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Digestibility of different multi-species native warm-season grass mixtures grown in varied harvest regimen

By

Janet Moromoke Ogunlade

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Animal Nutrition
in the Department of Animal and Dairy Science

Mississippi State, Mississippi

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Janet Moromoke Ogunlade

2013

Digestibility of different multi-species native warm-season grass mixtures grown in varied harvest regimen

By

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Study was conducted to evaluate *in vitro* digestibility of native warm-season grasses. Three grasses were used: big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* Nash), and indiangrass (*Sorghastrum nutans* Nash). There were no differences in NDF, ADF, FAT and OM of the three grass species. However, DM, hemicellulose and CP were slightly different in the three grass species. Also, the frequency nested in cutting effects was determined. *In vitro* dry matter disappearance of big bluestem, little bluestem and indiangrass was evaluated to determine rate of disappearance. The 100 % indiangrass revealed the greatest rate of disappearance for IVDMD and 100 % little bluestem grass the least, respectively. However, that of other proportion mixtures of treatments and 100 % big bluestem grass were in between. There were no differences in *in vitro* neutral detergent fiber disappearances among treatments.

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CHAPTER I

INTRODUCTION

The beef industry is greatly segmented. It can be subdivided into segments that represent distinct developmental phases including growth, production and reproduction. Production and efficient utilization of nutritive grass throughout the grazing season was critical for cost-efficient beef production (Owens et al., 2008a). Therefore, goals of livestock producers should be to increase overall efficiency of beef production, increase market meat availability, and maximize profits.

Native warm-season grasses serve as important habitat for wildlife animals such as birds (songbirds (*Eopsaltria australis*), bobwhite quail (*Colinus virginianus*), white-tailed deer (*Odocoileus virginianus*) and bison (*Bos bison*), and also as a good forage source for livestock production (USDA, 2002; USDA, 2011). Native warm-season grasses can be used for both, pasture and hay (Mitchell et al., 2005; USDA, 2002; USDA, 2011). An additional advantage presented by the use of warm-season grasses is their greater yield of DM even on soils of lesser fertility (Vona et al., 1984). A mix of indiangrass (*Sorghastrum nutans* Nash) and other native warm-season grasses and forbs provide nest, brood and escape cover for bobwhite quail (USDA, 2011). Currently, in terms of land availability, maintaining monocultures of improved forages in pasture may be costly in most parts of the world.

Because of the reduction in land availability for forage production, many native warm season grasses have been seeded (Forbes and Coleman, 1993). Doll et al. (2009) stated that restoration management may have positive effects on pasture health, such as better root production. Warm-season grasses may serve as the main source of forage for livestock during summer especially in the northern U.S. (Burns and Fisher, 2010). Native warm-season grasses can serve as the primary feed source for cattle during summer months, whereas hay is often a primary feed source during winter months. However, cattle grazing mature native grasses during the flowering season, may encounter many nutrient deficiencies (Bodine and Purvis, 2003). In order to improve animal production, there must be a constant and reliable forage supply (Belesky, 2006), which must be harvested before nutritional quality is compromised.

Some livestock farmers believe increasing the regrowth interval may prolong the availability of planted forages. However, this practice results with a decline of nutritive value of the forage (Binnie et al., 1996). Increasing regrowth interval did not lead to greater productivity of warm-season grasses (Belesky, 2006). Additionally, Gonzalez-Valenzuela, et al. (2008) reported that the longer the harvest intervals, the greater the NDF content of the plant.

Even though mono-specific forage feeding to livestock is often practiced, livestock performance information concerning big bluestem *Andropogon gerardii* Vitman monoculture is limited (Mitchell et al., 2005). Furthermore, the effect feeding different proportion mixtures of the native warm-season grass species has on animal performance and productivity is not fully understood.

Therefore, the objective of this study is to evaluate *in vitro* dry matter disappearance of different proportions mixtures of three native warm-season grasses: big bluestem, little bluestem (*Schizachyrium scoparium* Nash), and indiangass grown on two different soil types and harvested at varied regrowth regimens.

CHAPTER II

LITERATURE REVIEW

Native Warm-Season Grasses

Warm-season grasses are good forage resources for livestock especially during the summer period. Ranchers are continually striving to improve returns from livestock that are grazing native warm-season rangeland (Cline et al., 2010). From a conventional agronomic perspective, variability in grassland production and quality among different years and seasons is undesirable (Doll et al., 2009). Warm-season grasses can be classified into two basic categories: annual warm-season grass and perennial warm-season grass. An example of annual warm-season grass is Pearl millet (*Pennisetum glacum*), Sudangrass (*Sorghum bicolor*) and Foxtail millet (*Setaria italica*). Examples of perennial warm-season grasses are bermudagrass (*Cynodon dactylon* (L.) Pers.), bahiagrass (*Paspalum notatum* Flueggé), switchgrass (*Panicum virgatum*), Eastern gamagrass (*Tripsacum dactyloides*), indiangrass, big bluestem grass, and little bluestem grass.

Furthermore, big bluestem is a good quality forage species for livestock, with CP content between 16 and 18% during May through August, but below 6% in September and October (USDA, 2002). Although, Corson et al. (2007) reported that plant CP content varied according to plant nitrogen uptake. Big bluestem and indiangrass are native warm-season grasses commonly used for pasture in the western U.S. (Mulkey et

al., 2008). Warm-season grass such as bermudagrass is commonly used in southeastern U.S. in the form of pasture or hay for livestock (USDA, 2011). Switchgrass, big bluestem and indiangrass, are the dominant warm-season grasses native to the tall grass and Southeastern prairies of North America (Mulkey et al., 2008). Mitchell et al. (2005) reported that big bluestem pastures provide abundant quality forage during late spring and summer. Indiangrass is a major component of the tall grass vegetation which dominates the prairies of the central and eastern United States (USDA, 2011). Switchgrass, big bluestem, and indiangrass are native warm-season grasses commonly used for pasture, hay, and conservation (Mulkey et al., 2008). These plants can supply substantial amounts of simple carbon compounds to the soil through their root system (Van der krift et al., 2001). The United States Department of Agriculture (USDA, 2011) reported that indiangrass can be grown as a monoculture, or in mixtures with other native grasses to provide forage in the form of rangeland, pastureland or hay for livestock. However, maintaining of monospecific stands of sown forages in pasture is difficult in the Appalachian region (Belesky, 2006). Warm-season grasses assist by maintaining fewer weeds in pastures, possibly because they are deep-rooted grass (Mulkey et al., 2008). Caucasian bluestem (*Bothriochloa caucasica*) can form dense swards that offer few sites for volunteer species encroachment (Belesky, 2006). Yao et al. (2011) reported that bermudagrass is a relatively deep-rooted grass. Little bluestem is a moderate quality forage species compared to other native warm-season grasses that is readily grazed by livestock and widely distributed in North America (USDA, 2002). Corson et al. (2007) stated that warm-season grasses had a faster photosynthetic rate at warmer temperatures and light intensities, and nitrogen and water use was more efficient. Warm-season grasses

have increased microbial biomass activity in late spring due to increased temperatures and active grass growth (Yao et al., 2011). Mulkey et al. (2008) reported from an economic stand point that, in order for warm-season grasses to maintain their stand vigor, they should be managed properly.

In many parts of northern and western Europe, grass is the principal forage for beef production (Owens et al., 2008b). Ruminant production is based on forage which strongly dependent on forage maturity (Lima et al., 2008; Kozloski et al., 2005). However, it has been clearly shown that forage varies in nutrient composition with regard to season of the year and stage of maturity (Sun et al., 2010). Warm-season grasses have marginal amount of phosphorus, sulphur, zinc, and adequate amounts of magnesium (Vona et al., 1984). Switchgrass has been identified as a greater potential biomass energy crop (Mulkey et al., 2008).

Composition and Nutritive Value of Warm-Season Grasses

Many factors such as temperature, light intensity, water availability, latitude, maturity and harvest and storage methods affect forage production (Traxler et al., 1998). Madakadze et al. (1998) reported that native warm-season grasses, especially big bluestem and indiangrass, are characterized by needing warmer optimal temperature for photosynthesis, which helps survivability during the months of July and August. Warm-season grasses use the C4 photosynthetic pathway, whereas cool-season grasses use the C3 photosynthetic pathway. Redfearn et al. (1995) stated that C3 and C4 grass species differ from one another as a result of differences in the anatomical arrangement of their plant tissues. They further reported that this makes C3 and C4 grasses have variations in their protein fraction present in their mesophyll and bundle sheath cells. Big bluestem and

indiangrasses are C4 grasses which have greater nitrogen utilization capacity and water use efficiency compared to C3 grass species (Kakani and Reddy, 2007; Mulkey et al., 2008; Woodis and Jackson, 2008; Heggenstaller et al., 2009). Kakani and Reddy (2007) emphasized that C4 pathway grass species have capacity for greater DM accumulation due to their greater photosynthetic rate and also greater utilization of light, as well as longer growth duration. Nevertheless, more efficient use of this forage for animal production requires a better knowledge of plant nutrients and how they affect digestion, metabolism and efficiency of feed utilization (Kozloski et al., 2005). The animal utilization of better quality forage throughout the growing season has a great influence on cost efficiency of beef production in livestock systems (Owens, 2008a). Nitrogen, especially degradable intake protein (DIP), can be deficient in native range forage consumed by cattle later in the summer grazing season (Cline et al., 2010).

Ruminal degradability of forage proteins is variable among forage species (Mitchell et al., 1997). Cell walls are more or less digested by ruminants depending on plant tissue, whereas cell contents are theoretically entirely digested (Giger-Reverdin, 1995). Particle size within the digestible cell wall is relatively uniform in either early-or late-cut forage, but indigestible cell walls may increase markedly, contributing to gut fill and intake limitation (Robles et al., 1980). Lignification is the main factor influencing the extent of fiber degradation in the ruminant alimentary tract (Lima et al., 2008). Dietary protein consumed by ruminants is either degraded in the rumen by microorganisms or remains undigested and passed to the small intestine (Mitchell et al., 1997). Varga and Kolver (1997) reported that intake, dietary interactions, feeding strategies, and feed additives will, to some degree, influence microbial growth and subsequent fiber

digestion. The C4 grass species, especially big bluestem and switchgrass, have the ability to retain the undegraded protein fractions (escaped protein) in their bundle sheath cells for longer period in the rumen as compared to their degraded protein (Redfearn et al., 1995). Foster et al. (2010) stated that photosynthetic cells in the leaf tissues of C4 grasses are arranged in such a way that it collectively leads to increases in NDF concentration in the rumen; this was because the leaves anatomical structure are being protected from microbial degradation.

Regrowth Interval Influence

Much of the information available on the effects of length of regrowth interval and season on herbage yield and quality comes from swards which have been cut at a range of regular intervals throughout the entire season (Binnie et al., 1997). Omonode and Vyn (2006) emphasized that restoration of grasslands is believed to be an in-expensive, efficient and environmentally-friendly method to reduce the rate of increase of atmospheric carbon dioxide concentrations, stop soil erosion, increase soil nutrient and water retention and improve soil environmental quality. The nutritive value of forage generally changes according to the growth stage (Ashikaga et al., 2010). It was observed by Gonzalez-Valenzuela et al. (2008) that as grass matured, CP concentration decreased. But Doll et al. (2009) reported that farmers can influence forage production by choice of plant species, fertility amendments and defoliation management (mowing).

Forage production is the largest land use in the cattle producing region of south Florida (Newman etV al., 2009a). For optimum forage production, knowledge of factors that affect yield and nutritive value is very important (Gonzalez-Valenzuela et al., 2008). Nutritive value of forage can be affected by multiple factors such as environment (i.e.,

herbivores, insects, soil nutrients and erosion) and climate (i.e., precipitation, temperature, light and humidity). Belesky (2006) reported that non-living and living factors influence regrowth of plants. During the reproductive phase, air temperature influenced, to some degree, dormancy loss in the resulting seeds (Hoyle et al., 2008). Yao et al. (2011) stated that temperature and moisture are important environmental factors influencing soil microbial growth and activity. Doll et al. (2009) reported that forage production and quality were very responsive to fluctuations in weather conditions. As grass matures, the nutritive quality generally declines due to increased lignification (Owens, et al., 2008b). Robles et al. (1980) reported that increasing maturity of forage results in slower rates of digestion by animals, particularly if lignin contents of the cell wall increased. As plant maturity increased, cell wall proportion and lignification increased (Lima et al., 2008). Lignin is quantitatively the third most abundant constituent of the cell wall. With aging, the lignin content of forage may increase to 12 % of DM (Giger-Riverdin, 1995). Biligetu and Coleman (2010) stated that understanding of compensatory growth traits of plant species is very important, as this assists forage breeders to select superior lines with improved regrowth potential.

Many external factors can influence forage productivity, which can latter impact cattle performance and productivity (Stuedemann and Franzluebbers, 2007). Warm climate affects the timing and rate of germination of plant. Also, the maturity stage of a plant affects ruminal degradability (Mitchell et al., 1997). It was noticed by Owens et al. (2008a) that increasing the regrowth interval reduced ruminal ammonia concentration and amount of undigested feed nitrogen leaving the rumen and so leads to reduced nitrogen being excreted by the animal.

Climatic conditions in the Southeastern United States permit the growth of many different forage crops (Vona et al., 1984). Air temperature is generally optimal for warm-season grasses growth during July and August because there is a greater amount of carbon supply to the soil through the plant's roots (Yao et al., 2011). Indiangrass and big bluestem regrowth was from stem bases (Madakadze et al., 1998).

Estimating cell wall content of feed for ruminants is important because it is greatly related to its organic matter digestibility and thus its nutritive value (Giger-Reverdin, 1995). Increasing maturity of forage is associated with depressed forage intake (Robles et al., 1980). Belesky (2006) reported that long regrowth intervals do not necessarily lead to increase in grass nutrients. Increased regrowth interval leads to decline of nutritive values of forage because there was increased lignification (Owens et al., 2008b). It was reported by Mitchell et al., (1997) that as plant mature, cell wall sizes increases, and therefore a reduction in forage quality and CP occurs. Gozalez-Valenzuela et al. (2008) reported that as harvest interval increased, the NDF concentration increased. As plants matured there was an increase in lignification, and this led to a decreased digestibility by ruminant animals (Mitchell et al., 1997). However, Lima et al. (2008) stated that lignin-fraction has no inhibitory effects on ruminal degradability of the forage soluble components. Both nitrogen concentration and *in vitro* DM disappearance of bermudagrass have their greater concentration in the early parts of the grazing season which then decline as herbage matures (Starks et al., 2008). Although, a prolonged regrowth interval can reduce forage nutrient composition but, Owens (2008b) stated that regrowth interval of not greater than 24 to 26 d was good for spring-grazing beef cattle.

Also, it was reported by Gozalez-Valenzuela et al. (2008) that harvest interval of 40 to 60 days led to increased DM yields. Owens et al. (2008b) reported that under average grass growing conditions in Ireland, regrowth intervals of grass not greater than 24 to 26 d are recommended for spring grazing of beef cattle. Regrowth interval, harvest date and sown species interacted to influence DM distribution among grass sward components (Belesky, 2006). However, Binnie et al. (1997) reported that there was no advantage to be gained by lengthening the regrowth interval beyond 6 wk. Also, it was noted by Obour et al. (2009) that extended growing season and more or less frequent harvest for hay or grazing of forage grass can potentially recycle soil nutrients. The relative contribution of each seeded species total swards yields varied as a function of time during the grazing season, reflecting different requirements for growth (Belesky, 2006). In the field, individual plants may perform differently in response to topographical conditions because they grow in different geographical locations (Hoyle et al., 2008). Forage production and quality were very responsive to fluctuation in weather conditions (Doll et al., 2009). Warm-season grasses grow best when temperature are between 27 and 35^{o}C, but have limited growth during winter months (Yao et al., 2011).

Soil Type

Within species of native warm-season grasses there was variation in rate of growth and date of maturity (Madakadze et al., 1998). Plants can supply substantial amount of simple carbon compounds to the soil through their root (Vanderkrift et al., 2001). It was noticed by Uddin et al. (2011) that soil type directly affected stages of plant growth and development. Plant species differ widely in the quality and quantity of litter they produced (Vanderkrift et al., 2001). Different soils have different nutrients as a

result of parent material and soil microbial activities together with factors such as temperature and precipitation. Microbial populations change as a result of variations in environmental conditions between summer and early autumn (Yao et al., 2011) which may affect the plant production. However, it was reported by Obour et al. (2009) that application of manure to perennial pastures in combination with inorganic nitrogen during the crop growing season was beneficial to grass in that it helped to supply more nitrogen and phosphorus at the rate and ratio that helped it to increase its nutrient uptake ability.

Grasslands cover approximately 40 % of the land surface (Ann-Karhrin et al., 2011). There are different types of soil for forage production which include loam soil, sand soil and clay. Loams are classified as sandy, clay, or silt loams. This soil type is a rich soil composed of sand, silt, clay, and organic matter in evenly mixed particles of various sizes. It has less clay particles. It is more fertile than sandy soils; loam is not compacted and tenacious like clay soils. Its porosity allows more moisture retention and air circulation which greatly contributed to plant survivability. The majority of soil nitrogen is proteinaceous in nature and often associated with mineral (Nannipieri and Eldor, 2009). The mineralogical composition of loam is varied: the sandier loam has more quartz content such as s fine sandy loam soil, whereas the more clay type contains clay minerals, such as kaolinite, illite, and montmorillonite. The effect of fertilization alone was important for bacterial communities in clay loam soil (Ann-Kathrin et al., 2011). Sometimes there can be a mixture of silt clay loam. This clay type soil contains an element iron; iron was positively correlated with percent silt, but negatively correlated with soil pH (Ownely et al., 2003). Loam may be rich in organic substances, and in arid

regions, it may be rich in water-soluble salts. Most soils of agricultural importance are some type of loam.

Clay soil is a soil type that is composed of very fine particles, usually silicates of aluminum and (or) iron and magnesium. Clay soil impedes the flow of water because it absorbs water slowly and then retains it for a long time. Soil loss, and its associated impacts, is one of the most important and probably the least well known of today's environmental problems (Ekwue and Samaroo, 2009). Wet clay soil is heavy and sticky, and tends to swell from the added moisture. When dry, clay soil shrinks and settles. The top layer can bake into a hard, concrete-like crust which cracks. Some plants have difficulty growing in clay soil because their seedlings or roots are unable to penetrate through hard, dry soil, or can be waterlogged in wet soil. Adding organic material to clay soil is an effective method of improving growing conditions (Messiha et al., 2009).

Sandy soils are soil types that have less water-holding capacity. They are easily susceptible to soil erosion and leaching. They have less nutrient holding capacity (Messiah et al., 2009). Many sandy soils have minimal phosphorus retention capacity, making them susceptible to phosphorus loss (Newman et al., 2009b). When seeds are planted in a deeper sand soil, they have slow germination rate (Zul et al., 2006). However, seed germination rate varies with soil moisture (Hoyle et al., 2008). Plant production may be affected, as Zul et al. (2006) reported that moisture content in sand land is irregular and unpredictable. Water, tillage and wind erosion contribute significantly to the redistribution of soil and soil organic carbon. In relation to determining the influence of soil types on forage productivity and the soil nutrient

availability for native warm-season grass survivability under varied re-growth intervals, soil elements such as carbon cannot be overlooked.

Dormancy is the term to describe the time of biological rest or inactivity characterized by cessation of growth or development. Plant carbon supply was reduced due to warm-season grass dormancy (Yao et al., 2011). Management of native warm-season grasses in combination with their deep root production may lead to increased carbon sequestration (Woodis and Jackson, 2008; Doll et al., 2009). Different productivity and oxidation rates of soil organic carbon of eroded versus deposited soil also contributed to soil organic carbon spatial patterns (Ritchie et al., 2007). Other factors that can affect germination include erosion, temperature, and light. When erosion and leaching occur, it exposed the plant root to air and affected development and productivity. Vona et al. (1984) made it known that warm-season grasses can grow and survive on phosphorus-deficient soil. The soil environment is the key factor in determining the overall microbial community structure (Ann-Kathrin et al., 2011). Most important environmental factors influencing soil microbial growth and activities are temperature and moisture (Yao et al., 2011). Nitrogen plays a significant role in crop production (Belanger and Gastal, 1999). Although, soil microbes are strong competitors with plants for available nitrogen (Yao et al., 2011), Owens et al. (2008a) reported that livestock production, especially ruminant has been identified as a major source through which nitrogen is lost in the grazing environment.

Another crucial soil process regulating the amount of inorganic nitrogen for plant uptake is microbial biomass turnover (Yao et al., 2011). Microbes are key players in biogeochemical cycling, such as organic matter decomposition and turnover of carbon

fixation of atmospheric nitrogen and nitrification pathway (Ann-Kathrin et al., 2011). It was noticed by Yao et al. (2011) that there was less availability of nitrogen during late summer and early autumn, which might have been as a result of denitrification or irrigation processes. Management factors such as land use change through agricultural intensification and thus have strong effects on soil local microbial communities of grassland (Ann-Kathrin et al., 2011). Greater microbial activities increased nitrogen retention by warm-season grasses (Yao et al., 2011). Forage quality and productivity has been improved with the use of inorganic and organic fertilizers (Stuedemann and Franzluebbers, 2007).

Soil bacterial activities overwhelmed fungi during the summer months by using the easily degradable carbon provided by grasses (Yao et al., 2011). This resulted with shifts of dormancy within existing plant ecosystems (Milbau et al., 2009). However, increased nitrogen fertilizer led to increased microbial activities and also to increased available nitrogen which later increased plant growth (Yao et al., 2011). Newman et al. (2009b) stated that increasing the nitrogen application rate above the recommended rate resulted with increased DM yield. However, nitrogen in the soil-plant system exist in different oxidation forms as soluble and gaseous compounds and as both organic and inorganic compounds that may be associated with soil minerals (Nannipieri and Eldor, 2009).

The use of nitrogen fertilizer on forage production has been restricted by environmental schemes and legislation in some parts of the country (Owens et al., 2008a). Different plant species have different optimal temperature for reducing dormancy (Milbau et al., 2009). Microbial growth increased at optimal temperature and moisture

(Yao, et al., 2011). In terms of soil nutrients, switchgrass and big bluestem were competitors. Mulkey et al. (2008) reported this competition occurs only when they are both planted at the same location. These researchers stated that big bluestem is more competitive than switchgrass for soil nutrients and water. Limited amounts of rainfall do not seem to be important for improving soil moisture for favorable plant species growth. Therefore, good and adaptability soil type needs to be considered for establishment of native warm-season grasses because most plant species rely on moisture from the soil than rainfall.

In Vitro Dry Matter Disappearance

In vitro DM disappearance is determined by measuring the DM disappearance of a feed sample after it has been subjected to digestion by microorganisms present in rumen fluid. *In vitro* dry matter disappearance is a direct measure of digestibility, whereas ADF concentration is used to indirectly estimate digestibility (Starks et al., 2008). *In vitro* DM disappearance has been extensively used to evaluate the nutritional value of ruminants feeds (Mabjeesh et al., 2000). Disapearance rates among grass species is a function of their nutritive values, morphology and physiology (Foster et al., 2011). Ruminal degradability of forage proteins is variable among forage species (Mitchell et al., 1997). Some warm-season grasses such as limpograss are vegetatively propagated grasses and so have large and thick stems (Arthington and Brown, 2005) which decrease their *in vitro* dry matter disappearances when mixed with other warm-season grasses that are not vegetatively propagated. Forage and grain combination diets have *in vitro* dry matter disappearance which differ from forage diets alone because of differences in the structural and non-structural carbohydrates supplies. This has effects on microbial protein

synthesis. Firkin et al. (2007) reported that carbohydrate supply profoundly influenced the amounts of ruminal NH_3-N assimilated into microbial protein, and this led to suppression of OM digestibility. *In vitro* dry matter disappearance of herbage can help livestock managers to make timely decisions for adjusting stocking rate and managing pastures during the grazing season (Starks et al., 2007).

CHAPTER III

MATERIALS AND METHODS

Site Management

The research was conducted at two soil locations of the Mississippi State University Research Experiment Station, North Farm, Starkville, Mississippi. Site 1 was a Marietta fine sandy loam soil with no slope. Site 2 was a Sumter silty clay loam soil with slight slope. The soil pH was 6.9 for soil 1 and 7.8 for soil 2, respectively. Each soil location was burned in October, 2011. The three native warm-season grasses big bluestem, little bluestem and indiangrass were planted in spring. Fertilizer in the form of ammonium nitrate was applied by hand broadcasting at the rate of 7.71 kg of N/ha. Each plot received its nitrogen application in May 2012.

Forage Sample Collection and Treatments

Samples for each grass species were hand clipped at a height of 17.78 cm. Collection of 2 replicates per grass sample was made. There were four cutting times with four different regrowth intervals. The regrowth intervals were: every 28 d, every 56 d, at 84 d and at 112 d. The length of the project was 112 d. The first cutting was collected on May 1, 2012, which marked the end of the first 28 d period. On May 29, 2012 the second cutting was conducted. Two harvests were collected; one which marked the end of the second 28 d cutting and one which marked the end of the first every 56 d first cutting. On

June 24, 2012 the third cutting was conducted. Two harvests were at this time, one which marked the end of the third 28 d cutting and one which marked the end of the 84 d cutting. On July 26, 2012 the fourth cutting was conducted. Three harvests were done, one for the end of the fourth every 28 d cuts, another end of the second every 56 d cutting and the third which is 112 d cutting. Over all, four total cuttings were conducted, making 4 total cuttings for the every 28 d regrowth interval, 2 total cuttings for every 56 d regrowth intervals, 1 for 84 d and 1 for 112 d. Samples were weighed, dried at 65^{o}C for 48 hrs in a forced air oven, acclimatized to room temperature and humidity, and reweighed to determine DM. Dried samples were ground through a Wiley mill (Thomas Scientific, Philadephia) to pass a 2-mm pore size screen. The three native warm-season grasses collected were then combined to make 13 treatments of different proportions of big bluestem, little bluestem and indiangrass (Table 1).

Laboratory Analysis

Duplicate samples of approximately 0.25 g of each of the 13 treatments were weighed into ANKOM bags having an average pore size of 50 ± 15 µm. and then were heat sealed. Samples were incubated in a digestion vessel containing 25 bags per vessel (Mabjeesh et al., 2000). All laboratory containers necessary for ruminal fluid processing were filled with hot water 30 min prior to collection. Approximately 7,000 mL of ruminal fluid was collected from a ruminally fistulated steer which was fed hay, and was placed in pre-warmed insulated containers and immediately transported to the laboratory. An aluminum blender pre-warmed with warm water was flushed with CO_2 and used for 2 s to blend ruminal fluid after which ruminal fluid was strained through four layers of cheese cloth into a warmed 1L beaker that had been flushed with CO_2 for approximately 5 s

Ruminal fluid (375 mL) was then added to each digestion vessel. Each fluid jar was filled with CO_2, capped and then placed and maintained inside an ANKOM Daisy incubator at 33^{o}C for 48 hrs (Mabjeesh et al., 2000). Bags were then taken out of digestion vessels and rinsed thoroughly with tap water until the rinse was clear (Mabjeesh, et al., 2000). Bags were then air dried for 3 h and then placed in a forced-air oven at 110°C for 24 hrs to determine the residual DM weights (Mabjeesh et al., 2000). Samples were next placed in NDF solution to determine *in vitro* NDF disappearances AOAC (2003). Individual grass samples of big bluestem, little bluestem and indiangrass (Table 1) were analyzed for NDF, ADF, and CP , fat, dry matter was determined by putting it inside the forced-air oven at 110°C for 24 hrs , then removing it and place inside a desiccator to cool down prior to weighing. It was then put inside a muffle furnace at 550^{o}C for 8 hrs and then removed and re-weighed after cooling down to determine the ash content according to AOAC (2003) and after laboratory analysis, OM was calculated by subtracting ash % from 100 %. Hemicellulose was calculated by subtracting ADF from NDF.

Statistical Analysis

All data were analyzed using the general linear model procedures of SAS (2009). Data were arranged as a completely random design as 13 × 2 × 4 factorial. There were 13 treatments, 2 soil types and 4 regrowth intervals. The linear model used included main effects of treatment, soil, soil × frequency, treatments × soil and treatment x frequency , and frequency nested in cutting, frequency nested in cutting x treatment. There were no frequency nested in cutting × treatment, treatment × frequency and frequency × soil ($P >$ 0.05). Therefore, they were removed from the model. And models included only the main effects of the soil, treatment, treatment x soil, and frequency nested in cutting. Means

were considered different at $P < 0.05$. When significant, means were separated using Fisher's protected least significant difference.

CHAPTER IV

RESULTS AND DISCUSSION

Different proportions of big bluestem, little bluestem and indiangrass mixed to be analyzed for IVDMD are presented in Table 1. Nutrient composition of big bluestem, little bluestem and indiangrass grass species are presented in Table 2. The three grass species were similar ($P = 0.0667$; 0.1857; 0.2005; 0.9010; 0.1386) for CP, NDF, ADF, fat and OM, respectively. However, these grasses were different ($P = 0.0005$; 0.0007) for DM and hemicellulose. This could be due to changes in their nutrient composition as these grasses increased during their growing phases. Ashikaga et al. (2010) reported that the nutritive value of forage generally changes according to the growth stage.

There was frequency nested in cutting effects for DM, OM, CP, NDF, ADF, hemicellulose and fat among the three grass species (Table 3). Dry matter was different ($P < 0.0001$) among the cuttings. The first, second, and third cutting for every 28 d were similar in DM but greater than the fourth cutting. Also, cuttings at every 56 d differed from one another. For cutting time effects, crude protein was different among the cuttings (Table 4). At the May 29 cutting, the 56 d cutting had DM content greater than the 28 d cutting. Also, in June 26 cutting, 84 d cutting had DM content greater than 28 d cutting. Furthermore, at the July 24 cutting, 56 d and 84 d cutting were similar in DM while but greater than the 28 d cutting. This was supported by the findings of Belesky (2006), who

reported that regrowth interval, harvest date and sown species affected DM distribution. There were no differences for OM ($P = 0.9368$; Table 3) and ($P = 0.2359$; Table 4).

Crude protein was different ($P < 0.0001$) among the samples harvested at varied regrowth intervals (Table 3). For cutting every 28 d grass cut after the first 28 d had the greatest CP content. Second and third cuttings were similar for CP concentration with the fourth cutting being intermediate. At every 56 d regrowth interval the CP concentration was not different between first cut and second cutting. On cutting time effects, crude protein was different among the cuttings (Table 4). On May 29, the 28 d cutting had CP content greater than 56 d cutting. On June 26, the 28 d cutting had CP content greater than 84 d cutting. Likewise, on July 24, the 56 d cutting and 112 d cutting were similar in CP but less than the 28 d cutting. These results concur to the findings of Gonzalez-Valenzuela et al. (2008) who reported that as grass matured, CP content was reduced. Owens et al. (2008b) reported similar findings and concluded that the decrease of CP content may be due to an increase in the proportion of stem or a decrease of CP concentration of leaf or stem or both.

Neutral detergent fiber was different ($P = 0.0006$) among the cuttings (Table 3). At every 28 d regrowth interval, the greatest NDF concentration was in the fourth cutting while the first three cuttings were similar to each other. Also, for every 56 d the NDF concentration increased as maturity increased. This supported the findings of Gonzalez-Valenzuela et al. (2008) who reported that the longer the harvest interval, the greater the NDF content of the plant. Cline et al. (2010) also cited several studies which indicated that NDF increased as a result of increased regrowth interval. It has been shown that re-growth interval of not greater than 24 to 26 d was good for spring grazing beef cattle

(Owens et al., 2008b). On cutting time effects, NDF was different among cuttings (Table 4). At the May 29, the 28 d cutting had NDF greater than the 56 d cutting. Likewise, in June 26 cutting, the 28 d cutting had NDF greater than the 84 d cutting. Furthermore, at the July 24 cutting, the 28 d and 56 d cuttings were similar in NDF but greater than the 112 d cutting. This is in contrast to the findings of Gonzalez-Valenzuela et al. (2008) who reported that the longer the harvest interval, the greater the NDF content of the plant.

cid detergent fiber was different ($P = 0.0224$) among cuttings for regrowth intervals (Table 3). At every 28 days regrowth interval, ADF was the greatest at the second and fourth cutting, least at the third cutting with first cutting being intermediate. Also, at every 56 d, ADF increased as maturity increased. This was in support of the findings of Robles et al. (1980) who reported that in late season grass cell walls are thicker with larger particle size and therefore contributing to the gut fill and intake limitation of animal. For cutting time effects, ADF was different among the cuttings (Table 4). On May 29, the 28 d cutting had an ADF concentration greater than the 56 d cutting. Likewise, on June 26, the 28 d cutting had an ADF concentration greater than the 84 d cutting. This was consistent with the finding of Jung and Allen (1995) who reported that as forage matured, crude fiber content increased and ADF generally decreased. Furthermore, at the July 24 cutting, the 28 d, 56 d and 112 d were ($P = 0.0224$) different from one another in acid detergent fiber.

Fat was different ($P < 0.0001$) among the cutting of the different regrowth intervals (Table 3). For the every 28 d regrowth interval, fat was different among the cuttings. Fat was greatest at the third cutting of the 28 d cutting as compared to other cutting of every 28 d. At the every 56 d regrowth interval, fat was greater at second

cutting of every 56 days as compared to first cutting. It has been shown that there is an increase in fat deposition as the grass matured in 56 d regrowth intervals, but this trend is different in every 28 d regrowth interval. This may be a result of drought in June that leads to greater fat deposition compared to the fourth cutting in July. Fat was different among the cutting (Table 4). On May 29, the 28 d cutting and 56 d cuttings were similar in fat contents. Likewise, at the June 26 cutting, the 28 d cutting and the 84 d cuttings were similar in fat contents. Furthermore, at the July 24 cutting time, 28 d cutting and 112 d were similar in fat contents but greater than 56 d cutting. This may also be as result of maturity or water availability within existing plant ecosystems (Milbau et al., 2009).

Hemicellulose was different ($P = 0.001$) among cuttings of the different regrowth intervals (Table 3). For every 28 d regrowth interval, hemicellulose was greatest at third and fourth cutting, least for the second cutting and intermediating for the first cutting. At every 56 d regrowth interval, hemicellulose increased as the maturity increased. It has been shown that additional the polysaccharide material which is deposited during the secondary cell wall growth is richer in cellulose than xylans compared to during primary cell wall growth (Jung and Allen, 1995). On cutting time effects, hemicellulose was not different among the cutting ($P = 0.5526$) (Table 4).

Dry matter content of big bluestem was less ($P = 0.0041$) than little bluestem and indiangras from soil 1. Indiangrass and little bluestem had the same dry matter content in soil 2 with big bluestem being intermediate (Table 5). This may be as result of differences in their moisture contents. Microbial growth increased at optimal temperature and moisture (Yao et al., 2011). And, because moisture and temperature are one of the key factors which determine plant growth.

Organic matter ($P = 0.0028$) of big bluestem, little bluestem and indiangrass were only different when harvested from soil 2 (Table 5). Organic matter content was greatest in little bluestem and least in big bluestem with indiangrass at intermediate. This may be result of differences of soil pH which may have affected plant growth and this may finally have affected the proportion of plant leaves of the individual grass species thereby this lead to variation in OM. Soil pH plays an important role in the availability of various macro- and micro- nutrients (Ownely et al., 2003). The optimum pH for these grasses is 6. And since the site 2 pH is 7.8 this mighty be too high to support these grasses OM productivity.

Crude protein ($P = 0.0001$) and hemicellulose ($P = 0.0077$) of big bluestem, little bluestem and indiangrass were only different when harvested from soil 2 (Table 5). The two sites were of different soil type and pH Site 2 is silty clay loam with a pH of 7.8. Soil silt was negatively correlated with soil pH and zinc, while ammonium nitrogen was positively correlated with zinc (Ownley et al., 2003). The clay soil is heavy and sticky, and tends to swell from additional moisture thereby impeding the penetration of plant seedlings or roots. Additionally, site 2 is subtended by chalk marl which is impenetrable. This can affect the soil microbial activity which helps fixation of nitrogen for the production of plant protein and limit root spread for nitrogen interception. Also, increased temperature lead to increased microbial activity and plant growth, and this lead to greater hemicellulose deposition in primary cell wall (Jung & Allen, 1995). Furthermore, the soil pH (7.8) of site 2 may have affected the microbial activity. Alkaline phosphatase activity is negatively correlated to the soil pH and dehydrogenase activity with greater activity in soil of decreased pH (Ann-kathrin et al., 2011). Clay soil is rich in calcium, aluminum

and iron (Newman et al., 2009a). The majority of soil nitrogen is proteinaceous in nature and often associated with minerals (Nannipieri and Eldor, 2009).

Fat content of indiangrass was greater ($P = 0.0095$) than big bluestem with little bluestem being intermediate when harvested from site 1. Fat was not different among these three grass species when harvested from site 2 (Table 5). This may be a result of the texture of the soil 1 being a sandy loam soil. This was consistent with the finding of Madakadze et al. (1998) who reported that within species of native warm-season grasses there was variation in rate of growth and date of maturity. Sandy soil is susceptible to leaching, erosion and drought. Due to drought stress on the sandier soil, these grasses need to develop stem wax so as to survive at this soil condition.

Neutral detergent fiber content was not different ($P = 0.8477$) among the three grass species from the two sites. Also, the three grass species did not differ ($P = 0.1726$) from one another in ADF concentration from the two sites (Table 5).

In vitro DM disappearance for all 13 treatments is depicted in Table 6. The rate of DM disappearance was greatest for indiangrass (T3 = 61.81 %) whereas little bluestem (T2 = 54.02 %) was the least and big bluestem (T1 = 58.16 %) and other treatments were in between. It is apparent that as indiangrass increase in proportion of the mix the IVDMD of the mixture increased. Conversely, as little bluestem proportion of the mix decreased IVDMD increased. Finally, as big bluestem proportion of the mix increased IVDMD increased compared to grasses mixed in equal proportion. In addition, *in vitro* neutral detergent fiber was not different among these 13 treatments ($P = 0.7129$).

In vitro dry matter disappearances and *in vitro* neutral detergent fiber disappearance, for each cutting within harvest and are summarized in Table 7. From the

first cutting of the 28 d regrowth interval, there was a reduction of *in vitro* disappearance from subsequent cuttings likewise in the *in vitro* NDF indicating that cutting at regrowth interval greater than 28 d did not increase digestibility. This concurs with Owens et al. (2008b) who stated that re-growth interval of not greater than 24 to 26 d was good for spring grazing beef cattle. Disappearance rate among forages is a function of nutritive value, morphology and physiology (Foster et al., 2011).

Furthermore, some warm-season grasses such as limpograss (*Hemarthria altissima*) are vegetatively propagated and have large and thick stems (Arthington and Brown, 2005) which causes the IVDMD to decrease when mixed with other warm season grasses that are not vegetatively propagated (Arthington and Brown, 2005). Also, the findings of Binnie et al. (1997) suggested that there was no advantage to be gained by lengthening the regrowth interval beyond 6 wk. Length of regrowth interval was particularly critical at the 28 d first cutting with no advantages to be gained by increasing the cutting rate beyond first cut. There was little, if any advantage to increasing the rate of cutting, particularly after June. This was supported by the findings of Gonzalez-Valenzuela, et al. (2008) who stated that at least 40 d between harvests are required to avoid detrimental effects on plant density and to achieve maximum regrowth between harvests. On cutting time effects, IVDMD were different among the cutting ($P < 0.0001$; Table 8). At the May 29 cutting, the 28 d cutting IVDMD was greater than 56 d cutting. Likewise, at the June 26 cutting, the 28 d cutting IVDMD was greater than the 84 d cutting. Furthermore, at the July 24 cutting, the 56 d and 112 d cutting were similar in IVDMD but lesser than the 28 d cutting. These have been attributed to increased lignification of the cell wall of the forage and loss of quality from the forages (Mitchell et

al., 1997; Starks et al., 2007; Lima et al., 2008; Owens et al., 2008b). However, at the May 29 cutting, the 28 d cutting *in vitro* NDF disappearances was similar to the56 d cutting. But at June 26 cutting, 84 d cutting *in vitro* NDF disappearances was greater than the 28 d cutting. At the July 24 cutting, the 56 d and 112 d are similar in *in vitro* NDF but lesser than the 28 d cutting. Length of regrowth interval was particularly critical for the 28 d regrowth interval with little advantage to be gained when length of regrowth was increased to 112 d.

Furthermore, harvesting at a rate of either every 28 d or every 56 d during June and July period resulted in no increase of IVDMD, it was at the expense of quality. As the grass matured there may have been an increase in cell wall size and content. Foster et al. (2011) reported that an increase in lignified stems can lead to reduce *in vitro* disappearance.

In conclusion, accurate and timely estimation of the nitrogen concentration and IVDMD of pasture during the grazing season can help livestock managers make appropriate decisions concerning fertilizer application, stocking rate and feeding of supplements (Starks et al., 2008). Because forage grasses are mostly fed to ruminants, the nutritive value, particularly nitrogen concentration, is an important consideration (Belanger and Gastal, 1999). The results from this experiment, in conjunction with available literature, indicated that growing monocultures of native warm-season grasses especially indiangrass for forage production for livestock can be practiced without benefits for growing multispecies grasses. There may likely be soil treatments interaction when multi-species were planted on the same sites because each one of them

may respond to soil available nutrients differently, and this may affects expected collective productivity.

Although, increasing the regrowth interval may lead to reduction of rumen NH_3-N concentration and thus potentially reduces N excretion to the environment (Owens et al., 2008a) there may be limited CP supply for ruminal microbes to make microbial protein. The results from this experiment indicated there is reduction in CP, and DM, but increased in NDF, fat and hemicellulose as the regrowth interval increased beyond the 28 d first cutting. Also, the same trend occurred for the every 56 d cutting. This suggested that in order to have a greater quality of forage for livestock, 28 d regrowth intervals of not more than one cutting time can be practiced. This will make the forage retain CP concentration and less fiber content at this growing time.

Table 1 Proportions (%) of big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* Nash), and indiangrass (*Sorghastrum nutans* Nash) mixed to be analyzed for [a]IVDMD.

Treatment	Big bluestem	Little bluestem	Indiangrass
T1	100	0	0
T2	0	100	0
T3	0	0	100
T4	50	50	0
T5	50	0	50
T6	0	50	50
T7	33	33	33
T8	50	25	25
T9	25	50	25
T10	25	25	25
T11	75	12.50	12.50
T12	12.50	75	12.50
T13	12.50	12.50	75

[a] IVDMD = *in vitro* dry matter disappearance.

Table 2 Nutrient composition (DM basis, %) of big bluestem (BB), little bluestem (LB) and indiangrass (IG).

Grass species	[a]DM	[a]NDF	[a]ADF	[a]HC	[a]FAT	[a]OM	[a]CP
BB	29.11[b]	71.02	37.47	33.55[b]	10.00	92.80	6.89
LB	31.95[c]	72.02	36.16	35.83[c]	10.07	93.25	7.99
IG	30.04[bc]	71.37	37.51	33.86[b]	10.12	92.99	6.99
Std. Error	0.667	0.675	0.819	0.548	0.156	0.307	0.269
P <	0.0005	0.1857	0.2005	0.0007	0.9010	0.1386	0.0667

[a]DM = Dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; HC = hemicellulose; FAT = ether extract; OM = organic matter; CP = crude protein.
[b, c] Means within columns lacking common superscripts differ ($P < 0.05$).

Table 3 Effect of cutting native warm-season grasses at varied regrowth interval on [a]DM, [a]OM, [a]CP, [a]NDF, [a]ADF, [a]FAT, and [a]HC (DM basis, %).

		Cutting time			
	[b]Frequency	May 1	May 29	June 26	July 24
[a]DM	1	31.70[d]	30.85[d]	30.36[d]	23.29[c]
	2	-	37.28[d]	-	25.92[c]
	3	-	-	34.45	-
	4	-	-	-	26.36
		Std. Error ± 0.929; (P <.0001)			
[a]OM	1	93.12	93.38	93.05	92.89
	2	-	93.08	-	93.44
	3	-	-	92.98	-
	4	-	-	-	92.71
		Std. Error ± 0.428; (P = 0.9368)			
[a]CP	1	9.98[d]	8.87[c]	8.66[c]	9.04[cd]
	2	-	6.98[c]	-	6.70[c]
	3	-	-	6.51	-
	4	-	-	-	6.68
		Std. Error ± 0.375; (P < 0.0001)			
[a]NDF	1	71.36[c]	71.68[c]	71.13[c]	74.74[d]
	2	-	68.78[c]	-	75.17[d]
	3	-	-	69.18	-
	4	-	-	-	72.49
		Std. Error ± 0.9405; (P = 0.0006)			
[a]ADF	1	37.92[cd]	39.69[d]	35.53[c]	39.13[d]
	2	-	37.58[d]	-	39.56[c]
	3	-	-	34.02	-
	4	-	-	-	37.52
		Std. Error ± 1.1409; (P = 0.0224)			
[a]FAT	1	8.08[d]	6.98[c]	12.02[f]	10.79[e]
	2	-	6.77[c]	-	10.12[d]
	3	-	-	12.18	-
	4	-	-	-	10.17
		Std. Error ± 0.217; (P <.0001)			
[a]HC	1	33.44[cd]	31.83[c]	35.61[d]	35.61[d]
	2	-	31.20[c]	-	35.62[d]
	3	-	-	35.16	-
	4	-	-	-	34.96
		Std. Error ± 0.763; (P = 0.0010)			

[a]DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent; ADF = acid detergent fiber; FAT = ether extract ; HC = hemicellulose.
[b]Frequency = Different harvest regimen.1 = cut of forage at every 28 d; 2 = cut of forage at every 56 d; 3 = cut of forage at 84 d and 4 = cut of forage at 112 d.
[c, d] Means within rows lacking common superscripts differ ($P < 0.05$).

Table 4 Effect of cutting native warm-season grasses at varied regrowth interval on [a]DM, [a]OM, aCP, [a]NDF, [a]ADF, and [a]FAT (DM basis, %).

	[b]Frequency	Cutting time			
		May 1	**May 29**	**June 26**	**July 24**
[a]DM	1	31.70	30.85[c]	30.36[c]	23.29[c]
	2	-	37.28[d]	-	25.92[d]
	3	-	-	34.45[d]	-
	4	-	-	-	26.36[d]
		Std. Error ± 0.929; (P <.0001)			
[a]OM	1	93.12	93.38	93.05	92.89
	2	-	93.08	-	93.44
	3	-	-	92.98	-
	4	-	-	-	92.71
		Std. Error ± 0.428; (P = 0.2359)			
[a]CP	1	9.98	8.87[d]	8.66[d]	9.04[d]
	2	-	6.98[c]	-	6.70[c]
	3	-	-	6.51[c]	-
	4	-	-	-	6.68[c]
		Std. Error ± 0.375; (P < 0.0001)			
[a]NDF	1	71.36	71.68[d]	71.13[c]	74.74[d]
	2	-	68.78[c]	-	75.17[c]
	3	-	-	69.18[c]	-
	4	-	-	-	72.49[c]
		Std. Error ± 0.9405; (P = 0.0006)			
[a]ADF	1	37.92	39.69[d]	35.53[d]	39.13[c]
	2	-	37.58[c]	-	39.56[d]
	3	-	-	34.02[c]	-
	4	-	-	-	37.52[e]
		Std. Error ± 1.1409; (P = 0.0224)			
[a]FAT	1	8.08	6.98[c]	12.02[c]	10.79[d]
	2	-	6.77[c]	-	10.12[c]
	3	-	-	12.18[c]	-
	4	-	-	-	10.17[d]
		Std. Error ± 0.217; (P <.0001)			
[a]HC	1	33.44	31.83	35.61	35.61
	2	-	31.20	-	35.62
	3	-	-	35.16	-
	4	-	-	-	34.96
		Std. Error ± 0.763; (P = 0.5526)			

[a]DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; FAT = ether extract; HC = hemicellulose.
[b]Frequency = Different harvest regimen.1 = cut of forage at every 28 d; 2 = cut of forage at every 56 d; 3 = cut of forage at 84 d and 4 = cut of forage at 112 d.
[c, d,e] Means within column lacking common superscripts differ ($P < 0.05$).

Table 5 Soil nutrient content (DM basis, %) effects of big bluestem (BB), little bluestem (LB), and indiangrass (IG) grown on two different soils.

		Grass species		
	[b]Soils	BB	LB	IG
[a]DM	1	26.88[c]	30.62[d]	30.78[d]
	2	31.34[cd]	33.28[d]	29.29[c]
		Std. Error ± 0.877; (P = 0.0041)		
[a]OM	1	93.41	92.54	92.38
	2	92.19[c]	93.96[e]	93.59[d]
		Std. Error ± 0.404; (P = 0.0028)		
[a]CP	1	7.56	6.95	6.96
	2	6.22[c]	9.05[d]	7.02[c]
		Std. Error ± 0.354; (P = 0.0001)		
[a]NDF	1	70.43	71.03	70.80
	2	71.61	73.00	71.93
		Std. Error ± 0.888; (P = 0.8477)		
[a]ADF	1	36.55	36.88	36.52
	2	38.38	35.44	38.49
		Std. Error ± 1.077; (P = 0.1726)		
[a]HC	1	33.88	34.19	34.28
	2	33.22[c]	37.47[d]	33.44[c]
		Std. Error ± 0.721; (P = 0.0077)		
[a]FAT	1	9.64[c]	9.72[cd]	10.32[d]
	2	10.36	10.42	9.92
		Std. Error ± 0.205; (P = 0.0095)		

[a]DM = Dry Matter; OM = Organic Matter; CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; HC = Hemicellulose; FAT = Ether Extract.
[b]Soil 1 = Marietta fine sandy loam soil (pH = 6.9); Sumter silty clay loam soil (pH = 7.8)
[c,d,e] Means within rows lacking common superscript differs dry matter basis, ($P < 0.05$).

Table 6 *In vitro* dry matter disappearance (IVDMD) and *in vitro* dry matter neutral detergent fiber disappearances (IVNDFD) of different proportion of big bluestem, little bluestem and indiangrass mixed together (DM basis, %).

Treatments	IVDMD	IVNDFD
T1	58.16[bcd]	70.86
T2	54.02[a]	62.12
T3	61.81[e]	71.75
T4	57.21[abcd]	70.20
T5	54.75[ab]	66.14
T6	60.46[de]	71.11
T7	56.99[abcd]	69.33
T8	57.90[bcd]	70.05
T9	55.27[abc]	68.24
T10	56.74[abc]	70.38
T11	58.29[cde]	69.17
T12	55.75[abc]	66.42
T13	58.80[cde]	70.43
Std. Error	1.258	2.997
P <	0.0001	0.7129

[a, b, c, d, e] Means within column lacking common superscript differs ($P < 0.05$).
T1 = Mixtures of 100 % big bluestem, 0 % little bluestem and 0 % indiangrass.
T2 = Mixtures of 0 % big bluestem, 100 % little bluestem and 0 % indiangrass
T3 = Mixture of 0 % big bluestem, 0 % little bluestem and 100 % indiangrass.
T4 = Mixture of 50 % big bluestem, 50 % little bluestem and 0 % indiangrass.
T5 = Mixture of 50 % big bluestem, 0 % little bluestem and 50 % indinagrass.
T6 = Mixture of 0 % big bluestem, 50 % little bluestem, and 50 % indiangrass.
T7 = Mixture of 33 % big bluestem, 33 % little bluestem and 33 % indiangrass.
T8 = Mixture of 50 % big bluestem, 25 % little bluestem and 25 % indiangrass.
T9 = Mixture of 25 % big bluestem, 50 % little bluestem and 25 % indiangrass.
T10 = Mixture of 25 % big bluestem, 25 % little bluestem and 50 % indiangrass.
T11 = Mixture of 75 % big bluestem, 12.5 % of little bluestem, 12.5 % indiangrass.
T12 = Mixture of 12.5 % big bluestem, 75 % little bluestem and 12.5 % indiangrass.
T13 = Mixture of 12.5 % big bluestem, 12.5 % little bluestem and 75 % indiangrass.

Table 7 Effect of cutting native warm-season grasses at varied regrowth interval on [a]IVDMD and [a]IVNDFD (DM basis, %).

		Cutting time			
	[b]Frequency	**May 1**	**May 29**	**June 26**	**July 24**
[a]IVDMD	1	68.35[d]	62.69[c]	60.34[c]	61.88[c]
	2	-	59.81[d]	-	55.13[c]
	3	-	-	55.04	-
	4	-	-	-	53.76
		Std. Error ± 0.841; (P <.0001)			
[a]IVNDFD	1	75.69[d]	69.52[c]	65.95[c]	69.01[c]
	2	-	68.39[d]	-	60.46[c]
	3	-	-	79.15	-
	4	-	-	-	62.13
		Std. Error ± 2.004; (P <.0001)			

[a]IVDMD = *In vitro* dry matter disappearance; IVNDFD = *In vitro* neutral detergent fiber disappeara

[b]Frequency = Different harvest regimen.1 = cut of forage at every 28 d; 2 = cut of forage at every 56 d; 3 = cut of forage at 84 d and 4 = cut of forage at 112 d.

[c, d] Means within row lacking common superscript differs ($P < 0.05$).

Table 8 Effect of cutting native warm-season grasses at varied regrowth interval on [a]IVDMD and [a]IVNDFD (DM basis, %).

		Cutting time			
	[b]Freq.	**May 1**	**May 29**	**June 26**	**July 24**
[a]IVDMD	1	68.35	62.69[d]	60.34[d]	61.88[d]
	2	-	59.81[c]	-	55.13[c]
	3	-	-	55.04[c]	-
	4	-	-	-	53.76[c]
		Std. Error ± 0.841; (P <.0001)			
[a]IVNDFD	1	75.69	69.52[c]	65.95[c]	69.01[d]
	2	-	68.39[c]	-	60.46[c]
	3	-	-	79.15[d]	-
	4	-	-	-	62.13[c]
		Std. Error ± 2.004; (P <.0001)			

[a]IVDMD = *In vitro* dry matter disappearance; IVNDFD = *In vitro* neutral detergent fiber disappearance.

[b] Frequency = Different harvest regimen.1 = cut of forage at every 28 d; 2 = cut of forage at every 56 d; 3 = cut of forage at 84 d and 4 = cut of forage at 112 d.

[c, d] Means within column lacking common superscript differ ($P < 0.05$).

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