ABSTRACT

SAUVE, ALAINA KATHLEEN. Ruminant intake, digestibility, and nitrogen balance of gamagrass hay and baleage harvested during sunrise and sunset (Under the direction of Gerald B. Huntington).

Two experiments were conducted to evaluate the differences in TNC composition of gamagrass harvested in the afternoon (PM) compared to the morning (AM). In the first experiment gamagrass hay was fed to goats with half receiving a protein supplement while the other half received none in a crossover design with a 2 X 2 factorial arrangement of treatments. In the second experiment gamagrass baleage fertilized at 2 different nitrogenous levels 50 kg N/ha (LO) and 150 kg N/ha (HI) was fed to steers in a similar experimental design with half of the steers receiving LO and the other HI. In both experiments TNC concentration including monosaccharides was increased in PM compared to AM ($P < 0.001$). During the ad libitum phase, goats and steers both had similar DMI of PM and AM ($P < 0.88$). There was a tendency ($P < 0.14$) for digestible DMI (DDMI) to be increased by 4 % for PM compared to AM in the goat study; however, was decreased by 9 % in the steer study. During the balance trial, there was a tendency ($P < 0.09$) for goats to have increased hay and total (hay + supplement) intakes of PM compared to AM, but there were no differences for steers. DDMI was 7 % greater for PM than AM in the goat study, whereas the opposite held true for the steers. DDMI was 9.5 % greater for AM than PM. Compared to AM, PM improved dry matter digestibility (DMD) (55.7 vs. 53.1% DM) when fed to goats. There was a tendency for DMD to be improved in AM (53.2 vs. 51.0 % DM) when fed to steers. In both experiments, the higher protein level (supplement and HI) improved DMD, although the supplement did so to a greater extent. Goats and steers fed supplement and HI had increased N intakes and N digestibility ($P < 0.02$). In both experiments, the higher protein levels increased urinary N, urine urea N, and urea N as a percent of total urinary N ($P < 0.001$). Compared to no supplement and LO, supplementation and HI increased plasma urea N ($P < 0.05$), with overall levels being higher in goats (13.24 vs. 8.61 mM) than steers (6.04 to 4.12 mM). Due to all of these factors N retention was improved with supplementation (2.3 vs. 1.2 g/d), but was similar ($P < 0.89$)

between fertilization levels. We concluded from these results that PM forages do have increased TNC concentration and the differences are preserved through the drying process of making hay and fermentation during ensiling. However, ruminants do not always prefer the PM forages, especially when they are ensiled. N retention in goats can be improved with supplementation of low CP forages. To investigate the mechanism that resulted in an increased preference or DMI of PM forages we measured insulin and ghrelin concentrations. There was a tendency for insulin to be elevated in AM compared to PM ($P < 0.12$) and HI compared to LO ($P < 0.07$). Across all treatments, ghrelin concentration peaked at 1000 and decreased for the remainder of the day. We concluded from this data that ghrelin concentration in beef steers increases prior to feeding (animals were fed at 1000), which supports other data suggesting that it may be involved in meal anticipation. My hypothesis was that ghrelin and insulin concentrations would be affected by differences in macronutrient intake, specifically TNC. In the steer study, AM increased DMI, DDMI, and DMD, and the differences in plasma hormone concentration may suggest that regulation of intake between time of harvest may differ as well.

RUMINANT INTAKE, DIGESTIBILITY, AND NITROGEN BALANCE OF GAMAGRASS HAY AND BALEAGE HARVESTED DURING SUNRISE AND SUNSET

By

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APPROVED BY:

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Chair of Advisory Committee

DEDICATION

This work is dedicated to my fiancé, Jonathan Ciencewicki, who compassionately handled my stress while I was in school and provided the unconditional love and encouragement to help me learn that I can truly accomplish anything.

B**IOGRAPHY**

Alaina Kathleen Sauvé was born on November 5, 1980 in Brooklyn, New York to Marc and Kathleen Sauvé. Alaina's family, including an older sister Jacqueline and younger brother Joshua, moved to Howell, New Jersey when she was nine years old. At that time they moved onto a small 'farmette' where Alaina experienced for the first time the joy of owning horses. It was on this farm and her grandparents' farm in Montrose, PA that her interest in livestock developed.

Alaina attended Rutger's University from 1998-2002 where she obtained her Bachelor of Science degree in animal science with a focus in pre-veterinary medicine. After being admitted into Ross University veterinary school, she decided to defer for a year to pursue other interests and stay closer to home. In August 1998, Alaina moved with her boyfriend, Jonathan Ciencewicki, to North Carolina to establish residency and apply to the North Carolina State University (NCSU) veterinary school the following year. Jonathan was admitted into the toxicology program at the University of North Carolina and is currently pursuing a Ph.D. degree in toxicology. Alaina decided to work for a year at the National Institute of Environmental Health Sciences as a laboratory technician. After working closely with the scientists, she decided that she wanted to go back to school, and this time not for veterinary studies, rather she wanted to pursue a career in research in the animal science department at NCSU.

While attending NCSU, Alaina wanted to remain well-rounded, so she played on various intramural sports teams, became President of the Animal Science Graduate Student Association, and faced her fears of public speaking at the American Society of Animal Science national meetings in St. Louis, 2004, where she gave an oral presentation on her research. She is currently in pursuit of an M.S. degree in nutrition with a minor in physiology under the direction of Dr. Gerald Huntington.

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LITERATURE REVIEW

GENERAL INTRODUCTION

There are many factors that affect the voluntary intake of animals and narrowing down which ones are most important is very difficult. The problem is the factors are not mutually exclusive, and in fact, it is probably some combination or integration of them that is responsible for the overall control. Although, there are specific situations where one control may have dominance over others such as gut fill with high fiber diets or metabolic acidosis with high energy diets (Van Soest, 1994). This contributes to the difficulty in interpreting these types of experiments and drawing solid conclusions.

The ruminant is remarkable because of the symbiotic relationship that has evolved over centuries between the animal and the microbes residing within its rumen. Due to this relationship, they have adapted to live on a wide assortment of low quality feeds with minor consequences on their health or well being (Provenza and Balph, 1987). Since fermentation occurs before absorption, ruminants have the ability to digest and highly utilize forage material, or more specifically fiber, which is much less available to non-ruminants (Fisher, 2002). They can also utilize non-protein nitrogen (NPN) to meet amino acid requirements for metabolic functions, as first shown by Zuntz in 1891. In return they supply the microbial population with an ample energy source in the forms of readily fermentable carbohydrate (RFC) and fiber. However, with all of the digestive specializations in ruminants understanding regulation becomes more complicated as well.

"Differences in voluntary dry matter intake (VDMI) account for more than 50% of the variation in digestible nutrient consumption by ruminants" (Allen, 1996). Producers want livestock to digest feed efficiently and maximize their production outputs. Therefore, as researchers we need to understand intake regulation in order to decrease the variation in VDMI. Daily VDMI is measured by an animal's appetite in the short-term, which can be influenced by many factors such as photoperiod, social interactions, and changes in climatic conditions (Rhind et al., 2002).

Whether the animals are grazing or being fed individually, to maximize VDMI we need to understand the preferences they attribute to foods when given a choice. Researchers have found that identifying plant characteristics that animals deem as feeding stimulants has

been harder than identifying factors that animals avoid (Provenza and Balph, 1987). It has been suggested that the soluble energy in the plant may serve as a primary factor for diet selection (Mayland and Shewmaker, 1999).

Diet selection is controlled by a conglomeration of factors stemming from physical, chemical, and neuronal stimuli. Thus far, animal studies have identified rumen fill and blood metabolites as two of the major appetite regulatory controls. However, there are many factors that are integrated in this control system. Future research may focus on changes in hormone concentrations, neuronal signals originating in the gut, liver and fat depots, as well as crosstalk between regions of the brain (Rhind et al., 2002) in order to understand what is driving appetite stimulation and satiation. Developments in this area could elucidate new mechanisms that are aimed at maximizing feed intake and feed conversion efficiency, which have the potential for improving animal performance and thus producer profitability.

IMPORTANCE OF REGULATING AND OPTIMIZING FEED INTAKE

Metabolic/physiological

Understanding the regulation of feed intake is important because it enables livestock producers to effectively feed individual or groups of animals according to their nutrient requirements as well as their production outputs. Growing, lactating, gestating, and maintaining animals are all fed different nutrient concentrations based on their physiological state. Producers want to avoid decreases in feed intake, growth or milk outputs, or reproductive complications because these animals are not meeting their nutrient requirements. Therefore, it is necessary to identify regulators of intake, either endogenous or exogenous, and to use this information to our advantage. Early and Provenza (1998) discovered that animals given the same feed every day will eventually exhibit a decreased preference for that feed; therefore, changing ingredients in the feed may stimulate intake.

The control of intake should be examined in the short-term and long-term. Nutritional signals recognized over the short-term may not be directly related to the overall control of energy balance (Faverdin, 1999). If one is only studying short-term effects, they may miss changes that would have occurred over a longer period of time due to sensory feedback. This would be the case if the animal needs time to learn. On the other hand,

nutrients that cause large short-term effects may be eliminated if the animal had time to adapt (Faverdin, 1999).

Production/economic

In raising livestock, the main goal is to improve animal production with the least cost. Sheep that were given ad libitum access to a low protein diet, 8.2%, took twice as long to reach the same live weight as sheep consuming a high protein diet, 17% (Arsenos and Kyriazakis, 2001). This would have resulted in an extra four weeks of feed, housing, and health costs for sheep on the low protein diet. This means in order to benefit producers, nutritionists need to devise management tools that allow feed to be efficaciously transformed into products.

For meat animals it is necessary to improve feed intake because it affects efficiency of gain and therefore cost of production. Feed costs account for 40 to 70% of the total expense in beef cattle (Wertz et al., 2004). In order to increase profitability producers need to follow one strategy, either increase the amount of meat produced and keep feed costs the same or keep production levels the same and decrease feed costs.

FACTORS CONTROLLING INTAKE IN RUMINANTS

Physical

Neutral detergent fiber (NDF), including hemicellulose and cellulose, is the most limiting chemical constituent of VDMI for ruminants. Other physical limitations include: particle size, particle fragility, and rate and extent of NDF digestibility (as reviewed by Allen, 1996). Grinding and pelleting forages can increase VDMI due to the reduction in particle size and retention time in the rumen (Allen, 1996). This has been implemented as a standard practice in livestock production systems allowing for an increase in rate of passage, and resulting in less solute in the rumen and greater feed intake. Low quality forages are generally associated with lower digestibility and longer retention time, which can accelerate the onset of rumen fill and have a negative effect on VDMI. This can especially be a problem with some warm season (C_4) grasses.

Short-term control of intake is influenced by changes in gut distension, associated with rumen fill, as well as changes in the chemical concentration of nutrients. Animals respond to a variety of sensory cues regarding their diet selection. There can be physical

and chemical cues from the plant itself, which include color, texture, water content, flavor, and aroma (Mayland and Shewmaker, 1999).

Infusing HCl and NaOH in various amounts into the rumen of sheep can affect feed intake. In one study the infusions resulted in decreased intake with increasing treatment osmolality (Cooper, et al., 1995), either by becoming more acidic or basic than usual rumen conditions. The same animals who had previously selected a high energy density feed as a large portion of their diet when given a choice between high/low, or high/medium density, changed their selection during the infusion period by substituting the high energy density feed with one of the lower energy ones. This may be an attempt to decrease any further changes in osmolality. This is an example of ruminants trying to maintain stable rumen conditions by minimizing any feeds that may cause problems with fermentation. Others have shown that if treatments have similar osmolalities, than animals will develop preferences based on the chemical composition of the nutrients (Villalba and Provenza, 1997a). Sheep that were given the choice between the low/medium energy diets had the least variation in their daily intakes compared to the low/high and medium/high energy diet combinations (Cooper et al., 1995), which could be due to the fact that those two feeds offered in combination allowed for minimal fluctuations in digestion.

$NaHCO_3$ added to high energy diets (12.7 ME), in the range of 40 to 80 g/kg of fresh matter helped maintain a rumen pH greater than 6 (James and Kyriazakis, 2002). Disruptions below 6 have been documented to disrupt normal rumen fermentation of cellulose. When given a choice, the high energy density feed was avoided by sheep, which counteracts a theory that ruminants consume diets to maximize their intake of energy. However when buffer was added from 10 to 80 g$NaHCO_3$/kg fresh matter, they selected the high energy diet in much larger proportions (James and Kyriazakis, 2001). Given the fact that only the high levels of $NaHCO_3$ prevented dramatic drops in pH, there were other factors that influenced the decision of these sheep, especially at the lower level of $NaHCO_3$.

Supplementation

There are several reasons to limit supplementation of forages with readily fermentable carbohydrates such as grains. It can lead to a decrease in rumen pH by interfering with cellulolytic bacteria and thereby limiting fiber digestion. Reports have shown that supplementing forages with soluble carbohydrates, such as sucrose, may

decrease fiber digestion of forages in the rumen as well as total tract digestibility (Khalili and Huhtanen, 1991b). Importantly from this study, they determined the decrease in digestibility was due to a slower rate of digestion, which would increase rumen fill and potentially decrease voluntary intake of animals. Preference studies have shown that animals initially favor foods high in readily fermentable carbohydrates, but learn to avoid them if ingested in high amounts (Villalba and Provenza, 1997b) which could result in overproduction of VFA, changes in pH or osmolality, or an additive effect by all three (Forbes, 1996).

On the other hand, when feeding a low quality (5.5% CP), C_4 forage, it was shown that supplementing with a non-structural carbohydrate, regardless of level (.32, .64 or .96kg/d of NSC) improved intake as well as total tract digestibility of DM, OM, NDF, and CP (Bowman et. al, 2004). Supplementing forages with sugars may have different results when the sugars are combined with either insufficient levels (0.03% BW) of digestible intake protein or sufficient levels (0.12%BW). When protein was limiting, neither the supplementation of sugar nor starch resulted in differences in OM digestibility; however, fiber digestibility declined. Regardless of protein level, total OM and digestible OM intakes were increased with supplementation. When protein was fed at a sufficient level, supplementation increased OM digestibility, and in another study increased NDF digestibility (Heldt et al., 1999).

Instead of supplementing forages with energy supplements, which have been shown to cause a decrease in intake and digestibility of the forage (Bodine and Purvis, 2003) harvesting forage in the afternoon versus the morning is a management practice that takes advantage of plants' natural photosynthetic capabilities. The increase in TNC in the afternoon may decrease the fiber content in some of these low quality forages (Fisher et al., 1999)**,** although this is not always the case. Others have reported there are no differences in NDF, ADF, and cellulose composition due to time of day (Burns et al., 2005; Fisher et al., 2005; Orr et al., 2001).

Using a supplement that has high levels of starch, usually a concentrate feed, can decrease voluntary intake of forages as well as impair fiber digestion (Hoover, 1986). However, supplementing with soybean hulls has shown benefits in beef steers on high forage diets by increasing total digestible DMI and improving the rate and efficiency of

nitrogen retention (Magee et al., 2005). The use of high fiber supplements that are extensively fermented can improve animal utilization of low quality forage (Martin and Hibberd, 1990). The TDN from soybean hulls compared to corn has 15 to 30% greater value for improving animal performance of growing cattle (Garces-Yepez et al., 1997).

Palability/preference

In order to provide the most suitable environment for livestock considering social housing, feed levels and composition, as well as climatic conditions, it is important to understand animal preference, for individuals and amongst groups. Researchers are now learning how information is integrated in the periphery and brain. From here these concepts can be applied to determine what drives animal motivation.

Preference tests force the animal to make a choice based on what they would be inclined to eat. It has been shown that short-term preferences might outweigh long-term preferences because animals will make choices for what fits them best at that moment even if it results in negative consequences in the long run. If animals were asked to perform a task, for which they have learned an appropriate response, and thus base the choice of which tasks or how often they will perform the tasks on past experiences, this may be a more valid measurement of preference. This causes the animal to weigh the cost of performing the task against the benefit of a reward such as food. This could help us learn about animal motivation and the basis of preferences. Animals make choices differently when foods are offered alone or as a pair. Animals fed in confinement usually have one feeder, and thus can only adjust their diet selection by increasing or decreasing the total amount. However, when given a choice between two or more feeds animals can adjust their intake by sampling both feeds continuously, as well as ingesting one in greater quantities than another if it provides improved sensory feedback from the brain or gastrointestinal tract.

Lambs are able to associate different flavors with foods that vary in nutrient composition based on sensory feedback from digestion and absorption. This feedback combines food palability and nutrient composition. These preferences for flavors can become even stronger when they help ameliorate a toxicity or deficiency (Early and Provenza, 1998). Lambs exhibited stronger aversions to flavors associated with 90 and 110% TDN diets, compared to a flavor associated with 100% TDN, which would most accurately meet the lambs' energy requirements (Early and Provenza, 1998). These are

subtle differences that the animals are detecting. It was hard to determine whether or not the changes in preference could be related solely to differences in nutrient composition or differences in palability when changing flavors back and forth due to sensory feedback. It would have been more effective to look at one variable at a time and determine its role in preference before combining several interactions.

Goatcher and Church (1970) discovered that different species of animals have varying sensitivity to different chemicals. Carbohydrates, which are often associated with a sweet flavor, can be detected strongest by cattle, followed by goats and then sheep. Therefore, if there are subtle differences in carbohydrate concentration in a particular feed, such as morning versus afternoon harvested forages, one may assume that cattle may be the animal most proficient at picking up the differences. On the other hand, goats are dexterous at sorting through feeds and selecting the most preferred parts.

Learned vs. innate behavior

There is speculation over whether animals make choices based on innate preferences, such as a specific flavor, or if they are in fact selecting a balanced diet when two or more feeds are offered together. Several studies support the hypothesis that ruminants can learn to associate a flavor with alleviating a nutritional deficit or excess (Forbes and Provenza, 2000; Villalba and Provenza, 1997a, 1999; Early and Provenza, 1998). Animals also adjust their intake based on past experiences, or nutritional history. Following experimentation, force-fed animals often under-consume to compensate, whereas fasted animals will over-consume on feed (Konturek et al., 2004) to reach an overall appropriate energy balance.

This theory has been reevaluated over the years due to changes in the literature that suggest that animals do not always exhibit 'nutritional wisdom,' and thus do not always choose feeds that best meet their requirements. Growing sheep fed isocaloric diets, were given a choice between two feeds, a high protein diet, 23.5%CP paired with a lower protein choice, either 7.8%, 10.9%, 14.1%, or 17.2% (Kyriazakis and Oldham, 1993). The sheep always chose a mixture of feeds, despite the fact that one feed alone may have satisfied their protein requirement. Even animals offered the choice of 14.1%CP, which was greater than their requirement, chose a significant amount of the high protein feed (23.5%), which may be a result of animal sampling behavior. Animals prefer a mixture of feeds with varying nutrient composition compared to one feed alone (Early and Provenza, 1998). This would

be important for the evolution of these animals that needed to adapt to changing environmental conditions. In this same study they compared results of individual sheep. One sheep showed an immediate preference for the 14.1% CP diet, which seemed to be an innate response since it did not require post-ingestive feedback. Whereas another animal on the same treatment showed equal preferences for both feeds (14.1% and 23.5% CP) at the onset, and then increased preference for the more appropriate protein level (14.1%) over time (Kyriazakis and Oldham, 1993). This would appear to be a learned response.

Sheep whose physiological states were manipulated by either protein deprivation (8.2% CP) or repletion (17% CP), did not demonstrate an unlearned appetite for protein when subsequent diet selection was recorded for 30 minutes, one hour, or one day. Feeds were offered in pairs as a choice between high or low CP, and protein deprived animals did not compensate by ingesting more of the high CP feed (Arsenos and Kyriazakis, 2001). Measuring short-term intake is a great tool because it detects if the animal can make these alternative decisions instantaneously, rather than associating it with digestive or neural feedback, thus the need to learn in diet selection.

Another theory is that animals attempt to minimize any discomfort associated with the digestion of feeds (Forbes and Provenza, 2000). Discomfort can be described as a deficiency or excess of a particular nutrient, distension of the gut wall, or any other factor in which an animal seeks to reduce the imbalance by pursuing an opposite direction such as altering food intake. This implies that the animal is able to learn from past experiences and to alter behavior based on these experiences. Growing sheep offered a choice between diets that were either high protein (23.5% CP), or supplemented with urea (13.2% CP), chose a diet that primarily consisted of the higher protein, despite the fact that they consumed excess protein (Kyriazakis and Oldham, 1993). This suggests that when given a choice animals want to minimize any excess of rapidly degradable nitrogen, which could potentially cause discomfort.

Products of digestion/fermentation

Ruminal fermentation of carbohydrates, both structural and non-structural, results in the production of short-chain fatty acids (SCFA), which are quickly metabolized in the body, and provide 50 to 75% of total energy for the ruminant (Faverdin, 1999). Infusing acetate or propionate into the rumen can manipulate intake (cessation of intake) based on the

amount and ratio of the infusion. Lambs were conditioned with two flavors of wheat straw while receiving intraruminal infusions of sodium acetate and sodium propionate at 4, 8, or 12% daily DE. The lambs showed preferences for the 4% DE, but avoided the flavors associated with the higher levels (Villalba and Provenza, 1997a). The pH values did not fluctuate over time, and NaCl was infused in one of the trials to demonstrate that changes in intake were not a result of osmolality. Since SCFA are a natural product of fermentation, they may be acting as satiety signals to the CNS.

Ruminants have been shown to select diets higher in energy density (Villalba and Provenza, 1997b, Cooper et al., 1995). When offered diets containing different A:P ratios, lambs preferred the higher propionate ratio, 55:45 compared to 75:25 (Villalba and Provenza, 1997b). Propionate is the primary SCFA produced in the rumen from digestion of soluble sugars, starch, hemicellulose and cellulose. Lambs may select this diet since there is a larger concentration of substrate that can be utilized towards gluconeogenesis. Conditioning in these infusion studies is imperative to draw conclusive results because it may take a while for the animal to receive sensory feedback from the body. Changes in the proportion of SCFA produced in the rumen can affect diet selection and voluntary intake.

NEURAL AND ENDOCRINE HUNGER AND SATIETY SIGNALS

Importance of ghrelin

Although it is important to study changes in intake due to chemical and morphological changes in plant properties, it is equally imperative to understand the physiological changes occurring within the animal. These can include neural, metabolic, and hormonal intake signals. Due to sensors located in the gastrointestinal tract that respond to mechanical, chemical, and neuro-hormonal stimuli, the central nervous system is in constant communication with the enteric nervous system (Konturek et al., 2004), and this link helps distinguish the overall energy balance in the body.

Ghrelin, a 28 amino acid peptide, was recently discovered in 1999, as a growth hormone secretagogue receptor ligand (Kojima et al., 1999). Growth hormone (GH) was known to be regulated by two hormones, somatostatin and growth hormone releasing hormone (GHRH). It was recently shown to be stimulated by ghrelin and is involved with

the regulation of overall growth of tissues, and carbohydrate-protein-lipid metabolism. Ghrelin's potential involvement with GH demonstrated the importance of this hormone and presented a possibility that it may be involved in overall energy homeostasis. Ghrelin is produced primarily in the oxyntic glands of the stomach, but also is secreted from the intestines, kidneys, hypothalamus and pituitary gland.

One of the most exciting aspects about discovering ghrelin is that it offers a link between the gut where it is primarily produced and the brain where its receptors are located. Ghrelin was first studied in rats and humans and identified as one of the controls of energy homeostasis through the hypothalamus. Intracerebroventricular injections of ghrelin increased food intake and body weight gain in free-feeding rats, as well as dwarf rats, a growth-hormone deficient model; therefore, suggesting that ghrelin's action on food intake is independent of GH stimulation (Wren et al., 2000; Nakazato et al., 2001).

Ghrelin and eating behavior

Body composition rather than body weight may influence plasma ghrelin levels (Beck et al., 2004); therefore, it would seem reasonable to study plasma leptin levels, which are indicative of the body's energy status at the same time as measuring ghrelin levels. In addition studies should take various measurements of body composition to see if the changes in plasma ghrelin are due to that or other factors.

Cummings et al. (2001) decided to determine physiological ghrelin levels in humans given meals three times a day to see if there were patterns in secretion throughout the day. By taking blood samples every 30 min, they noted that plasma ghrelin levels increased on average 78% before each meal and fell to a nadir approximately one hour postprandially. There were large standard errors associated with each time point, which suggested considerable variation among individuals within a 24-h period. Since the meals were offered at specific times each day, it would be difficult to assess whether or not the rise in ghrelin was due to feeding behavior or an anticipation of a meal.

In an experiment where sheep were offered feed once daily in a nylon bag, thus prohibiting access to the feed, there was a tendency for an increase in plasma ghrelin levels at the onset of feeding. This was followed by a temporary decline and then an even greater increase that remained high for the remainder of the pseudo-feeding period (Sugino et al., 2002). Therefore, the increase in ghrelin level was a result of anticipation of the meal.

However, fasting rats delivered a 50% dextrose solution had decreased serum ghrelin levels compared to rats that received water as a control (Tschop et al., 2000; Shiiya et al., 2002). This suggests that the actual ingestion of nutrients, not psychological factors, were affecting ghrelin serum levels.

Ghrelin and physiological status

Another important implication for ghrelin is that levels may vary according to physiological state. Obese rats at two months of age have decreased concentrations of ghrelin relative to normal rats; however, the effect is diminished by the time they reach 6 months of age (Beck et al., 2004). Similar observations were documented in anorexic humans (negative energy balance) having higher ghrelin levels, compared to obese patients (positive energy balance) having lower circulating levels (Shiiya, et al., 2002). This implies that ghrelin levels may change during various growth stages of life, and perhaps may be altered during other physiological states such as pregnancy or lactation. Three month old Holstein calves had lower plasma ghrelin concentrations than mature cows and did not have fluctuations in secretion surrounding feeding times (Miura et al., 2004). This information is perplexing since a young ruminant's stomach is composed primarily of the abomasum, which is where ghrelin mRNA has been shown to exist in mature animals (Miura et al., 2004). Ghrelin mRNA expression in beef cattle digestive tracts is found primarily in the abomasum with some expression also found in the small intestine; however there is no mRNA found in any of the other stomach compartments (Wertz et al., 2004). This is similar to what has been shown in non-ruminant species (Kojima et al., 1999). The number of animals was limited in the Holstein study and due to the large variation in individual animals the results from this study should be reviewed with caution.

Tschop et al. (2000) concluded that the increase in weight gain due to exogenous ghrelin administration was a result of decreasing fat utilization by adipocytes in rats and mice. Dairy cows in early lactation are in negative energy balance, and nutritionists are interested in finding methods to decrease their fat utilization and avoid future related reproductive inefficiencies. Since circulating ghrelin levels fluctuate throughout the day, primarily around feeding times, it would seem conclusive that this hormone plays a role in stimulating food intake.

Ghrelin in ruminants

Presently, there is not much information on the appetite stimulating effects of ghrelin in ruminants. Blood samples were collected every ten minutes for three hours from sheep fed once per day. They had a mean ghrelin concentration that increased over time from 1.7 ng/mL, one hour prior to the meal, up to 4.7 ng/mL at the onset of the meal (Sugino et al., 2002). These levels dropped considerably one hour postprandially to 0.8 ng/mL. Throughout the night ghrelin levels gradually increased with fasting and the next morning were similar to initial levels the previous day, 1.9 ng/mL. Finishing steers that were fasted for 48-h had more than five times greater ghrelin concentrations (649 pg/mL) than those fed ad libitum (115 pg/mL) for twelve hours of the day (Wertz et al., 2004). The fed steers had peak concentrations immediately prior to feeding with levels around 200pg/mL, whereas fasted steers' ghrelin levels remained elevated.

It was also demonstrated that the increase in ghrelin levels prior to the start of a meal influenced GH secretion, which increased approximately four-fold during feeding in both fed and pseudo-fed sheep (Sugino et al., 2002). Since then, others have shown that ghrelin is directly involved in stimulating GH release into the circulation by injecting ghrelin either into the artery of goats or hypothalamus of calves (Hashizume et al., 2005). However, another study using Holstein cows and calves noted that there was no correlation between ghrelin and GH plasma levels (Miura et al., 2004). GH is an important hormone to study in ruminant nutrition since it is involved in the metabolism of proteins, sugars, and fats, as well as having a role in muscle accretion which is necessary for the growth of animals.

To investigate further that ghrelin's ultradian rhythm is centered on feeding times, Sugino et al. (2004) fed mature sheep (51 kg) either two or four times per day or ad libitum. When fed two meals ghrelin levels peaked at 3.5 and 3.7 ng/mL from an initial 0.5 and 0.9 ng/mL one hour prior. There were no significant differences between peaks of each meal; however, the peak concentrations were decreased compared to the sheep fed once a day. When fed four times per day the peak ghrelin levels gradually decreased over the course of the day from 2.1 to 1.1 ng/mL, and the fourth peak was did not differ from baseline value, 0.4 ng/mL (Sugino et al., 2004). This suggests that the importance of ghrelin in stimulating feed intake decreases with an increase in number of meals. Sheep fed ad libitum did not have significantly different peak values, just minor fluctuations throughout the day.

Ghrelin affected by macronutrient intake

Several studies have looked at the difference in ghrelin plasma levels with rats fed predominantly carbohydrate diets compared to fat based diets (Beck et al., 2002, Sanchez et al., 2004). After 14 weeks, rats on high fat diets had the lowest plasma ghrelin concentration in their blood, averaging 30% lower than the control group fed a mixed diet (Beck et al., 2004). Rats consuming the high carbohydrate diet had 26% greater plasma ghrelin concentration than the control, and 81% greater concentration than those on the high fat diet.

When rats were given a choice between the high fat or high carbohydrate diets the overall plasma concentration of ghrelin was decreased, below 1000 pg/mL, whereas before the control diet had levels approximately 1800 pg/mL and the macronutrients induced changes well above and below that level (Beck et al., 2002). Plasma ghrelin levels were lowest in rats that had a strong preference for the high fat diet and greatest in rats that had a strong preference for the high carbohydrate diet. The rats that chose a more balanced combination had plasma ghrelin levels in between the two extremes (Beck et al., 2002). However, due to the stronger satiation effects that carbohydrates are supposed to induce compared to fat it would seem that the rats consuming the high carbohydrate diet would have lower ghrelin levels, thus decreasing the drive to eat.

When rats were fasted and subsequently re-fed, gastric ghrelin mRNA levels decreased on both carbohydrate and fat diets (Sanchez et al., 2004). However, 45 minutes following feeding the ghrelin mRNA levels remained low on the carbohydrate diet, but were recovered to fasting levels in those fed the fat diet. The reduction in ghrelin mRNA is thus more persistent when rats were fed the high carbohydrate diet.

In conclusion, there are probably many dietary control mechanisms occurring at the same time and some of them may work together to obtain the final end result. More research needs to be done in this area to see if ghrelin is more greatly affected by specific nutrient composition rather than energy intake as a whole.

Insulin

Insulin concentration has been negatively correlated with plasma ghrelin level in rats (Beck et al., 2002). Although insulin has been determined to be a strong regulator of glucose homeostasis in monogastric species (Rezek et al., 1978) its role in ruminant

metabolism remains less explicit. Ruminants rely primarily on gluconeogenesis, even in a fed state, to provide metabolizing tissues with glucose. Blood glucose, which has a lower total concentration in ruminants compared to monogastrics has been shown to decrease in goats the first hour post-feeding, and then subsequently rise until a plateau is reached (de Jong, 1981). This study used goats that greatly varied in body weight, ranging from 20 to 50 kg and authors did not use BW as a covariate in the statistical analysis. However, others have shown that blood glucose levels are not affected by food intake (as reviewed by Van Soest, 1994). If blood glucose is not regulated nearly as rigorous in ruminants, it is necessary to ask ourselves if insulin is an important regulator of feed intake.

In monogastric species insulin is critical for uptake of glucose into cells and its production is increased with a greater carbohydrate load. When feeding goats various combinations of hay and concentrate there were small increases in insulin concentration in the blood and remained elevated for hours (de Jong, 1982). There were no changes in concentration when the goats were fasted, thus the increase in insulin concentration was due to ingestion of nutrients. It has also been shown that tissues' responsiveness to insulin decreases with age in steers (Eisemann et al., 1997). The hypothalamus is involved with regulation of food intake and body weight through several mechanisms including gastric hormones and neuropeptides, as well as insulin and glucagon (de Jong, 1986). Therefore, insulin may play a role in regulation of feed intake or overall energy balance. Similar to leptin from adipose, insulin released from the pancreas also decreases the expression of NPY and AgRP, both neuro-peptides involved with appetite stimulation (as reviewed by Leibowitz and Wortley, 2004). In this manner it is thought to be a satiety signal.

Insulin may be increased for up to 2 hours postprandially (de Jong, 1982). This could be due to the production of volatile fatty acids (VFA) within the rumen from digestion of fibrous material. VFAs, such as acetate, propionate, n-butyrate, and 3-methylbutyrate increase rapidly in the systemic circulation within 10 to 20 minutes of feeding and to an even greater extent in the portal vein (de Jong, 1982). Even though goats do not absorb glucose directly from the diet, they do have insulin released, either in relation to feed intake, diet composition, or both. No changes in blood concentration were seen in fasted goats.

Feeding lactating dairy cows 33% of their maintenance requirements decreased their plasma leptin and insulin levels, which remained low after a 48-h fast (Block et al., 2003).

Based on these results, hyperinsulinemic-euglycemic clamps were placed in dairy cows in positive energy balance and an increase in plasma leptin level was observed. Insulin's role in controlling voluntary intake appears to be indirect through its regulation of leptin. Insulin has also been suggested to have a role in the decrease in ghrelin levels observed post-prandially.

Leptin

Leptin is a hormone involved with satiation after food is consumed. The acruate nucleus has specific neurogenic peptides: neuropeptide Y (NPY), and agouti-related protein (AGRP), which are responsive to leptin. Leptin is known to inhibit the synthesis as well as the release of NPY (Schwartz et al., 1996). In contrast, ghrelin increases the gene expression of NPY. Both anti-NPY IgG and anti-AGRP IgG administered 4 hours before ghrelin infusions, inhibited the increase in food intake that is normally seen with increased ghrelin concentrations (Nakazato et al., 2001). These results show the potential for an antagonistic relationship between ghrelin and leptin due to the fact that they might be mediated via the same pathway. In addition this suggests that ghrelin's main stimulatory effect on appetite may stem from its action in the acruate nucleus.

COMPOSITION OF FORAGES AM/PM

Carbohydrates are the main energy source in feeds, and can be supplied through pastures, hays, silages, and grains. If energy is limited it can negatively impact the digestion of other nutrients. The primary difference in morning cut versus afternoon cut forages is the accumulation of total non-structural carbohydrates (TNC) later in the day. The forages that have been exposed to the sun all day have maximal time for photosynthesis to occur, so they can trap the sun's energy to synthesize glucose and transport it as soluble sugars, including glucose, fructose, and galactose. Previous studies looked at the differences in TNC in cool season grasses, C_3, which tend to have greater overall concentrations (Fisher et al., 1999). Fresh alfalfa samples collected at 1400 versus 0830 had increased total sugars (6.09 vs. 5.15 g/kg DM), starch (3.58 vs. 1.43 g/kg DM), and the largest change was represented by the difference in sucrose (3.23 vs. 2.45 %) (Melvin, 1965). It is also important to document

whether this difference in harvest time can have a positive effect on forage quality and animal performance using a warm season grass, C_4.

C_4 compared to C_3

One of the positive attributes of plants with C_4 metabolsim is that they can tolerate higher temperatures, which makes them an important forage resource, especially in the southern US. These grasses usually have lower protein content and higher fiber content than C_3 grasses. Therefore, we will discuss if the increase in TNC during the day can help counteract some of the negative qualities associated with these forages.

Plants with C_4 metabolism usually have increased lignified tissues, which means slower rate of passage, decreased digestibility, and a potential decrease in voluntary intake compared to C_3 forages. In order to improve usage of these grasses it is necessary to improve their nutritive value. This could be accomplished by increasing the soluble portion of the plant thereby increasing digestibility. A positive characteristic of C_4 plants is that they are more efficient at fixing light into energy at a rate of 5 to 6%, whereas C_3 plants operate at a lower efficiency of less than 3%, become light saturated at 25 to 50% of full sunlight, and undergo photorespiration wasting up to 40% of the energy that was already captured by the plant (Ball et al., 1998). One of the most appealing features of the C_4 grasses is that they produce high forage yields, which provides more feed per hectare for animals.

CHANGES IN FORAGE COMPOSITION AM/PM

Due to photosynthesis, green plants accumulate carbohydrates during the day and decrease at night while the plant uses its energy reserves for metabolism. Although stems have the highest concentrations of soluble sugars, the TNC concentration in the leaf portion actually cycles diurnally. Photosynthesis takes place in the leaves and the carbohydrates formed are then translocated to various parts of the plant to be used for metabolism, growth, respiration or storage. Forages store these reserve carbohydrates in different locations such as roots, rhizomes, stolons, and stem bases depending on the species and their anatomy. In C_3 grasses the majority of energy is stored as fructans, whereas C_4 grasses store it as starch.

There are several factors that may affect the rate of photosynthesis in the plant such as nitrogen availability, moisture level, maturity, and temperature. When water stress first

ensues there is a decrease in the rate of photosynthesis followed by an accumulation of carbohydrates, minerals, as well as potentially toxic substances such as nitrates (Ball et al., 1998).

Table 1. Composition of TNC fractions (% DM) found in a variety of forages from the studies of Fisher et al., 2002, 2005; Mayland et al. 2003b, ; Melvin, 1965; Orr et al., 1997; Tava et al., 1995

	Carbohydrate Fraction	Time of Harvest	
Item		PM	AM
Tall fescue grass	WSC[a]	13.30	10.80
Tall fescue grass[e]	Soluble sugars	9.30 14.60	5.80 15.00
Ryegrass	WSC[a] Starch	18.30 4.10	15.60 3.00
White clover	WSC[a] Starch	7.30 8.70	5.40 3.60
Fresh alfalfa	Total sugars[b] Starch Sucrose	6.09 3.58 3.23	5.15 1.43 2.45
Alfalfa hay[f]	TNC[c] Monosaccharides Disaccharides SCPS[d] Starch	5.62 1.45 2.26 1.00 0.93	4.61 1.04 0.84 0.09 0.80
Switchgrass hay[g]	TNC[c] Soluble sugars Starch	7.80 5.30 2.50	7.1 4.3 2.8

[a]WSC = water soluble carbohydrates.
[b]Total sugars = sum of sucrose, fructose, and glucose. Glucose and fructose were similar and sucrose contributed greatest increase in total sugars for the PM forage, which is why it is represented here.
[c]TNC = total non-structural carbohydrates.
[d]SCPS = short chain polysaccharides.
[e]Leaves and stems
[f]fed to sheep
[g]fed to cattle

Tall fescue, a C_3 plant, stores its reserve carbohydrates in the stem bases. Tall fescue that was clipped following light exposure had greater leaf:stem ratios than those exposed to darkness (Mayland et al., 2003b). Despite the stems having a greater concentration of soluble sugars 150g/kg DM, the only changes in carbohydrate level occurred in the leaf from 58g/kg DM following a dark period to 93 g/kg DM after exposure to light (Mayland et al., 2003b). Gamagrass, a C_4 perennial, also stores its carbohydrates in the stem bases; therefore, the same diurnal cycling may be exhibited only in the leaves. This diurnal cycling of soluble sugars that occurs in the plant has been documented for many years (Youngberg et al., 1972).

Perennial ryegrass harvested in the afternoon contained an increase in DM and water soluble carbohydrate concentration without affecting digestibility or NDF concentration (Orr et al., 2001). When grazing sheep consumed afternoon harvested ryegrass and clover with increased water soluble carbohydrate (WSC) levels they had increased OM digestibility (Orr et al., 1997). Both monocultures of ryegrass and white clover showed increases in WSC and starch over the day.

Afternoon harvested tall fescue and alfalfa hay had increased TNC, monosaccharide, disaccharide, and short-chain polysaccharide concentrations (Fisher et al., 1999; Fisher, 2002); however, starch content was only increased in alfalfa. The results for starch have been variable thus far. This could be explained by the fact that C_3 grasses typically accumulate fructosans rather than starch as a storage carbohydrate. Sucrose concentrations follow a similar diurnal pattern of cycling with PM alfalfa/crested wheatgrass hays containing 42% sucrose, compared to AM hays with 36% sucrose (MacKay et al., 2003). Additionally, lettuce was documented to have increased TNC, disaccharides, starch and fructose concentrations when cut in the afternoon, although there were no differences in the monosaccharide concentration (Mayland et al., 2002).

In experiments using goats, sheep and cattle there were decreases in NDF, ADF, and cellulose in the tall fescue experiments, whereas the results with alfalfa were more variable (Fisher et al., 1999; Fisher, 2002). The decrease in fiber fractions in the PM hays could be a result of dilution with TNC. In the experiment with alfalfa that was fed to sheep there was a decrease in all fiber fractions, yet when fed to goats and steers there were only significant

decreases in the hemicellulose portion of the plant. High levels of fiber are often associated with decreases in DMI or digestibility. Therefore, the PM harvested forages have the potential to have decreased fiber and increased soluble sugar content, hence, increasing rate of passage, and decreasing rumen fill in order for the animal to consume more feed and improve digestibility of the forage.

The results for fluctuations in N concentration during the day have been variable. Nitrogen levels in monocultures of ryegrass and clover remained constant over a 12-h time period throughout the day (Orr et al., 1997) similar to alfalfa hays in which CP levels did not change. Despite the fact that the tall fescue had high CP levels (20% CP), there was still an increase in CP concentration for the PM harvested forage. Others have seen a decrease in the nitrogen concentration of the PM forages, which has been attributed to their dilution during the day by accumulating non-structural carbohydrates (Youngberg et al., 1972).

PREFERENCE FOR PM FORAGES

A producer's main goal in raising livestock is to improve animal performance with the least cost method, and harvesting forages in the afternoon would be an ideal way. The soluble sugars are the portion of the plant that exhibit diurnal cycling, increasing in concentration over the day and decreasing throughout the night.

Grazing Behavior

The preference for afternoon harvested forages is in accordance with natural behaviors that have been documented for grazing animals. Cows consume the majority of their daily intake after afternoon milking up until sunset (Rook et al., 1994). This afternoon preference has also been documented in sheep studies, where 25 to 48% of their total daily grazing time occurs during the four hours just previous to the sun setting (Penning et al., 1991). In a study using Holstein-Friesian cows that were given a new daily grass allowance in a strip grazing system either after morning or afternoon milking, it was found that the animals exhibited different grazing patterns (Orr et al., 2001). There were no differences in total daily DMI based on when they were allotted new pasture to graze (18 and 17.8 kg DM). However, the cows that were given a fresh grazing opportunity in the afternoon consumed approximately 8 times more feed during their evening meals. The cows that were

given a fresh grazing opportunity in the morning only consumed about 2 times more feed for their morning meal, despite the novelty of new pasture being offered. They spent the majority of time grazing in the afternoon. The AM animals spent more time ruminating (454 versus 433 min/24h) and approximately equal time spent consuming forage for both meals. The PM cows spent about 3 times the length of time eating during the afternoon session when compared to the morning (Orr et al., 2001). Therefore, when animals are fed in a strip-grazing management system, producers should move fencing after afternoon milking instead of morning milking to give the animals' maximum exposure to higher sugar concentrations and the potential to consume more feed and hopefully produce more milk. This supports what was previously documented, that intake rates of sheep grazing ryegrass and clover were increased in the evening (Orr et al., 1997).

Rotationally stocked sward depletion conditions were simulated to test if the "timing of herbage allocation in pastures may impact the daily balance of sward photosynthetic gain and respiratory loss and therefore energy intake by livestock" (Griggs et al., 2003). In the control samples, differences in TNC level for AM and PM forages did exist, and differences were greater in the top half of the canopy, (40-27 cm) compared to the lower half (12-18 cm). This supports previous literature that states most of the differences occur in the leaf portion and not the stem (Mayland et al., 2003b). However, following sward depletion in the morning and at night, to symbolize allocation of pasture to animals grazing in a rotationally stocked orchardgrass pasture following 24-h sward depletion, there was no evidence of an increase in TNC concentration in PM versus AM grasses (Griggs et al., 2003). This suggests that under these conditions there is no advantage to PM grazing. This might be explained by the fact that the larger differences in TNC are seen at the higher portion of the canopy, and thus after sward depletion those differences are vastly reduced.

Preference measured as DMI

Scientists started out trying to characterize the compositional differences between forages based on what time of day they were grazed by animals, harvested for hay, or ensiled. Next they looked at whether or not these changes in composition could be detected by the animals themselves, as a potential factor for improving voluntary intake. Preference for the afternoon harvested forages has been documented using cattle, sheep, horses, goats, and rabbits. In order to document short-term preference, forages were offered side by side,

giving animals a choice and allowing them to use their natural instincts to make a decision. In a series of studies they allotted 2.5 h to sheep and goats, and 30 min to steers to measure DMI (g/meal) and intake rates to measure short-term dietary choices. The tall fescue (Fisher et al., 1999) and alfalfa (Fisher et al., 2002) hays harvested in the afternoon versus the morning increased DMI for all three species and greater initial intake rates for sheep and goats, but not for steers. Multidimensional scaling revealed negative correlations of NDF, ADF, cellulose, and acid-detergent lignin concentration with voluntary intake. Intake was positively correlated with disaccharide, fructan, and TNC concentration, as well as in-vitro true DM digestibility. There was no correlation with CP, starch, or acid-insoluble ash concentration for all three species (Fisher et al., 1999). In both experiments steers had the largest increase in DMI compared to sheep and goats.

Due to the improvements in DMI of short-term meals using C_3 grasses and legumes (Fisher et al., 1999; 2002), another study was performed to see if this diurnal cycling of soluble sugars was also present in plants with C_4 metabolism. This time they used switchgrass hay harvested in three different years at two different times of day, morning and afternoon, to determine preference among sheep, cattle, and goats (Fisher et al., 2005). In 1997 hays, goats showed a preference for the PM hays, whereas sheep and cattle were not able to detect the subtle differences. This may be due to the decrease in overall concentration of TNC in warm-season when compared to cool-season plants, which on average, showed only a difference of 7g/kg DM. However, due to the lower concentration in C_4 grasses, that 7 g/kg difference still translates into a 10 percent difference between AM and PM hays. The other factor may be that there was not an overall decrease in the fiber fractions of the PM hays, whereas in past studies this may have played an important role in the increase in intake. Interestingly, hays harvested in 1998 had decreased fiber fractions, similar to previous reports (Fisher et al., 1999); however, an increase in DMI for PM hays was only observed in sheep. Using the 1998 hays they also looked at the effect of N-fertilizer, and found that all three species preferred the high fertilization levels, 150 kg N/ha over the lower one, 75 kg N/ha. This higher fertilization level contained lower soluble TNC, as well as lower fiber fractions (Fisher et al., 2005). Therefore, changes in intake may be more closely related to changes in the structural part of the plant versus the non-structural portion.

Non-ruminants also select a diet predominantly composed of PM forage when PM and AM forages were offered in pairs. Horses consumed 630 g of a PM mixture of alfalfa and crested wheatgrass compared to 310 g of the AM forage (MacKay et al., 2003). However, the test hay was only offered for 10 minutes twice a day, so this would be an example of very short-term preference. Rabbits had greater short-term preference for PM versus AM lettuce when they were given a choice. Conversely, when PM and AM were not offered in pairs there was no difference in preference (Mayland et al., 2003a).

Application to production situations

Nowadays farmers are receiving decreased profit margins from their animal products, thus they have increased the production expectations from individual animals, which has resulted in changes in feeding management. Supplementation of non-structural carbohydrates has increased dramatically over the years to ensure that energy is not limiting the digestion of other macronutrients, specifically protein which is expensive. Feed producers market grain as an animal product, and the greater supplementation levels have resulted in increased production costs for the farmer. Harvesting in the afternoon enables the plants to accumulate soluble sugars during the day, which provides additional energy to the animal from the plant itself rather than from a costly supplement. This is not to suggest that these forages can replace the use of supplements, merely just decrease their use. More recently, scientists have been trying to equate this increase in soluble sugars with an improvement in animal performance. Milk yields tended to be higher for animals grazing in the afternoon, but the number of cows was too low for the effects to be significant (Orr et al., 2001). The potential of higher milk yields demonstrates yet another area of improvement in animal performance from making this simple change in forage management.

Adoption of this new management strategy

A large portion of this AM/PM research has been done in western states where the weather is vastly different from the humid southeast. Drying time does affect the extent to which these sugars are captured, since sugars continue to break down through respiration once forage is cut. Therefore, the cost of extra drying time needs to be weighed against the increase in quality associated with these forages. Harvesting in the afternoon is a cost effective method that the agronomist can utilize to improve animal performance since

energy is often the factor limiting digestion and absorption of nutrients in the rumen. It was estimated that an increase in $15 per ton of forage would be expected.

SILAGE COMPOSITION

Nitrogen Levels

The amount of non-protein N (NPN), dietary form of carbohydrate, and source and level of dietary N present may affect microbial utilization of ensiled forage N (Dewhurst et. al, 2000). Silages usually contain a large portion of total N as NPN and structural carbohydrates with slow fermentation rates. When feeding silage, these large fractions of soluble protein are not matched by an ample supply of soluble carbohydrates in the rumen, which could lead to absorption of ammonia. Ensiled forages fed without supplementation are often associated with decreased microbial protein synthesis compared to those fed with supplements. Gamagrass, a C_4 perennial, has a large proportion of the N associated with the neutral detergent insoluble fraction, and thus may decrease the amount of NPN available after fermentation (Van Soest, 1994). In a study feeding mixed cultures of microorganisms, either gamagrass silage or hay, it was found that there were no differences in the rumen of molar proportions of VFA, NH_3-N, nor NDF digestibilities between the different harvesting methods (Eun et al., 2004). Therefore, gamagrass silage supported ruminal fermentation to the same extent as gamagrass hay.

Carbohydrate Levels

The diurnal differences in carbohydrate levels in forages, including fresh grass and dried hays, in the morning and evening has been documented. However, it has not been explored whether these differences would be maintained during the fermentation process of making silage. Silages play an important role when drying conditions become limited, but there are negative characteristics associated with ensiling. Mechanically, these include maintaining a completely anaerobic environment and storage of feed without it spoiling. From an animal production standpoint the efficiency of energy utilization in a fermented feed by rumen microbes is estimated to be 30-40% lower than would be expected from an unfermented feed (ARC, 1984). In a previous report, there were no differences in the initial pH of fresh silage when harvested in the morning compared to the afternoon, yet after

ensiling in miniature Polythene silos for 7 days the 1400 PM harvested forages had a lower pH (4.87) versus the 0830 AM pH (5.55). After ensiling there were only faint traces of sugars left in the plant material (Melvin, 1965). The lower pH in the afternoon harvested forage could be explained by the increase in soluble carbohydrates, and thus an increase in lactic acid formation which decreases the pH. By taking advantage of the plants natural photosynthetic capacity to accumulate TNC during the day, it may be possible to capture a greater amount of TNC during the ensiling process, which could improve the quality of the silage.

ENERGY AND PROTEIN SYNCHRONIZATION

Improving the synchronization of energy and nitrogen offered to the rumen may have positive benefits for animal performance, such as increase in growth rates, milk production, and the like. Previous research has shown benefits for feeding sources of carbohydrate and protein that have similar degradation rates, in order to provide ample energy to the microbes to maximize microbial protein synthesis. In order to improve the capture of DIP, the supply of nitrogen and energy should be synchronized in delivery to the microbes (Johnson et al., 2001). This ensures that ammonia accumulated in the rumen will be utilized by the microbes to synthesize microbial protein, thereby minimizing the total amount of ammonia absorbed across the rumen wall. Decreasing ammonia absorption is also beneficial to the animal since it can be toxic at high levels, and decreasing the amount absorbed reduces the amount of energy that would otherwise be expended by the liver in converting ammonia to urea. Leng (1967) determined that when high NPN levels were administered into the rumen, the microbes did not have ample time to utilize all of the ammonia generated, and the excess ammonia was absorbed into the blood. This resulted in an increase in plasma urea and urinary N excretion. However, providing enough starch to complement the level of protein entering the rumen has been shown to lower the absorption of ammonia and increase nitrogen retention in steers (Taniguchi et al., 1995).

Using lactating dairy cows, Kolver et al. (1998) formulated diets to match the TNC degradation rate of supplemental corn with the nitrogen degradation rate of pasture. These feeds were supplied either together for a synchronous delivery of nutrients or

asynchronously by offering corn four hours after pasture. They did not see any improvement in nitrogen retained in synchronously fed cows, which could potentially be used for milk production or growth. However, they did observe decreases in rumen ammonia levels with cows on the synchronous diet at 3 (19.9 vs. 25.5mg/dl) and 5 h post-feeding (13.5 vs. 23.6mg/dl). In this study there was an increase in the capture of ruminal N, although the protein status of the cow, exemplified by growth and milk characteristics was unchanged (Kolver et al., 1998). Microbial utilization of N in the forage was increased since there was an ample energy supply in the rumen. The protein status of the cow did not change because the N may have been excreted in the urine or the amount of N in the diet was not meeting their requirement.

Supplementation of sucrose to a silage-based diet has resulted in decreases in rumen ammonia, with the largest drop observed when sucrose was infused continuously (Khalili and Huhtanen, 1991a). Microbial protein reaching the small intestine was increased however there was no significant improvement in efficiency of synthesis due to the increase in fecal N output. Supplying sucrose twice a day versus continuous infusion did not result in further benefits of silage N utilization, even though rates of synchronization were more closely matched under this feeding regimen.

Adding molasses, a readily fermentable carbohydrate source, and canola, as a protein source, in combination to a silage based diet did not produce any positive effects. Supplementing molasses alone produced decreases in rumen ammonia levels, but they were attributed to a slight decrease in CP digestibility of the silage rather than an increase in microbial protein synthesis (Petit et al., 1994). The molasses did not affect nitrogen retention nor plasma urea concentration. In another study, plasma urea was decreased 2 h after ammonia levels peaked in cows fed a diet where energy and protein were released synchronously into the rumen (Kolver et al., 1998). This complements the idea that synchronizing energy and protein leads to improved microbial utilization of nitrogen and decreased urea production.

The ruminant animal has exceptional capabilities for recycling nitrogen as urea from the liver back into the rumen when nitrogen becomes a limiting factor. The microbial population relies on this nitrogen for continuous cell growth. According to Kennedy and Milligan (1980), recycling of urea back into the rumen is positively correlated with the

soluble carbohydrate concentration present in the rumen. Infusing carbohydrate continuously into the rumen increases microbial N outflow from the rumen and microbial growth efficiency by 14-17% compared to pulse dosing, which delivers a load of carbohydrate all at once to the microbes (Henning et al., 1993). Similarly, pulse dosing nitrogen into the rumen resulted in a sharp increase in rumen NH_3-N levels and urinary N excretion, as well as decreased N retention and microbial N outflow. Synchronizing the energy and protein from either the pulse dose or continuous infusion treatments did not increase microbial yield. The extensive recycling of nitrogen may explain why there were no benefits seen with synchronous diets.

Unless producers are feeding high producing animals with large energy and nitrogen requirements, the amount of nitrogen recycling in the body may be sufficient to support maximal microbial growth and thus synchronization won't provide any further improvements. In a situation where the animals have large metabolic demands placed on them, such as growth and milk production, the recycled N may not support maximal microbial growth when the energy levels in the rumen are high. Dairy cows fed a synchronized diet that combined high amounts of DIP and rumen available TNC provided the greatest outflow of bacterial N; whereas the lowest outflow was seen when high TNC was combined with the lowest CP diet (Aldrich et al., 1993). On the low CP diet, there was ample energy for growth, but not enough nitrogen to maximize microbial protein synthesis. In another study mid-lactation dairy cows were fed a UIP source along with a rumen degradable NSC source and observed minimal benefits when they were synchronized (Casper et al., 1999).

With all of this discussion concerning microbial protein utilization, one must not forget that carbohydrates provide the energy necessary for microbial digestion of protein and thus microbial growth. The form that the energy is offered in the diet affects the amount of protein utilized (Oldham, 1984). Barley was combined with cottonseed meal and fed to mid-lactation Holstein cows to simulate a rapidly fermented synchronous supply of nutrients, and there was an increase in microbial yield compared to other treatments (Herrera-Saldana et al., 1990). However, it is difficult to draw any solid conclusion from this study since they changed dietary ingredients as well as concentrations and degradabilities of nutrients among treatments. Due to the differences in available nutrients

and the fact that it was only a 4-d collection period the results may be confounded by other factors besides synchronization rates.

Contradicting many of the results just presented, Valkeners and colleagues determined in Belgian Blue bulls that as long as the nutrient supply offered is balanced over 48 h then there are no further benefits to synchronization of carbohydrate and protein in the rumen (Valkeners et al., 2004). The nitrogen recycling is so extensive that it almost self-synchronizes the energy and protein going into the rumen. Unlike Belgian Blue bulls, asynchrony can be detrimental in growing lambs. Lambs fed an asynchronous diet retained less energy and exhibited lower energy efficiency compared to intermediate or synchronized fed lambs (Richardson et. al, 2003).

SUMMARY

A shorter time period between feeding and post-ingestive feedback will result in a stronger preference, according to Forbes and Provenza (2000). This is a potential reason why animals prefer the PM harvested forages over the AM. The accumulation of the rapidly fermented non-structural carbohydrates may provide more rapid feedback to the animal. Supplementation of forages with grains is a common process with livestock, but leads to increased costs for the producer. Processing the supplements so that the nutrients are more available can increase the costs even further. The in fermentable carbohydrates in the afternoon harvested forage may provide benefits for animals while escaping the increase in cost. By harvesting forages through different methods such as ensiling, hay or fresh forage, we may be able to determine which method best conserves the fermentable carbohydrates and is directly translated into an increase in animal performance.

Although it is exciting to understand what causes short-term changes in feed intake in livestock, the more relevant questions deal with control in the long-term to determine if we can sustain improved intake levels. In the following study we attempted to address this question by determining whether various ingredients or macronutrients harvested in the PM compared to AM, will result in differing plasma concentrations of ghrelin, a hormone involved in intake regulation.

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The Effects of Total Non-Structural Carbohydrates and Nitrogen Balance on Voluntary Intake of Goats and Digestibility of Gamagrass Hay Harvested at Sunrise and Sunset

ABSTRACT

The objective was to evaluate the differences in TNC composition of Iuka gamagrass (*Tripsacum dactyloides L.*) harvested at 0530 (AM) or 1730 (PM), and to measure how TNC concentration and CP supplement affect the voluntary intake, digestibility, and N balance of goats fed gamagrass when field-cured and stored in square bales. Boer X Spanish wethers (24 ± 3 kg) were randomly assigned to supplement (31% CP, fed at 11% of DMI, 14 goats) or no supplement (14 goats) treatments. Within supplement or no supplement, goats were randomly assigned to a crossover design of AM (7 goats) or PM (7 goats) harvested hays. Goats were individually housed in metabolism crates with free access to H_2O and mineral blocks. They were fed twice daily, with supplement offered once a day 30 min prior to morning feedings. After a 7-d adaptation, voluntary intake (goats were fed 110% of previous day's intake) was measured for 14 d, followed by a 4-d adjustment period (to equalize DM offered between periods) and a 5-d balance trial to measure DM digestibility (DMD) and N retention. After Period 1 the goats were switched to their new diets, and the protocol was repeated. The data presented here are main effects and unless stated otherwise, means differ at $P < 0.03$. Compared to AM, the PM harvest had greater TNC (70.8 vs. 59.0 g/kg DM), monosaccharide (37.0 vs. 28.6 g/kg DM), and di- and polysaccharide (18.5 vs. 15.4 g/kg DM) concentration. The DMD was greater for PM vs. AM (55.7 vs. 53.1%) and for supplement vs. no supplement (56.6 vs. 52.2%). Crude protein (79g/kg DM) and starch (15.2g/kg DM) concentrations were similar ($P < 0.88$) for PM and AM. Compared to no supplement, supplement increased total (hay + supplement) DDMI (DMI multiplied by DMD from the balance trial) during the voluntary intake phase (339 vs. 301g/d) and balance trial (334 vs. 288g/d). However, gamagrass DMI was not affected ($P > 0.10$) by supplement during the voluntary intake phase (530 vs. 576g/d) or balance trial (522 vs. 551g/d). Voluntary gamagrass DMI (552 vs. 554g/d, $P < 0.85$) and voluntary total DDMI (327 vs. 313g/d, $P < 0.14$) were similar for PM and AM; however, total DDMI during the balance trial was greater for PM vs. AM (322 vs. 299g/d). In spite of similar N concentration in the feeds, PM increased ($P < 0.04$) N intake (9.0 vs. 8.5g/d), N digested as a percent of intake (57.1 vs. 54.8%), and N retention (1.9 vs. 1.6g/d). By design, N intake was higher for supplement (10.3g/d) than no supplement (7.2g/d). Supplementation increased outputs of

fecal N (4.06 vs. 3.51g/d), urinary N (3.93 vs. 2.55g/d), urine urea N (2.67 vs. 1.32g/d), urea N as a percent of total urinary N (67.84 vs. 51.57%), and plasma urea N (13.2 vs. 8.6mM). Compared to no supplement, supplement improved N digestion (6.2 vs. 3.7g/d) and retention (2.3 vs. 1.2g/d). We conclude that PM gamagrass had a greater DMD and DDMI than AM gamagrass due to increased TNC and not due to differences in intake by the goats. Supplementation had small effects on DMI and DMD of gamagrass. Supplementation and PM fed together was designed to provide a more synchronous nutrient flow to the rumen and thus increase N retention, however, we concluded that adding a supplement to the diet improved N retention, but no further benefits were noted.

Key Words: Total non-structural carbohydrates (TNC), Voluntary Intake, N Metabolism, Goats, *Tripsacum dactyloides*

INTRODUCTION

The nutritive value of feeds is conventionally classified into three general areas: digestibility, feed consumption, and energy efficiency (Raymond, 1969). It is desirable to optimize these three components for animals to perform at their most efficient production levels. However, the difficulty is that providing proper nutrition to the ruminant means providing appropriate nutrition to the microbial population residing in the rumen. The efficiency of microbial protein production may be improved by balancing the overall daily ratio of ruminally available energy and N in the diet (Henning et al, 1993). Improving the synchronization of available carbohydrate and nitrogen in the rumen will promote the capture of forage N as microbial N.

There has been an increased interest in the differences of the TNC portion of plants harvested in the PM compared to the AM. The TNC, which include starch and soluble sugars, are energy sources that are readily fermentable in the rumen and are preferred by cattle, sheep and goats (Orr et al., 1997; Mayland et al, 2000). Cattle, sheep, and goats preferred afternoon harvested tall fescue (Fisher et al., 1999) and alfalfa (Fisher et al., 2002) when pairs of AM and PM hays were offered side by side. In these short-term studies, preference was measured as an increase in DMI over several meals. Based on the results from the experiments listed above and others (Buntinx et al., 1997), the increased preference for forages harvested or grazed in the afternoon was attributed to the TNC fraction, which is readily available to the animal and higher in concentration in the afternoon.

Forages with an increased amount of TNC have greater apparent digestibility, voluntary intake, and may enhance energy efficiency as well. Subtle changes in the non-structural components of plant tissue, such as TNC, can have a profound effect on the microbial processes of both attachment and penetration (McAllister et al, 1994). This could result in an increase in the efficiency at which ruminants digest a particular feed. Whenever energy and nitrogen are not provided in an appropriate balance, the feed may not be utilized by the microbes to its full potential; therefore, the feed is not being utilized by the animal to its full potential.

In studies using cattle, sheep and goats fed tall fescue and alfalfa hays the preference for PM harvests compared to AM harvests were consistently associated with the increase in

TNC, and I also believe the same conclusions hold true for warm season grasses (C_4). The objective of this study was to determine the effect of time of harvest on TNC in gamagrass and to evaluate whether these differences can improve intake and digestibility when fed to goats. The second objective was to determine the effects of supplementation on N balance in goats fed PM compared to AM harvested hays.

MATERIALS AND METHODS

Animal and Experimental Procedures

The experimental design was a crossover with a 2 X 2 factorial arrangement of treatments. There were four experimental treatments derived from a field of Iuka gamagrass (*Tripsacum dactyloides (L.) L.*). Before the start of the experiment a 0.81 hectare field of gamagrass was fertilized with N at two different levels, 50 or 100 kg per hectare. Preliminary analysis of hay from the field on August, 2003 showed that there was no difference in CP concentration between the two fertilization levels; therefore hays from the two fertilization levels were combined within AM and PM harvests. A protein supplement was developed and consisted of soybean hulls, soybean meal, corn-gluten meal, corn, and urea. It was formulated to mimic the protein fractions previously measured in gamagrass hay (Archibeque et al., 2001). This was achieved by combining the A and B_1 fractions as well as the B_2 and B_3 fractions (Table 1). The four final treatments were PM hay + supplement (**PM/S**), PM hay + no supplement (**PM/NS**), AM hay + supplement (**AM/S**), and AM hay + no supplement (**AM/NS**).

Since there were no apparent differences between N fertilization levels, each half of the field was randomly divided into AM and PM sections based on the time of harvest in a split-split plot arrangement. For two weeks in July, 2003, forage samples were collected in a random manner at 1400, and 1800, and the following morning at 0600 and 1000 from all sections of the field. Whole canopy samples were taken and subsequently hand-separated into leaf blade, stem and sheath, and dead/other tissue to provide qualitative data on its chemical composition and to document any changes over time. Electric clippers were used to cut a 6 in diameter plot to a 3 in stubble. Samples were collected and placed in a freezer (-20°C) about 1.5 h after sampling.

On August 24, 2003, the PM section of the gamagrass field was cut with a mower conditioner at 1800. The ambient temperature was 80° F and the sky was mostly sunny with few clouds. The following morning the AM section was cut at 0650. The ambient temperature was 65° F with no rainfall during harvesting. Samples were taken in front of the mower and handled in the same manner as described above. The fields were tedded daily and allowed to dry for 3 d. All treatments were wind rowed with a side delivery rake and baled with a conventional square baler on the afternoon on August 27. The ambient temperature was 95° F and the sky was sunny. On this day, whole canopy samples of dried hay were collected directly in front of the baler. The hay was stored in the experimental hay barn at the Metabolism Educational Unit at North Carolina State University, in Raleigh NC.

Initially thirty-six Boer-cross wethers from the Small Ruminant Educational Unit at North Carolina State University were selected for the experiment. The goats were treated to eliminate internal parasites with Cydectin (Fort Dodge Laboratory, Kansas City, MO) and transferred to the Metabolism Educational Unit to be acclimated to a diet of gamagrass hay. The experiment began in October, 2003, and twenty-eight goats (24 ± 3 kg) were selected for the study based on similar BW and soundness. Eight replacement goats were kept throughout the first 3 wk of the experiment. Handling, sampling, and care of the goats were approved by the North Carolina State University Animal Care and Use Committee (IACUC # 01-029-A).

On the first day in the barn, goats were weighed and fecal samples were collected from random goats to check for parasite load. Blood samples were collected via jugular venipuncture. Within one hour blood samples were centrifuged and the plasma was transferred into storage tubes and stored in a freezer (< -4°C) for later analysis in the lab. Goats were housed in two separate rooms, each with no windows and similar hours of artificial lighting and similar temperatures. There were two types of crates (wooden and metal) and treatments were balanced across crate type. There were an unequal number of goats in rooms 1 and 2, but treatments were balanced across rooms. In the metal crates the goats wore collars around their necks and were chained to the crate. In the wooden crates the goats had their heads restrained in stanchions. Electric lighting provided 14 h of light and 10 h of darkness in both rooms. Goats had ad libitum access to a trace-mineralized salt block and water. Fecal egg counts, blood packed cell volumes, and rectal temperatures were

recorded for each goat during the experiment to check for parasite resistance and overall health status. All values were within the acceptable range. During acclimation to gamagrass hay all 36 goats received 39 kg of hay daily, which averaged about 1.1 kg per head. Therefore, diets were formulated so that if goats consumed at least 800 g of DM/d, gamagrass alone would meet their daily protein requirement for maintenance and 100g of weight gain (NRC, 1981).

Periods lasted 30 d, with a 7-d adaptation to crates, a 14-d ad libitum intake phase, a 4-d adjustment to a restricted feed level and fecal harnesses, followed by a 5-d balance trial. The crossover was between the AM and PM hays, so a given goat switched between AM and PM harvest, but did not switch between S and NS. Goats remained in metabolism crates throughout period 1, and were then allotted a 9-d period of exercise in individual pens (21.5in x 36.5in) until the start of Period two.

Hay was offered in two equal portions at 0800 and 1530, and the supplement was fed 30 min prior to the 0800 feeding. The supplement was always consumed by the time hay was fed. Prior to feeding, hay was pressed in a Van Dale Bale Processor, Model S600 (J-Star Industries, Ft. Atkinson, WI) with knives spaced 12.5 cm apart. Each goat was initially fed 748g/d of hay and subsequently offered at 110% of the previous day's intake during the ad libitum period. Supplement was offered at 11% of forage intake, and thus fluctuated from day to day following hay intake. During the ad libitum phase, hay and supplement grab samples were collected daily and composited weekly. Orts were weighed once daily in the morning, sampled daily and composited weekly. During the first adaptation period 3 goats refused to eat more than 1% of their BW, so they were replaced with 3 of the replacement goats.

For the 5-d balance trial the amounts offered were the same in both periods to minimize any effects intake would have on digestibility. Goats were fed their individual average intakes over the 14-d ad libitum phase plus an extra 5% if they would be crossing over from PM to AM treatments in Period 2. They were expected to eat less of AM than PM during the ad libitum phase based on previous research. If they were crossing over from an AM treatment to PM, an extra 10% was added to the allotted feed amount since they were expected to eat more in Period 2. Prior to collections for the balance trial all crates were thoroughly scrubbed and washed. Crates were placed at intervals in the room to

prevent any cross-contamination between feces or feed. Over the 5 d there was a total collection of orts, feces and urine, as well as daily grab samples of the hay offered and supplement fed. Feces were collected daily into fecal buckets and a 5 % aliquot of the total wet weight was saved and dried in a forced air oven at 60°C each day. At the end of 5 d they were dried further for 48 h. Orts and hay samples were dried in the same manner, except that hot weights were obtained over 48 h and then they were allowed to air equilibrate for another 48 h before final weights were obtained. Urine was collected daily, brought up to a volume of 1000 mL, weighed and specific gravity was calculated. Urine pH was measured daily on samples before they were brought up to volume using pH-sensitive paper to assure proper acidification ($5 \leq pH \geq 6$). At first, the urine was collected in a bucket having 20 mL of 6N HCl acid and 100 mL of water. The first day goats' urine had a pH of 2, so the amount of HCl acid used was decreased to 5mL. 100 mL aliquots were collected daily and composited over the entire balance trial to obtain a representative sample. This was placed into a 30 mL plastic bottle and frozen (-4°C) until analyzed. Scurf was collected daily in plastic bags. Goats were weighed and bled at the beginning of the adjustment phase and end of the balance trial for both periods. Temperatures were taken daily for each goat during the balance trial. Goat # 3155 refused to eat and developed abnormal feces, so he was removed from the balance trial portion of the experiment.

Chemical Analysis

All pre-harvest samples were dried in a freeze drier (VirTis Model, Gardiner, NY). However, the majority of the samples were not weighed after drying; therefore, DM for these samples will not be reported. Hay, orts, supplement and feces were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 5mm screen. All of these samples were mixed and subsampled, ground through an Udy Mill (Udy Corporation, Fort Collins, CO) to pass through a 1 mm screen, and stored in plastic whirl pack bags at room temperature until analyzed. All samples were scanned in a near-infrared spectrophotometer, NIRS Model 5000 (FOSS NIRSystems Inc., Laurel, MD) and a global H statistic of ≤ 0.3 was used to define the spectral boundaries of the population. A neighborhood H statistic of ≤ 0.6 was used to identify samples with different spectra, and these samples were selected as calibrators and analyzed in the laboratory to develop prediction equations. Descriptive values corresponding to the NIR analysis will appear in appendix A. These include the

number of samples used in each calibration, the range, SEC, SEC/RSQ, SECV, and SECV/1-VR for each variable measured.

Pre-harvest Forage

All pre-harvest forage samples were scanned (n=80) and used as the initial base population. Then, 48 were selected as having different spectra, analyzed in the laboratory, and used for calibration to create NIR prediction equations. TNC and its components, starch, monosaccharides, and di- plus polysaccharides were analyzed using the following method (see appendix B for detail). A 0.5 g sample was weighed into each of two separate 125 mL flasks and 15 mL of H_2O was added and boiled on a hot plate for 2-3 minutes. After cooling, each flask received 10 mL of buffer (pH = 4.45) made up of 0.2N acetic acid and 0.2N sodium acetate in a 3:2 ratio. In one flask the sample was hydrolyzed using 10 ml of the enzyme amyloglucosidase (EC – 3.2.1.3, Rhizopus mold, Sigma-Aldrich, St. Louis, MO) while the other had no enzyme added and was thus left un-hydrolyzed. Both samples were incubated for 44 h at 38 to 40°C and occasionally swirled. On day 3, samples were filtered through filter paper into a 100 mL volumetric flask. The flask and paper were rinsed, and the volumetric was then brought to volume with deionized H_2O.

Enzyme blanks containing water, buffer, and enzyme were included in each analysis. Soluble starch and ground corn samples were used as standards in each assay to test the activity of the enzyme. All sample extracts were analyzed using the appropriate chemistry cartridge (Appendix B) in a Technicon Auto Analyzer (Technicon Industries Systems, Tarrytown, NY). The TNC were analyzed from the hydrolyzed extract using the total sugar cartridge according to Bran and Luebbe's colorimetric method G-227-99, Rev. 2. The extracts were hydrolyzed with HCl at 90°C to break down the di- and polysaccharides to reducing sugars. All reducing sugars were reacted with p-hydroxy benzoic acid hydrazide, which forms a yellow osazone in alkaline medium at 85°C. The absorbance was measured at 420 nm and the TNC concentration determined. The concentration of naturally occurring monosaccharides was determined from the un-hydrolyzed extract, using the same procedure as TNC, but without the HCl hydrolysis. Glucose concentration was analyzed from both extracts using the glucose cartridge according to Bran and Luebbe's colorimetric method G-142-95, Rev. 1. Starch concentration was calculated as [(mg glucose from hydrolyzed extract – mg glucose from the enzyme blank) – (mg glucose from the un-hydrolyzed

extract)] * 0.9 (0.9 is the factor to convert glucose to starch, AOAC, 1990). The di- and polysaccharides were calculated by subtracting starch and monosacchardies from the TNC.

DM was determined as outlined by the AOAC procedure (1990). All 80 samples were then predicted based on equations developed from lab values for DM, TNC, starch, monosaccharides, and free glucose. Di- and poly- saccharides were calculated by difference.

Hay and orts

All feed and ort samples (n=198) were scanned by NIRS and used as the initial base population. Nine samples were identified as outliers, and therefore, were dropped from the population (n=189). Of the 189 samples, 75 were selected for carbohydrate analysis. After chemical analysis, equations were developed and concentrations of DM, TNC, starch, monosaccharides, and free glucose were predicted for all 189 samples. Di- and poly-saccharides were calculated by difference. Actual laboratory values were used from chemical analysis for the nine outliers.

Nitrogen and fiber analysis was determined from the same 198 samples, spectral scans were compared against a previous gamagrass hay database (n=40) compiled by Leonard and Burns at North Carolina State University, Raleigh, NC. Only 3 samples from this trial were chosen as spectrally different; however, 12 additional samples were added. Therefore, of the total number of samples in this study (n=198), only 15 were selected to be analyzed in the laboratory, and used for calibration to create NIR prediction equations. Based on the equations developed, N, NDF, ADF, cellulose, ADL, and AIA were predicted for all 189 samples. In the laboratory, the fiber fractions were run sequentially according to the procedures outlined by Van Soest et al., (1991) in a batch processor (Ankom Technology Corp., Fairport, NY). They were determined in the order of NDF, ADF, cellulose, and ADL. AIA was calculated from ADL. Crude protein was estimated as 6.25 times the percentage of Kjeldahl N and determined using (AOAC, 1990) procedures. Protein fractions of the hay (n = 12) and supplement (n = 3) were determined in duplicate as described by Licitra et al. (1996). True protein was measured using trichloroacetic acid (TCA) as the precipitate and from this NPN, the A protein fraction, was calculated. Insoluble protein was determined through filtration using sodium azide as the reagent. True soluble protein, the B1 fraction was derived from the difference between true protein and

insoluble protein. The subsequent protein fractions, B2, B3, and C were determined using standard NDF and ADF procedures stated previously followed by the Kjeldahl N procedure (AOAC, 1990). Hydrogen peroxide was added to help break down the filter paper during digestion.

Feces

All 54 fecal samples were analyzed in the laboratory for DM, fiber components, ADL, AIA and Kjeldahl N. They were then added to a previous file of data from fecal samples from goats, sheep, and cattle fed gamagrass to provide a total of 122 samples. Based on the calibration equations constituents of all fecal samples were predicted.

Supplement

All of the supplement grab samples were analyzed by chemical procedures and were not scanned by NIRS due to the minimal number of samples. They were analyzed for DM, NDF, ADF, cellulose, ADL, AIA as well as Kjeldahl N (AOAC, 1990). When analyzing for NDF and ADF, amylase was added since corn was one of the ingredients (Van Soest et al., 1991). Protein fractions of the supplement were determined in the same manner as described above, except that amylase was added in the batch processor.

Urine and Blood

Urine and plasma were analyzed for urea N using the diacytyl monoxime method of Marsh et al. (1957) on a Technicon Auto Analyzer (Technicon Instruments Corporation, Tarrytown, NY). Urine was also analyzed using an auto analyzer (AOAC, 1990).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model accounted for supplement, harvest, supplement x harvest, period, period x supplement, and residual sources of variation. Goat within supplement was used as the random effect since supplement was not balanced across goats. Supplement was tested by the test term goat within supplement while the remaining variables were tested against the residual sum of squares. The BW was run as a covariate and remained in the model when $P < 0.20$ for BW. Period x supplement remained in the model when $P < 0.20$ for effect of the interaction. Statistical significance was determined at $P \leq 0.05$ and tendencies were identified at $0.05 \leq P \leq 0.15$.

Composition data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model accounted for supplement, harvest, harvest x supplement, period, period x supplement, and residual sources of variation. Statistical significance was determined at $P \leq 0.05$ and tendencies were identified at $0.05 \geq P \leq 0.15$.

RESULTS

Pre-harvest gamagrass and hay composition

In the pre-harvest gamagrass samples taken from July 16 to August 25, the TNC concentration in the whole canopy samples increased during the day from 0600 to 1800 (Figures 1, 2 and 3). During mid-July, TNC increased from 6.6% to 9.6% of DM in the field that was fertilized with 50 kg N/ha (LO) and from 6.6% to 9.3% of DM the following week. The field that was fertilized with 100 kg N/ha (HI) exhibited a slightly greater increase in TNC throughout the day ranging from 6.0% to 10.1% of DM and from 5.9% to 9.6% of DM the following week. Starch, di-, and polysaccharides all followed a similar trend for the same weeks (Figures 1 and 2). Monosaccharides, which contribute the largest proportion to TNC, remained steady throughout the day (Figure 1 and 2). August 24 to 25 samples were obtained on the day the fields were mowed, and exhibited a large decrease in overall TNC concentration and eliminated any differences between LO (4.7% to 6.6% of DM) and HI levels (5.3% to 6.1% of DM) (Figure 3). Starch and monosaccharide concentration increased in LO, whereas starch, di- and polysaccharides increased in HI.

Leaf tissue exhibited a similar pattern with increased TNC throughout the day (Figures 4, 5 and 6). The TNC increased in concentration over the day regardless of N fertilization level, ranging from 6% to 10% of DM, a 4 percentage unit difference. The pre-harvest sample and the leaves had similar overall concentration of TNC throughout the day, indicating that the primary source of TNC found in gamagrass plants is present in the leaf portion. In the leaves, all of the constituents of TNC increase steadily over the course of the day. In August there was a decrease in the overall concentration of TNC compared to July, and the difference over the course of the day was about 2 percentage units ranging from 5.3% to 7.5% of DM over both N fertilization levels.

Although the concentrations of TNC were greater in the stems compared with the leaves and increased during the day from 12.2% to 16.7% of DM in LO and 10.6% to 16.2%

of DM in HI (Figure 7), stems made up only a small proportion of the plant DM. In the following week of July, the stems exhibited a similar 25% increase in TNC throughout the day (Figure 8). This is the portion of the plant that contains the highest level of starch, which is a 25-50 fold increase over the levels found in the leaf. In the pre-harvest forage and the leaves, monosaccharides comprised the largest portion of TNC, whereas in the stem, starch levels were found in equivalent proportions to the monosaccharide levels (Figures 7, 8, and 9). In August, the overall levels dropped with the highest percentage being 12.6% for LO and 10.5% of DM for HI (Figure 9) found at the end of the day.

Two different levels of N fertilization were used in order to see whether there would be a protein x energy interaction from the diets. We attempted to maximize protein utilization in the rumen by providing the animal with a diet higher in CP accompanied by a highly fermentable carbohydrate source, the PM hays. However, preliminary analysis showed no difference in CP concentration between HI and LO (Table 1). Consequently, a protein supplement was provided to half of the goats in order to maintain two different levels of N intake. The protein supplement was designed to contain 32.97% CP (Table 2), and after chemical analysis was determined to contain 30.8% CP (Table 3).

There were no interactions between time of harvest and supplementation, so the data presented here will be a discussion of the main effects. Compared to AM hay, PM hay was higher in TNC ($P < 0.001$), monosaccharide ($P < 0.001$), and di/polysaccaride ($P < 0.001$) concentration (Table 1). There were also small decreases in NDF, ADF, and cellulose concentrations ($P < 0.05$). This was attributed to the increase in TNC concentration. The hays were similar in DM, lignin, and starch (Table 1). There was a tendency for PM hay to be lower in B_3 and C protein fractions ($P < 0.09$); however there were no differences in the A, B_1, or B_2 fractions (Table 4). The protein fractions of the supplement were designed to match the combination of A+B_1, B_2+B_3, and C fractions of gamagrass (Table 2). The supplement was higher in B_1 and B_2, while lower in B_3 than the hays (Table 4), but overall matched the fractions in the manner in which it was intended.

Hay intake and digestibility

Ad libitum hay and total (hay + supplement) intakes in grams per day and on a BW basis for goats were similar between PM and AM hay (Table 5). There were also no differences in NDF, ADF, cellulose, lignin, and CP intakes. Using the DM digestibility

coefficient for each goat obtained during the balance trial (Table 6) and multiplying it by intake in the ad libitum trial showed that there was a tendency ($P < 0.14$) for digestible DMI to be greater in goats when fed PM compared to AM hay (Table 5). Goats when fed PM had increased TNC, monosaccharide, di- and polysaccharide intakes ($P < 0.001$) compared to AM (Table 7); however, there were no differences in starch intakes.

Goats fed a protein supplement tended to have decreased ad libitum hay intake in grams per day ($P < 0.10$) and decreased intake as a percentage of BW ($P < 0.01$) due to substitution of the hay with supplement (Table 5). Ad libitum total intakes in grams per day and as a percentage of BW were unaffected by supplementation. Goats fed supplement had increased CP intake ($P < 0.001$) and digestible DMI ($P < 0.03$) calculated in a similar manner as stated above (Table 5). There was a tendency for supplementation to decrease TNC ($P < 0.15$), starch ($P < 0.10$), and monosaccharide ($P < 0.14$) intakes (Table 7). There were no interactions between hay and supplement for any of the variables during the ad libitum phase of the trial.

During the balance trial there was a tendency for goats when fed PM to have increased hay intake and total intake ($P < 0.09$) compared to AM, but there were no significant differences in DMI as percentage of BW (Table 6). Goats when fed PM hay had a tendency for lower fecal DM output ($P < 0.13$), resulting in an increase in DDMI and DM digested as percentage of intake ($P < 0.001$) (Table 6). Intakes of TNC, mono-, di-, and polysaccharides were increased in goats when fed PM ($P < 0.001$) (Table 8). Starch intakes were also increased ($P < 0.003$) in goats when fed PM (Table 8).

Intakes of NDF, ADF, and cellulose were similar for goats when fed PM or AM hays (Table 9). Compared to goats when fed AM hay, those when fed PM hay had decreased fecal NDF output ($P < 0.05$), and thus increased NDF digested ($P < 0.02$) and NDF digested as a percentage of intake ($P < 0.01$) (Table 9). There was a tendency for goast when fed PM to have decreased fecal ADF output ($P < 0.07$), thus increasing ADF digested and ADF digested as a percentage of intake ($P < 0.05$) (Table 9). There was also a tendency for goats when fed PM to have decreased fecal cellulose output ($P < 0.06$) and an increase in cellulose digested as a percent of intake ($P < 0.01$).

N balance

Compared to goats when fed AM, those when fed PM hay had increased N intake ($P < 0.001$) (Table 6). There were no differences in fecal N, urinary N, urinary N excretion in the form of urea, or urea N as a percentage of total urinary N (Table 6). Goats when fed PM had a greater amount of N digested ($P < 0.001$), N retained ($P < 0.04$), and N digested as a percentage of intake ($P < 0.002$) (Table 6). Compared to AM, goats when fed PM had similar N retained as a percentage of N intake or percentage of N digested, as well as similar blood urea N concentrations (Table 6).

During the balance trial there were no differences in hay or total intakes in grams per day or as a percentage of BW of goats either consuming a protein supplement or not receiving any supplementation (Table 6). There were no differences in fecal DM output. Supplementation did increase the amount of DM digested ($P < 0.02$) and DM digested as a percentage of intake ($P < 0.001$) compared to no supplementation. Supplementation did not affect TNC, monosaccharide, starch, di-, or polysaccharide intakes (Table 8). There was a tendency for an interaction between goats when fed PM hays with a protein supplement to have greater TNC ($P < 0.15$) and monosaccharide intakes ($P < 0.122$), which is due to the additive effect of the higher levels of TNC in the PM hay in addition to the TNC in the supplement.

Intakes of NDF, ADF and cellulose were similar for goats despite supplementation (Table 9). Goats fed supplement had decreased fecal NDF ($P < 0.05$), and increased digestibility of NDF ($P < 0.003$). There were no differences in fecal ADF or cellulose output. There was a tendency for supplementation of goats to increase the amount of ADF and cellulose digested ($P < 0.09$). It also increased the amount of ADF and cellulose digested as a percent of intake ($P < 0.001$) (Table 9).

Goats fed supplement had increased N intake ($P < 0.001$), fecal N ($P < 0.02$), urine N, urine urea N, and urine urea N as a percentage of total urine N ($P < 0.001$) (Table 6). These goats also had an increase in the amount of N digested, N retained, and N digested as a percent of intake ($P < 0.001$). The amount of N retained as a percent of intake was increased with supplementation ($P < 0.02$). Goats that were fed the protein supplement also had higher blood urea N levels ($P < 0.001$) (Table 6).

DISCUSSION

In this experiment, gamagrass hay was chosen to determine if the diurnal shift in TNC would alter animal responses when C_4 hay was fed, despite that C_4 forages have lower TNC concentration compared to C_3 forages. Intake was measured over a 14-d ad libitum period to document whether potential preference was short-term or if it could be sustained over longer periods. Goats were chosen as the experimental unit since they have been documented to be selective in their feed choices.

There is a lack of extensive research characterizing gamagrass as a potential feed source for livestock. It is a subtropical perennial bunchgrass (Ball et al., 1998) with moderate digestibility ranging from 40 to 57 % of DM. It contains its greatest proportion of DM as leaf and when fed to steers supported ADG similar to flaccidgrass and almost double that of Bermuda grass (Burns et al., 1992). In this experiment the DMD was 55.7% for gamagrass harvested in the afternoon (PM) and 53.1% for gamagrass harvested in the morning (AM) (Table 6). This grass is primarily produced from June through August (Ball et al., 1998). In July, the leaves of pre-harvest gamagrass had a 4 percentage unit increase (5.5% to 9.5% DM) in TNC from 0600 to 1800 (Figure 4). During this time there was also an increase in starch, monosaccharide, di-, and polysaccharide concentrations. The difference in TNC concentration throughout the day decreased to a 3 percentage unit difference the following week (6% to 9% DM), and in August immediately before the fields were harvested (Figures 5 and 6) there was only a 2 percentage unit difference (5.3% to 7.3%) from 0600 to 1800. In order to obtain the full benefit from harvesting in the afternoon C_4 grasses may need to be harvested before August due to the drop in TNC late in its growing season. In August starch, di- and polysaccharide concentrations increased as well throughout the day; however, monosaccharide concentration remained constant (Figure 6). Similar patterns occurred in the pre-harvest whole canopy and stem samples. Melvin (1965) reported that although there was an increase in total sugars and starch in fresh alfalfa sampled in the afternoon, the primary increase was in sucrose, a disaccharide. The overall concentration of TNC is higher in the stem (11% to 16% DM) and increases throughout the day (Figures 7, 8, and 9). The 5 percentage unit difference is maintained through the July

samples. In this experiment the stem portion of the plant substantially increased in TNC concentration throughout the day, whereas others have found the diurnal variation to exist only in the leaf portion (Mayland et al., 2003a). However, in the Mayland study, they used tall fescue, a C_3 grass that was grown in artificial lighting. Differences may exist between C_3 and C_4 grasses.

It is difficult to draw any conclusions of differences in TNC composition in the stem portion in August because LO did not have enough sample to be analyzed for the earlier time 0600, and HI showed no difference with time (Figure 9). In the pre-harvest whole canopy sample taken in July there was a 4 percentage unit difference in TNC (6% to 10% DM) between 0600 and 1800, which decreased at the end of August to only a 2 percentage unit difference (4.5% to 6.5% DM) and an overall decrease in total concentration (Figures 1 and 3). Tall fescue contained its lowest TNC concentration in August when compared to May, June, and September over a two year period (Mayland et al., 2000), and there may be a similar trend for gamagrass. In this experiment harvesting of the gamagrass field was delayed into August due to inclement weather.

Soyhulls were chosen to comprise 65% of the supplement composition since it was a readily fermentable fiber source (Table 2). The other four ingredients were selected because they were commercially available and the supplement was formulated based on the N solubility of gamagrass (Archibeque et al., 2001) (Table 2). This was done to minimize any changes in N degradability in the rumen. The protein fractions had to be combined into three fractions rather than five, due to the complicated nature of trying to match a protein supplement to forages, which are higher in the B3 fraction.

The supplement mimicked the gamagrass protein fractions as it was intended, by combining the A and B_1 fractions and the B_2 and B_3 fractions (Table 2). The fractions were combined for the reason that a protein supplement could never have a high enough B_3 fraction similar to forages. Upon analysis, the combination of A and B_1 was slightly higher, about 5 percentage units than the original design and the combination of B_2 and B_3 was slightly lower, about 4 percentage units (Table 4). The protein fractions of both the AM and PM hays used in this study were similar to those reported by others for gamagrass hay (Archibeque et al., 2001; Magee, 2004). The A and B_1 fractions were slightly greater in gamagrass from Archibeque et al. (2001); however, in that study gamagrass was dried inside

of a barn which may change the nutrient composition. Although the total CP levels were similar between AM and PM, there was a tendency for a difference in the B_3 and C protein fractions (Tables 1 and 4). This was due to the fact that in the AM hays, the B_3 fraction increased in HI compared to LO, whereas in the PM hays the B_3 fraction decreased in concentration in HI compared to LO. In the AM hays there were no differences in the C fraction between HI and LO. The C protein fraction in the PM hays decreased in concentration in HI.

During the ad libutum phase, goats when fed AM and PM hays had similar hay DMI and total DMI (hay + supplement) in grams per day or as a percentage of BW (Table 5). Previous research that has seen improvement with PM forages has focused on testing preference in animals offered AM and PM hays side-by-side and given a limited amount of time to consume a meal (Fisher et al., 1999, 2002, 2005; Mackay et al., 2003; Mayland et al., 2003b). In those studies animals were exposed to all hays prior to testing. Others have observed a PM preference in grazing animals looking at intake rates over a 1-h period (Orr et al., 1997). My results indicate that livestock do not exhibit an increase in DMI for PM forages over prolonged periods of time. According to Forbes and Provenza (2000) animals learn associations between foods due to feedback signals, or 'discomfort' they endure after digesting a feed. Animals offered a choice between AM and PM forages side-by-side may be better able to associate a particular feed with a metabolic comfort level. When they are offered this feed in a switchback design the animal may not associate the comfort level with a particular feed due to the long length of time between periods. Therefore, in a feeding situation, such as this experiment, the animal may regulate its intake based on something that is affecting it at that moment, such as fiber content.

There was a tendency for a 4% increase in digestible DMI in goats when fed PM during the ad libitum phase (Table 5). The digestibility coefficient used to calculate this variable was determined during the balance trial. Restricting intake during the balance trials reduced standard error among treatments. During the balance trial there was a tendency for goats when fed PM hays to have increased hay and total DMI when compared to goats when fed AM hays (Table 7). Digestible DMI was improved approximately 7%, by PM harvest. The digestibility of PM, compared to AM hays, was increased by 4.77%, so even though these goats had higher intakes they had a tendency for a decrease in fecal DM output (Table

6). These results are congruent with research conducted by Burns et al. (2005), who measured ad libitum DMI of alfalfa over a 14-d period and obtained variable results with different species. Goats fed the PM hays had greater DMI and digestible DMI, whereas sheep showed no differences between PM and AM. Cattle consumed more of the PM hays, but had no differences in digestible DMI between AM and PM hays.

Compared to AM, PM had increased TNC, monosaccharide, di-, and polysaccharide intakes during both the ad libitum and balance trial phases (Tables 5 and 6). This was due to the greater concentration of these constituents in the PM hays (Table 1). Starch intakes were only increased by PM hays during the balance trial (Table 6). Despite the fact that there were no differences in starch concentration between the average of PM and AM hays (Table 1), there could have been slight changes in starch concentration over time with a larger decrease in AM hay. On the contrary, it may just be due to variation between bales fed in the different phases of the experiment.

Intakes of NDF, ADF, cellulose and lignin were similar among AM and PM during both ad libitum and balance trial phases of the experiment (Tables 5 and 9). This suggests that even though there were statistical differences in NDF, ADF, and cellulose composition between AM and PM hays they were most likely not biologically relevant (Table 1). The differences were one percentage unit or less. Compared to AM, PM had increased cellulose, ADF, and NDF digestibility as a percent of intake, ranging from a 2.5 percent to a 3.5 percent improvement, respectively (Table 9). This was due to their decreased fecal NDF output, and a tendency for a decrease in fecal ADF and cellulose output (Table 9).

Fisher et al., (1999) reported decreases in NDF, ADF, and cellulose composition of PM hays compared to AM hays, which they concluded was due to dilution with TNC. However, our results tended to support others that have reported there are no differences in NDF, ADF, and cellulose composition due to time of day (Orr et al., 2001; Fisher et al., 2005; Burns et al., 2005). Burns et al. (2005) determined that goats and cattle fed PM alfalfa hays had increased NDF and ADF digestibilities as a percent of intake, whereas sheep were similar between hays.

PM had greater N intakes than AM (Table 6). There was no difference in CP concentration between AM and PM hays (Table 1), which included samples collected from the entire experiment. However, N intake during the balance trial was calculated using the

composition of the hay fed during those weeks only, in which PM hays had a slightly higher N concentration compared to AM hays. A similar finding was reported by Fisher et al. (1999). Others have concluded that forages harvested in the afternoon have decreased CP concentration due to dilution with TNC (Orr et al., 2001; Burns et al., 2005; Fisher et al., 2005) or no change in CP between harvest times (Orr et al., 1997). PM compared to AM, increased the amount of N digested (g/d and % intake) as well as N retained (Table 6). The urinary N output was 3.32g in PM compared to 3.16g in AM hays, which is a 0.16 g/d difference. Therefore, the improvements in digestibility and retention must be primarily a result of the increase in N intake by 0.5 g/d for PM compared to AM hays.

When supplement was added to the hays, hay intake was reduced during the ad libitum phase, but was not significantly affected during the balance trial (Tables 5 and 6). On average for both periods the substitution of hay for supplement was approximately 50%. This substitution rate is in agreement with substitution rates previously reported for gamagrass (Magee, 2004). Supplementation increased digestible DMI by 11 to 13% in the ad libitum and balance trial phases, respectively. DM digestibility was improved by 15% with supplementation. This would be expected since the digestibility of the supplement was calculated to be 85%, which was much greater than the average digestibility of the hay which was about 54%.

Supplementation of goats tended to decrease TNC, starch, and monosaccharide ad libitum intakes (Table 7) and had no effect during the balance trial (Table 8). I do not have non-structural carbohydrate composition data for the supplement. However corn, an ingredient in the supplement, has a high concentration of starch, which is highly digestible. The TNC intakes were only represented by the hays, and thus followed the same trend as the decrease in hay intake during the ad libitum phase.

Fiber and lignin intakes were similar between supplemented and non-supplemented goats (Tables 5 and 9). Supplementation increased fiber digestibility as a percent of intake by 2.5 to 3.5 percentage units. Although total DM intake (hay + supplement) was not affected by supplementation, the NDF, ADF, and cellulose concentrations in the supplement were decreased by about 30, 9, and 8 percentage units respectively, compared to the hays. Soyhulls, which comprised the majority of the DM in the supplement, are a readily fermentable fiber source. Therefore, I would expect goats given a supplement to have

improved fiber digestibility. Also, there were no significant differences in fecal excretion of these components; however, mathematically interpreting these data suggests a decrease with supplementation.

By design, goats receiving supplement had higher CP intakes compared to those receiving none in both the ad libitum and balance trial phases (Tables 5 and 6). Goats fed supplement had increased fecal N, urine N, urine urea N (g/d and % of intake), as well as plasma urea N (Table 6). The increase in urine urea N and plasma urea N indicates that supplementation may have exceeded the goats' protein requirements or more likely that the energy levels in the rumen were too low for the microbes to fully utilize the N and thus, excess N entered the blood stream. It has been suggested that efficiency of microbial protein production may be improved by balancing the overall daily ratio of ruminally available energy and N in the diet (Henning et al., 1993). This excess release of urea indicates that the supplemented goats did not have synchronization of energy and protein available in the rumen. When ample amounts of N are available to the microbes residing in the rumen, but not balanced by an adequate energy concentration, the proportion of urine N in the form of urea increases (Huntington and Archibeque, 1999).

Goats that did not receive supplement had TDN intakes of 350.5 g/d (calculated from the equation TDN = 92.5135-.7965*ADF concentration; used avg. ADF between PM and AM since they were similar). According to the NRC (1981) goats weighing approximately 23kg growing at a rate of 50g/d need 370g TDN/d to satisfy maintenance plus low growth rates. Goats that did not receive supplement had CP intakes of 45 g/d. According to the NRC (1981) goats weighing approximately 23 kg growing at a rate of 50 g/d need 54 g CP/d to satisfy maintenance plus low growth rates. Therefore, gamagrass fed alone without supplementation failed to meet the goats' energy and protein requirements. However, the decrease in urine urea N and plasma urea N, indicates that the lower protein and energy levels found in the rumen may have been more adequately synchronized, allowing the microbes to capture a greater amount of N, resulting in less ammonia absorption and therefore, less urea production.

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Figure 1. The effects of N fertilization level and time of day on TNC and its constituents concentrations in pre-harvest gamagrass. Samples were collected July 16-17, 2003.

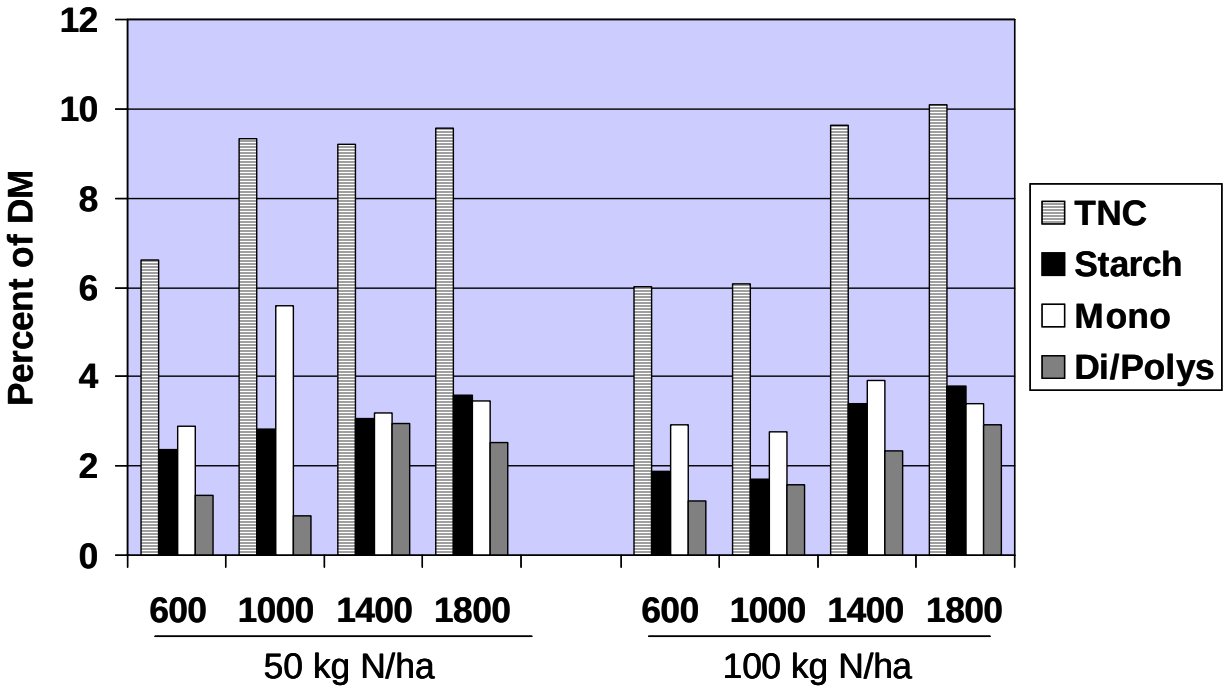

Figure 2. The effects of N fertilization level and time of day on TNC and its constituents concentrations in pre-harvest gamagrass. Samples were collected July 21-22, 2003.

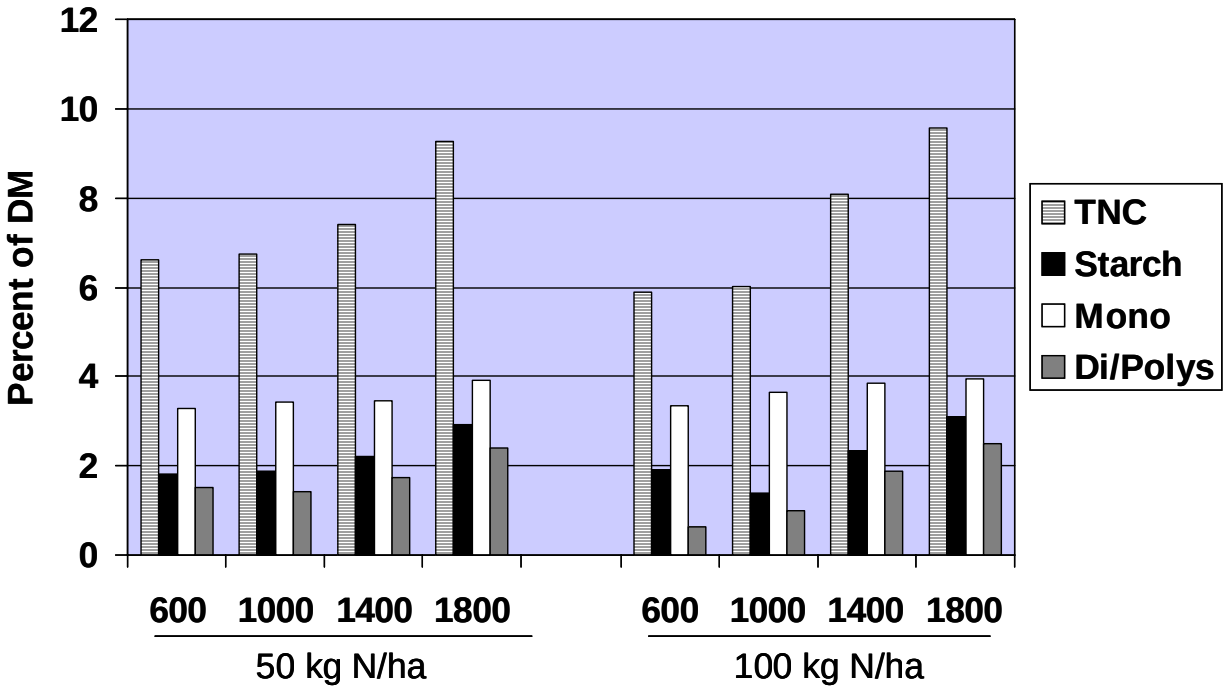

Figure 3. The effects of N fertilization level and time of day on TNC and its constituents concentrations in pre-harvest gamagrass. Samples were collected August 24-25, 2003.

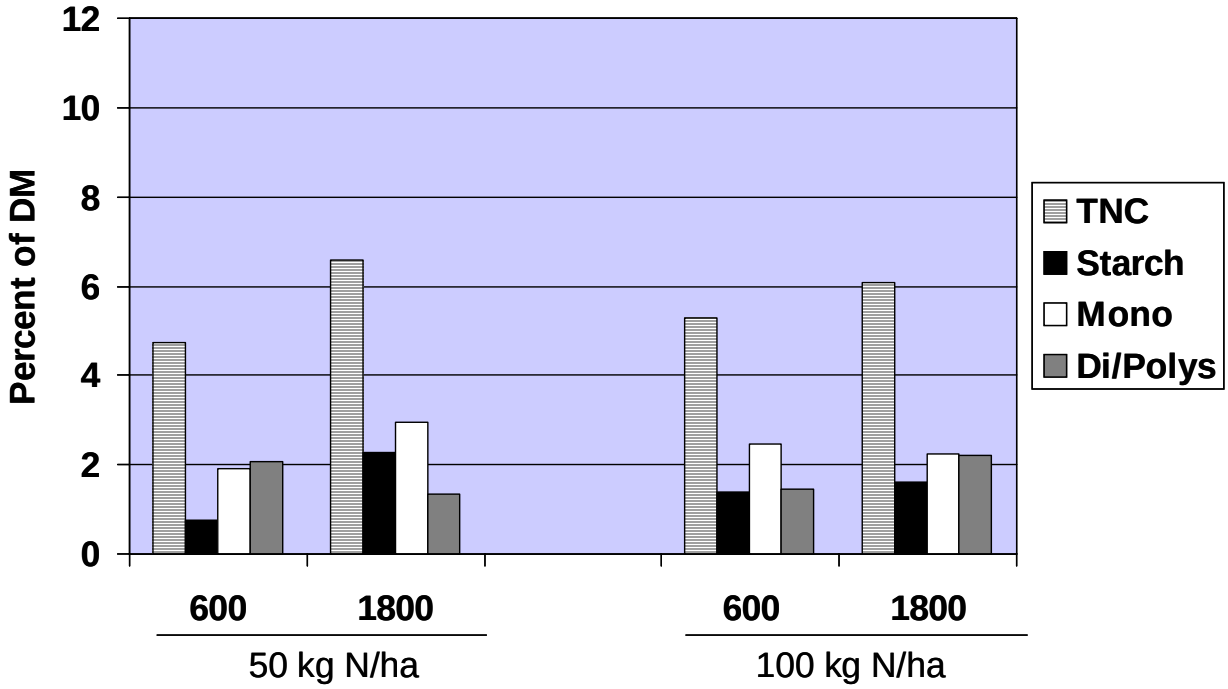

Figure 4. The effects of N fertilization and time of day on TNC and its constituents concentrations in the leaves of gamagrass. Samples were collected July 16-17, 2003.

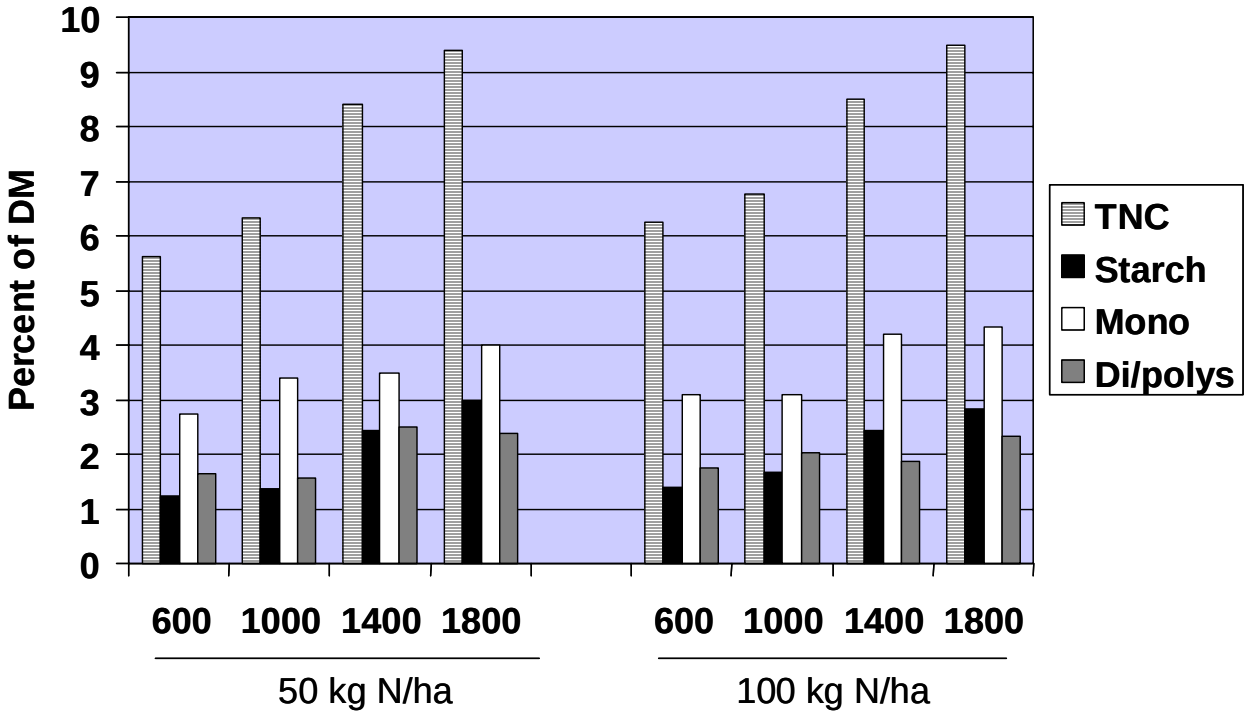

Figure 5. The effects of N fertilization level and time of day on TNC and its constituents concentrations in the leaves of gamagrass. Samples were collected July 21-22, 2003.

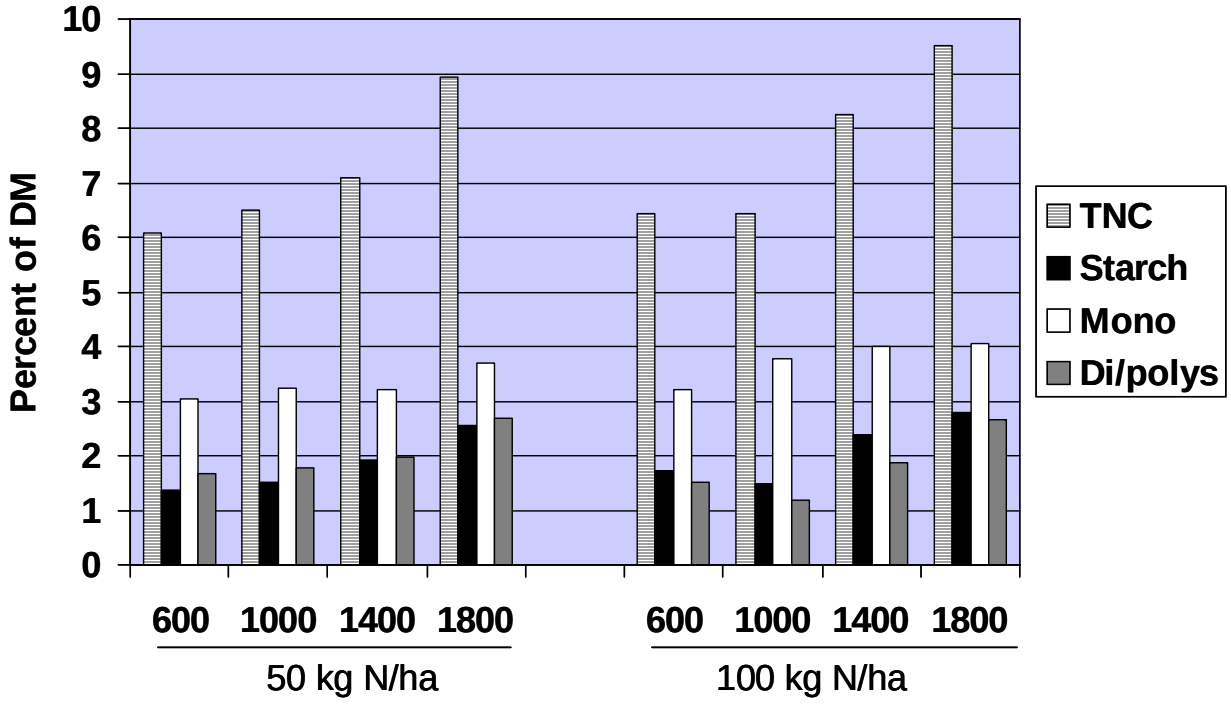

Figure 6. The effects of N fertilization level and time of day on TNC and its constituents concentrations in the leaves of gamagrass. Samples were collected August 24-25, 2003.

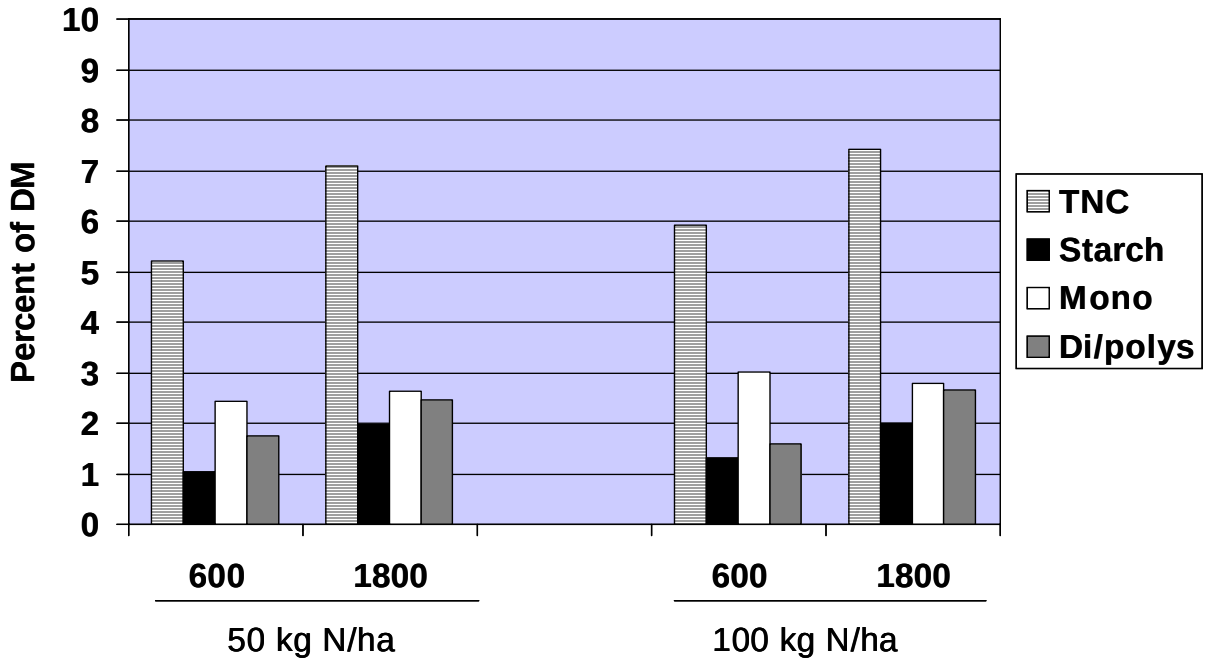

Figure 7. The effects of N fertilization level and time of day on TNC and its constituents concentrations in the stems of gamagrass. Samples were collected July 16-17, 2003.

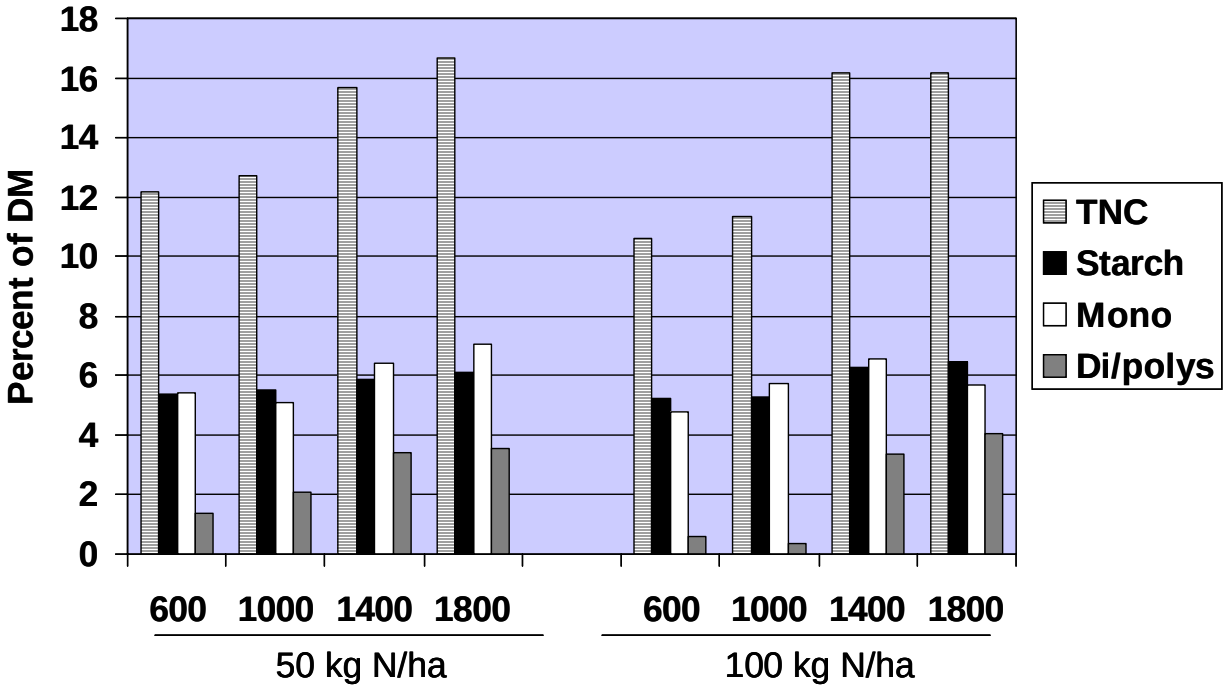

Figure 8. The effects of N fertilization level and time of day on TNC and its constituents concentrations in the stems of gamagrass. Samples were collected July 21-22, 2003.

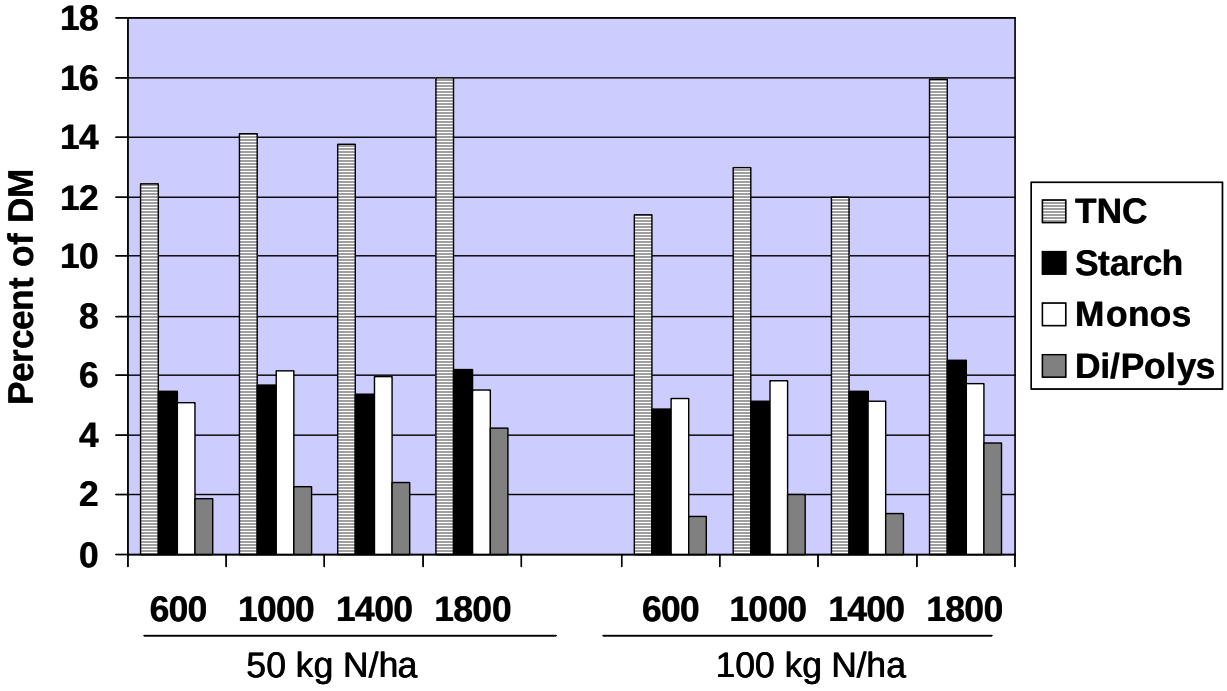

Figure 9. The effects of N fertilization level and time of day on TNC and its constituents concentrations in the stems of gamagrass. Samples were collected August 24-25, 2003.

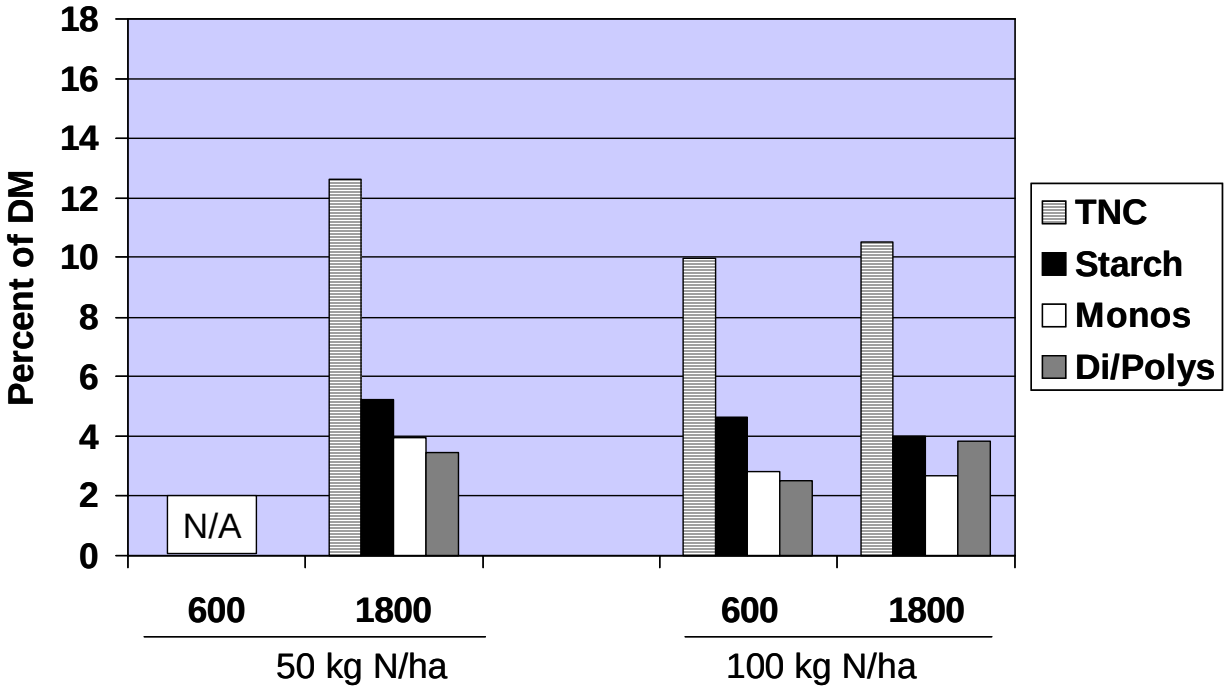

Table 1. Nutrient value of gamagrass hays[a] harvested in the morning (AM) or afternoon (PM).

Item	PM	AM	SE	*P*-value
DM %	93.9	93.8	0.05	0.175
	% of DM			
CP	7.89	7.94	0.23	0.880
NDF	70.0	71.0	0.20	0.007
ADF	35.7	36.3	0.17	0.048
Cellulose	31.6	32.2	0.19	0.051
Lignin	3.85	3.88	0.04	0.638
TNC	7.08	5.90	0.08	0.001
Starch	1.53	1.50	0.03	0.456
Monosaccharides	3.70	2.86	0.07	0.001
Di/Polysaccharides	1.85	1.54	0.03	0.001

[a]n = 8

Table 2. Ingredients and design of protein supplement. Protein fractions were designed to mimic the protein fractions previously determined in gamagrass hay (Archibeque et al., 2001).

Source	% of DM	CP, % of DM	A	B_1	B_2	B_3	C
			Proportion of CP, g/d				
SBM[a]	5	2.77	0.43	0.22	2.03	0.01	0.09
Soy hulls	65	9.36	2.30	0.48	4.43	1.32	0.83
Corn	3.5	0.34	0.04	0.03	0.25	0.00	0.02
CGM[b]	25	16.25	0.65	0.33	14.27	0.16	0.85
Urea	1.5	4.25	4.25	0.00	0.00	0.00	0.00
Total	100	32.97	7.67	1.06	20.98	1.49	1.79
			% of Total CP				
Supplement			23.25[c]	3.18[c]	63.64[c]	4.51[c]	5.42
Gamagrass Hay			23.50[d]	3.30[d]	24.50[d]	43.50[d]	5.20
			26.80		68.00		5.20

[a]SBM = soybean meal
[b]CGM= corn gluten meal
[c]Supplement: combined A and B_1 (26.43% of CP) and B_2 and B_3 fractions (68.15% of CP)
[d]Gamagrass hay: combined A and B_1 (26.8% of CP) and B_2 and B_3 fractions (68.0% of CP)

Table 3. Nutrient composition of protein supplement[a].

Item	Supplement
DM %	91.3
	% of DM
CP	30.8
NDF	40.3
ADF	27.3
Cellulose	25.7
Lignin	1.21

[a]n = 8

Table 4. Protein fractions of gamagrass hays[a] and supplement[b].

Item	Supplement	PM hay	AM hay	SE $_{hays}$	*P*-value $_{hays}$
CP, % DM	30.8	7.89	7.94	0.23	0.880
		% of CP			
A	22.75	19.1	19.4	1.47	0.904
B_1	8.99	1.37	1.31	0.61	0.944
B_2	54.31	23.5	22.7	0.78	0.471
B_3	9.42	46.4	51.3	1.73	0.071
C	4.59	5.96	6.68	0.27	0.084

[a]n = 6
[b]n = 3

Table 5. DM, fiber, and CP intake[a] of goats during ad libitum phase of trial.

	Supplement				Harvest			
Item	NO	YES	SE	*P*-value	PM	AM	SE	*P*-value
BW, kg	23.5	23.8	0.58	0.740	23.71	23.56	0.41	0.444
DM intake, g/d								
Hay intake	576.0	530.0	19.1	0.095	551.6	554.41	15.0	0.844
Hay + Supplement intake	576.0	601.9	20.6	0.375	587.9	590.02	16.2	0.888
Digestible DM intake[bc]	301.0	339.3	12.0	0.030	327.1	313.21	9.48	0.141
Hay DMI, % BW	2.45	2.22	0.06	0.008	2.33	2.34	0.05	0.950
Total DMI, % BW	2.45	2.52	0.06	0.406	2.48	2.49	0.06	0.959
NDF intake	405.2	400.1	14.8	0.885	399.2	406.17	14.8	0.498
ADF intake	206.5	207.5	7.77	0.927	205.4	208.70	6.01	0.534
Cellulose intake	183.0	185.1	6.88	0.828	182.1	185.99	5.33	0.412
Lignin intake	21.52	20.28	0.80	0.279	20.91	20.89	0.62	0.974
CP intake	46.07	65.04	2.27	0.001	54.61	56.50	1.74	0.210

[a]Intakes are represented as g/d

[b]Digestible DMI (DDMI) was calculated using the digestion coefficients for each goat during the balance trial.

[c]n = 13

[d]n = 14 for all other variables

Table 6. Dry Matter and N intake, digestion, retention, and blood urea N in goats fed hay harvested in the PM or AM and fed with or without a protein supplement.

	Supplement[a]				Harvest[b]			
Item	NO	YES	SE	*P*-value	PM	AM	SE	*P*-value
BW, kg	22.5	23.4	0.411	0.127	23.0	22.8	0.40	0.713
DM intake, g/d								
Hay	551.4	522.0	19.60	0.291	543.6	529.8	14.2	0.087
Hay + Supplement	551.3	590.4	20.61	0.185	577.8	564.0	14.8	0.087
DMI, % of BW	2.45	2.52	0.065	0.473	2.51	2.47	0.05	0.250
Fecal DM, g/d	263.9	256.2	10.70	0.606	255.5	264.6	7.96	0.123
DM digested, g/d	288.2	333.5	12.21	0.013	322.3	299.4	8.77	0.001
DM digested, % intake	52.2	56.60	0.54	0.001	55.74	53.08	0.46	0.001
N intake, g/d	7.24	10.29	0.37	0.001	9.02	8.52	0.26	0.001
Fecal N, g/d	3.51	4.06	0.16	0.020	3.78	3.79	0.12	0.896
Urine N, g/d	2.55	3.93	0.10	0.001	3.32	3.16	0.09	0.162
Urine urea N, g/d	1.32	2.67	0.08	0.001	2.03	1.96	0.07	0.465
Urine urea N, % of total	51.57	67.84	1.49	0.001	59.75	59.67	1.18	0.948
N digested, g/d	3.74	6.24	0.24	0.001	5.24	4.73	0.17	0.001
N retained, g/d	1.22	2.30	0.19	0.001	1.92	1.61	0.15	0.037
N digested, % intake	51.41	60.50	0.77	0.001	57.11	54.80	0.63	0.002
N retained, % intake	16.00	22.05	1.67	0.015	19.96	18.09	1.41	0.251
N retained, % digested	31.50	36.15	2.57	0.202	35.32	32.32	2.22	0.259
Blood Urea N, mM	8.61	13.24	0.40	0.001	11.08	10.77	0.31	0.291

[a]n = 13
[b]n = 14

Table 7. Ad libitum intake[a] of non-structural carbohydrates in goats consuming gamagrass hay.

	Supplement[b]				Harvest[b]			
Item	NO	YES	SE	*P*-value	PM	AM	SE	*P*-value
TNC intake	38.07	35.17	1.40	0.149	40.14	33.11	1.09	0.001
Starch intake	8.91	8.13	0.33	0.100	8.54	8.34	0.26	0.566
Monosaccharide intake	18.92	17.41	0.70	0.134	20.64	15.69	0.55	0.001
Di & polysaccharide intake	10.24	9.64	0.37	0.254	10.88	8.99	0.29	0.001

[a]Intakes are represented as g/d
[b]n = 14

Table 8. Total non-structural carbohydrate intake of goats consuming gamagrass hay during the balance trial.

	Supplement[a]				Harvest[b]			
Item	NO	YES	SE	*P*-value	PM	AM	SE	*P*-value
TNC intake	34.32	32.45	1.31	0.315	36.69	30.07	0.95	0.001
Starch intake	7.66	7.10	0.30	0.191	7.60	7.15	0.22	0.003
Monosaccharide intake	17.65	16.70	0.69	0.333	19.40	14.95	0.50	0.001
Di & Polysaccharide intake	9.01	8.66	0.34	0.441	9.69	7.97	0.25	0.001

[a]n = 13
[b]n = 14

Table 9. Fiber intakes and their digestibilities by goats during the balance trial.

	Supplement[a]				Harvest[b]			
Item	NO	YES	SE	*P*-value	PM	AM	SE	*P*-value
NDF intake, g/d	392.0	398.1	15.26	0.775	395.1	395.1	11.0	0.998
Fecal NDF, g/d	176.5	168.6	7.36	0.045	168.4	176.7	5.47	0.045
NDF digested, g/d	215.5	229.5	8.62	0.254	226.7	218.3	6.19	0.015
NDF digested, % intake	55.03	57.73	0.59	0.003	57.39	55.37	0.50	0.002
ADF intake, g/d	198.2	204.5	7.82	0.567	201.0	201.7	5.63	0.844
Fecal ADF, g/d	92.3	87.9	3.90	0.430	88.1	92.1	2.90	0.069
ADF digested, g/d	106.0	116.6	4.39	0.093	113.0	109.6	3.16	0.051
ADF digested, % intake	53.53	57.08	0.67	0.001	56.19	54.41	0.54	0.004
Cellulose intake, g/d	176.8	183.8	6.98	0.482	179.7	180.9	5.02	0.622
Fecal cellulose, g/d	73.1	69.5	3.10	0.408	69.6	73.0	2.32	0.059
Cellulose digested, g/d	103.7	114.3	4.27	0.088	110.1	108.0	3.08	0.211
Cellulose digested, % intake	58.73	62.28	0.64	0.001	61.27	59.73	0.52	0.010

[a]n = 13
[b]n = 14

Intake, digestibility, N balance and Endocrine Regulation of Beef Steers Fed Gamagrass Baleage Harvested at Sunrise and Sunset

ABSTRACT

The objective of this study was to evaluate the differences in TNC composition of Iuka gamagrass (*Tripsacum dactyloides L.*) harvested at 0600 (AM) or 1800 (PM). Gamagrass was fertilized with N at 2 different levels of 50 kg N/ha (LO) and 150 kg N/ha (HI) to determine if the increase in TNC in PM could affect the utilization of the increased N in HI. We measured how TNC and N concentration affect the voluntary intake, digestibility, N balance, and metabolic hormone concentrations of beef steers fed gamagrass. Gamagrass was direct-cut, immediately wrapped into large round bales, and wrapped with plastic to ensile for 90 d before being fed. Within fertilization level, steers (320 ± 57 kg) were randomly assigned to HI or LO. Within fertilization level, steers were randomly assigned to a crossover design of AM or PM. Steers were individually housed in tie-stalls with free access to H_2O and mineral blocks. They were fed twice daily. After a 14-d adjustment to adaptation diets, voluntary intake (steers were fed 110% of previous day's intake) of the experimental treatments was measured for 17 d, followed by a 4-d adjustment (to equalize DM offered between periods) and a 5-d balance trial to measure DM digestibility (DMD) and N retention. After Period 1 the steers were switched to their new diets, and the protocol was repeated. Means differ at $P< 0.05$. Compared to AM, PM had greater TNC (3.5 vs. 2.3%), starch (0.71 vs. 0.52%), and monosaccharide (2.3 vs. 1.2%) concentrations. There was an interaction ($P < 0.003$) for TNC, starch, and monosaccharide concentration since they were increased in the AM/LO treatment to similar levels found in PM treatments (3.05, 0.76, and 1.6%). Compared to AM, PM had a greater pH (5.4 vs. 4.5), ethanol (15.1 vs. 6.8 g/kg DM), and isopropanol (5.3 vs. 0.48 g/kg DM) concentrations. However, AM had greater propionate (0.80 vs. 0.59 g/kg DM) and lactate (41.4 vs. 8.7g/kg DM) concentrations. AM responded differently than PM in regards to fertilization with decreased pH, acetate and lactate concentrations in HI compared to LO ($P < 0.08$). PM had an increased pH and lactate and no change in acetate for HI compared to LO. Ad libitum DMI was not affected by time of harvest ($P < 0.88$) or fertilization level ($P < 0.23$). However, compared to PM, AM increased DDMI during the ad libitum (3.3 vs. 3.0 kg/d) and balance trial (3.3 vs. 2.9 kg/d). There was a tendency ($P < 0.12$) for DMD to be greater for AM than PM (53.2 vs. 51.0%), yet there were no differences in NDF, ADF, or cellulose digestibilities

($P < 0.93$). HI improved cellulose digestibility (65.6 vs. 61.7%) and tended ($P < 0.13$) to improve NDF digestibility (52.8 vs. 50.2%). Despite the similar intakes between AM and PM, AM increased N digested (53.1 vs. 43.4 g/d). There were no differences in N retention ($P = 0.33$) since AM also increased urinary N (48.3 vs. 42.3 g/d), and tended ($P < 0.10$) to increase plasma urea N (5.4 vs. 4.7 mM), and thus urea N excreted in the urine (28.4 vs. 23.9 g/d). By design, HI tended ($P < 0.07$) to have greater N intakes (106.0 vs. 95.2 g/d). Compared to LO, HI increased N digested (55.0 vs. 41.5 g/d), urinary N (52.3 vs. 38.3 g/d), urea N as a percent of total urinary N (64.7 vs. 44.3%), and plasma urea N (6.0 vs. 4.1 mM). Insulin concentrations tended to be greater in AM ($P < 0.12$) and HI ($P < 0.07$) from 0900 to 1530. Plasma ghrelin concentrations were increased on LO compared to HI in the AM diets, and showed no changes in PM regardless of fertilization level ($P < 0.001$). Plasma ghrelin concentration peaked at the 1000 feeding time, and then decreased for the remainder of the day. Compared to PM, AM insulin concentration peaked at 1100, stayed elevated until 1200, and then decreased.

Key Words: Total non-structural carbohydrates (TNC), Voluntary Intake, N Metabolism, Steers, *Tripsacum dactyloides,* ghrelin, insulin

INTRODUCTION

Agronomists have discovered that harvesting hay in the PM compared to the AM leads to an accumulation of TNC. Studies showed that the increased soluble sugars are associated with increased short-term intake, a measurement being used to determine preference by cattle, sheep and goats (Fisher et al., 1999, 2002). Preference for PM hay was also documented in non-ruminant species such as rabbits and horses (Mackay et al., 2003; Mayland et al., 2003). However, in the study presented in the previous chapter there were no differences in voluntary intake by goats consuming gamagrass hay harvested in the PM and AM and fed for 14 d. We did observe an increase in apparent digestibility and digestible DMI in goats consuming PM harvested hays.

There are metabolic hormones that are important regulators between appetite and satiety and serve as a connection between the brain and the gut. Insulin and leptin levels are indicative of the body's energy status and are released into the blood in proportion to the amount of fat stored in the body or glucose present in the blood. Leptin is usually involved in long-term control, whereas insulin is more important for short-term control. These both limit food intake. On the other hand, some hormones act to stimulate appetite. Ghrelin concentration has been shown to increase one hour prior to feeding and peak immediately before the meal (Sugino, 2002) suggesting it may serve as an appetite stimulus. Hunger signals stimulate intake of feed, whereas satiety signals trigger meal termination, and thus both of these together control frequency of eating and meal size and hence total energy intake (Druce et al., 2004). These metabolic hormones can be sensitive to the nutritional state of the animal, and may be receptive to the amount and type of nutrient in the diet, specifically TNC.

The objective of this study was to determine if benefits of harvesting forages in the PM can be sustained for the long-term for C_4 grasses as previously noted for C_3 grasses, and if the increase in TNC can be preserved through ensiling. Forages harvested in the PM have the potential for increasing preference and voluntary intake by livestock as well as improving digestibility, providing an easily adoptable management tool for farmers at no extra costs.

MATERIALS AND METHODS

Animal and Experimental Procedures

The experimental design was a crossover with a 2 X 2 factorial arrangement of treatments. There were four experimental treatments derived from a field of Iuka gamagrass (*Tripsacum dactyloides (L.) L.*). Before the start of the experiment a 0.81 hectare field of gamagrass was fertilized with N at two different levels, 50 or 150 kg per hectare. Each fertilization level was harvested at two different times of day, PM or AM to create a total of four treatments. The four treatments were PM baleage + 50 kg N (**PM/LO**), PM baleage + 150 kg N (**PM/HI**), AM baleage + 50 kg N (**AM/LO**), and AM baleage + 150 kg N (**AM/HI**). Fresh forage samples were collected randomly at 1400, 1800 and the following morning at 0600, and 1000 from all sections of the field one week prior to harvest. At this time there were many other species growing in the field, which we estimated to be about 25% ryegrass and others. Whole canopy samples were taken and subsequently hand-separated into leaf, stem and sheath, and dead/other to provide qualitative data on the chemical composition. Electric clippers were used to cut a 6 in. diameter plot to a 3 in stubble. Samples were collected from the field and placed in a freezer (-20 to -30°C) within 1.5 h.

On May 25, 2004, the PM sections of the gamagrass field were cut with a mower-conditioner at 1800. The wind row was directly baled with a large round baler fit to deliver plastic netting when the bale reached 48 in diameter. The bales were immediately transported to the storage site and wrapped with four layers of white plastic. The wrapped bales were labeled and stored outside on the ground and adjacent to the Metabolism Educational Unit. The ambient temperature was 95°F during the PM harvest. An Iuka gamagrass field adjacent to the experimental field was harvested at 1620 on the same day to provide adaptation feed since the feed supply from the experimental field was limited. Overnight there were thunderstorms and in the morning the ambient temperature was 70°F. The AM harvest from the experimental field was cut at 0600 and the adaptation field was cut at 0800. On both days whole canopy samples were randomly collected immediately after mowing and before baling. The bales fermented for 90 d prior to their use in feeding trials. Each bale was given an identification number and sampled individually throughout the experiment.

Nine Angus steers (initial BW 302 ± 73 kg) from the Beef Educational Unit at North Carolina State University were acclimated to working closely with people, familiarized with the handling facilities, and trained to lead by halter. Handling, sampling, and care of the steers was approved by the North Carolina State University Animal Care and Use Committee (IACUC # 04-043-A). Seven of the steers developed pink eye and were treated with injections of oxytetracylcine and a nitrofurazone powder in their infected eye. They were all treated to eliminate internal parasites with a pour-on treatment of Cydectin (Fort Dodge Laboratory, Kansas City, MO). Eight steers were selected for the experiment on the basis of BW and demeanor, and blocked into two groups on the basis of BW. Group 1, the heavier steers (BW 327 ± 28 kg) and group 2, the lighter steers (BW 259 ± 44 kg) were transported from the beef unit to the Metabolism Educational Unit on August 24, 2004. They were randomly allotted to their respective treatments, but both groups of steers were included in each treatment (heavy and light in each of 4 treatments). This was an attempt to evenly distribute steers according to weight. They were placed in individual tie-stalls (114.9 x 177.8 cm) for the entire experiment with individual feeders and automatic water dispensers. Steers were adapted to the facilities with a diurnal pattern of 14 h of light and 10 h of darkness. Before the start of the experiment, one light weight steer developed a large growth near its jugular vein and was removed from the study. Its replacement into the light group was the heaviest steer out of the original nine (BW = 375 kg).

Upon arrival, steers were fed switchgrass hay for one week until a bale splitting method for the large round bales was resolved. During the experiment gamagrass bales were split using a hay saw, half of the bale rewrapped tightly, turned over to minimize any oxygen exposure, and left at the storage site. The other half was placed in a single blade, PTO driven, grinds three-point hitch tub (Tomahawk Model 5050, Teagle Machinery Ltd., Cornwall, UK) and ground. The baleage was stored in large plastic bags in a freezer until it was fed-out. Bags were taken out of the freezer a day in advance to thaw before feeding. Records were kept on the dates when each bale was opened and ground.

Periods lasted 40 d, with a 14-d adjustment to adaptation diets, a 17-d ad libitum intake period, 4-d adjustment to a restricted feed level and urine funnels, followed by a 5-d balance trial. Steers were allowed to exercise on a regular basis, 3 times per week at 1 to 2 h per day. At the end of period 1 the steers had an 8-d adaptation to their new diets and on

day 9 they started their ad libitum intake period. The crossover was between the AM and PM hays, regardless of fertilization level.

Baleage was offered twice a day with 1/3 of the daily allotment fed at 1000 and 2/3 at 1530. This split was based on length of time to consume forage and capacity of the steers' feeders. Initially steers were fed at 2% BW on a DM basis and subsequently were offered 110% of the previous day's intake during the ad libitum period. A loose mineral mix designed for beef cattle (Southern States, Richmond, VA) was offered at 50g/d before forage was offered, and steers had ad libitum access to water. Steers and stalls were cleaned daily. For the 5-d balance trial the amount of baleage offered to a given steer was the same in both periods to minimize any effects that intake would have on digestibility. Steers were fed based on their individual average intakes over the first 14-d of ad libitum plus an extra 5% if they were crossing over from PM to AM treatments since they would be expected to eat less. If they were crossing over from AM to PM an extra 10% was added to the allotted feed amount since they would be expected to eat more in period 2. They were acclimated to these set intakes during the adjustment phase preceding the balance trial.

During the entire experiment a 1% aliquot of the amount of daily feed offered on an as fed basis was taken and composited weekly by bale. At the end of the week, two subsamples were collected. One was freeze-dried for nutritive value analysis while the other remained frozen for short-chain fatty acid analyses. If there was a change in bale number within a day, then the sample was weighted based on how much of each bale was fed, and samples for different bales were kept separate. A 5% aliquot of orts on an as fed basis was collected daily in the morning and composited weekly by animal.

Prior to collections for the balance trial, all stalls were thoroughly scrubbed and washed. Wooden panels were attached to stalls to prevent any cross contamination of feces or feed, but were designed to allow visual contact between steers. Tarps were placed behind stalls to ensure total recovery of all fecal material. Steers' coats were clipped, and they had urine collection harnesses placed on them during the adjustment phase. During the balance trial, urine was collected by aspiration from an electric vacuum pump into polypropylene jugs. The jugs were connected to plastic tubing and the urine harnesses on the animal, and initially contained 200 mL of 6 N acetic acid and 1 L of water. Urine pH was measured daily using pH-sensitive paper to maintain proper acidification ($4 \leq pH \leq 6$) and amounts of

acid were adjusted accordingly. Urine was weighed daily and a 5% aliquot was retained. It was composited over the 5-d balance trial to obtain a representative sample, and a 30 mL subsample was frozen (< -21°C) until analyzed. Feces were weighed daily and a 5% aliquot was retained. On day 5, steers were removed from their stalls and the stalls were meticulously scraped and the material was added to the last day's collection. The feces were dried in a forced air oven at 60°C. Due to the limited feed supply three steers did not complete the balance trials, two on the AM/LO and one on the PM/LO treatments. Steers were weighed at the beginning of the ad libitum and balance periods as well as at the end of the balance trial. Blood samples were collected at the same time via jugular venipuncture. Daily rectal temperatures were recorded throughout the balance trial for each steer and values fell within an acceptable range (99 to 103°F).

On day 15 of ad libitum intake, steers were fitted with indwelling jugular catheters to measure circulating hormone levels. The area over the jugular vein was clipped, scrubbed and anesthetized with 5 cc of lidocaine 30 min before sterile catheters were inserted. A sterile scapel was used to create a 1 cm incision in the skin. A sterile 10 gauge needle was used to puncture the jugular vein and then the catheter was inserted. To check for patency, the catheter tubing was flushed with 5 cc of sterile citrate in isotonic saline to keep the blood from coagulating. The catheter was sutured to the skin, covered with gauze, and wrapped with an elastic bandage. On days 16 and 17 of ad libitum intake, blood samples were collected in duplicate into 4 mL Vacutainer (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) tubes with EDTA as the anticoagulant. The sampling procedure consisted of drawing 10 mL of blood and anticoagulant which was discarded, then an 8 mL blood sample was saved, and 10 mL of citrate was used to flush the catheter afterwards. Blood was collected every 30 min from 0800 to 0900, then every 15 min from 0900 to 1200, which centered around the morning feeding (1000). From 1200 to 1430 steers were sampled every 30 min, which increased once again to every 15 min for the last hour before the afternoon feeding. Within one hour of collection, plasma was harvested, transferred in duplicate into 4 cc storage tubes, and frozen at -21°C until analyzed. The time of feeding and amount the animal consumed during the morning hours was recorded on both days. Rectal temperatures were recorded both days to evaluate the health status of each animal, and values fell within 100 to 104°F.

To minimize the stress associated with this invasive procedure, steers underwent an adaptation period so we could accustom them to having their necks wrapped and the presence of groups of people. The catheter tubing was 3.05 m long, so that during sampling days a person could stand away from the head and not affect the normal eating habits of the steer.

Chemical Analysis

All pre-harvest forage samples, orts, and one of the two feed samples were dried in a freeze drier (VirTis Model, Gardiner, NY). Pre-harvest forage, feed, orts, and feces were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1 mm screen. All of these samples thoroughly mixed, subsampled, and stored in plastic whirl packs until analyzed. Freeze dried samples were stored in a freezer (-20 to -30 °C). All samples were scanned in a near-infrared spectrophotometer, (NIRS Model 5000, FOSS NIRSystems Inc., Laurel, MD) and a global H statistic of ≤ 0.3 was used to define the spectral boundaries of the population. A neighborhood H statistic of ≤ 0.6 was used to identify samples with different spectra. These samples were selected and analyzed in the laboratory for the development of prediction equations. Descriptive values corresponding to the NIR analysis will appear in appendix C. These include the number of samples used in each calibration, the range, SEC, SEC/RSQ, SECV, and SECV/1-VR for each variable measured.

Pre-harvest Forage

Pre-harvest forage samples were scanned (n = 32) and used as the initial base population. Due to the small number of samples, all were analyzed in the laboratory and used to develop NIR prediction equations for NDF and constituent fractions, DM, and N. In the laboratory, the fiber fractions were run sequentially according to the procedures outlined by Van Soest et al., (1991) in a batch processor (Ankom Technology Corp., Fairport, NY). They were determined in the order of NDF, ADF, cellulose, and ADL, with modifications to the calculations (Leonard et al., personal communication) and AIA was calculated from ADL. DM was run with modifications as outlined by AOAC procedures (1990). Crude protein was estimated as 6.25 times the percentage of Kjeldahl N and determined using (AOAC, 1990) procedures.

Results from these samples were added to the database from the first trial using goats (n = 80), and the total number of samples (n = 112) were used to develop prediction

equations for carbohydrate estimations. TNC and its components, starch, monosaccharides, and di- plus polysaccharides were analyzed using the following method (see appendix B for detail). Procedures for this method are the same as those used in the experiment with goats (Chapter 1).

DM was determined as outlined by the AOAC procedure (1990). All 80 samples were then predicted based on equations developed from lab values for DM, TNC, starch, monosaccharides, and free glucose. Di- and poly- saccharides were calculated by difference.

Feed and orts

All the feed and ort samples were scanned by NIRS (n = 112) and used as the initial base population. Of the 112, 48 were selected for carbohydrate analysis in the laboratory and analyzed using the method described above. The selected samples were then combined with previously analyzed gamagrass silage from a small database (n = 19) compiled by Leonard and Burns at North Carolina State University, giving a total population of 67 and a more robust equation to increase the confidence in the prediction. Prediction equations were generated with the NIR for the total population and all feed and ort samples were predicted for carbohydrate analysis.

The original 112 feed and ort samples were selected against previous gamagrass silage from a database (n = 159) compiled by Leonard and Burns at North Carolina State University, Raleigh, NC. Based on this large database, only 19 samples from this trial were selected for laboratory analysis of fiber components and N. Two samples were identified as outliers and therefore, were dropped from the total population (n = 176; 159 + 19 - 2). The 176 samples were used to develop NIR equations, from which the composition of all feed and ort samples was predicted. Actual laboratory values from the chemical analysis were used for the outliers. All fiber components, ADL, AIA, DM and Kjeldahl N were run using the same techniques as stated above.

Protein fractions of the feed were determined in duplicate (n = 12) as described by Licitra et al. (1996). SCFA, alcohols, and lactic acid concentrations were determined by gas-liquid chromatography (Varian model 3800 model, Chromotography Systems Business, Sugar Land, TX). The SCFA and alcohols were analyzed using a NUKOL FUSED SILICA capillary column with a 30m X 0.25mm x 0.5um film thickness (Supelco, Supelco Park,

Bellefonte, PA). Lactic acid was determined using a 6 ft x ¼" glass column packed with Supelco 4% CARBOWAX 20 M with a mesh size of 80/120 Carbopack B-DA (Supelco, Supelco Park, Bellefonte, PA). Both methods required samples to be prepared and extracted in a similar manner. For both assays there were duplicate injections of duplicate extractions, which were eventually averaged for each sample (bale).

15g of the frozen baleage sample was blended with 100 mL of deionized water for 30 s and then placed in a beaker overnight in a refrigerator. The following day samples were filtered through cheese cloth, mixed, and then filtered again through filter paper. At this point samples were placed in 30 mL bottles and frozen until further analysis.

For SCFA and alcohol determination, 5 mL of this extract was mixed with 1 mL of 25% metaphosphoric acid, vortexed, and then set on the bench top for 30 min. The samples were then centrifuged and the supernatant was decanted into storage tubes. On the day of analysis extractions were run in duplicate. One milliliter of sample was combined with 100 µL of 1% valerate, the internal standard, glass vials. The gas-liquid chromatographer was calibrated with methanol, isopropanol, ethanol, butanol, acetate, propionate, butyrate, isobutyrate, and valerate as standards, which were all also diluted with 25% metaphosphoric acid. All standards received 100 µL of the internal standard. The total run time for each sample to travel through the column was 7 min. 1µL of sample was injected in duplicate with a 1:25 (sample:carrier gas) split . The injector, where the sample was volatilized the sample and the detector, where it was read were both set at 240°C. N_2 was used as the carrier gas. The pressure in the column was set at 25 psi and the initial start temperature in the column was 40°C for 30s, increased to 155°C at the rate of 100°C/min and held there for 5 min. The temperature was then raised to 180°C for an additional minute. Results were obtained from the integrator and back-calculated to a DM basis. On the chromatogram there was an additional peak between the methanol and ethanol peaks. The retention time for isopropanol was tested and found to be the same as this extra peak.

Extracts from the 30 mL bottles were prepared in duplicate for lactic acid determination. 900 µL of sample was combined with 100 µL of 0.1% pivalic acid, the internal standard, and 100 µL of 0.3M oxalic acid into glass vials. Prior to this step, only the AM samples were diluted in a 1:4 ratio with 1 part sample to 3 parts water. This was done to get the results in the linear range of the machine 20, 60 and 80 mg/100mL. The machine

was calibrated with pivalic and lactic acid as standards. The lactic acid standard also received 100 μL of internal standard. The total run-time for each sample to travel through this column was 16 min. The oven was 175°C and the entire reaction was run under isothermal conditions. The injector used to volatilize the sample was set at 200°C and the detector where its read was set at 225°C. The flow of the carrier gas, N, was set at 25 mL/min. Results were obtained from the integrator and back-calculated to a DM basis.

Feces

Fecal samples were scanned by NIR (n = 13) and analyzed in the laboratory for NDF and constituent fiber fractions, ADL, AIA, DM, and Kjeldahl N. They were then added to a previous file of data for fecal samples from goats, sheep, and cattle fed gamagrass to provide a total of 135 samples. Based on the prediction calibration equations all fecal samples were predicted for the constituents listed above.

Urine and Blood

Urine and plasma were analyzed for urea N using the diacytyl monoxime method of Marsh et al. (1957) on a Technicon Auto Analyzer (Technicon Instruments Corporation, Tarrytown, NY). Urine was also analyzed using an auto analyzer (AOAC, 1990).

Blood (hormone meaurements)

Plasma ghrelin concentration was determined in duplicate by radioimmunoassay. A human (Active) ghrelin commercial RIA kit was used (LINCO Research, St. Charles, Missouri), which is specific for the biologically active form of ghrelin with the octanoyl group on Serine 3. The concentration of ^{125}I labeled ghrelin was detected from a Packard II model gamma counter (Packard Instrument Company, Sterling, Virginia). In our study, the lower and upper detection limits for this assay were 8.3 and 2000 pg/mL, respectively. The quality control from the supplier of the analytical kit contained a known amount of ghrelin. In addition, we used a plasma pool sample from a random steer, and this pooled sample was included in each assay to determine the inter- and intra-assay coefficient of variation. At first, all time points in a given day (n = 24) were analyzed in the laboratory, but then due to money constraints the number of samples analyzed was reduced (n = 18). The sampling protocol that was selected used time points that focused around feeding time due to the nature of the hormones' activity, but also spanned the entire length of the day. It included

these times: 0800, 0830, 0900, 0915, 0930, 0945, 1000, 1015, 1030, 1100, 1200, 1300, 1330, 1400, 1430, 1500, 1515, and 1530.

Due to the low sensitivity of the assay (human kit) the amount of plasma used in each run was increased from 100 to 200 μL. We did not change the volume in the standards. Therefore, the concentration obtained when using the new volume was multiplied by a factor of 0.62 to equalize the volumes in both standards and unknown samples.

After reviewing the preliminary results, it appeared that steers' ghrelin patterns throughout the day were similar for day 16 and 17. Since it was more biologically relevant to use an average number for both days, all samples run at this point were run with days as duplicates. Samples were placed into batches so that each steers' plasma for period 1 and 2 would be included in each assay minimizing any inter-assay variation.

Plasma insulin was measured using a commercial solid-phase radioimmunoassay. Coat-A-Count insulin kits (Diagnostic Products Corporation, Los Angeles, CA) were used. The concentration of ^{125}I labeled insulin was determined using a Packard II model gamma counter (Packard Instrument Company, Sterling, Virginia). The samples were low on the standard curve and were not detected by the gamma counter. The standards in the kit were made with human insulin. The cross reactivity of bovine insulin to human insulin antibody is only 60%. Thus, a bovine insulin standard was created using 10 mg of bovine insulin in 10 mL of 0.5N acetic acid to give a concentration of 26.6 U/mg. This was diluted to create a standard curve that included very low concentrations. The standards were made in PBS gel, which is common for the dilution of hormones. In our study, the lower and upper detection limits for this assay were 0.86 and 666 μIU/mL, respectively. A plasma pool sample from a random steer was included in each assay. The sampling protocol that was selected used time points that focused around time of feeding due to the nature of the hormones' activity, but it also spanned the entire length of the day. It included these times: 900, 930, 1000, 1015, 1030, 1045, 1100, 1115, 1130, 1200, 1230, 1300, 1330, 1400, 1445, and 1530.

Similar to ghrelin, after preliminary analysis it appeared that steers' insulin patterns throughout the day were similar for day 16 and 17. Since it was more biologically relevant to use an average number for both days, all samples run at this point were run with days as duplicates. Samples were placed into batches so that each steers' plasma for period 1 and 2 would be included in each assay, thereby minimizing inter-assay variation.

Statistical Analysis

All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model accounted for fertilization, harvest, harvest x fertilization, period, period x fertilization, and residual sources of variation. Steer within fertilization was used as the random effect since fertilization was not balanced across steers. Fertilization was tested by the test term steer within fertilization while the remaining variables were tested against the residual. Steer BW was used as a covariate when applicable and remained in the model when $P < 0.20$. The period x fertilization interaction remained in the model when $P < 0.20$. Statistical significance was determined at $P \leq 0.05$ and tendencies were identified at $0.05 \leq P \leq 0.15$.

Composition data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model accounted for fertilization, harvest, harvest x fertilization, period, period x fertilization, and residual sources of variation. The number of elapsed days from the harvesting of each bale until the day it was opened was run as a covariate and remained in the model when $P < 0.20$. Statistical significance was determined at $P \leq 0.05$ and tendencies were identified at $0.05 \leq P \leq 0.15$.

RESULTS

Gamagrass and baleage composition

The pre-harvest gamagrass whole canopy samples that were obtained 1 wk prior to harvesting showed that TNC concentration increased throughout the day from 0600 to 1800 (Figure 1). In the sward that was fertilized with 50 kg N/ha (LO), TNC increased from 10.8% to 12.6% of DM, a 2 percentage unit increase. Whereas there was a slightly larger increase in TNC, 10.4% to 13.5% of DM, in the forage that was fertilized with 150 kg N/ha (HI), a 3 percentage unit increase. Concentrations of starch and monosaccharides in LO did not increase, but remained steady throughout the day, whereas di- and polysaccharide concentrations increased. In HI starch, di- and polysaccharides increased with time of day, whereas monosaccharides decreased.

The leaf proportion, which comprised 64.9% of the DM of the gamagrass plant in this experiment, had TNC levels that reached a maximum at 1400, increasing from 8.2% to 11.9% of DM in LO and 8.5% to 11.4% of DM in HI (Figure 2). The levels slightly

decreased in the sample collected at 1800. In both HI and LO, starch, di- and polysaccharides increased until 1400. In LO, monosaccharides increased throughout the day, whereas in HI level they remained steady over all sample times.

The stem proportion, which comprised 22.3% of the DM of the gamagrass plant, had the greatest concentration of TNC as a percentage of DM. TNC increased from 15.4% at 600 to 18.8% of DM at 1800 in LO, whereas TNC increased from 16.0% to 19.7% of DM during the same time in HI (Figure 3). Starch was found to be higher in concentration in the stem compared to the leaf or the total sward, and increased slightly throughout the day. Di- and polysaccharides also increased over time. Monosaccharides consistently contributed the majority to TNC concentration; however, their pattern of production throughout the day remains less clear. The stem, leaf, and whole canopy monosaccharide concentration remained similar at all time points with only minor fluctuations.

In the pre-harvest whole canopy and leaf samples, DM increased throughout the day by about 2 percentage units for LO and 3 percentage units for HI (Figures 4 and 5). These levels reached a maximum at 1400. CP levels remained similar throughout the day. However, they differed between LO, which was 11.5% CP in the whole canopy and 12.0% CP in the leaf, compared to HI which contained 13.5% CP in the whole canopy and 15.2% CP in the leaf (Figures 4 and 5). In both the leaf and whole canopy samples, NDF, ADF, and cellulose concentrations slightly increased in the beginning of the day and decreased to their lowest level at 1800 (Figures 6 and 7).

The stem portion also only had a small increase in DM throughout the day, a 2 percentage unit increase for LO and a 1 percentage unit increase for HI (Figure 8). The overall CP levels were lower than those found in the leaf and did not increase over time. In the stem fraction there was only a 1 percentage unit difference between the LO treatment 7.1% CP and the HI treatment 8.2% CP (Figure 5). The NDF, ADF, and cellulose concentrations followed a similar trend to the concentrations found in the leaf and whole canopy, with a temporary increase in the middle of the day and reached nadir by 1800 (Figure 9). The overall concentration of these components was slightly higher than obtained from the leaf or the sward.

Compared to baleage harvested in the morning (AM), baleage harvested in the afternoon (PM) had increased concentrations of DM ($P < 0.001$), NDF ($P < 0.01$), and a

tendency for an increase in cellulose ($P < 0.06$) (Table 1). AM and PM baleage were similar in CP, ADF, and lignin. Compared to LO, HI baleage (HI) had increased CP concentration, yet decreased NDF, ADF, cellulose, and lignin ($P < 0.001$) (Table 1). There were no differences in DM for HI versus LO. There was an interaction between time of harvest and fertilization level for DM and lignin ($P < 0.001$). The interaction for DM was due to HI having greater DM in the PM baleage and lower in the AM baleage, whereas LO DM was unaffected by time of day. The lignin interaction was due to the unparallel changes between the AM and PM baleage within the HI and LO treatments.

Although the AM and PM baleages were similar in CP concentration, they differed in protein fractions (Table 2). PM baleage had decreased B_1 ($P < 0.002$), and increased B_2 ($P < 0.03$) and C ($P < 0.002$) fractions. HI decreased the C fraction ($P < 0.001$), and tended to increase the B_1 fraction ($P < 0.12$). There was a time of harvest by fertilization interaction for B_2 ($P < 0.02$), in which HI decreased the fraction in PM baleage but increased it in AM baleage. There were no differences between AM and PM baleages for the A or B_3 fractions (Table 2).

TNC, including starch and monosaccharides were increased in the PM baleage compared to the AM baleage ($P < 0.001$) (Figure 10). HI decreased TNC, including monosaccharides, starch, di-, and polysaccharides ($P < 0.01$). The interaction between time of harvest and fertilization level was significant for TNC, starch and monosaccharides ($P < 0.003$), and was due to HI causing a larger decrease in these carbohydrate fractions in the AM baleage (Figure 10).

Due to the fermentation process within the bales, PM baleage had increased levels of isopropanol and ethanol ($P < 0.001$) and a tendency for an increase in isobutyrate ($P < 0.09$) compared to AM baleage (Table 3). PM baleage had a higher pH ($P < 0.001$), whereas AM baleage had increased concentrations of lactate ($P < 0.001$) and propionate ($P < 0.05$). Compared to LO, HI decreased methanol and acetate ($P < 0.05$) and tended to decrease butanol ($P < 0.15$) and propionate ($P < 0.14$). There was a time of harvest x fertilization interaction (Table 3) for pH ($P < 0.001$), methanol ($P < 0.02$), and lactate ($P < 0.02$). The interactions for pH and lactate were due to the fact that the HI caused an increase in levels in the PM baleage and a decrease in the AM baleage. The methanol interaction arose due to

the larger response of AM baleage to HI. There were no differences in butyrate concentration between treatments (Table 3).

Baleage intake and digestibility

There were no differences among treatments in ad libitum DMI measured either as kilogram per day or percentage of BW (Table 4). Compared to steers when fed PM baleage, steers when fed AM baleage had greater digestible DMI ($P < 0.02$) and tended to be heavier in BW ($P < 0.11$). Steers fed HI had a decrease in ADF ($P < 0.03$), cellulose ($P < 0.05$), lignin ($P < 0.01$), and CP intake ($P< 0.01$). These steers also had a tendency for a decrease in NDF intake ($P < 0.06$). There was a time of harvest x fertilization interaction (Table 4) for lignin intake ($P < 0.01$) and CP intake ($P = 0.04$). The HI fertilization elicited a decrease in lignin intake in steers when fed PM baleage, whereas steers when fed AM baleage were unaffected by fertilization level. The CP interaction was due to the greater response to fertilization level for AM baleage than for PM baleage.

During the ad libitum phase of the trial, when steers were fed PM baleage they had increased TNC, starch and monosaccharide intakes ($P < 0.001$) (Table 5). Steers fed HI had decreased intakes of TNC, starch, monosaccharides, di- and polysaccharides ($P < 0.001$) when compared to steers fed LO. There was a time of harvest x fertilization interaction (Table 5) due to the HI causing greater decreases in TNC and starch intakes in PM baleage compared to AM baleage ($P < 0.02$).

During the balance trial, steers when fed AM baleage had increased DM digested per day ($P < 0.05$) and a tendency to increase DM digested as a percentage of intake ($P < 0.12$) (Table 6). There were no differences in DMI or DMI as percentage of BW between AM and PM baleage. Intakes of NDF, ADF, and cellulose were similar between AM and PM baleages (Table 7). Compared to steers when fed AM baleage, steers when fed PM baleage had a decrease in lignin intake ($P < 0.04$). There were no differences in NDF, ADF, cellulose or lignin fecal output, amounts digested in kilogram per day or as a percentage of intake between AM and PM baleage (Table 7).

Steers fed LO had increased fecal DM output ($P < 0.04$) and a tendency for greater DMI ($P < 0.08$) than steers fed HI (Table 6). Steers fed HI had increased DM digested as percentage of intake ($P < 0.05$). When compared to steers fed HI those fed LO had increased NDF ($P < 0.03$), ADF ($P < 0.03$), cellulose ($P < 0.04$), and lignin ($P < 0.003$)

intakes (Table 6). Steers fed LO also had increased fecal NDF, ADF, cellulose, and lignin outputs ($P < 0.03$). Steers fed HI had an increase in cellulose digested as a percent of intake ($P < 0.02$). There was a tendency for the steers fed HI to have an increase in the amount of NDF digested as a percent of intake ($P < 0.13$) and ADF digested in kg per day ($P < 0.09$). NDF and cellulose digested per day were similar for HI and LO.

N balance

Compared to steers when fed PM baleage, steers when fed AM baleage had increased urine N concentration ($P < 0.05$), N digested g/d ($P < 0.01$), and N digested as a percent of intake ($P < 0.01$) (Table 6). There was a tendency ($P < .10$) for steers when fed AM baleage to have increased urine urea N and blood urea N (Table 6). N intake, fecal N, urine urea N as a percentage of total urinary N, N retained, and N retained as a percentage of intake were similar for steers fed AM and PM baleages.

During the balance trial, steers fed HI had increased N intake ($P < 0.06$), urine N ($P < 0.001$), urine urea N concentration and as a percentage of total urinary N ($P < 0.001$), N digested kilogram per day and as a percentage of intake ($P < 0.02$), and blood urea N ($P < 0.05$) compared to steers fed LO (Table 6). Fecal N, N retained, and N retained as a percentage of intake were similar for both fertilization levels.

Intakes of TNC ($P < 0.006$) and monosaccharide ($P < 0.01$) were higher in steers when fed PM baleage compared to when fed AM baleage (Table 8). Starch, di- and polysaccharide intakes were similar between AM and PM baleage. Steers fed LO had increased TNC ($P < 0.001$), starch ($P < 0.001$), monosaccharide ($P < 0.05$), di-, and polysaccharide ($P < 0.02$) intakes. There were interactions between time of harvest and fertilization level (Table 8) for TNC ($P < 0.002$), starch ($P < 0.002$), and monosaccharide ($P < 0.04$) intakes. The interaction indicates that there was a decrease in intake of TNC constituents in AM compared to PM at HI, but a smaller decrease in monosaccharide intake in AM compared to PM at LO.

Insulin and ghrelin concentrations

Steers when fed AM baleage tended to have greater concentrations of insulin ($P < 0.12$) than when fed PM. There was an interaction between time of harvest and sample time (Figure 11). Insulin concentration in steers when fed AM baleage peaked 1 h post-

prandially, remained elevated, and began to return to initial levels approximately 2 h post-prandially (Figure 11). The insulin concentration in steers when fed PM baleage remained steady with only minor fluctuations. Initial and final concentrations of insulin were similar for PM and AM baleages. There was an interaction between fertilization level and sample time (Figure 12). The insulin concentration in steers fed LO increased post-feeding, and peaked 1 h post-feeding. The insulin concentration in steers fed HI increased at the time of feeding, decreased to baseline levels, and later peaked 1.5 h post-prandially (Figure 12). Initial and final insulin concentrations were similar for both HI and LO.

There were no interactions between harvest by sample time, fertilization by sample time, and harvest by fertilization by sample time. There were differences in ghrelin concentration among sample times ($P < 0.01$) (Figure 13). Ghrelin concentration increased until 1000, which was the initiation of the meal, and immediately began to decrease post-prandially and reached a nadir 2 hours post-feeding. There was no subsequent increase in concentration before the second meal of the day (Figure 13). There was a harvest by fertilization interaction for ghrelin concentration (Figure 14). Steers when fed PM baleage did not differ in ghrelin concentration on either fertilization level. On the other hand, steers when fed AM baleage had a large decrease in ghrelin concentration on HI compared to LO (Figure 14).

DISCUSSION

Similar to the rationale reported in Chapter 1, this experiment was performed to obtain information on changes in chemical composition of C_4 forages harvested in the morning compared to the afternoon. This was an attempt to connect changes in plant metabolism with improvements in animal performance using growing beef steers as a model. Gamagrass harvested as baleage was used to determine if differences in TNC concentration would persist through fermentation. Based on the data reported in the previous experiment it was expected that PM harvested baleage would have improved digestibility, and when fed to steers would increase their digestible DM intake.

Nitrogen fertilization has been reported to improve the quality of C_4 forages by increasing DM yield and N concentration (Johnson et al., 2001). The higher N fertilization level (150 kg N/ha) combined with the afternoon harvest should thereby optimize microbial

fertmentation in the rumen by providing a readily available energy source to ensure total capture of forage N into microbial N. This would improve beef steers' utilization of nutrients, specifically by increasing N retention.

Metabolic hormones were measured during the ad libitum phase to determine if they were associated with changes in intake in steers when fed PM compared to AM baleage. Certain hormone concentrations such as: ghrelin, leptin, CCK, and insulin can be sensitive to the nutritional state of the animal, and may be receptive to the amount and type of nutrient in the diet, specifically TNC. By studying the concentration of these hormones over Periods 1 and 2 of the experiment we hoped to link preferences for PM baleage with differences in plasma concentration. This would allow researchers to begin to understand how these animals are able to detect the subtle differences in forage quality and why they seek the higher carbohydrate diets. Upon analysis of the data, we decided in the end to only complete the characterization of ghrelin and insulin.

Morphological and chemical composition can affect utilization of forages by livestock. In pre-harvest samples collected in mid-May 2004, the leaf proportion of the gamagrass plant comprised approximately 64.9% of the total DM whereas the stem fraction comprised 15.7%. Forages with a higher leaf:stem ratio have been associated with improved intakes and digestibilities (Cherney et al., 1990). Gamagrass has previously been reported to have approximately 58% of DM as leaf and 25% of DM as stem (Burns et al., 1992; Magee, 2004). The leaf proportion of pre-harvest gamagrass had a 3 percentage unit increase in TNC concentration from 0600 to 1800, regardless of fertilization level (Figure 1). TNC, starch, monosaccharide, di- and polysaccharide concentrations all reached maximum levels at 1400 with 2 to 3 percentage unit increases. Monosaccharides contributed the highest proportion to the total TNC concentration; however, the largest fluctuation throughout the day occurs in the di- and polysaccharide constituents of TNC in both HI and LO. These results were in agreement with Melvin (1965) who stated that the largest increase in TNC in the afternoon was associated with sucrose, a disaccharide. There was a 3 percentage unit increase in DM throughout the day (Figure 4). CP did not fluctuate during the day and maintained a 3 percentage unit difference between HI and LO fertilization levels, which equates to the HI fertilization having a 20% increase in CP concentration. NDF, ADF, and

cellulose concentration decreased over the course of the day from 2 to 3 percentage units (Figure 7).

Compared to pre-harvest gamagrass, the baleage had a large decrease in overall TNC concentration in both PM and AM (Figure 10). PM baleage remained greater in TNC, starch, and monosaccharide concentration, but there were no longer any differences in di- and polysaccharide concentration between AM and PM. The soluble sugars in the pre-harvest plant exhibited the greatest fluctuation over the course of 12 hours. The decrease in TNC was a result of the bacterial fermentation of the readily accessible sugars and starch to production of organic acids and alcohols. This is primarily performed by lactic acid bacteria (Davies et al., 1998). The HI fertilization produced a greater decrease in TNC and its constituents, in AM compared to PM baleage. The use of N fertilization decreases the soluble sugar content by increasing the forages' growth rate (McDonald, 1981), and thus greatly affects the concentration of the carbohydrate fraction primarily used for excess storage. In the case of C_4 forages this would be the starch fraction. The AM/LO treatment was more similar in TNC composition to the PM/HI and PM/LO treatments. This indicates that the sugars in the PM/HI, PM/LO, and AM/HI were fermented to a greater extent during ensiling than the sugars in AM/LO, which seemed to be conserved.

PM baleage had a greater pH and percentage DM than the AM baleage (Table 3). A higher pH in low DM silages generally indicates proteolysis as well as increased butyrate concentration (Van Soest, 1994). However, there were no differences in butyrate or CP concentration between times of harvest. HI fertilization increased the pH and DM in PM baleage, and produced the opposite effect in AM baleage. These data directly contradict the results of Melvin (1965) who determined that the pH was decreased in silages harvested in the afternoon due to the greater initial concentration of water-soluble carbohydrates (WSC). His rationale was that increased WSC concentration provided an immediate substrate for lactic acid bacteria, which resulted in a faster drop in pH. There is a lot of variability in harvesting silage, but typically a pH < 4 is considered well preserved silage (McDonald, 1981). Differences in temperature and DM can affect optimal conditions for making silage, and therefore, interpretation of the results in this experiment are difficult due to the void of information available concerning gamagrass silage. Van Soest (1994) states that most tropical grasses do not make good silages because they are lower in sugar content and higher

in water content, which are both negative factors for fermentation. Wilting is commonly practiced when ensiling grasses to increase the DM and stabilize the forage. Therefore, I would have expected the PM baleage, which was higher in DM and TNC to provide superior quality forage by providing the anaerobic microbes with a larger readily available energy source to promote a drop in pH.

Fermentation converts the TNC fraction to organic acids and alcohols. Compared to AM, PM baleage had increased ethanol and isopropanol concentration, and a tendency for increased isobutyrate (Table 3). HI tended to decrease these two alcohols in PM and increase them in AM baleage. Ethanol was the primary alcohol produced regardless of treatment, although the concentration was at least two-fold greater in PM baleage. Methanol and butanol were found in the lowest concentration out of all of the alcohols and HI tended to cause larger decreases in these constituents in PM compared to AM baleage.

Acetate and butyrate concentration were similar between AM and PM baleage (Table 3). HI caused greater decreases in concentration in AM compared to PM baleage. Propionate and lactate were increased in concentration in AM baleage. This is contradictory with other research that reported propionate and lactate were increased when ryegrass silages contained high concentrations of WSC, 25 % of the DM, compared to low WSC, 6.6% of the DM (Davies et al., 1998).

The chemical composition differences that are occurring between treatments may be explained by the different fermentation pathways of lactic acid bacteria. There are the homolactics which ferment hexoses primarily to lactic acid, and the heterolactics whose end products are a combination of lactic acid and other products, including ethanol (McDonald, 1981). I think the differences in baleage composition suggest that homolactics are the primary fermenters in AM baleage due to the fact that a high lactate to acetate ratio is indicative of this type of fermentation (Davies et al., 1998). The PM baleage had slightly higher concentrations of acetate compared to lactate and also contained a higher proportion of alcohols, and thus may be produced by the heterolactics.

Ad libitum intake data of this experiment included all 8 steers for both periods. During the balance trial there was not enough feed supply for the AM/LO and PM/LO treatments, hence the second period only included 5 steers. Ad libitum DMI of steers as a percentage of BW was similar when steers were fed AM or PM baleage (Table 4). HI

increased DMI as a percentage of BW, in steers when fed AM and had no effect on steers when fed PM. During the balance trial steers fed LO had increased DMI (kg/d), which was enhanced further in steers consuming AM/LO compared to PM/LO (Table 6). In this phase HI caused a decrease in DM digested in steers when fed AM and an increase in DM digested in steers when fed PM. This improvement for PM baleage was a result of the improvement in the synchronization of energy and protein levels in the rumen in the PM/HI treatment. For similar reasons, steers fed AM/LO had the greatest amount of DM digested. The overall DM digestibility of the forage was increased by 2 percentage units for steers when fed AM baleage primarily due to greater DMI with similar fecal DM outputs. DM digestibility was improved by 3 percentage units in steers fed HI because fecal DM output was reduced. In contrast, Archibeque et al. (2001) reported decreased IVTDMD with increased levels of N in gamagrass hay.

Fiber intakes and digestibility of steers when fed AM or PM baleages were similar in both phases of the experiment, despite the PM baleage being 2 percentage units greater in NDF concentration (Tables 1, 4 and 7). Fisher et al. (1999) reported decreases in NDF, ADF, and cellulose composition of PM hays compared to AM hays, which they concluded was due to dilution with TNC. Others have reported that there are no differences in NDF, ADF, and cellulose composition due to time of day (Orr et al., 2001; Fisher et al., 2005; Burns et al., 2005). Burns et al. (2005) determined that goats and cattle fed PM alfalfa hays had increased NDF and ADF digestibility as a percent of intake, whereas sheep were similar between hays. Steers fed LO had increased fiber and lignin intake and fecal output during both phases of the experiment (Tables 4 and 7). This was primarily due to the increase in concentration in all fiber components and lignin in LO compared to HI fertilization (Table 1). Cellulose digested as a percent of intake was improved by 4 percentage units, and there were tendencies to improve NDF digested as a percent of intake and ADF digested in kilograms per day. In direct contrast with the findings from this trial, Archibeque et al. (2001) reported increases in cellulose and lignin concentration in gamagrass hay fertilized with a high level of N. However, another study characterizing C_4 grasses, determined that those fertilized with increasing N levels had decreased NDF%, ADF%, and increased IVOMD (Johnson et al., 2001).

Compared to steers when fed AM baleage, steers when fed PM baleage had increased TNC and monosaccharide intakes during both phases of the experiment (Tables 5 and 7). Starch intakes were increased only during the ad libitum phase. Steers fed LO had increased intakes of TNC, including all of its constituents. This supports previous research stating that a high N fertilization application to switchgrass lowers the TNC content and sometimes lowers the starch or soluble sugar content as well (Fisher et al., 2005). The interaction for TNC and its constituents was due to the steers fed the AM/LO treatment having similar responses to those fed the PM/HI and PM/LO treatments (Table 5). During the balance trial steers fed the AM/LO treatment had even greater TNC, starch, di-, and polysaccharide intakes than those fed either PM treatment.

By design, the HI fertilization increased CP intakes in both PM and AM fed steers, by 10.3 % and 20.5 %, respectively, during the ad libitum phase. Fecal N was similar amongst all treatments. However, urine N, N digested in grams per day and as a percentage of intake were all increased in steers when fed AM compared to PM baleage. Most likely the improvement in digestibility was due to the 5 g/d decrease in fecal N in the AM baleage. It could also be a result of the increased B_1 protein fraction found in AM baleage since there was no difference in the A fraction. This fraction is soluble and readily available to the animal. There was no difference in the A (NPN) fraction between fertilization levels, which was intriguing since there were different levels of substrate available (50 vs. 150 kg of N) for the plants to uptake and build protein. Therefore, there must be a maximal level at which plants can fix this N into plant protein. The C protein fraction was higher in PM baleage, which should also lower N digestibility since this fraction is completely indigestible in the rumen. LO fertilization tended to decrease urine N output to a much greater extent in steers when fed PM compared to steers when fed AM. HI fertilization primarily increased the B_2 protein fraction in AM baleage and B_1 and B_3 in PM.

There was a tendency for urine urea N and plasma urea N to be increased in steers when fed AM compared to PM baleage (Table 6). This is due to the numerical increase in the A protein fraction of the forage without an ample carbohydrate source to ensure the total capture of forage N as microbial N. The NPN in fermented silages is not efficiently utilized by bacteria because it is not accompanied by a rapidly available source of carbohydrate, which is used up for the most part during fermentation (Van Soest, 1994). Steers fed LO

had a greater decrease in urine and plasma urea N when it was harvested in the PM as opposed to the AM. There were no differences in N retained among all treatments (Table 6). This may be due to the large SE associated with this variable since there was only a small number of steers used. Therefore, the hypothesis that synchronization of readily fermentable carbohydrates with an increased N concentration would increase the amount of N retained for growth of beef steers was not congruent with the results found in this experiment. Overall, the TNC were not conserved well in baleage, thereby decreasing the energy available to microbes residing within the rumen since they can not utilize organic acids and alcohols, which are the end products of their fermentation. In addition, the initial concentration of TNC in gamagrass may be too low to drop the pH rapidly, which is necessary for proper fermentation.

Insulin has a similar role in ruminant metabolism compared to non-ruminants. The major difference is that ruminants have a steady supply of glucose produced from gluceneogenesis of amino acids and organic acids coming in from the diet. Insulin's role as a regulatory mechanism of ruminant intake still needs to be elucidated. In this experiment, steers when fed AM baleage tended to have greater insulin concentrations than when fed PM baleage (Figure 11). Although the PM baleage had a greater concentration of TNC, we have observed that the products of fermentation formed during ensiling can be quite different between harvest times. Since TNC will be utilized by the microbial population for energy, the resulting lactate, acetate, propionate, and butyrate may actually be the primary regulators of insulin production in ruminants. It was previously reported that propionate and butyrate could stimulate insulin secretion, but only at supra-physiological levels (Stern et al., 1970). Since lactate is the major component varying in concentration between AM and PM baleage this organic acid may be a regulator of insulin in the rumen. I have not uncovered any data to support this conclusion.

The interaction between time of harvest and sample time suggests that the insulin concentration in AM fed steers reached a maximum value 1 h post-prandially. Insulin concentration remained elevated until 2 hours post-prandially in which it began to taper off. The PM fed steers insulin concentration never increased. On the contrary, levels remained steady throughout the day. The PM baleage fed to these steers had increased alcohols and decreased lactate concentration. These changes in bale composition may account for

changes in hormone activity. However, it would have been beneficial to have determined the concentrations of SCFA in the rumen to see if there were any changes in rumen fermentation due to the different diets. The overall DMI of steers when fed AM baleage was greater compared to when fed PM. Insulin release may be stimulated by increases in intake, regardless of sugar concentration. Compared to ruminants on high roughage diets, ruminants on low roughage diets tended to have greater insulin concentrations (Evans et al., 1975). Regardless of the amount of roughage in the diet, insulin concentrations were increased in sheep and cattle 30 min post-prandially and again 5.5 hours post-feeding when compared to levels 30 min pre-prandially (Evans et al., 1975).

Steers fed LO had increased insulin concentration 1 hr post-prandially, whereas steers fed HI had an increase 1.5 h post-feeding (Figure 12). It may be that the microbes have rapid utilization of N in steers fed LO since there is less N available, which would be followed by a quicker formation and release of organic acids. The interaction between fertilization level and time of harvest was due to the large increase in insulin concentration in steers when fed AM/HI compared to AM/LO, yet steers when fed PM had similar insulin concentrations from both N fertilization levels.

Ghrelin concentration was elevated prior to feeding and reached a maximum level immediately before the time of feeding (Figure 13). This was followed by an immediate decrease of 20 pg/mL in steers' ghrelin concentration, which fell below initial levels 2 h pre-feeding. Based on prior research with sheep (Sugino et al., 2004), a larger response in ghrelin concentration would have been elicited if the steers were fed discrete meals rather than ad libitum. The release of ghrelin has been associated with the anticipation of a meal, and decreases in concentration by increasing frequencies of meals, and is virtually eliminated in sheep fed ad libitum (Sugino et al., 2004). Mature Holstein cows have greater ghrelin levels that fluctuate during the day, which was not seen with 3 month old calves (Miura et al., 2004). The steers used in this study were still growing, and as a result their ghrelin concentrations may have been decreased.

HI produced greater increases in ghrelin concentration in steers when fed AM compared to PM baleage (Figure 14). The change from LO to HI fertilization in AM steers resulted in almost a 40 pg/mL increase in ghrelin concentration, whereas in PM fed steers it was less than a 5 pg/mL increase in ghrelin. HI also increased DMI as a percentage of BW

in steers when fed AM baleage and had no effect in steers when fed PM. One hypothesis is the increase in ghrelin concentration on this AM/HI diet stimulated an increase in DMI in these steers. There was a considerable amount of variation in ghrelin plasma concentration between steers and therefore, a study using a larger amount of animals would need to be conducted to accurately quantify changes in ghrelin levels focused around feeding time.

GENERAL SUMMARY OF CHAPTERS 1 AND 2

Many of the papers addressing animal preference of afternoon forages use diet selection as their criterion and focus primarily on C_3 grasses and legumes. Due to the metabolic differences in C_4 plants we decided to measure TNC composition in gamagrass, because of its high leaf:stem ratio and digestion kinetics that have the potential to favor animal performance (Burns et al., 1992). One of the positive attributes of C_4 plants is that are favored by higher temperatures, which make them an important forage source, specifically in the southern US. Similar to Burns et al. (2005) we received variable results between experiments measuring sustained intake of a C_4 grass. By comparison it may be a result of the difference in species used or the different harvesting methods.

In both experiments TNC increased from 0600 to 1800 in leaf, stem, and whole canopy samples. In the first experiment the overall TNC concentration decreased with maturity of the plant from mid-July through late August. This also decreased the difference between PM and AM forages, which started out as a 4 percentage unit difference and decreased to less than a 2 percentage unit at time of harvest. This decreased to a 1.5 percentage unit difference when gamagrass hay was baled. This hay was harvested late August following a period of inclement weather. In the second experiment the grass was harvested in May and the overall TNC concentration was double the amount of the previous year. This suggests that producers may want to harvest grass earlier to maintain the greatest TNC concentration and to maximize the quality of PM compared to AM forages. Trade-offs between yield and TNC concentration would have to be a money making decision by the forage producer. They could also choose to set aside a portion of their crop as high quality forage. Monosaccharides comprise the largest portion of TNC; however, the diurnal fluctuation seems to occur primarily in the di- and polysaccharide portion. Determination of the precise time in the afternoon that provides the maximal benefit still needs elucidation.

In the first experiment there were no differences in ad libitum DMI of goats; however, due to the increase in digestibility of PM baleage, DDMI was increased from 4 to 7% compared to AM. Steers had decreased DDMI from 9 to 9.5% when fed PM baleage compared to AM. The overall concentration of TNC was decreased drastically in the fermented baleage, and there was only a 1.2 percentage unit difference (3.5 vs. 2.3%) between harvesting in the PM and AM, respectively. The AM baleage also had a higher lactate concentration and a lower pH, which are characteristics associated with better ensiling. The pH of PM baleage was greater than 5, which means it was a lower quality product. The increase in TNC and lower moisture level did not enhance the fermentation of PM baleage compared to AM, which we had expected. PM and AM baleages were similar in butyrate concentration, which has been shown to cause decreases in intake. There was a greater amount of alcohols produced in the PM baleage, which may be the reason for the decrease in DMI. Alcohols may affect the palability of silages. Fiber digestibilities of goats were improved due to increased TNC in PM hays, which are rapidly fermented in the rumen thereby improving digestibility. As seen in the experiment with steers however, fermentation decreased the overall TNC concentration and the difference between AM and PM, hence similar fermentation in the rumen and fiber digestibility.

Compared to AM, PM improved N retention in goats but had no effect in steers. The addition of a protein supplement improved N retention in goats since gamagrass was limited in protein. However, the combination of PM and supplementation produced no further benefits in spite of the attempt to synchronize rapidly fermentable carbohydrate with protein in the rumen. Differences between HI and LO for N retention in steers was not obtained because of the large SE. There were low animal numbers in this study, and period had a tendency to be significant ($P < 0.08$) since there were 8 steers in period 1 and 5 steers in period 2. Supplementation of C_4 forages may be necessary to meet the energy and protein requirements of growing animals.

Previous research documenting preferences for PM harvested forages has focused on animals being offered choices side by side. Provenza and Forbes (2000) suggested that animals learn associations between foods due to the discomfort they cause or the feedback signals they get after digesting a feed. This may indicate that they can detect subtle differences between feeds offered as pairs because they can associate a particular feed with a

particular metabolic sensation. When feed is offered in a switchback design the animal may not remember the sensation for the particular feed offered in the previous period. Therefore, in this type of feeding situation the animal may be regulating intake based on something that is affecting it at that moment rather than through a feedback loop (ie. fiber content or organic acid and alcohol concentrations).

The other purpose of this project addressed endocrine regulation of voluntary intake. Ghrelin levels in steers increased 1 h prior to the morning feeding and decreased post-prandially. There were no differences in concentration between times of harvest (AM or PM) or fertilization level (HI or LO). There was an increase in AM/LO, but no changes in PM between HI and LO. Samples were collected on the last 2 days of ad libitum intake, and within a steer ghrelin levels were similar for both days suggesting that it was a physiological control regulating intake. The fluctuations prior to feeding would have been greater if more steers were used due to the large variation among animals. Some animals had two distinct peaks in ghrelin concentration before both feeding times, whereas others had what seemed to be random fluctuations or only a slight increase. Previous reports using sheep have documented similar patterns with peak concentrations at feeding time (Sugino et al., 2002, 2004). However, in sheep and horses fed ad libitum there were no differences in ghrelin concentration throughout the day (Sugino et al., 2004; Gordon and McKeever, 2005). Even though our steers were fed ad libitum, the anticipation of new feed at 1000 and 1530 may have stimulated the release of ghrelin.

Low quality diets are often first limited by physical characteristics of the diet; therefore, it may be more beneficial to study the role of ghrelin, insulin, and other metabolic hormones in ruminants fed a higher quality forage or concentrate. Levels have also been shown to change with growth and physiological state. Therefore, research should focus on studying changes in concentration from calving to weaning, since ghrelin levels are expected to increase with age. Also this would allow livestock producers to take advantage of ghrelin's stimulatory effects on appetite and provide focus on increasing feed intake during these specific times in life.

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Figure 1. The effects of N fertilization and time of day on TNC and its constituents concentrations in pre-harvest gamagrass.

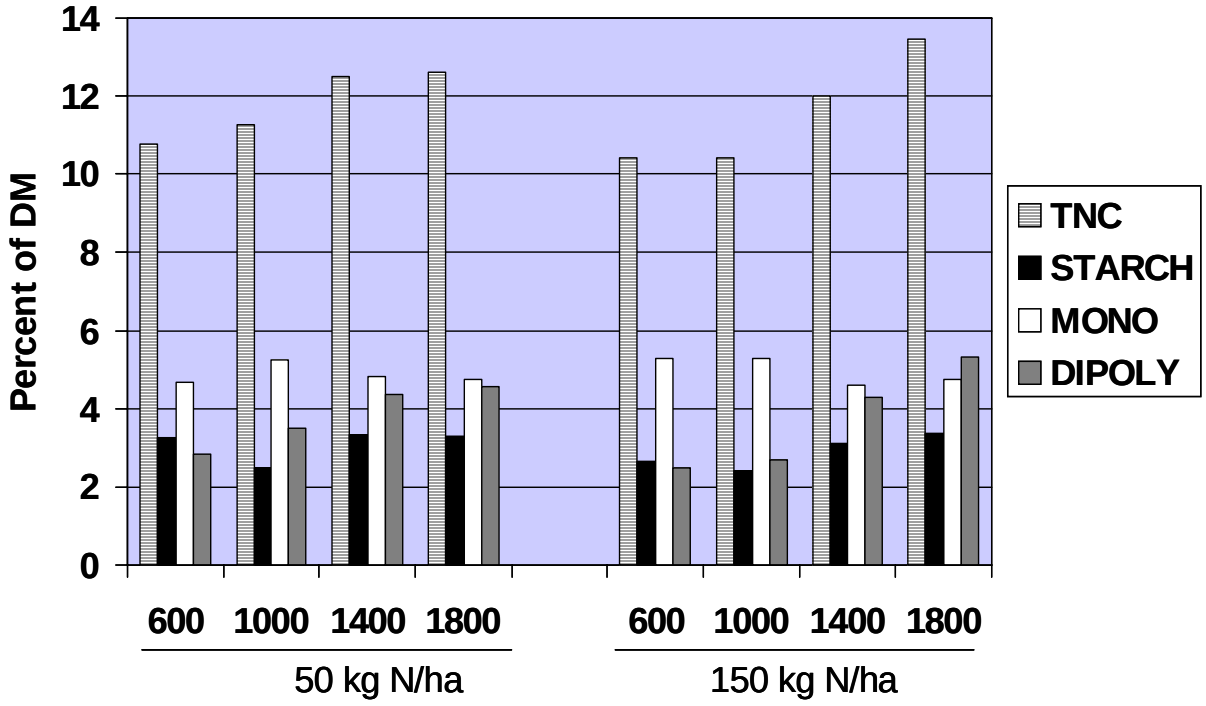


Figure 2. The effects of N fertilization and time of day on TNC and its constituents concentration in the leaves of gamagrass.

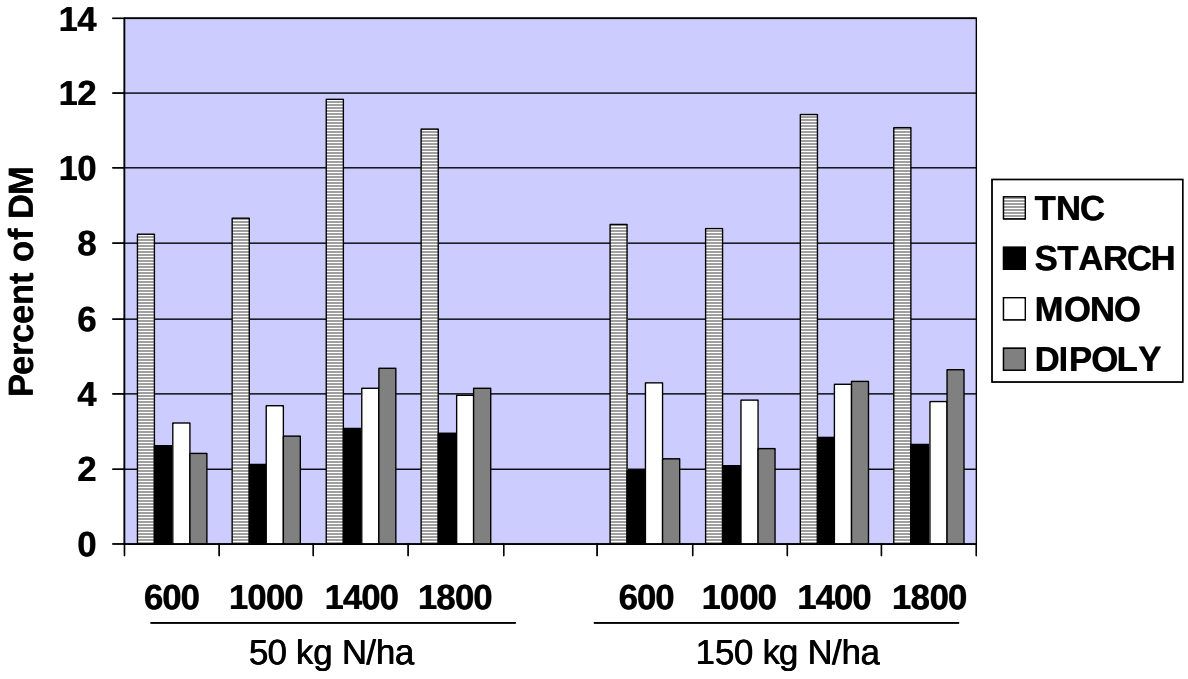


Figure 3. The effects of N fertilization and time on TNC and its constituents concentration in the stems of gamagrass.

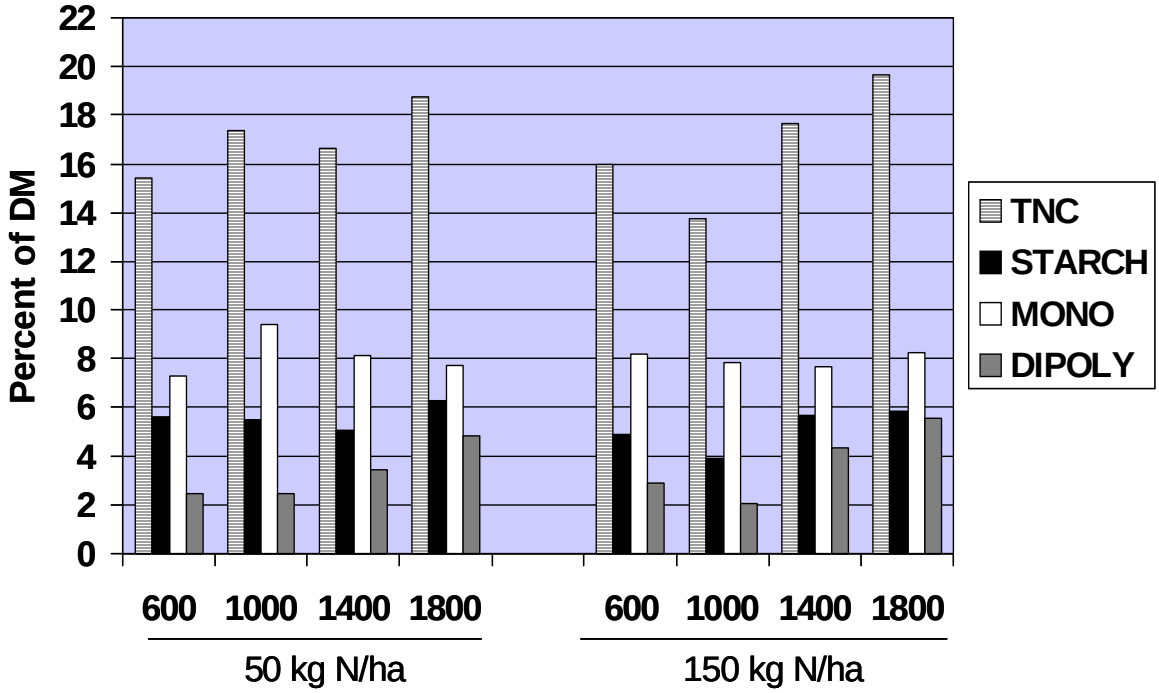


Figure 4. The effects of N fertilization and time of day on DM and CP in the leaves of gamagrass.

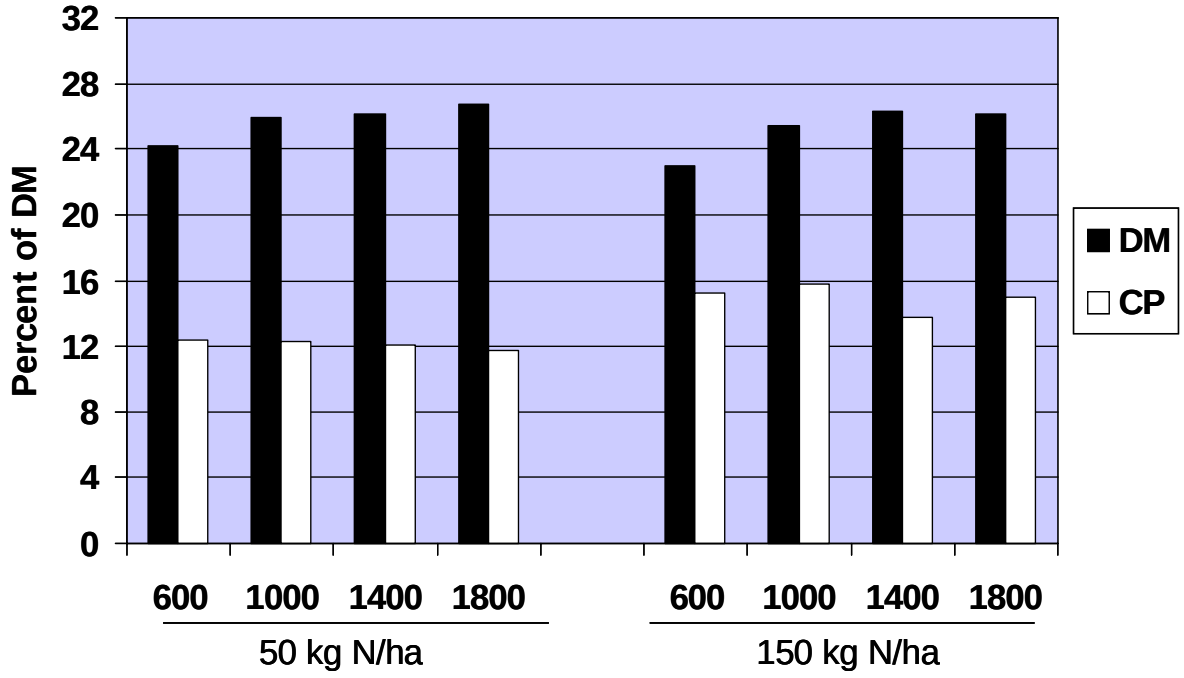


Figure 5. The effects of N fertilization and time of day on DM and CP in pre-harvest gamagrass.

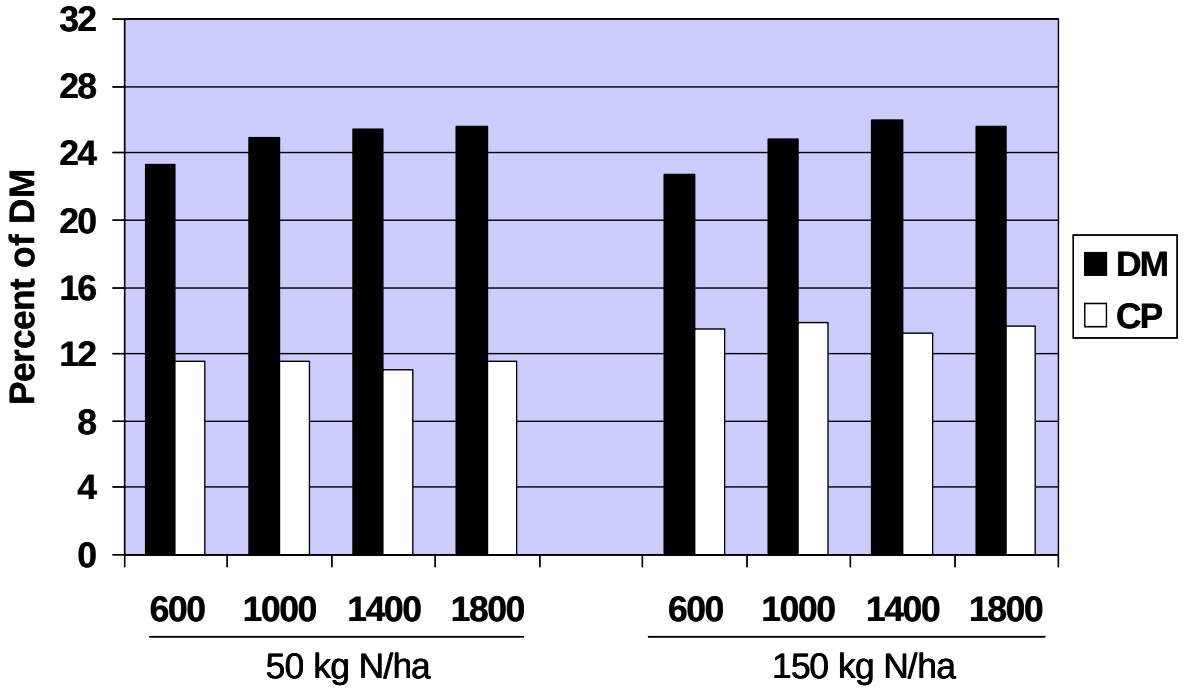


Figure 6. The effects of N fertilization and time of day on NDF, ADF, and cellulose in the leaves of gamagrass.

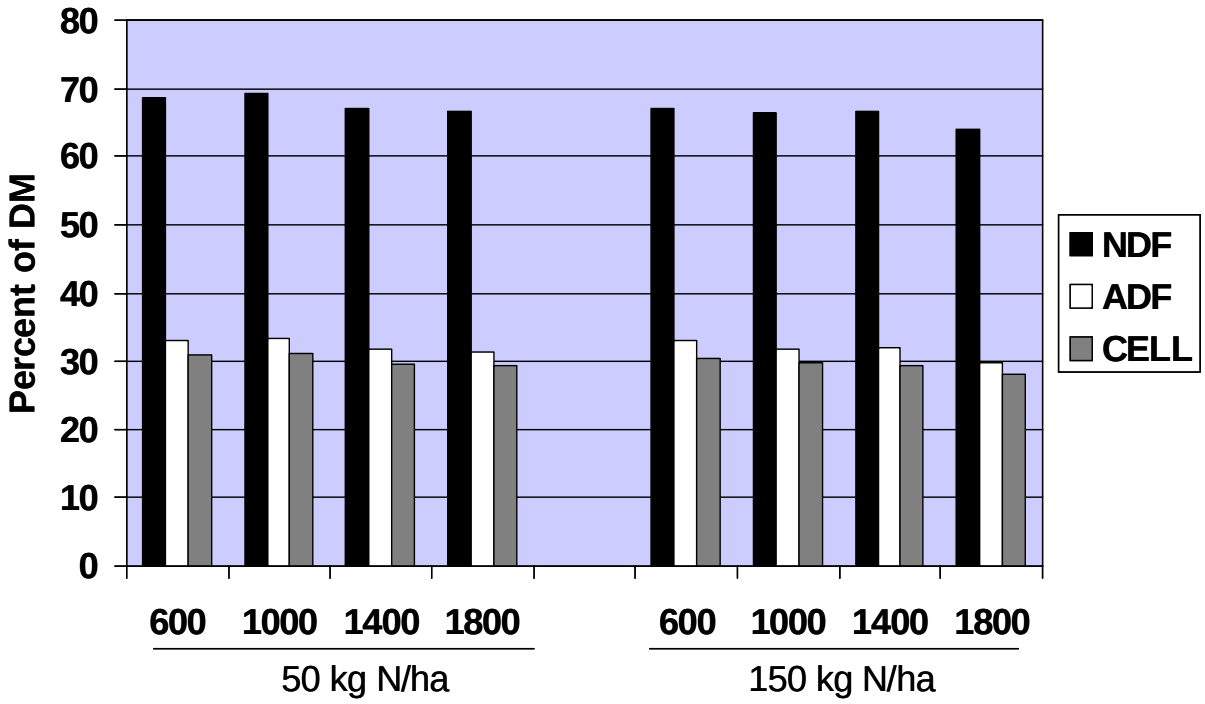


Figure 7. The effects of N fertilization and time of day on NDF, ADF, and cellulose in pre-harvest gamagrass.

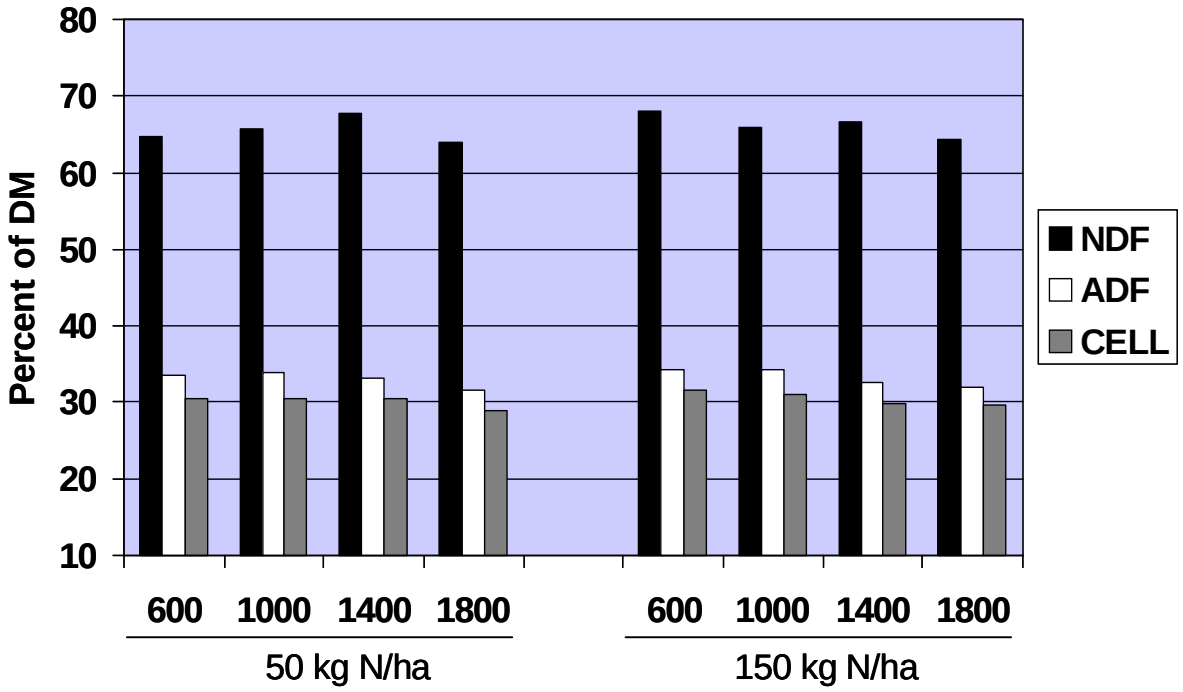


Figure 8. The effects of N fertilization and time of day on DM and CP in the stems of gamagrass.

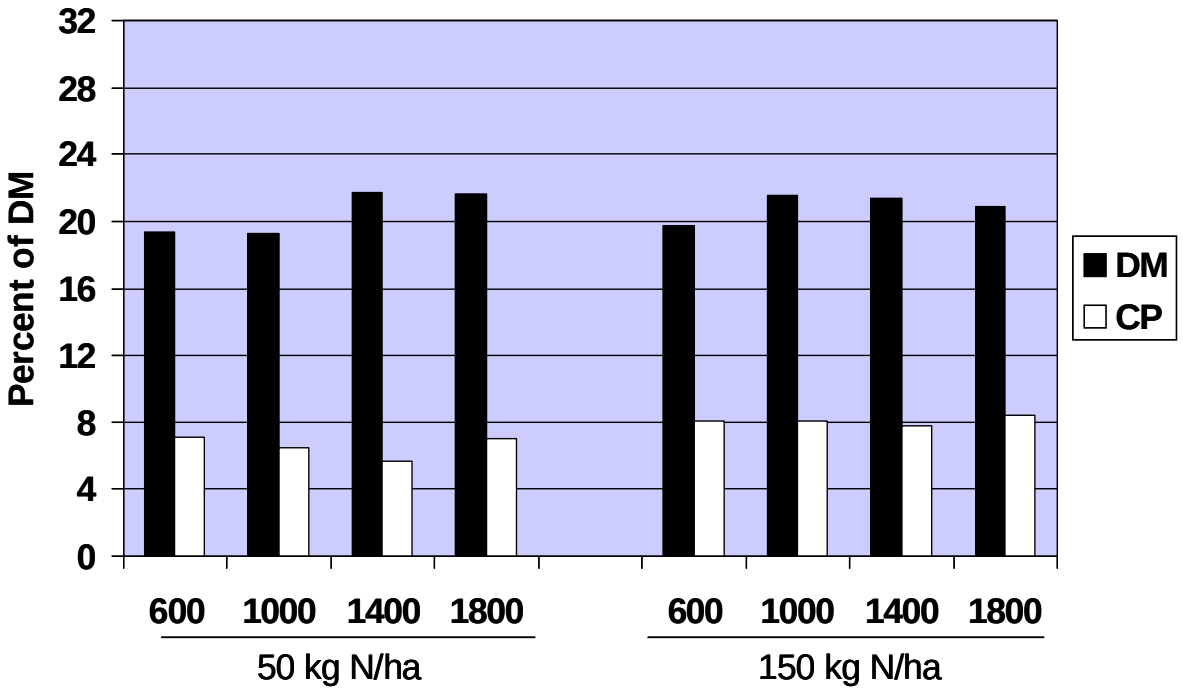


Figure 9. The effects of N fertilization and time of day on NDF, ADF, and cellulose in the stems of gamagrass.

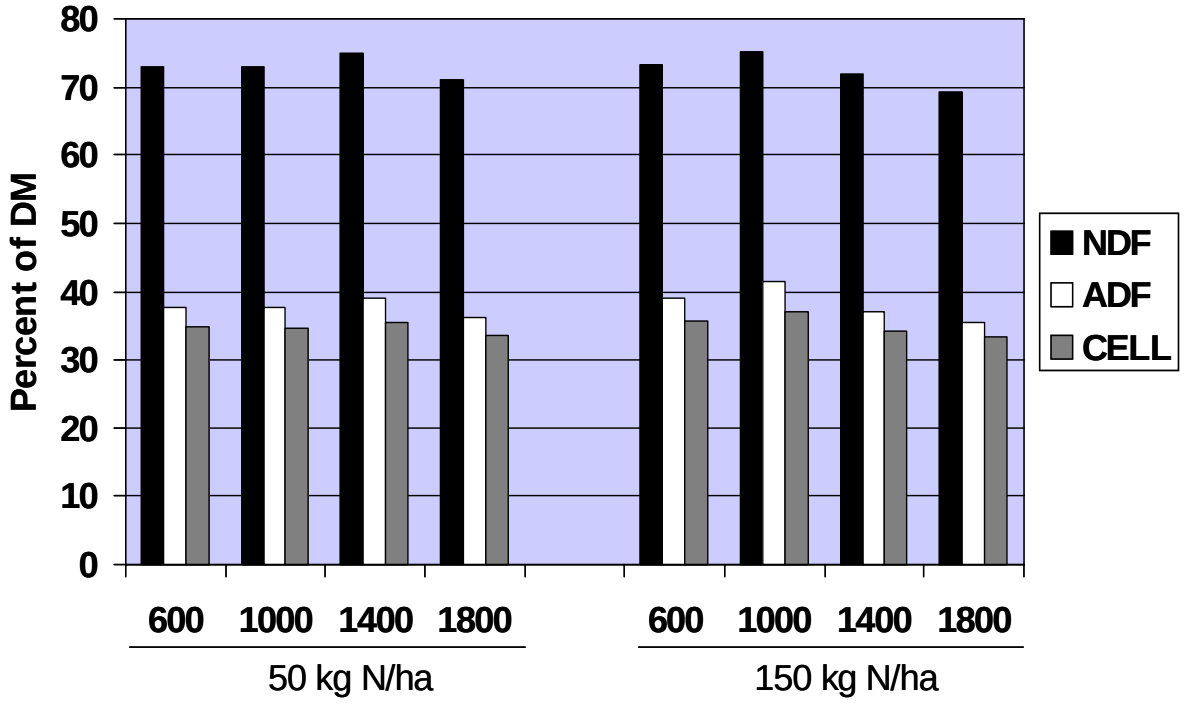


Table 1. Nutrient Composition of Baleage.

	PM		AM			*P*-value		
Item	HI	LO	HI	LO	SE	AM/PM	HI/LO	X
DM %	30.2	27.7	24.4	27.5	0.49	0.001	0.475	0.001
	% of DM							
CP	11.7	9.69	11.9	9.49	0.14	0.932	0.001	0.124
NDF	67.0	68.8	66.4	67.6	0.33	0.008	0.001	0.319
ADF	39.0	39.6	38.2	39.4	0.25	0.332	0.001	0.843
Cellulose	34.3	34.8	33.6	34.7	0.22	0.061	0.001	0.135
Lignin	4.00	4.78	4.36	4.48	0.07	0.638	0.001	0.001

[a]SE is for the interaction.
n = 8 for PM/HI
n = 7 for PM/LO
n = 8 for AM/HI
n = 6 for AM/LO

Figure 10. Baleage composition of TNC and its components (P < 0.003; SE = 0.12).

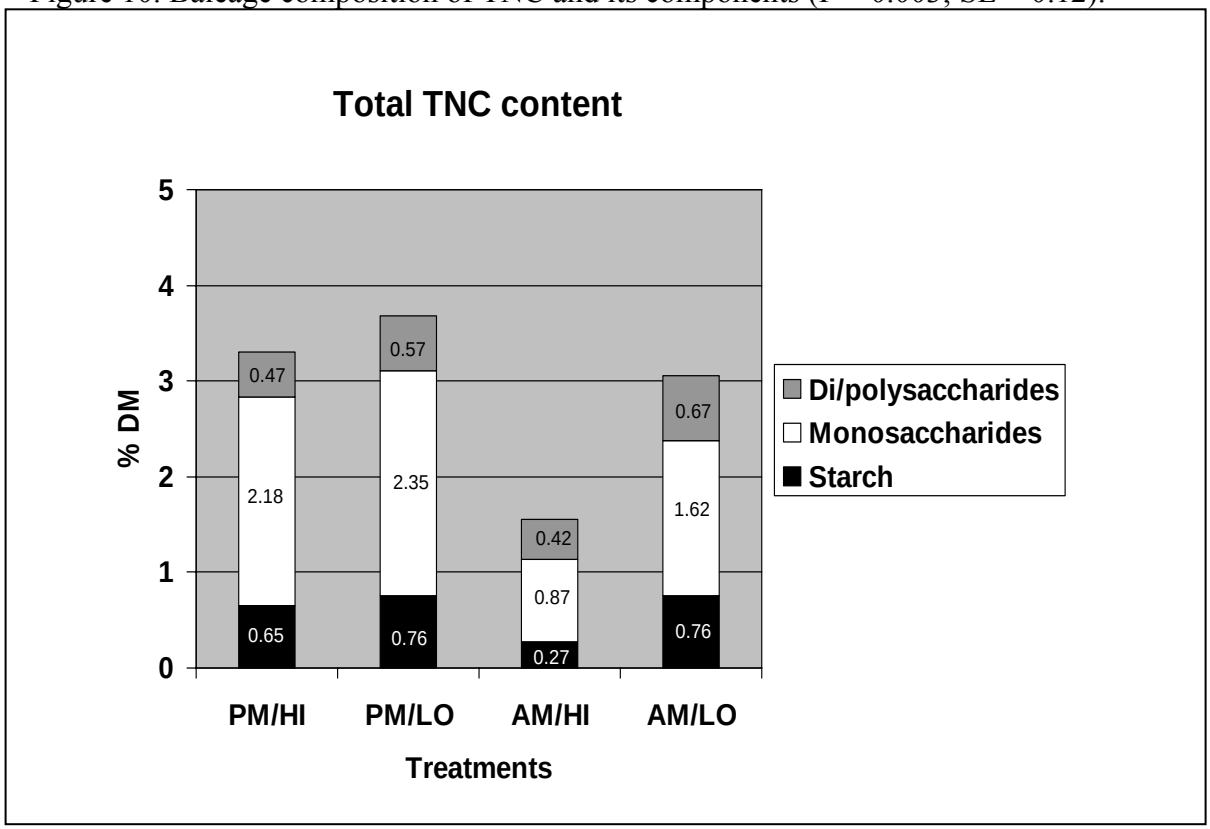


Table 2. Protein fractions of baleage.

	PM		AM			*P*-value		
Item	HI	LO	HI	LO	SE	AM/PM	HI/LO	X
CP, % DM	11.7	9.69	11.9	9.49	0.14	0.932	0.001	0.124
	% of CP							
A	55.2	57.2	57.0	57.1	0.88	0.362	0.259	0.304
B_1	1.98	0.23	3.37	3.38	0.49	0.002	0.113	0.109
B_2	21.9	23.0	22.1	19.8	0.56	0.029	0.353	0.015
B_3	16.4	13.9	14.9	14.8	0.83	0.721	0.168	0.193
C	4.65	6.76	3.55	4.70	0.32	0.002	0.001	0.169

n = 8 for PM/HI.
n = 7 for PM/LO.
n = 8 for AM/HI.
n = 6 for AM/LO.

Table 3. pH, alcohol, SCFA, and lactate concentration of baleage.

	PM		AM			*P*-value		
Item	HI	LO	HI	LO	SE[a]	AM/PM	HI/LO	X
pH	5.51	5.30	4.36	4.56	0.06	0.001	0.868	0.001
	g/kg of DM							
Methanol	0.12	0.70	0.48	0.52	0.11	0.399	0.005	0.014
Isopropanol	4.56	5.95	0.80	0.16	0.52	0.001	0.441	0.044
Ethanol	13.58	16.52	7.52	5.72	1.38	0.001	0.658	0.073
Butanol	0.77	1.28	0.27	1.00	0.46	0.361	0.149	0.799
Acetate	12.04	12.58	11.57	15.83	1.12	0.190	0.028	0.083
Propionate	0.44	0.74	0.80	0.80	0.11	0.046	0.136	0.159
Butyrate	3.67	4.60	4.11	4.43	0.64	0.821	0.297	0.614
Isobutyrate	0.20	0.05	0.03	0.03	0.06	0.092	0.158	0.187
Lactate	9.63	7.73	37.84	45.00	2.00	0.001	0.165	0.021

[a]SE used is largest for the unbalanced data since the # of bales was not equal on all treatments.

n = 8 for PM/HI.

n = 7 for PM/LO.

n = 8 for AM/HI.

n = 6 for AM/LO.

Table 4. Ad libitum DM, CP, and fiber intakes of steers.

	HI fertilization		LO fertilization			*P*-value		
Item	PM baleage	AM baleage	PM baleage	AM baleage	SE	Baleage	Fert	HxS
BW, kg	315	318	338	343	24.7	0.104	0.521	0.678
Ad lib DM intake, kg/d	5.85	6.05	6.23	6.06	0.13	0.877	0.228	0.167
DMI, % BW	1.87	1.93	1.86	1.81	0.11	0.928	0.713	0.105
Digestible DM intake	3.05	3.27	2.99	3.29	0.08	0.015	0.820	0.660
NDF intake	3.86	3.97	4.29	4.13	0.10	0.754	0.059	0.144
ADF intake	2.21	2.28	2.46	2.41	0.06	0.857	0.028	0.260
Cellulose intake	1.98	2.01	2.16	2.12	0.05	0.896	0.047	0.494
Lignin intake	0.22	0.26	0.30	0.27	0.01	0.530	0.004	0.003
CP intake	0.68	0.73	0.61	0.58	0.02	0.667	0.005	0.041

Digestile DMI uses digestion coefficient from balance trial for each steer.
n = 4 for all treatments

Table 5. Ad libitum intake of non-structural carbohydrates of steers consuming gamagrass baleage.

	HI fertilization		LO fertilization			*P*-value		
Item	PM baleage	AM baleage	PM baleage	AM baleage	SE	Baleage	Fert	HxS
TNC intake, g/d	200.7	89.8	240.7	186.2	0.008	0.0001	0.0003	0.015
Starch intake	39.9	14.6	48.9	46.4	0.002	0.0004	0.0002	0.001
Monosaccharide intake	133.9	51.5	154.9	97.8	0.007	0.0001	0.005	0.09
Di & polysaccharide intake	26.9	23.5	36.9	41.7	0.004	0.869	0.007	0.357

n = 4 for all treatments

Table 6. Dry Matter and N intake, digestion, retention, and blood urea N in steers fed gamagrass baleage harvested in the PM or AM and either fertilized with LO or HI N levels.

	Fertilization Level				Harvest			
Item	HI	LO	[a]SE	*P*-value	PM	AM	[a]SE	*P*-value
BW, kg	314	335		0.541	321	329		0.223
DM intake, kg/d	5.66	6.25	0.23	0.075	5.77	6.14	0.22	0.217
DMI, % of BW	1.83	1.84	0.12	0.937	1.84	1.83	0.11	0.900
Digestible DM intake, per 100kg of BW	0.98	0.93	0.06	0.574	0.94	0.97	0.05	0.640
Fecal DM, kg/d	2.63	3.08	0.15	0.037	2.83	2.89	0.13	0.742
DM digested, kg/d	3.02	3.16	0.11	0.352	2.94	3.25	0.10	0.049
DM digested, % intake	53.53	50.58	1.07	0.055	50.97	53.15	0.98	0.120
N intake, g/d	106.0	95.2	0.01	0.064	98.1	103.0	0.01	0.342
Fecal N, g/d	51.02	53.39	0.01	0.571	54.70	49.71	0.01	0.215
Urine N, g/d	52.28	38.30	2.29	0.001	42.25	48.32	2.10	0.053
Urine urea N, g/d	33.86	18.42	2.06	0.001	23.86	28.42	1.89	0.096
Urine urea N, % of total	64.65	44.27	2.23	0.001	52.92	56.00	1.93	0.209
N digested, g/d	54.95	41.51	2.08	0.001	43.37	53.08	1.91	0.004
N retained, g/d	2.67	3.21	2.95	0.885	1.12	4.76	2.71	0.324
N digested, % intake	51.99	44.06	1.83	0.019	44.24	51.81	1.45	0.006
N retained, % intake	2.41	2.90	2.88	0.893	0.75	4.56	2.64	0.292
Blood Urea N, mM	6.04	4.12	0.59	0.051	4.72	5.44	0.47	0.103

[a]SE is the largest number for any treatment.
n = 4 for PM/HI
n = 3 for PM/LO
n = 4 for AM/HI
n = 2 for AM/LO

Table 7. Fiber intakes and digestibilties of steers during the balance trial.

	Fertilization Level				Harvest			
Item	HI	LO	SE	*P*-value	PM	AM	SE	*P*-value
NDF intake, kg/d	3.75	4.26	0.16	0.032	3.89	4.11	0.15	0.289
Fecal NDF, kg/d	1.77	2.12	0.10	0.023	1.90	1.99	0.09	0.468
NDF digested, kg/d	1.98	2.14	0.08	0.155	1.99	2.12	0.08	0.241
NDF digested, % intake	52.82	50.21	1.25	0.126	51.28	51.74	1.15	0.764
ADF intake, kg/d	2.17	2.47	0.10	0.032	2.25	2.39	0.09	0.275
Fecal ADF, kg/d	1.02	1.22	0.06	0.031	1.08	1.15	0.06	0.331
ADF digested, kg/d	1.15	1.26	0.03	0.089	1.17	1.23	0.04	0.319
ADF digested, % intake	52.94	50.97	1.22	0.224	52.07	51.84	1.12	0.871
Cellulose intake, kg/d	1.92	2.18	0.09	0.038	2.00	2.10	0.08	0.346
Fecal cellulose, kg/d	0.66	0.83	0.04	0.014	0.73	0.77	0.04	0.456
Cellulose digested, kg/d	1.26	1.34	0.05	0.184	1.27	1.33	0.05	0.337
Cellulose digested, % intake	65.61	61.69	1.10	0.019	63.71	63.59	1.01	0.927
Lignin intake, kg/d	0.24	0.29	0.01	0.003	0.25	0.28	0.01	0.037
Fecal lignin, kg/d	0.28	0.35	0.02	0.018	0.31	0.31	0.02	0.913

[a]SE is the largest number for any treatment.
n = 4 for PM/HI
n = 3 for PM/LO
n = 4 for AM/HI
n = 2 for AM/LO

Table 8. TNC intake of steers during the balance trial.

	PM baleage		AM baleage			*P*-value		
Item	HI	LO	HI	LO	SE[a]	Baleage	Fert	HxS
TNC intake, g/d	201.6	212.2	89.6	223.5	0.018	0.006	0.001	0.002
Starch intake, g/d	39.2	44.2	17.3	57.1	0.003	0.208	0.001	0.002
Monosaccharide intake, g/d	134.2	131.1	50.0	107.5	0.008	0.012	0.044	0.038
Di & Polysaccharide intake, g/d	28.2	36.7	22.3	54.2	0.007	0.403	0.019	0.113

[a]SE is the largest number for any treatment.
n = 4 for PM/HI
n = 3 for PM/LO
n = 4 for AM/HI
n = 2 for AM/LO

Figure 11. Insulin concentration in steers fed gamagrass baleage harvested in the morning (AM) or afternoon (PM). There was a tendency for an interaction ($P < 0.12$; SE = 1.2) between harvest and time of sampling. Steers were fed at 1000.

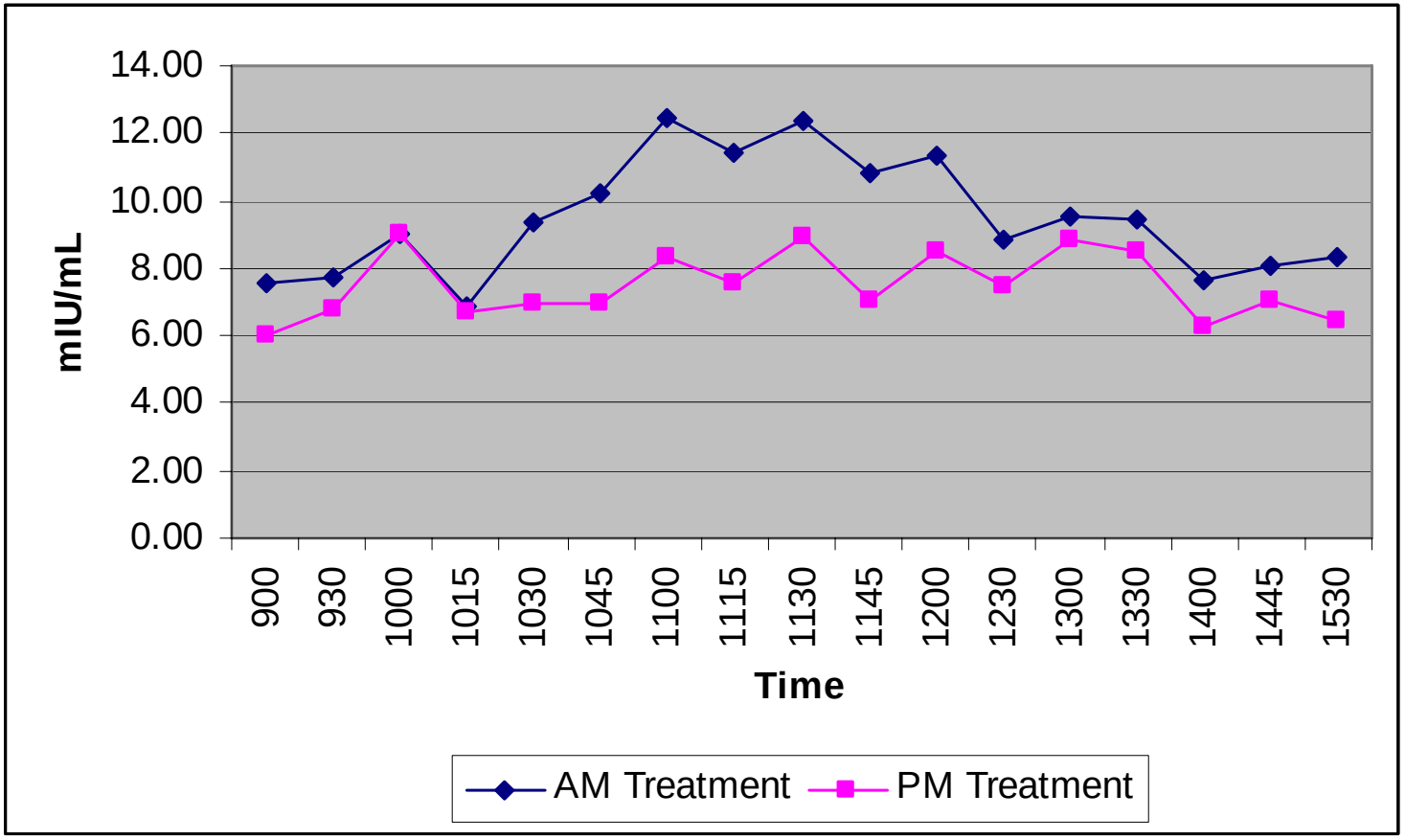


Figure 12. Insulin concentration in steers fed gamagrass baleage fertilized with either 50 kg N/ha (LO) or 150 kg N/ha (LO) levels. There was a tendency for an interaction between fertilization level and time of sampling ($P < 0.07$; SE = 1.6). Steers were fed at 1000.

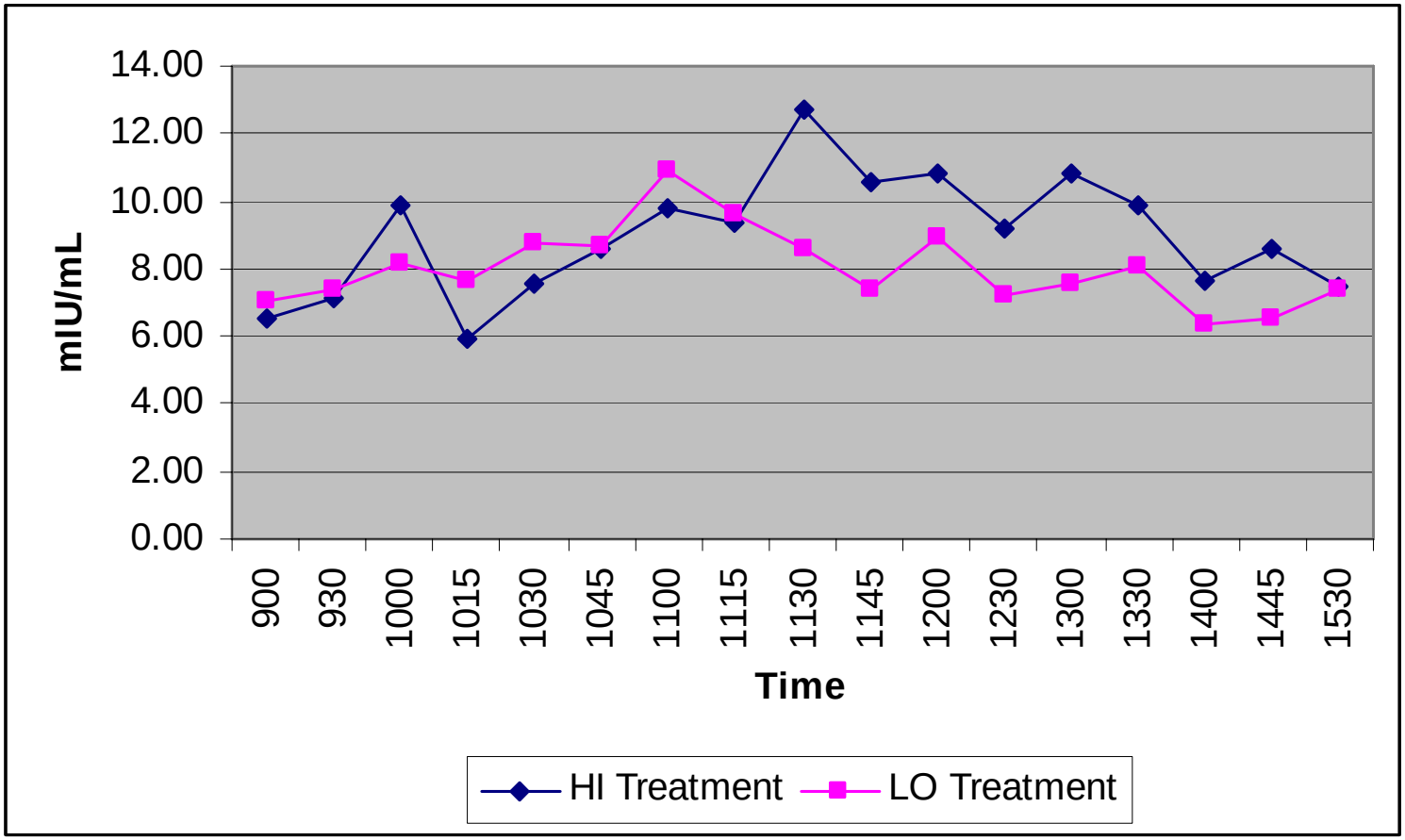


Figure 13. The average ghrelin concentration for steers across all sample times. Sample times differ ($P < 0.007$; SE = 7.9). Steers were fed at 1000.

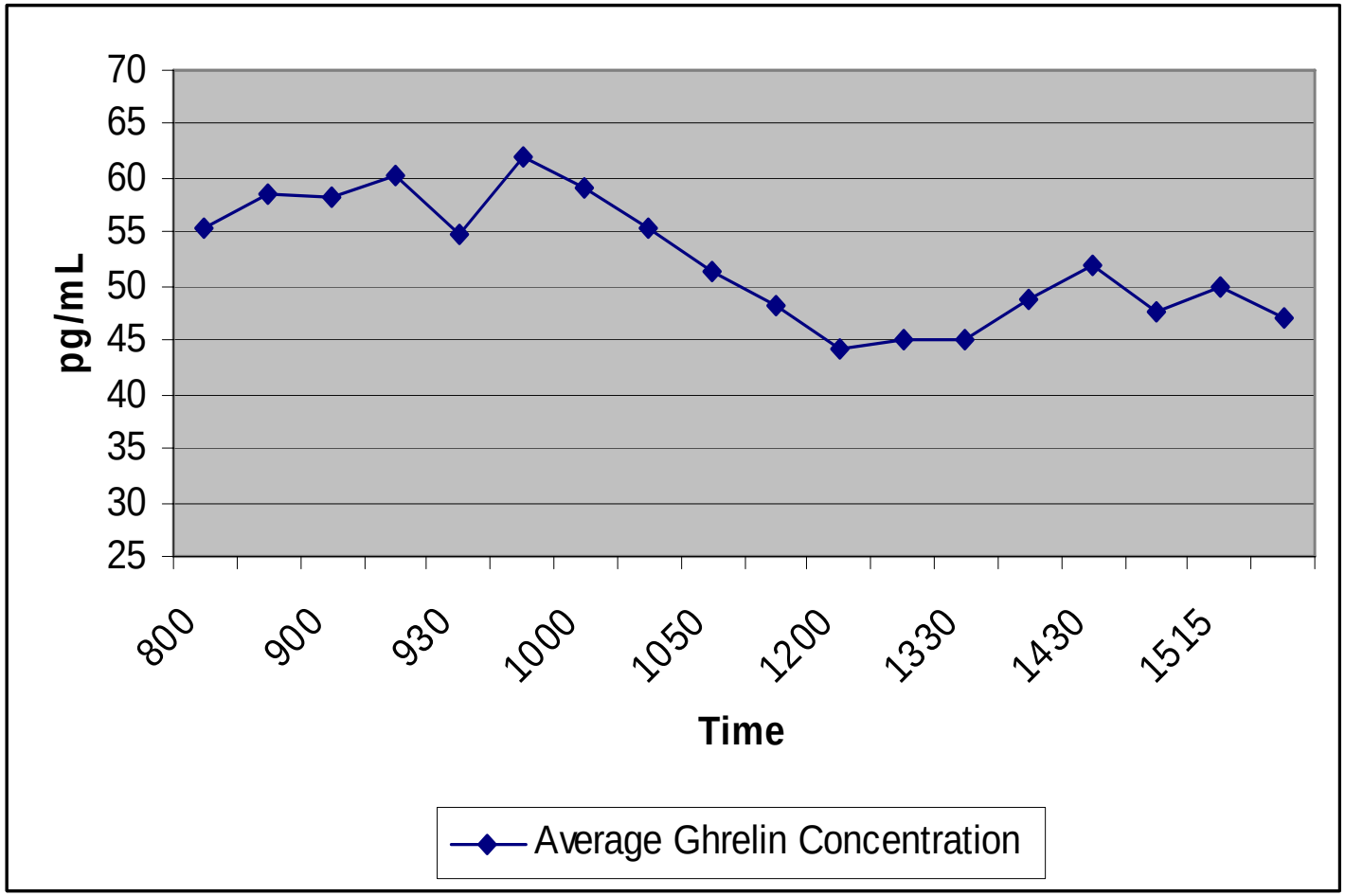
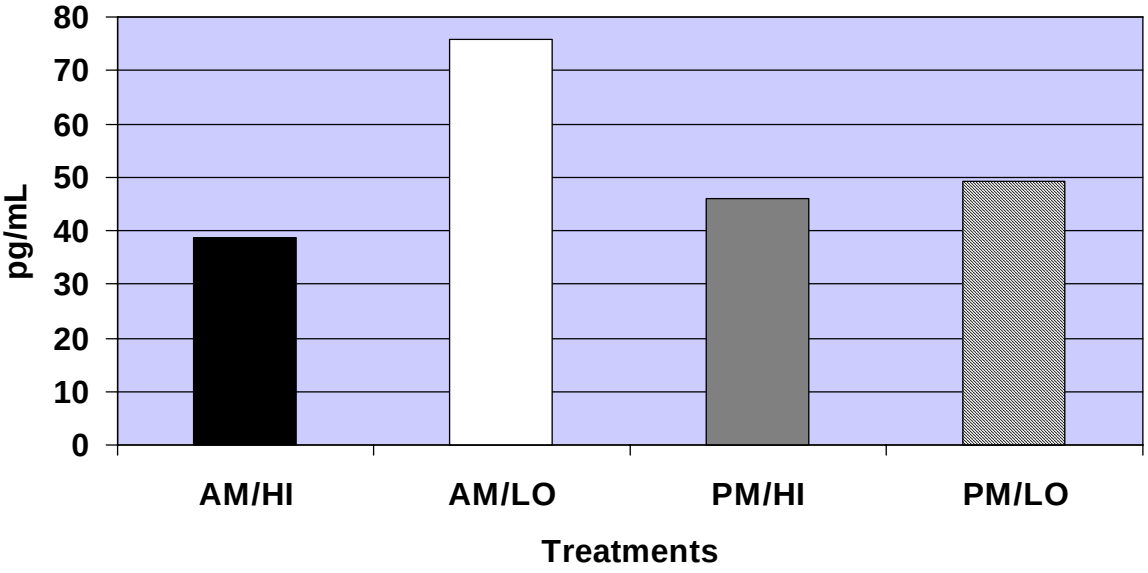


Figure 14. The interaction ($P < 0.001$; SE = 9.2) for average plasma ghrelin concentration between harvest and fertilization.



APPENDICES

Appendix A. NIR equation descriptive information for gamagrass hay fed to goats.

Data set	Variable	# Samples in calibration	Mean	Range	SEC	SEC RSQ	SECV	SECV 1-VR
AF + ORTS	MONO	72	3.11	1.94 - 4.56	0.080	0.976	0.118	0.948
AF + ORTS	STARCH	72	1.54	.83 - 2.19	0.082	0.931	0.111	0.875
AF + ORTS	TNC	71	6.07	3.66 - 8.03	0.132	0.979	0.203	0.950
AF + ORTS	FREE_GLU	71	1.39	.96 - 1.78	0.038	0.965	0.064	0.905
AF + ORTS	DM	53	94.27	93.32 - 96.48	0.091	0.989	0.187	0.953
AF + ORTS	NDF	52	73.68	68.36 - 79.74	0.797	0.929	1.007	0.886
AF + ORTS	ADF	52	38.94	34.01 - 47	0.657	0.953	0.762	0.937
AF + ORTS	CELL	53	33.92	29.79 - 41.45	0.477	0.964	0.684	0.926
AF + ORTS	LIGNIN	51	4.82	3.64 - 7.17	0.155	0.967	0.231	0.926
AF + ORTS	ADF ASH	50	0.21	0.02 - 0.94	0.068	0.856	0.102	0.678
AF + ORTS	NITROGEN	55	1.21	.7 - 1.70	0.044	0.969	0.046	0.966
FECALS	DM	119	94.32	91.36 - 97.26	0.375	0.942	0.491	0.900
FECALS	NDF	119	66.65	60.08 - 74.29	1.003	0.872	1.120	0.840
FECALS	ADF	117	34.86	30.11 - 39.48	0.612	0.904	0.760	0.851
FECALS	CELL	117	26.67	21.37 - 29.79	0.436	0.940	0.538	0.909
FECALS	LIGNIN	113	7.67	6.16 - 10.19	0.291	0.905	0.343	0.868
FECALS	ADF_ASH	114	0.58	0.14 - 1.41	0.123	0.801	0.145	0.725
FECALS	NITROGEN	118	1.54	1.17 - 2.12	0.027	0.984	0.037	0.968
PLT SEPS	TNC	46	7.83	1.70 - 16.94	0.182	0.998	0.498	0.986
PLT SEPS	STARCH	47	2.70	0.22 - 6.51	0.123	0.996	0.234	0.986
PLT SEPS	MONO	46	3.26	0.82 - 7.06	0.160	0.989	0.309	0.959
PLT SEPS	FREE GLU	46	1.53	0.66 - 3.47	0.045	0.996	0.164	0.947

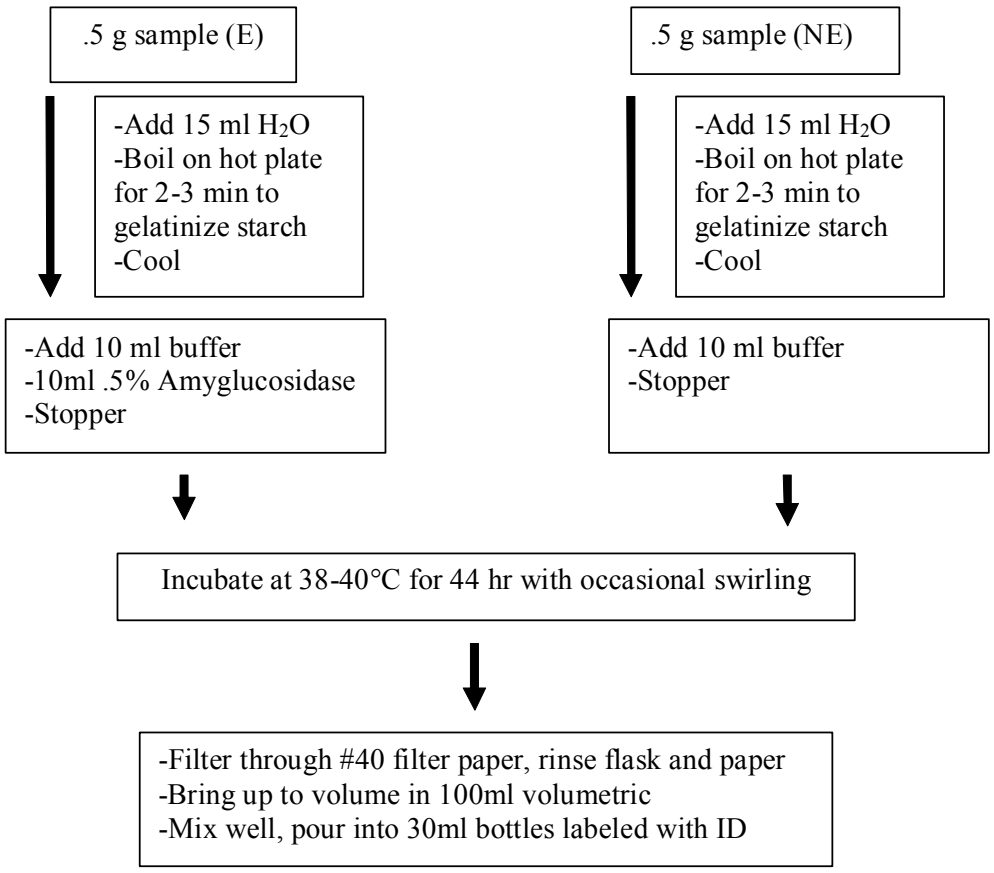
SEC = Standard Error of Calibration
SECV = Standard Error or Cross Validation
SEC RSQ = fraction of explained variance for SEC
SECV 1-VR=similar to RSQ (one minus the variance ratio (explained variance divided by total variance)) for each validation group

Appendix B. Flow chart of laboratory analysis of TNC, starch, monosaccharide, and free glucose concentration.

FLOW CHART FOR TNC

Two different Technicon (Bran+Luebbe) auto-analyzer cartridges were used to determine starch, as glucose (method G-142-95 Rev. 1) and Total Sugar/Reducing Sugars (method G-227-99 Rev. 2).

E = Amyglucosidase added to convert starch to glucose
NE = No amyglucosidase added



NE →	Glucose Cartridge	'free' or endogenous glucose
NE →	Reducing Sugar Cartridge (no hydrolysis)	soluble sugars only; monomers
E →	Glucose Cartridge	glucose from starch + 'free' glucose
E →	Total Sugar Cartridge (hydrolysis with HCl)	TNC- contains starch as glucose, monomers di- and short-chain polysaccharides

Appendix C. NIR equation descriptive information for gamagrass baleage fed to steers.

Data set	Variable	# Samples in calibration	Mean	Range	SEC	SEC RSQ	SECV	SECV 1-VR
AF + ORTS	MONO	63	1.62	0.23 - 3.33	0.212	0.903	0.289	0.825
AF + ORTS	STARCH	64	0.70	0.15 - 1.31	0.070	0.936	0.103	0.865
AF + ORTS	TNC	66	2.71	.86 - 4.47	0.187	0.953	0.298	0.884
AF + ORTS	FREE_GLU	63	0.45	.11 - 1.10	0.066	0.911	0.088	0.843
AF + ORTS	DM	174	94.43		0.495	0.937	0.646	0.892
AF + ORTS	NDF	171	72.86	64.9 - 81.95	0.938	0.947	1.126	0.924
AF + ORTS	ADF	172	40.21	34.01 - 47.97	0.732	0.933	0.855	0.909
AF + ORTS	CELL	169	34.89	29.79 - 41.64	0.490	0.962	0.590	0.945
AF + ORTS	LIGNIN	168	5.03	3.65 - 7.17	0.268	0.828	0.310	0.778
AF + ORTS	ADF ASH	166	0.27	.02 - 1.1	0.100	0.816	0.140	0.641
AF + ORTS	NITROGEN	172	1.36	0.58 - 1.98	0.032	0.987	0.042	0.978
FECALS	DM	130	91.14	91.36 - 98.19	0.368	0.944	0.504	0.894
FECALS	NDF	132	66.74	60.08 - 74.29	1.089	0.810	1.226	0.798
FECALS	ADF	129	35.19	30.11 - 41.45	0.672	0.910	0.828	0.863
FECALS	CELL	131	26.54	21.37 - 29.79	0.516	0.919	0.600	0.892
FECALS	LIGNIN	123	7.88	5.89 - 11.71	0.251	0.959	0.350	0.920
FECALS	ADF_ASH	124	0.70	0.14 - 2.84	0.172	0.888	0.210	0.834
FECALS	NITROGEN	129	1.58	1.17 - 2.12	0.024	0.989	0.035	0.977
PLT SEPS	TNC	75	10.06	2.55 - 19.68	0.308	0.995	0.489	0.988
PLT SEPS	STARCH	77	3.02	0.48 - 6.51	0.199	0.987	0.293	0.971
PLT SEPS	MONO	75	4.26	0.82 - 8.44	0.237	0.985	0.454	0.944
PLT SEPS	FREE GLU	73	1.81	0.66 - 3.66	0.110	0.981	0.173	0.952
PLT SEPS	DM	32	92.46	90.00 - 94.83	0.105	0.994	0.370	0.933
PLT SEPS	NDF	32	65.95	52.51 - 75.14	0.497	0.993	0.740	0.985
PLT SEPS	ADF	32	34.04	29.09 - 41.47	0.391	0.980	0.684	0.936
PLT SEPS	CELL	30	30.86	26.24 - 35.57	0.372	0.978	0.555	0.949
PLT SEPS	LIGNIN	29	3.22	2.05 - 5.39	0.252	0.904	0.375	0.787
PLT SEPS	ADF ASH	28	0.16	0.04 - 0.27	0.057	0.294	0.067	0.039
PLT SEPS	NITROGEN	29	1.88	0.89 - 2.47	0.036	0.994	0.053	0.987

SEC = Standard Error of Calibration
SECV = Standard Error or Cross Validation
SEC RSQ = fraction of explained variance for SEC
SECV 1-VR=similar to RSQ (one minus the variance ratio (explained variance divided by total variance)) for each validation group