $P < 0.10$.

Results

The average steer rectal temperature during period one was 38.8 ± 0.08ºC (n = 8, 5 temperatures per steer) with an average barn temperature of 26.6 ± 1.2ºC (n = 6). The average steer rectal temperature during period 2 was 38.6 ± 0.24ºC (n = 8, 5 temperatures per steer) with an average barn temperature of 23.3 ± 2.0ºC (n = 3).

Compared to steers fed orchardgrass, steers fed gamagrass had greater ($P < 0.10$) hay ad libitum DM intake (Table 2). Compared to steers fed supplement, steers not fed supplement had increased hay intake ($P < 0.01$).

Ad libitum total DM intake (hay plus supplement) increased ($P < 0.10$) for steers fed gamagrass compared to steers fed orchardgrass (Table 2). Total DM intake was increased ($P < 0.01$) for steers fed supplement when compared to steers without supplementation. When steers were fed supplement, the ad libitum intake as percentage of BW increased ($P < 0.01$) compared to when steers were not supplemented. Ad libitum intake, as a percentage of BW, was similar between hays and there was no interaction between hay source and supplement (Table 2).

There was a period within hay effect on substitution rates (100 * (decrease in hay intake with supplement, kg d^{-1}/ supplement intake, kg d^{-1}) when steers were fed supplement. The substitution rates were greater ($P < 0.06$) for period one compared to period two. However, there was no significant difference in substitution rates between gamagrass (46.91 ± 9.03) and orchardgrass (36.88 ± 9.03).

Compared to steers fed orchardgrass, steers fed gamagrass had decreased N intake ($P < 0.06$), decreased urine N output ($P < 0.05$), decreased urine urea N in both g d^{-1} and as a percentage of total urinary N ($P < 0.01$), decreased N digested ($P < 0.06$), similar fecal N output, and similar N retained (Table 3). Compared to steers fed orchardgrass, steers fed gamagrass had increased N retention as a percentage of N intake ($P < 0.05$), increased N retained as a percentage of N digested ($P < 0.01$), and similar N digested as a percentage of N intake. Compared to steers fed orchardgrass, steers fed gamagrass had increased DM digestibilities but similar hay DM intake, total DM intake or fecal DM. Compared to steers fed orchardgrass, steers fed gamagrass tended to have greater ($P = 0.11$) OM intake, greater OM digested ($P < 0.05$), greater OM digested as a percentage of intake ($P < 0.01$), and similar fecal OM (Table 4). Compared to steers fed orchardgrass, steers fed gamagrass had similar NDF and ADF intake, fecal NDF and ADF, NDF and ADF digested, and similar NDF and ADF digested as a percentage of intake. Compared to steers fed orchardgrass, steers fed gamagrass had lower blood urea N (BUN) ($P < 0.01$) (Table 3).

When steers were fed supplement, N intake increased ($P < 0.01$), N digested increased ($P < 0.01$), fecal N increased ($P < 0.01$), urine urea N decreased ($P < 0.05$), urine urea N as a percentage of total urinary N decreased ($P < 0.05$), and urinary N tended to increase ($P = 0.16$) (Table 3). When steers were fed supplement, N retained increased ($P < 0.01$), N retained as a percentage of intake increased ($P < 0.05$), and N retained as a percentage of digested increased ($P < 0.01$). When steers were fed supplement, BUN decreased ($P < 0.01$) (Table 3).

When steers were fed supplement, fecal DM increased ($P < 0.01$), hay DM intake decreased ($P < 0.01$), DM digested increased ($P < 0.01$) and DM digested as a percentage of intake increased ($P < 0.01$). When steers were fed supplement, OM intake decreased ($P < 0.01$), fecal OM increased ($P < 0.05$), OM digested increased ($P < 0.01$), and OM digested as a percentage of intake decreased ($P < 0.01$). When steers were fed supplement, NDF and ADF intake decreased ($P < 0.01$), NDF and ADF digested decreased ($P < 0.01$), NDF and ADF digested as a percentage of intake decreased ($P < 0.01$), and had similar in fecal NDF and ADF (Table 4).

There were hay source x supplement interactions (Table 3) for urine urea N ($P < 0.05$), urine urea as a percentage of total urinary N ($P < 0.05$), N retained as a percentage of N digested ($P < 0.10$), and BUN ($P < 0.03$). There was a trend ($P < 0.11$) for a hay source x supplement interaction for N retained as a percentage of N intake. The interactions were due to a greater response to supplement for orchardgrass than for gamagrass.

Compared to orchardgrass, gamagrass was lower in CP ($P < 0.01$), lower in NDF ($P < 0.01$), lower in ADF ($P < 0.10$), and greater in OM ($P < 0.01$) (Table 1). The hays were similar in DM. (Table 1). The leaf and stem proportions of each hay were slightly different with gamagrass having numerically less leaf and more stem than orchardgrass (Table 5). Gamagrass was lower than orchardgrass in both leaf and stem CP (Table 5). Compared to whole orchardgrass, whole gamagrass was lower in $B_1$and B_3, greater in A and B_2, and similar in protein fraction C. Compared to orchardgrass leaf, gamagrass leaf was lower in A, B_1, and C, greater in B_2, and similar in protein fraction B_3. Compared to orchardgrass stem, gamagrass stem was greater in A, B_1 and B_2, lower in B_3, and similar in protein fraction C.

In vitro disappearance rate of cell wall for the soybean hull corn supplement was approximately four times faster than IVCWD for either hay (Table 7, Figure 1). Compared to orchardgrass, gamagrass cell wall disappeared at a slower rate (Table 7, Figures 2, 3). The disappearance rates for orchardgrass and gamagrass leaf cell wall were similar as were the rates for orchardgrass and gamagrass stem cell wall (Figures 4-7, Table 7). The NDFN percentages were formulated using the same exponential equation as IVCWD (Table 8). Supplement NDFN had a disappearance rate of 1.85% h^{-1} that was slower than NDFN for either hay (Table 8, Figures 8, 9, 10). Compared to orchardgrass, gamagrass NDFN disappeared at a lesser rate for whole plant (Figure 9, 10, Table 8), greater rate for leaf (Figure 11, 12, Table 8), and at a lesser rate for stem (Figure 13, 14, Table 8).

Discussion

The hays used in this experiment were chosen based on previous experimental data that suggest differences in leaf to stem ratios (Cherney et al., 1990), protein fractions (Sniffen et al., 1992), and disappearance rates between cool-season grasses and warm-season grasses and their effects on N metabolism (Redfearn et al., 1995).

The ratio of leaf to stem in each grass has the potential to alter fermentation in the rumen due to the differences in degradability. The highly fermentable leaf protein produces ammonia which is absorbed, converted to urea in the liver and either excreted or recycled as urea-N. Redfrearn et al. (1995) reported that while switchgrass, big bluestem, and smooth bromegrass had similar protein fractions, ruminal degradation rates were different at identical incubation times and were influenced by anatomical structures. Burns et al. (1992) reported the leaf to stem ratio for gamagrass to be higher than other warm-season grasses such as bermudagrass and flaccidgrass. We chose gamagrass and orchardgrass for this experiment

because they were available and we expected the gamagrass to have a higher leaf to stem ratio than orchardgrass. However, the hand separations of gamagrass and orchardgrass used in this experiment revealed that the ratios were not as different as expected and that the orchardgrass actually had a higher leaf to stem ratio than that of the gamagrass (Table 5).

Warm-season grasses and cool-season grasses differ in the percentage of protein fractions; warm-season grasses contain a smaller percentage of B_2 protein fraction and a greater percentage of B_3 protein fraction as a percent of CP than cool-season grasses. Archibeque et al. (2001) reported that gamagrass had about 25% of CP as B_2 fraction and 40 to 47% of CP as B_3 fraction. Warm-season switchgrass (Archibeque et al., 2002) had similar concentrations of B_2 and B_3 protein fractions as those reported for gamagrass. Archibeque et al. (2002) reported cool-season grass protein fractions for tall fescue with the B_2 protein fractions fescue ranging from 38 to 44% of CP and 20 to 29% for B_3 protein fraction of CP. Krishnamoorthy et al. (1982) reported similar values for tall fescue to those reported by Archibeque et al. (2002) and also reported protein fraction values for timothy. Timothy had a B_2 fraction ranging from 34 to 38% and a B_3 fraction ranging from 22 to 29 % of CP. Based on these previously reported values, the gamagrass and orchardgrass hays were expected to have differing B_2 and B_3 protein fractions. However, the laboratory analysis of the hays in the present experiment did not show the differences previously discussed. The B_2 fractions of the orchardgrass and the gamagrass differed by approximately 6 percentage units, the B_3 protein fractions differed by only 5.2 percentage units, and the C fractions differed by only 0.2 percentage units (Table 6). The similarities found between the orchardgrass and gamagrass B_2, B_3, and C protein fractions reiterates the fact that the hays were more similar to each other than was anticipated.

The protein fractions of the gamagrass used in this study were similar to those reported by others; however, the orchardgrass was lower in B_2 fractions and higher in B_3 and C protein fractions than those reported previously. The post harvest treatment of the orchardgrass hay may have contributed to some of the differences found. Both grasses were harvested at early maturity. The orchardgrass, however, may have had some heat damage due to residual moisture at the time of baling. The heat damage may have shifted some of the protein fractions from B_2 to B_3. The preliminary analysis of the orchardgrass reported that 1.59% of DM was unavailable protein. Comparing the preliminary C fraction of orchardgrass used in this study to those reported by other authors places this orchardgrass within the reported range of C protein fractions. Therefore, the heat damage that occurred with this orchardgrass may have shifted some of the B_2 protein to B_3 protein but not enough to completely render the protein unavailable, such that is found in the C protein fraction.

In vitro cell wall digestion was also analyzed for both the orchardgrass and gamagrass hays and there were no differences found (Tables 7, 8), which was not what was expected. The gamagrass and orchardgrass were expected to differ in leaf and stem and thus the degradation of the whole plant should have been different. The exponential formula used to for IVCWD predicted the NDF and rate of disappearance for the samples. The timed IVCWD revealed that essentially even the rate at which the hays disappeared was similar for each timed-interval indicating that the fiber fermentability was similar between hays. The predicted 0-time values for cell wall (Table 7) were similar to compositional values of %NDF (Table 1) for both gamagrass and orchardgrass demonstrating that the hays had were not unusual in characteristics and that the IVCWD were reasonable results.

The leaf portion of the plant degraded at a rate almost twice as fast as the stem for both gamagrass and orchardgrass. These results are in agreement with the concept that leaf is composed of less fiber and is more digestible than stem and that the leaf to stem ratio of the whole plant will have a direct affect on the energy and protein release in the rumen.

The supplement cell wall was degraded twice as fast as the hay and by 72 h approximately 1% of the sample remained (Table 7). This indicates that the supplement was highly degradable and low in indigestible fractions.

The disappearance rate of NDFN for whole orchardgrass and orchardgrass stem was greater than the whole gamagrass and gamagrass stem (Table 8). The rate of NDFN disappearance of gamagrass leaf, however, was greater than orchardgrass leaf. The NDFN after 72 h provides some estimate of C protein fraction. Compared to the calculated values of C protein fraction from table 6, the predicted CP as a percentage of DM after 72 h overestimates the amount of C protein fraction for the hays. The predicted and calculated values for gamagrass were 1.18 and 0.49 C fraction as %DM. The predicted and calculated values for orchardgrass were 1.12 and 0.66 C fraction as %DM, for gamagrass leaf were 1.15 and 0.47 C fraction as %DM, for orchardgrass leaf was1.97 and 0.77 C fraction as %DM, for gamagrass stem were 0.68 and 0.27 C fraction as %DM, and for orchardgrass stem were 1.04 and 0.36 C fraction as %DM. The predicted and calculated values for the supplement, however, were similar with values of 0.6 and 0.8 C fraction as %DM. As mentioned above, the NDFN after 72 h incubation provides some estimate of C fraction for the forages but after comparing the calculated and predicted values, it is apparent that after 72 h there is still some N other than C protein left in the sample.

The leaf NDFN of orchardgrass appears to have a relatively high C fraction compared to the gamagrass leaf, whole and stem cellulose fractions of orchardgrass (Table 6). This, I believe, may relate to the heat damage that occurred after baling. I think that most of the damage occurred in the leaf and therefore increased the amount remaining after 72 h and lowering the overall disappearance rate of orchardgrass.

The preliminary analysis of gamagrass and orchardgrass showed the hays to be different in CP with gamagrass at 9.6% CP and orchardgrass 12% CP. Our experimental design was to have similar N intake across hays; however, this was not the case. The gamagrass hay declined in N concentration from 8.8 % in period one to 7.8 % CP in period two. The orchardgrass also declined in CP as the experiment progressed. Orchardgrass declined from 12.3 % in period one to 11.4 % CP in period two. One explanation for the decline in CP may be that the storage of the hay overtime somehow caused the N to drop; however, because the storage of the hay was three months, we do not believe this to be the cause. Another explanation for the decline in CP may be the way the hays were selected to be fed. The N concentration of the hay may have varied according to the location of the grass in the field. The order in which they hay was baled and stacked in the barn remained consistent to the order in which it was harvested. Therefore, the hay should have been randomly selected and fed to the steers to ensure that the N of the hay fed was representative of the entire lot of hay.

Steers fed orchardgrass had greater N intakes than steers fed gamagrass (Table 3). This was due to the greater N concentration in the hay. The increased N intake was also associated with increased urinary N and urinary urea N excretion but similar fecal N amounts, indicating that the N above need was not utilized but excreted in the urine (Table

3). The N intake difference between the hays was approximately 15 g. The difference in urinary N between the hays was also approximately 16 g, reiterating the point that although the steers fed orchardgrass had higher N intakes, all of the extra N was excreted in the urine. The greater amount of N retained as a percentage of intake and the amount of N retained as a percent digested for gamagrass fed steers is consistent with the lower amounts of N excretion when animals are consuming N at levels near their maintenance requirements. Davis (2001) used NRC (1996) equations to calculate nutrient requirements for growing beef steers (272 kg BW, 545 kg BW with 28% body fat at finishing). A growing steer with ADG of 0.23 or 0.45 kg d^{-1} requires 491 or 595 g CP d^{-1}. In this experiment, steers fed gamagrass alone averaged 513 g CP d^{-1} which is between these requirements.

When supplement was added to the hays, hay intake was reduced for both the ad libitum (Table 2) and balance trial DM intakes (Table 3). The reduction of hay, however, was minimal. The substitution rate for steers fed gamagrass was 47% of the amount of supplement fed and for steers fed orchardgrass, the substitution rate was 37%. The difference between the two hays was not significant. Period one of this experiment had increased temperatures and humidity compared to period two. The temperature differences between the two periods caused a decrease in hay intake and an increase in substitution rates for period one when compared to period two of this experiment. Unexpectedly, one steer in period two, fed orchardgrass, had an increase in hay DM intake when fed supplement. Because the animal numbers were low in this experiment, the steer that ate more hay in response to supplementation decreased the substitution rate overall for steers fed orchardgrass. Again, there was no significant difference in substitution rates for steers fed orchardgrass compared to steers fed gamagrass. The low substitution rates seen in this

experiment are in agreement with substitution rates found in previous work (Garces-Yepez et al., 1997, Moore et al., 1999). In this experiment, the supplement TDN intake was 0.50% BW, the voluntary forage intake for gamagrass and orchardgrass was 1.91 % BW and 1.79 % respectively, and the forage TDN:CP ratio for gamagrass and orchardgrass was 7.5 and 5 respectively. These values are in agreement with a literature review by Moore et al. (1999) indicating that supplements decrease voluntary forage intake when supplemental TDN intake is > 0.7 % BW, when voluntary forage intake is > 1.75 % BW, or when forage TDN:CP ratio is > 7. Furthermore, when the amount and source of protein in the concentrate adequately supplements the forage, the substitution rates were found to be at a minimum (Jarrige et al., 1986). Adding supplement to the forages increased total DM digested and total DM digested as a percentage of intake (Table 3). Assuming 90% digestibility of the supplement, I calculated from data table 3 that supplement decreased DM digestibility of gamagrass from 61.7 to 56.0% and decreased DM digestibility of orchardgrass from 58.8 to 52.2%. This is in agreement with the OM and fiber digestibilities. Adding supplement to the forage decreased OM, NDF, and ADF intake, OM, NDF, and ADF digested, OM, NDF, and ADF digested as a percentage of intake and increased fecal OM while fecal NDF and ADF remained similar (Table 4). Adding supplement to the forage decreased the fiber intake and digestion while increasing the DM digestion overall, thereby increasing the digestibility of the forage plus supplement treatment.

Urinary N was similar between those fed supplement and those without supplement; however, urea N, BUN, and urine urea N as a percentage of total N decreased with the addition of supplement. The decrease in urea N and BUN indicates that supplemented steers had greater synchronization of protein and energy release in the rumen and that the

microorganisms were capturing a greater amount of N and less ammonia was absorbed. Blood urea levels are a function in part of the absorption of ammonia and conversion to urea. When high dietary N is available but not captured as microbial N, the ammonia is absorbed and blood urea levels increase. When large amounts of N are available but not accompanied by an energy source, the proportion of urea N in the urine is higher compared to when energy is available (Huntington and Archibeque, 1999). The interaction among hay and supplement for BUN coincided with the interaction of urinary urea N excretion. Steers fed orchardgrass had a greater decrease in blood urea N with the addition of supplement than steers fed gamagrass. Steers fed orchardgrass responded better to supplementation because the orchardgrass hay alone had an unsynchronized release of energy and protein in the rumen and the addition of supplemental energy in coordination with available protein helped microorganisms capture N. Steers fed gamagrass did not respond as much to supplement because the hay alone was synchronous in energy and protein release in the rumen.

Implications

A corn, soybean hull mix supplement can serve a variety of purposes depending on the chemical and morphological composition of the hay. Supplementation with corn and soybean hulls at a rate of 0.75% BW to hays containing 60% TDN and ranging from 9 to 12% CP will reduce hay intake, but increase the total DM intake. Supplementation will improve the rate and efficiency of N captured as body tissue in steers, which should increase overall steer performance.

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Table 1. Composition of hays and supplement.

	Supplement[a, b]	Gamagrass[c]	Orchardgrass[c]	SE_{hays}
DM %	87.0	91.9	91.8	0.05
% of DM				
CP %[d]	11.3	8.4	11.9	0.14
OM %[d]	96.1	95.0	91.9	0.04
NDF %[e]	34.2	70.6	71.9	0.47
ADF %[d]	22.9	36.2	37.9	0.32

[a]Contained 50 g molasses per kg of supplement.
[b]n = 2 for supplement.
[c]n = 7 for each hay.
[d]Hays differ ($P < 0.01$)
[e]Hays differ ($P < 0.10$)

Table 2. Ad libitum hay DM intake and treatment DM intake.

	No Supplement		Supplement			*P* - value		
Item	Gamagrass	Orchardgrass	Gamagrass	Orchardgrass	SE	Hay	Supp	HxS
BW, kg	303	276	304	276	1.5	0.292	0.945	0.972
Ad lib Hay DM intake, kg/d	5.78	4.95	4.97	4.3	0.111	0.10	0.002	0.475
Ad lib total DM intake, kg/d	5.78	4.95	6.71	6.04	0.111	0.10	0.001	0.475
DMI % BW	1.91	1.79	2.22	2.21	0.05	0.64	0.001	0.33

Table 3. Dry matter and N intake, digestion, retention, and blood urea N in steers fed gamagrass or orchardgrass hay with or without supplement during the balance trial.

	No Supplement		Supplement			*P*-value		
Item	Gamagrass	Orchardgrass	Gamagrass	Orchardgrass	SE[a]	Hay	Supp	HxS
DM Intake, kg/d								
Hay	5.37	4.72	4.55	4.10	0.119	0.18	0.004	0.45
Hay + Suppl.	5.37	4.72	6.13	5.68	0.119	0.18	0.003	0.45
Fecal DM, kg/d	2.06	1.95	2.15	2.12	0.029	0.67	0.010	0.25
DM digested, kg/d	3.32	2.77	3.97	3.56	0.105	0.08	0.005	0.52
DM digested, % intake	61.69	58.79	64.87	62.67	0.669	0.02	0.007	0.63
N intake, g/d	73.9	88.7	90.4	105.5	1.54	0.06	0.001	0.93
Fecal N, g/d	30.9	35.2	39.3	41.7	1.33	0.19	0.005	0.51
Urine N, g/d	17.4	35.9	17.0	31.6	1.35	0.003	0.160	0.22
Urine urea N, g/d	6.6	28.1	5.0	20.6	1.07	0.001	0.014	0.05
Urine urea N, %total N	34.5	77.7	28.3	65.5	2.20	0.001	0.014	0.05
N digested, g/d	43.02	53.43	51.07	63.74	1.60	0.06	0.005	0.52
N retained, g/d	25.60	17.50	34.00	32.10	1.76	0.29	0.003	0.16
N digested % intake	57.84	60.29	56.34	60.13	1.37	0.13	0.580	0.65
N retained, % intake	34.46	19.80	37.62	30.17	1.73	0.03	0.020	0.11
N retained, % digested	59.80	32.40	66.70	49.90	2.38	0.008	0.008	0.09
Blood urea N, mM	2.73	8.20	2.59	5.99	0.31	0.002	0.02	0.03

[a]n = 4 for each mean.

Table 4. Organic matter, NDF, and ADF intake, digestion, and retention in steers fed gamagrass or orchardgrass hay with or without supplement during the balance trial.

	No Supplement		Supplement			*P*-value		
Item	Gamagrass	Orchardgrass	Gamagrass	Orchardgrass	SE[a]	Hay	Supp	HxS
OM Intake, kg/d	5.10	4.35	4.32	3.77	0.114	0.11	0.01	0.43
Fecal OM, kg/d	1.93	1.80	2.03	1.97	0.029	0.53	0.02	0.26
OM digested, kg/d	3.17	2.55	2.29	1.80	0.099	0.04	0.01	0.57
OM digested, % intake	62.09	58.71	52.99	47.68	0.914	0.01	0.1	0.35
NDF Intake, kg/d	3.79	3.33	3.22	2.89	0.085	0.17	0.01	0.47
Fecal NDF, kg/d	1.48	1.28	1.46	1.35	0.035	0.24	0.56	0.26
NDF digested, kg/d	2.31	2.05	1.76	1.54	0.075	0.16	0.01	0.80
NDF digested, % intake	60.91	61.54	54.58	53.32	0.920	0.80	0.01	0.37
ADF Intake, kg/d	1.92	1.74	1.63	1.51	0.043	0.29	0.01	0.55
Fecal ADF, kg/d	0.79	0.74	0.80	0.77	0.015	0.51	0.19	0.47
ADF digested, kg/d	1.13	1.00	0.83	0.74	0.037	0.21	0.01	0.68
ADF digested, % intake	58.9	57.75	50.83	48.80	0.920	0.29	0.01	0.66

[a]n = 4 for each mean.

Table 5. Leaf and stem composition[a].

	Gamagrass		Orchardgrass	
Item	Leaf	Stem	Leaf	Stem
% of whole plant	57.5.	25.3	69.8	16.5
DM %	92.9	93.3	93.1	93.4
CP %	9.8	4.3	12.5	5.8
OM %	94.9	95.9	91.6	92.7
NDF %	69.4	75.8	71.7	79.3
ADF %	32.4	41.1	37.0	46.0

[a]mean of one analysis for samples collected before hay was processed through the hay press. n = 4

Table 6. Protein fractions of supplement, gamagrass and orchardgrass hay[a].

		Gamagrass			Orchardgrass		
Item	Supplement	Leaf	Stem	Whole	Leaf	Stem	Whole
CP, % DM	11.32	9.80	4.27	8.36	12.48	5.80	11.95
A, % CP	13.05	16.36	39.95	16.30	18.85	33.76	16.71
B_1, % CP	4.42	1.32	4.97	2.33	3.71	1.88	2.61
B_2, % CP	56.42	27.72	43.19	28.31	21.08	32.90	21.95
B_3, % CP	19.47	50.29	5.73	44.83	50.64	25.30	50.02
C, % CP	7.13	4.76	6.26	5.31	6.17	6.26	5.52

[a] mean of one analysis for samples collected.
n = 1

Table 7. Predicted initial NDF concentration and in vitro rates of disappearance for leaf, stem, and whole plant for gamagrass and orchardgrass[a]

		Gamagrass			Orchardgrass		
Item	Supplement	Leaf	Stem	Whole	Leaf	Stem	Whole
Predicted Initial NDF, %DM	43.1	72.4	72.5	70.8	75.0	76.1	74.2
Rate, % h^{-1}	4.42	1.22	0.72	1.06	1.21	0.70	1.20
R^2	0.90	0.96	0.90	0.93	0.99	0.82	0.92

[a]Calculated a, x, and R^2 values for in vitro cellulose digestion using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

Table 8. Predicted initial NDFN concentration and in vitro rates of disappearance for leaf, stem, and whole orchardgrass and gamagrass[a]

		Gamagrass			Orchardgrass		
Item	Supplement	Leaf	Stem	Whole	Leaf	Stem	Whole
Predicted Initial NDF, %DM	1.97	7.97	1.47	5.56	8.14	2.89	7.32
Rate, % h^{-1}	1.85	2.66	1.04	2.19	1.82	1.34	2.70
R^2	0.74	0.96	0.89	0.95	0.99	0.85	0.93

[a]Calculated a, x, and R^2 values for NDFN using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

Figure 1. Regression of supplement NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.

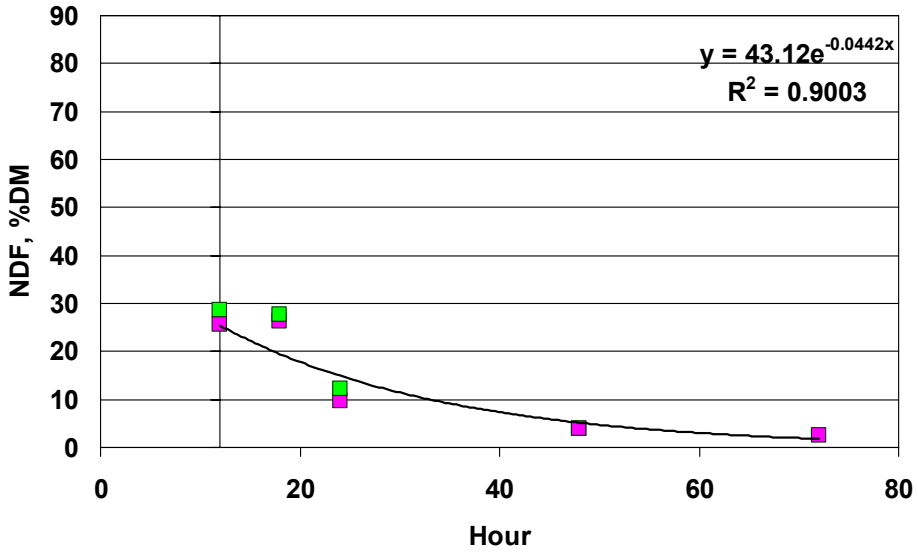

Figure 2. Regression of gamagrass NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.

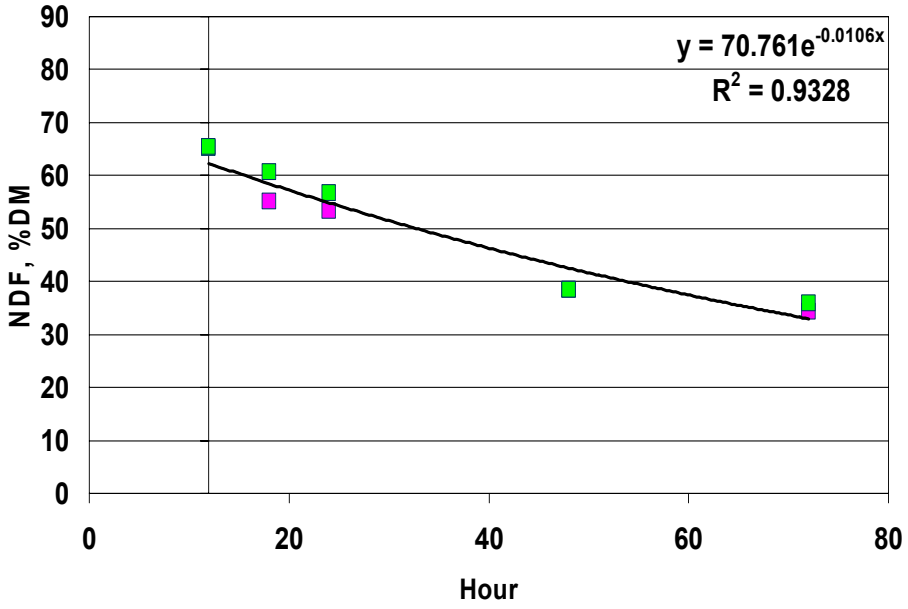

Figure 3. Regression of orchardgrass NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.
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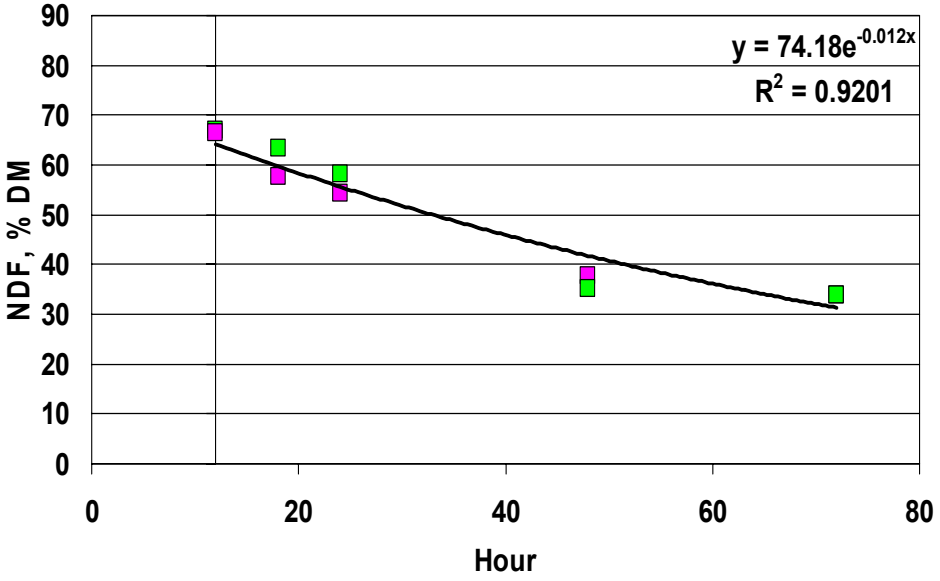


Figure 4. Regression of gamagrass leaf NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

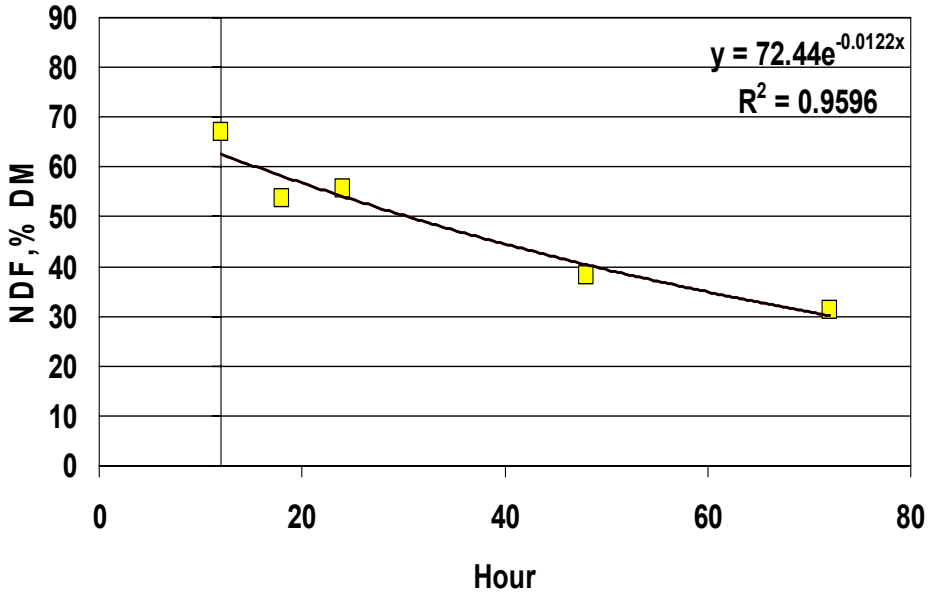


Figure 5. Regression of orchardgrass leaf NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

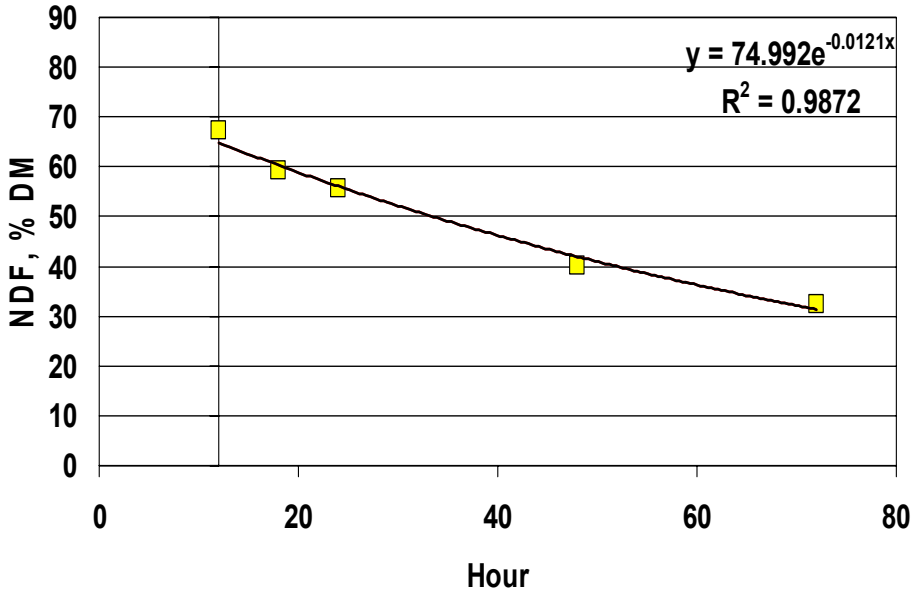


Figure 6. Regression of gamagrass stem NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

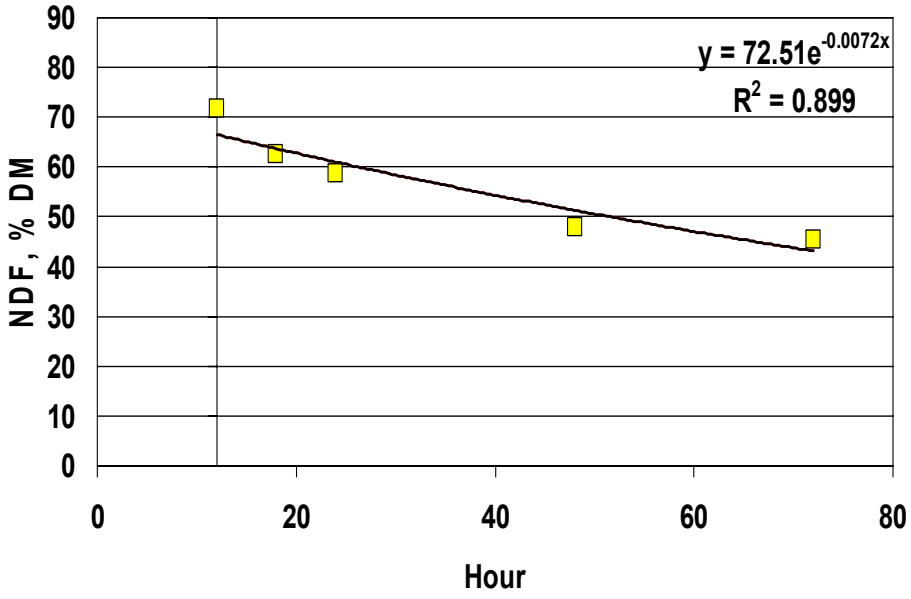


Figure 7. Regression of orchardgrass stem NDF, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

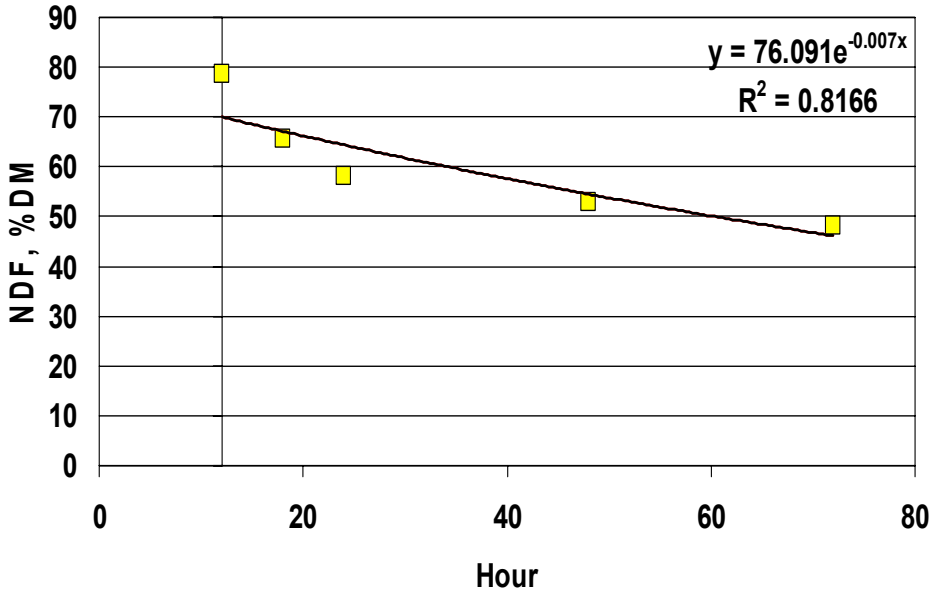


Figure 8. Regression of supplement CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.

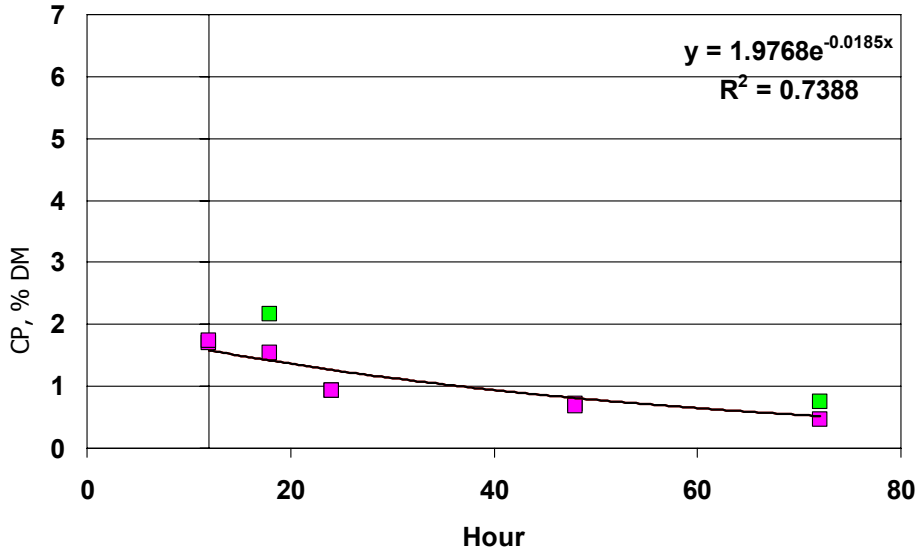


Figure 9. Regression of gamagrass CP, %DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.

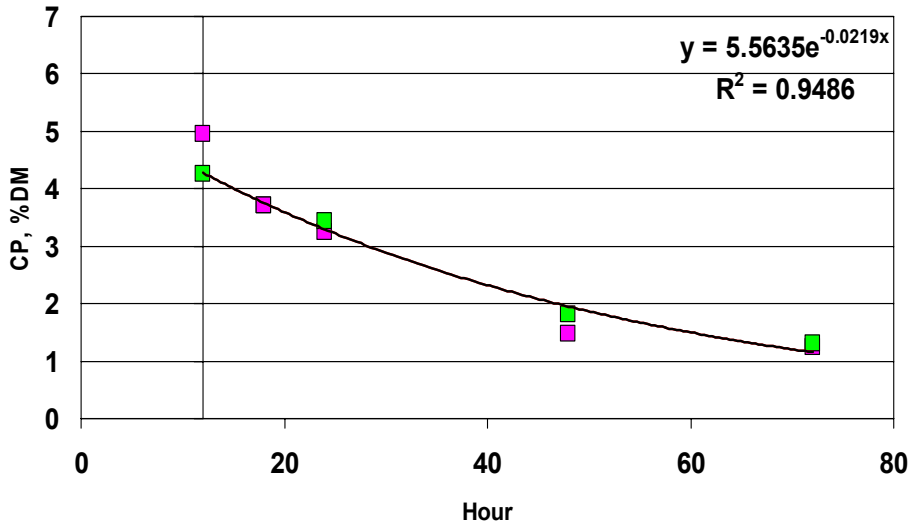


Figure 10. Regression of orchardgrass CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.
■ represents period one. ■ represents period two.

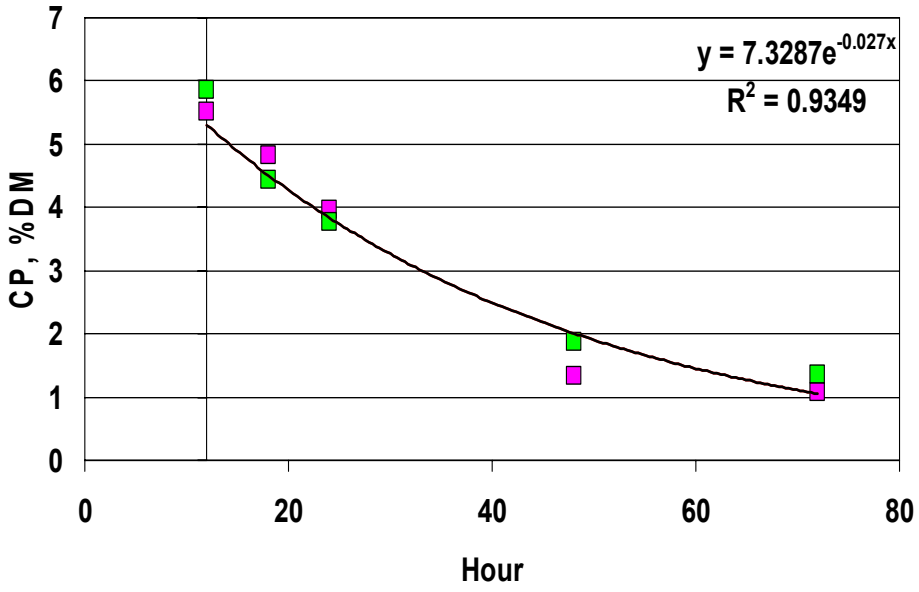


Figure 11. Regression of gamagrass leaf CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

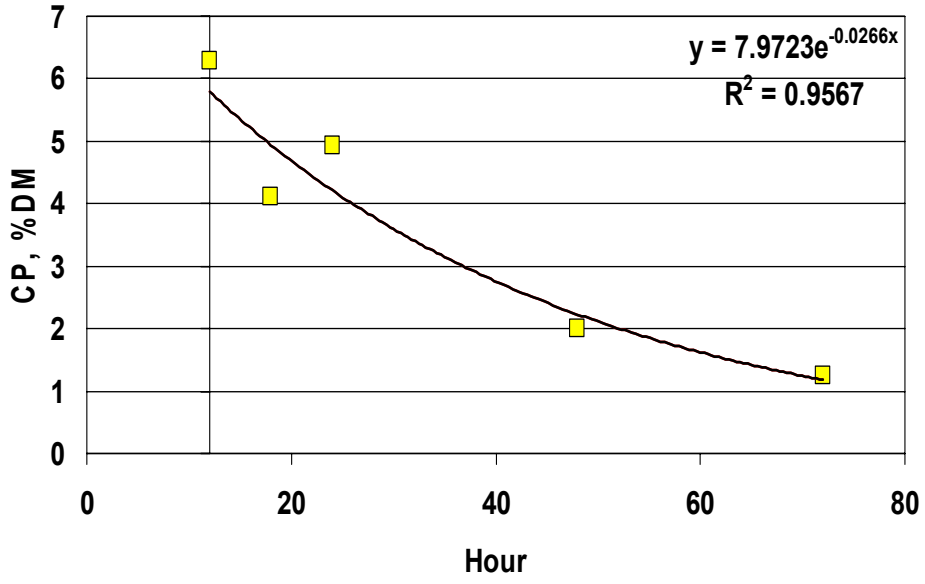


Figure 12. Regression of orchardgrass leaf CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

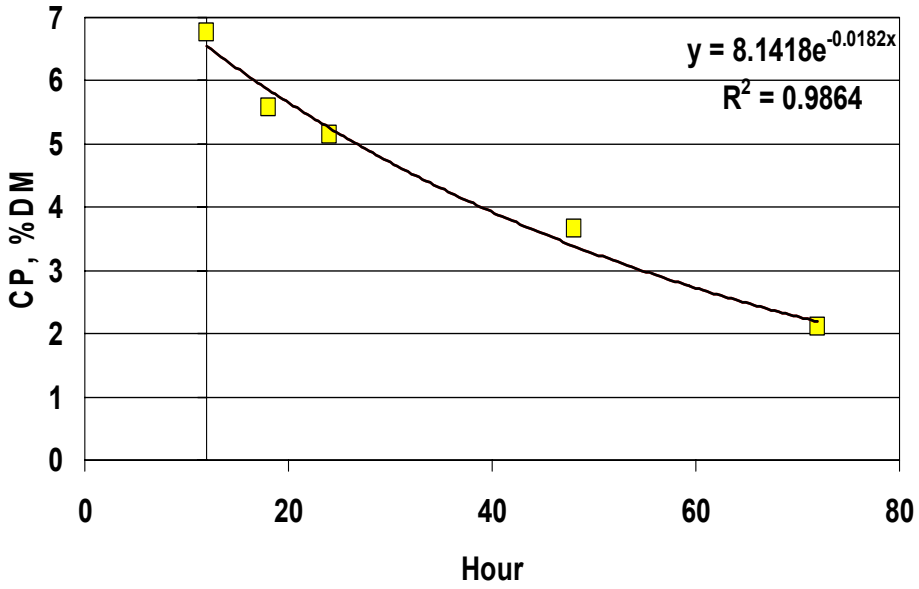


Figure 13. Regression of gamagrass stem CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance.

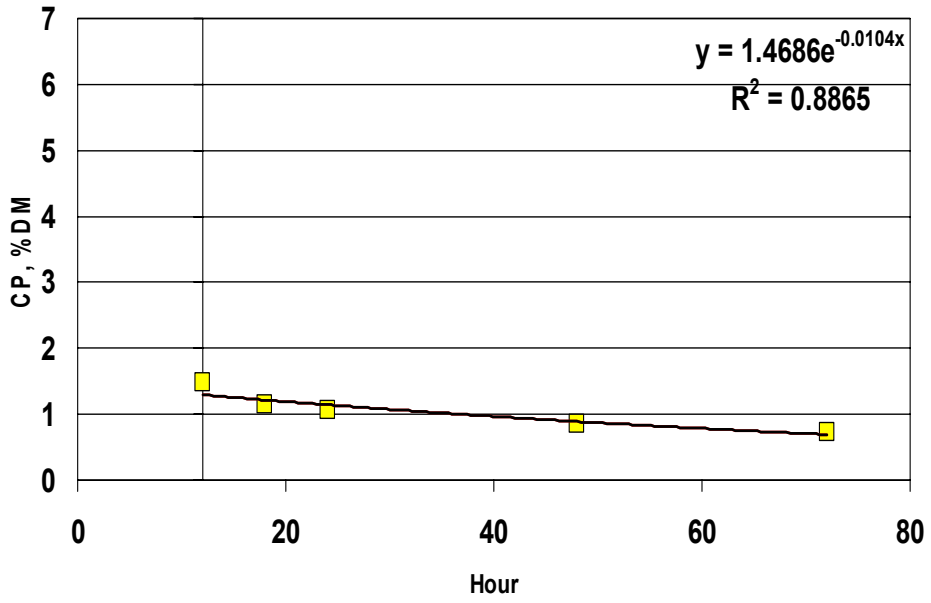
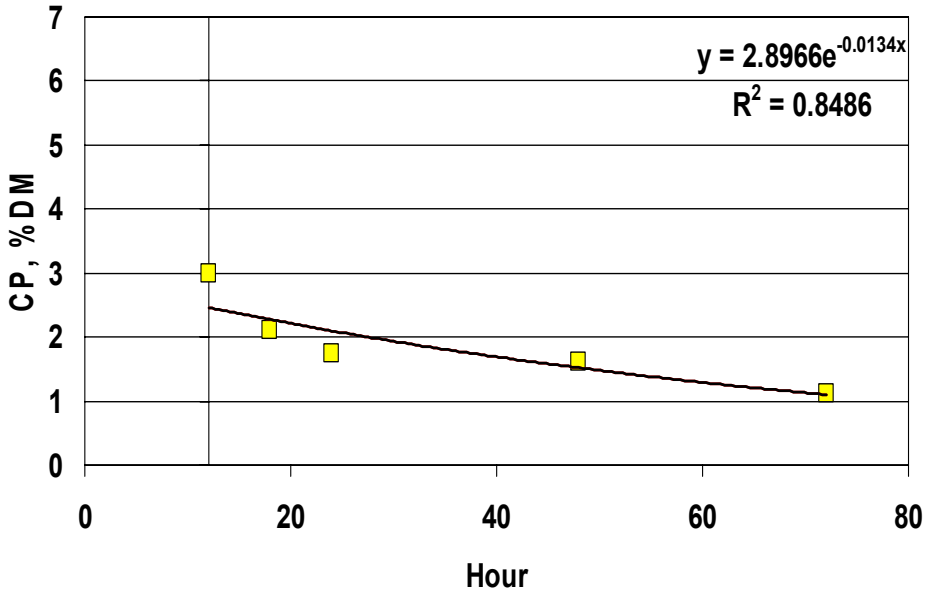


Figure 14. Regression of orchardgrass stem CP, % DM (y) vs time of incubation (x). Calculated a, x, and R^2 values for in vitro cell wall disappearance using equation $y = ae^x$ where 'a' is predicted % of DM, 'x' = rate h^{-1} of disappearance. .



^{15,15}N – UREA TRACER

^{15}N Introduction

Ammonia produced from rumen fermentation of the diet can be absorbed and converted to urea or used for bacterial protein synthesis and then subsequently digested and absorbed in the small intestine. Urea synthesized in the liver has two fates; an amount is excreted in the urine and the remainder enters the gastrointestinal tract. Once urea enters the gastrointestinal tract, it is hydrolyzed in the lumen by bacteria. The free ammonia can then be excreted as nitrogen products in the feces, reenter the ornithine cycle or undergo anabolic use by the animal through microbial and mammalian metabolites of ammonia (Lobely et al., 2000). Determining how much urea-N enters the gastrointestinal tract has proven to be a difficult task. There are essentially two major approaches to measuring urea-N. One approach is the measurement of net flux across splanchnic tissues (Huntington, 1989) and the other approach is through tracer isotopes such as ^{15}N, ^{14}C, and ^{13}C (Nolan and Leng, 1972).

Walser et al. (1954) developed a tracer technique based on the systemic infusion of $^{15}N^{15}N$ and the formation of $^{14}N^{15}N$ urea from ammonia after its hydrolysis in the gut. This technique was used in humans extensively by Jackson et al. (1993) and adapted for ruminant use by Sarraseca et al. (1998). Sarraseca et al. (1998) based the model for ruminants on the assumption that two molecules of $^{15}NH_3$ are produced when urea enters the gut as $^{15}N^{15}N$. Tracing urea movement through the ruminant begins with the compartmentalization of the system into specific body pools. Infusion of ^{15}N urea enters the body urea pool at a known rate and from there is taken to the liver. Once urea leaves the liver it can then be excreted in the urine or enter the gastrointestinal tract. The amount of ^{15}N entering the gastrointestinal tract can then be calculated by the difference between ^{15}N urea entry rate and the amount excreted in the urine. The equation to quantify the amount of ^{15}N urea entering the gut

comes from Lobley et al. (2000). In continuous infusions, when steady state conditions are achieved, or at least the urea pool size does not change, urea entry rate is quantified by the infusion rate multiplied by the enrichment of ^{15,15}N-urea. Urine urea excretion is the measurement of urea in the excreted amount of urine over time.

Materials and methods

Day one of the balance trial coincided with day one of the isotope infusion trial for half of the steers. Four steers, one from each treatment, were infused on days one through three of the balance trial and the remaining four steers were infused on day six of the balance trial. Prior to infusion of the isotope, baseline blood, via jugular venipuncture into heparinized tubes, was collected for each steer. Baseline urine samples were also collected for each steer. Steers were infused with ^{15}N^{15}N-urea (Cambridge Isotope Laboratories, Inc. Andover, MA CA #2067-80-3) prepared in 0.9% NaCl saline solution. Isotope infusion rates were maintained at 85 mL/hr using a peristaltic pump (Model 1000, International Medical Systems Inc. Huntington, NY) and the ^{15}N^{15}N-urea infusion rate was 0.272 mMoles urea N per hour. Urine was collected every 6 h starting at hour 32 after initiation of infusion. A separate aliquot of 125 mL was taken for ^{15}N analysis. The amount and pH of urine during these intervals was recorded and pooled for the daily collection.

Chemical Analysis

Urea analysis of urine was processed through cation exchange resin columns (BioRad AG 50W-x8 lot). Cation exchange columns consisted of plastic columns about 1.5 cm in diameter filled with resin. During the ion exchange procedure, sample ions with the same charge replace the counterions present in the resin. The sample ions are exchanged when introduced onto the resin and can then be eluted by introducing an ion with a higher affinity

for the resin (Bio-Rad Laboratories, Hercules, CA). Once the urea concentration of the urine sample was determined, the necessary volume to provide 1.5 mg urea-N was calculated for each sample. Urine from four steers was randomly chosen. Zero, 38, 44, 50, and 56 h samples for period one and two were processed for each steer in numerical order on randomly assigned columns for each steer beginning with the 0-h. New columns were prepared for the remaining samples. Resin beds were charged by the following method before each use. The new resins were rinsed with 10 mL of 1 N NaOH, twice with 10 mL of N-free water, twice with 10-mL rinses of 1 N HCL, followed by two more rinses of 10 mL N-free water. To collect the sample, an amount of urine was added to the column after calculation, the column was washed with 7 mL of N-free water, which was collected and discarded. A 20-mL rinse of N-free water eluted the urea into 50-mL glass beakers rinsed with N-free water. Samples were then dried with forced air at 60ºC overnight. Samples were reconstituted with 1 mL of phosphate buffer solution. Two hundred microliters of buffer and sample were transferred to the urease beaker apparatus.

The beaker apparatus was constructed from a 50 mL Erlenmeyer flask with a rubber stopper apparatus that suspends the filter disc above solution. The stopper apparatus consisted of the rubber stopper (Kontes, 882310-0000) with suspended open-ended plastic buckets (Kontes, 882320-0000). From the plastic buckets, a wire hook (Y-GWXX-090, 0.009 in stainless steel Type 316V) was constructed from which the paper filter disc (Whatman glass microfiber filters 55mm, Type 934-AH, Fisher 09-873 E) was pierced and suspended above solution to capture ammonia.

Three mL of 0.1 M phosphate buffer (pH = 7) was added to each flask in addition to the sample. One hundred microliters of urease solution was then added to the flask. Five

microliters of 2.5 M $KHSO_4$ was pipetted onto each filter paper and then placed in the flasks and sealed immediately. The flasks were then shaken for 20 min in the Dubnoff agitator at 25ºC, then injected with 200 mL of 3 N NaOH with a 20 gauge needle through the stopper apparatus. The flasks were then continuously shaken for 1 h. Twenty-four hours after the injection of NaOH, filters were removed from the flasks and placed in the dessicator along with an open container of concentrated H_2SO_4. After the filter paper was completely dry, it was rolled into tin cups (Elemental Microanalysis, Devon, United Kingdom) and analyzed by a Thermo Finnigan DELTA^{plus} mass spectrometer (Thermo Finnigan, San Jose CA) equipped with a CE Elantech NA 2500 (CE Elantech Inc. Lakewood NJ).

Statistical Analysis

Statistical analysis of data was performed using analysis of variance using the GLM procedure of SAS (1999-2001). The model for the balance trial data included independent variables hay, steer(hay), supplement, hay x supplement, periods(hay), and residuals as a source of variation. Dependent variables included UUE, $\%^{15}N$, and atoms percent excess. Hay was tested by the test term steer(hay) while the remaining variables were tested against the residual. Statistical significance was declared at $P < 0.05$.

Results

Steers fed gamagrass had lower ($P < 0.05$) UUE than steers fed orchardgrass. Steers fed supplement tended to have lower ($P = 0.06$) UUE than steers not fed supplement. There tended to be a hay by supplement interaction for UUE. Steers fed orchardgrass with supplement had a greater decrease ($P < 0.08$) in UUE than steers fed gamagrass with supplement

Percent ^{15}N was significantly different among hays. Steers fed gamagrass had higher percentages of ^{15}N-in urea than steers fed orchardgrass. There were no differences in $\%^{15}N$ due to supplement or the hay by supplement interactions.

Discussion

The Hoffman procedure analyzes N_2 gas produced from the degradation of urea treated with hypobromite. Within the mass spectrometer, under electron impact conditions, N_2 gas liberated from pure $^{14}N^{14}N$, $^{15}N^{14}N$, and $^{15}N^{15}N$ urea molecules yield mass/charge (*m/z*) values 28, 29, and 30 (Sarraseca et al., 1998). A correction factor is necessary for measurements under standard conditions because in solution, the close proximity of molecules can contribute to the production of N_2 gas. Therefore, in samples low in $^{15}N^{15}N$ urea, the *m/z* yield of 29 may be increased at the expense of the 30 peak (Sarraseca et al., 1998).

From the measurements of the Hoffman procedure, urea entry rates can be calculated (UER). UER is calculated from the dilution of infused $^{15}N^{15}N$ urea in the urine compared to the concentration of $^{15}N^{15}N$ urea in the infusate. This calculation allows for the correction factor and produces a value assumed to be equal to total synthesis (Sarraseca et al., 1998).

In my experiment, we trapped the ^{15}N-urea but did not separate the isotopomers using the Hoffman reaction. Because the $^{15}N^{15}N$ and $^{15}N^{14}N$ were not separated, urea entry rate and urea recycling could not be calculated (Lobley, 2000). Previous work have indicated that the ratios of 29 and 30 (*m/z*) vary across animals (Archibeque et al., 2002) and thus a specific correction factor for peak 29 and 30 can not be produced. Therefore, in order to calculate a UER and re-entry to the ornithine cycle, the Hoffman reaction must be used to separate the peaks on samples produced from this experiment.

Natural abundance was determined from the analysis of urine samples at 0 h prior to infusion. Natural abundance determined the amount of ^{15}N-urea present in the animal before ^{15}N-urea was infused and established a specific background percent for each animal. Analysis of natural abundance resulted in a range from 0.364 to 0.371 atoms %^{15}N which is within the range of abundance reported by Archibeque et al., (2001) and Sarraseca et al. (1998). Subtracting the natural abundance from the measured amount of ^{15}N-urea present in urine over the infusion period determined the infusion atoms percent excess (a.p.e.) urea present in the animal.

Measurements of urinary urea excretion rate (UUE) were calculated using measured urine excreted and urea N present in sampled urine. UUE was calculated using the grams of urea-N analyzed in the urine sample by the amount of urine excreted and the mass of N. There were two possible ways of calculated UUE for my experiment. Samples of urine were taken for 5 d over the experimental periods, aliquoted, and pooled. The pooled samples were analyzed and urinary excretion amounts were recorded. From these components, I could calculate and average UUE for each steer per day. Another way to calculate UUE was to use the four samples of urine collected during the infusion period. These four samples were collected every 6 h for a 24-h period. Each sample was aliquoted and the urine excretion amounts were recorded. From these measurements average daily UUE could also be calculated. Statistical analysis of the pooled samples and 6-h samples for UUE revealed that there was no difference between the two.

The results of analysis for the UUE measurements indicate a possible interaction ($P <$ 0.08) of the hay by supplement (Table 1). The interaction of hay by supplement for UUE is in correlation with blood urea levels. Orchardgrass fed steers experienced a greater decrease

in BUN in the presence of supplement than steers fed gamagrass indicating better capture of available N. A reduction of urea excreted in the urine indicates that the N was also better captured and available N was used and not lost in urine.

The hourly samples of urine were analyzed for urea and ^{15}N from urea. The concentration of ^{15}N urea in these samples produces a slight wave in the plateau. The time of day these samples were taken explains the wave. Four 6-h urine samples were taken at 1600, 2200, 0400, and 1000 respectively. The first and fourth urine samples were taken during times of activity and the second and third urine samples were taken during times of relative inactivity for ruminants (Lefcourt et al., 1999). The 1600 and 1000 urine samples represent the lower points on the wave and the 2200 and 0400 urine samples represent the higher points in the wave. The atoms percent excess and $\%^{15}N$ concentration measurements are opposite than those of UER. The UER increases when intake increases (Archibeque et al., 2001 and Sarraseca et al., 1998) and decreases during low intakes. The slight fluctuation of concentration found in these 6-h samples, however, is a viable estimation for daily urea entry. Lobely et al. (2000) reported that an enrichment plateau can be reached within 24 h of infusion therefore the samples taken in this experiment during from 38 to 56 h of infusion are representative of steady state and the fluctuation within this period is due to diurnal patterns and not due to fluctuations in steady state.

Implications

Although the 29 and 30 peak of urea were not separated in my experiment, I believe the overall results produced from my measurements are still valid. Archibeque et al., (2001) and Sarraseca et al., (1998) reported that with increased intake, UER increases. In my experiment, steers fed gamagrass had higher a.p.e than steers fed orchardgrass which can be

interpreted to mean lower UER which coincides with these previous reports. I expected to see more of a supplement effect on a.p.e because of the added fermentable energy in the diet and with further analysis of my samples, may see a significant difference in a.p.e for steers fed supplement. Overall, the results from my experiment support previous findings.

Table 1. Percent ^{15}N, urine urea excretion, and urinary urea N, for hours 38, 44, 50, and 56.

		Hay							
		Gamagrass		Orchardgrass					
Item	Hour after start of infusion	Supplement		Supplement			$P <$		
		No	Yes	No	Yes	SE	Hay	Supp	HxS
% 15N[b]	38	0.637	0.675	0.522	0.505	0.03	0.01	0.7	0.4
	44	0.680	0.708	0.524	0.563		0.01	0.6	0.9
	50	0.627	0.749	0.528	0.561		0.01	0.1	0.3
	56	0.635	0.661	0.511	0.523		0.05	0.3	0.7
Atoms percent Excess[b,c]	38	0.267	0.309	0.158	0.143	0.03	1.0	0.5	0.7
	44	0.310	0.341	0.159	0.200		1.0	0.8	0.6
	50	0.257	0.382	0.162	0.199		0.5	0.2	0.3
	56	0.265	0.205	0.146	0.161		0.6	0.04	0.5
Urinary Urea N mM/h[b]	38	49.3	40.50	267.68	150.39	20.0	0.01	0.03	0.05
	44	32.17	33.75	238.08	137.48		0.01	0.17	0.12
	50	49.06	44.97	221.3	176.17		0.04	0.6	0.4
	56	53.88	47.74	275.56	160.00		0.01	0.14	0.09
Total Urine N, mmol/h	38	180.22	140.26	341.96	223.16	30.0	0.01	0.11	0.36
	44	101.57	112.74	249.47	179.55		0.01	0.44	0.30
	50	124.7	141.44	262.53	232.55		0.01	0.66	0.26
	56	157.04	150.88	325.81	215.70		0.01	0.12	0.15

SE values are for hay by supplement interactions.
[b] Times differ ($P < 0.05$)
[c] Atom percent excess = % ^{15}N – preinfusion %^{15}N from urea.

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General Summary

Supplementation of a highly fermentable energy source to moderate quality hays containing from 9 to 12% CP and 60% TDN may improve the rate and efficiency of N capture as body tissue in steers. Soybean hulls are a fermentable fiber source that is relatively high in energy and low in lignin. A supplement of soybean hulls in combination with ground corn and a very small amount of molasses provide a quick source of energy and digestible fiber.

In this experiment, steers fed orchardgrass responded more to the supplement than steers fed gamagrass. We believe the response to supplement was due to an unsynchronized release of energy and protein in the rumen from orchardgrass alone. As hypothesized, we believe that the leaf to stem ratio of the forage will affect N metabolism in the rumen. The leaf portion of the plant is usually higher in N and lower in fiber making N quickly available and the leaf highly digestible. The orchardgrass had greater amounts of leaf than stem, and thus digestion of orchardgrass resulted in readily available N in the rumen. The addition of a highly fermentable energy supplement allowed the microorganisms in the rumen to better capture the available N provided by the leaf portion of the orchardgrass and helped to synchronize the rumen thus increasing N efficiency.

In this experiment, steers fed gamagrass showed little response to the supplement. The response to the supplement by steers fed gamagrass was also in agreement with the hypothesis that leaf to stem ratios affect N metabolism. In the case of gamagrass, the leaf to stem ratios were better balanced providing a more synchronized protein and energy release in the rumen by the gamagrass alone. Because of the synchronized release of energy and protein provided by gamagrass, the addition of a fermentable energy source did not provide a

significant increase in N efficiency to the 8.4 %CP in gamagrass. In summary, the addition of the supplement was beneficial to the steers fed unsynchronized hay through better N capture in the rumen and thus increased N efficiency.

^{15,15}N urea-N tracer is used to follow the movement of urea through a ruminant. This tracer technique is useful in determining how much N a ruminant uses, excretes, and recycles. Infusion of ^{15}N urea enters the body urea pool at a known rate and is taken to the liver. Once the urea leaves the liver, it can be excreted in the urine or enter the gastrointestinal tract. The amount of ^{15}N entering the gastrointestinal tract can then be calculated by the difference between ^{15}N urea entry rate and the amount excreted in the urine. Chemical analysis of ^{15}N consists of analyzing N_2 gas produced from the degradation of urea treated with hypobromite (Hoffman reaction). Then using a mass spectrometer, ^{14}N^{14}N, ^{15}N^{14}N, and ^{15}N^{15}N urea molecules will yield mass/charge values. From these measurements produced from the Hoffman reaction, urea entry rates can be calculated. In my experiment, ^{15}N urea was trapped, but the isotopomers were not separated. Therefore, the molecules will need to be separated and analyzed to ensure that the urea entry rates and the re-entry rates to the ornithine cycle are accurate.