THE EFFECT OF SEASONAL VARIATION AND FERTILIZATION ON THE NUTRITIONAL CONTENT OF C4 GRASSES

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Rachel Marie Miller

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APPROVED:

Jessica Suagee-Bedore, PhD, PAS
Committee Director

Philip Urso, PhD
Committee Member

Katelyn Kaufman, PhD, PAS
Committee Member

John B. Pascarella, PhD
Dean, College of Science and Engineering Technology

ABSTRACT

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Horses are an herbivorous species of mammal that evolved to consume grass and other forages for their nutritional needs. They quality of forage consumed is important to the health of the animal, and there can be an effect of season and fertilization seen in the nutritional content of the grass. Many studies have looked at the nutritional content of C3 grasses, but few have looked at C4 grasses common in Texas. Therefore, the focus of this research was to analyze the nutritional content of 3 varieties of warm season grasses in response to season, time of day, and fertilization. Plots were set up in a grid design to prevent cross-contamination. Half of the plots were fertilized at a rate of 260 kg/ha (FERT) and half were unfertilized (CON). Samples were collected at 6AM and 6PM on, or as close to the first of the month as possible, from June through September, which encompassed the entire growing season for these grasses. Samples were analyzed for NDF, ADF, crude protein, and the water-soluble sugars fructose, glucose, and sucrose using standard laboratory procedures. The fiber content of all three varieties of grass was lowest in June and highest in August and September. There was an average NDF content of 63.8 ± 0.5 %DM and an average ADF content of 34.0 ± 0.6 %DM in June, versus an average NDF content of 68.1 ± 0.5 %DM and an average ADF content of 40.2 ± 0.6 %DM in September. The crude protein in all three varieties of grass was higher in FERT samples than CON samples, with an average protein content of 10.5 ± 0.5 %DM in CON samples and an average protein content of 13.4 ± 0.5 %DM in FERT samples. There

were no significant effects of time, treatment, or month in the sugars, or any effects of interactions. This indicates that C4 grasses grown in East Texas have a lower overall nutritional content than C3 grasses, especially in the sugar content. Therefore, horses that may be sensitive to rich pasture may be safely grazed throughout the growing season of these C4 grasses.

KEY WORDS: Equine nutrition; Sugars; ADF; NDF; Crude protein; C4 grasses

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CHAPTER I

Introduction

Horses are an herbivorous species of mammal from the family Equidae that have long been used by humans to assist in work, transportation, and companionship (Endenburg, 1999). Horses are part of a specific class of herbivores known as hind-gut fermenters (Alexander, 1993). Their digestive tract is characterized by a rapid digestion in the stomach, a quick enzymatic digestion in the small intestine, and a long fermentation in the large intestine (Santos et al., 2011). Fermentation in the large intestine breaks down indigestible fibers, such as cellulose and hemicellulose, into volatile fatty acids (VFA), which is one of the primary energy sources of the horse (Suagee et al., 2010).

For 5 million years, the fermentation of fibrous plants was the only nutrition horses received. Feeding grains to supplement energy did not become common until the 1800's, when horses began doing more work and spending less time at pasture (Reynolds, 1882). Feeding grain began to be more common than feeding forages, with some people recommending a 1:1 ratio of grain to forage (Williams and Ellis, 1945). Producers began searching for ways to improve animal efficiency while also decreasing the amount of forage needed in the diet, leading to the improvement of forage species through selective breeding and management strategies, such as mowing, fertilizing, and weeding. These methods have increased the nutritional content of the forages commonly grazed by horses.

The purpose of this study was to investigate how the above methods have influenced the nutritional content of forage species commonly grazed by horses in East Texas.

CHAPTER II

Literature Review

Nutrients in the Equine Diet

Horses need six classes of nutrients to survive: proteins, fats, carbohydrates, vitamins, minerals, and water. For optimal health, these nutrients must be present in appropriate quantities in the diet. The most important of these is water, for without water, a horse will only survive for 3-4 days (Pagan, 2008; Hampson et al., 2010).

Protein. Amino acids, which make up proteins, are known as the building blocks of the body. In horses, protein is the largest non-water component of the body (Stratton, 2018). There are 20 amino acids predominantly found in animal proteins, enabling each protein to be made of a unique sequence of these amino acids. In all animals, amino acids are broken into two classes: essential amino acids and nonessential amino acids. Essential amino acids are those that cannot be synthesized *de novo* by mammalian enzymes and must be present in the diet (Stratton, 2018). Nonessential amino acids can be made *de novo* from other amino acids and other constituents and are not required to be in the diet if sufficient precursor molecules are present. Typically, equine diets supply all amino acids to prevent deficiencies. Of the 20 amino acids, 9 are considered essential in the horse (NRC, 2007). Of the 9 essential amino acids, 3 are considered limiting in common horse diets. Limiting amino acids are those amino acids supplied in minimal amounts in the diet, and therefore are most likely to limit growth or performance in a horse. The first limiting amino acid in horses is lysine, while the second and third are believed to be threonine and methionine, respectively (NRC, 2007; Winsco et al., 2011). Producers

know these amino acids are limiting in the horse, yet lysine is the only limiting amino acid for which there is enough research to support a daily recommended intake (Graham et al., 1994; NRC, 2007).

Dietary Fat. Lipids are a broad category of molecules that are loosely defined as biological molecules that are hydrophobic in nature and potentially soluble in organic solvents (Fahy et al., 2005). There are many different lipids, such as phospholipids and sterols, but the primary form of concern in equine nutrition are triglycerides. Triglycerides are also known as dietary fat and are primarily used as an energy source for muscle to prevent depletion of glucose in endurance-type exercise (Holloszy and Kohrt, 1996). These molecules can also be stored as body fat to serve as energy storage between meals or when resources are unavailable. Dietary fats are used as carriers to transport fat-soluble vitamins throughout the digestive tract in order for absorption into the body (NRC, 2007), and also provide essential fatty acids that are necessary for proper immune function (Hall et al., 2004). Natural horse diets rarely contain more than 5% fat on a dry matter basis, and average around 3% fat (Pagan, 1998; Frape, 2004). Fat supplementation is becoming a more common method for treating a variety of nutritional concerns, such as an inability to maintain or gain weight or for meeting caloric needs in cold climates. They are also used for aesthetic reasons such as improving coat quality. Despite this, the National Research Council does not have a stated requirement for total fat in the diet (NRC, 2007).

Carbohydrates. Carbohydrates serve primarily as a source of energy for the horse. They can be broken down into two further categories: Structural and Nonstructural carbohydrates.

Structural. Structural carbohydrates are found in plant material and include cellulose, hemicellulose, and pectins. They are known as structural carbohydrates due to their use as structural support for the plant within the cell walls. Cellulose and hemicellulose are abundant in grasses, while pectins are found in non-traditional roughages such as beet pulp. Mammals do not produce the enzyme necessary to digest the β1-4 glycosidic linkages that make up structural carbohydrates, however, hindgut fermenters and ruminants host a microbial population with this capability (Silva et al., 2016). The fermentation of these carbohydrates produces volatile fatty acids (**VFAs**), an important source of energy (Suagee et al., 2010). Previous research has found that VFAs can provide up to 70% of the horse's energy requirements each day (Ralston et al., 1983).

Cellulose, the portion of plant cell walls that provides structural support, is considered the most abundant organic material on earth. Cellulose is a water-insoluble carbohydrate, and its fermentation in the digestive tract of herbivores produces large quantities of VFAs, particularly acetate (O'sullivan, 1997; Jansen et al., 2000), which is used to produce ATP or body fat. It is also considered an insoluble fiber due to its inability to be dissolved in water. Insoluble fibers function to provide scratch factor to the horse, helping to scrape away dead cells throughout the digestive tract, assisting in enzyme secretion; they also assist in maintaining the shape and function of the large intestines, preventing twists and impactions (Warner, 1991; NRC, 2007; Collins, 2015).

Hemicellulose is a class of polysaccharide found in the cell walls of plants, though it is of a simpler form than cellulose. It is also fermented by the hindgut to produce VFAs, and in particular, produces large quantities of acetate (Pauly et al., 2013; Singh et al., 2018). It is considered a soluble fiber, which along with other soluble fibers,

are necessary for maintaining the water balance in the horse's digestive tract due to their high water holding capacity (Warren et al., 1999). Previous research has suggested that the large intestine of the horse serves as a reservoir for water and electrolytes, making the consumption of hemicellulose vital (Carlson, 1987; Meyer et al., 1987; Meyer, 1995).

Nonstructural. Nonstructural carbohydrates (**NSCs**) are found in plant material and are defined as the carbohydrates that do not provide structure to the plant. These carbohydrates instead comprise the stored energy for the plant, and are primarily starches, sugars, and fructans (Martínez-Vilalta et al., 2016). The primary component of all NSCs is glucose, a simple six carbon sugar. Glucose is combined with either other glucose or other simple sugars to make sucrose, fructans, amylose, amylopectin, and all other carbohydrates found within the plant. Starch is a storage form of glucose and encompasses two forms: amylose and amylopectin. Amylose has a linear structure characterized by α(1→4) linkages between units, while amylopectin is highly branched, containing a large number of α(1→6) bonds (Buleon et al., 1998). In plants used for equine grazing, NSCs is primarily found within the leaf, either as free sugars being used for energy by the plant or stored as starch granules for future use (Martínez-Vilalta et al., 2016).

Vitamins. Vitamins are organic compounds required by the body in minute amounts (NRC, 2007). They are classified into two groups, those that are fat-soluble and those that are water soluble (Crandell, 2000). The fat-soluble vitamins are Vitamin A, Vitamin D, Vitamin E, and Vitamin K. The water-soluble vitamins are the Vitamin B complex and Vitamin C. Requirements for vitamins have only been estimated, not fully determined (NRC, 2007). Fat-soluble vitamins are present in fresh pastures and all the

requirements of the horse can be met by free access to fresh pasture and sunlight, or access to high quality hay that is less than 6 mo old (Kalač, 2012; Manthe and Youngs, 2013). B-complex vitamins are produced by the microbial populations of the horse *in vivo*, therefore supplementation through diet is not necessary when horses consume enough forage (Carroll et al., 1949; Duren and Crandell, 2001). Vitamin C is assumed to be produced endogenously in the horse, and this production provides all daily requirements (Pearson et al., 1943; Stillions et al., 1971).

Minerals. Minerals are single elements that serve a variety of roles in the body, including serving as cofactors for enzymes, enabling muscle contractions, and allowing nerves to send signals (Biricik et al., 2005). Minerals are classified into 2 groups based on the requirements in the diet: macrominerals, which are required in larger amounts and measured in g/kg, and microminerals, which are required in minimal amounts and measured in mg/kg (Pagan, 2000; NRC, 2007). There are 7 macrominerals needed by the horse -- Calcium, Phosphorous, Sodium, Chlorine, Sulfur, Magnesium, and Potassium. There are also 7 microminerals required by the horse -- Cobalt, Copper, Iodine, Iron, Manganese, Selenium, and Zinc (NRC, 2007). Many of these 14 minerals have their requirements met by grazing and concentrate supplementation, with deficiencies considered rare in the equid (Frape, 2004). According to the National Research Council (2007), all 14 minerals required by the horse have recommendations on what is required; however, the most important minerals in growth and maintenance are calcium and phosphorous.

Calcium. Calcium in the equine body is primarily found within bone. While the main function of calcium is the building and maintenance of bones, calcium also plays a

vital role in regulating muscle contractions, the function of cell membranes, blood coagulation, and the regulation of many different enzymes (NRC, 2007). Approximately 99% of all calcium in the equine body is found in the skeleton, with the remaining 1% found in the soft tissue cells and the extracellular fluid (0.9% and 0.1%, respectively) (Toribio, 2011). Calcium would ideally be fed at twice the rate of phosphorous (Frape, 2004), but must be fed at a rate of 1.1:1 to prevent fractures, lameness, and other bone issues. Compared with other species, horses absorb a larger proportion of dietary calcium (up to 75%) (Toribio, 2011), meaning true deficiencies in calcium are very rare. With proper forage access and supplementation with an appropriate concentrate feed, calcium requirements are easily met for horses in maintenance (NRC, 2007; Toribio, 2011).

Phosphorous. Phosphorous in the equine body is also primarily found in bone. In the equine body, phosphorus is vital for energy production, is found in RNA and DNA, and is an integral part of all cell membranes (Sjaastad et al., 2010; Ögren, 2013). Phosphorous should be consumed at half the rate of calcium to prevent metabolic issues, such as nutritional secondary hyperparathyroidism (Stewart et al., 2010). Forages can supply the full phosphorus requirements of horses, therefore owners primarily need to be concerned with the ratio of calcium to phosphorous rather than meeting a specific daily amount (NRC, 2007).

Table 1. Nutritional content of common feedstuffs in equine diets (%DM)[1]

Feed Name	CP	Fat	NDF	ADF	Ca	P
Corn Grain	9.4	4.2	9.5	3.4	0.04	0.3
Rolled Oats	13.2	5.1	30.0	14.6	0.11	0.4
Grass Hay	18.0	3.3	49.6	31.4	0.72	0.34
Legume Hay	20.5	2.1	36.3	28.6	1.56	0.31
Grass+ Legume Hay	19.7	2.5	45.4	30.8	1.20	0.31
Beet Pulp	10.0	1.1	45.8	23.1	0.91	0.09

[1]Values obtained from the 2007 NRC

Common Health Concerns Arising from Incorrect Nutrition

Diseases and metabolic disorders related to incorrect nutrition, including colic, obesity, insulin resistance, and founder, are the most common causes of mortality and morbidity in horses (NAHMS, 2016). Though these diseases can be deadly, they are also partially preventable through management practices. A proper understanding of both equine nutrition and the mechanics of each disease are needed to prevent a horse from suffering.

Colic is a generic term for abdominal pain. It can have either nutritional or non-nutritive causes. Nutritional causes are wide ranging, but one of the most common is changing type or amount of grain too quickly (Hudson et al., 2001). Quick feed changes lead to excess starch fermentation in the hind gut, potentially resulting in endotoxemia and colitis (Hudson et al., 2001). Another cause for colic is feeds high in fiber. The increased fiber predisposes the horse for impaction colic, which can be difficult to treat

(Gonçalves et al., 2002).Though many studies have looked for a singular cause of colic, none has been identified at this point.

Obesity in horses is defined as a horse whose body condition score (**BCS**) is greater than 8. Body condition is assessed by looking at 6 areas where fat deposition occurs on the horse and scoring each one on a scale of 1 – 9, with a score of 1 indicating extreme emaciation and a score of 9 indicating morbid obesity. The scores for all 6 areas are then averaged to determine the overall BCS. Current estimates report that 45-51% of the horse population is overweight or obese (Thatcher et al., 2008; Wyse et al., 2008). Obesity in horses increases their risks of heat stress, joint damage, insulin resistance, and laminitis (Quinn et al., 2008). Laminitis in turn increases a horse's risk of mortality and reduces their usefulness to their owners. The work done by many horses does not require supplemental energy in the form of grain, yet today's horse owners primarily feed diets high in grain and low in hay or pasture (Hoffman et al., 2009; Murray et al., 2015). This increases the risk of obesity, insulin resistance, and laminitis.

Insulin resistance refers to a reduced response of insulin-sensitive cells, particularly muscle, adipose, and liver to insulin stimulation (Kronfeld et al., 2005). This reduced response can be caused by a disruption in insulin receptors or an inability for the cell to respond to insulin once it is transported inside (Kronfeld et al., 2005). Insulin resistance is affected by the weight, activity level, and diet of the horse (Kronfeld et al., 2005), and has been shown to predispose the affected horse to a number of diseases. In particular, obese horses consuming high levels of NSC are more prone to insulin resistance, and insulin resistance has been shown to increase the risk of laminitis (Geor and Harris, 2009).

Founder, also known as laminitis, is a debilitating, metabolic disease of the hoof. It is the second leading cause of death in horses and is also a highly prevalent disease, with some research surveys indicating as many as 34% of horses are afflicted with it at some point during their lifetime (Wylie et al., 2011). Laminitis specifically affects the laminae - specialized structures of the equine hoof that attach the skeleton to the hoof wall. These structures are responsible for suspending the entire weight of the horse within the hoof capsule while resisting the forces applied to them during standing and locomotion. Laminitis can manifest in either an acute or a chronic form, depending on the underlying cause. Acute cases may develop in less than 24h, resulting in a horse walking out of their hoof wall and leaving the bone exposed. Alternatively, the chronic form may take days to months to develop and is generally treatable through pain management and removal of the underlying cause. Horses suffering from laminitis typically begin to show symptoms of pain, including difficulty walking, laying down more frequently, and removing weight from the affected leg, prior to full bone-hoof wall separation (Dyson, 2011). There are three broad categories of causal agents of laminitis: physical trauma (e.g. galloping on concrete), sepsis (e.g. systemic bacterial infection), and endocrine pathology (e.g. obesity, diet, age, breed). The latter tends to result in a chronic form of laminitis and is the most easily preventable and treatable as long as horse owners pay attention to the management of their horses and treat at the first sign of disease (Patterson-Kane et al., 2018). A specific subtype of laminitis, Pasture-Associated Laminitis (**PAL**), is the most insidious form and has been shown to be responsible for 46-61% of all laminitis cases (Hinckley and Henderson, 1996; Geor, 2009). Pasture-Associated Laminitis is defined as laminitis that occurs in horses and ponies kept

exclusively on pasture. The exact mechanisms that cause PAL are unknown, but it is believed that fresh, lush forages high in NSC are responsible (Geor, 2009).

Factors Affecting NSC in Grasses

The NSC content of grass varies between plant species due to a variety of factors, including season, climate, amount of light during the day, nutrient levels in the soil, and genetic capacity to produce and store NSC. The carbon pathway utilized by the grass also affects capacity to accumulate NSC and is the definitive cause for the differences in NSC accumulation.

C3 vs. C4 Grasses. The two main carbon pathways found in photosynthetic plants are labeled as C3 and C4 due to the number of carbons present in the product of the first photosynthesis reaction. Cool-season (northern) grasses are also known as C3 grasses, as the first product of photosynthesis in these plants is a 3-carbon molecule, 3-phosphoglycerate (Ehleringer and Cerling, 2002), which is then used to create glucose. In these C3 grasses, each step of the photosynthetic process occurs in the same location, the bundle sheath cells (Gowik and Westhoff, 2011). Cool-season grasses fix CO_2 in the bundle sheath cells of the leaf. Due to the location, oxygen can compete with CO_2 for binding spots on the Rubisco enzyme that is responsible for "fixing" the carbon into phosphoglycerate. If Rubisco binds oxygen instead of CO_2, the cell goes through a process known as photorespiration, which results in a net loss of energy for the plant. In warmer climates, photorespiration can inhibit photosynthesis up to 50% of the time, making the plant itself extremely inefficient (Sage, 2001). This occurs for two reasons: First, the specificity of Rubisco for CO_2 decreases as temperatures increase. Secondly, the solubility of O_2 decreases more slowly than CO_2 at warmer temperatures, leaving

more O_2 available for reaction with Rubisco than CO_2 (Dusenge et al., 2019). Example species of C3 grasses are Timothy (*Phleum pratense*), Tall Fescue (*Festuca arundinacea*), and Perennial Ryegrass (*Lolium perenne*).

Warm-season (southern) grasses are also known as C4 grasses due to their unique photosynthetic pathway that evolved as an adaptation to high light intensities, high temperatures, and lower quantities of rainfall (Gowik and Westhoff, 2011). Photosynthesis in C4 grasses takes place across 2 different cells within the leaf, the bundle sheath cells and the mesophyll cells. Rather than fix carbon in the same place where photosynthesis occurs, C4 plants fix carbon into oxaloacetate using phosphoenolpyruvate (**PEP**) carboxylase in mesophyll cells. This 4-carbon molecule is then pumped into the bundle sheath cells, where it undergoes decarboxylation and is refixed using Rubisco (Ehleringer and Cerling, 2002). This modification to the photosynthetic pathway allows Rubisco to operate in an environment filled with CO_2, preventing the inefficient photorespiration pathway and increasing the efficiency of the plant, resulting in a lower concentration of Rubisco in C4 plants (Gowik and Westhoff, 2011). Additionally, this photosynthetic modification allows C4 plants to exhibit more water efficiency, as they can maintain CO_2 concentrations around Rubisco while keeping their stoma mostly closed (Long, 1999). Example species of C4 grasses are Bahiagrass (*Paspalum notatum*) and Bermudagrass (*Cynodon dactylon*).

Cool-season grasses tend to accumulate NSC at a greater rate than warm-season grasses, and the primary form of this NSC accumulation is fructans rather than starch, the latter being abundant in warm season grasses. Fructans provide protection from freezing temperatures and are necessary for cool season plants to survive frosts that can occur

during the early spring growing season (Watts, 2004; Zhao et al., 2008). Cool season grasses do not have a regulatory mechanism for fructan production, so they are able to accumulate high levels within their tissues (in excess of 400 g/kg of dry matter) (Longland and Byrd, 2006). Along with the previously mentioned differences between cool- and warm-season grasses, season and time of day also influence NSC accumulation.

Seasonality. Seasonality refers to the phenomenon that occurs when NSC accumulation increases during certain seasons, specifically spring and fall in northern grasses. In one study, the average NSC content of cool season grasses were greater in the summer, at 11.3 ± 0.6 %, than in the fall, at 9.9 ± 0.4 % (DeBoer et al., 2018). An earlier study found NSC content to be greatest during April, at 19.6 ± 0.3 %, vs. October, at 7.6 ± 5.7 % (Byrd, 2006) in tall fescue. The difference in content is due to temperature differences between seasons. Previous research has found that northern grasses accumulate NSC at a rate of 1.9 times that of southern grasses when grown at 10°C/5°C (day/night) (Byrd and Staniar, 2005). These temperatures coincide with spring and fall in the northern United States, indicating a higher NSC content in these seasons. In contrast, NSC production in C4 grasses may be driven more by drought conditions than temperature. Drought conditions are common in the southern United States, and grasses that grow in similar conditions have been shown to be well adapted to periods of drought, and as drought length increases so does the NSC content of the grass (Volaire and Lelievre, 1997). This research suggests that NSC content of southern grasses could be highest during the summer, when conditions are more drought-like, than in the spring and falls, as seen in northern grasses.

Diurnal Variation. An interesting phenomenon observed in northern grasses is diurnal variation in NSC content. Diurnal variation refers to the changes in NSC content that occur regularly within a 24-hour period. Studies on ryegrass (*Lolium perenne*) have shown that NSC content increases as the day goes on, with peak NSC occurring between 12:00 PM and sunset, depending on the season (Longland et al., 1999). Warm-season grasses that are common in the southern United States have not been extensively studied, but it is believed that they follow the same diurnal variation pattern as northern grasses (Undersander, 2013).

Species of Grass Studied

The three species of grass used for this study were Jiggs Bermudagrass [*Cynodon dactylon (L.)*], Tifton 85 Bermudagrass [*Tifton 68 bermudagrass* X *PI 290884*], and Pensacola Bahiagrass (*Paspalum notatum* Flüggé var. *saurae* Parodi).

Jiggs Bermudagrass is a naturally occurring ecotype that was discovered in East Texas in the 1980s and initially distributed by a private company (Aguiar et al., 2014). Jiggs has been shown to perform well in adverse weather conditions, such as extended high temperatures combined with low rainfall, poor soil (Brandstetter et al., 2018), and poorly draining soils (Scaglia and Boland, 2014). This variety of bermudagrass out competes weeds through all growing seasons and responds favorably to fertilization. It also has a nutritional advantage due to the structure of the cell walls promoting a higher NDF content and lower ADF content, which in turn increases digestibility and energy intake (De Rezende et al., 2015). However, research has indicated that Jiggs is not as productive as other varieties such as Tifton 85, and can also be susceptible to drought.

Tifton 85 Bermudagrass was created by Dr. Burton at the USDA-ARS research center in Tifton, Georgia in 1993 by crossing a plant introduced from South Africa (PI 290884) with Tifton 68 bermudagrass (Burton, 2001). It has been shown to be more productive than other varieties of bermudagrass (Hill et al., 1993) and also scores higher in digestibility trials done in beef and dairy cattle (Hill et al., 2001). Tifton 85 is very resistant to both drought and overstocking, making it a good choice for pasture. However, the thick stems of Tifton 85 can cause drying issues when making hay from it. Tifton 85 has also been found to lack cold tolerance and tends to die off during the winter (Corriher and Redmon, 2011).

Pensacola Bahiagrass is a naturally occurring species of the *Paspalum* genus discovered in Southern Florida in 1941 (Burton, 1967). It is believed to originate from Argentina, having washed into the Perdido Wharf prior to 1926, and has since spread throughout the Southern United States. It is commonly used as a pasture forage due to its greater tolerance for grazing, drought, and cold when compared to other C4 grasses (Gates et al., 2004).

Methods for Analyzing Forages

There are two primary methods for analyzing forage samples: wet chemistry and Near-Infrared Spectroscopy (NIR). Each method has both its benefits and drawbacks depending on what is needing to be analyzed, the speed with which results are needed, and the accuracy expected within the results.

Wet chemistry uses a variety of chemicals to extract and analyze compounds in a sample, such as fiber, crude protein, sugars, minerals, and fat. Each compound must be

analyzed individually, making wet chemistry extremely time consuming. The process of analyzing the compounds is both extensive and detailed, compiled of multiple steps that must be completed with precision and accuracy.

Near-Infrared Spectroscopy is a form of forage analysis that is less costly and labor intensive than traditional wet chemistry. It analyzes multiple compounds in the sample in ~30 seconds and can be run by anyone who has been taught to use the machine. It is accomplished by a machine that shines near-infrared light at the sample being analyzed. This light is then absorbed or reflected by the chemical bonds in the sample. What is reflected back at the computer is stored, analyzed, and used to estimate the levels of chemicals in the sample (Stuth et al., 2003). Though quicker and cheaper than wet chemistry, NIR is only as accurate as the equations used by the computer. These equations should be created using hundreds or thousands of samples but can be created using fewer. The fewer samples used in creating the equation, the less accurate the equations will be.

Research Objective

There is sufficient evidence linking starch and sugar intake to laminitis in horses (Kronfeld and Harris, 2003), and this event can occur in horses that are maintained on high NSC pasture. According to a survey conducted by the USDA, pasture-associated laminitis accounts for 54% of cases of equine laminitis for which the initial cause is identifiable (Wineland, 2000). This indicates that pasture alone is capable of inducing laminitis in horses. Previous research has also indicated that pre-laminitic horses kept on pasture can develop laminitis, but this research was conducted using pastures seeded with northern grasses that do not grow well in the south (Treiber et al., 2006). While these

studies have been beneficial to equine science, they do not help horse owners in southern climates make decisions on best management practices for their pre-laminitic or previously laminitic horses. Anecdotal evidence suggests that horses in southern climates are more at risk for pasture-associated laminitis during the months of August and September (Dr. Benjamin Buchanan, *personal communication*). Therefore, the focus of this research is to quantify the NSC level of southern grasses throughout their growing season.

CHAPTER III

Materials and Methods

Forage Species and Treatments

Three varieties of warm-season grasses were used for this research: Jiggs Bermudagrass [*Cynodon dactylon (L.)*], Tifton 85 Bermudagrass [*Tifton 68 bermudagrass* X *PI 290884*], and Pensacola Bahiagrass [*Paspalum notatum* Flüggé var. *saurae* Parodi]. The two varieties of Bermudagrass were collected from cultivated hay fields at the Gibbs Ranch of Sam Houston State University, located in Huntsville, TX (30.7456728, -95.620214). Bahiagrass was collected from a non-cultivated field at the same facility. This field was not used for grazing but was mowed regularly during the grazing season during previous years and did not have weed invasion. All grasses were grown in Arol fine sandy loam soil.

Two treatments were applied to each forage species: fertilization and time of sample collection. The fertilizer (treatment group; **FERT**) treated plots received 260 kg/ha of 16-6-12 (manufacturer, etc.) while control (**CON**) plots remained unfertilized during the growing season. Half of plots were harvested at 0600 hours (**AM**) and the remaining plots were harvested at 1800 hours (**PM**). Samples were collected on or as close to the first of the month as possible, from June 2019 to September 2019. The variance in collection date was due to weather conditions, as these plots were outside.

Research Plot Design

Each plot measured 49 m^2 and was divided into sixteen subplots that each measured 0.58 m^2 (Figure 1). Subplots were marked with flags at each corner and the flag

in the southeast corner was labeled with the plot treatment, replicate number, and sampling time (AM vs. PM). Cross contamination was prevented by leaving a space of one mower width around the edge of each plot. The day after sample collection, all plots were mowed with a Briggs & Stratton 550E series push mower (Briggs & Stratton, Wauwatosa, WI) to a height of 5.08 cm above the ground. No additional water was added to any of the plot areas beyond rainfall (Table 2).

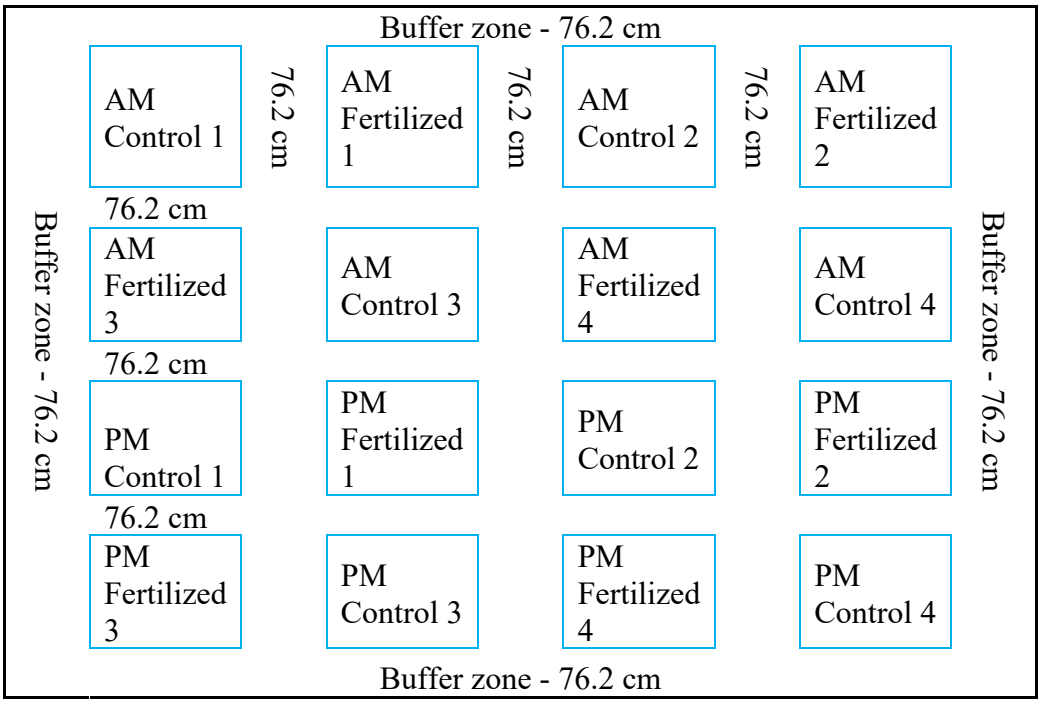

Figure 1. Research Plot Map

Table 2. Temperature and rainfall during study period

Month	Daily rain, mm	Min air temp, C	Max air temp, C	Avg air temp, C
April	95.3	15.6	26.9	21.3
May	137.2	20.3	30.2	25.3
June	183.1	22.1	33.1	27.6
July	16.8	23.1	34.5	28.8
August	13.0	24.7	36.7	30.7

Sample Collection and Preparation

All grass inside the subplot was cut to a height of 5.08 cm above the ground and placed inside a labeled collection bag. Samples were transported to the laboratory, where each sample was cut to approximately 5.08 cm in length, thoroughly mixed, and frozen for at least 24 h at -20°C until drying. A small sample (~3 g) was reserved for dry matter analysis in a separate labeled bag. Samples were dried at 60-90° C for 18-24 h. Once dried, samples were cooled in a desiccator and placed back into labeled bags. All samples were ground to pass through a 1mm screen using a MF 10 basic Microfine grinder (IKA-Werke, Staufen, Germany). Ground samples were stored at 21° C until analysis.

Nutrient Analysis

Samples were analyzed for ADF, NDF, crude protein, calcium, phosphorous, starch, and sugars via wet chemistry methods described below.

Fiber Analysis. Fiber analysis was conducted using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). For NDF analysis, 0.45-0.5 g of dried,

ground sample was precisely weighed into labeled F57 fiber bags (ANKOM Technology, Macedon, NY), then heat sealed (ANKOM Technology, Macedon, NY) within 3mm of the edge of the bag. Bags were tapped to evenly distribute the sample, then placed on the racks. A blank correction bag was used in each run of the machine. Once filled, racks were placed into the fiber analyzer, and 2 L of NDF solution (sodium lauryl sulfate, EDTA disodium, sodium borate, and sodium phosphate, (ANKOM Technology, Macedon, NY), 20 g of sodium sulfite (ANKOM Technology, Macedon, NY), and 4.0 mL of alpha-amylase solution (ANKOM Technology, Macedon, NY) were added to the chamber. The chamber was closed, heat and agitation turned on, and left to run for 75 minutes. Once finished, samples were rinsed three times for 5 min each, using deionized water heated to 75° C. The first 2 rinses included 4.0 mL of alpha-amylase solution, while the third consisted of only deionized water. Once rinsed, all bags were soaked in acetone (ANKOM Technology, Macedon, NY) for 5 min, and dried at room temperature (20° C) for 15 min. Samples were then dried in a drying oven for 2 h at 100° C. When dry, samples were placed in a desiccator to cool, and then weighed. Percent NDF was calculated by dividing the sample residue by the original sample weight and multiplying by 100. All results will be presented as a % of DM.

ADF was analyzed following NDF analysis, whereby samples were re-loaded onto the racks, and 2 L of ADF solution (water, sulfuric acid, cetyltrimethylammonium bromide, ANKOM Technology, Macedon, NY) added to the chamber. The chamber was closed, heat and agitation turned on, and left to run for 60 minutes. Once finished, three 5 min rinses were completed using deionized water heated to 75° C. Before draining the 3rd rinse, the pH was tested to ensure there was no acid remaining on the sample bags. If the

pH was not neutral, another rinse was completed as described above. Once rinsed, all bags were soaked in acetone for 5 min, and dried at room temperature (20° C) for 15 min. Samples were then dried in a drying oven for 2 h at 100° C. When dry, samples were placed in a desiccator to cool, and then weighed. Percent ADF was calculated by dividing the sample residue by the original sample weight and multiplying by 100. All results will be presented as a % of DM.

Protein Analysis. Crude protein was determined using the Flash 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA). The Elemental Analyzer operates by flash combusting each sample and analyzing the resultant gas. For analysis, 5-10mg of sample was weighed into a tin cup (Thermo Fisher Scientific, Waltham, MA) and placed on the machine. The machine automatically loaded each sample using a loading chamber and oxygen gas. For nitrogen determination, after combustion, the produced gases were carried by a helium flow to a second reactor filled with copper, then swept through CO_2 and H_2O traps, a gas chromatography column and finally detected by a Thermal Conductivity Detector (TCD). Percent crude protein was automatically calculated by the Eager*Smart* software (Thermo Fisher Scientific, Waltham, MA) that is necessary to run the elemental analyzer.

Sugar Analysis. Sugars were analyzed using an Agilent 1100 Series High-Performance Liquid Chromatography machine (Agilent Scientific Instruments, Santa Clara, CA). For water-soluble carbohydrate extraction, 500 mg of dried ground sample was placed in a 100mL beaker and then 40mL of 80° C water was added to the beaker. The beaker was then placed on a stirring hotplate heated to 80° C and stirred at 500rpm for 15 min. After 15 min had elapsed, the beaker was cooled to room temperature and the

total volume was brought to 50mL. Two subsamples of 12.5mL each were taken and placed into Falcon tubes for centrifugation. Samples were centrifuged for 5 min at 2600rpm at 22° C and the clear supernatant was filtered through a Millex-GP 0.22µm syringe-driven filter unit (Merck KGaA, Darmstadt, Germany). The filtered supernatant was measured, the volume recorded, and was then placed in Falcon tubes and frozen at -2° C until analysis.

Before analysis via HPLC, frozen samples were heated to 80° C and then cooled to room temperature. 1,790µL of samples were placed into a 2mL gas chromatography tube and then 10µL of a xylose spike solution (250mg/mL) were added as an internal standard. Samples were then vortexed and placed on the Agilent 1100 Series High-Performance Liquid Chromatography machine (Agilent Scientific Instruments, Santa Clara, CA). Samples were read at 195nm with a 1.5mL/min flow rate and a 16min run time using a Restek™ Ultra HPLC Columns with Amino Column Packing, 5µm Particle Size column fitted with a Restek™ Ultra Guard Cartridge with Amino Packing (Restek Pure Chromatography, Bellefonte, PA). Peak placement for the sugars of interest were identified by running standards made of ultra-pure water (Cayman Chemical Company, Ann Arbor, MI) and a known amount of either glucose, sucrose, or fructose (Alfa Aesar, Haverhill, MA). After analysis, reports for each sample were downloaded from the Agilent Chemstation Software (Agilent Scientific Instruments, Santa Clara, CA) and peaks identified and quantified, and all results will be presented as a % of DM.

CHAPTER IV

Results

Dry Matter

Bahiagrass. There were no effects of the interactions of month by TRT by time, month by time, or TRT by time (P>0.7). There was a month by TRT interaction (P<0.01; Table 3), where, for CON plots, August DM (42.5 ± 1.4 %) was greater than June DM (23.1 ± 1.4 %; P<0.01), July DM (24.6 ± 1.4 %; P<0.01), and September DM (30.8 ± 1.4 %; P<0.01). June DM (23.1 ± 1.4 %) was also lesser than September DM (30.8 ± 1.4 %; P=0.01) for CON plots. For FERT plots, August DM (50.5 ± 1.4 %) was greater than June DM (31.7 ± 1.4 %; P<0.01) and July DM (34.3 ± 1.4 %; P<0.01) but was not different from September DM (54.5 ± 1.4 %; P>0.5). July DM (34.3 ± 1.4 %) was lesser than September DM (54.5 ± 1.4 %; P<0.01) but was not different from June DM (31.7 ± 1.4 %; P>0.8) and June DM (31.7 ± 1.4 %) was lesser than September DM (54.5 ± 1.4 %; P<0.01). Within month, August DM was lower in CON (42.5 ± 1.4 %) than FERT plots (50.5 ± 1.4 %; P<0.01), July DM was lesser in CON (23.1 ± 1.4 %) than FERT plots (34.3 ± 1.4 %; P<0.01), June DM was lesser in CON (23.1 ± 1.4 %) than FERT plots (31.7 ± 1.4 %; P<0.01), and September DM was lesser in CON (30.8 ± 1.4 %) than FERT plots (54.5 ± 1.4 %; P<0.01). There was also a tendency for an effect of time, with samples collected in the morning (37.7 ± 0.7 %) being greater in dry matter than samples collected in the evening (35.3 ± 0.7 %; P=0.07).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT (P>0.3). There was a main effect

of month (P<0.01; Table 3), with August DM (55.4 ± 1.1 %) being greater than that of July DM (41.4 ± 1.1 %; P<0.01), June DM (36.8 ± 1.1 %; P<0.01), and September DM (45.2 ± 1.1 %; P<0.01). July DM (41.4 ± 1.1 %) was also greater than that of June DM (36.8 ± 1.1 %; P=0.03) but was not different from September DM (45.2 ± 1.1 %; P>0.09). June DM (36.8 ± 1.1 %) was lesser than that of September DM (45.2 ± 1.1 %; P<0.01). There was a main effect of TRT, with FERT plots (49.0 ± 0.8 %) being greater in DM than CON plots (40.4 ± 0.8 %; P<0.01). There was also an effect of time, with samples collected in the morning (45.9 ± 0.8 %) being greater in dry matter than samples collected in the evening (43.5 ± 0.8 %; P=0.04).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT (P>0.1). There was also no effect of time (P>0.1). There was a main effect of month (P<0.01; Table 3), with August DM (52.9 ± 1.3 %) being greater than July DM (35.8 ± 1.3 %; P<0.01), June DM (34.1 ± 1.3 %; P<0.01), and September DM (42.9 ± 1.3 %; P<0.01). July DM (35.9 ± 1.3 %) was not different from June DM (34.1 ± 1.3 %; P>0.7) but was lesser than September DM (42.9 ± 1.3 %; P<0.01). June DM (34.1 ± 1.3 %) was lesser than September DM (42.9 ± 1.3 %; P<0.01). There was a main effect of TRT, with FERT plots (45.2 ± 0.9 %) having a greater dry matter than CON plots (37.7 ± 0.9 %; P<0.01).

Table 3. Dry matter content of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 1.4)									
CON	23.4	22.8	25.5	23.6	44.4	40.6	32.8	28.7						
FERT	33.6	29.8	35.3	33.4	51.1	50.0	55.2	53.8						
CON MO mean	23.1[a]		24.6[a]		42.5[c]		30.8[b]		<0.01	0.03	<0.01	NS	<0.01	NS
FERT MO mean	31.7[a]		34.3[a]		50.5[b]		54.5[bc]							
Time mean	37.7[a]	35.3[b]	-	-	-	-	-	-						
					Jiggs Bermudagrass (SEM ± 1.1)									
CON	33.3	29.9	41.0	36.1	52.9	50.0	40.2	40.1						
FERT	44.5	39.6	42.9	45.7	61.4	57.3	51.1	49.5						
MO mean	36.8[a]		41.4[b]		55.4[d]		45.2[bc]		<0.01	0.04	<0.01	NS	NS	NS
CON mean	40.4[a]		-	-	-	-	-	-						
FERT mean	49.0[b]		-	-	-	-	-	-						
Time mean	45.9[a]	43.5[b]	-	-	-	-	-	-						

(continued)

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
	Tifton 85 Bermudagrass (SEM ± 1.3)													
CON	34.8	28.7	32.4	30.9	53.1	46.7	37.6	37.3						
FERT	37.3	35.5	42.6	37.8	55.7	56.0	46.3	50.2						
MO mean	34.1[a]		35.9[a]		52.9[c]		42.8[b]		<0.01	NS	<0.01	NS	NS	NS
CON mean	37.7[a]		-	-	-	-	-	-						
FERT mean	45.2[b]		-	-	-	-	-	-						

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

Yield

Bahiagrass. There were no effects of the interactions of month by TRT by time, TRT by month, or TRT by time ($P>0.1$). There was a month by time interaction ($P<0.01$; Table 4), whereby, for AM plots, August yield (260 ± 71.8 kg/ha) was lesser than September yield (556 ± 71.8 kg/ha; $P<0.01$) and June yield (684 ± 71.8 kg/ha; $P<0.01$) but was not different from July yield (289 ± 71.8 kg/ha; $P>0.05$). Additionally, July yield (289 ± 71.8 kg/ha) was lesser than June yield (684 ± 71.8 kg/ha; $P<0.01$) and September yield (556 ± 71.8 kg/ha; $P<0.01$). September yield (556 ± 71.8 kg/ha) was lesser than June yield (684 ± 71.8 kg/ha; $P<0.01$). Conversely, for PM plots, there were no differences across months ($P>0.05$). There was an effect of TRT ($P<0.01$), whereby fertilized samples (515 ± 71.8 kg/ha) had a greater yield than CON samples (359 ± 71.8 kg/ha).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, month by time, or TRT by time ($P>0.08$). There was a month by TRT interaction ($P=0.02$; Table 4), whereby, for CON plots, August yield (444 ± 148 kg/ha) was lesser than June yield (1230 ± 128 kg/ha; $P<0.05$) but was not different from July yield (847 ± 128 kg/ha; $P>0.05$) and September yield (448 ± 128 kg/ha; $P>0.05$). July yield (847 ± 128 kg/ha) was greater than September yield (448 ± 128 kg/ha; $P<0.05$) but was not different from June yield (1230 ± 128 kg/ha; $P>0.05$), and June yield (1230 ± 128 kg/ha) was greater than September yield (448 ± 128 kg/ha; $P<0.05$). For FERT plots, August yield (569 ± 128 kg/ha) was lesser than July yield (1756 ± 128 kg/ha; $P<0.05$) and June yield (2034 ± 128 kg/ha; $P<0.05$) but was not different from or September yield (640 ± 128 kg/ha; $P>0.05$). July yield (827 ± 60.8 kg/ha) was greater than September yield (640

± 128 kg/ha; P<0.05) but was not different from June yield (2034 ± 128 kg/ha; P>0.05) and June yield (2034 ± 128 kg/ha; P>0.05) was greater than September yield (640 ± 128 kg/ha; P>0.05). There were no differences seen between CON and FERT samples within the same month (P>0.05). There was no effect of time (P>0.05).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time, month by TRT, or TRT by time (P>0.7). There was a month by time interaction (P=0.04; Table 4), whereby, for AM samples, September (494 ± 95.6 kg/ha) had a lesser yield than June (1142 ± 95.6 kg/ha; P<0.05) and July (1209 ± 95.6 kg/ha; P<0.05) but was not different from August yield (697 ± 95.6 kg/ha; P>0.05). August (697 ± 95.6 kg/ha) had a lesser yield than June (1142 ± 95.6 kg/ha; P<0.05) and July (1209 ± 95.6 kg/ha; P<0.05) but was not different from September (494 ± 95.6 kg/ha; P>0.05). June (1142 ± 95.6 kg/ha) had a greater yield than August (697 ± 95.6 kg/ha; P<0.05) and September (494 ± 95.6 kg/ha; P<0.05) but was not different from July (1209 ± 95.6 kg/ha; P>0.05). July (1209 ± 95.6 kg/ha) had a greater yield than August (697 ± 95.6 kg/ha; P<0.05) and September (494 ± 95.6 kg/ha; P<0.05) but was not different from June (1142 ± 95.6 kg/ha; P>0.05). For PM samples, June (1372 ± 95.6 kg/ha) had a greater yield than July (919 ± 95.6 kg/ha; P<0.05), August (533 ± 95.6 kg/ha; P<0.05), and September (580 ± 95.6 kg/ha; P<0.05). There were no other differences between PM samples (P>0.05). There was no effect of TRT seen between CON and FERT plots (P>0.3).

Table 4. Yield in kg/ha of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 71.8)									
CON	677	424	282	273	241	286	385	299						
FERT	691	627	295	364	279	628	728	506						
MO mean	605		304		358		479							
Time*MO AM	684[c]	—	289[a]	—	260[a]	—	556[b]	—						
Time*MO PM	—	526[a]	—	319[a]	—	457[a]	—	402[a]	<0.01	NS	<0.01	NS	NS	<0.01
CON mean	359[a]				—									
FERT mean	515[b]				—									
					Jiggs Bermudagrass (SEM ± 148)									
CON	1090	1369	978	716	388	500	384	512						
FERT	1964	2103	1654	1857	513	624	595	685						
CON MO mean	1230[b]		847[ab]		444[a]		448[a]		<0.01	NS	<0.01	NS	0.02	NS
FERT MO mean	2034[b]		1756[b]		569[a]		640[a]							

(continued)

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Tifton 85 Bermudagrass (SEM ± 95.6)									
CON	1115	1172	1106	518	593	397	463	574						
FERT	1170	1573	1312	1321	802	669	525	585						
MO mean	1257		1064		615		536							
Time*MO AM	1142[b]	—	1209[b]	—	697[a]	—	494[a]	—						
Time*MO PM	—	1372[b]	—	919[a]	—	533[a]	—	580[a]	<0.01	NS	<0.01	NS	NS	0.04
CON mean	742[a]				—									
FERT mean	995[b]				—									

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

Neutral Detergent Fiber

Bahiagrass. There were no effects of the interactions of month by TRT by time, month by time, month by TRT, or TRT by time (P>0.06). There was an effect of month (P<0.01; Table 5), whereby samples collected in August (66.4 ± 0.5 %DM) had a lesser %NDF than samples collected in September (71.0 ± 0.5 %DM; P<0.01) but had a similar %NDF to samples collected in June (65.7 ± 0.5 %DM; P>0.8) and July (68.0 ± 0.5 %DM; P>0.1). July (68.0 ± 0.5 %DM) had a greater %NDF than June (65.7 ± 0.5 %DM; P=0.01) but a lesser %NDF than samples collected in September (71.0 ± 0.5 %DM; P<0.01). June had a lesser %NDF than September (71.0 ± 0.5 %DM; P<0.01). There was also an effect of time (P<0.01; Table 5), in which samples collected in the AM (68.7 ± 0.5 %DM) had a higher %NDF than samples collected in the PM (66.8 ± 0.5; P<0.01). There was no effect of TRT seen (P>0.06).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, month by TRT, or TRT by time (P>0.4). There was an effect of the month by time interaction (P<0.01; Table 5), whereby, for samples collected in the AM, August NDF (65.0 ± 0.7 %) was greater than June NDF (59.0 ± 0.7 %; P<0.01) but was not different from July NDF (64.7 ± 0.7 %; P=1.0) or September NDF (65.1 ± 0.7; P=1.0). July NDF (64.7 ± 0.7 %) was greater from June NDF (59.0 ± 0.7 %; P<0.01) but was not different from September NDF (65.1 ± 0.7 %; P>0.9). June NDF (59.0 ± 0.7 %) was lesser than September NDF (65.1 ± 0.7; P<0.01). For samples collected in the PM, August NDF (70.3 ± 0.7 %) was greater than July NDF (63.6 ± 0.7 %; P<0.01) and June NDF (59.9 ± 0.7 %; P<0.01) but was not different from September NDF (68.5 ± 0.7 %; P>0.5). July NDF (63.6 ± 0.7 %) was greater than June NDF (59.9 ± 0.7 %; P<0.01) but

was lesser than September NDF (68.5 ± 0.7 %; P<0.01). June NDF (59.9 ± 0.7 %) was lesser than September NDF (68.5 ± 0.7 %; P<0.01). Within month, August NDF was lesser in samples collected in the AM (65.0 ± 0.7 %) than samples collected in the PM (70.3 ± 0.7 %; P<0.01) and September NDF was lower in samples collected in the AM (65.1 ± 0.7 %) than samples collected in the PM (68.5 ± 0.7 %; P=0.02). There were no other differences seen within month (P>0.9).

Tifton 85 Bermudagrass. There was no effect of the interactions of month by TRT by time, month by TRT, or month by time (P>0.07). There was an effect of TRT by time (P=0.04; Table 5), whereby, for FERT samples, samples collected in the AM (64.3 ± 0.5 %) had a lesser % NDF than samples collected in the PM (66.2 ± 0.5 %; P=0.06). There were no other differences seen between time points and treatments.

There was a main effect of month (P<0.01), where August % NDF (68.0 ± 0.5 %) was greater than June % NDF (62.8 ± 0.5 %; P<0.01) and July % NDF (63.9 ± 0.5 %; P<0.01) but was not different from September % NDF (66.8 ± 0.5 %; P>0.3). July % NDF 63.9 ± 0.5 %) was lesser than September % NDF (66.8 ± 0.5 %; P<0.01) but was not different from June % NDF (62.8 ± 0.5 %; P>0.4). June % NDF (62.8 ± 0.5 %) was lesser than September % NDF (66.8 ± 0.5 %; P<0.01).

Table 5. Neutral Detergent Fiber (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON)**.** [1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 0.5)									
CON	66.1	65.8	69.1	69.1	68.5	65.4	72.4	69.8						
FERT	65.2	65.9	70.5	63.4	66.4	65.2	71.9	70.0						
MO mean	65.7[a]		68.0[b]		66.4[ab]		71.0[c]		NS	<0.01	<0.01	NS	NS	NS
Time mean	68.7[b]	66.8[a]	-	-	-	-	-	-						
					Jiggs Bermudagrass (SEM ± 0.5)									
CON	59.1	59.7	65.2	63.1	65.0	69.7	64.5	68.3						
FERT	59.0	60.1	64.2	64.2	64.9	70.9	65.8	68.6						
MO mean	62.8		63.9		67.6		66.7							
Time* MO AM	59.0[a]	-	64.7[b]	-	65.0[b]	-	65.1[b]	-	NS	<0.01	<0.01	NS	NS	<0.01
Time* MO PM	-	59.9[a]	-	63.6[b]	-	70.3[c]	-	68.5[c]						

(continued)

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
	Tifton 85 Bermudagrass (SEM ± 0.5)													
CON	64.6	63.1	64.7	62.3	67.1	67.7	66.0	68.0						
FERT	62.1	61.2	63.0	65.5	66.8	70.4	65.5	67.5						
MO mean	62.8[a]		63.9[a]		68.0[b]		66.7[b]							
CON mean	65.6[a]	65.3[a]	-	-	-	-	-	-	NS	NS	<0.01	0.04	NS	NS
FERT mean	64.3[a]	66.2[b]	-	-	-	-	-	-						

[1] No interactions of month x TRT x time were detected for any species $P > 0.1$

Acid Detergent Fiber

Bahiagrass. There were no effects of the interactions of month by TRT by time, month by time, month by TRT, or TRT by time ($P>0.1$). There was a main effect of month ($P<0.01$; Table 6), whereby, August % ADF (36.6 ± 0.9 %) was lesser than September % ADF (41.9 ± 0.9 %; $P<0.01$) but was not different from July % ADF (36.0 ± 0.9 %; $P>0.9$) or June % ADF (33.5 ± 0.9 %; $P>0.1$). July % ADF (36.0 ± 0.9 %) was lesser than September % ADF (41.9 ± 0.9 %; $P<0.01$) but was not different from June % ADF (33.5 ± 0.9 %; $P>0.2$). June % ADF (33.5 ± 0.9 %) was lesser than September % ADF (41.9 ± 0.9 %; $P<0.01$). There was no main effect of TRT or time ($P>0.1$).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, month by time, month by TRT, or TRT by time ($P>0.07$). There was a main effect of month ($P<0.01$; Table 6), whereby, August % ADF (35.4 ± 0.3 %) was greater than July % ADF (33.0 ± 0.3 %; $P<0.01$), June % ADF (30.6 ± 0.3 %; $P<0.01$), and September % ADF (30.7 ± 0.7 %; $P<0.01$). July % ADF (33.0 ± 0.3 %) was greater than June % ADF (30.6 ± 0.3 %; $P<0.01$) and September % ADF (30.7 ± 0.7 %; $P<0.01$). June % ADF (30.6 ± 0.3 %) was not different from September % ADF (30.7 ± 0.7 %; $P>0.9$). There was also an effect of TRT ($P<0.01$) where CON samples had a greater % ADF (32.8 ± 0.2 %) than FERT samples (31.9 ± 0.2 %; $P<0.01$). There was no effect of time ($P>0.7$).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time or month by time ($P>0.1$). There was an effect of the month by TRT interaction ($P=0.04$; Table 6), whereby, for August, August CON % ADF (36.4 ± 0.7 %) was greater than July CON % ADF (32.2 ± 0.7 %; $P<0.01$), June CON % ADF (32.5 ±

0.7; P<0.01), and September CON % ADF (33.3 ± 0.7 %; P=0.05). August FERT % ADF (37.3 ± 0.7 %) was greater than June FERT % ADF (30.9 ± 0.7 %; P<0.01) and September FERT % ADF (33.4 ± 0.7 %; P<0.01) but was not different from July FERT (34.8 ± 0.7 %; P>0.1). For samples collected in July, July FERT % ADF (34.8 ± 0.7 %) was greater than June FERT % ADF (30.9 ± 0.7 %; P<0.01). No other differences were seen.

Table 6. Acid Detergent Fiber (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 0.9)									
CON	33.9	34.1	36.0	39.3	38.0	36.0	41.8	39.8						
FERT	33.4	32.7	36.0	32.8	36.5	35.7	46.2	39.8	NS	NS	<0.01	NS	NS	NS
MO mean	33.5[a]		36.0[a]		36.6[a]		41.9[b]							
					Jiggs Bermudagrass (SEM ± 0.3)									
CON	32.0	30.6	33.6	33.0	34.9	35.5	30.2	29.8						
FERT	30.1	29.5	32.2	33.0	35.3	35.9	30.1	29.7						
MO mean	30.6[a]		33.0[b]		35.4[c]		30.7[a]		0.004	NS	<0.01	NS	NS	NS
CON mean	32.8[b]		-		-		-							
FERT mean	32.0[a]		-		-		-							

(continued)

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
	Tifton 85 Bermudagrass (SEM ± 0.7)													
CON	33.0	32.1	32.6	31.8	36.4	36.5	33.4	33.2						
FERT	31.2	30.7	32.1	37.4	36.3	38.3	33.0	33.8						
CON MO mean	32.5[a]		32.2[a]		36.4[b]		33.3[a]							
FERT MO mean	30.9[a]		34.8[b]		37.3[b]		33.4[a]		NS	NS	<0.01	0.03	0.04	NS
TRT*Time CON	33.8[a]	33.4[a]	-	-	-	-	-	-						
TRT*Time FERT	33.2[a]	35.1[b]	-	-	-	-	-	-						

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

Crude Protein

Bahiagrass. There were no effects of the interactions of month by TRT by time or TRT by time interaction (P>0.1) for crude protein % in Bahiagrass. There was a month by TRT interaction (P<0.01; Table 7), where, for CON plots, August CP (6.8 ± 0.4 %) was lesser than July CP (9.7 ± 0.4 %; P<0.01) and June CP (9.4 ± 0.4 %; P<0.01) but was not different from September CP (6.7 ± 0.4 %; P=1.0). July CP (9.7 ± 0.4 %) was greater than September CP (6.7 ± 0.4 %; P<0.01) but was not different from June CP (9.4 ± 0.4 %; P>0.9). June CP (9.4 ± 0.4 %) was greater than September CP (6.7 ± 0.4 %; P<0.01). For FERT plots, August CP (10.2 ± 0.4 %) was lesser than July CP (14.5 ± 0.4 %; P<0.01) but was not different from June CP (10.2 ± 0.4 %; P=1.0) or September CP (8.4 ± 0.4 %; P=0.1). July CP (14.5 ± 0.4 %) was greater than June CP (10.2 ± 0.4 %; P<0.01) and September CP (8.4 ± 0.4 %; P<0.01). June CP (10.2 ± 0.4 %) was not different from September CP (8.4 ± 0.4; P=0.09). Within month, August CP was lesser in CON plots (6.8 ± 0.4 %) than FERT plots (10.2 ± 0.4 %; P<0.01) and July CP was lesser in CON plots (9.7 ± 0.4 %) than in FERT plots (14.5 ± 0.4 %; P<0.01). There were no differences in June CP between CON plots (9.4 ± 0.4 %) and FERT plots (10.2 ± 0.4 %; P>0.9) or in September CP between CON plots (6.7 ± 0.4 %) and FERT plots (8.4 ± 0.4 %; P>0.1).

There was a month by time interaction (P=0.03; Table 7), whereby, for AM samples, August CP (9.6 ± 0.4 %) was lesser than July CP (12.6 ± 0.4 %; P<0.01) but was greater than September CP (7.3 ± 0.4 %; P=0.01). August CP (9.6 ± 0.4 %) was not different from June CP (10.6 ± 0.4 %; P>0.7). July CP (12.6 ± 0.4 %) was greater than June CP (10.6 ± 0.4 %; P=0.04) and September CP (7.3 ± 0.4 %; P<0.01). June CP (10.6 ± 0.4 %) was greater than September CP (7.3 ± 0.4 %; P<0.01). For PM samples, August

CP (7.4 ± 0.4 %) was lesser than July CP (11.6 ± 0.4 %; P<0.01) but was not different from June CP (9.0 ± 0.4 %; P>0.1) or September CP (7.8 ± 0.4 %; P>0.9). July CP (11.6 ± 0.4 %) was greater than June CP (9.0 ± 0.4 %; P<0.01) and September CP (7.8 ± 0.4 %; P<0.01). June CP (9.0 ± 0.4 %) was greater than September CP (7.8 ± 0.4 %; P<0.01). Within month, August CP was greater in AM samples (9.6 ± 0.4 %) than in PM samples (7.4 ± 0.4 %; P=0.02). There were no other differences seen within June, July, or September (P>0.05).

Jiggs Bermudagrass. There was no effect of the month by TRT by time interaction (P>0.9). There was a month by TRT interaction (P<0.01; Table 7), whereby, for CON plots, August CP (10.8 ± 0.5 %) was lesser than June CP (13.5 ± 0.5 %; P<0.01) and September CP (13.1 ± 0.5 %; P=0.03) but was not different from July CP (11.2 ± 0.5 %; P>0.9). July CP (11.2 ± 0.5 %) was lesser than June CP (13.5 ± 0.5 %; P=0.03) but was not different from September CP (13.1 ± 0.5 %; P>0.1). June CP (13.5 ± 0.5 %) was not different from September CP (13.1 ± 0.5 %; P>0.9). For FERT plots, August CP (13.7 ± 0.5 %) was lesser than June CP (19.1 ± 0.5 %; P<0.01) but was not different from July CP (15.1 ± 0.5 %; P>0.5) or September CP (15.1 ± 0.5 %; P>0.4). July CP (15.1 ± 0.5 %) was lesser than June CP (19.1 ± 0.5 %; P<0.01) but not different from September CP (15.1 ± 0.5 %; P=1.0). June CP (19.1 ± 0.5 %) was greater than September CP (15.1 ± 0.5 %; P<0.01). Within month, August CP was lesser in CON plots (10.8 ± 0.5 %) than in FERT plots (13.7 ± 0.5 %; P<0.01), July CP was lesser in CON plots (11.2 ± 0.5 %) than in FERT plots (15.1 ± 0.5 %; P<0.01), June CP was lesser in CON plots (13.5 ± 0.5 %) than in FERT plots (19.1 ± 0.5 %; P<0.01), and September CP was not different between CON plots (13.1 ± 0.5 %) and FERT plots (15.1 ± 0.5 %; P>0.08).

There was a month by time interaction (P=0.02; Table 7), whereby, for AM samples, August CP (12.8 ± 0.5 %) was lesser than June CP (15.6 ± 0.5 %; P<0.01) but not different from July CP (12.9 ± 0.5 %; P=1.0) or September CP (14.9 ± 0.5 %; P>0.07). July CP (12.9 ± 0.5 %) was lesser than June CP (15.6 ± 0.5 %; P<0.01) but was not different from September CP (14.9 ± 0.5 %; P>0.1). June CP (15.6 ± 0.5 %) was not different from September CP (14.9 ± 0.5 %; P>0.9). For PM samples, August CP (11. 7 ± 0.5 %) was lesser than June CP (17.0 ± 0.5 %; P<0.01) but was not different from July CP (13.3 ± 0.5 %; P>0.2) or September CP (13.4 ± 0.5 %; P>0.2). July CP (13.3 ± 0.5 %) was lesser than June CP (17.0 ± 0.5 %; P<0.01) but was not different from September CP (13.4 ± 0.5 %; P=1.0). June CP (17.0 ± 0.5 %) was greater than September CP (13.4 ± 0.5 %; P<0.01). There were no differences seen between AM and PM samples within the same month (P>0.05). There was an effect of the TRT by time interaction (P=0.02; Table 6), whereby, for AM samples, CON samples were lower in CP (11.8 ± 0.4 %) than FERT samples (16.3 ± 0.4 %; P<0.01). For PM samples, CON samples were lower in CP (12.4 ± 0.4 %) than FERT samples (15.2 ± 0.4 %; P<0.01). There were no differences seen between AM and PM samples within the same treatment (P>0.05).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time or month by time (P>0.5). There was an effect of the month by TRT interaction (P<0.01; Table 7), whereby, for CON samples, August CP (9.5 ± 0.5 %) was lesser than September CP (13.4 ± 0.5 %; P<0.01) but was not different from July CP (11.4 ± 0.5 %; P>0.1) or June CP (9.9 ± 0.5 %; P>0.9). July CP (11.4 ± 0.5 %) was not different from June CP (9.9 ± 0.5 %; P>0.4) or September CP (13.4 ± 0.5 %; P>0.1). June CP (9.9 ± 0.5 %) was lesser than September CP (13.4 ± 0.5 %; P<0.01). For FERT

samples, August CP (10.4 ± 0.5 %) was lesser than July CP (15.4 ± 0.5 %; P<0.01) and June CP (16.0 ± 0.5 %; P<0.01) but was not different from September CP (12.8 ± 0.5 %; P>0.07). July CP (15.4 ± 0.5 %) was greater than September CP (12.8 ± 0.5 %; P=0.02) but was not different from June CP (16.0 ± 0.5 %; P>0.9). June CP (16.0 ± 0.5 %) was greater than September CP (12.8 ± 0.5 %; P<0.01). Within the same month, July CP was lesser in CON plots (11.4 ± 0.5 %) than in FERT plots (15.4 ± 0.5 %; P<0.01) and June CP was lesser in CON plots (9.9 ± 0.5 %) than in FERT plots (16.0 ± 0.5 %; P<0.01), but there were no differences seen between treatments in August CP or September CP (P>0.9). There was an effect of the TRT by time interaction (P<0.01; Table 7), whereby, for AM samples, crude protein was lesser in CON plots (11.0 ± 0.4 %) than in FERT plots (14.8 ± 0.4 %; P<0.01). For PM samples, crude protein was lesser in CON plots (11.1 ± 0.4 %) than in FERT plots (12.5 ± 0.4 %; P=0.05). Within the same TRT, FERT AM samples (14.8 ± 0.4 %) were greater in CP than FERT PM samples (12.5 ± 0.4 %; P<0.01), but there were no differences in CP between CON AM samples and CON PM samples (P>0.9).

Table 7. Crude protein (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		August		September		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME *MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 0.4)									
CON	10.3	8.5	10.6	8.8	7.3	6.3	6.7	6.7						
FERT	10.8	9.5	14.6	14.3	11.8	8.5	7.9	8.8						
CON MO mean	9.4[b]		9.7[b]		6.8[a]		6.7[a]							
FERT MO mean	10.2[a]		14.5[b]		10.2[a]		8.4[a]		<0.01	<0.01	<0.01	NS	<0.01	0.03
Time*MO AM	10.6[b]	–	12.6[c]	–	9.5[b]	–	7.9[a]	–						
Time*MO PM	–	9.0[a]	–	11.5[b]	–	7.4[a]	–	7.8[a]						
					Jiggs Bermudagrass (SEM ± 0.5)									
CON	12.2	14.7	10.6	11.7	10.9	10.6	13.4	12.7						
FERT	19.0	19.2	15.1	15.0	14.6	12.8	16.3	14.0						
CON MO mean	13.5[b]		11.2[a]		10.7[a]		13.1[b]							
FERT MO mean	19.1[b]		15.1[a]		13.7[a]		15.1[a]							
TRT*Time CON	11.8[a]	12.4[a]	–	–	–	–	–	–	<0.01	NS	<0.01	0.02	<0.01	0.02
TRT*Time FERT	16.3[b]	15.2[b]	–	–	–	–	–	–						
Time* MO AM	15.6[b]	–	12.9[a]	–	12.8[a]	–	14.9[a]	–						
Time*MO PM	–	17.0[b]	–	13.3[a]	–	11.7[a]	–	13.3[a]						

(continued)

Treatment (TRT)	June		July		August		September		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME *MO
	AM	PM	AM	PM	AM	PM	AM	PM						
	Tifton 85 Bermudagrass (SEM ± 0.5)													
CON	9.7	10.1	11.2	11.7	9.8	9.2	13.4	13.4						
FERT	16.8	15.1	17.2	13.7	11.2	9.7	13.9	11.6						
CON MO mean	9.9[a]		11.4[b]		9.5[a]		13.4[b]							
FERT MO mean	16.0[b]		15.4[b]		10.4[a]		12.8[a]		<0.01	<0.01	<0.01	<0.01	<0.01	NS
TRT*Time CON	11.0[a]	11.1[a]	–	–	–	–	–	–						
TRT*Time FERT	14.8[b]	12.5[a]	–	–	–	–	–	–						

[1]No interactions of month x TRT x time were detected for any species P > 0.1

Fructose

Bahiagrass. There were no effects of the interactions of month by TRT by time, TRT by time, or month by TRT ($P>0.1$). There was an effect of the month by time interaction ($P<0.01$; Table 8), whereby, for AM samples, August fructose (0.3 ± 1.1 %) was lesser than June fructose (6.2 ± 1.1 %; $P<0.01$) but was not different from July fructose (0.4 ± 1.1 %; $P=1$) or September fructose (0.6 ± 1.1 %; $P=1$). July fructose (0.4 ± 1.1 %) was lesser than June fructose (6.2 ± 1.1 %; $P<0.01$) but was not different from September fructose (0.6 ± 1.1 %; $P=1$). June fructose (6.2 ± 1.1 %) was greater than September fructose (0.6 ± 1.1 %; $P<0.01$). Conversely, for PM samples, no differences were seen in fructose concentrations ($P>0.05$).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.7$). Interestingly, there were also no effects of time, TRT, or month ($P>0.4$).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.2$). Interestingly, there were also no effects of time, TRT, or month ($P>0.4$).

Table 8. Fructose content (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 1.1)									
CON	9.7	0.2	0.7	0.8	2.2E-16	0.4	1.0	1.1						
FERT	2.7	0.3	2.8E-02	0.3	0.5	0.1	0.2	0.6						
MO Mean	3.2		0.4		0.3		0.7		NS	NS	<0.01	NS	NS	<0.01
MO*Time AM	6.2[b]	-	0.4[a]	-	0.3[a]	-	0.6[a]	-						
MO*Time PM	-	0.2[a]	-	0.5[a]	-	0.3[a]	-	0.9[a]						
					Jiggs Bermudagrass (SEM ± 0.8)									
CON	0.7	1.4	0.04	1.1E-17	1.3	1.2	0.02	1.3						
FERT	1.7	3.1	0.02	2.2E-16	1.7	0.07	1.2	1.3	NS	NS	NS	NS	NS	NS
MO mean	1.7		0.02		1.0		1.0							
					Tifton 85 Bermudagrass (SEM ± 0.7)									
CON	0.03	0.4	1.9	0.03	-8.3E-17	2.3	0.5	1.5						
FERT	2.6	0.2	0.3	0.2	0.9	0.5	0.7	1.7E-03	NS	NS	NS	NS	NS	NS
MO mean	0.8		0.6		0.9		0.7							

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

Glucose

Bahiagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.3$). Interestingly, there were also no effects of time, TRT, or month ($P>0.2$).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, or month by time ($P>0.1$). There was an effect of the month by TRT interaction ($P=0.01$; Table 9), whereby, for CON samples, August glucose (3.0 ± 0.4 %) was greater than June glucose (0.6 ± 0.4 %; $P<0.01$) and September glucose (0.5 ± 0.4 %; $P<0.01$) but was not different from July glucose (1.2 ± 0.4; $P=0.9$). There were no other differences seen between CON samples ($P>0.05$). Conversely, there were no differences seen between FERT samples across all months ($P>0.05$). Interestingly, August CON samples (3.0 ± 0.4 %) had a greater glucose concentration than August FERT samples (0.6 ± 0.4; $P<0.01$).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.3$). There was an effect of TRT, with CON samples (1.8 ± 0.3 %) having a greater glucose concentration than FERT samples (1.0 ± 0.3 %; $P=0.03$). There were no effects of month or time ($P>0.1$).

Table 9. Glucose content (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 0.8)									
CON	1.5	2.4	1.4	1.5	0.9	0.4	0.9	1.6	NS	NS	NS	NS	NS	NS
FERT	1.6	0.8	0.2	0.2	1.5	0.2	0.8	1.4						
					Jiggs Bermudagrass (SEM ± 0.8)									
CON	0.3	0.9	1.6	0.9	2.0	3.9	0.7	0.2						
FERT	0.4	0.6	1.3	0.3	0.7	0.5	0.6	0.7						
CON MO mean	0.6[a]		1.2[b]		3.0[b]		0.4[a]		0.02	NS	0.01	NS	0.01	NS
FERT MO mean	0.5[a]		0.8[a]		0.6[a]		0.7[a]							
					Tifton 85 Bermudagrass (SEM ± 0.3)									
CON	0.8	1.3	3.2	2.2	1.7	2.4	0.9	1.8						
FERT	0.3	0.5	0.7	0.6	1.0	2.1	0.9	1.7						
CON mean	1.8[b]		-	-	-	-	-	-	0.03	NS	NS	NS	NS	NS
FERT mean	1.0[a]		-	-	-	-	-	-						

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

Sucrose

Bahiagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.1$). Interestingly, there were also no effects of time, TRT, or month ($P>0.1$).

Jiggs Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, month by time, or month by TRT ($P>0.4$). Interestingly, there were also no effects of time, TRT, or month ($P>0.1$).

Tifton 85 Bermudagrass. There were no effects of the interactions of month by TRT by time, TRT by time, or month by TRT ($P>0.1$). There was an effect of the month by time interaction (Table 10), where, for samples collected in the AM, there were no differences seen across months ($P>0.05$). For samples collected in the PM, August sucrose (2.35 ± 0.4 %) was greater than June sucrose (0.3 ± 0.4 %; $P<0.01$). There were no other differences seen within PM samples ($P>0.05$). Interestingly, samples collected in August in the AM (0.4 ± 0.4 %) had a much lower sucrose concentration than samples collected in August in the PM (2.35 ± 0.4 %; $P<0.01$). There was no effect of TRT seen ($P>0.5$).

Table 10. Sucrose content (%DM) of three species of C4 grasses across a four month (MO) growing season and two times of day (time), that were either fertilized monthly with 206 kg/ha of 16-6-12 (FERT) or remained unfertilized (CON).[1]

Treatment (TRT)	June		July		Aug		Sept		TRT	TIME	MO	TRT* TIME	TRT* MO	TIME* MO
	AM	PM	AM	PM	AM	PM	AM	PM						
					Bahiagrass (SEM ± 0.8)									
CON	0.1	1.1	2.2	4.8	2.6	1.1	3.0	2.6	NS	NS	NS	NS	NS	NS
FERT	1.2	1.0	0.3	0.6	2.5	0.2	0.9	2.3						
					Jiggs Bermudagrass (SEM ± 0.8)									
CON	1.6	1.3	1.2	0.8	0.5	2.2	0.4	1.7	NS	NS	NS	NS	NS	NS
FERT	0.7	1.7	0.4	0.02	0.3	1.4	1.2	2.1						
					Tifton 85 Bermudagrass (SEM ± 0.3)									
CON	1.2	0.3	1.0	1.0	0.1	1.6	0.7	1.7						
FERT	0.5	0.3	0.1	0.2	0.7	3.1	1.4	0.3	NS	NS	NS	NS	NS	0.02
Time*MO AM	0.9[a]	-	0.6[a]	-	0.4[a]	-	1.1[a]	-						
Time*MO PM	-	0.3[a]	-	0.6[a]	-	2.4[b]	-	1.0[a]						

[1]No interactions of month x TRT x time were detected for any species $P > 0.1$

CHAPTER V

Discussion

To recap, the objectives of this research were to identify the roles of month, time of day, and fertilization on nutrient profiles of three common grass species used for equine nutrition in southeast Texas. Some of the key findings included increasing fiber content across the growing season, the impact of time of day on crude protein concentrations, and the minimal effect of any variable on sugar concentrations.

The results of this study indicate that NDF and ADF concentrations increase across the growing season despite being harvested every 4 weeks. Previous research using C3 grasses indicated that regular harvesting would prevent the seasonal rise in NDF and ADF as compared to grasses allowed to grow continuously. The disparate findings between the current and previous studies could be due to mechanistic differences between C3 and C4, whereby C4 have adapted to extreme heat and low rainfall. One report indicated that there is a positive correlation between temperature and the NDF and ADF concentrations found in C4 grasses regardless of regular grazing or clipping (Henderson and Robinson, 1982). Previous studies have also found the same increasing NDF and ADF concentrations across the growing season, with lower values present in the spring than in the summer (Kering et al., 2011). These results indicate that grazing during June will provide a lower fiber forage to a horse than grazing the same pasture in July through September. This also indicates that early cutting hay could be of higher nutritional value to the horse than late cuttings of hay from the same pasture.

Fiber content did not vary from AM to PM in this study, which is an inconsistent finding with previous research showing tall fescue, a C3 grass, to have higher ADF and NDF contents in the AM as compared to the PM (Mayland et al., 1998). Previous research in perennial ryegrass has also found that NDF concentrations were higher in the morning than in the afternoon (Delagarde et al., 2000). Plants assimilate carbon into simple sugars during sunny days and then convert these sugars into complex carbohydrates overnight, leading to these higher ADF and NDF contents in the mornings. A possible explanation for differences between C3 and C4 plants is that of rainfall. It is possible that C4 grasses maintain a higher fiber content throughout the day in response to low levels of rainfall, but further research is needed to confirm this, as well as comparisons across years with differing rain fall amounts. Fertilization decreased ADF content in Jiggs Bermudagrass but had no effect on ADF in the other species or on NDF contents in this study. This conflicts with previous research, which indicated that fertilization would decrease both NDF and ADF concentrations in C4 grasses (Johnson et al., 2001). Findings from this study could be related to the rate of fertilization, as Johnson et al. (2001) found the lowest NDF and ADF concentrations in grasses fertilized at a rate of 157 kg of N/ha, which was 4.7 times the amount used in the present study.

Crude protein content of all grasses was improved through fertilization, however a steady decline through the growing season was also observed. The improvement in crude protein through fertilization is well characterized for C3 grasses and has also been demonstrated in C4 (Mutz and Drawe, 1983). In regard to the decline during the growing season, C4 grasses have been shown to have a rapid decline in crude protein throughout the growing season whether or not there is repeated clipping or mowing (Perry Jr and

Baltensperger, 1979). With regard to growing season, a previous study found that nitrogen fertilization increased herbage crude protein content throughout the growing season (Bittman and Kowalenko, 1998). However, the former study was conducted on C3 grasses and occurred in a high rain-fall environment, which does not mimic the conditions under which the present study was conducted.

A second key finding was that crude protein contents were highest in the morning. One study done in tall fescue, a C3 grass, found crude protein content to be highest in the afternoon (Mayland et al., 1998). A different study done in timothy grass hay found higher crude protein percentages at sunrise (Silva et al., 2020). The disparate findings between studies could be explained by allowing the grass to dry on the field instead of being immediately processed or frozen for later analysis. The longer grass is left on the field to dry, the greater the risk of leaf shatter and loss of protein.

There were minimal impacts of time or treatment on sugar content in these C4 grasses, and minimal differences between species. This is in opposition to C3 grasses which have higher sugar contents in the PM than AM and show large variation across species (Byrd and Staniar, 2005; DeBoer et al., 2018). This discrepancy is due to the fact that fructose in C3 grasses can be translocated to the stem, leading to a greater capacity to store fructose. C4 grasses cannot transport sugars to the stem, and are therefore self-limiting in their ability to store NSC, leading to lower sugar contents (DeBoer et al., 2018).

Previous research has shown that C3 grasses accumulate sugars during the day, with highest levels seen in the evening hours (Downing and Gamroth, 2007). The same trend was not seen in the current study, with little variation found between AM and PM

samples. One possible explanation for this discrepancy is the biomechanical differences between C3 and C4 grasses. To protect against excessive water loss during high temperatures, C4 grasses keep their stoma closed. By closing their stoma, C4 grasses limit their ability to assimilate CO_2, thereby limiting their ability to produce sugars (Bellasio et al., 2018).

Fertilization increased total sugar content in Tifton 85 Bermudagrass and had a small effect on Jiggs Bermudagrass in the fall. The mechanisms that allow for fertilization to increase sugar content are not well understood, but it is believed that increasing available nitrogen in deficient plants increases overall growth. This overall growth increase leads to a higher proportion of chloroplasts in the leaves of the plant, allowing for a greater capability for sugar production via photosynthesis (White, 1973).

There was a high level of fructose seen in the AM samples of Bahiagrass in the month of June that was not seen in the PM samples of the same species and month. We believe this to be an anomaly caused by the presence of weeds and perennial ryegrass in the plots. These plants normally die off by the beginning of June, but some persistence does occasionally occur. This finding is in opposition to previous research, which found low levels of sugars, specifically fructose, in Bahiagrass early in the growing season (Housley and Pollock, 1993; Beneragama and Kumara, 2018).

CHAPTER VI

Conclusion

This study has shown that C4 grasses not only have lower overall sugars than C3 grasses, but also that there is minimal diurnal variation or seasonality seen. This is in direct opposition to previous research on C3 grasses and was not the expected finding of this study. In northern climates, horses prone to laminitis or other metabolic diseases can only be safely grazed during the morning hours to avoid a high sugar intake due to the diurnal variation and increase in sugar content across the day.

With a lack of diurnal variation in C4 grasses, there are no specific times that a horse prone to laminitis needs to avoid grazing to reduce sugar intake. Therefore, horses prone to laminitis or other metabolic diseases impacted by high sugar content in feed can be safely grazed on C4 grasses throughout the day and grazing season.

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VITA

Rachel Marie Miller

EDUCATION

Master of Science student in Agriculture at Sam Houston State University, August 2019 – present. Thesis title: "The effect of Seasonal Variation and Fertilization on the Nutritional Content of C4 Grasses."

Bachelor of Science (May 2019) in Animal Science, Sam Houston State University, Huntsville, Texas.

ACADEMIC EMPLOYMENT

As Masters Student at Sam Houston State University

3. Fall 2020. EQSC 4379: Equine Nutrition. 3 credits. Lecture Teaching Assistant.
2. Spring 2020. EQSC 2364: Equine Science. 3 credits. Lecture and Laboratory Teaching Assistant.
1. Fall 2019. EQSC 2364: Equine Science. 3 credits. Lecture and Laboratory Teaching Assistant.

COMMUNITY SERVICE

4. Spring 2021. Private Tutor. Tutored students in equine science courses to improve their grades. Attendance: 10 students
3. April 2019. Area IX CDE Contests. Facilitated movement of contestants around equine facility. Attendance: 200 contestants
2. October 2019. SHSU Children's Barnyard. Assisted with equine portion of animal exploration day for local elementary school children. Huntsville, TX. 5-hour event. Attendance: 300 students and teachers
1. October 2018. SHSU Children's Barnyard. Assisted with equine portion of animal exploration day for local elementary school children. Huntsville, TX. 5-hour event. Attendance: 300 students and teachers

ARTICLES IN PREPARATION FOR SUBMISSION TO PEER REVIEWED JOURNALS

1. **Miller, Rachel**, J. Suagee-Bedore, K. Thompson, L. McFarland, T. Thomson, K. Stutts, M. Anderson, A. Wagner, I. Girard. Comparison of physiological responses to exercise in horses on a conditioning regimen versus inactive horses receiving a yucca and fenugreek dietary supplement. In preparation for submission to *J. Equine Vet. Sci.*

ABSTRACTS PRESENTED AT CONFERENCES

6. **R. M. Miller**, J. K. Suagee-Bedore. Use of a life-sized horse replica to teach haltering in a 2000 level equine course. Presented at the 2021 NAEAA Virtual Annual Conference.
   Presenter (poster)
5. **R. M. Miller**, P. M. Urso, J. K. Suagee-Bedore. The effect of growing season and fertilization on ADF and NDF levels in three species of warm-season grass. Presented at the Equine Science Society 2021 Virtual Symposium.
   Presenter (oral)
4. J. K. Suagee-Bedore, **R. M. Miller**, T. L. Thomson, K. K. Fikes. Experimental learning in an equine nutrition laboratory promotes applied nutrition decisions. Presented at the 2021 NAEAA Virtual Annual Conference.
3. E. A. Snyder, N. Shost, **R. M. Miller**, K. K. Fikes, R. Smith, B. A. Corl, A. L. Wagner, I. D. Girard, J. K. Suagee-Bedore. Late gestation supplementation of long chain fatty acids increases foal docosahexaenoic acid concentrations at birth. Presented at the Equine Science Society 2021 Virtual Symposium.
2. **Miller, Rachel**, J. K. Suagee-Bedore, K. Thompson, L. McFarland, T. Thomson, K. Stutts, M. Anderson, A. Wagner, I. Girard. Comparison of physiological responses to exercise in horses on a conditioning regimen versus inactive horses receiving a yucca and fenugreek dietary supplement. Presented at the Ninth Annual ACT Student Research Symposium.
   Presenter (Poster)
1. **Miller, Rachel**, J. K. Suagee-Bedore, K. Thompson, L. McFarland, T. Thomson, K. Stutts, M. Anderson, A. Wagner, I. Girard. Comparison of physiological responses to exercise in horses on a conditioning regimen versus inactive horses receiving a yucca and fenugreek dietary supplement. Presented at the 2019 ASAS Southern Section Annual Meeting.
   Presenter (Oral)
   In: 2019 J. Anim. Sci. 97 (Suppl. 1):30. Abstract # 93.

GRANTS

3. Title: Graduate Student Research Grant
   PI: Rachel Miller. Co-PI: Jessica Suagee-Bedore

   Submitted to The Graduate School at Sam Houston State University

   Amount requested: $949.00
   Amount awarded: $949.00
   Grant submitted: May 2021

2. Title: The NSC content of warm season grasses throughout their growing season and its potential impact on equine metabolic diseases
   PI: Rachel Miller. Co-PI: Jessica Suagee-Bedore

   Submitted to the School of Agricultural Sciences at Sam Houston State University

   Amount requested: $1,920.00
   Amount awarded: $500.00
   Grant submitted: October 2019

1. Title: Undergraduate Travel Grant
   PI: Rachel Miller. Co-PI: Jessica Suagee-Bedore

   Submitted to the College of Science and Engineering Technology at Sam Houston State University

   Amount awarded: $500.00