EFFECTS OF CRUDE PROTEIN CONTENT ON INTAKE AND DIGESTION OF COASTAL BERMUDAGRASS HAY BY HORSES

A Thesis

by

CHELSEY LYNN SPURGIN

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

MASTER OF SCIENCE

May 2010

Major Subject: Animal Science

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Approved by:

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ABSTRACT

Effects of Crude Protein Content on Intake and Digestion of

Coastal Bermudagrass Hay by Horses. (May 2010)

Chelsey Lynn Spurgin, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Tryon Wickersham
Dr. Josie Coverdale

This study was conducted to determine the effect of forage CP level on intake and digestion of Coastal bermudagrass hay by horses. Four cecally fistulated geldings were used in a 4 × 4 Latin square design with four treatments and four periods. Horses were fed one of four Coastal bermudagrass hays consisting of 7, 10, 13, or 16% CP during each of the 4 15-d periods. Intake and apparent digestibility were determined for each horse at the end of each period by total fecal collection. In addition, cecal fluid and blood samples were collected from each horse on the last day of each period for determination of cecal ammonia, cecal pH, plasma urea nitrogen, and plasma glucose concentrations.

Crude protein concentration of Coastal bermudagrass hay influenced equine intake and digestion. Increasing CP concentration linearly increased digestible OM intake (**DOMI**) from 3.79 to 5.98 kg/d for 7 and 16% CP hay, respectively ($P = 0.04$). Furthermore, as forage CP level increased, CP intake increased linearly ($P < 0.01$). Forage CP level had no effect on forage DM intake. Quadratic effects ($P \leq 0.05$) were

observed for forage OM, NDF, ADF, and digestible energy. Overall digestibility was lowest for the 7% CP hay and highest for the 10% CP hay.

Cecal pH remained above 6.62 irrespective of treatment and time, indicating that cecal pH was suitable for microbial growth. As forage CP level increased, cecal ammonia concentration increased linearly from 0.03 m*M* for the 7% to 1.74 m*M* for the 15% CP hay ($P < 0.01$). Concentration of plasma glucose also linearly increased ($P = 0.04$) from 68.77 to 73.68 mg/dL as CP concentration increased from 7% to 16% CP. Plasma urea nitrogen exhibited a quadratic effect as concentration increased ($P < 0.01$) from 4.34 to 5.61 m*M* for the 7 and 16% CP hays, respectively.

DEDICATION

I dedicate this thesis to my parents, Benny and Deanie Spurgin, without whom, I would not stand a chance. And to my grandmother, Lavern Smith, whom I adore.

ACKNOWLEDGEMENTS

To thank God in this type of setting is not like me. However, I feel the course of events that has led me to write this paragraph has most certainly been divinely orchestrated. Not because graduate school has, in any way, resembled Eden or provided bliss. Rather, because this experience and the challenges it has presented are unlike anything I had ever planned for myself or previously encountered. So, I thank God for offering this path in front of me, which presented me with many new experiences and for which I am grateful.

For the opportunity to begin the journey that is scarcely summarized by this thesis, I would like to thank my major professor, committee co-chair, and friend, Dr. Wickersham. Thank you for teaching me about cows, fiber, and other topics laced in good conversation. I would also like to thank my committee co-chair, Dr. Coverdale for her patience and assistance during my research. Also, thanks go to the remaining member of my committee, Dr. Redmon, for his participation and support.

I would like to acknowledge my wonderful friends. Special thanks go to Amanda and Amy whose visits to College Station are some of the most memorable and hilarious moments of my graduate career. I cannot tell you how grateful I am for your friendship. Amy, I am sorry about your losing a contact at the barn (I still owe you a Starbucks beverage). Also to Jared who should be acknowledged for his gasoline bill alone. Thank you for coming to see me when I could not come to see you, for lifting

heavy barrels of hay, and for you relentless support and encouragement. I am so happy that I met you so long ago and that you are a part of my life today.

At last, I would like to acknowledge my parents. Thank you, Mom and Dad. Your love and support have never gone unnoticed. The sacrifices you have made for my benefit are countless and I owe each of my achievements to you. I am very thankful to have been blessed with parents who are so encouraging, generous, and fun. I love you.

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CHAPTER I

INTRODUCTION: REVIEW OF LITERATURE

Introduction: Forage quality is the potential of forage to be utilized by an animal in order to meet its requirements or from the perspective of a producer the ability of forage to allow an animal to meet performance/production goals. Measurement of forage quality is difficult because it requires feeding the forage in question and subsequently determining animal performance. To avoid the cost of performance trials, researchers and nutritionists often measure forage utilization by intake and digestion to describe forage quality and then extrapolate these results to animal performance. Forage utilization projects involve significant costs and require the forage to be fed before its quality can be determined; therefore, measures of nutritive value (e.g., crude protein, neutral detergent fiber, and acid detergent fiber) are often related to measures of animal performance and forage utilization. Ultimately, these measures of nutritive value can be used to predict animal performance without having to feed the forage resource. While relationships to nutritive value and forage quality are well characterized in other species, namely ruminants, there is a dearth of information describing this relationship in equines.

Equine performance is measured differently from food animal species because the goals of livestock and equine producers are most often different. Sound growth and structural development, athletic performance, health, and longevity are primary

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This thesis follows the style of the Journal of Animal Science.

management objectives of horse producers (Aiken et al., 1989a,b). Production of horses is also typically measured by the performance of individual animals as opposed to groups or herds. In contrast to the apparent difference, both equine and livestock producers depend on high-quality, forage-based diets to meet the requirements of their animals. Therefore, the focus of this review is to quantify our current understanding of forage utilization by equines and relate that to our understanding in other livestock species, primarily ruminants.

Intake: Forage quality is often determined by an animal's willingness to consume the offered forage. Accordingly, measurement of intake is useful in describing forage quality. Intake is a measure of the interaction between both the plant and animal and how the animal responds to the offered diet. Forage characteristics such as caloric density, nitrogen content, and digestibility can positively or negatively impact intake (Van Soest, 1994). The physiologic status (e.g., pregnancy, stage of lactation, age, sex) of the animal also contributes to observed intake responses. Physical restraints due to gut fill and changes in rate of passage have a significant effect on intake in ruminants (Van Soest, 1994). Forages high in fiber are bulky and have a slower rate of passage due to their lower digestibility, which limits intake. Low fiber forages travel faster through the gastrointestinal tract due to less fibrous bulk and faster digestion and allow for a greater intake by the animal.

Caloric density is the concentration of energy in a feedstuff and has been reported to modulate intake in swine. Intake of diets high in digestibility energy (**DE**) are often less than diets containing less DE because energy requirements have been addressed and

metabolic constraints limit intake. The ability of DE intake to constrain or limit intake is most often observed in diets that are highly digestible and have a high DE concentration without excess fiber (Van Soest, 1965). Henry (1985) observed increases in intake by pigs when the caloric density of the diet was low. This data indicates that swine attempt to meet their energy requirements when caloric density is low by increasing intake. Correspondingly, Lofgren and Warner (1972) fed swine low-, medium-, and high-energy diets and reported that the swine consumed the same amount of energy per day regardless of the caloric density of the diet. In other words, animals fed the low-energy ration increased dry matter consumption to compensate for decreased caloric density and thus achieved a desired caloric intake. Animals consuming the low-energy diet had significantly lower plasma insulin levels post-feeding, indicating a possible metabolic effect on intake. Insulin may stimulate a neurologic response center to increase appetite in swine.

Caloric density has also been linked to intake in horses. Bush et al. (2001) used fat supplementation to vary the caloric density of equine diets and reported horses consuming the lowest caloric density diet had the greatest dry matter intakes. Additionally, Cymbaluk and Christison (1989) reported growing horses offered forage- or concentrate-based diets adjusted intake to achieve similar digestible energy DE intakes. Horses fed forage-based diets consumed 18% more dry matter (**DM**) in order to reach comparable DE intakes of horses fed the concentrate-based diet. In contrast to swine diets, equine diets often contain a higher percentage of forage; therefore, meal size and gut-fill may prevent horses from achieving the same level of DE intake by increasing

consumption. This difference in diets may complicate management of competitive equine athletes which require large amounts of DE to maintain a satisfactory level of performance. To overcome the limitations of gut fill and high fiber diets, horses may benefit from smaller, more frequent meals in order to meet DE requirements.

In contrast to metabolic suppression of intake in diets containing high levels of DE, intake of high fiber diets can be constrained by gut capacity (Waldo, 1986). Accordingly, forage cell wall content has been negatively correlated to voluntary intake in ruminants (Van Soest, 1965). This is the result of decreased rates of digestion with high fiber diets and the subsequent accumulation of digesta in the gastrointestinal tract. Elevated levels of gastrointestinal fill result in decreased consumption (Jung and Allen, 1995). This explains the higher intakes of ruminants and equines consuming alfalfa compared to grass hay. While alfalfa is often higher in lignin than grasses, intake of alfalfa is higher because of a smaller cell wall fraction and higher digestibility (Van Soest, 1965; LaCasha et al., 1999). Van Soest (1965) reported intake in ruminants becomes constrained by fill when cell wall material is in excess of 55-60% of DM. Despite anatomical differences between ruminants and horses, Dulphy et al. (1997) reported that horses have voluntary dry matter intakes (**VDMI**) comparable to sheep. Additionally, La Casha et al. (1999) reported yearling horses increased intake as cell wall concentration declined when offered forages of similar digestibilities. Horses offered alfalfa, Matua, and Coastal bermudagrass hay consumed more of the Matua hay that was 17% lower in cell wall components but only 4% lower in apparent digestibility than the Coastal bermudagrass hay.

Although greater intake is generally associated with higher forage quality, increasing forage intake may compromise the performance of some competitive equine athletes. Diets of these horses are supplemented with concentrate to increase caloric density without adding bulk (Bush et al., 2001). Forage-based rations hold more water in the gastrointestinal tract and cause horses to increase water consumption, increasing gastrointestinal weight and bulk undesirable for elite equine athletes (Pagan et al., 1998; Warren et al., 1999; Rice et al., 2001). The added weight increases energy expenditure required to meet performance goals (Rice et al., 2001). However, equines competing in endurance events benefit from the water and electrolyte reservoir created from high-forage diets (Warren et al., 1999). High-quality forage should be fed to endurance-type equine athletes to prevent unmet nutrient requirements due to poor digestibility and reduce the likelihood of increased gastrointestinal weight.

Forage provides energy in the form of hydrolyzable and fermentable carbohydrates. Hydrolyzable carbohydrates (**CHO-H**) are those susceptible to foregut hydrolysis by mammalian enzymes. The CHO-H fraction includes the nonstructural carbohydrates (**NSC**), monosaccharides, disaccharides, and starch (Hall, 2003) and is digested and absorbed in the small intestine. If the amount of CHO-H consumed by the animal exceeds enzymatic or transporter capacity to digest the CHO-H, the remaining CHO-H is fermented in the hindgut (Hintz et al., 1971; Dyer et al., 2002). Although abundant in concentrate feeds, hydrolyzable carbohydrates are typically not a substantial component of forages. Hoffman et al. (2001) reported CHO-H to account for only 19 and 38% of NSC in grass-legume hay and pasture samples, respectively.

Carbohydrates not hydrolyzed by mammalian enzymes and absorbed into the enterocyte have the potential to be fermented by microbes in the equine hindgut to yield volatile fatty acids (**VFA**; Hintz et al., 1971; Argenzio et al., 1974; Hoffman et al., 2001), which provide between 30 and 80% of the energy to an equine (Glinsky et al., 1976; Vermorel et al., 1997). Fermentable carbohydrates are classified as rapidly fermentable (**CHO-F_R**) or slowly fermentable (**CHO-F_S**) (Hoffman et al., 2001). Pectins, fructans, oligosaccharides, and any starch escaping enzymatic hydrolysis, make up the CHO-FR fraction (Hoffman et al., 2001; Hall, 2003). Slowly fermentable carbohydrates include the structural polysaccharides found in the cell wall fraction, hemicellulose, cellulose, and lingo-cellulose (Hoffman et al., 2001).

Hydrolyzable carbohydrates are more energy efficient than CHO-F because they may be digested and directly absorbed in the foregut as monosaccharides. Due to higher amounts of CHO-H, grain-supplemented diets result in the absorption of more free glucose from enzymatic digestion in the foregut. However, equine diets are forage based and horses consuming grain-forage rations rely predominantly on hindgut fermentation as their primary energy supply (Argenzio and Hintz, 1972). Glucose absorbed in the foregut accounted for less than 14% of DE in ponies consuming a diet of 69% oats, and less than 7% in those consuming an all-roughage diet (Argenzio and Hintz, 1972). As the proportion of CHO-H decreases in the diet, the percentage of glucose absorbed from the foregut decreases, and a greater percentage of glucose is synthesized from fermentation products. Gluconeogenesis of propionate absorbed from the hindgut contributes to the maintenance of plasma glucose levels in the horse when there is limited direct absorption

of glucose form the foregut (Simmons and Ford, 1991). Argenzio and Hintz (1970) demonstrated intravenous infusions of propionate resulted in an increase in plasma glucose in fasted ponies. Volatile fatty acid production, and ultimately energy availability from VFAs is dependent on diet. Addition of high-quality forage to equine diets has the potential to increase VFA production in the cecum, thus elevating energy availibility. Kern et al. (1973) fed timothy and red clover hay to ponies consuming diets with or without oats. Feeding the more digestible red clover forage increased VFA concentrations in both unsupplemented and grain-supplemented ponies. Hintz et al. (1971) reported total cecal VFA concentrations were lowest for horses consuming a 1:4 forage-grain diet than for horses consuming either 1:0 or 3:2 forage-grain diets. High concentrate diets and the subsequent escape of starch from the small intestine facilitate lactic acid production in the cecum and consequently change the cecal microbial environment. This alteration in microbial populations changes VFA concentrations in the cecum (Medina et al., 2002).

In addition to energy balance, VFAs appear to have a physiologic effect on intake. Ralston et al. (1983) investigated a possible metabolic effect of intracecal VFA concentration on intake in ponies. Intracecal infusions of acetate and propionate increase satiety, and the highest infusion of propionate decreased meal size, indicating cecal VFA concentration alters intake in equines. Because volatile fatty acids produced from hindgut fermentation serve as the primary energy supply for horses consuming all forage diets, the energy value of forages is largely determined by the amount and accessibility of its fibrous parts.

Digestibility: The extent of forage digestion, in part, determines the availability of nutrients for maintenance, production, and performance. Therefore, forage quality is partially dependent on digestibility. Plants contain structures, compounds, and associations between compounds to inhibit digestion, which decreases forage quality while improving the likelihood of plant survival. Leaf and stem are two most basic anatomical divisions of plants and are helpful when discussing nutrient composition and forage digestibility. Leaves contain less fiber and are therefore more digestible than stems (Buxton and Redfearn, 1997; Moore and Jung, 2001). Stem epidermis, sclerenchyma, and xylem tissues are more resistant to microbial fermentation (Akin, 1989; Wilson, 1993). The epidermis is the outer cellular covering of the plant and is thicker and thus less digestible on the stems of plants. Sclerenchyma tissue is found adjacent to vascular bundles in leaves and stems and develops a thick cell wall which becomes lignified with plant maturity. Xylem is a vascular tissue with thick, lignified walls found throughout the plant (Wilson, 1993). Conversely, phloem, mesophyll, and immature parenchymal tissues are highly digestible (Akin, 1989). Pholem is a smaller portion of the vascular tissue and is found throughout the plant (Wilson, 1993). Mesophyll cells make up the bulk of leafy tissue but also occur in small disconnected clusters along the outer edge of the stem (Wilson, 1993). Parenchymal cells are chiefly found in stems and midrib of grasses, but also comprise a small portion of leaves (Wilson, 1993). Tissues intermediate in digestibility include the leaf epidermis and parenchymal bundle sheath cells (Akin, 1989). The parenchyma bundle sheath is a specialized group of cells surrounding vascular tissues of C_4 grasses, where it is used in

the C_4 photosynthetic pathway (Wilson, 1993). Because of the anatomical arrangement of these tissues, leaves are generally more digestible than stems (Albrecht et al., 1987). Consequently, as the ratio of leaf to stem area increases, forage quality increases.

Cell contents are more digestible than those found in the cell wall fraction of plants, due to the fibrous nature of plant cell walls. Cell contents include non-structural carbohydrates (**NSC**) and crude protein (**CP**). Non-structural carbohydrates include CHO-H and CHO-F_R constituents, monosaccharides, oligosaccharides, fructans, and starch (Hall, 2003). Due to their extensive digestion, cell contents provide a source of readily available energy and nutrients (Fonnesbeck, 1969; Hoffman et al., 2001). The high digestibility of cell contents is relatively consistent between and within forage species and positively contributes to forage quality by translating into increased digestibility (Fonnesbeck, 1969). Forage digestibility will reflect the amount of cellular contents present in that forage: forages higher in NSC will, generally, be higher in digestibility (Harbers et al., 1981). However, cell contents make up a much smaller portion of the plant than cell wall material, especially in warm-season grasses, and therefore have a lesser overall impact on forage quality (Moore and Hatfield, 1994).

Forage digestibility is largely dependent on the amount and digestibility of cell wall constituents due to their abundance within the plant. Plant cell walls are multilayered, consisting of primary, secondary, and tertiary walls deposited at different times during growth and maturity (Iiyama et al., 1993). The primary cell wall is laid down during cellular growth and is constructed of cellulose microfibrils, hemicellulose, pectin, xylans and limited amounts of protein (Jung and Allen, 1995). Primary cell walls

of neighboring cells are separated by thin middle lamella. In young, actively growing cells, the middle lamella and primary cell wall are thin and not yet lignified and thus highly digestible (Iiyama et al., 1993). Lignin deposition begins in the middle lamella and primary cell wall only after expansion has ceased (Terashima et al., 1993).

Once cellular expansion is complete, the secondary wall begins thickening along the inner circumference of the primary cell wall. Secondary cell walls are much thicker than primary cell walls, consisting of up to three layers of cellulose, xylans, and varying amounts of lignin. The thickness and rigidity of secondary cell walls provides structural strength to the plant but reduces the overall digestibility of the forage cell wall portion (Wilson, 1993; Wilson and Mertens, 1995). The tertiary cell wall is a thin layer lining the luminal side of the secondary cell wall. Tertiary cell walls appear to be entirely indigestible when subjected to microbial fermentation (Wilson, 1993). Because cell wall expansion occurs as plants mature, younger plants are higher quality forage because the ratio of cell contents to cell wall is highest.

Cell wall components vary in digestibility. Pectins are highly digestible by microbial fermentation (Hatfield and Weimer, 1995; Jung and Engels, 2002). Hatfield and Weimer (1995) reported digestibilities of 92 and 83% for alfalfa leaf and stem cell walls respectively. Hemicellulose and cellulose true digestibilities have been estimated at 49.5 and 43.4 % respectively (Fonnesbeck, 1969). Lignin is effectively indigestible and its association with cell wall carbohydrates may decrease their digestibilities as well. The effects of cell wall portions on forage quality are generally negative in both ruminant and equine studies. However, ruminants digest fiber more effectively than horses; therefore,

reliance on ruminant data to estimate fiber digestion in horses may overestimate forage utilization in horses (Koller et al., 1978). These differences in equines and ruminants are attributed to the greater quantity of ruminal microbes (Kern et al., 1973; Koller et al., 1978) and the advantageous arrangement of the ruminant gastrointestinal tract. The additional foregut fermentation in the ruminant increases exposure of digesta to microbial fermentation prior to enzymatic and hindgut fermentation, the only two opportunities for digestion in equines.

Of the cell wall constituents, the structural polysaccharides hemicellulose and cellulose most significantly contribute to forage quality due to their large share of total cell wall material. Cellulose is the most abundant constituent of the cell wall fraction (Terashima et al., 1993). Cellulose is initially deposited along the primary cell wall in long straight microfibrils of β-1, 4 linked glucose molecules (Terashima et al., 1993; Wilson, 1993). The secondary cell wall contains cellulose arranged at a rotated angle to the primary microfibrils to form a strong polysaccharide lattice (Harris, 1990). The β linkages that contribute to the textile strength of cellulose are resistant to enzymes secreted by the gastrointestinal tracts of higher mammals. Consequently, horses rely on microbial fermentation to degrade cellulose from forage material passing through the hindgut. Cellulose digestibility in the equine appears to be relatively consistent between forage species. Fonnesbeck (1968) reported apparent cellulose digestibilities of 44.7, 49.1, 41.5, 42.8, 47.5, 49.9% for horses consuming alfalfa, Lincoln bromegrass, reed canarygrass, alta fescue, timothy, and red clover, respectively. Vander Noot and Gilbreath (1970) reported 52.1, 54.4, 48.3, and 37.8% apparent digestibilities for horses

consuming orchardgrass alfalfa, timothy, and bromegrass, respectively. The lower cellulose digestibility for bromegrass in this study is likely due to its lower quality resulting from 5.9% CP level and later maturity at harvest when compared to the other forages used. Hemicellulose differs from cellulose in composition and structure. Hemicellulose is comprised of branched β-1, 4 linked chains of predominantly xylose residues with lesser amounts of arabinose, glucuronic acid, and galactose, and acetyl groups (Hatfield, 1989). Horses appear to digest hemicellulose of grasses more effectively than of legumes. Like cellulose, hemicellulose fermentation provides a significant source of energy for horses (Fonnesbeck, 1968). Due to their large contribution to energy status, the amount and digestibility of the cell wall polysaccharides is an important component of forage quality.

The digestibility of cellulose and hemicellulose is most significantly limited by lignification (Jung and Deetz, 1993). Lignin is essential for the structural integrity of the plant, but limits the digestibility of cell wall polysaccharides (Moore and Jung, 2001). Lignification begins once secondary cell wall accretion has begun in the primary cell wall and middle lamella regions and spreading throughout the secondary layers (Jung and Allen, 1995; Terashima et al., 1993). Lignin propagation begins at the onset of secondary wall thickening at the middle lamella and expands outward into the cell wall layers, following polysaccharide deposition (Jung and Allen, 1995; Terashima et al., 1993). As the quantity of cell wall increases with plant growth, lignin concentration increases exponentially (Jung and Vogel, 1986). Lignin concentration is greatest in the outer middle lamella and primary cell wall regions (Jung and Allen, 1995). However,

quantitatively, the greatest amount of lignin is found within the secondary cell wall do to the overall size of the secondary cell wall. The extent of expansion and aromatic composition of lignin differ among plant tissues, species, and maturities. The ether and ester crosslinkages of the lignin matrix invade cell wall polysaccharides, physically blocking enzymatic attack of the polysaccharides (Jung and Deetz, 1993). In this way, cell wall content negatively affects forage quality because of the limited digestibility of cell wall polysaccharides and its association with negative effects on intake and animal performance.

Jung and Vogel (1986) reported lignin decreased cell wall and DM digestion of C_3 and C_4 grasses and inhibited digestion of cellulose more than hemicellulose. Muller et al. (1972) fed brown midrib (**BRM**) corn silage to lambs to demonstrate effects of lignin on digestibility and VDMI. The BRM ration was similar in cell wall material but had 34% less permanganante lignin than the control corn silage. Lambs fed the BMR ration had 29% greater VDMI and greater hemicellulose and cellulose digestibility than those offered the control rations. Greater intakes and digestibilities were attributed to reduced gastrointestinal fill associated with lower lignin concentrations (Muller et al., 1972; Jung and Allen, 1995). Poorly digestible forage is slowly degraded; therefore, has a slower rate of passage through the reticulorumen, which has been reported to decrease intake of ruminants offered low-quality forage (Lippke, 1980). Harbers et al. (1981) reported horses offered three types of grass consumed the hay lowest in digestibility the least, and the most digestible hay showed the highest intake values. Decreasing indigestible cell

wall material decreases gastrointestinal fill and promotes forage intake and digestibility (Jung and Allen, 1995).

Animal Performance: Forage utilization may also be derived from changes in animal performance or production. As expected, both intake and digestibility play a significant role in determining animal performance. Higher intakes in growing horses resulted in increased animal performance, measured as average daily gain (Cymbaluk et al., 1989). Lippke (1980) reported forage DM intake was a better predictor of body weight gain than forage digestibility for ruminants consuming low-quality forage. However, the importance of intake on animal performance may be overestimated due to variation in intake between animals (Heany et al., 1968). Forage digestibility is an indicator of animal performance as it describes the availability of energy (Coleman and Moore, 2003). Anderson et al. (1988) measured animal performance of beef yearlings grazing high-, medium-, and low-in vitro dry matter digestibility switchgrass varieties and reported higher gains for cattle grazing pastures with higher in vitro digestibilities. Aiken et al. (1989a) reported a decrease in average daily gain of Quarter horse yearlings grazing bermudagrass at varying stocking rates as the animals increased consumption of fibrous carbohydrates. Higher stocking rates forced horses to consume larger quantities of the lower layer stem and senescing plant tissues that contain higher cell wall percentages than the leafy grass tissues. This reduced ability to select a higher quality diet translated into the consumption of less digestible forage that ultimately compromised animal performance. Available

In addition to body weight gain, equine performance is measure by rates of heart girth, hip, wither, and body length gain. Ott and Kivipelto (2002) compared these parameters in yearling horses fed a 12% CP concentrate and either alfalfa or Coastal bermudagrass hay. Horses consuming the alfalfa forage out gained an average 0.15 kg • d^{-1} more than those consuming Coastal bermudagrass. Yearlings on the alfalfa ration also had greater gains in heart girth, hip, wither, and body length than those on the Coastal bermudagrass ration. The higher gains resulting from the alfalfa diet are due to the higher CP content (Ott et al., 1979) and digestibility. Horses on the alfalfa diet consumed an average of 25.8% more protein than horses on the Coastal bermudagrass diet. When forage quality is inadequate, horses may regress in performance. Guay et al. (2002) reported mares consuming low-quality Timothy hay lost weight during gestation while those fed alfalfa or Matua hay gained. The Timothy hay used on this trial was 4.1% CP compared to15.5 and 24.9% CP of the Matua and alfalfa hays, respectively. Also, Timothy was 13.0 and 40.1% higher in poorly digestibly NDF than Matua and alfalfa, respectively.

Furthermore, equine performance is also a function of sound gastrointestinal health. Forage is necessary in equine diets to maintain a healthy hindgut pH, decreasing the likelihood for hindgut acidosis and laminitis (Medina et al., 2002). Dietary forage has been linked to a decrease of gastric ulcers in weanlings. Flores et al. (2009) reported high grain diets increased the number of gastric ulcers in weanling horses by as much as 3 fold compared to horses consuming hay diets. Coenen (1990) examined the gastric lining of ponies fed either a mixed feed or all hay diet. None of the ponies consuming the hay diet

showed any visible gastric ulcers compared to 37% of the ponies on the mixed feed diet. Furthermore, forage-deficient diets have been linked to a higher incidence of equine colic (Tinker et al., 1997).

Forage Types: Forage quality is largely dependent on plant species. The most prominent plant groups produced as livestock forage include the legume and grass families, which belong to Dicotyledon and Monocotyledon subclasses, respectively (Moore and Jung, 2001). Legumes and grasses differ in composition, morphology, and nutritive value. Differences in quality and nutritive value between forage types are attributable to the physiologic and anatomical differences between the subclasses and species.

Grasses and legumes share physiologically distinct pathways of carbon fixation. Their metabolic differences account for the majority of the variation in their composition and morphology. Both legumes and grasses harvest light energy via photosynthesis to manufacture biomolecules imperative for growth. In both types of plants, photosynthesis occurs in the chloroplasts and consists of two sets of reactions: the light-dependent reactions and the light-independent reactions, or carbon reactions (Portis, 1982; Taiz and Zeiger, 1998). Light-dependent reactions occur in the thylakoid of the chloroplast and convert light energy into energy available for use by the plant (Portis, 1982; Taiz and Zeiger, 1998). Carbon reactions occur in the stroma of the chloroplast and use this energy for the assimilation of atmospheric CO_2 into carbohydrate precursors (Portis, 1982; Taiz and Zeiger, 1998).

Higher plants utilize a common method to harvest light energy, during which light energy is absorbed by pigments in the thylakoids of the chloroplast (Bassham and Buchanan, 1982). Absorbed energy is used to transfer electrons from H_2O to $NADP^+$, producing O_2 and NADPH (Taiz and Zeiger, 1998). As H_2O is oxidized during electron transfer, a proton gradient forms across the thylakoid membrane (Taiz and Zeiger, 1998). As protons flow down their concentration gradient from the thylakoid lumen into the stroma, ATP is produced (Taiz and Zeiger, 1998). Together, NADPH and ATP fuel the carbon fixation pathway of photosynthesis, also called the Calvin cycle (Taiz and Zeiger, 1998).

The Calvin cycle is used by all green plants to synthesize carbohydrate precursors of starch and sugar molecules used by the plant for growth and energy storage (Taiz and Zeiger, 1998). However, the mechanism of carbon assimilation differs between plant species. Legumes and grasses native to areas of moderate temperatures and light intensities initially fix CO_2 into the 3-carbon molecule, 3-phosphoglycerate, and is consequently named the C_3 pathway (Bassham and Buchanan, 1982; Taiz and Zeiger, 1998). The first and most important enzyme in this pathway is ribulose-1,5-bisphophate carboxylase/oxygenase (Rubisco), which catalyzes the reaction of CO_2 with ribulose-1,5-bisphosphate to yield two molecules of 3-phosphoglycerate (Taiz and Zeiger, 1998). Rubisco alone accounts for approximately 40% of the total soluble protein of most green plant leaves (Tiaz and Zeiger, 1998). Following CO_2 assimilation, the ATP and NADPH produced in the light-dependent reactions drive the phosphorylation and subsequent reduction of 3-phosphoglycerate, generating glyceraldehyde-3-phosphate (Taiz and

Zeiger, 1998). In order to complete this stage of the Calvin cycle, 3 molecules of ribulose-1,5-bisphophate combine with 3 molecules of CO_2 ultimately producing 6 molecules of glyceraldehydes-3-phosphate (Bassham and Buchanan, 1982). The constant CO_2 influx demands the repletion of ribulose-1,5-bisphosphate (Taiz and Zeiger, 1998). Thus, through a series of reactions, 5 molecules of glyceraldehydes-3-phosphate are used to regenerate the 3 substrate molecules of ribulose-1,5-bisphosphate Bassham and Buchanan, 1982). The remaining glyceraldehyde-3-phosphate is used for starch synthesis or exported into the cytoplasm (Bassham and Buchanan, 1982) where it is usually converted to sucrose for distribution throughout the plant (Geiger and Giaquinta, 1982).

The efficacy of photosynthesis is dependent on the atmospheric partial pressure gradient that drives CO_2 into the cell (Ogren and Chollet, 1982). Although atmospheric pressures at moderate temperatures are enough to drive the carboxylation activity of rubsico, due to Henry's law the partial pressure of CO_2 is always less than that of O_2 because the total amount of atmospheric O_2 is higher than that of CO_2. As temperature increases, however, the ratio of O_2 to CO_2 in the air also increases and the carboxylation activity of Rubisco becomes increasingly less efficient (Taiz and Zeiger, 1998). Higher concentrations of atomospheric O_2 encourage the oxygenase activity of rubsico because O_2 competes with CO_2 for the same active site, resulting in the production of 2-phosphoglycolate, a 2-carbon molecule with no metabolic potential. This process is called photorespiration (Ogren and Chollet, 1982). Rubisco binds O_2 in the chloroplast and reacts with ribulose-1,5-bisphosphate to produce 2-phosphoglycolate and 3-phosphoglycerate (Taiz and Zeiger, 1998). Without CO_2 assimilation, the plant has no

source of carbon to synthesize starch or other carbohydrates it requires for growth. In order to recover CO_2 from 2-phosphogylcerate, plants must employ an energetically costly carbon recovery pathway involving three organelles (Taiz and Zeiger, 1998). Ultimately, CO_2 is recovered from a glycine residue resulting in a 75% recovery of carbon (Taiz and Zeiger, 1998).

Rising temperatures cause an increase in the proportion of photorespiration to photosynthesis. Furthermore, at higher atmospheric O_2 the kinetic properties of Rubisco shift toward the oxygenase activity (Ogren and Chollet, 1982). In other words, O_2 affinity of Rubisco increases as temperatures increase. Thus, in order to thrive, grasses native to tropical and temperate temperatures and higher light intensities have evolved to employ a modified version of the C_3 photosynthetic pathway (Moser et al., 2004). This C_4 photosynthetic pathway, so named because of its 4-carbon acid intermediates, includes a CO_2-concentrating mechanism adapted by plants to avoid photorespiration (Taiz and Zeiger, 1998; Moser et al., 2004). Plants utilizing this modified pathway are distinctive in their leafy tissues in order to accommodate CO_2 accumulation up to 10-times the atmospheric levels (Furbank and Hatch, 1987). Mesophyll is the only chloroplast containing tissue of C_3 leaves; however, C_4 leaves have also adapted to incorporate chloroplast-containing bundle sheath cells in addition to their mesophyll tissue (Taiz and Zeiger, 1998). The "Kranz anatomy" of C_4 plants allows for biochemical cooperation between specialized tissues involved in C_4 photosynthesis (Bassham and Buchanan, 1982).

The C_4 pathway begins with the carboxylation of phosphoenolpyruvate (PEP) with HCO_3^- by PEP carboxlyase in the cytoplasm of mesophyll cells (Bassham and Buchanan, 1982). This produces the first 4-carbon acid, oxaloacetate which is subsequently converted to the second 4-carbon acid, malate in the mesophyll chloroplast (Bassham and Buchanan, 1982). Malate is passively transported to the specialized bundle sheath cells where it is actively decarboxylated, yielding CO_2 and pyruate (Bassham and Buchanan, 1982). The CO_2 enters the Calvin cycle within the bundle sheath cell and the pyruvate is passively shuttled back to the mesophyll cytoplasm to regenerate phosphoenolpyruvate (Bassham and Buchanan, 1982; Taiz and Zeiger, 1998). The overall result of the cycle is the concentrating mechanism of CO_2 into the bundle sheath cells where Rubisco is located and the Calvin cycle takes place. Therefore, even at high temperatures when atmospheric concentration of CO_2 is low, enough CO_2 is concentrated in the bundle sheath cells to keep the carboxylation activity of Rubisco and the Calvin cycle operating efficiently. This allows C_4 grasses to thrive in warm climates, such as the southeastern region of the United States.

In addition to photosynthetic differences, legumes and cool- and warm-season grasses differ metabolically in their methods of nitrogen fixation. Initally, the enzyme nitrate reductase actively catalyzes the conversion of nitrate to nitrite in the cytosol of root cells (Taiz and Zeiger, 1998). In most plants, nitrate conversion is located in the root plastid or within the shoot chloroplast, but may be possible in the both locations when nitrate concentrations in the plant are abundant (Taiz and Zeiger, 1998). The highly reactive nitrite is swiftly relocated to the plastid in root cells where it is converted via

nitrite reductase into ammonium (Taiz and Zeiger, 1998). Ammonium is also a byproduct of photorespiration and is readily metabolized by nitrogen assimilation enzymes in the chloroplasts (Taiz and Zeiger, 1998). Nitrate assimilation in root plastids is more common in cool-season plants, whereas warm-season plants most often utilize chloroplasts for nitrate assimilation (Taiz and Zeiger, 1998). Ammonium is further metabolized into amino acids by enzymes in the plastid or chloroplast (Taiz and Zeiger, 1998). This nitrogen assimilation pathway is not common to all types of forage. Legumes develop symbiotic relationships with a nitrogen-fixing rhizobacteria to acquire nitrogen (Taiz and Zeiger, 1998). Nodules found on legume roots house the nitrogen-fixing bacteria which assimilate nitrogen (N) into ammonia (Taiz and Zeiger, 1998). The ammonia is converted to organic N before leaving nodule (Taiz and Zeiger, 1998). Grasses may have root contamination with rhizobia; however, nodules are not produced and exchange of fixed nitrogen is negligible (Taiz and Zeiger, 1998). By acquiring N through microbial symbiosis, legumes bypass the energetically costly reactions of biological nitrogen assimilation. This allows legumes to obtain soil N without the energy expenditure normally required for N acquisition.

Metabolic differences between C_3 and C_4 plants have direct effects on their chemical composition and, consequentially, on forage quality. Greater digestibility of legumes than both types of grasses and the generally observed greater digestibility of cool-season grasses than warm-season grasses is explained by the arrangement and structure tissues within the leaf (Wilson et al., 1983; Moser et al., 2004). Leaves are the primary sites of photosynthesis in both C_3 and C_4 plants (Taiz and Zeiger, 1998). Leaves

are more digestible than stems and positively affect forage quality, but leafy tissues are not equal in digestibility between C_3 and C_4 forages (Akin, 1989). Although C_3 and C_4 leaves are proportionately similar in amounts of lignified tissues, the Kranz anatomy of C_4 grasses contains more poorly digestible bundle sheath cells and epidermal tissue and fewer highly digestible mesophyll cells (Akin, 1989; Wilson et al., 1983). Furthermore, mesophyll tissues of C_4 plants are tightly packed within the leaf, limiting their susceptibility to microbial attack (Akin, 1989; Hanna et al., 1973). The C_4 mesophyll cell wall appears to also be more lignified than that of C_3 species. Akin, (1989) discussed findings that proclaim bermudagrass contains significantly more phenolic compounds than that of two cool season species, orchardgrass and tall fescue. The mesophyll of cool-season leaves is more accessible to digestion due to the widely spaced vasculature in C_3 leafy tissues (Akin, 1989). The C_4 pathway also allows a greater N-use efficiency than the C_3 pathway (Moser et al., 2004). Due to the concentration of CO_2 within bundle-sheath cells, less Rubisco is required for adequate photosynthetic activity. This lowers the total N content resulting in lower CP levels in C_4 plants compared to C_3 varieties (Moser et al., 2004).

Structural and chemical differences between forage types are evident and explain differences in cell wall digestibility. Unlike legumes, grasses develop a lignified midrib section in their leaves, which contributes to the higher fiber content and lower digestibility of grasses compared to legumes (Buxton and Redfearn, 1997). Generally, legumes are higher in digestibility than both types of grasses due to a higher proportion of cell contents to cell wall material (Fonnesbeck, 1968). Brown and Pitman (1991)

reported legumes had 240-250 g/kg of digestible DM compared to 150-160 g/kg for warm-season grasses. Legume cell walls contain a larger fraction of highly-digestible pectin than the cell walls of grasses (Åman, 1993; Chesson, 1993). Fibrous components of legume cell walls are less digestible than those of grasses, but due to the higher volume of cell wall material in grasses, the overall digestibility of legumes is superior to grasses (Buxton and Redfearn, 1997). Although both legume and grass cell walls contain similar amounts of cellulose, grasses have up to four times more hemicellulose than legumes (Van Soest, 1967). However, hemicellulose of grass cell walls is more digestible than that of legumes; therefore, in some cases, grass quality may be underestimated when compared to legumes (Fonnesbeck, 1968).

Warm-season grasses are typically lower in quality than cool-season grasses. Leafy tissues of cool-season grasses are generally more easily degraded because of the widely spaced arrangement of poorly digestible vascular bundles (Akin, 1989) and higher proportion of mesophyll tissue (Buxton and Redfearn, 1997). Cross section analysis of cool- and warm-season grass leaves revealed cool-season varieties contained approximately 20% moderate to poor digestibility tissues compared to 60% in warm-season specimens (Akin, 1989). In addition, warm-season grasses are higher in lignin (Jung and Vogel, 1986) and lower in NSC (Moore and Hatfield, 1994) than cool-season species. Cell wall differences among forage types include the amount and distribution of lignin. As a proportion of cell wall, legumes are higher in lignin, and therefore generally lower in fiber digestibility, than grasses (Moore and Jung, 2001). Grasses, however, are higher in total cell wall concentration than legumes and thus contain a greater total

amount of lignin (Moore and Jung, 2001). Although legume fiber may be less digestible compared to grass fiber, the greater quantity of fiber in grasses allows lignin to have a more negative effect on the overall digestibility of grasses (Moore and Jung, 2001). Similar observations have been made between cool- and warm-season grasses. The composition of lignin is similar between the two grasses (Jung and Vogel, 1986), but warm-season species typically contain more fiber and therefore more lignin (Moore and Jung, 2001).

Nutritive Value: In order relate forage quality to quantitative values and compare divergent forage types, chemical components must be grouped into measureable fractions. These fractions are forage nutritive values and relate the chemical composition of forage type to forage quality. Each analytical fraction represents positive and negative contributions to forage quality and productivity. Fibrous cell wall constituents are partitioned using the detergent fiber system which is composed of neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and acid detergent lignin (**ADL**) fractions (Van Soest, 1963). The NDF fraction consists of cell wall polysaccharides, hemicellulose and cellulose, and lignin; ADF represents cellulose and lignin, or NDF less hemicellulose (Van Soest and Wine, 1967). Acid detergent lignin, describes the indigestible lignin fraction separate from the potentially degradable cell wall polysaccharides. This divides the fibrous components into those contributing to animal performance and forage nutritive value (cellulose and hemicellulose) and non-nutritive lignin (Fonnesbeck, 1969). The amount and ratio of the fibrous fractions varies among forage species.

Forages differ in concentration and digestibility fibrous components. In a review by Reid et al. (1988) of 428 forages fed to sheep and cattle, legumes and cool- and warm-season forages contained approximately 36, 37, and 43% ADF, respectively. Warm season grasses also have a higher percentage of NDF than their cool season and legume counterparts (Brown and Pitman, 1991; Moore and Hatfield, 1994). Neutral detergent fiber makes up as much as 85% the plant in C_4 grasses compared to 40-55% in legumes and up to 70% C_3 species (Buxton et al., 1995). Previous research has reported values of 67.6 and 49.9% NDF for warm-season and cool-season grasses, respectively (Barton et al., 1976; Van Soest, 1973). Grasses, however, seem to have larger portions of digestible NDF than their legume counterparts due to the extensive lignification of legume cell walls (Brown and Pitman, 1991; Buxton and Redfearn, 1997). This constitutes a possible underestimation of the nutritive value of grasses compared with legume species. Neutral detergent fiber has been attributed to reducing the effects of starch overload in the equine hindgut when fed at a 1:1 ratio with starch (Medina et al., 2002). This emphasizes the need for forage-based diets in both laminitis prone horses and performance horses consuming meals which approach the upper limits of dietary starch concentration (0.4% BW; Potter et al., 1992).

Despite the benefits of NDF in proper ratio to concentrate, increasing NDF and ADF concentrations has been reported to decrease forage intake and digestibility in ruminants (Mandebvu et al., 1999). Reid et al. (1988) reported negative correlations between ADF and NDF percentages and dry matter digestibility (**DMD**), DMI, and digestible dry matter intake (**DDMI**). Previous research describes the digestibility of

NDF as self-limiting in the horse: as the percentage of NDF increases, digestibility of NDF decreases due to declining intake, gut fill, and other physiologic constraints (Darlington and Hershberger, 1968; Hintz et al., 1971; Glade, 1984). Increasing NDF reduces digestibility, and thus intake will decline. Van Soest (1965) reported NDF content was negatively correlated to VDMI in sheep for both grass and legume forage. Furthermore, Waldo (1986) suggested NDF components to be the most significant predictor of forage VDMI for ruminants. This explains why intake of legume species is generally greater than that of grass species, and of the grasses, C_3 DMI is typically higher than that of C_4 forages (Minson, 1990; Pearson et al., 2001). Acid detergent fiber is less digestible than NDF due to its higher proportion of lignin (Tamminga, 1993). Due to this constraint, ADF is more significantly correlated with forage DMD than NDF (Reid et al., 1988; Van Soest, 1993). Legumes contain less ADF than grasses but this difference is less significant than differences of NDF concentration (Brown and Pitman, 1991).

Lignin negatively affects forage digestibility as well (Mandebvu et al., 1999). Jung and Vogel (1986) demonstrated a negative linear effect of lignin on in vitro DM, cell wall, hemicellulose, and cellulose digestibility in both cool and warm season grasses. Webster et al. (1965) reported a negative relationship between lignin content and DMD of bermudagrass forage in cattle. Diets in this study with the highest DMD were lowest in lignin concentration, and cattle gained the least while grazing bermudagrass during the summer season of peak lignin content. Lignin limits forage digestion in animals due to its detrimental effects on the digestibility of cell wall polysaccharides. Lignin is a polyphenol matrix of hydroxycinnamyl alcohols, which forms chemical bonds with cell

wall polysaccharides rendering them less digestible or indigestible by microbial fermentation (Jung and Allen, 1995; Moore and Jung, 2001). Ferulate cross-linkages between lignin components and cell wall polysaccharides and protein are formed as lignin is deposited in early plant cell differentiation (Iiyama et al., 1993; Moore and Jung, 2001). The lignin matrix reduces cell wall digestibility by sterically hindering cell wall constituents being digested by enzymes (Moore and Jung, 2001). Lignification in some plant tissues is greater than in others, and thus digestibility will vary among tissues. Highly lignified tissues, xylem and epidermis of grasses, are much lower in digestibility than relatively non-lignified tissues such as mesophyll and parenchyma (Akin, 1989).

Forage nutritive value is also indicated by CP concentration. Protein contributes to growth and maintenance of animal tissue, and is required by the equine at BW × 1.26 g CP per kg BW per d (NRC, 2007). Similar to fibrous nutritive values, CP content varies by location within the plant, between forage types, and is impacted by plant maturity. Protein is found in greater concentrations in the leaves of grasses and legumes. Protein is located in the cell contents and is typically highly digestible. Fonnesbeck (1969) found the average true CP digestibility among twelve types of equine forages was 81.7 %. Among forage types, CP is highest in legumes, followed by cool-season and finally warm-season varieties (LaCasha et al., 1999; Sturgeon et al., 2000).

The effects of CP concentration on digestibility and intake of forages are variable and not well documented. Research supports a positive correlation between CP content and forage digestibility. Fonnesbeck et al. (1967) reported legume forages of higher digestible CP content had higher DMD values and resulted in greater intake compared to

forages with less digestible CP. These results are similar to those of Alexander et al. (1961) which reported increasing digestible CP content was associated with increased forage digestibility. Edouard et al. (2008) reported that DMD of both fresh grass and hays increased significantly in equines as the CP content of the forage increased. Additionally, CP digestibility increased as CP content increased. Results from LaCasha et al. (1999) and Sturgeon et al. (2000) indicated CP digestibility increased in equines as forage CP content increased. Other studies have concluded CP has little to no effect on forage digestibility or intake (Pearson et al., 2001). However, it appears that digestion may be compromised and, consequently, intake limited by low CP diets. Pearson et al. (2001) reported equids consuming low-quality forage diets with low CP content, exhibited decreased intake, perhaps due to inhibition of the fermentative microbial system in the hindgut. In addition, behavioral vices of horses consuming low protein diets may be alleviated by increasing dietary CP. Schurg et al. (1977) reported horses consuming a diet of 6.2% CP were consistently observed practicing coprophagy and wood biting. These behaviors were completely eradicated by increasing the dietary CP content to 10% by supplementation with soybean meal.

Coastal Bermudgrass: In the southern United States, warm-season grasses are the predominant forage source for livestock (Galyean and Goetsch, 1993; Moser et al., 2004; Hanna and Sollenberger, 2007). Of these, Coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) is the most prevalent source of livestock forage (Moser et al., 2004; Hannah and Sollenberger, 2007). The success of Coastal bermudagrass is attributable to many characteristics including cold-tolerance and ease of establishment relative to other

bermudagrass hybrids (Conrad et al., 1981). Heat and drought tolerance, high biomass yield, palatability, and grazing tolerance make both fresh and clipped Coastal bermudagrass a valuable forage resource for livestock producers (Taliaferro et al., 2004). In contrast to native systems, producer management can significantly alter crop yield and nutritional quality of Coastal bermudagrass.

Coastal bermudagrass's popularity is largely resultant from robust stand development and sustainability. Conrad et al. (1981) compared bermudagrass hybrids under 4 different grazing pressures and was able to maintain Coastal bermudagrass pastures at a 33% higher average stocking rate over 3 years than on S-16 or S-54 hybrid pastures. Additionally, Coastal bermudagrass produced consistent average daily gains at a 50% higher stocking rate due its superior hardiness (Conrad et al., 1981). Coastal bermudagrass is able to withstand higher grazing pressures compared to other forage species because of its structural rigidity provided by an abundance of cell wall material. Though prolific, Coastal bermudagrass contains more poorly digestible tissues including lignified vascular tissue, parenchymal bundle sheath cells, and epidermis and less highly digestible mesophyll tissue than other warm-season grasses (Akin, 1989). Harbers et al. (1981) found horses were able to readily utilize mesophyll and phloem tissues but were unable digest epidermis and bundle sheath cells.

Coastal bermudagrass is lower in quality than both cool-season grasses and legumes at similar maturities. Sturgeon et al. (2000) compared the digestibility of bermudagrass to Matua and alfalfa hays among horses. Dry matter digestibility was lowest for bermudagrass, highest for alfalfa and intermediate for matua. Bermudagrass

also had the lowest CP content, 8.28%, compared to 10.90 and 16.44% for Matua and alfalfa respectively; ADF and NDF digestibilities were not significantly different between the forages. These results correspond with results of LaCasha et al. (1999) who reported 60, 64, and 64% dry matter digestibilities and 11.3, 13.5, and 20.0% CP values for Coastal bermudagrass, Matua bromegrass, and alfalfa, respectively. Intake of Coastal bermudagrass by horses is also lower compared with other forage types. LaCasha et al. (1999) reported voluntary DM intake of horses expressed as g/kg, %BW, and g/kg $BW^{.75}$ was lower for bermudagrass compared to both Matua bromegrass and alfalfa. Low digestibility and intake values for Coastal bermudagrass are explained by the high NDF content of this forage. Of the forages used in the digestibility and intake trial by Sturgeon et al. (2000), Coastal bermudagrass was highest in NDF, and contained 29% more NDF than alfalfa. In accordance, LaCasha et al. (1999) reported Coastal bermudagrass NDF content was 41.8% higher than alfalfa. However, producer management strategies may be employed to increase the quality of Coastal bermudagrass and its utilization by equines.

Nitrogen Fertilization: The chemical properties and biomass yield of forage are greatly influenced by fertilization (Masters and Mitchell, 2007). Increasing crop yield and nutritive value through effective management allows hay producers to meet economic goals and product quality standards. Nitrogen fertilization increases the quantity and quality of Coastal bermudagrass (Snyder and Leep, 2007; Taliaferro et al., 2004). Application of N containing fertilizers supports growth and biomolecule assimilation. Johnson et al. (2001) increased biomass production of bermudagrass, star

grass, and bahiagrass 129% by fertilizing with 78 kg of N• ha^{-1}• $cutting^{-1}$. Additionally, Trenholm et al. (1998) found fertilization with N rates up to 9.8 g • meter $squared^{-1}$• $month^{-1}$ increased shoot growth in two bermudagrass hybrids. Previous research by Prine and Burton (1956) reported Coastal bermudagrass stands fertilized with 1008 kg N • ha^{-1} yielded an average of 6.7 and 10.0 times more forage during a wet and dry season, respectively, compared to unfertilized stands. Compared with other forage species, Coastal bermudagrass is paramount in its ability to produce in response to N fertilization (Johnson et al., 2001; Snyder and Leep, 2007).

In addition to increasing forage yield, N application affects nutritive values of Coastal bermudagrass. Fertilization increases CP content, producing higher quality forage for livestock consumption (Taliaferro et al., 2004). Burton and Jackson (1962) investigated the effects of 5 different N sources on Coastal bermudagrass and found all sources increased CP as fertilization rate increased. Early investigations by Prine and Burton (1956) and Alexander et al. (1961) reported increases in protein content of Coastal bermudagass as high as 30 and 19% when N fertilization rate reached 1008 kg N • ha^{-1} and increased from 56 to 112 kg N • ha^{-1}, respectively. Doss et al. (1966) reported N content of bermudagrass increased as fertilizer application increased to 627 kg N • ha^{-1}. Johnson et al. (2001) reported fertilization with 157 kg N • ha^{-1}• $cutting^{-1}$ increased CP content by an average of 55% in bermudagrass. This corresponds with results of Burns et al. (2009) who reported CP content of Coastal bermudagrass increased linearly with increasing N application.

Observations of N fertilization effects on fiber content have also been reported. The quantity of NDF in bermudagrass has been reported to linearly decline as N fertilization increases (Johnson et al., 2001). Galdámez-Cabrera et al. (2003) found that concentrations of NDF and ADF in Coastal bermudgrass decreased linearly as N fertilization rates were incrementally increased from 0 to 168 kg • ha^{-1}. However, Burns et al., 2009 reported no effect of N fertilization on NDF content. Johnson et al. (2001) reported an increase in ADF concentration in bermudagrass when N was applied up to 78 kg of N/(ha·cutting); however, additional fertilization decreased ADF concentration. Fertilization is an effective form of crop management to improve the yield and nutritive value of Coastal bermudagrass.

Maturity at Harvest: Along with fertilization, harvesting interval affects the quality of forage (Taliaferro et al., 2004). For this reason, recommended harvest intervals for bermudagrass are 4 to 6 weeks to optimize yield and forage quality (Taliaferro et al., 2004; Hanna and Sollenberger, 2007). Prine and Burton (1956), harvested fertilized Coastal bermudagrass at 1, 2, 3, 4, 6, and 8 week intervals to determine effects of clipping frequency on yield, CP content, and plant morphology. This study reported near maximum yield for the 6 week harvests, but a decline in percent protein and in leaf percentage as harvest intervals increased, especially between 4 and 6 weeks. Though the percentage of CP declines as the plant matures, the total protein content of the harvest may remain relatively equal due to the increased biomass yield (Alexander et al., 1961). Also, the apparent digestibility of protein decreases with increasing maturity, especially after 6 weeks (Alexander et al., 1961). More recently, Mandevbu et al. (1999) reported

declining CP concentrations in bermudagrass corresponded to increasing maturities at harvest. Darlington and Hershberger (1968) reported CP digestibility, along with DM and crude fiber digestibilities, also decreases as maturity at harvest increases. This data agrees with that of Alexander, et al. (1961) which found decreasing TDN with increasing maturity in fall-harvested Coastal bermudgrass fed to yearling heifers. Measurements of percentage CP and digestible CP are more relevant to forage quality and animal performance than measurements of total CP per harvest.

Cell wall concentration and proportions are also effected by harvesting intervals. Mandebvu et al. (1999) found levels of ADF, ADL, and total cell wall concentration in two types of bermudagrass increased as the age at harvest increased. Rouquette et al. (1972) reported higher percent of NDF and leaf hemicellulose:cellulose with the aging of Kleingrass. Additionally, Barton et al. (1976) reported Coastal bermudagrass concentrations of NDF and ADF increased by 10% as maturity at harvest increased from 4 weeks to 8 weeks. Hemicellulose concentration remained relatively consistent between harvests, suggesting the increase in fiber content is the result of an increase in cellulose and/or lignification. This supports results of Jung and Engels (2002) who investigated effects of maturity on alfalfa cell wall development. As maturity increased, rapid lignin accumulation in xylem secondary cell walls was observed while slower lignification on phloem and parenchymal tissues along with increases in cellulose and hemicellulose were also reported (Jung and Engels, 2002). Even when harvesting intervals are kept equal, cell wall proportions may increase as the growing season progresses. When harvested at 28-d intervals, the NDF and ADF concentrations of Coastal bermudgrass have been

reported to express a quadratic response to maturity, increasing until reaching a peak concentration and then showing a slight decline in the latest cutting (Johnson et al., 2001).

The increase in cell wall material and lignin concentration during maturity causes forage digestibility to decrease as age at harvest increases (Akin, 2008; Jung and Casler, 2006). Darlington and Hershberger (1968) reported the digestibility of alfalfa, timothy, and orchardgrass by equines decreased significantly as maturity increased. Lower digestibility of bermudgrass associated with increases in maturity may also be explained by an increase in the proportion of poorly digestible tissues. Akin et al. (1977) reported leaf blades of bermudagrass increased in the amount of parenchyma bundles sheath cells and decreased in mesophyll cells with increasing maturity. Furthermore, the leaf:stem ratio of forages decreases with maturity (Buxton and Redfearn, 1997).

Even when harvest intervals are kept within the recommendations, forage quality may continue to deteriorate as the growing season progresses due to changes in plant nutritive value. Galdámez-Cabrera et al. (2003) harvested bermudgrass on May 30 and August 18 and reported the later cutting had a higher NDF concentration largely due to an increase in hemicellulose concentration. This was attributed to increasing temperature and sun exposure associated with the later harvest date. Concentrations of ADF in warm-season grasses have also been reported to increase as temperature increases during the growing season (Johnson et al., 2001). Therefore digestibility, intake, and overall performance of animals may be decreased when fed warm-season forage harvested during the warmest summer months.

Conclusion: Forage is an essential component of the equine diet. Forage provides nutrients and energy to the horse and promotes sound growth and behavior as well as gastrointestinal health. By selecting and managing forage for optimal quality, intake and digestion may be improved. Increasing intake and digestibility is important maximize forage utilization in order to meet performance goals. Through management strategies, production of Coastal bermudagrass high in CP is possible. However, relatively little is known regarding the effects of CP level of Coastal bermudagrass hay on intake and digestion responses in horses. The effects of feeding various levels of CP will provide insight on the ability of horses to utilize this forage and on the extent of management required to provide high-quality Coastal bermudagrass hay.

CHAPTER II

MATERIALS AND METHODS

Four previously cecally fistulated geldings (average initial BW 548.2 kg ± 23.3 kg; average age 7.5 ± 2 yr) were used in a replicated 4 × 4 Latin square experiment. Geldings were housed in individual dry lots (6 m × 14 m) and provided ad litbitum access to fresh water and a trace mineral/salt block (composition: ≥ 96.0% NaCl, 0.16% Fe, 0.40% Zn, 0.32% Mn, 0.01% I, 0.04% Cu, and 0.004% Co; Producers Co-op, Bryan, TX). Body weight was measured at the beginning of each period to monitor weight gain or loss. All horses received dental care, vaccinations, and deworming according to standard farm protocol. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University (AUP# 2008-102).

Dietary treatments were four qualities of Coastal bermudagrass hay (*Cynodon Dactylon* (L.) Pers.) (7, 10, 13, and 16% CP). All hays were produced on an established stand of Coastal bermudagrass in College Station, Texas and harvested at the same maturity. Hay (Table 1) was chopped through at 75 cm × 75 cm screen and fed at approximately 120% of the previous 4-d average intake to ensure access to forage did not constrain intake. Hay was offered in two equal feedings at 0600 and 1800 each day. The experiment was divided into 4 periods of 15 d each in which the horses received the designated treatment, each horse receiving each treatment exactly one time. Experimental periods were divided into 3 phases: 1) d 1 through 10 for adaptation dietary to treatments; 2) d 11 through 14 for measurement of hay intake and digestion; 3) d 15

cecal sampling. During the 10 d adaptation, horses were housed in individual dry lot pens (6 m × 14 m). During the 4 d collection phase, horses were housed in individual stalls (3 m × 3 m) with concrete floors covered with rubber mats and tied to restrict movement and facilitate the determination of intake and total collection of feces. Horses were allowed 1 hr of walking exercise at 1000 each day using a free stall walker.

Calculations of intake and digestion were made from observations on d 11 through d 14. Feed and ort samples were collected on d 10 through d 13 to correspond with fecal samples collected on d 11 though d 14. Hay was sampled as it was being fed, 400 g of each hay type was retained by grab sample daily and immediately dried for subsequent analysis. Orts were removed at 0600 and, approximately, 200 g were retained for analysis. Fecal bags (Bun-Bag, Inc., Sagle, ID) were removed and contents weighed at 0600, 1200, 1800, and 2400 daily. Feces collected over each 6-h period were thoroughly mixed and, approximately, 400 g from each horse was retained by grab sample and immediately dried for later analysis.

Cecal fluid samples were collected on d 15 of each period just before feeding (0 h) and 4, 8, 12 h after feeding. To facilitate fluid collection, the fistula was opened and cecal fluid was collected by suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh). Immediately after sampling, cecal pH was measured using a hand-held pH meter (VMR sypmpHony SP21; VWR International, Inc., Westchester, PA) with a Beckman 3-in-1 combination electrode (Beckman Coulter, Inc., Fullerton, CA). Following collection, 8 mL of sample fluid was transferred into an empty vial containing 2 mL meta-phosphoric acid and frozen at -20˚ C. Approximately 10 ml blood

was collected via jugular venipuncture into an evacuated tube containing 15% EDTA (Tyco Healthcare Group LP, Mansfield, MA) and into a heparinized Vacutainer blood collection tube containing a minimum of 120 USP units of sodium-heparin (BD Vacutainer, Franklin Lakes, NJ) prior to feeding (0 h) and 4, 8, and 12 h after feeding. Samples were placed on ice immediately after collection and centrifuged at 2.8×10^4 rpm for 20 min within 1 h after collection. Plasma was frozen for subsequent determination of plasma urea N and glucose concentrations.

Partial DM of hay, orts, and fecal samples were performed by drying at 55°C in a forced-air oven for 96 h. All dried samples were then ground with a Wiley mill to pass a 1-mm screen (Thomas Scientific, Swedesboro, NJ). Hay samples collected during the measurement period were pooled across days on an equal weight basis. Ort and fecal samples were composited in proportion to their daily refusal or output, respectively, by horse across days. Hay, ort, and fecal samples were dried for 24 h at 105°C in a forced-air oven to determine DM and then combusted for 8 h at 450°C in a muffle furnace for percent ash determination. Percent OM was determined by subtracting percent ash from 100. Nitrogen content of hay was determined by total combustion (Rapid N-Cube, Elementar Americas, Inc, Mt. Laurel, NJ). Gross energy of hay and fecal samples was determined using an oxygen bomb calorimeter (Parr 6300; Parr Instrument Company, Moline, IL). Digestibility of energy was calculated as the remainder of consumed minus fecal excreted energy expressed as a percentage of intake. Crude protein was calculated as N × 6.25. The ANKOM-Fiber Analyzer was used to determine NDF and ADF of all hay, ort, and fecal samples were (ANDOM-Technology, Fairport WY). To determine

acid detergent insoluble ash (ADIA) of hay, ort, and fecal samples, the bags containing the ADF residues were combusted for 8 h at 450°C in a muffle furnace. Total tract digestion coefficients for DM, OM, and NDF were determined using total collection, as described by Cochran and Galyean (1994). Colormetric determination of cecal ammonia (Broderick and Kang, 1980), plasma glucose (Sigma-Alrich Inc., St. Louis, MO), and plasma urea (Marsh et al., 1965) were made using an UV/VIS (DU730 UV/VIS Spectrometer, Beckman Coulter, Inc., Fullerton, CA).

Intake, digestion, and plasma urea N concentration were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Terms in the model were treatment and period with horse included as a random effect. Fermentation profile variables were analyzed using the MIXED procedure of SAS. Terms in the model were treatment, period, hour, and hour × treatment with horse and treatment × period × horse included as random terms. The repeated term was hour with treatment × horse as the subject. Compound symmetry was used for the covariance structure. The LSMEANS option was used to calculate treatment means. Orthogonal polynomial contrast (linear, quadratic, and cubic) were used to partition treatment sums of squares. Statistical significance was determined as $p \leq 0.05$ and trends toward significance were determined as $p \leq 0.10$.

CHAPTER III

SUMMARY OF RESULTS

Results and Discussion: Increasing the CP concentration of Coastal bermudagrass resulted in a linear increase in digestible OM intake (**DOMI**) from 3.79 to 5.98 kg/d for 7 and 16% CP treatments, respectively ($P = 0.04$; Table 2). As expected, increasing forage CP content resulted in a linear increase in CP intake ($P < 0.01$). In contrast to DOMI and CP intake, Coastal bermudagrass CP concentration did not affect any other measures of intake ($P \geq 0.11$). Increasing hay CP concentration resulted in quadratic effects ($P \leq 0.05$) on digestion of OM, NDF, ADF, and gross energy. In general, the lowest digestibilities were observed when the 7% CP hay was fed, with the exception of ADF digestibility which was numerically lower for the 16% CP hay than the 7% CP hay. Digestibilities were greatest when the 10% CP hay was offered before declining for the 13 and 16% CP forages.

There were no treatment × time interactions; thus, only overall treatment means are presented (Table 4). Cecal pH remained above 6.62 irrespective of treatment and time, indicating that cecal pH was suitable for microbial growth. While there was a cubic effect ($P < 0.01$; Table 4) of CP content on cecal pH, the biological significance of these differences is negligible. There were no significant postprandial peaks or troughs that would indicate an effect of treatment on cecal pH. Concentration of cecal ammonia linearly increased ($P < 0.01$) from 0.30 m*M* for 7% to 1.74 m*M* for the 16% CP hay.

Plasma glucose linearly increased ($P = 0.04$) from 68.77 to 73.68 mg/dL as forage CP concentration increased. Plasma urea nitrogen exhibited a quadratic effect ($P < 0.01$) from 4.34 to 5.61 m*M* for 7 and 10% CP hays, respectively.

Aiken et al. (1989b) fed mature horses Coastal bermudagrass hay (10.7% CP) and had a reported DM intake of 2.0% BW compared to 1.67, 1.64, 1.83, and 2.16% BW reported in our study for 7, 10, 13, and 16% CP hays, respectively. The Coastal bermudagrass used on our study was 26.3, 9.6, 4.2, and 1.24% higher in ADF and 15.5, 13.1, 15.5, and 13.1% lower in gross energy for the 7, 10, 13 and 16% CP hays, respectively, than the forage used by Aiken et al. (1989b). However, Aiken et al. (1989b) reported apparent DM digestibility of 43.0% compared to our 39.6, 54.2, 48.7, and 49.2% and NDF concentration of 70.5% DM compared to 74, 69.2, 67.8, and 66.0% DM for our 7, 10, 13, and 16% CP hays, respectively. The higher ADF and lower gross energy of our hay may have decreased intake by contributing indigestible bulk along with supplying less available energy for digestion to the hindgut. Additionally, dry matter intake (DMI) for the 7, 10, and 13% CP hays were lower than the average DMI of 2.09% BW reported by LaCasha et al. (1999) for yearling horses consuming 11.3% CP Coastal bermudagrass. Apparent DM digestion reported by LaCasha et al. (1999) was 46.0%, which is lower than that of our 10 and 13% CP forages. In contrast, intake in our study was greater than the observations of Harbers et al. (1981) when equines were fed warm-season prairie hay primarily consisting of big bluestem (*Andropogon geradri*). Horses on this trial were offered hay at 2% BW per day on DM basis and had an average DM intake of approximately 1.6% BW. Intake on this trail is likely lower than our lowest reported

intake due to the low CP (4.7%) of the prairie hay. Although the CP of this hay is notably lower than the 7% of the lowest CP forage used on our study, the prairie hay was 4.6% lower in NDF. Also, likely due to the lower NDF concentration, digestibility of the prairie hay DM was slightly higher at 41.52% compared to 39.63% of our 7% CP hay.

Organic matter digestion was lower for all hays than the 60% reported by LaCasha et al. (1999). The 52.8% NDF digestibility of the 10% CP forage is similar to apparent NDF digestibilities of 52.0 and 51.7% reported by LaCasha et al. (1999) and Sturgeon et al. (2000) for horses consuming 11.3 and 8.28% CP Coastal bermudagrass hay, respectively. The 33.06, 43.95, 34.72, and 30.29% ADF digestibilities of the 7, 10, 13, and 16 % CP hays were lower than the 47.32% reported by Harbers et al. (1981) for warm-season forage and higher than the 26% reported by LaCasha et al. (1999) for Coastal bermudagrass hay. Aiken et al. (1989) reported a 35.7% apparent digestibility for ADF of Coastal bermudagrass hay, which is higher than that of all forages on our study except the 10% CP hay.

Overall apparent digestibilities were similar to those of previous equine research for comparable Coastal bermudagrass hays (Aiken, 1989; LaCasha et al., 1999). The lowest DM digestibility observed for the 7% CP forage is likely due to the higher NDF (Glade, 1984; Hinz et al, 1971; Darlington and Hershberger, 1968) and ADF (Reid, 1988; Van Soest, 1993) content in this hay. Additionally, the 7% CP forage did not meet the 15.2 Mcal/d digestible energy (DE) requirement for a 500 kg mature horse at rest (NRC, 2007). Dry matter intake of the 10, 13, and16% CP forages exceeded the DE requirement at 19.4, 20.4, and 20.5 Mcal/day, respectively. The highest DM digestibility of the 10%

CP forage may be explained by the highest digestible energy observed for this forage. Increasing energy available to cecal microbes may increase forage digestion in the equine hindgut. Unexpectedly, the hay highest in CP and lowest in fiber content was intermediate in apparent digestibility. This was possibly due to a higher rate of passage and therefore lesser time interval for thorough digestion of this forage. Furthermore, the forage highest in digestibility was intermediate in CP (10% CP). This is explained by the markedly higher OM digestibility of this forage. In our study, as forage OM intake increased, digestible OM intake also increased. However, a point is reached at which intake will exceed the physiologic capacity for digestion, at which time, OM digestibility will decline with increasing digestible OM intake. Consequently, apparent OM digestion was lower for the 7, 13, and 16% hays, CP, 41.1, 48.9 and 48.5%, respectively, than for the 10% CP forage, 55.0%.

Resting plasma glucose levels in our study are lower than those previously reported by Ralston et al. (1979) for ad libitum fed horses. Ralston et al. (1979) used a 15.2% CP and 10.7% total fiber complete pelleted ration and obtained an average plasma glucose level of 111.4 mg/dL for ponies following a 19 hr period of ad libitum feed access. The lower fiber ration would result in a higher percentage of digestion in the foregut and thus a greater amount of glucose absorption in the small intestine, resulting in higher plasma glucose values. Our results showed no consistent post prandial peaks or troughs in plasma glucose. This is consistent with results of Stull and Rodiek (1988) who reported horses consuming an alfalfa hay diet had post prandial plasma glucose levels that were within 5% of resting plasma glucose levels. All-forage diets will produce lesser

changes in plasma glucose due to limited foregut digestion of available polysaccharides. Plasma urea nitrogen (PUN) increased post feeding for all treatments except the 10% CP hay. The 16% CP hay produced a much higher increase in PUN post feeding than other treatments and higher resting PUN, indicating an intake of protein above dietary requirements (Kumata and Harper, 1961). Previous equine studies have also reported a corresponding increase in PUN with increasing dietary CP concentration (Reitnour and Treece, 1971; Prior et al., 1974). The resting PUN of the 7% CP forage, 3.65 m*M*, was lowest, but was only slightly lower than the 13% CP forage, 4.08 m*M*, indicating the quality of protein in even the lowest CP hay was satisfactory to prevent an increase in PUN due protein turnover within the horse.

The average cecal pH for all treatments was similar to the average cecal pH of 6.97 reported by Willard et al. (1977) for horses consuming a grass-legume mixture. However, results from this study showed a 3% decrease in cecal pH 4 hr post feeding while our results were somewhat different. The only decrease in cecal pH 4 hr post feeding was recorded for the 10% CP hay and was only 1% lower than the 0 hr reading. This is likely due to the fact that horses this study were fed at 12 hr intervals which may have resulted in restricted intake due to limited amount of forage. Horses on our trial were given ad libitum access to hay in order to provide intake that would not be limited by forage availability. Ad libitum access to hay would result in decreased differences between hr 0 and hr 4 cecal pH readings because horses are actively digesting hay in the hindgut up until the new feeding at hr 0, assuming horses are eating the forage they are exposed to, which results in a higher initial cecal pH. Medina et al. (2002) reported an

average cecal pH of 7.15 for horses consuming a diet of high fiber pellets and straw. This is slightly higher than the average cecal pH of 6.98, 6.81, 6.84, and 7.00 for horses on our study consuming 7, 10, 13, and 16% CP hay. Medina et al. (2002) reported an average cecal pH at hr 0 of approximately 7.8, much higher than those reported on our study. Again, this is likely due to the feeding intervals of the study. Medina et al. (2002) fed two equal feedings at 0800 and 1800 daily. This allowed for an extended amount to pass between feedings, and, consequently, for active hindgut digestion to decrease and cecal pH increase prior to the hr 0 measurement. Cecal pH measured at 5-7 hours post feeding averaged approximately 6.9, which is similar to those reported on our study for horses consuming forage ad libitum. Cecal ammonia concentration increased as dietary CP concentration. Increasing dietary CP concentration increases the amount of substrate available for ammonia production in the hindgut.

Conclusion: Forage utilization has been defined as the product of intake and digestion and in ruminants is, in part, driven by forage CP concentration (Moore and Kunkle, 1994). In contrast to the described relationship in ruminants, data in hoses is largely absent from the literature, especially for warm-season forages. Therefore, the primary objective of this project was to determine the effect of forage CP content on the utilization of Coastal bermudagrass hay by horses. As previously described, the forages used in this project where taken from the same location and harvested at the same maturity, the only difference was the level of nitrogen fertilization provided. Ideally, forage CP concentrations would have been more divergent, allowing data to be collected on low CP Bermudagrss hay (less than 6%). In this study, the lowest CP hay used was

6.9% CP which is very close to the 7% CP breakpoint reported by Moore and Kunkle (1994) to be the point at which forage intake decreases in ruminants as CP concentration is reduced. In accordance with the observations in ruminants, forage intake did not significantly increase with increasing CP content. However, intake of the 16% CP was 22% greater than the intake of the 7% CP hay, indicating at least some benefit to increased CP content.

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APPENDIX

Table 1. Forage composition.

Item	7	10	13	16
	----------% of DM----------			
OM	92.7	93.0	92.7	90.5
CP	6.9	9.8	12.7	15.6
NDF	74.0	69.2	67.8	66.0
ADF	43.3	35.3	33.3	32.3
Gross Energy, Mcal/kg	3.6	3.7	3.7	3.6

Table 2. Effect of forage crude protein concentration on intake by horses.

	Forage Crude Protein, % of DM					Contrast *P*-value		
Item	7	10	13	16	SEM[1]	Linear	Quadratic	Cubic
No. of Observations	3	4	4	4				
Intake, kg/d								
Forage DM	9.58	9.19	10.25	12.08	1.38	0.16	0.37	0.90
Digestible DM	3.79	4.71	4.93	5.98	0.51	0.02	0.88	0.44
Forage OM	8.97	8.60	9.53	10.98	1.30	0.21	0.43	0.87
Digestible OM	3.70	4.47	4.61	5.35	0.49	0.04	0.98	0.50
NDF	7.14	6.50	7.07	8.14	1.02	0.39	0.35	0.85
Digestible NDF	2.76	3.22	3.27	3.73	0.39	0.11	1.00	0.58
ADF	4.05	3.28	3.43	3.91	0.54	0.89	0.20	0.75
Digestible ADF	1.31	1.34	1.17	1.20	0.17	0.46	1.00	0.54
CP Intake	0.67	0.91	1.30	1.88	0.15	< 0.01	0.25	0.92
Energy Intake, Mcal/d								
Gross	40.98	40.00	44.97	45.08	7.99	0.61	0.94	0.73
Digestible	14.86	19.37	20.36	20.47	3.04	0.21	0.45	0.82

[1]For n=3.

Table 3. Effect of forage crude protein concentration on apparent total tract digestion by horses.

	Forage Crude Protein, % of DM					Contrast *P*-value		
Item	7	10	13	16	SEM[1]	Linear	Quadratic	Cubic
No. of Observations	3	4	4	4				
Total tract digestion, %								
DM	39.63	54.23	48.67	49.23	3.02	0.09	0.03	0.04
OM	41.11	55.01	48.85	48.52	2.92	0.22	0.03	0.05
NDF	38.84	52.76	46.62	45.55	2.99	0.28	0.03	0.06
ADF	33.06	43.95	34.72	30.29	3.53	0.23	0.04	0.09
Gross Energy	36.04	51.65	45.70	45.33	3.69	0.19	0.05	0.10

[1]For n=3

Table 4. Effect of forage crude protein concentration on cecal fermentation and plasma metabolite concentration in horses.

	Forage Crude Protein, % of DM					Contrast *P*-value		
Item	7	10	13	16	SEM[1]	Linear	Quadratic	Cubic
No. of Observations	3	4	4	4				
Cecal pH	6.98	6.81	6.84	7.00	0.07	0.11	0.66	< 0.01
Cecal ammonia, m*M*	0.30	0.55	0.82	1.74	0.38	< 0.01	0.32	0.65
Plasma Glucose, mg/dL	68.77	70.65	72.85	73.68	0.29	0.04	0.76	0.82
Plasma Urea Nitrogen, m*M*	4.34	4.12	4.03	5.61	0.32	0.01	< 0.01	0.21

[1]For n=3.

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