

**Fiber and Protein Degradation Characteristics of Stockpiled Tifton 85
Bermudagrass**

by

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ABSTRACT

A 2-yr study was conducted to determine effects of rate of N fertilization on kinetic parameters of fiber digestion and protein degradability in stockpiled Tifton 85 bermudagrass. Six 0.76-ha pastures of stockpiled Tifton 85 bermudagrass were cut to a 10-cm stubble height on Aug 1 and fertilized with either 56 (56N), 112 (112N), or 168 (168N) kg N/ha (2 pastures/treatment). Kinetic parameters of fiber digestion included 72-hr potential extent of NDF digestion (PED), rate of NDF digestion, and lag time. The PED did not differ among N fertilization treatments in Yr 1 or Yr 2. In Yr 1, PED was greatest ($P < 0.05$) on Oct 24, intermediate on Nov 28 and Dec 13, and least ($P < 0.05$) on Jan 16. In Yr 2, PED was greatest ($P < 0.05$) on Nov 11 and Nov 25, intermediate on Jan 7, and least ($P < 0.05$) on Jan 21. Rates of NDF digestion did not differ ($P > 0.05$) among N fertilization treatments in either Yr 1 or Yr 2. In Yr 1, rate of NDF digestion was greater ($P < 0.05$) on Oct 24 than Dec 13 and Jan 16, but not different ($P > 0.05$) from Nov 28; and was greater ($P < 0.05$) on Nov 28 than Jan 16, but not different ($P > 0.05$) from Dec 13. In Yr 2, rate of NDF digestion was greater ($P < 0.05$) on Nov 25 than Jan 7 and Jan 21, but not different ($P > 0.05$) from Nov 11; and was greater ($P < 0.05$) on Nov 11 than Jan 7, but not different ($P > 0.05$) from Jan 21. In Yr 1, lag time was greater ($P < 0.05$) for the 112N than 56N and 168N fertilization treatments, and less ($P < 0.05$) for the Oct 24 than Nov 28, Dec 13 and Jan 6 sampling dates. In Yr 2, there were no differences ($P > 0.05$) among N fertilization treatments; lag time was least ($P < 0.05$) on

Nov 11 and Nov 25, intermediate on Jan 7 and greatest ($P < 0.05$) on Jan 21. In Yr 1, there was a negative correlation ($P < 0.05$) between forage lignin concentration and both PED ($r = -0.91$) and rate of NDF digestion ($r = -0.60$); lignin concentration tended ($P > 0.05$) to be positively correlated with lag time ($r = 0.39$). In Yr 2, there was a negative correlation ($P < 0.05$) between forage lignin concentration and PED ($r = -0.87$), and a positive ($P < 0.05$) correlation ($r = 0.91$) with lag time; rate of NDF digestion tended ($P > 0.05$) to be negatively correlated ($r = -0.25$) with lignin concentration. In Yr 1, DIP was greatest ($P < 0.05$) on Oct 24 and Dec 13, intermediate on Nov 28, and least ($P < 0.05$) on Jan 16. In Yr 2, DIP was greatest ($P < 0.05$) for the 56N and 168N treatments, and least ($P < 0.05$) for 112N; DIP was greatest ($P < 0.05$) on Jan 21, intermediate on Nov 25 and Jan 7, and least ($P < 0.05$) on Nov 11. Mean monthly air temperature was correlated ($P < 0.05$) with all parameters of NDF digestion; PED ($r = 0.69$, $r = 0.91$) and rate ($r = 0.56$, $r = 0.51$) were positively correlated, whereas lag time ($r = -0.54$, $r = -0.85$) was negatively correlated in Yr 1 and 2, respectively. Results of this study suggest that kinetic parameters of NDF digestion in stockpiled Tifton 85 bermudagrass were influenced more by temporal changes over the stockpile season than by N fertilization level. Furthermore, changes in protein degradation characteristics were less pronounced than changes in kinetic parameters of NDF digestion. Supplementation formulations should utilize kinetic parameters of fiber digestion to insure that energy-yielding components of NDF are sufficient to meet requirements throughout the stockpile season. The highly degradable CP fraction in stockpiled bermudagrass produces sufficient degradable intake protein to support fibrolytic activity and growth of ruminal microorganisms throughout the stockpile season.

Supplementation with sources of digestible fiber and undegradable intake protein could be expected to increase metabolizable protein supply to the host animal, depending on changing forage quality throughout the stockpiled grazing season.

Let us run with endurance the race that is set before us, fixing our eyes on Jesus, the author and finisher of our faith.
Hebrew 12:12

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I. LITERATURE REVIEW

Tifton 85 bermudagrass

Variety development

Tifton 85 bermudagrass (*Cynodon dactylon*) was developed by the USDA-ARS and the University of Georgia Coastal Plain Experiment Station in Tifton as a hybrid of PI 290885/Stargrass (*Cynodon nlemfuensis*) from South Africa and Tifton 68 bermudagrass (Burton et al., 1993). Tifton 68 is characterized by a darker green color, larger stems and broader leaves than other commercially available bermudagrass hybrids. It is highly digestible and high-yielding; however, due to its low cold tolerance, it is not as widely used for production (Hill et al., 2001) in the southeastern region. Compared with other bermudagrass varieties (hybrid or seeded types), Tifton 85 is taller, has larger stems and broader leaves, and is darker in color. Also, it produces large rhizomes, corms, and rapidly spreading stolons. Tifton 85 is one of the highest yielding and highest nutritive-value varieties of bermudagrass. Whereas Tifton 85 has greater cold tolerance than Tifton 68, it is not especially cold hardy. Therefore, Tifton 85 is recommended in climates typical of the Lower Gulf Coast region (Hill et al., 2001).

Tifton 85 can be established by broadcasting and disking green stems that have been cut at an advanced growth stage into a moist soil, or by planting sprigs with a mechanical planter (Burton, 1993).

Lignification and ferulic acid

Lignification is the single most important plant characteristic limiting the digestibility of forages (Van Soest, 1982), and ferulic acid linkages between cell wall polysaccharides and core lignin limit cell wall digestion (Jung and Allen, 1995). Lignin is a complex polymer synthesized from aromatic acids. Arabinoxylan, a component of forage hemicellulose, bonds via ester linkages with ferulic acid which in turn bonds with the chemically mature lignin core via ester or ether linkages (Jung and Allen, 1995). Ruminant microorganisms possess phenolic esterases that are able to hydrolyze ferulate-ester linkages; yet, cleavage of the ether linkages does not occur by action of these organisms (Jung and Allen, 1995). Therefore, a greater concentration of ether linkages rather than core lignin *per se* is associated with decreased digestibility (Mandebvu, 1999).

As forage matures, lignin concentration increases as the cell wall thickens, which is negatively associated with digestibility (Van Soest, 1982). When lignin-containing forages are consumed by ruminant animals, numerous factors result in the associated negative correlation with digestibility. From a stereochemical perspective, accumulation of lignin can cause steric hindrance of rumen microorganisms, preventing them from accessing the structural carbohydrates for degradation. Also, the aromatic acids from which lignin is synthesized (primarily *p*-coumaric, ferulic, and sinapic acids) accumulate in some forages, specifically grasses, and are inhibitory to rumen microorganisms, resulting in decreased fiber degradation. Furthermore, aromatic acids can be polymerized into other types of phenolic compounds such as tannins that can also decrease carbohydrate and protein degradation (Van Soest, 1982).

Tifton 85 bermudagrass improvements over other bermudagrass varieties

Coastal bermudagrass is a hybrid developed and released by the USDA and the Georgia Coastal Plain Experiment Station. It is coarsely-stemmed, tall-growing, and produces rhizomes and stolons. Coastal bermudagrass is the most widely planted bermudagrass throughout the Southeast; however, winterkill can occur in northernmost areas of the region. Tifton 68 is a hybrid cultivar, released in 1983, that spreads rapidly by large stolons. It is similar in winter hardiness to Coastal; however, it has greater yield and digestibility (Ball, 2002). Tifton 78 is another bermudagrass hybrid, released in 1984, which is more digestible than Coastal. Similar to Tifton 68, it is more stoloniferous and rapidly spreading than Coastal (Ball, 2002).

In a comparison of Tifton 85 with other hybrid bermudagrass varieties such as Coastal, Tifton 68 and Tifton 78, Hill et al. (1993) found that Tifton 85 had greater nutritive quality and DM accumulation. Tifton 85 produced 26% more DM, 110 g/kg more digestible DM, and 80 g/kg more digestible NDF (Mandebvu et al., 1999). Compared with Tifton 44, a more cold-tolerant cultivar, Tifton 85 had greater concentrations of cellulose and ADF, but lesser concentrations of hemicellulose and lignin. Further studies showed that Tifton 85 had greater IVDMD than Tifton 44 (Burns et al., 2007).

Tifton 85 and Coastal bermudagrass differ in concentrations of NDF, ADF, hemicellulose, cellulose and ADL, and percentage IVDMD (Mandebvu et al., 1999). Concentrations of all cell-wall constituents are greater in Tifton 85, except for ADL that is less (Mandebvu et al., 1999). Tifton 85 has less concentration (g/kg cell wall) of acid-insoluble lignin and greater concentration of total cell wall (g/kg DM). The total ester

ferulic acid concentration is greater in Tifton 85, but the total ethereal ferulic acid is less (Mandebvu et al., 1999). The decreased concentration of ferulate-ether linkages is thought to be the cause of the increased digestibility exhibited by Tifton 85.

Forage quality analysis methodology

The traditional method of determining forage quality is through wet chemistry analysis. Wet chemistry refers to analytical techniques that utilize chemicals and drying agents, based on both chemical and biochemical principles, to determine forage components. The forage fiber components that are most commonly analyzed are NDF, ADF, and lignin; DM and CP are also routinely reported.

The old proximate analysis (Weende) system of feed/forage analysis has long since been replaced by the Van Soest detergent-fiber fractionation system (Van Soest et al., 1991). This system uses detergent solutions of varying pH and ionic strength to separate the digestible and indigestible structural components of the plant cell wall. The fibrous feed is first extracted with a neutral detergent solution, which separates the soluble (cell contents) fraction from the insoluble (cell wall) fraction. Using a sequential approach, the NDF residue is then extracted with acid detergent solution, which solubilizes the hemicellulose. The cellulose is then removed by digesting the ADF residue with sulfuric acid, leaving only the lignin and acid-insoluble ash; this residue is then combusted, leaving only the acid insoluble ash.

The NDF is the insoluble residue that results from extraction with neutral detergent solution. The NDF fraction represents the "bulk" in a forage, which is negatively correlated with DM intake (Van Soest et al., 1991). The ADF is the insoluble

residue resulting from extraction with acid detergent solution, consisting of lignin, cellulose and acid-insoluble ash; as ADF concentration increases, forage digestibility and intake of DE decrease (Van Soest et al., 1991). Lignin is the indigestible cell-wall fraction; as lignin concentration increases, digestibility of cellulose and hemicellulose decrease (Van Soest et al., 1991). Digestibility of DM is determined either in vitro or in situ/in vivo. In vitro DM digestibility is determined by incubation of the forage in buffered rumen fluid for a prescribed period of time, typically 48 hr, followed by extraction with neutral detergent solution. In situ digestibility of forage DM is determined by incubating the forage in a rumen-cannulated animal.

Forage protein concentration is most commonly reported as crude protein (CP; $\%N \times 6.25$) that includes both true protein and non-protein nitrogen. Whereas CP is the commonly reported value, it can be further subdivided into fractions based upon ruminal degradability. Degradable intake protein (DIP) is the portion of CP that is degraded by ruminal microorganisms. The DIP fraction contains the true protein and NPN that are used by rumen microorganisms to synthesize microbial protein, and is typically expressed as a percentage of the CP (NRC, 1996). The other fraction is the undegradable intake protein (UIP) that is not degraded in the rumen, but rather in the small intestine; it is sometimes also referred to as rumen undegradable protein, escape protein, or by-pass protein (NRC, 1996). Thus, DIP and UIP provide the ruminant animal with amino acids derived from a composite of microbial and unaltered feed proteins.

Digestion kinetics

Whereas traditional fiber analysis provides insight into the nutritive value parameters of forage, limitations are evident from its application to newer, improved varieties such as Tifton 85 bermudagrass. Kinetic parameters such as the potential extent of NDF digestion, rate of NDF digestion, and fermentative lag time can provide insight into forage nutritive quality and utilization that cannot be discerned readily from static measures of chemical composition derived from conventional laboratory analysis.

The potential extent of NDF digestion is the percentage of NDF that is available for digestion in the rumen. The extent of digestion can be influenced by chemical and physical properties of plant cell walls such as lignification (Van Soest, 1982) and silication (Mertens, 1977). The crystallinity of fibrous carbohydrates and morphological characteristics of plant tissues may also affect the fraction that is potentially digestible (Mertens, 1977). The rate of NDF digestion is that quantity of NDF that can be digested in the rumen within a prescribed period of time, expressed as %/h (Van Soest, 1982). The rate of digestion is influenced by diet composition, quality, and nutrient availability. Fermentation of complex carbohydrates is dependent upon the availability of adequate microbial nutrition. Structural carbohydrates are fermented at a slower rate than storage carbohydrates (Van Soest, 1982). Lag time is the elapsed time before NDF digestion begins, during which forage particles are masticated and colonized by rumen microorganisms following ingestion (Mertens, 1977). Lag time may also be affected by factors such as hydration rate, chemical and physical alterations of fiber (Mertens, 1977), and nutrient limitations (Pell and Schofield, 1993).

To determine these parameters, forage samples are subjected to fermentation either in vitro (Mandebvu et al., 1998) or in vivo (Miller and Muntifering, 1985) for discrete periods of time, typically up to 72 hours. The potential extent of NDF digestion is assumed to be complete at the end-point of the incubation, and fermentation residues are assayed for residual NDF.

Quantitative analyses of digestion kinetic parameters have received considerable attention and have resulted in a variety of mathematical models of fiber digestion. The fermentation (i.e., digestion) rate can be determined via in vitro batch culture, in vivo incubation in the rumen, enzyme solubilization, and the zero-time method. Digestion must be measured at various times to estimate the rate of change (Van Soest, 1982). The zero-time procedure (Hungate, 1966) aims to assay the fermentation rate at the time of sampling. The zero-time value is the maximum initial rate and is obtained through graphical extrapolation. This technique is useful in measuring the rapidly available substrate, but not for estimating the digestion of cellulosic carbohydrates (Van Soest, 1982). Fiber digestion parameters can be derived by fitting data to a nonlinear equation (Ørskov et al., 1980) or first-order kinetic equation (Mertens, 1977). The nonlinear equation used is:

$$p = a + b(1 - e^{-ct})$$

where p = actual degradation after time 't', a = intercept of the degradation curve when t is equal to 0, b is the potential degradability of the substrate, and c is the rate constant for the degradation of b (Ørskov et al., 1980). Thus, a + b equals the potentially digestible fractions, and 100 - (a + b) equals the indigestible fraction (Mandebvu et al.,

1998). In this system, lag effects can be confounded with extent of digestion, and 'a' can be underestimated when there is a lag time (Van Soest, 1982).

Fiber digestion kinetics in vivo may be conceptualized by the following equation:

$$AED = PED * K_d / (K_d + K_p) * e^{-K_p L}$$

where AED = apparent extent of forage fiber digestion, PED = potential extent of digestion, K_d = rate constant of digestion, K_p = rate of passage, and L = discrete lag time. Thus, $e^{-K_p L}$ represents the potentially digestible fiber remaining at the end of the lag time, $K_d / (K_d + K_p)$ represents the theoretical maximum proportion of fiber disappearance when lag is equal to 0, and the product of these two terms represents the fraction of potentially digestible fiber which was actually digested (Miller and Muntifering, 1985). The first-order kinetic equation is determined utilizing a logarithmic transformation, or nonlinear regression approach, in which rate of NDF digestion is the percent potentially digestible NDF remaining versus time. The resulting first-order kinetic equation is then solved for 100% potentially digestible NDF remaining to derive an estimate of lag time. Furthermore, lag can be estimated as either discrete lag or kinetic lag. Discrete lag is the most commonly applied model, but is not a true mechanistic model as it does not involve causation. Discrete lag is derived from regression of the logarithmic rate, y-axis, vs time, x-axis. The assumption made in this model is that no ingesta leave the rumen during the lag period (Van Soest, 1982). A nondiscrete lag model (van Milgen et al., 1991) acknowledges that lag pool size decreases over time. The size of this pool is assumed to decrease according to first-order kinetics and likely involves microbial attachment, hydration, and enzyme introduction (Van Soest, 1982). It appears to be more effective in describing the

disappearance curves resulting from nylon bag digestion than the discrete lag system (van Milgen et al., 1991). Discrete lag is used in most practical systems, as it can be easily estimated through regression (Van Soest, 1982).

Galdámez-Cabrera et al. (2003) evaluated ruminal degradation of DM and fiber from bermudagrass among differing (0, 56, 112, and 168 kg N/ha) N fertilization levels. It was reported that both potential extent and rate of NDF digestion in common bermudagrass were increased with increased N fertilization. Effective NDF degradability was increased by 10 g/kg for each 100 kg N/ha increase.

Mandebvu et al. (1998) evaluated digestion kinetics of Tifton 85 bermudagrass and Coastal bermudagrass, and reported that Tifton 85 had greater potential extent of NDF digestion than Coastal. Additionally, they reported a numerical increase in rate of NDF digestion of Tifton 85 over Coastal at similar stages of maturity. Despite Tifton 85 having a greater extent of digestion of both NDF and DM and a greater potentially digestible NDF fraction, concentrations of NDF, ADF, and lignin were similar between the two varieties. The discrepancy between the similar lignin concentration and digestibility is due to the ether-linked ferulic acid concentration. Tifton 85 has a lesser concentration of ether ferulic acid linkages compared with Coastal, which many explain the greater digestibility (Mandebvu et al., 1999).

Forage quality parameters have been used to develop various prediction systems, with relative feed value (RFV; Rohweder et al, 1978) being the most widely used. Forage RFV is based upon dry matter intake (DMI) and digestible dry matter intake (DDMI). Hackmann et al. (2008) evaluated the correlation between degradation parameters and RFV. Potentially digestible DM, NDF, and hemicellulose were

significantly correlated with RFV for grass-legume mixtures. The correlation between DDMI and degradation parameters was further investigated, and determined to be related for grass forages. The correlation between DDMI and degradation parameters, but not RFV, indicates the shortcomings of the RFV system. Limitations of the system include the ability of RFV to account for variation in plant-related factors such as forage species, growth conditions, and stage of maturity.

Protein fractionation

Crude protein provides an approximate estimate of available protein; however, further characterization of CP has been used recently to better enable its application to animal feeding strategies. The protein fractions are designated as A, B, and C. Protein fraction A is the non-protein nitrogen (NPN) fraction that is considered to be instantaneously soluble when time is equal to zero (Ferguson, 2016). Fraction B is the potentially degradable true protein portion that can then be further subdivided into fractions characterized by fast (B_1), medium (B_2), and slow (B_3) rates of rumen degradation (Lalman et al., 2000). The fraction B_2 has a degradation rate which is similar to the rate of passage from the rumen (Ferguson, 2016). Fraction C is the cell-wall bound or insoluble fraction (NRC, 1996), which is considered to be undegradable and indigestible. These proteins are those associated with lignin, tannins, and heat-damaged proteins (Ferguson, 2016), and are then broadly characterized in the DIP and UIP fractions.

Johnson et al. (1999) found that Tifton 85 bermudagrass fractions were increased by 136.7 (A), 84.6 (B_1), 109.5 (B_2), 38.7 (B_3) and 53.8 (C) percent when

fertilized with 157 kg N/ha compared with the control (0 kg N/ha). The protein fractions representing NPN, fast, and moderate rates of degradation were increased to a greater extent than the indigestible and slow-rate fractions in response to increased N fertilization (Lalman et al., 2000).

Vendramini et al. (2007) studied the effect of N fertilization and regrowth interval on CP fraction concentrations in Tifton 85 bermudagrass. As N fertilization increased, a linear increase in potentially degradable protein and linear decrease in rumen undegradable protein was observed. Additionally, the authors noted that increasing N fertilization increased effectively degradable DM. They suggested that the large fractions of degradable and undegradable protein, when compared with the rapidly degradable fraction, may result in inadequate rumen degradable protein, especially when N fertilization levels are low, suggesting that supplementation with rumen-degradable protein supplementation may address this limitation.

Stockpiled forage

Stockpiling

Stockpiled forage, often referred to as standing hay, is forage that is left in the field to accumulate for later use. The forage is clipped, fertilized, and allowed to accumulate in the field until dormancy, after which it is utilized at a predetermined future time (Allen et al., 2011). The stockpiled forage can then be grazed or mechanically harvested for hay. A forage-management plan that includes stockpiling can be executed for any period of forage production deficit; however, it is most often executed prior to dormancy at the end of the growing season (Ball et al., 2015). The stockpiling period

typically occurs during the fall and winter in the Southeast, which allows for a forage option during the gap between cool-season and warm-season growing seasons. The climate of the southeastern U.S. lends itself especially well to the incorporation of stockpiling into a forage management plan that must also take forage species and growth characteristics into consideration in order to be successful.

Implementation of stockpiling into a forage management plan can reduce costs associated with feeding hay such as harvesting, storage, and waste. The cost of feed and feeding is the greatest expense to cattle producers (Lawrence and Strohhahn, 1999). Extending the grazing season is able to reduce the cost of feed and labor associated with wintering the cattle herd. Ball et al. (2015) have stated that the use of stockpiled forage can extend the grazing season by an average of 70 days.

An important consideration when stockpiling forage is the changes that occur in nutritive value during the stockpiling and grazing period. Maturation of forage in the earlier part of the stockpiling period results in increased lignification. As forage becomes more fibrous and lignified, digestibility and CP concentration decrease. Additionally, stockpiled forage is exposed to environmental factors in the latter part of the stockpiling/grazing period that further decrease nutritive value. Stockpiled bermudagrass has been reported to decline in quality over the stockpiling period, with TDN decreasing from 81% to 48%, and CP concentration decreasing from 23% to 6% between October and February (Jennings et al., 2009). Tifton 85 is an especially good candidate for stockpiling because the ferulic acid-lignin relationship is primarily ester-linked, and therefore digestibility remains favorable (Mandevu et al., 1999).

Forage species

When selecting a forage species to be used in a stockpiling system, it is important to consider both climate and goals of the forage management plan. Forage species comprise cool-season (C_3) and warm-season (C_4) types that vary in their pattern of growth, growing period, and productivity. Cool-season perennial grasses such as tall fescue (*Lolium arundinacea*) and orchardgrass (*Dactylis glomerata*) fix CO_2 solely by the enzyme ribulose biphosphate carboxylase (Rubisco) into 3-phosphoglycerate (3-PGA), a 3-carbon compound. Preceding synthesis of 3-PGA by Rubisco, warm-season perennial grasses such as bermudagrass and bahiagrass (*Paspalum notatum*) initially fix CO_2 by the enzyme phosphoenolpyruvate carboxylase into oxaloacetate, a 4-carbon compound. Fixation of CO_2 in C_4 plants is the less energetically efficient, but C_3 plants lose up to 40% of their fixed CO_2 through photorespiration, whereas this loss does not occur as extensively in C_4 plants. Consequently, warm-season grasses that utilize the C_4 pathway have greater energetic efficiency of net CO_2 fixation and are able to be more productive, resulting in greater forage yield (Ball et al., 2015).

Cool-season grasses are often stockpiled in locations where they are adapted to fit the needs of local production systems. The most common cool-season species used are tall fescue, orchardgrass, and Kentucky bluegrass (*Poa pratensis*). Tall fescue is known for its ability to maintain quality throughout the stockpiled period. Orchardgrass is similar to tall fescue; however, it yields less and does not maintain quality to the same extent as tall fescue (Ball et al., 2015). Kentucky bluegrass tends to have superior nutritive value but does not achieve sufficient yield to be an especially suitable forage species for stockpiling. A forage species needs to be able to produce a minimum of

2,000 kg of DM/ha for a stockpiling process to be successful (Ball et al., 2015). The primary limitation to the use of tall fescue is the presence of the endophytic fungus, *Neotyphodium coenophialum*, that has negative effects on animal performance due to the production of ergot alkaloids (Tucker et al., 1973). Delayed grazing of stockpiled tall fescue is one mitigation strategy used to limit exposure of cattle to lower ergot alkaloid concentrations, as these are reduced during the stockpiling period (Burns et al., 2006). Whereas tall fescue often is superior in nutritive value, the warm-season forages often stockpiled are bermudagrass and bahiagrass (Ball et al., 2015).

Perennial warm-season grasses are the primary forages grazed by beef cattle in the Southeast, the most common species being bermudagrass and bahiagrass which are suitable for fall stockpiling (Scarborough et al., 2006). The availability of these forages for grazing is less than that observed with tall fescue, as warm-season grasses have more rapid dry matter deterioration. Bermudagrass is a primary warm-season grass due to its stand persistence and ability to remain productive for over 35 yr under proper management. Additionally, bermudagrass is popular in the Southeast due to its ability to withstand heavy grazing pressure and variable rainfall, and its tolerance of acidic and sandy soils (Hill et al., 2001). The stockpiling process involves allowing the forage to grow and accumulate mass until senescence. Plant senescence is often associated with decreased quality in regards to DM digestibility and CP concentration, and the use of stockpiled bermudagrass for winter grazing has not been widely practiced due to a perception of inadequate quality. However, studies have recently been conducted which indicate adequate forage quality for various classes of livestock. Lalman et al. (2000) conducted an experiment in Oklahoma which showed that fertilized bermudagrass

stands maintain adequate CP concentrations to sustain gestating beef cows.

Additionally, McNamee (2014) conducted an experiment in Alabama which showed that stockpiled Tifton 85 produced sufficient forage mass and maintained a level of nutritive value to support lactating beef cows without supplementation.

Forage species variety should also be considered when choosing a forage for stockpiling, as they vary in DM production capacity and cold tolerance. It is important to consider the duration of the forage gap that the stockpiled forage is expected to fill. For example, Lalman et al. (2000) found that Coastal bermudagrass accumulates more DM and continues growing later into the fall months than common bermudagrass.

Another consideration in choosing a plant variety is the above-ground sward structure. Physical plant characteristics such as stem strength must be considered, as lodging is a common problem with stockpiled forage. Additionally, the persistency of the stand must be considered, which makes bermudagrass an especially good candidate for stockpiling. Improved grazing management strategies can also be implemented to better increase forage utilization in stockpiled grazing systems. Strip grazing is a strategy in which cattle are given access to a small area using temporary fencing and allowed to graze for a predetermined amount of time. The fence is then moved to expand the area which can be grazed. Strip grazing can decrease losses associated with trampling, defecation, and uneven grazing.

Fertility considerations for stockpiling

Prior to the stockpiling period, the forage is either grazed or cut for hay so that forage height is between 8 and 10 cm by mid- to late summer, at which time N fertilizer

is applied to maximize forage accumulation (Scarborough et al., 2006). Lalman et al. (2000) found that the use of N fertilizer reduces the land area required per grazing animal. Whereas soil testing is the most accurate way to determine the amount of N needed for a given stand, it has been shown that 110 to 132 kg/ha is appropriate. Fertilizing with N at the appropriate level is crucial to forage accumulation and productivity of the stockpiled forage. Additionally, the timing of fertilization is a key factor. Nitrogen fertilizer should be applied at the beginning of the stockpiling period, as delayed fertilization has shown to provide minimal benefit to forage accumulation. (Barnhart, 2013).

Implications to further research

Beef production in the Gulf Coast region, and throughout the Southeast, relies heavily on both warm- and cool-season forages. Supplemental feeds are utilized to bridge the gap between these growing seasons. Stockpiling is a strategy used to mitigate this gap. Synchronizing degradability characteristics of forage fiber with those of supplemental feeds can maximize fiber digestion from stockpiled forage. Kinetic characteristics can improve quality predictions from chemical composition because they may provide insight into forage quality that cannot be determined solely from traditional laboratory analysis, which is increasingly important as improved forage varieties are implemented in production. Incorporating degradability characteristics with current forage quality prediction systems may result in the development of more accurate systems for predicting forage quality (Hackmann et al., 2008).

Tifton 85 bermudagrass is a bermudagrass cultivar with increased digestibility and decreased ferulic acid concentration. The compositional change has the ability to affect forage quality characteristics. Research has been conducted investigating the fiber degradability characteristics of common bermudagrass and Tifton 85 bermudagrass during the conventional summer-growing season, but no research has been conducted to investigate these characteristics with stockpiled Tifton 85 bermudagrass. Both the productivity and nutritive value of stockpiled bermudagrass are influenced by N fertilization and environmental factors.

The research presented herein is novel because it investigates Tifton 85 bermudagrass utilized in a stockpiled production system. Reconciling the kinetic characteristics of NDF and protein degradability with those of supplemental feedstuffs may be used to develop more accurate supplementation strategies.

II. FIBER AND PROTEIN DEGRADATION CHARACTERISTICS OF STOCKPILED Tifton 85 BERMUDAGRASS

INTRODUCTION

Stockpiling forage is an effective strategy for minimizing winter-feed costs in beef cattle production systems in the Southeast. Stockpiling involves allowing forage to accumulate beginning in mid-summer, but not utilizing it for grazing until fall, a time of typical forage deficit in the region. This management practice is cost-effective, as it reduces feed costs and does not require the same input costs associated with hay production. Bermudagrass (*Cynodon dactylon*), a warm-season perennial, is one of the primary forage species utilized in the Southeast in this system. Tifton 85 is a bermudagrass cultivar selected for increased nutritive value, digestibility, yield and cold tolerance compared with other commercially available hybrids, making it an ideal forage for fall-stockpiling in the Lower Gulf Coast region (Hill et al., 2001).

Conventional laboratory analysis provides useful insight into forage nutritive value parameters; however, limitations are evident in its application to improved varieties such as Tifton 85. Because protein degradation of high-quality forage is generally more rapid and degradation of energy-yielding components of NDF is generally much slower, kinetic parameters such as the potential extent of NDF digestion, rate of NDF digestion, and fermentative lag time can provide insight into forage nutritive quality and predicted animal utilization that cannot be easily discerned

from static chemical composition measures. Likewise, characterization of forage protein in terms of rumen degradable and undegradable fractions can provide greater insight than is available solely from CP. Reconciling kinetic characteristics of NDF degradation and protein degradability of forage with those of supplemental feedstuffs may result in more accurate supplementation strategies. Incorporating degradability characteristics with traditional forage quality prediction systems may result in the development of more accurate forage quality models (Hackmann et al., 2008). For these reasons, a study was conducted to determine the effects of N fertilization and seasonal changes on the fiber and protein degradation characteristics of stockpiled Tifton 85 bermudagrass.

MATERIALS AND METHODS

Research Site

Forage for the study was grown and harvested at the Wiregrass Research and Extension Center (WREC) in Headland, AL (31.35' N, 85.34' W) from an existing, established Tifton 85 bermudagrass pasture that had been utilized previously for hay production. The pasture soil-type was a Dothan fine sandy loam (fine-loamy, kaolinitic, and thermic Plinthic Kandiudults).

Forage treatments

Six 0.76-ha paddocks were used in 2012 (Yr 1) and 2013 (Yr 2) for stockpiling. Forage was clipped to a 10-cm stubble height on Aug 1 and then fertilized on Aug 17 and Aug 28 in Yr 1 and 2, respectively. Two paddocks/treatment were fertilized with 56 (56N), 112 (112N), or 168 (168N) kg N/ha of ammonium nitrate (NH₄NO₃).

Forage harvest, sampling, and traditional laboratory analyses

Four forage samples were harvested from each paddock on 4 separate days between October 24, 2012 and January 2, 2013 (Yr 1), and between November 11, 2013 and January 21, 2014 (Yr 2) using hand clippers to leave a 5-cm above ground stubble height within a 0.25-m² quadrat. Samples were then placed in sealed plastic bags and stored on ice during transport to the Ruminant Nutrition Laboratory at Auburn University. Samples were dried at 50° C for 48 hr and air-equilibrated to ambient room temperature and relative humidity. Samples were then weighed, pooled into individual paddock composites, mixed thoroughly for uniformity, and ground to pass a 1-mm screen in a Wiley Mill (Thomas Scientific, Philadelphia, PA). Forage concentrations of DM and CP were determined according to procedures of AOAC (1990), and concentrations of NDF were determined according to the procedures of Van Soest et al. (1991). Forage harvest and traditional laboratory analysis were completed by McNamee (2014).

In vitro NDF digestion

Forage IVDMD was determined by the Van Soest et al. (1991) modification of the Tilley and Terry (1963) procedure using the Daisy II incubation system (Ankom Technology Corporation, Fairport, NY). Forage samples from Yr 1 and Yr 2 were assayed on separate days. Ruminal fluid was collected in mid-afternoon at the Auburn University College of Veterinary Medicine from a cannulated Holstein steer that was offered ad libitum access to bermudagrass hay and was limit-fed a 19% CP supplement

consisting of soy hull pellets, corn gluten feed, and whole cottonseed. Ruminal fluid was then transported to the Ruminant Nutrition Laboratory in pre-warmed thermos containers where it was further processed for batch-culture IVDMD. Four hundred mL of strained rumen fluid and 1,600 mL of warmed (39°C) phosphate-carbonate buffer (pH 6.8) were added to each of 8 fermentation jars containing ground forage (250 mg) sealed in Dacron filter bags (25- μ porosity). Each fermentation jar contained a single sample from each sampling date \times N-level experimental unit (paddock) within yr (24 samples total). Jars were designated for termination of fermentation at 3, 6, 9, 12, 24, 48, and 72 h post-inoculation for determination of digestion kinetic parameters. Samples were frozen to terminate fermentation, and NDF concentrations in fermentation residues were determined subsequently according to the procedures of Van Soest et al. (1991).

Fiber degradation characteristics were determined according to Mertens (1977) and Miller and Muntifering (1985). The potential extent of NDF digestion was assumed to be complete at 72 hr. Rates of digestion were determined by least-squares regression of \ln % potentially digestible fiber remaining vs. time. Lag time was determined by solving the first-order kinetic equation equal to 100% potentially digestible NDF remaining.

Protein fractionation

Forage concentrations of degradable intake protein (DIP) were determined using the procedure by Mathis et al. (2001) using a *Streptomyces griseus* protease (Type XIV Bacterial; Sigma-Aldrich, CO., St. Louis, MO). Sufficient mass of each sample that provided 15 mg N was placed in 125-mL Erlenmeyer flasks. Forty milliliters of a borate-

phosphate buffer (pH 6.8 – 7.0) were added to each flask and then incubated at 39° C for 1 h in a shaking water bath. Ten milliliters of the buffer-protease solution were then added to each flask and incubated at 39° C for 48 h in a shaking water bath. Upon completion of incubation, samples were filtered under vacuum through Whatman #541 filter paper using either a Buchner or Gooch funnel. Residues were rinsed with 400 mL distilled water to remove residual incubation solution, and dried at 100° C for 48 h to determine DM mass. The residues were analyzed for N by the Kjeldahl method (AOAC, 1990). The percentage of forage CP as undegradable intake protein (UIP) was determined by dividing mg of residual N by mg of N in the pre-incubated sample. This value was then subtracted from 100 to determine the percentage of forage CP that was degradable intake protein (DIP).

Statistical analyses

Forage chemical composition, protein fractionation, and NDF digestion kinetics data were analyzed as a completely randomized design with two replicates per treatment. Due to extreme climatic differences between years (Figure 1), data from each year were analyzed separately as a completely randomized design using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). The experimental unit was pasture. Independent variables included sampling date, N fertilization level, and the sampling date × N level interaction. Digestion kinetic parameters included rate of NDF digestion, discrete lag time, and potential extent of NDF digestion. The dependent variables analyzed pertaining to CP fractionation were DIP as percent of CP and as percent of forage DM. Digestion kinetic parameters were also analyzed using the PROC

CORR procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Differences were considered significant when $P < 0.05$, and trends when $P > 0.05$ and $P < 0.10$.

RESULTS AND DISCUSSION

Temperature and Precipitation

Monthly mean air temperatures (Figure 1, calculated as average of all highs and lows) during forage-sampling periods varied between Yr 1 and Yr 2, and in both yr relative to 30-year average temperatures. In Yr 1, temperatures were comparable to 30-yr average values in Aug, Sept, Oct, and Nov; however, temperatures in Dec and Jan were 29% and 62% higher than average, respectively. Likewise, temperatures in Yr 2 were comparable to 30-yr average values in early- to late fall (Aug to Nov) and 14% higher in Dec. However, in contrast to Yr 1, temperature in Jan of Yr 2 was 39% below the 30-yr average.

Monthly precipitation (Figure 2) also varied during forage-sampling periods between Yr 1 and Yr 2, and in both yr relative to 30-yr average values. In Yr 1, monthly precipitation totals were comparable to 30-yr average values in Sept and Dec; however, monthly totals in Oct, Nov, and Jan were 30, 73 and 78% less than average, respectively, and Aug precipitation was 62% greater than average. In Yr 2, precipitation in Sept was comparable to the 30-yr average. However, Aug, Oct, Nov, and Jan precipitation totals were 43, 94, 39 and 64% less, respectively, and Dec precipitation was 97% greater than average.

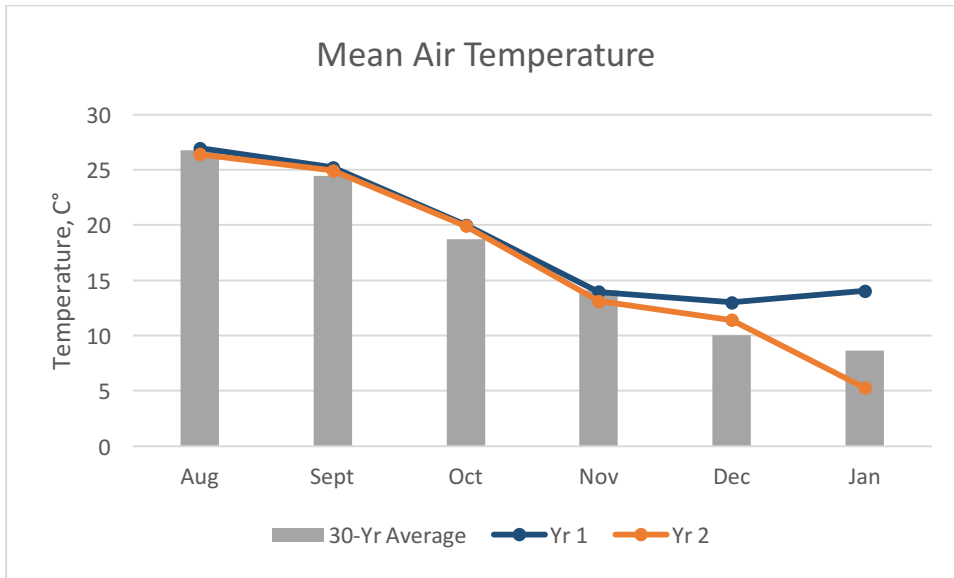


Figure 1. Monthly and 30-yr average mean air temperature from August to January by yr at Wiregrass Research and Extension Center, Headland, AL.

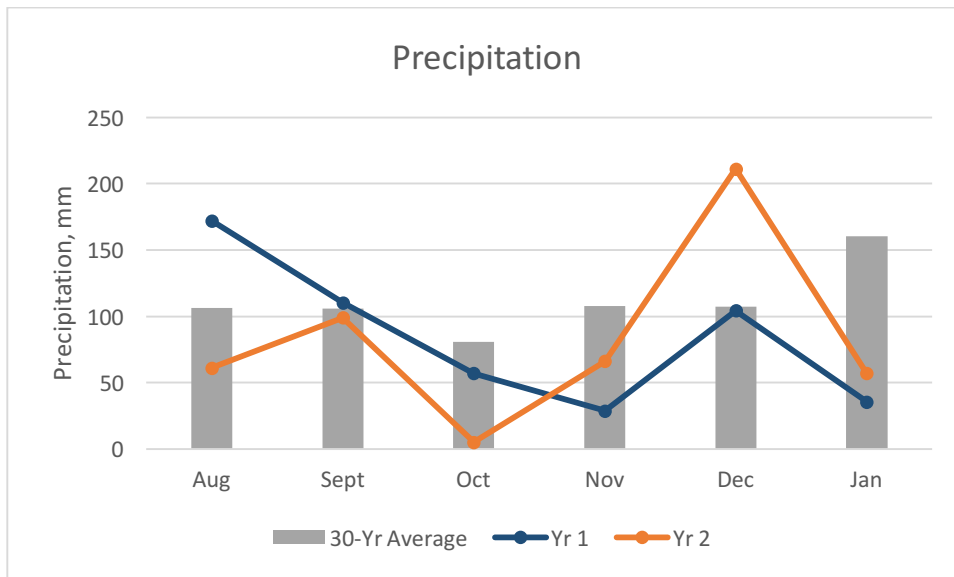


Figure 2. Monthly and 30-yr average precipitation from August to January by yr at Wiregrass Research and Extension Center, Headland, AL.

Digestion Kinetics

Potential extent of NDF digestion

Potential extent of NDF digestion (PED; Table 1) did not differ among N fertilization levels in Yr 1 ($P = 0.41$) or Yr 2 ($P = 0.54$). Similarly, Galdamez-Cabrera et al. (2003) observed no differences in potentially digestible NDF (g/kg forage DM) in common bermudagrass fertilized at 0 (764 g/kg), 56 (752 g/kg), 112 (768 g/kg), and 168 (768 g/kg) kg N/ha; relative response to N fertilization varied between harvest dates during the late spring/summer growing season (May 10 and Aug 18). Messman et al. (1991) also reported that N fertilization had little to no effect on PED of NDF in bromegrass (*Bromus inermis*), a cool-season perennial, fertilized with 0 or 89 kg N/ha. These findings support collectively the generalization that N fertilization across forage species, fertilization levels, and growing seasons does not effect the potential extent of NDF digestion.

In Yr 1, the potential extent of NDF digestion was greatest ($P < 0.05$) on Oct 24, intermediate on Nov 28 and Dec 13, and least ($P < 0.05$) on Jan 16. In Yr 2, the potential extent of NDF digestion was greatest ($P < 0.05$) on Nov 11 and Nov 25, intermediate on Jan 7, and least ($P < 0.05$) on Jan 21, illustrating a decrease over time. The PED of NDF decreased over the entire grazing season by 34% in Yr 1 and by 70% in Yr 2, in agreement with Mandebvu et al. (1998) who reported that PED of NDF was less for 3.5-wk and 7-wk regrowth than 3.5-wk primary-growth of Tifton 85 bermudagrass hay in the summer growing season. Samples in the present study averaged 10-wk regrowth from time of N fertilization in each yr; with 12-wk and 14-wk total regrowth from time of clipping in Yr 1 and Yr 2, respectively. Potential extent of

NDF digestion in our study ranged from 76% to 50% in Yr 1, similar to values of 78% to 55% reported by Mandebvu et al. (198), and from 67% to 20% in Yr 2. The decrease in PED of NDF observed in both studies indicates that PED is negatively affected by both the length of time prior to hay harvest in actively growing summer forage and the length of time before utilization in fall-stockpiled systems. The decrease in PED of NDF is associated with lignification that results from plant maturation in actively growing forage during the summer, and weathering in stockpiled forage during the fall/winter.

Forage concentration of potentially digestible NDF (PDNDF; Table 2) did not differ among N fertilization treatments in Yr 1 ($P = 0.94$) or Yr 2 ($P = 0.67$). In Yr 1, concentration of PDNDF was greater ($P < 0.05$) on Oct 24 than Dec 13 and Jan 16, and was greater ($P < 0.05$) on Nov 28 than Jan 16. Concentration of PDNDF in Yr 2 was greatest ($P < 0.05$) on Nov 11 and Nov 25, intermediate on Jan 7, and least ($P < 0.05$) on Jan 21. Forage concentration of PDNDF decreased throughout the grazing season by 29% in Yr 1 and 63% in Yr 2. Mandebvu et al. (1998) also reported that the potentially digestible fraction of NDF in Tifton 85 during the summer growing season decreased with maturity and was similar between 3.5-wk primary-growth and regrowth, which collectively had greater PDNDF than 7-wk regrowth, further demonstrating the inverse relationship between the potentially digestible NDF fraction and length of time preceding harvest or grazing.

Table 1. Potential extent of NDF digestion (%) from stockpiled Tifton 85 bermudagrass in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	74.0	77.1	76.8	76.0 ^a
	Nov 28	64.6	68.0	65.7	66.1 ^b
	Dec 13	60.9	59.2	68.8	63.0 ^b
	Jan 16	52.8	48.6	49.0	50.1 ^c
	Mean	63.1	63.2	65.1	
2	Nov 11	66.4	67.7	67.8	67.3 ^x
	Nov 25	59.8	65.7	65.0	63.5 ^x
	Jan 7	42.9	24.1	26.9	31.3 ^y
	Jan 21	21.9	14.2	23.8	20.0 ^z
	Mean	47.8	42.9	45.9	

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 1.3).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 3.5).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

Table 2. Concentration of potentially digestible NDF (% DM basis) in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	43.2	50.1	47.7	47.0 ^a
	Nov 28	45.6	45.6	44.2	45.1 ^{ab}
	Dec 13	43.0	40.4	45.6	43.0 ^b
	Jan 16	36.7	33.9	33.3	34.6 ^c
	Mean	42.1	42.5	42.7	
2	Nov 11	43.6	43.6	43.3	43.5 ^x
	Nov 25	36.2	39.1	38.5	37.9 ^x
	Jan 7	30.7	19.2	20.4	23.3 ^y
	Jan 21	16.3	16.1	15.9	16.1 ^z
	Mean	31.7	29.5	29.5	

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 1.3).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 2.3).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

Rate of NDF digestion

In both Yr 1 and Yr 2, rate of NDF digestion (Table 3) did not differ ($P > 0.05$) among the 56, 112, and 168 kg N/ha fertilization treatments. Galdamez-Cabrera (2003) reported that digestion rate increased linearly when N fertilization levels were increased from 0 to 168 kg N/ha. However, the rate of increase was minimal, less than 1% for every 100 kg N/ha increase. Messman et al. (1991) also reported increased rate of NDF digestion in bromegrass with increased N fertilization from 0 to 89 kg N/ha. In Yr 1, rate of NDF digestion was greater ($P < 0.05$) on Oct 24 than Dec 13 and Jan 16, but not different ($P > 0.05$) from Nov 28; and was greater ($P < 0.05$) on Nov 28 than Jan 16, but not different ($P > 0.05$) from Dec 13. In Yr 2, rate of NDF digestion was greater ($P < 0.05$) on Nov 25 than Jan 7 and Jan 21, but not different ($P > 0.05$) from Nov 11; and was greater ($P < 0.05$) on Nov 11 than Jan 7, but not different ($P > 0.05$) from Jan 21. A decrease in rate of NDF degradation was also observed by Galdamez-Cabrera et al. (2003) for bermudagrass hay harvested on the later of 2 different dates during the summer grazing season in Arkansas. Scarbough et al. (2006) reported that rate of DM disappearance declined linearly across harvest dates of fall-stockpiled common and Tifton 44 bermudagrass fertilized with 0, 37, 74, or 111 kg N/ha, in Fayetteville and Batesville, AR, respectively. Rate of NDF digestion of bromegrass decreased from the late-boot stage to full-head stage, or with increasing maturity, in a study by Messman et al. (1991). Rates of NDF digestion observed in the present study were greater than those reported by Galdamez-Cabrera et al. (2003) for common bermudagrass and by Mandebvu et al. (1999) for Tifton 85 bermudagrass during the summer growing season. The ADL values reported by Mandebvu et al. (1999) were greater for both common and

Tifton 85 bermudagrass than those observed in this study (Appendix II). Forage lignin concentration is negatively correlated with rate of NDF digestion and may thus explain the decreased rates observed.

Table 3. Rate of digestion of NDF (%/hr) for stockpiled Tifton 85 bermudagrass in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	7.2	6.8	6.6	6.9 ^a
	Nov 28	6.6	6.4	6.6	6.5 ^{ab}
	Dec 13	6.4	6.5	6.3	6.4 ^{bc}
	Jan 16	6.2	6.1	6.2	6.2 ^c
	Mean	6.6	6.5	6.4	
2	Nov 11	6.7	6.9	6.8	6.8 ^{xy}
	Nov 25	6.9	7.3	6.9	7.1 ^x
	Jan 7	6.2	6.3	6.7	6.4 ^z
	Jan 21	6.7	7.0	6.5	6.7 ^y
	Mean	6.7	6.9	6.7	

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.13).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.12).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

Discrete lag time

In Yr 1, lag time (Table 4) was greater ($P < 0.05$) for the 112N than 56N and 168N fertilization treatments across all sampling dates, and less ($P < 0.05$) for the Oct 24 than Nov 28, Dec 13 and Jan 6 sampling dates across all N-fertilization treatments. In Yr 2, there were no differences ($P > 0.05$) among N fertilization treatments. Lag time was least ($P < 0.05$) on Nov 11 and Nov 25, intermediate on Jan 7 and greatest ($P < 0.05$) on Jan 21. Lag time increased 18% over the Yr 1 grazing season and 49% in Yr 2. Mandebvu et al. (1998) reported a numerical increase in lag time with increasing maturity of Tifton 85, although it did not differ significantly among maturity dates in their experiment. The predicted effect of lag time on digestibility of grasses as theorized by Mertens (1977) is consistent with PED values observed in the present study. Scarbrough et al. (2006) reported shorter lag times, determined via in situ procedures, of fall-stockpiled common and Tifton 44 bermudagrass than those reported herein, which may be explained in part by the difference in fermentation system used. Varel and Kreikemeier (1995) reported that lag time determined by in situ procedures were, on average, 3.5 hr shorter than those determined by in vitro procedures. Also, the varieties of bermudagrass investigated by Scarbrough et al. (2006) were different from those used in the present study. The lag times reported by Mandebvu et al. (1998) for common bermudagrass were comparable to those found by Scarbrough et al. (2006); those reported for Tifton 85 were more closely related to those observed in Yr 1 of the present study, suggesting an effect of bermudagrass variety on lag time.

Table 4. Discrete lag time of digestion (hr) for stockpiled Tifton 85 bermudagrass in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	5.6	6.2	5.6	5.8 ^a
	Nov 28	7.5	7.5	6.1	7.0 ^b
	Dec 13	6.3	7.4	6.2	6.7 ^b
	Jan 16	6.7	7.7	6.9	7.1 ^b
	Mean	6.5 ^c	7.2 ^d	6.2 ^c	
2	Nov 11	9.6	8.2	9.2	9.0 ^x
	Nov 25	9.3	9.7	9.1	9.4 ^x
	Jan 7	12.7	13.0	10.7	12.1 ^y
	Jan 21	14.5	13.7	15.4	14.5 ^z
	Mean	11.5	11.1	11.1	

^{a,b} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.28).

^{c,d} Within a row, means without a common superscript differ ($P < 0.05$; SEM = 0.24).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.33).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

Correlation of lignin and kinetic parameters of NDF digestion

In Yr 1 and Yr 2, forage lignin concentration (Table 5) was correlated with all parameters of NDF digestion. In Yr 1 and Yr 2, forage lignin concentration was negatively correlated ($P < 0.05$) with 72-hr potential extent of NDF digestion ($r = -0.91$, $r = -0.89$) and concentration of potentially digestible NDF ($r = -0.85$, $r = -0.87$). Forage lignin concentration was negatively correlated ($P < 0.05$) with rate of NDF digestion in Yr 1 ($r = -0.60$), whereas the negative correlation in Yr 2 ($r = -0.25$) was not significant ($P > 0.05$). In Yr 1 and Yr 2, lignin concentration was positively correlated ($P = 0.06$, $P < 0.05$) with lag time ($r = 0.39$, $r = 0.91$). Lignification (forage lignin concentration as a percentage of NDF; Table 6) was similarly correlated with kinetic parameters of NDF digestion. In Yr 1 and Yr 2, lignification was negatively correlated ($P < 0.05$) with 72-hr potential extent of NDF digestion ($r = -0.90$, $r = -0.87$) and concentration of potentially digestible NDF ($r = -0.86$, $r = -0.88$). Lignification was negatively correlated ($P < 0.05$) with rate of NDF digestion in Yr 1 ($r = -0.60$), whereas the negative correlation in Yr 2 ($r = -0.24$) was not significant ($P > 0.05$). In Yr 1 and Yr 2, lignification was positively correlated ($P = 0.06$, $P < 0.05$) with lag time ($r = 0.39$, $r = 0.92$). The accumulation of lignin in the cell wall causes steric hindrance of the rumen microorganisms in their accessibility to structural carbohydrates, inhibits rumen microorganisms due to aromatic acids, and produces low-molecular-weight phenolic compounds that can negatively affect carbohydrate and protein degradation, decreasing the extent and rate of NDF digestion. Lag time is positively associated with lignification, as lignin limits the ability of the microorganisms to colonize and begin degradation of fiber particles.

Mertens (1977) reported separate correlations within temperate grasses and legumes between forage lignin concentration and rate of digestion; however, the global correlation was not significant when both grass and legume data were combined. Mertens (1973, referenced by Mertens, 1977) utilized a larger, more diverse data set and observed a non-significant correlation between lignin and rate of NDF digestion. Also, Smith et al. (1972) reported a moderate positive correlation between lignin and rate of digestion across 15 forage species. Their reported rates varied from 7 to 18 %/hr, with lignin concentration varying from 3 to 10% of DM; rates in the present study ranged from 6 to 7%/hr, and lignin concentrations ranged from 1.5 to 6% of DM. Whereas the magnitude and significance of correlation differed among studies, the direction was consistent throughout, implying possibly that the strength of the relationship between forage lignin concentration and rate of NDF digestion may be related to forage species.

Correlation of mean air temperature and kinetic parameters of NDF digestion

Mean air temperature (Table 7) was correlated with all parameters of NDF digestion. In Yr 1 and Yr 2, mean monthly air temperature was positively correlated ($P < 0.05$) with 72-hr potential extent ($r = 0.69$, $r = 0.91$) and rate ($r = 0.56$, $r = 0.51$) of NDF digestion. Likewise, Galdamez-Cabrera et al. (2003) reported that increased ambient temperature positively affected PED of NDF in bermudagrass; monthly air temperatures increased from 13.3 to 28.3 °C over the summer growing season in their study. Mean air temperature was negatively correlated ($P < 0.05$) with lag time ($r = -0.54$ and $r = -$

0.85). In both Yr, PED of NDF was the kinetic parameter most highly correlated with mean air temperature.

Mean monthly air temperature was negatively correlated ($P < 0.05$) with lignin concentration in both Yr 1 ($r = -0.57$) and Yr 2 ($r = -0.87$). As mean air temperature increases over the summer growing season, forage lignin concentration also increases (Van Soest, 1994). However, in a fall stockpiled system, mean air temperatures are decreasing over time, whereas forage lignin concentration increases. The cause of lignification, as related to temperature, is therefore the result of differing physiological mechanisms. In the summer growing season, lignification is the result of forage maturation; in fall-stockpiled systems, the forage is less or no longer actively growing/maturing, and lignification is the result of weathering.

Differences in lag time between Yr 1 and Yr 2 may be related in part to mean air temperatures, as temperatures and lag times over the first 2 sampling periods of Yr 2 were similar to the lag times and temperatures in Yr 1. The second 2 sampling dates of Yr 2 had greater lag times and decreased temperatures than Yr 1. Air temperature was similar between Yr 1 and Yr 2 from Aug through Nov; Dec and Jan remained relatively unchanged from Nov in Yr 1, but decreased in Yr 2. Also, the first killing frost of Yr 2 was observed on Nov 10 of Yr 2, prior to sampling, but did not occur until after the stockpile season in Yr 1. The negative correlation between lag time and temperature would explain the lower lag times reported by Mandebvu et al. (1998) for Tifton 85 bermudagrass grown during the summer season.

Table 5. Correlation between kinetic parameters of NDF digestion and forage lignin concentration¹ in stockpiled Tifton 85 in Yr 1 and Yr 2

Year	Statistics	Parameter			
		PED	Rate	Lag time	PDNDF
1	<i>r</i>	-0.911	-0.602	0.394	-0.847
	<i>P</i> -value	<0.0001	0.002	0.06	<0.0001
2	<i>r</i>	-0.887	-0.252	0.914	-0.87
	<i>P</i> -value	<0.0001	0.24	<0.0001	<0.0001

¹Forage concentration of ADL (% of DM).

Table 6. Correlation between kinetic parameters of NDF digestion and cell-wall lignification¹ in stockpiled Tifton 85 in Yr 1 and Yr 2

Year	Statistics	Parameter			
		PED	Rate	Lag time	PDNDF
1	<i>r</i>	-0.901	-0.599	0.384	-0.859
	<i>P</i> -value	<0.0001	0.002	0.06	<0.0001
2	<i>r</i>	-0.866	-0.238	0.916	-0.875
	<i>P</i> -value	<0.0001	0.26	<0.001	<0.0001

¹Forage concentration of ADL (% of NDF).

Table 7. Correlation between kinetic parameters of stockpiled Tifton 85 and mean air temperature¹ in Yr 1 and Yr 2

Year	Statistics	Parameter			
		PED	Rate	Lag time	PDNDF
1	<i>r</i>	0.668	0.559	-0.541	0.422
	<i>P</i> -value	0.0004	0.005	0.006	0.04
2	<i>r</i>	0.914	0.507	-0.851	0.875
	<i>P</i> -value	<0.0001	0.01	<0.0001	<0.0001

¹Mean monthly air temperature.

Protein fractionation

Degradable intake protein

The degradable intake protein (DIP; Table 8) fraction as a percentage of total forage CP was greatest ($P < 0.05$) on Oct 24 and Dec 13, intermediate on Nov 28, and least ($P < 0.05$) on Jan 16. In Yr 2, DIP percentage of CP across all sampling dates was greatest ($P < 0.05$) for the 56N and 168N fertilization treatments, and least ($P < 0.05$) for the 112N treatment. Degradable intake protein percentage of CP was greatest ($P < 0.05$) on Jan 21, intermediate on Nov 25 and Jan 7, and least ($P < 0.05$) on Nov 11. The DIP fraction had a net decrease of 2% in Yr 1 and an increase of 6% in Yr 2 with progression of the stockpile season. Despite changes in the DIP fraction, values remained relatively high throughout the stockpile season. There was a N fertilization x sampling date interaction such that there were no differences ($P > 0.05$) among N fertilization treatments on Jan 7; however, the 56N treatment was greater ($P < 0.05$) than the 112N treatment on Nov 11, the 56N and 168N treatments were greater ($P < 0.05$) than the 112 treatment on Nov 25, and the 56N and 112N treatments were greater ($P < 0.05$) than the 168N treatment on Jan 21.

Rogers et al. (1996) conducted a study with a mixture of Tifton 44, Guymon, and common bermudagrass cultivars that were fertilized with either 448 or 896 kg N/ha. They reported that the soluble protein fraction (% of CP) decreased over the actively growing season (May to Aug). They reported an average soluble protein fraction across the growing season of 34.8 (448 kg N/ha) and 38.2% (896 kg N/ha), which may be compared with the mean degradable protein fraction in stockpiled Tifton 85 of 94.5 (Yr 1) and 92.45 % (Yr 2) in the current study. In addition, the in situ-derived pool size of the

B fraction of bermudagrass hay reported by Mathis et al. (2001) averaged 40% of total N across four locations in eastern Kansas. Johnson et al. (1999) observed a quadratic response of the B₁ (fast rate of degradation) and B₂ (medium rate of degradation; comparable to ruminal rate of passage) protein fractions across harvest dates (summer to early fall). A linear decrease was reported for the B₃ (slow rate of degradation) fraction. The degradable intake protein fraction found in our study decreased linearly over the growing season. When comparing this effect with those reported by Johnson et al. (1996), it could be speculated that the slowly degraded (B₃) fraction is the most prevalent in stockpiled Tifton 85.

Rogers et al. (1996) observed an increase in the soluble protein fraction of bermudagrass with increasing level of N fertilization. Johnson et al. (1999) reported that all subsets of the potentially degradable true protein fraction (B) of Tifton 85 bermudagrass increased linearly with increasing N fertilization levels of 0, 39, 78, 118, and 157 kg N/ha during the summer growing season. Similarly, Vendramini et al. (2007) observed an increase in the potentially degradable fraction of Tifton 85 as N fertilization was increased at levels of 0, 50 and 160 kg N/ha. Our findings do not indicate a notable difference in the degradable protein fraction among N fertilization levels, and the DIP fraction as a percentage of forage total CP remained relatively high over all sampling periods.

In Yr 1, concentration of DIP as a percentage of forage DM (Table 9) tended ($P = 0.09$) to be greater for the 56N and 112N than 168N treatment. Concentration of DIP was greatest ($P < 0.05$) on Oct 24, intermediate on Nov 28 and Dec 13, and least ($P < 0.05$) on Jan 16. Concentration of DIP decreased 38% between Oct 24 and Dec 13,

then another 17% by Jan 16. This pattern was also observed in Yr 2; i.e., a 38% decrease in concentration of DIP between Nov 11 and Jan 7, followed by a large decrease of 23% by Jan 21. Concentration of DIP was greater ($P < 0.05$) on Nov 11 than on Nov 25, Jan 7, and Jan 21, and tended ($P = 0.07$) to be greater for the 168N than the 56N and 112N treatments. The concentration of degradable intake protein throughout the grazing season remained at a level sufficient for maintaining fibrolytic activity of ruminal microorganisms (Van Soest, 1994) and microbial CP synthesis (NRC, 1996).

Johnson et al. (1999) applied 5 N fertilization levels (0, 39, 79, 118, and 157 kg N/ha) to summer-grown Tifton 85 and observed an increase in CP concentration as N level increased. In a similar study was conducted by Webster et al. (1965), CP concentration in 'Midland' bermudagrass increased with increasing N fertilization across multiple harvest dates between May and Oct. This pattern of increased protein concentration with increasing N fertilization was also reported by McNamee (2014) for stockpiled Tifton 85, and was also observed for degradable intake protein concentration of stockpiled Tifton 85 in the present study.

Table 8. Degradable intake protein fraction (% of CP) from stockpiled Tifton 85 bermudagrass in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	95.7	96.0	95.6	95.8 ^a
	Nov 28	93.0	92.1	93.1	92.7 ^b
	Dec 13	95.6	95.4	95.5	95.5 ^a
	Jan 16	93.5	94.2	93.8	93.8 ^c
	Mean	94.8	94.4	94.5	
2	Nov 11	91.4 ^d	88.7 ^e	90.1 ^{de}	90.1 ^x
	Nov 25	92.6 ^d	87.4 ^e	94.5 ^d	91.5 ^y
	Jan 7	91.2	93.0	92.9	92.4 ^y
	Jan 21	96.5 ^d	96.7 ^d	94.2 ^e	95.8 ^z
	Mean	92.9 ^g	91.5 ^h	92.9 ^g	

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.32).

^{d,e,f} Within a row, means without a common superscript differ ($P < 0.05$; SEM = 1.1).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.44).

^{g,h} Within a row, means without a common superscript differ ($P < 0.05$; SEM = 0.38).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

Table 9. Concentration of degradable intake protein fraction (% DM basis) in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2

Year	Sampling Date	Treatment ¹			Mean
		56N	112N	168N	
1	Oct 24	15.6	15.9	17.8	16.5 ^a
	Nov 28	9.3	9.9	11.5	10.2 ^b
	Dec 13	10.4	9.6	10.9	10.3 ^b
	Jan 16	7.3	8.6	9.7	8.5 ^c
	Mean	10.7	11.0	12.5	
2	Nov 11	16.2	14.2	19.2	16.5 ^x
	Nov 25	10.6	9.3	14.6	11.5 ^y
	Jan 7	8.4	10.5	10.6	9.8 ^y
	Jan 21	7.9	9.9	10.5	9.4 ^y
	Mean	10.8	11.0	13.7	

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 0.64).

^{x,y,z} Within a column, means without a common superscript differ ($P < 0.05$; SEM = 1.0).

¹ 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

SUMMARY AND CONCLUSIONS

Results of this study suggest that fiber and protein degradation characteristics of stockpiled Tifton 85 bermudagrass are influenced by temporal changes during the stockpiling period and by level of N fertilization. Fiber and protein degradation characteristics were more greatly affected by temporal changes over the stockpile season than by N fertilization. Increased lignification was observed over the fall/winter stockpile season as the result of weathering, similar to that associated with maturation of actively growing forage during the summer season. Both PED and rate of NDF digestion were negatively correlated with lignification, whereas lag time was positively correlated. Kinetic parameters varied between Yr 1 and Yr 2, indicating an effect of meteorological conditions; mean monthly air temperature was correlated with all parameters. Protein degradation characteristics were influenced by N fertilization and sampling time during the grazing season, but remained at a level sufficient for maintenance of fibrolytic activity of ruminal microorganisms and microbial CP synthesis.

By synchronizing fiber and protein degradation characteristics of forages with those of supplemental energy and/or protein feedstuffs, more accurate supplementation strategies may be developed. Digestion kinetic parameters are related to digestibility and intake and can be used to modify supplementation strategies throughout the growing season as nutritive quality declines. Protein degradation characteristics are becoming increasingly important as metabolizable protein, DIP and UIP replace CP in expressing animal requirements and use by nutritionists in diet formulation. Formulations should meet both rumen degradable protein, equal to bacterial crude protein synthesis (NRC, 1996), and undegradable intake protein requirements to

maximize animal performance. Supplement formulation is typically determined a single time for any given growing season, especially for grazed pasture, without taking changing forage quality or animal needs into account. Changes in the characteristics of fiber and protein degradation can be utilized to formulate more accurate need- and time-dependent supplementation strategies.

A supplementation strategy must take into account the decreasing concentrations of potentially digestible NDF and degradable intake protein with increasing animal needs over the stockpile season. At the beginning of the stockpile season protein, supplementation should be based on inclusion of an undegradable protein source because Tifton 85 has high PED of NDF and can provide DIP to support ruminal BCP synthesis at levels greater than the required 13 g/100 g DOM (NRC, 1996). As time into the stockpiling/grazing season progresses, concentrations of degradable intake protein and total CP decrease and should be supplemented with a source of both undegradable and degradable intake protein. Furthermore, energy supplementation should be utilized to increase utilization of the rumen degradable protein. Particularly when air temperature is decreased, or a killing frost occurs during the stockpile season, a source of highly digestible NDF should be included at increased rates over the season. Alternatively, under these conditions it might be beneficial to supplement with limited quantity of a readily digestible energy source to better capture available protein when lag times are greatly increased. Developing a more specific supplementation program using these approaches may provide a more targeted approach for producers to achieve production goals in forage-based management systems.

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APPENDIX I

Concentration of NDF (% DM basis) in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2¹

Year	Sampling Date	Treatment ²			Mean
		56N	112N	156N	
1	Oct 24	64.8	65.0	62.0	63.9
	Nov 28	70.5	67.0	67.3	68.3
	Dec 13	70.6	68.2	66.3	68.3
	Jan 16	69.5	69.8	68.0	69.1
	Mean	68.9	67.5	65.9	
2	Nov 11	65.7	64.5	63.8	64.7
	Nov 25	62.4	61.1	60.7	61.4
	Jan 7	67.8	69.1	66.5	67.8
	Jan 21	68.0	72.7	65.3	68.6
	Mean	66.0	66.8	64.1	

¹ Adapted from McNamee (2014).

² 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

APPENDIX II

Concentration of ADL (% DM basis) in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2¹

Year	Sampling Date	Treatment ²			Mean
		56N	112N	156N	
1	Oct 24	1.6	1.4	1.3	1.4
	Nov 28	1.8	1.9	1.9	1.9
	Dec 13	3.1	3.0	3.0	3.0
	Jan 16	4.5	4.9	4.9	4.8
	Mean	2.8	2.8	2.8	
2	Nov 11	1.8	1.7	1.9	1.8
	Nov 25	3.7	3.4	3.9	3.7
	Jan 7	4.2	4.3	5.1	4.5
	Jan 21	5.7	5.6	6.7	6.0
	Mean	3.9	3.8	4.4	

¹ Adapted from McNamee (2014).

² 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

APPENDIX III

Percentage of IVDMD in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2¹

Year	Sampling Date	Treatment ²			Mean
		56N	112N	156N	
1	Oct 24	69.2	74.4	74.3	72.6
	Nov 28	61.4	63.9	65.7	63.7
	Dec 13	56.8	58.3	64.4	59.8
	Jan 16	53.7	52.0	51.0	52.2
	Mean	60.3	62.2	63.9	
2	Nov 11	71.8	73.5	74.5	73.3
	Nov 25	62.0	63.0	69.6	64.9
	Jan 7	49.5	51.5	51.0	50.7
	Jan 21	51.0	52.5	55.4	53.0
	Mean	58.6	60.1	62.6	

¹ Adapted from McNamee (2014).

² 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.

APPENDIX IV

Concentration of CP (% DM basis) in stockpiled Tifton 85 bermudagrass receiving different rates of N fertilization in Yr 1 and Yr 2¹

Year	Sampling Date	Treatment ²			Mean
		56N	112N	156N	
1	Oct 24	19.1	16.1	19.0	18.1
	Nov 28	10.1	11.2	12.3	11.2
	Dec 13	12.2	10.2	13.2	11.9
	Jan 16	10.0	9.7	12.0	10.6
	Mean	12.9	11.8	14.1	
2	Nov 11	17.7	16.0	22.3	18.7
	Nov 25	11.5	11.0	16.1	12.9
	Jan 7	9.1	11.2	11.4	10.6
	Jan 21	9.0	10.1	11.4	10.2
	Mean	11.8	12.1	15.3	

¹ Adapted from McNamee (2014).

² 56N = 56 kg N/ha; 112N = 112 kg N/ha; 168N = 168 kg N/ha.