Alternative Forage Systems for Developing Replacement Heifers

by

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Abstract

Heifer development systems are an essential phase of beef production due to lifelong production implications. Reproductive success of a heifer's first breeding season is critical to the sustainability of cow-calf production systems. Understanding the effects that different management practices have on a heifer's ability to conceive to artificial insemination or a clean-up bull are essential to the sustainability of beef cattle production systems. Cattle producers experiencing increased costs might consider alternative heifer development strategies without sacrificing growth or pregnancy rates. Heifer development systems utilizing grazed forage systems could be an option for some producers. Understanding the impacts of different forage species and mixtures could help provide a lower input method of developing heifers in these systems. The objective of this dissertation was to evaluate alternative forage systems for developing replacement heifers. A multi-year heifer development demonstration project at the Sand Mountain Research and Extension Center in Crossville, AL supported local beef heifer development using cool-season annuals, and provided 13 consignors with herd production data that can be used to further herd potential over time. Integration of legumes into warm-season grasses is an important step in producing a better quality forage with less N inputs as a potential grazing option for developing heifers. Fall-planting of alfalfa into bermudagrass systems may better favor production and persistence characteristics of mixed stands compared with spring plantings, where a spring-planted mixture of 'Bulldog 505' alfalfa and 'Tifton 44' bermudagrass sustained a less than 20% contribution from alfalfa in the year-after-planting. Summer perennial forage systems, such as native warm-season grasses or bahiagrass, may support heifer growth during the summer months prior to winter breeding for fall-born calves; however, growth rate of heifers may be relatively slow during

this time period (0.4 to 0.7 kg/day). For the metabolomics analysis, this limited number (n = 27) animal project, differences were not observed between heifers conceived to AI vs. natural conception to clean-up bull and no differences were observed across forage treatments. These projects demonstrate that using alternative forage options such as cool-season annuals, legumes in warm-season forage systems, and native species may diversify systems management options for beef producers evaluating heifer development strategies on-farm.

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List of Abbreviations

ADG	Average daily gain
AI	Artificial Insemination
ANOVA	Analysis of Variance
BCS	Body Condition Scoring
BW	Body Weight
˚C	Degrees Celsius
C_3	Cool-season grasses
C_4	Warm-season grasses
CIDR	Controlled Internal Drug Release
cm	Centimeter
FDR	False Discovery Rate
GnRH	Gonadotrophin Releasing Hormone
Ha	Hectare
IACUC	Institutional Animal Care and Use Committee
kg	Kilogram
n	number
NWSG	Native warm-season grasses
PGF	Prostaglandin F2α
RTS	Reproductive Tract Score
USA	United States of America
USDA	United States Department of Agriculture

CHAPTER 1

INTRODUCTION

Forage-based cattle production is an integral part of the southeastern United States (USA) agriculture economy. Approximately 94.8 million cattle and calves make up the 2019 inventory in the USA, which represents $67 billion in cash receipts (USDA, 2019). Beef cattle production systems in the southeastern USA have the potential to use grazed forages for more than 300 days per year. Land that is too poor or erodible for cultivation can become productive when utilized for grazing livestock. Grazed beef systems across the region are diverse and have been developed implementing both perennial and annual forage systems to ensure forage availability across multiple seasons. Southeastern USA grasslands are primarily used for beef cow-calf production, while stockering weaned calves to feeder weight on high-quality pastures is another important enterprise (Hoveland, 2000). Heifer development is also a key component of beef operations in the southeast USA. Replacement females are crucial to the cow-calf sector for replacement of culled cows and improving genetic potential (Bagley, 1993). The period of time most often indicated by the term heifer development is from weaning to confirmation of pregnancy after the first breeding season (Rhinehart, 2017). With the amount of time and money invested in the development of heifers, reproductive efficiency and efficacy are essential to a sustainable system. The heifer development phase is a critical period in selection of females for fertility early in their productive lifespan. Attention to management of heifers during development can have a sustained impact on long-term fertility efficiency. Development of replacement females to fit their production environment is vital for proficiency of the cow-calf sector. Utilizing production practices that minimize input costs and increase selection of reproductive performance may provide a viable opportunity for increased long-term efficiency of beef cattle production. Forage management, genetic and phenotypic selection, and nutritional management must all be part of the heifer development plan to ensure sustainability and profitability in the cow-calf system. Optimizing forage systems management in the Southeast may help provide beef cattle producers with on-farm management practices that support beef heifer development.

Heifer development in grazed, forage-based systems may be improved in the Southeast through diversifying species contribution in the region where species such as tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.), bermudagrass [*Cynodon dactylon* (L.) Pers.], and bahiagrass (*Paspalum notatum* Flügge) are the predominant forages grown by stakeholders. Integration of annuals, legumes, or alternative perennial species options in beef operations may improve forage production potential and forage quality needed to support animal gain in growing beef heifers. The humid summer characteristics of the Southeast are advantageous to C_4 plants of sub-tropical origin, such as bermudagrass, that tend to survive mild winters by becoming dormant. Some disadvantages to bermudagrass are: poor to moderate forage quality, pest pressure and fertility cost (Johnson et al., 2001). One method of improving nutritive quality of warm-season perennials is interseeding legumes such as alfalfa. Legumes have the potential to increase overall nitrogen to the system as well as increase yearly dry matter (DM) yields (Ledgard and Steele, 1992). Overseeding alfalfa into sod-forming warm-season perennial grass (WSPG) can improve forage quality, while increasing annual dry matter (DM) yields in the spring and fall months when bermudagrass and bahiagrass are the least productive (Ball et al., 2015). Warm-season and cool-season perennial grasses can be stockpiled beginning in August or September, respectively, to provide grazing from October through February. Research and demonstration work in Arkansas has shown that grazing seasons can be extended to 300+ days per year using planned management practices with perennial and complementary annual forages and stockpiled forages. That approach was successful for spring and fall-calving beef herds, but can also be implemented for stocker or heifer development programs (Jennings et al., 2017).

The overall objective of the dissertation research was to evaluate alternative forage systems and their impact on developing heifers performance and fertility.

CHAPTER 2

LITERATURE REVIEW

Beef Heifer Development Systems in the Southeast USA

Overview

Studies in numerous animal species provide evidence that diet during beef heifer development can partially control physiological changes necessary for puberty (Frisch, 1984). Numerous studies have reported inverse correlations between post-weaning growth rate and age at puberty (Arije and Wiltbank, 1971; Ferrell, 1982; Short and Bellows, 1971; Wiltbank et al., 1966, 1969, 1985). Pregnancy rates in heifers have been shown to be dependent upon the number of animals displaying estrus prior to or early in the breeding season (Byerley et al., 1987; Short and Bellows, 1971). Thus, rate of post-weaning growth was determined to be an important factor affecting age of puberty, which influenced pregnancy rates. Previous research has demonstrated the timing of weight gain has minimal consequence on heifer fertility, but the weight a heifer reaches by the start of her first breeding season heavily affects her reproductive success (Funston and Deutscher, 2004).

Selection and development of replacement heifers improves the likelihood that heifers entering development programs will conceive early in the breeding season followed by increased stayability (Snelling et al., 1995). Beef heifers should be managed to achieve puberty early, conceive early in the first breeding season, calve unassisted, and breed back quickly for their second calf (Wiltbank et al., 1966; Patterson et al., 1992; Funston and Deutcher, 2004; Summers et al., 2014). Heifer development system goals include increasing the percentage of heifers reaching puberty before the start of the breeding season and decreasing input costs associated with development. Heifer development is a key component to a beef management system. When thinking about the appropriateness of the heifer development paradigm used over the last several decades, some critical factors need to be considered: drastic reduction in supply of relatively inexpensive feeds traditionally used in heifer development programs, genetic changes in the cattle population over the last 40 yr, and the need to understand the implication that management practices may have on lifetime production efficiency rather than on an annual production cycle (Endecott, 2013).

The period of time most often indicated by the term heifer development is from weaning to confirmation of pregnancy after the first breeding season (Rhinehart, 2017). By the time a heifer has calved for the first time, significant resources have been invested in her to reach this point. In addition to the significant cost of retaining or purchasing and developing heifers, there is also opportunity cost and time invested. To quantify the investment in days, Rhinehart (2017) illustrates a time period investment of 1,200 d from the start of the breeding season to confirmation of pregnancy with a second calf. With the amount of time and money invested in the development of heifers, reproductive efficiency and efficacy are essential to a sustainable system.

The heifer development phase is a critical period in selection of females for fertility early in their productive lifespan. Replacement females are crucial to the cow-calf sector for the replacement of culled cows and improving the genetic potential of the cow herd (Bagley, 1993). The traditional recommendation has been to develop beef heifers to reach 65% of mature BW by the onset of the breeding season (Varner et al., 1977; Patterson et al., 1992). More recent research has demonstrated heifers reaching less than 55% of mature BW by breeding do not display impaired reproductive performance (Funston and Deutscher, 2004; Martin et al., 2008; Roberts et al., 2009; Funston and Larson, 2011; Funston et al., 2011). Reports suggest that the “target weight” system for developing heifers may have become outdated and costly (Endecott et al., 2014). As beef cattle producers switched from calving heifers as three-year-olds to calving as two-year-olds, more emphasis has been placed on selecting heifers that reach puberty at an earlier age and lighter weight in relation to their expected weight as a mature cow.

To optimize production, heifers must be bred by 15 months of age to calve as 2-yr olds. Heifers that calve early in the season tend to calve early in subsequent calving seasons (Short and Bellows, 1971; Beck et al., 2005). Clanton et al. (1983) found that age of puberty was not affected by differing rates of growth by heifers consuming the same diets as long as BW was similar at the start of the breeding season. Interestingly, heifer development protocols may influence resulting behavior traits associated with the environment in which the heifer was developed. Research has reported range-developed heifers may retain better grazing skills and be more productive during the subsequent summer (Olson et al., 1992; Perry et al., 2009).

The nutritional program of the heifer development phase is influenced by regional availability of feedstuffs and adapted forage resources. Various strategies have been employed to achieve this end point such as constant body weight gains from weaning to breeding or nutritional restriction followed by greater nutrient intake and compensatory gain. Findings from multiple experiments suggest that flexibility exists in how this target weight is attained to achieve acceptable pregnancy rates. Feeding to pre-breeding weights as low as 51 percent of mature weight have shown to be more cost effective than development to 57 percent of mature weight, even though lighter heifers were allowed a 15 d longer (45 vs. 60 d) breeding season (Martin et al., 2007).

Post-weaning management of heifers to achieve traditional target weights, particularly by feeding high-energy diets, is not supported by current research. Heifers developed on forage-based systems, however, generally require additional protein supplementation to achieve even modest gains (Funston et al., 2017). One reason reproductive performance has not been drastically impaired by feeding to lower target weights may relate to genetic changes in age at puberty (Funston et al. 2017). While some disagreement exists as to the ideal target weight for heifers at the start of their first breeding season, nutritional management during the post-weaning phase is crucial to breeding success.

An overarching factor that influences age at puberty in heifers is the nutritional management that occurs during the development of the heifer. Management practices should not be changed suddenly and implementation of low-input development should be done with careful attention to detail and when genetic potential of the specific group of heifers is known. In the Southeast, nutritional systems for heifer development may consist of a drylot or grazing-based system or a combination of those systems.

Drylot System

Retaining and developing replacement heifers present a significant expense to the cow-calf producer, only surpassed by feed expense (Springman et al., 2017). When considering a dry-lot (DL) system, commodity feed costs and availability must be evaluated. In the Southeast, annual rainfall averages 127 to 152 cm, which may pose challenges for concentrating heifers in a DL scenario. Depending on the number of heifers being developed, barns may be employed to assist with mire and nutrient management.

Previous research comparing a DL system to a grazing system has demonstrated that both methods are effective in achieving acceptable growth and pregnancy results, but the cost of development may differ. Summers et al. (2014) compared grazing corn residue and DL systems in Nebraska that demonstrated heifers in the DL [fed brome hay (70.5%), wet corn gluten

(16.2%), and cracked corn (9.9%) where nutrient analysis recorded as crude protein (12.8%), crude fat (2.7%), and Mcal/kg (0.9%)] gained more during the development period than on corn residue. However, heifers developed on corn residue experienced increased post-AI average daily gain (ADG) on summer range compared with DL-developed heifers. The difference may be due to compensatory gain or retained learned grazing behavior as suggested by Summers et al. (2014).

Funston and Larson (2011) compared heifer development using a DL system or a more extensive winter grazing system (WG) that included a combination of corn residue and winter range. During the winter grazing period, WG heifers gained less ($P = 0.01$) BW than DL heifers and WG heifers had lighter ($P = 0.02$) BW at breeding. Fewer ($P < 0.01$) WG heifers reached puberty before breeding. Conception to AI was not different ($P = 0.23$); however, AI pregnancy rate tended ($P = 0.08$) to be less in WG heifers. Final pregnancy rates were not different ($P = 0.38$) between treatment groups. Although WG heifers had lighter ($P = 0.02$) BW at pregnancy diagnosis, they compensated for this with greater ($P = 0.05$) ADG after breeding, resulting in similar ($P = 0.22$) pre-calving BW. A Winter grazing system did not influence ($P > 0.10$) percentage of calving in the first 21 d, calf birth date, and calf birth BW, or dystocia score. Pregnancy rate after the second breeding season was not different ($P = 0.56$) between treatments. Heifer development using extended winter grazing reduced ($P < 0.01$) the cost of producing a pregnant heifer by $45 compared with DL.

Lardner et al. (2014) evaluated two heifer development systems using a DL and bale grazing system. Spring-born Angus heifers (253 ± 0.7 kg) were randomly allocated over 2 consecutive yr (yr 1, n = 80; yr 2, n = 96) to be developed to either 55% (350 kg) of mature BW (moderate gain, MG) or 62 percent (395 kg) of mature BW (high gain, HG). Each MG and HG group was further assigned to 1 of 2 replicated systems: (1) bale graze bromegrass-alfalfa round bales in field paddocks (BG) or (2) fed bromegrass-alfalfa round bales in DL pens. During the winter development period, MG heifers had lower (*P* = 0.01) ADG than HG heifers and MG heifers had lighter (*P* = 0.01) BW at breeding. The proportion of heifers attaining puberty by 14.5 mo of age was less (*P* = 0.05) in MG (20 ± 4%) than HG heifers (52 ± 3%). From the end of the 202-d development period to pregnancy diagnosis, ADG was greater (*P* = 0.04) in MG heifers than HG heifers (0.83 vs. 0.71 kg/d). First-calf pregnancy rates were 86 and 88% for MG and HG heifers, respectively (*P* = 0.41). Second- and third-calf pregnancy rates of cows, developed in either a MG or HG system as heifers, were not different (*P* = 0.74; 94.7 vs. 95.9% and 93.8 vs. 93.9%, respectively). Economic analysis revealed a $58 reduced development cost for heifers developed to 55% compared with 62% of mature BW without a loss in reproductive performance (Lardner et al., 2014).

The previous studies demonstrated a cost savings when developing heifers in a grazing system rather than a DL system without sacrificing reproductive performance. Salverson et al. (2005) reported similar results for growth and pregnancy, but reported similar system costs ($122/hd for forage with supplement; $117/hd DL) between a DL and grazing system with supplement in South Dakota.

In large part, the DL systems reported herein benefited from a favorable climate for maximizing animal performance, availability of roughage, and proximity to regions where feed grains are grown for feed rations. When considering a DL system, nutrient and waste management are concerns in the Southeast, which is a region of high precipitation across seasons. A DL system can be an effective means of reaching growth and reproductive goals for beef heifers. Previous research has demonstrated a difference in cost of gain that needs to be considered when selecting a development system.

Grazing-Based Systems

A grazing system is defined as an integrated combination of animal, plant, soil, and other environmental components and the grazing method(s) by which the system is managed to achieve specific results or goals (Allen et al., 2011). Each grazing system is unique, but the principles that function within systems can be transferred to other locations and situations. Desired outcomes include economic objectives, production goals for plants and animals, and environmental quality and may include objectives such as recreation, preservation, and aesthetics of open space (Allen and Collins, 2003).

Climate and soil conditions largely define where different forage types are grown and divide the USA into different ecoregions. In southeastern states, near-year-round grazing systems are possible. Systems are often based on warm-season perennial grasses such as bermudagrass and bahiagrass, or cool-season perennials such as tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) and orchardgrass (*Dactylis glomerata* L.). They usually involve sequential use of several forage species during the year, and may be grown alone or in combination with a complementary species to lengthen the grazing season and meet livestock nutritional requirements. Cool-season annuals, either seeded directly into warm-season perennial sods or planted separately, lengthen the grazing season. Warm-season annuals or native warm-season grass systems are used as alternative grazing sources in tall fescue-based systems in the summer months or on transitional fields.

Livestock grazing systems are defined by multiple factors, including climate, soil and forage resources, type of livestock enterprise(s), proximity to and timing of markets, and environmental concerns. They are also fashioned by the lifestyle goals and experience of the cattle producer or manager. Within systems, an array of different grazing methods is employed to optimize animal performance, to meet their varying nutritional needs, and to manage the forage resource.

Tall Fescue

Tall fescue is not native to the USA or even North America, but it originated in Europe (Buckner et al., 1979). Tall fescue most likely found its way to the USA as a seed contaminant in seed of other forage species. The variety 'KY-31' tall fescue started being widely planted in the USA in the 1940s (Ball et al., 2019). Tall fescue is a cool-season perennial bunch grass with a vigorous root system, it has a long growing season. Tall Fescue greens up in late winter or early spring, is highly productive in mid-to-late spring, stays green during summer, and makes a substantial amount of growth in the autumn. Tall fescue comes closer to being a year-round grass than any other native or introduced plant available (Ball et al., 2019).

In the USA, tall fescue generally grows in the area that encompasses a good portion of what is called the transition zone that lies between areas in the North where cool-season species dominate and areas in the South where warm-season grasses dominate (Ball et al., 2019). Tall fescue is well suited for stockpiling due to a waxy coating on the leaves and stems that resist deterioration from exposure to the elements in late autumn and early winter, allowing cattle to graze during the winter months when the growth potential of the plants has declined.

Total seasonal production of tall fescue is affected by weather, fertilization (especially nitrogen), and cutting or grazing management. Yields of 900 to 1800 Kg of DM/ha are common, with the higher yields associated with proper fertilizer applications and harvest management. Tall fescue nutritional value exhibits a seasonal change in total digestible nutrients (TDN) and digestibility. Crude protein content in green, leafy tall fescue leaves can be high throughout the growing season (spring, 22% CP; summer, 18% CP; and fall, 19% CP on a DM basis). Digestibility and sugars are greatest in fall, intermediate during spring, and least in summer. Palatability follows essentially the same trend as digestibility and sugar concentration. The *in vitro* dry matter disappearance (IVDMD) of tall fescue in the spring measures 58.1% and in the fall 56.2% (Lacefield et al., 2003).

Pratt and Hayes (1950) noted poor performance of animals grazing ‘KY-31’ tall fescue, which started the evaluation of the forage for livestock toxicity. The isolation and occurrence of ergot alkaloids from tall fescue was documented by Lyans et al. (1986). Fescue toxicosis is an umbrella term commonly used to encompass all tall fescue-related animal disorders such as bovine fat necrosis, fescue foot, fescue toxicity or summer slump, aglactia, decreased pregnancy rates, and lack of hair shedding (Ball et al., 2019). Fescue toxicosis in livestock is due to ingestion of endophyte-infected tall fescue. The endophyte (*Epichloë coenophiala* Bacon and Schardl.) spreads exclusively via seed and is found throughout the plant structural components at different concentrations (Ball et al., 1993; Bacon and Siegel, 1988). Fescue toxicosis is linked to physiological changes in beef cattle such as decreased intake, decreased ADG, vasoconstriction or reduced blood flow to extremities, and elevated body temperature (Hemken et al., 1979; Hurley et al., 1981; Hammond et al., 1982; Daniels et al., 1984; Hemken et al., 1984; Boling, 1985; Thompson et al., 1987; Stuedemann and Hoveland, 1988).

Reduced calving rates have been attributed to endophyte-infected fescue. In one study, calving rates for cows grazing low endophyte fescue (var. Kenhy) were 86%, compared with 67% for cows grazing high-endophyte fescue (Boling, 1985). Similarly, 96% of beef heifers raised on low-endophyte fescue (0 to 5% plants infected) conceived, compared with 55% of those raised on high-endophyte fescue (80 to 90% plants infected) (Schmidt et al., 1986). Only 33% of the primiparous cows grazing the highly infected pastures were successfully rebred compared with 93% on the low-endophyte pastures. Conception rates decreased 3.5% for each 10% increase in fungal infection. In a related study, 2-yr-old heifers exposed to bulls beginning on d 31 after grazing either endophyte-infected or uninfected fescue had 80 and 90% calving rates, respectively, with a 15% decrease in calving weights (Beers and Piper, 1987). Gay et al. (1988) reported in a 3-yr study which calving rates were 94.6 and 55.4% for cows grazing uninfected and infected fescue, respectively. Washburn and Green (1991) described similar results in a 3-yr study in which 39% of the animals on high endophyte-infected tall fescue became pregnant and raised a calf vs 65% of those on low-endophyte forage. Heifers on high or low endophyte forage respectively, with surviving calves were 11 vs. 58% (1st yr); 63 vs 84% (2nd yr); and 42 vs 53% (3rd yr). First-service artificial insemination (AI) conception was reduced for the high-endophyte animals (45 vs. 74%) for the first 2 yr of the study (Washburn et al., 1989); however, overall first-service conception among those animals inseminated for the 3-yr period was 74 vs. 78% (high vs. low endophyte, respectively).

Bermudagrass and Bahiagrass

Bermudagrass [*Cynodon* (L.) Pers.] likely originated in tropical Africa; however, Australia, Eurasia, the Indo-Malaysian area, and the Bengal region of India/Bangladesh have also been proposed as its origin (Holm et al., 1979). Bermudagrass was introduced to the USA prior to the middle 1700s. Bermudagrass is a rapidly spreading perennial, having both rhizomes and stolons. It is a warm-season plant, making little growth when temperatures drop below 10°C. Drought tolerance of bermudagrass is much better than that of bahiagrass or dallisgrass (*Paspalum dilatatum*). Bermudagrass is best adapted to moderately well-drained soils with medium to high fertility. It will tolerate considerable flooding, but makes little growth under these conditions. When adequate nutrients are available, it grows well in a soil pH range from 5.0 to up to 8.0.

Bermudagrass is productive from spring until fall and is well-suited for grazing or hay production. It is a high-yielding that produces grass 4.5 to 6.4 metric tons of hay per hectare with moderate nutritive quality (10 - 14% CP; 33 -3 8% acid detergent fiber (ADF); 63 - 68% neutral detergent fiber (NDF); 52 - 58% TDN on a dry matter (DM) basis) and persistence (Ball et al., 2012). Significant advancements have improved bermudagrass yields and forage quality. Dr. Glenn Burton, principal geneticist at the Georgia Coastal Plain Experiment Station in Tifton, released several hybrid bermudagrasses for use in the South. Including 'Coastal', 'Midland', 'Coastcross I', 'Tifton 44', 'Tifton 78' and 'Tifton 85'.

'Coastal' bermudagrass (CB) is the major warm-season grass grown across the U.S. Upper South. Coastal, an F_1 hybrid of 'Common' bermudagrass and a bermudagrass introduced from Asia, was released more than 55 years ago and has been established for hay and grazing on approximately 6,070,284 ha in the southern United States (Hancock et al., 2017). More recent hybrids of 'Tifton 44' (T44) and 'Tifton 85' (T85) offer improved nutritive value and yield characteristics over 'common' and 'coastal'.

Burns and Fisher (2007) compared DM intake and digestion of CB, T44, and T85 hays grown under different soil and climate conditions and harvested at either the same or different maturities. In the comparison of CB and T44 with steers, DM intake was greater for CB in one of three experiments, whereas intakes did not differ in the other two. Greater intake for CB was associated with greater DM digestion.

In the other two experiments, T44 had greater DM digestion than did CB in one trial but did not differ in the other. In a 2 yr experiment, hays of CB, T44, and T85, were compared by means of sheep (*Ovis aries*). In Year 1, sheep consumed more CB than either T44 or T85, whereas in Year 2, no differences in intake were detected. ‘Coastal’ was digested least in both experiments compared with T44 and T85, T85 had the greatest DM digestion in one of the two years. Samples of masticate of CB had the least in vitro true dry matter disappearance (IVDMD) with T44 intermediate and T85 greatest. In general, animal response data showed little advantage of T44 compared with CB; however, Tifton 85 appears to have greater digestible fiber and offers potentially greater DM digestion and digestible intake compared with CB (Burns and Fisher, 2007).

Bahiagrass (*Paspalum* Flügge) is a perennial grass with shallow rhizomes (Sampaio et al., 1976; Beaty et al., 1977), glabrous leaves with blades varying from 3 to 30 cm in length and 3 to 12 mm in width, and a characteristic inflorescence with two racemes. Bahiagrass is a morphologically diverse species indigenous to South America, where it is found growing on light-textured soils and is widely used in lawns, sports turf, and roadsides (Gates et al., 2004). Bahiagrass was first introduced into the USA by the Bureau of Plant Industry and grown by the Florida Agricultural Experiment Station in 1913 (Burton, 1967). Bahiagrass is best adapted to sandy soils and tolerates low soil fertility and low pH. Bahiagrass has a yield potential of 6,700 – 11,000 kg DM/ha and moderate nutritive quality [9-11% CP; 50-56% TDN; Ball et al., 2012].

In the southeastern USA, bahiagrass is most productive from April to October (Mislevy and Dunavin, 1993; Ball et al., 2015), and 85 to 90% of bahiagrass forage is produced from April through September (Mislevy and Everett, 1981, Kalmbacher, 1997). Stewart et al. (2007) reported herbage DM accumulation rates of 30, 62, and 15 kg/ha in May, mid-July, and October, respectively, for continuously stocked ‘Pensacola’ bahiagrass pastures receiving 120 N kg/ha/yr. Similarly in subtropical Japan, above-ground bahiagrass pasture productivity decreased as daylength decreased in autumn (Hirata et al., 2002).

Bahiagrass is the base of cow-calf operations in the lower portion of the subtropical zone, USA. Various bahiagrass cultivars have been released over the years; however, an evaluation of their performance under no N fertilization has not been reported. There is some evidence that bahiagrass can associate with free-living bacteria and fix atmospheric N_2. This study investigated the performance and potential biological N_2 fixation (BNF) of six bahiagrass cultivars (‘Argentine’, ‘AU Sand Mountain’, ‘Pensacola’, ‘TifQuik’, ‘Tifton-9’, and ‘UF-Riata’) under no N fertilization during the growing seasons of 2014, 2015, and 2016. Results showed no differences among cultivars for total herbage accumulation (2835 kg DM ha), total N aboveground (28 N kg/ha/yr). ‘Pensacola’ had greater CP than ‘Tifton-9’ (84 vs. 78 g kg, respectively), whereas ‘AU Sand Mountain’ had greater in vitro digestible organic matter (494 g kg) than other cultivars, except for Argentine. Argentine had greater root-rhizome mass (24,914 kg organic matter ha) than UF-Riata and TifQuik (15,367 and 14,682 kg organic matter/ha, respectively, Santos et al., 2019.

Bermudagrass and bahiagrass are adapted and thrive in the Southeastern, USA but not without challenges and set-backs. Insect pests cause problems for bermudagrass [i.e., bermudagrass stem maggot (*Atherigona reversura),* fall armyworms (*Spodoptera frugiperda),* chinch bugs (*Blissus leucopterus),* grubworms (*Cyclocephala spp.*)] and bahiagrass [i.e., mole crickets (*Gryllotalpa orientalis*), billbugs (*Sphenophorus spp.)*], as well as diseases such as dollar spot, rust, and blight (Mislevy and Dunavin, 1993). Fertility costs for production of bermudagrass are more than bahiagrass and other adapted forages to the Southeast (Ball et al., 2012). Weed pressure from other warm-season grasses are challenging to control through herbicide treatment and certainly costly to apply.

Alternative Forage Systems for Developing Heifers

Integration of Legumes into Warm-Season Grass Systems

Development of compatible, persistent, warm-season grass-legume mixtures could increase forage yield and quality during the summer months in the Southeastern, USA. Research has shown that warm-season grasses such as bermudagrass (*Cynodon* [L.] Pers.) respond productively to N fertilization (Evers, 1998; Osborne et al., 1999; Johnson et al., 2001; Beck et al., 2017); however, increased costs of N fertilizers have led producers to consider reincorporating legumes into pasture-based systems (Rouquette and Smith, 2010). Warm-season grass pastures in combination with legumes can improve forage quality, and at the same time, reduce the need for N fertilizer (Rosier et al., 1993; Sleugh et al., 2000; Springer et al., 2001). Forage-based heifer development systems require high-quality, persistent forages and warm-season grass-legume mixtures could be an option to achieve nutritional goals for developing heifers.

Atmospheric N_2 is fixed by legumes through a symbiotic relationship with *Rhizobium* bacteria located in nodules on the roots (Heichel, 1985). The bacteria in the nodules provide the legume with N, while the legumes provide the bacteria with photosynthetic assimilate. This relationship allows the legume to acquire necessary N without fertilizer application (Heichel, 1985). Heichel and Henjum (1991) and West and Wedin (1985) identified a positive correlation with N fixed with the amount of legume dry matter in a sward. The study found that N fixation provided an average 93% of N in legumes. Therefore, legumes reduce the necessity for applied N fertilizers. Legumes can maintain their growth and improve N availability to neighboring plants, such as grasses. The decomposition of legume residue increases the availability of N for surrounding grasses, thereby, increasing the potential for grass growth. Tissues from legume leaves and roots, as well as nodules, decompose. As the tissues decompose, N is released and made available for nearby grasses. Transfer of legume-N has been found to account for 6 - 79% of N uptake in grasses (Brophy et al., 1987; Heichel and Henjum, 1991; Walley et al., 1996).

Gil and Pick (2001) found that including alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) in eastern gamagrass (*Tripsacum dactyloides* L.) pastures increased mineralized soil N (15-62 kg N/ha/yr) compared with eastern gamagrass monoculture (2-15 kg N/ha/yr). Mixtures of smooth bromegrass (*Bromus inermis*) and kura clover (*Trifolium ambiguum*) or birdsfoot trefoil (*Lotus corniculatus*) showed fertilizer N replacement values ranging from 74-325 kg N/ha (Zemenchik et al., 2001). Fixation of N by legumes improves the growth of legumes and surrounding grasses.

Keyser et al. (2015) evaluated interseeding legumes into native warm-season grass (NWSG) mixture pastures as a way to increase forage quality and determine competitive nature of mixing NWSG and legumes. Interseeding pastures is a common practice, but has received only limited attention for NWSG. Switchgrass (SG; *Panicum virgatum* L.) and a big bluestem (BB; *Andropogon gerardii* Vitman) indiangrass (IG; *Sorghastrum nutans* Nash) blend (BB/IG), each with and without interseeded red clover (RC), were grazed (46 to 44 and 38 to 46-cm canopy heights for SG and BB/IG, respectively) by bred dairy heifers in a three-year evaluation. Establishment of RC was inconsistent leading to limited influence on forage mass and concentration of CP, and NDF. Similarly, RC had minimal influence on average daily gain (ADG; kg/d), animal days (AD)/ha, and total gain (GAIN; kg/ha). The ADG was 1.03 (SG), 1.23 (SG+RC), 1.25 (BB/IG), and 1.33 (BB/IG+RC) kg/d during the early season and 0.36 (SG+RC), 0.37 (SG), 0.54 (BB/IG), and 0.86 (BB/IG+RC) kg/d later in the season. Greater stocking density was possible with SG (234, 330, and 222 AD/ha in 2010, 2011, 2012, respectively) than BB/IG (196, 240, and 162 AD/ha in 2010, 2011, 2012, respectively), but total gain (kg BW/ha) was not consistently different. Switchgrass and BB/IG both provided acceptable forage quality and good animal performance and could be used for summer forage for bred heifers; RC had limited benefit and competed with NWSG.

Legumes improve forage quality of pastures and animal gain through relatively high yield, animal intake, and high CP (Marten et al., 1988; Van Keuren and Hoveland, 1985; Van Soest, 1982). When compared with grasses, legumes possess greater digestibility and greater CP concentration (Nelson and Moser, 1994; Van Soest, 1982). Legumes increased CP concentration in warm-season grass-legume mixtures compared with grass alone (Rosier et al., 1993). Kura clover-grass and birdsfoot trefoil-grass mixtures resulted in lower NDF and ADF and higher CP concentration compared with cool-season grass monocultures (Zemenchik et al., 2002).

Challenges exist when integrating legumes into warm-season grass systems, including establishment, pest management, and implementing a grazing system to reduce selectivity or preferential grazing of legumes. Carlassare and Karsten (2003) noted pasture composition and dynamics were influenced by plant sensitivity to warm temperatures in a mixed sward. Species composition and potential competitiveness is also influenced by available soil nutrients and moisture. Legumes are able to compete with grasses and dominate pastures in soils with low N fertility, but the reverse is true with high N fertility (Schwinning and Parsons, 1996). Planting density and competition for light, water and soil nutrients were reported by Rajaniemi (2002) as factors influencing the success of integrating legumes into warm season grass pastures.

Alfalfa Interseeded into Bermudagrass

Bermudagrass tends to have poor to moderate nutritive value when N input remains minimal (Johnson et al., 2001). Pastures containing alfalfa (*Medicago sativa* L.) can produce herbage with greater nutritive value than grass monocultures (Haby et al., 1999; Cassida et al., 2006; Beck et al., 2017). Data reported by Beck et al. (2017) indicate that at equal stocking rates, rotational grazing can maintain greater alfalfa persistence, forage nutritive quality, forage allowance, and supported increased animal performance compared with continuous grazing. Across sampling date, rotationally grazed pastures contained 2.1 percentage unit greater (P = 0.03) CP, 5.2 percentage unit less NDF, and tended to have 2.2 percentage unit greater TDN than continuously grazed pastures. In contrast, Bertelsen et al. (1993) reported no differences in CP, but greater NDF concentrations in diets selected by heifers grazing mixed pastures (tall fescue, alfalfa, orchardgrass) managed with rotational stocking compared with continuous stocking management. Likewise, Aiken (1998) reported that diets of steers grazing bermudagrass managed using continuous or rotational stocking contained similar IVDMD and CP during the summer grazing season. The improved forage nutritive quality (increased CP and TDN and decreased NDF concentration) is likely the result of the greater alfalfa content in the rotationally grazed swards due to improved alfalfa persistence.

Biermacher et al. (2012) reported similar ADG for steers grazing bermudagrass fertilized with 112 kg N/ha or bermudagrass interseeded with alfalfa; yet alfalfa yielded fewer grazing-d/ha and BW gain/ha than fertilized bermudagrass in south central Oklahoma. Beck et al. (2017) reported that steers grazing alfalfa interseeded into bermudagrass pastures in northern Arkansas had similar ADG to steers grazing bermudagrass fertilized with 56 kg N/ha, but alfalfa produced 48% greater steer grazing-d/ha and 39% greater BW gain/ha than bermudagrass fertilized with 112 kg N/ha.

White and Lemus (2015) evaluated establishment, performance and persistence of alfalfa when planted into a bermudagrass sward in Mississippi and reported that tillage did not affect any of the variables observed. However, seeding rate and time affected DM yield, forage nutritive value and plot composition. The increasing alfalfa seeding rate increased alfalfa yield in the plot but this was isolated to only the first year. Dry matter yields decreased over the three years due to the decrease in alfalfa composition, but DM yields increased throughout the growing season after the first year, suggesting bermudagrass recolonization within plots. Forage nutritive value was positively affected with as little as 20% alfalfa contribution, suggesting that even thinning stands by the third year might offer positive forage attributes.

Alternative Grass-Based Systems for Heifers

Native Warm-Season Grasses

Native warm season grasses (NWSG) are bunchgrasses indigenous to North America that actively grow during the warm months of the year, March and April through September and October. In the Southeast, weather patterns require livestock producers to continually evaluate their grazing management plans. Forage-based heifer development requires a consistent and dependable forage to utilize during the development phase. NWSG has shown to be a viable option for meeting the nutritional needs of stocker cattle (Keyser et al., 2019) and have the potential do meet requirement of growing heifers. Introduced forage species, especially C_3 grasses, are typically not well adapted to prolonged drought. In contrast once established, NWSG establish deep roots and their C_4 photosynthesis allow them to remain productive and survive prolonged drought events. These NWSG also have a lower nutrient requirement and can grow in more acidic and less fertile soil conditions than most introduced forage species (Keyser et al., 2019).

Native warm-season grasses have a reputation for being difficult to establish and easy to overgraze. Forage NWSG have not been through extensive breeding programs to enhance germination rate; therefore, they are slower to germinate, emerge and fully establish. Selecting the species best suited for grazing and growing together is essential to success. Big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), eastern gamagrass (*Tripsacum dactyloides*), indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*) are the primary NWSG utilized in the Southeast for grazing systems. These NWSG can be planted as monoculture or mixed stands, but some of these species grow in a mixture better than others. Big bluestem, little bluestem, and indiangrass grow well in mixed stands due to similarity in growth habits and palatability. Switchgrass and eastern gamagrass are suggested to be grown in monocultures due to their earlier maturing and aggressive growth characteristics (Keyser et al., 2019).

When most cool-season grasses are slowing production in June, warm-season grasses increase in production. Warm-season grass production is greatest in June, July and August, when cool-season grasses are less productive. The optimum temperature for warm-season grasses is 35°C, which is the maximum temperature for cool-season growth (Nelson and Moser, 1994). In addition to the adaptation to summer solar radiation and temperatures, many NWSG also have developed deep root systems which enable them to reach water not available to shallow rooting species, which is an additional advantage in times of drought.

Studies have shown that livestock production can be sustained NWSG pastures (Anderson, 2000; George et al., 1997; Krueger and Curtis, 1979). Backus et al. (2017) evaluated the management of NWSG for beef cattle and livestock growth potential. Experiments were conducted at 2 locations in Tennessee comparing weaned beef (*Bos taurus*) steers (268 ± 25 kg initial BW) during early-season grazing (Early; 30 d, typically corresponding to May, followed by post-dormancy biomass harvest) and full-season grazing (Full, mean duration = 98 d). For Exp. 1, which compared switchgrass, a blend of big bluestem (BBIG) and indiangrass (IG), and eastern gamagrass (EG), ADG was greater ($P < 0.05$) for BBIG (1.02 kg/d) than SG (0.85 kg/d), and both were greater ($P < 0.05$) than EG (0.66 kg/d). Grazing days for SG and EG were not different (389 and 423 animal unit days [AUD]/ha, respectively) and exceeded ($P < 0.05$) that of BBIG (233 AUD/ha) during Full. In Exp. 2 (SG and BBIG only), rates of gain were comparable to those in Exp. 1, but AUD were 425 (SG) and 299 (BBIG) AUD/ha. Such rates of gain and grazing days indicate that these grasses can provide desirable summer forage for growing cattle, and may be applicable for use in a beef heifer development system.

Optimal use of NWSG, in pasture systems, involves stocking rate suitable for livestock growth and plant persistence. Parish et al. (2019) evaluated forage nutritive value and heifer ADG at two stocking rates on mixed-sward pastures of big bluestem, little bluestem, and indiangrass. Pastures (3 replications) were stocked for 56 d during June and July in 2 yr with crossbred heifers (n = 24 heifers/yr) stratified by initial BW (288.3 ± 1.7 kg) to one of two continuous stocking rates: 1.9 heifers/ha (HIGH) and 1.2 heifers/ha (LOW). Mean forage nutritive values on a DM basis were not different between HIGH and LOW stocking rates. There was a year effect and stocking rate × day effect for TDN. At LOW, TDN decreased across days and at HIGH, TDN decreased from day 0 to day 28 but remained unchanged until day 56. There was a stocking rate × day interaction with ADG: LOW day 28 to 56 (1.20 ± 0.08 kg/day), HIGH day 0 to 28 (0.89 ± 0.08 kg/day, HIGH day 28 to 56 (0.44 ± 0.08 kg/day), and LOW day 0 to 28

(0.30 ± 0.08 kg/day). Understanding stocking rate implications to long term persistence of NWSG is essential to the sustainability of the forage.

Native warm-season grasses are attractive due to their decreased need for N application during the growing season. Chappell et al. (2020) evaluated the effects on above ground sward characteristics and beef heifer performance from NWSG blends with different nitrogen application treatments. A 2-yr study was conducted at Black Belt Research and Extension Center in Marion Junction, AL, to evaluate the effect of N fertilizer application rate on forage production characteristics, nutritive value, and animal performance of beef heifers grazing a mixture of NWSG including big bluestem, little bluestem, and indiangrass. Six, 2-ha plots were randomly assigned to one of two treatments (0 or 67 kg N/ha applied in early April; n = 3 replications per treatment). Paddocks were continuously stocked with four weaned Angus × Simmental beef heifers (initial BW 288 ± 7 kg) from late May/early June through mid-to-late August during 2018 (73 grazing-d) and 2019 (70 grazing-d), respectively. There were no differences among N-fertility treatments for mean forage mass, heifer ADG, or BCS across the 2-yr study. These data illustrate that NWSG systems may provide a viable grazing system in the summer months under reduced N inputs.

There is apprehension among beef producers about using warm-season grasses for livestock production. Native warm-season grasses are not as tolerant to intensive grazing as cool season grasses. The growing point of NWSG is much higher on the plant than on most cool-season grasses. Morphology of these grasses makes management of grazed pastures extremely important (Anderson et al., 2000). These grasses should not be grazed below 38-40 cm of growth, and be allowed to rest for approximately 30 days before the next subsequent grazing event (Keyser et al., 2019). Rest periods between grazing events are extremely important to

NWSG. This time allows for regrowth of vegetative tissue and replenishment of stored nutrients to rhizomes. Gerrish et al. (1994) reported death of big bluestem plants due to poorly managed stocking method and rest period.

Annual Forage Systems

Warm-season annual grasses are utilized in the summer months from May to August or September. Advantages of warm-season annual grasses include fast germination and emergence, rapid growth, high productivity, and flexibility of utilization. Disadvantages include the cost of annual establishment and the increased risk of stand failure due to variable rainfall in late spring and early summer. Warm-season annual grasses are best used in a rotation with small grains or annual ryegrass to optimize productivity per unit of land area. They also have great utility as transition crops prior to the establishment of improved perennial forage species. The sorghum species have prussic acid potential and are hosts for the sugarcane aphid. In addition, nitrate toxicity can be a problem during drought conditions and high N fertilization (Teutsch et al., 2018). Aldrich et al. (1990) found that rotating steers to sorghum-sudan grass (*Sorghum drummondii*) pastures significantly increased forage intake over that of steers grazing tall fescue alone; however, no differences were reported in overall steer performance. In recent years, sugarcane aphid (*Melanaphis sacchari*) has become more problematic for sorghum species raised for forage or grain. Heavy infestations of this aphid can reduce forage quality and yield (Teutsch et al., 2018).

Cool-season annual grasses are utilized for livestock grazing from December to June with adequate gains for all stages of production for beef cattle. Cool-season annuals such as annual ryegrass, wheat (*Triticum aestivum)* or cereal rye (*Secale* cereal) can be used as part of a pasture rotation within a tall fescue system or as a substitute for tall fescue winter grazing. In addition, feeding supplemental hay and concentrate can be reduced with the incorporation of cool-season annuals in cow-calf production systems (DeRouen et al., 1991). Beck et al. (2008) found that steers grazing wheat/cereal rye and annual ryegrass gained 0.88 and 0.84 kg/d, respectively, whereas those grazing toxic tall fescue gained 0.64 kg/d. The steers grazing cool-season annuals gained comparably to steers grazing novel-endophyte tall fescue (0.86 kg/d). Beck et al. (2005) reported similar results with even greater gains on the wheat/rye and annual ryegrass (1.18 and 1.07 kg/d, respectively). In addition to greater animal gains, Sanson and Coombs (2003) reported an improved pregnancy rate in cows grazing annual ryegrass (87.4 %) versus those grazing endophyte-infected (E+) tall fescue (64.2%). Poor cool-season annual stands can reduce animal gains to a level below that of toxic tall fescue (Beck et al., 2008).

Drought or excessive rain can cause establishment problems forcing late planting of cool season annual forages. Marks et al. (2019) evaluated different late planting dates to determine the impact of late-planting annual ryegrass on seasonal DM production, number of grazing days, and forage nutritive value as part of a beef heifer development program at the Sand Mountain Research and Extension Center in Crossville, AL. Two annual ryegrass varieties [‘Winterhawk’ (early maturing) or ‘Marshall’ (late maturing) ryegrass] were planted across three planting dates in winter 2016/2017 (December 15; February 1; March 1) into a prepared seedbed. Each variety × planting date combination was replicated in two 0.8-ha paddocks. Seasonal herbage accumulation, nutritive value and forage utilization were measured across the grazing season. Overall, annual ryegrass herbage accumulation ranged from 4,482 to 12,328 kg DM/ha across planting dates. December planting dates provided two to three grazing events for heifers in this project. February and March planting dates only supported one grazing event beginning in May. There were 70 d (March – June) of grazing provided for heifers in this trial. During the 1-yr evaluation, delayed planting of annual ryegrass provided adequate forage DM production to support late-spring grazing for growing heifers. The combination of relatively mild climatic conditions and adequate rainfall supported favorable growing conditions for ryegrass. February plantings were most affected by frost causing yield loss.

Gain Potential Using Forages to Develop Heifers

Developing heifers to become productive females in the cow herd is a tremendous investment in a cow-calf operation that takes several years to return a profit. Development of replacement females contributes a significant expense to beef producers due to feed costs and innate opportunity costs. The primary cost of developing heifers is the supplemental feed required to reach sufficient gains to attain puberty before breeding (Roberts et al., 2009). McFarlane et al. (2018) noted the total cost of producing the first calf from a heifer using three forage-based (big bluestem and indiangrass mixture or switchgrass) systems was $1,079/ head to $1,149/head, with tall fescue being the most expensive forage-based heifer development system, and the total cost to produce a calf from heifers developed in a drylot system ranged from $574 to $644/head greater than the forage-based systems. Fortunately, there are several options for developing heifers on forage-based systems that can reduce costs while meeting the required performance targets. Mild winters in the Southeast allow the use of cool-season forages that may significantly enhance the performance of grazing heifers. During the warm season, integration of forage legumes into grazing systems provides additional nutrients to meet the replacement heifer’s requirements, allowing her to become pregnant and enter the cow herd.

Weaned calves face weaning stress, a warm summer, and lower-quality forages, which result in lower gains. However, there are ways to overcome these factors and recover weight later in the season. Researchers have compared steady gains vs. lower gains in the initial phase and greater gains when approaching the breeding season. The results indicate that both programs can be adopted and yield similar results in reproductive performance of heifers, as long as heifers gain and achieve a minimal body weight prior to the breeding season (Freetly et al., 2001).

Stockpiling forages does increase the total herbage mass available for grazing; however, forage nutritive value is reduced in response to increased forage maturity (Wheeler et al., 2002). Therefore, a concern with stockpiled forages in heifer development systems is that BW gain may be inadequate for heifers to attain 60 to 65% of mature BW prior to breeding (Poore et al., 2006). However, Funston and Deutscher (2004) reported that developing heifers to a lower target BW (approximately 55% mature BW) reduced input costs without impairing reproductive function or subsequent calf performance.

A 3-yr study utilized 300 Angus-based, spring-born heifers to evaluate postweaning heifer development systems on gain, reproductive performance, and feed efficiency as a pregnant heifer. Heifers were blocked by BW and randomly assigned to graze corn residue (CR), upland range (RANGE), or were fed 1 of 2 diets in a drylot differing in energy levels: high (DLHI) or low (DLLO). Heifers developed on DLHI and DLLO were managed within the drylot for 166 d in yr 1, 150 d in yr 2, and 162 d in yr 3. Heifers developed on RANGE grazed winter range for an equivalent amount of days each yr as the DLHI and DLLO heifers. Heifers assigned to CR grazed for 103 d in yr 1, 84 d in yr 2, and 97 d in yr 3 before being transported to graze winter range for the remainder of the treatment period. All heifers were managed as a single group following the treatment period. Artificial insemination and natural mating were utilized during breeding. Percent of mature BW prior to the breeding season was greater for DLHI (67%) than RANGE (59%) and CR (58%). Pregnancy rates to AI were not different among treatments (59 ± 6%), and final pregnancy rates were also not different (87 ± 4%). A subset of AI-pregnant heifers from each treatment were placed in a Calan gate feeding system. Heifers were allowed a 20-d acclimation period before beginning the 90 d trial at approximately 170 d in gestation. Heifers were offered ad libitum hay; amount offered was recorded daily and orts collected weekly. Initial BW was not different among treatments (459 ± 11 kg). Body weight at the end of the trial (497 ± 17 kg) was also not different. Intake was not different, either as DMI (10.00 ± 1.07 kg) or residual feed intake (0.018 ± 0.190 kg). There was no difference in ADG (*P* = 0.36, 0.42 ± 0.23 kg/d) among treatments. Although the total development cost was not different among treatments, there was a $41 difference between the mean of the most expensive diet (DLHI) and the mean of the two least expensive diets (CR and RANGE). Developing heifers to a greater prebreeding BW did not influence subsequent AI or overall pregnancy rates or feed efficiency as a pregnant heifer (Springman et al., 2017).

McFarlane et al. (2017) developed a study to determine the effect of stockpiled forage type and protein supplementation on VFA production, serum metabolites, and BW in yearling beef heifers. Over 2 yr, spring-born, Angus crossbred yearling beef heifers (n = 42; 305 ± 2.9 kg initial BW) were randomly assigned to 1 of 3 forage pasture types: 1) endophyte-infected tall fescue (TF), 2) a big bluestem and indiangrass combination (BI), or 3) switchgrass (SG). Each pasture was then randomly assigned to receive either 1 of 2 isonitrogenous CP treatments: 1) 0.68 kg heifer/d of dried distiller's grains with solubles (DDGS; 28% CP and 88% TDN) or 2) 0.22 kg heifer/d of blood meal and fish meal (BF; 72.5% CP and 69.5% TDN), resulting in a 3 × 2 factorial arrangement of treatments. Treatments were initiated in January and terminated in April in both years of the study. Body weights and blood samples were collected approximately every 28 d from initiation of grazing until the end of the trial. Heifer BW change from January to February and overall BW change were greater for TF heifers. However, BW change from March to April was not different among forage types. Supplement type did not influence BW or BW change from January to February and from January to April; however, heifers fed DDGS had greater BW gain from March to April. Heifer BW change from February to March exhibited a forage type × supplement interaction, with BF-fed heifers gaining more BW on BI pastures than DDGS-fed heifers. Serum glucose concentrations, ruminal acetate, and the acetate:propionate ratio were greater for SG heifers. However, circulating serum non-esterified fatty acids (NEFA) and urea N (SUN) concentrations were not different among forage types. Serum glucose and NEFA concentrations were not influenced by supplement type. Circulating SUN concentrations were greater in BF-supplemented heifers. Ruminal acetate tended to be greater and butyrate concentrations were greater for BF-supplemented heifers. The acetate:propionate ratio was not influenced by supplement type. These results suggest that a compensatory gain period prior to breeding would be needed for these native warm-season species to be a viable option for growing and developing replacement heifers in the southeastern, USA.

Reynolds et al. (2018) investigated the effects of post-weaning forage grazing on heifer development and fertility. Weaned (7.5-mo-old) crossbred heifers (n = 139) were strip-grazed on alfalfa (A) or grass (G) pastures for 56 d with 2 replicates per treatment. Heifers were weighed every 2 wk and forage samples were collected on wk 1, 4, and 8. After grazing, all heifers were managed similarly. At 10 d before estrous synchronization, heifers weighed 381.7 ± 2.9 kg, and reproductive tract score (RTS) and antral follicle count (AFC) were determined by ultrasonography. Heifers were synchronized using the 14-d CIDR Split-Time AI protocol. Serum progesterone concentrations were determined at ultrasonography and synchronization. Heifers were considered pubertal when progesterone concentration was >1 ng/mL. A mixed model was used to determine effects of forage, wk, and their interaction on diet composition, and forage effects on heifer bodyweight at the end of treatment (BWT) and at ultrasonography (BWU), ADG, RTS and AFC. Correlations between variables were also tested. Crude protein was affected by forage and wk, and averaged 15.5% of DM for A and 8.87% for G. There was a forage × wk interaction on acid detergent fiber concentration. Forage did not affect BWT or BWU, but A heifers gained more than G heifers (0.41 ± 0.03 vs. 0.20 ± 0.03 kg/d) Both BWT and BWU were correlated with RTS, regardless of treatment. There was no effect of forage type on pubertal status or pregnancy rate. Replacement heifers were grazed on alfalfa or grass pastures after weaning, which resulted in different growth rates but did not compromise fertility measures (Reynolds et al., 2018).

Metabolomic Profiles and Their Association with Fertility Indication in Heifers

Heifer Reproductive Failure

Lamb et al. (2014) calculated an average ratio for the economic impact that one infertile heifer can have on a herd. The average loss due to failure to become pregnant during the breeding season is ~$165/heifer. Taking this into consideration, the NASS estimated total heifer infertility in the United States exceeding $4.7 billion annually (Lamb, 2014). Reproductive failure is one of the major factors that affects the profitability of the beef cattle production industry (Diskin, 1980). Poor fertility remains the major reason for culling in the beef cattle industry, which reduces the longevity of the herd (Wathes et al., 2014).

Fertility can be defined as the natural capability to produce offspring. In cattle, a number of metrics exist by which “fertility rate” is reported, including the number of days open, or calving to conception interval, and pregnancy rate to first and/or subsequent services. Regardless of the variation in measures of reproductive success, pregnancy is the endpoint and it is the culmination of precisely ordered, well-orchestrated events that which commence with the timely resumption of ovarian activity post-calving. The onset of cyclicity should initially result in the selection and growth of a healthy follicle that encloses a competent oocyte, and ultimately in, oestrus, ovulation, fertilization and uterine attachment by a viable embryo (Leroy et al. 2011). The concomitant development of a functional corpus luteum should provide an appropriate environment, through optimal progesterone secretion, in which the embryo can grow and develop (Diskin & Morris 2008).

Abraham (2017) defined fertility as a heifer that shows the desire to mate, the capacity to conceive, nourish a growing embryo, and successfully expel a live calf at 12-month intervals Studies show the estimated fertilization rate (natural or AI) for oocytes is 90% for beef cattle. However, the estimated calving rate from a single service is between 40% – 55%, which suggests an overall embryonic and fetal mortality rate (not including fertilization failure) of 35% – 50%, with the majority of embryonic losses (70% – 80%) occurring during the first three weeks of pregnancy (Diskin et al., 2006). Functional causes of infertility tend to affect individual heifers, but when combined, these infertile heifers can make a large impact on the overall herd (Abraham, 2017). Most functional causes can occur from an endocrinological abnormality, which can reflect fertility issues. These abnormalities include, but are not limited to: nondetected estrous (silent estrous), anestrus, ovulatory defects, persistent corpus lutea, luteal deficiencies, cystic ovaries, and repeat breeders (Abraham, 2017).

Metabolomics

Metabolomics is a rapidly growing field that has the potential to play a major role in improving the diagnosis and treatment of complex issues in health and disease. The term “metabolome” was first defined by Oliver et al. in 1998 as the “quantitative complement of all of the low-molecular-weight molecules present in cells in a particular physiological or developmental state” (Oliver et al., 1998). Metabolomics reflects events that are well down-stream of gene expression, and it gives valuable information about the metabolism of cells that other “-omics” technologies cannot accomplish (Bracewell-Milnes et al., 2017).

A better understanding of the metabolic effects of various etiologies of subfertility may aid the development of targeted therapeutics or lead to the identification of non-invasive biomarkers for diagnostic and prognostic purposes (Baskind et al. 2011). The collection of low-molecular weight compounds in an organism or biological sample is defined as the ‘metabolome’ (Wishart 2007). Low-molecular weight metabolites represent the intermediates of the cell’s regulatory processes; their individual profile is referred to as a ‘metabolic fingerprint’ (Kell 2005). Since the metabolome is related to an organism’s genotype, physiology and environment, it provides a powerful tool to assess the physiological state and to assist in the identification of possible biomarkers for fertility (Baka & Malamitsi-Puchner 2006, Sinclair et al. 2008).

From a conceptual level, the genome gives rise to the transcriptome, and the transcriptome gives rise to the proteome. The proteome acts on small molecules within an organism (both endogenous and exogenous) known as the metabolome. There is evidence that feedback interactions exist at all levels, and these levels are sensitive to environmental cues, environmental influences, nutrition, disease states, toxicants, etc. The physiological status of an organism is ultimately affected by the varying combination of feedback interactions throughout the genome, transcriptome, proteome, and metabolome (Phillips, 2018).

Phillips et al. (2018) utilized metabolomic profiling to identify metabolites in the blood plasma that may be useful in identifying infertile heifers at the time of artificial insemination (AI). Prior to AI, phenotypic parameters including body condition, weight, and reproductive organ measurements were collected. These were determined to be ineffective at differentiating between fertile and infertile heifers. Analysis of the resulting metabolomic profiles revealed 15 metabolites at significantly different levels (t-test $P \leq 0.05$), with seven metabolites having a greater than 2-fold difference (t-test $P \leq 0.05$, fold change ≥ 2, ROC-AUC ≥ 0.80) between infertile and fertile heifers. Phillips et al. (2018) further characterized the utility of using the levels of these metabolites in the blood plasma to discriminate between fertile and infertile heifers.

Bender et al. (2010) evaluated metabolite concentrations in follicular fluid and blood to explain differences in fertility between heifers and lactating cows (Bender et al., 2010). Within the last three decades, there has been a rapid decline in the fertility of dairy cows. Bender et al.(2010) utilized metabolomics to investigate metabolic differences between the follicular fluid of the dominant follicle of lactating cows and the follicular fluid of the dominant follicle of heifers. Follicular fluid was collected over three phases of follicular development: newly selected dominant follicles, pre-ovulatory follicles prior to estrous, and post-LH surge follicles (Bender et al., 2010). Twenty-four fatty acids and nine aqueous metabolites were found significantly different when comparing cows with heifers. Palmitic acid and stearic acid (saturated fatty acids) were higher in the follicular fluid of cows, and docosahexaenoic acid (saturated fatty acid) was higher in the follicular fluid of heifers. Bender discovered that, if there is a higher concentration of saturated fatty acids in cows, oocyte maturation and early embryo development will be negatively impacted. Results suggested that the overall follicular microenvironment in cows places oocytes at a developmental disadvantage compared with the microenvironment for heifers. This overall conclusion could contribute to fertility differences in heifers and cows alike (Bender et al., 2010).

University or Extension Heifer Development Programs

Overview

Heifer development programs provided or facilitated by Land Grant Universities emerged as one possible solution for addressing the negative impact of poor replacement female development on economic and generational sustainability for individual beef cattle producers and statewide beef cattle herds. While the general objective of these programs is similar, they vary widely among states in methodology and scope. One common approach is to arrange for centralized locations, on either university- or privately-owned property, to which producers consign heifers for development through the first breeding season with resulting pregnant heifers sold or returned to the farm of origin. Within this approach, age of heifers at delivery and management protocols also differ widely among programs. Another common approach is to offer university-developed standards by which producers, who make heifer development part of their business model, rear heifers that are eventually marketed under that specific program’s branding. Yet again, genetic standards and management protocols vary among programs with some programs also having multiple protocols under which heifers are sub-branded for marketing (Rhinehart, 2018).

Centralized Location Heifer Development

The “Miss Premium” heifer development program was initiated in Mississippi in 2008 where 65 heifers were consigned and delivered in late November of the inaugural year. On arrival, the heifers received booster vaccinations (modified live) for respiratory diseases and an initial vaccination for *Campylobacter fetus* (Vibrio) and leptospirosis (Lepto). They also received a Vibrio/Lepto booster prior to breeding. It is important to vaccinate against Vibrio and Lepto to prevent early abortions and delayed conception. Weights were also recorded at delivery and used as a baseline to calculate average daily gain (ADG). Nutritional management was based on a total mixed ration of ryegrass baleage, corn gluten feed, dried distillers grain, soy hulls, peanut skins and a complete mineral mix. The heifers were kept in grass traps with adequate bunk space, shade and shelter. The original goal of this program was to maintain an ADG of roughly 0.68 kg. Due to hybrid vigor, exceptional performance of the consigned heifers and excellent management, the actual ADG was more than 0.91 kg. Pelvic area measurements and reproductive tract scores were taken in mid January. The width and height of the pelvis were measured, using a specially designed caliper, at the narrowest point. Those distances are multiplied together to estimate the pelvic area in square centimeters. Originally, this measurement was related back to the age and weight of the heifer through a series of calculations to determine the maximum size calf that heifer could calve without assistance. Now, the most common use for pelvic area measurements is to set a cutoff and cull the heifers that do not meet or exceed it. For instance, the cutoff value for "Miss. Premium" heifers is 150 cm^2 when measured at 12 months of age and roughly 363 kg. Estrous synchronization for this project began in mid February with CIDR® application and injection of Cysterelin® (GnRH). Seven days later, the CIDR® was removed and an injection of Lutalyse® (Prostaglandin) was given. Each heifer was artificially inseminated (AI), to a bull the consigner chose, 12 hours after the first display of standing heat. They were put with a clean-up bull 10 days after insemination. For this particular group of heifers, 8 did not show standing heat and received another injection of Lutalyse® ten days after the first. One heifer responded to that injection and the others were put with the clean-up bull without having been artificially inseminated. Pregnancy was diagnosed, by ultrasound, 30 days after AI. The single-service AI conception rate for this group was 79.6%

(43/54). The heifers were returned to their farm of origin 50 days after the latest diagnosed pregnancy to avoid pregnancy loss from shipping stress (Rhinehart, 2009).

The University of Tennessee developed the Tennessee Beef Heifer Development Program with a partnership between the Tennessee Department of Agriculture to construct heifer development facilities on the AgResearch & Education Center in Lewisburg, TN. The Tennessee Farmers Cooperative was also a partner through donation of the working and feeding facilities and continued consultation for management of health and nutrition. At weaning, heifers were either weaned and backgrounded then sent to the program, or they are weaned and backgrounded at the facility, then development is initiated. Heifers are raised through their first breeding season and at the conclusion of the development, the producers have the option to retain the heifer(s) as replacements or market them through the value-added sale.

When the heifers are delivered, they should have received at least one round of vaccinations. They are vaccinated again at delivery and given another booster vaccination two weeks later. Also at delivery, blood is drawn and sent to the Kord Diagnostics Laboratory to be tested for BVD-PI, BLV and anaplasmosis. Internal and external parasites are controlled throughout the entire program.

The program is not a "gain test," so heifers are managed to reach approximately 70% of their expected mature weight before the start of the breeding season. For most heifers, that averages to 0.68 kg/d from weaning until breeding. Target weight is accomplished by grazing tall fescue in the spring and fall, native warm season grasses in the summer, and corn silage in the winter when forages are dormant. Supplemental feed is utilized when needed to balance nutrient requirements if forages alone are not adequate. High-quality mineral supplement is offered year-round. Prior to the breeding season, reproductive tract scores are collected to ensure heifers are pubertal. At that time, their pelvic area is measured to make sure they can deliver a normal-size calf if it is presented correctly at calving. Their first breeding is performed by timed AI to a synchronized estrus. Heifers are inseminated again, up to two more times, if they show heat during the breeding season. Clean-up bulls are not utilized in this program. Any sire can be used as long as it meets Tennessee Agriculture Enhancement Program (TAEP) genetic requirements as a calving-ease bull for that breed and semen may be purchased from a certified collection facility. Pregnancy status is checked (by ultrasound) as needed throughout the breeding season.

Records are maintained and sent as reports on growth (average daily gain and weight per day of age), hip height, frame score, disposition score, pelvic area measurement, and carcass ultrasound (rump fat, rib fat, ribeye area, intermuscular fat). For consigners who plan to sell their heifers, these data can also be a valuable marketing tool. Spring born heifers (January – March) are delivered to the facility in early October, bred in April through mid-June, and returned the following September. Fall born heifers (September – November) are delivered in early July, bred in December through February, and returned in May. The cost of the program is set prior to delivery for each season, and payment is due at the end of the program when the heifers are returned back to the consigners. Additionally, the TAEP provides a scholarship covering a significant amount of the development cost for up to four heifers per owner.

Some university and Extension systems lack infrastructure or lose a facility due to university expansion or zoning to host a heifer development program, and rely on producers to accommodate a heifer development program. Iowa State demonstrated a heifer development project that was a five-year project conducted on the site of the former Jackson County Farm north of Andrew, Iowa, for four years and on an area producer's farm for the fifth year. Heifers arrived around December 1 each year and the average number of heifers each year was 43 with a low of 37 and high of 47. After a 30+ day warm-up period the heifers were put on a 112-day test from early January to late April. They were fed a shelled corn and legume-grass hay ration consisting of between 13% and 14% crude protein and a range of 0.2 to 0.26 Mcal/kg of NEg over the five years. During the 112-day test, heifers gained 0.84, 0.81, 0.68, 0.74 and 1.0 kg/d, respectively, for years 1992 through 1996. The actual average breeding weight was less than the target weight in three years by 2.3, 5.4 and 10 kg, and exceeded the target weight in two year by 7.7 and 12.7 kg. Estrus synchronization used a combination of MGA feeding and Lutalyse injection. Heifers were heat detected and bred 12 hours later for a three-day period. On the fourth day, all heifers not bred were mass-inseminated. Heifers then ran with the cleanup bull for 58 d. The average synchronization response rate during the project was 79%. The overall pregnancy rates based on September pregnancy averaged 92%. The five-year average total cost per head for heifer development was $286.18 or about $.85 per d. Feed and pasture costs averaged 61% of the total costs (Harvey, 1998).

CHAPTER 3

INFLUENCE OF HARVEST STRATEGY ON YIELD AND NUTRITIVE VALUE OF ALFALFA-BERMUDAGRASS MIXTURES IN THE SOUTHEAST[1]

Introduction

There are over 1,618,742 ha of pasture and hayland in Alabama. This acreage supports 1.2 million head of beef cattle that are found throughout the state. In the Southeast, warm-season perennial grasses are a predominant part of year-round forage systems in the region. However, these forages are relatively high in fiber and low in digestible energy concentrations, making it necessary to provide additional supplementation when nutritional needs are not met for livestock. Bermudagrass (*Cynodon dactylon* L. Pers.) is the primary warm-season perennial forage in the Southeast due to its yield potential and moderate forage quality. Bermudagrass requires increased concentrations of N and K to achieve desired forage biomass yields. Increased costs of N and K fertilization, both synthetic and organic, have led producers to explore alternative management strategies such as the incorporation of legumes as an alternative source of N inputs (Rouquette and Smith, 2010).

Challenges exist in legume establishment, management, and persistence when integrated into warm-season perennial grasses (Muir et al., 2011). However, continued work on this topic in the Southeast has focused on identifying legume species that may be a fit for this system as a potential way to improve forage nutritive value and reduce the need for N fertilizer in warm-season perennial grass-based systems (Rosier et al., 1993; Sleugh et al., 2000; Springer et al., 2001). Although there are many benefits to including alfalfa in a forage program, disease and

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pest pressures have historically limited the adoption of alfalfa in southeastern states (Lacefield et al., 2009). However, this trend has changed with the release of new semi- and non-dormant alfalfa varieties. These newer varieties have greater tolerance to disease and pests, making them suitable for production in the Southeast (Bouton et al., 1997).

The addition of any legume to a grass monoculture has been demonstrated as a method to improve forage quality of the stand. Beck et al. (2017) found that the inclusion of alfalfa and/or clover to a bermudagrass monoculture increased concentrations of CP and TDN, especially during the early season, and helped extend the forage growing season. However, there is little published information on how newer varieties of alfalfa perform and persist when incorporated into bermudagrass in the Southeast. The objective of this research was to determine the influence of harvest intensity and frequency combinations in alfalfa-bermudagrass systems on aboveground sward canopy yield, nutritive value, and persistence characteristics under simulated grazing. This research will help establish more defined management parameters that can be further evaluated in grazing trials to develop recommendations for mixed alfalfa-bermudagrass systems in the Southeast.

Materials and Methods

Research Site

A 2-year, simulated-grazing, small-plot trial was conducted during the 2018 and 2019 growing seasons at the SMREC in Crossville, AL (32°29'07.1"N latitude, 85°96'67.9"W longitude). Research plots were located in a previously established ‘Tifton 44’ bermudagrass hayfield comprising of Hartselle fine sandy loam and Hartsell (Wynnville) fine sandy loam (Web Soil Survey, 2020).

Treatments and Experimental Design

Thirty-six plots (1.5 m × 4.6 m) were organized into four blocks, each comprised of nine plots representing a 3 × 3 factorial of harvest height (5, 10, and 15-cm) and harvest frequency (2, 4, and 6-wk) treatments (Figure 1). Treatments represent common defoliation management practices on perennial warm-season grass pastures in the Southeast.

101-1	102-3	103-2	201-3	202-1	203-2
104-3	105-2	106-1	204-1	205-2	206-3
107-2	108-1	109-3	207-2	208-3	209-1
301-1	302-3	303-2	401-1	402-3	403-2
304-2	305-1	306-3	404-3	405-2	406-1
307-3	308-2	309-1	407-2	408-1	409-3

Treatments

Havest Frequency	Harvest Height
1- 2 wk	1- 2 inch / 5 cm
2 - 4 wk	2 - 4 inch / 10 cm
3 - 6 wk	3 - 6 inch / 15 cm

Figure 1. Plot map for ‘Bulldog 505’ Alfalfa- ‘Tifton 44’ Bermudagrass Study.

Plot Establishment and Maintenance

Prior to alfalfa establishment in the spring of 2018, ‘Tifton 44’ bermudagrass was clipped to a 5-cm stubble height, and chemical dormancy was induced with glyphosate (0.61 kg a.i.ha^{-1}) on February 9, 2018. On March 6, 2018 ‘Bulldog 505’ alfalfa seed was planted into dormant ‘Tifton 44’ bermudagrass using a no-till drill (John Deere) on 36-cm rows at a rate of 28 kg ha^{-1}, and no deeper than 1.3 cm following alfalfa establishment recommendations for the Southeast (Hancock et al., 2015).

A soil test conducted in fall 2017, results were P (VH 120), K (M 75), Mg (H 126), Ca (H 1240) and average soil pH (5.6). During the previous harvest season in 2017, soil amendments were applied for hay production: ammonium nitrate (246 kg ha^{-1}), muriate of potash (373 kg ha^{-1}), and limestone (2,241 kg ha^{-1}) according to soil test recommendations. In March 2018, 34 kg ha^{-1} of P, 67 kg ha^{-1} K, and 2 kg ha^{-1} B were applied at the time of planting. During the experimental growing season, plant tissue samples were taken from 'Tifton 44'-alfalfa plots prior to the August cutting in 2018 to determine possible nutrient deficiencies, and macro- and micronutrient applications were determined as needed. Plant tissue samples indicated a need for Mb. Sodium molybdate was applied on July 6, 2018 at a rate of 0.56 kg ha^{-1}.

After initial alfalfa emergence and throughout the life of the stand, plots were screened for insect presence using the sweep net method (5 sweeps/plot), and insecticide applications were applied as needed to control common alfalfa pests. The only pest identified in the stand was blister beetles in August, 2018. These beetles contain cantharidin in their blood, which, if consumed, is potentially toxic to horses. Once identified, they were treated with carbaryl at 3.5 liters ha^{-1}.

To control annual grass weeds, pendimethalin (Prowl H20; BASF Ag Products, Floram Park, NJ) was applied on Jun 14, 2018 at a rate of 2.1 kg a.i. ha^{-1}. In the summer of 2019, rainfall was limited, and 2.54 cm of irrigation was applied on May 30. Plots were clipped according to their respective harvest intensity × frequency treatment using a Wintersteiger forage harvester (Wintersteiger Inc., Salt Lake City, UT) on June 15, June 29, July 13, July 27, August 15, August 24, and September 7, 2018. Based on alfalfa persistence, only visual stand ratings were collected in 2019. Visual assessment of alfalfa persistence in May 2019 revealed less than 5% alfalfa persistence across the treatment area across all plots, regardless of treatment.

Response Variables

Forage Yield

Forage yield was measured as part of the simulated grazing trial by harvesting a yield strip (1.2 m × 6 m) from the center of each plot to the randomly assigned harvest height treatment level of 5, 10, or 15 cm, respectively. Plots were harvested every 2, 4, or 6 weeks depending on their respective harvest frequency treatment assignment. All harvested material was collected, and fresh weights were recorded following field sampling. A subsample was collected from each strip for further analysis and moisture correction calculations. Subsamples were weighed fresh and dried in a forced air oven at 60˚C for at least 48 hours until a constant weight was reached to determine DM concentration. Subsample data were used to convert the total aboveground fresh weight to total DM yield within plot.

Nutritive Value

Subsamples were ground to pass through a 1-mm screen using a Wiley mill (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) and analyzed for nutritive analysis. Ground forage samples were scanned for concentrations of CP, ADF, NDF, and TDN using Near Infrared Reflectance Spectroscopy (NIRS) installed with equations developed by the NIRS Forage and Feed Testing Consortium (Hillsboro, WI) with a 10% check conducted using wet chemistry analysis for CP and fiber constituents at the Auburn University Soil Laboratory.

Canopy Cover, Botanical Composition, and Stand Density

Canopy cover, botanical composition, and stand density were measured at the initial, mid-point, and final harvests. Canopy cover was measured pre-harvest by visually estimating the percent stand of each component (alfalfa, bermudagrass, weeds and/or bare area) to the nearest

5% within three randomly placed 0.1-m^2 quadrats within the plot. Within each quadrat, material was harvested via hand clipping to a 2-cm height and collected for botanical composition analysis. Material was separated into botanical components (alfalfa, bermudagrass, and weeds), dried, and each individual component was weighed to determine component yield. Alfalfa stand density was rated by counting the number of alfalfa plants within three 0.1-m^2 quadrats placed at random locations within the plot.

Dry Weight Rank

Dry weight rank was measured at every harvest according to methods of Mannetje and Haydock (1963). Five 0.1-m^2 quadrats were placed randomly within each plot, and canopy height was measured within quadrat using a pasture ruler. Species within quadrat were visually assessed and ranked as first, second, or third based on relative contribution to stand. Each component was multiplied by 70.2, 21.1, and 8.7, respectively, which represent fixed multipliers described by Mannetje and Haydock (1963) and added to give the dry-weight percentage of each species in the stand.

Weather Data

Weather instruments operated by AWIS Weather Services, Inc. collected daily average ambient temperatures and daily total precipitation data throughout the experimental period. Weather instruments were located in Crossville, AL. Temperature and 100-yr average data and total precipitation are reported in Figures 2 and 3.

Statistical Analysis

Forage yield, nutritive value, canopy cover, botanical composition, stand density, and dry weight rank were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC) for a randomized complete block design with a 3 × 3 factorial arrangement of treatments.

Independent variables for harvest height, harvest interval, date, and their interactions. Block was considered a random variable. Treatment means were separated using the PDIFF option of the LSMEANS procedure (SAS Institute, 1994) and were determined to be significant when $\alpha = 0.05$; tendencies were described when $\alpha \geq 0.05$ and ≤ 0.10.

Results and Discussion

Monthly total precipitation and 100-yr average monthly total precipitation are presented in Figure 2. Monthly mean temperatures and 100-yr average monthly mean temperatures in Crossville, AL are presented in Figure 3. In 2018, rainfall followed the 100-yr average, except in August when rainfall was below average. Monthly mean temperatures tended to follow the pattern of the 100-yr average, except in Sep, when temperatures were greater. Fall growth of hybrid bermudagrass has shown to decrease as a response to decreasing photoperiod length, even when water, fertilizer and temperature were adequate (Burton, 1988).

Figure 2. Average rainfall in Crossville, AL for 2018 project months.

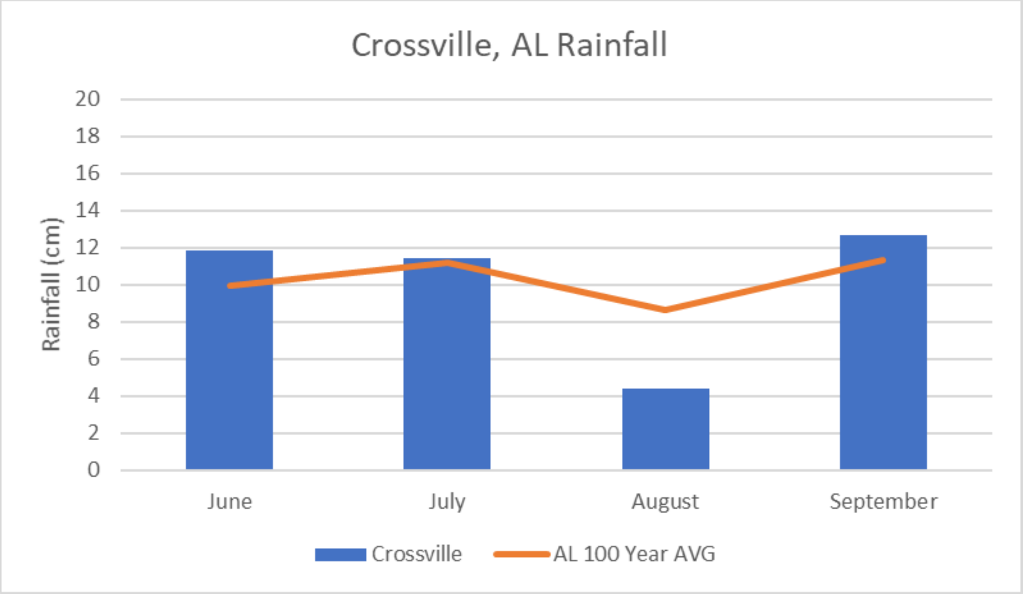
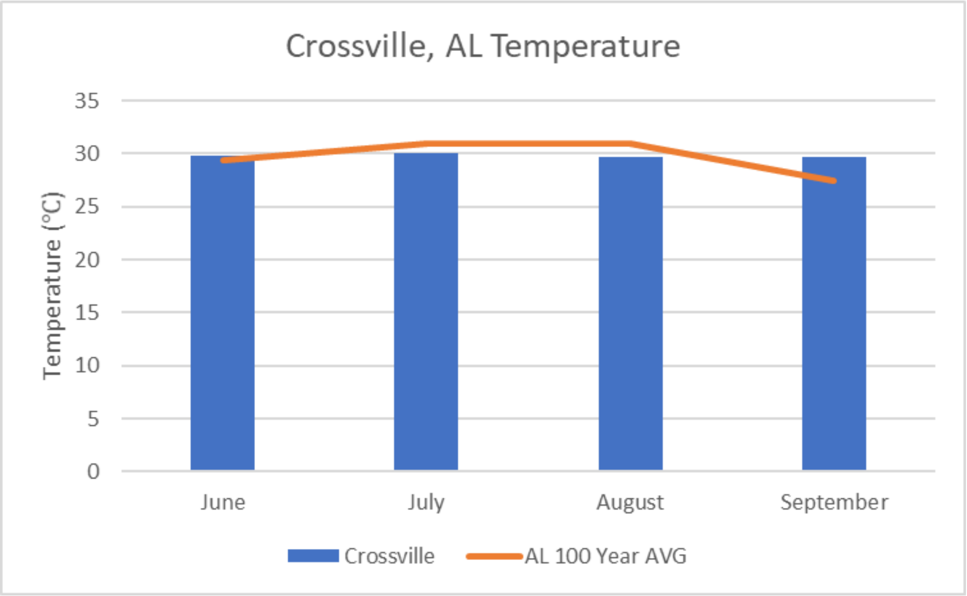


Figure 3. Average Temperature in Crossville, AL for 2018 project months.



Forage Yield

There were significant effects of harvest height ($P < 0.0001$) and harvest frequency ($P < 0.0001$) on forage yield of the alfalfa-bermudagrass mixture, but the interaction of these management parameters was non-significant ($P = 0.7803$). Forage yield decreased as harvest height increased ($P < 0.0001$; 12,969 for 5 cm, 9,182 for 10 cm, 6,137 kg DM ha^{-1} for 15 cm, respectively). When alfalfa-bermudagrass was harvested every 2 wk, forage yield was less (7,672 kg DM ha^{-1}) than longer regrowth intervals of 4 and 6 wk. However, there was no difference ($P = 0.6336$) in yield when the mixture was harvested every 4-wk (10,171 kg DM ha^{-1}) or 6-wk (10,445 kg DM ha^{-1}). With longer regrowth intervals between harvests, forage yield increased. When harvesting at a short stubble height (5 cm), yield would be expected to increase relative to other cutting heights due to less residual forage remaining post-clipping and more forage mass being physically removed during the harvest process. This short stubble height also encourages vegetative regrowth from remaining residual forage post-clipping. However, long-term implications of low harvest heights in mixed alfalfa-bermudagrass stands would be lack of persistence for alfalfa and bermudagrass. Yield characteristics observed for mixtures harvested at 10 to 15 cm may be related in part more dense, stemmy material remaining as residual forage post-harvest than the 5 cm clipping height, which represent a significant portion of the total plant canopy forage mass characteristics (Sbrissia et al., 2003). Sbrissia et al. (2003) observed increasing tiller mass, leaf mass per tiller, and tiller volume with increasing residual forage remaining post-grazing in Tifton 85 bermudagrass stands. Harvest intervals of 4- and 6-wk in this study supported greater yield than the 2-wk harvest frequency. Holt and Conrad (1986) reported that bermudagrass forage yield increased 0.15 Mg ha^{-1} for each day of advancing age between 14 and 56-days post-harvest. Studies in the southern Piedmont region of the US on vegetatively propagated ‘Midland 99’ and ‘Tifton 44’ bermudagrasses illustrated that under adequate moisture, harvest intervals of 4 and 6 wk maximized DM yields on clay loam and sandy soils, respectively (Fike et al., 2005). Similar results also were observed by Mandebvu et al. (1999), who found that longer harvest intervals (49 and 56 d) maximized Tifton 85 and Coastal bermudagrass yields, producing on average 6 and 6.4 Mg DM ha^{-1}. Ethredge et al. (1973) found that both harvest interval and stubble height had a major influence on Coastal DM yields, noting that shorter cutting heights (0 cm) resulted in greater DM yield than taller stubble (14 cm; 9.6 vs. 6.5 Mg DM ha^{-1} for 0 and 14 cm, respectively). Likewise, they found that longer harvest intervals resulted in the highest annual DM yields (6.9 vs. 8.2 Mg DM ha^{-1} for 21 and 35-d intervals, respectively). Aiken et al. (1995) evaluated the effects of harvest frequency and height for a crabgrass × ‘Coastal’ bermudagrass mixture over 2 consecutive years in Alabama with 3 different cutting heights and 4 different harvest intervals. The authors reported a linear decrease in DM yield as cutting height increased, but yield increased linearly with harvest frequency intervals as the growing season progressed from mid-to-late summer, which is similar to the results in the present study.

Nutritive Value

Harvest intensity effects on CP and TDN (5 cm, 10 cm, 15 cm) are presented in Table 1. When harvested at 5 cm in height, the alfalfa × bermudagrass mixture had greater CP concentration than at 10-cm and 15-cm. However, there was no difference ($P = 0.1069$) between the 10 cm and 15 cm harvest intensity levels. Forage TDN concentration was not different ($P = 0.2240$) between the 5-cm and 10-cm harvest intensities, but 5 cm ($P = 0.0027$) and 10 cm ($P = 0.0683$) had greater forage TDN concentration than 15-cm harvest intensity. Effect of harvest frequency on forage concentrations of CP ($P < 0.005$) and TDN ($P < 0.005$; g kg^{-1} DM basis, respectively) in alfalfa × bermudagrass mixtures is presented in Table 2. Forage CP concentration at 2-wk frequency was greater than at 4-wk and 6-wk . Similiarly, forage TDN concentration at the 2 wk harvest interval increased compared to the 4 wk and 6 wk frequencies of defoliation. There were no interactions of harvest intensity × harvest frequency for CP ($P = 0.6913$) or TDN ($P = 0.6045$) concentration. Stringer et al. (1996) reported CP concentrations of 215, 207, and 194 g kg^{-1} DM for alfalfa × bermudagrass mixtures with alfalfa planted in row spacings of 20 cm, 40 cm and 60 cm, respectively. Forage CP concentrations reported in the present study were less than those reported by Stringer et al. (1996), most likely due to the weed pressure and lack of alfalfa persistence throughout the season. Forage N and IVDDM concentrations reported by Rao et al. (2007) indicate an increase in values when cool-season annual legumes [grass pea (*Lathyrus sativa* L.) or lentil (*Lens culinaris* Med. 'Indianhead')] are interseeded into bermudagass compared to monoculture stands of bermudagrass. The 3-yr study reported forage N and IVDDM concentrations for the bermudagrass-grass pea treatment were 34 and 6 g kg^{-1} DM greater than for bermudagrass alone, but N and IVDDM concentrations of the forage mixture were intermediate between the increased N rates.

Table 1. Effect of harvest intensity on forage concentrations of CP (g kg^{-1} DM basis) and TDN (g kg^{-1} DM basis) in alfalfa×bermudagrass mixtures.

Harvest Intensity	CP*	TDN †
5 cm	116^{a}	605^{a}
10 cm	107^{b}	598^{a}
15 cm	100^{b}	587^{b}

a-b Within a column, least squares means with differing superscripts differ ($P < 0.05$).
*CP = Crude protein (% DM basis).
†TDN = Total digestible nutrients (% DM basis).

Table 2. Effect of harvest frequency on forage concentrations of CP (g kg^{-1} DM basis) and TDN (g kg^{-1} DM basis) in alfalfa×bermudagrass mixtures.

Harvest Frequency	CP*	TDN†
2 wk	121^{a}	619^{a}
4 wk	106^{b}	592^{b}
6 wk	97^{c}	579^{c}

a-b Within a column, least squares means with differing superscripts differ ($P < 0.05$).
*CP = Crude protein (% DM basis).
†TDN = Total digestible nutrients (% DM basis).

Canopy Cover

Canopy cover change throughout the season is presented in Table 3 and is a visual estimate of contribution of each species component in the stand. Alfalfa contribution was greatest ($P < 0.0001$) in the early part of the season and declined through the end of the season in 2018. Similarly, bermudagrass presence decreased ($P < 0.0001$) throughout the summer, whereas weed pressure increased ($P < 0.0001$) across the growing season. Harvest height (5, 10, or 15 cm) was not significantly different for alfalfa ($P = 0.3667$), bermudagrass ($P = 0.4822$), or weeds

(P = 0.5890). Harvest frequency effects and date × frequency interactions for alfalfa contribution to canopy cover are presented in Table 8. Alfalfa contribution was least (P < 0.0001) at the 2-wk interval compared with longer regrowth intervals following harvest. However, alfalfa quantity in the stand did not differ between the 4- and 6-wk harvest frequencies. The estimated N contribution from alfalfa is 224 kg ha^{-1} with a N fertilizer cash value of $0.45 kg of N when established as 30% of a mixture (Jennings, 2016). This target level of stand contribution was not achieved at the first sampling date for the study, and alfalfa component percentage decreased with subsequent harvest management. Date × harvest frequency tended (P = 0.0809) to impact alfalfa contribution. Early-season there was a tendency (P = 0.0834) for alfalfa contribution to be greater when harvested every 4 wk compared with every 2 wk. Alfalfa contribution was greater when harvested every 6 weeks compared with every 2 wk (P = 0.0069), but not different (P = 0.3101) compared with harvesting every 4 wk. Mid-season there was a tendency (P = 0.0566) for alfalfa contribution to be greater when harvested every 4 wk compared with every 2 wk. Alfalfa contribution increased (P = 0.0415) when harvested every 4 wk compared with every 6 wk. However, there was no difference (P = 0.8897) for presence of alfalfa between harvest frequencies of 2 and 6 wk. Late season, there were no differences (P = 0.1052) in alfalfa contribution among harvest frequencies. Harvesting at a 4 or 6-wk frequency in the early season and 4-wk frequency in the late season may be beneficial to the longevity of alfalfa and bermudagrass contribution in the stand. Shading from the grass component may negatively contribute to alfalfa stand contribution at longer growth intervals in late summer, which negatively impacts yield contribution from alfalfa in the mixture. Forage growth is most rapid until early flowering stage for alfalfa. Alfalfa growth continues until full flower, but often leaf losses from lower stems slow yield increase after first flower (Sheaffer, 1989), illustrating that longer regrowth intervals may negatively impact alfalfa contribution as a result of a combination of plant leaf retention characteristics and competition effects in mixed stands. Alfalfa plantings made between early March and mid-April may be successful but will likely require irrigation and a very aggressive defense against weeds. When planting into cool- or warm-season perennial grass sods, alfalfa establishment in the spring is generally not recommended due to anticipated cool-season annual weed competition (Hancock et al., 2015), although management situations influenced by drought or herbicide residual may influence planting timing decisions.

Harvest frequency effects and date × frequency interactions for bermudagrass canopy cover are presented in Table 4. Bermudagrass presence was not different among harvest heights ($P = 0.4822$) or harvest frequencies ($P = 0.1451$), although bermudagrass canopy cover remained low throughout the season. Low contribution was likely due to weed pressure, which largely consisted of crabgrass (*Digitaria sanguinalis*), buckhorn plantain (*Plantago lanceolata*), curly dock (*Rumex crispus*), and horse nettle (*Solanum carolinense*). There was a tendency for a harvest date × harvest frequency interaction ($P = 0.0944$), where early in the season there was no difference in species contribution ($P = 0.9893$) among harvest intervals for bermudagrass. During the beginning of the summer growing season, there was more bermudagrass than in the middle or later part of the season (27 vs. 17 and 3% respectively), where the stand contribution declined as the season progressed. Late in the season there was minimal bermudagrass presence across all plots, with no difference ($P = 0.3182$) among harvest frequencies. Weed pressure increased throughout the season ($P < 0.0001$). Although this was an established stand of 'Tifton 44' bermudagrass, early emergence of cool-season and warm-season weeds created a competitive environment for growth of the desirable forage species in the mixture. In more robust stands of bermudagrass monocultures, greater persistence has been reported for selected bermudagrass cultivars harvested at 4- or 8-wk harvest frequencies (Monson and Burton, 1982). These cultivars include Tifton 44, Coastal, and Midland bermudagrass, and exhibited superiority in aboveground spread of stolons following harvest conditions, which would directly relate to canopy cover estimates. In the case of mixed alfalfa-bermudagrass stands, post-emergence weed control options are relatively limited due to the mode of action of most pasture herbicides. Residual soil activity, or selectivity for broadleaf weeds may negatively impact the legume component in mixed stands, which limits their applicability in this system. Therefore, pre-emergent control of cool-season weeds may be important to reduce weed competition in spring-planted stands. There was no difference in canopy cover from weeds among harvest intervals ($P = 0.8239$) or heights ($P = 0.5890$). In 2019, treatment plots were evaluated for alfalfa and bermudagrass persistence. Upon inspection of the site, less than 5% stand of alfalfa was present and the decision was made to not continue the study for a second year due to lack of persistence of the alfalfa component.

Table 3. Harvest frequency and date effects on alfalfa contribution to canopy cover (% of DM).

Item	Harvest Frequency (wk)			
Date*	2	4	6	Mean
Early-Season	$9^{a,e}$	$13^{a,e,f}$	$16^{a,f}$	13^{a}
Mid-Season	$6^{b,e}$	$11^{a,f}$	$6^{b,e}$	7^{b}
Late-Season	1^{c}	1^{b}	4^{c}	2^{c}
SEM	1.16	1.15	1.20	1.17

$^{a\text{-}c}$ Within a column, means differ ($P < 0.05$, n = 3).
$^{e\text{-}f}$ Within a row, means differ ($P < 0.05$, n = 3).
*Early-season (6/15/2018); Mid-Season (7/27/2018); Late-Season (9/7/2018).

Table 4. Effect of date on species contribution to canopy cover (% of DM).

Item	Alfalfa	Bermudagrass	Weeds
Date*		%	
Early-Season	13^{a}	27^{a}	61^{a}
Mid-Season	7^{b}	17^{b}	75^{b}
Late-Season	2^{c}	3^{c}	95^{c}
SEM	1.16	2.66	3.24

$^{a\text{-}c}$ Within a column, means differ $P < 0.05$ (n = 3).

*Early-season (6/15/2018); Mid-Season (7/27/2018); Late-Season (9/7/2018).

Table 5. Harvest frequency and date effects on bermudagrass contribution to canopy cover (% of DM).

Item	Harvest Frequency (wk)			
Date*	2	4	6	Mean
Early-Season	28^{a}	28^{a}	24^{a}	27^{a}
Mid-Season	$28^{a,e}$	$13^{b,f}$	$12^{b,f}$	17^{b}
Late-Season	1^{b}	3^{c}	4^{c}	3^{c}
SEM	2.66	2.63	2.73	2.65

$^{a\text{-}c}$ Within a column, means differ ($P < 0.05$, n = 3).

$^{e\text{-}f}$ Within a row, means differ ($P < 0.05$, n = 3).

*Early-season (6/15/2018); Mid-Season (7/27/2018); Late-Season (9/7/2018).

Botanical Composition

Botanical composition change throughout the season is presented in Table 6. Following the same pattern as canopy cover, alfalfa and bermudagrass decreased ($P < 0.0001$) and weeds increased ($P < 0.0001$) over the season. Harvest height did not significantly impact alfalfa ($P = 0.2437$), bermudagrass ($P = 0.4726$), or weed contribution ($P = 0.4061$) in the stand. Alfalfa and bermudagrass as a percentage of the total forage yield was low in this study compared with other reported trials in the region. Brown and Byrd (1990) conducted 2 field experiments to compare the yield and botanical composition of alfalfa-bermudagrass mixtures with each species grown alone. In the first experiment, ‘Apollo’ alfalfa was grown alone and in mixtures with ‘Tifton 44’ bermudagrass fertilized at differing rates, and compared with bermudagrass fertilized with N at differing rates. In a second experiment, ‘Apollo’ alfalfa was grown alone in 15-cm rows and in 15- and 30-cm rows in mixtures with ‘Coastal’ bermudagrass. Bermudagrass was also grown alone and fertilized with differing rates of N. Alfalfa dominated a Tifton-44-alfalfa mixture in both experiments, comprising 100% of the forage in the spring and 53% in the fall.

Alfalfa-bermudagrass mixtures exhibit alternating contributions throughout the growing season. Alfalfa will generally be the predominant species in the late spring and early summer in mixed stands and, as the forage is grazed or harvested, opening the canopy, bermudagrass becomes the more dominant species of the mixture throughout the summer (Hancock, 2015). In the fall, alfalfa presence generally begins to remerge and may help increase the length of the growing season compared with bermudagrass alone. This pattern was not observed in the current study due to intense weed pressure throughout the study.

Table 6. Effect of date on aboveground sward botanical composition (% of DM).

Item	Alfalfa	Bermudagrass	Weeds
Date*	--	%--	
Early-Season	13	27	60
Mid-Season	7	17	75
Late-Season	2	3	95
SEM	1.36	2.76	9.14

$^{a\text{-}c}$ Within a column, means differ ($P < 0.05$, n = 3).
*Early-season (6/15/2018); Mid-Season (7/27/2018); Late-Season (9/7/2018).

Stand Density

Stand density was measured as number of alfalfa crowns per m^2 in the mixed forage stand. Following a similar pattern to visual and botanical composition estimates, alfalfa crown numbers decreased ($P < 0.0001$) throughout the season. Stand density for alfalfa changed throughout the season where alfalfa crown numbers were more prevalent earlier in the season (53 plants/m^2) than mid-season (18 plants/m^2) and late season (2 plants/m^2). Neither harvest height

(P = 0.9941) nor harvest frequency (P = 0.7139) significantly impacted alfalfa crown numbers throughout the season. As a monoculture, alfalfa has a tremendous ability to produce maximum yield over a wide range of stand densities. New seedlings should have at least 25 to 30 plants per 0.09 m^2 in the seedling year (Undersander et al., 2011). Using the 25 to 30 alfalfa plants per 0.09 m^2 outlined by Undersander (2011), an estimation for an alfalfa-bermudagrass stand could be 50% of those values (12.5 to 15 alfalfa plants per 0.09 m^2). Our results indicated lower alfalfa crown counts during the summer season, possibly due to spring planting and weed pressure.

Dry Weight Rank

Dry weight rank was measured at every harvest according to methods of Mannetje and Haydock (1963). Five 0.1-m^2 quadrats were placed randomly within plot, and canopy height was measured within quadrat using a pasture ruler. Species within quadrat were visually assessed and ranked as first, second, or third based on relative contribution to stand. Each component was multiplied by 70.2, 21.1, and 8.7, respectively, and added to give the dry-weight percentage of each species in the stand. This method provides a visual estimate that can be used to rank the relative contribution of each species in the stand, and is an alternative method that can be used in addition to visual canopy cover estimates. The effect of date on dry weight rank is presented in Table 7. Alfalfa persisted similarly in both early and mid season, but declined in the late-season. Bermudagrass and weeds followed the same pattern as alfafla where they persisted similarly across early and mid-season, but declined in the late season.

Table 7. Effect of date on dry weight rank of stand components (%).

Item	Alfalfa	Bermudagrass	Other
Date*		%	
Early-Season	22^{a}	37^{a}	42^{a}
Mid-Season	18^{a}	39^{a}	43^{a}
Late-Season	6^{b}	7^{b}	86^{b}
SEM	2.00	2.24	6.21

$^{a-c}$ Within a column, means differ ($P < 0.05$, n = 3).

*Early-season (6/15/2018); Mid-Season (7/27/2018); Late-Season (9/7/2018).

Conclusions

Whereas recommended planting time of alfalfa is in the fall, drought and/or unseasonably warm winters, or previous herbicide treatment can delay or affect successful stand establishment. Adjusting stand establishment to late winter/early spring can be an option, but as observed in this experiment, may decrease alfalfa persistence and increase contribution from undesirable grass and weed species. New technology may offer potential solutions to weed control such as Round-Up Ready® alfalfa varieties or time-release herbicide technology. Information obtained from this project aims to move the cattle industry in the southeastern region towards a year-round grazing management system through effective utilization of higher quality forage combinations and decreased dependence on costly fertilizer and feed inputs. Understanding the growth patterns and persistence of alfalfa-bermudagrass mixtures is an important step in producing a better quality forage with fewer N inputs. Additional research with fall planting, more grazing tolerant varieties, and additional pest control could allow for more success and eventual adoption of interseeded alfalfa into bermudagrass stands in North Alabama.

CHAPTER 4

ALTERNATIVE FORAGE SYSTEMS FOR DEVELOPING HEIFERS IN THE SOUTHEAST[2]

Introduction

Endophyte-infected tall fescue is the predominant perennial forage used by beef cattle producers in the Black Belt prairie soil region of Alabama; therefore, most beef heifers are developed using this system as the primary forage source during the development phase of their production. The mutualistic endophyte (*Epichloë coenophiala*), which is often found in tall fescue, increases drought tolerance and pest resistance making tall fescue a reliable perennial forage; however, the endophyte also produces toxic alkaloids which can negatively affect animal gains, reproductive performance and overall herd productivity (Danilson et al., 1986; Schmidt et al., 1986; Washburn et al., 1991; Gay et al., 1988; Brown et al., 1992). Fescue toxicosis is reported to contribute to over $2 billion in annual economic loss to the USA livestock industries (Poole et al., 2019). Research has reported that reproductive readiness is affected positively with enhanced nutrition (Short and Bellows, 1971) and negatively by toxic tall fescue (Washburn, 1991). The nutritional program of the heifer development phase is influenced by regional availability of feedstuffs and adapted forage resources. Various strategies have been employed to achieve this end point such as constant body weight gains from weaning to breeding or nutritional restriction followed by greater nutrient intake and compensatory gain. Findings from multiple experiments suggest that flexibility exists in how this target weight is attained to achieve acceptable pregnancy rates (Funston et al., 2011, 2017; Beck et al., 2017). Warm-season perennial grasses could be an alternative forage option to reduce the impacts of endophyte-infected tall fescue during the heifer development phase. Specific systems-management

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strategies to manage negative reproductive effects must be established, such as developing a grazing system with alternative forages during the “summer slump” of endophyte-infected tall fescue.

The objective of this study was to evaluate forage production and performance of weaned beef heifers developed grazing a fertilized or unfertilized mixture of big bluestem, little bluestem, and indiangrass (NWSG), ‘Pensacola’ bahiagrass (PB) or endophyte-infected (‘KY-31’) tall fescue (ETF) and their potential use in heifer development systems at the Black Belt Research and Extension Center (BBREC), Marion Junction AL.

Materials and Methods

All animals sourced in this study belonged to AAES. All procedures with animals were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee (PRN 2019-3591).

Research Site

A summer grazing trial was conducted at the Black Belt Research and Extension Center (BBREC) in Marion Junction, AL (32º28’50.29”N latitude, 87º15’26.61”W longitude) during the 2018 and 2019 growing seasons to evaluate forage production, persistence, nutritive value and beef heifer performance through breeding using 1) a native-warm season grass (NWSG) mixture of big bluestem (‘KY ecotype’ *Andropogon gerardii*), little bluestem (‘Cimarron’ *Schizachyrium scoparium*), and indiangrass (‘Boone’ *Sorghastrum nutans*) with no nitrogen (N) fertilization, 2) NWSG fertilized with 56 kg N/ha, 3), endophyte-infected ‘KY-31’ tall fescue or 4) bahiagrass under continuous stocking.

For NWSG, six plots (2 ha each) comprising Houston clay were demarcated in early 2018 for the project (Figure 4). The NWSG mixture was established in April 2016 using a seeding rate of 11 kg/ha (pure live seed) for the tertiary blend, consisting of 6 kg/ha big bluestem, 4 kg/ha indiangrass, and 1 kg/ha of little bluestem. Paddocks were sampled on a 2-ha grid basis in mid-April 2016 during the establishment year and amended with variable rates of P and K in July 2017 according to soil test recommendations of the Auburn University Soil Testing Laboratory. During the 2016 and 2017 growing seasons, both mowing and mob grazing were utilized to manage the forage to a target residual height of 25 cm. A prescribed burn was performed in late February 2018 and 2019 to reduce the amount of thatch present before the growing season initiated in May during the years of the grazing trial.

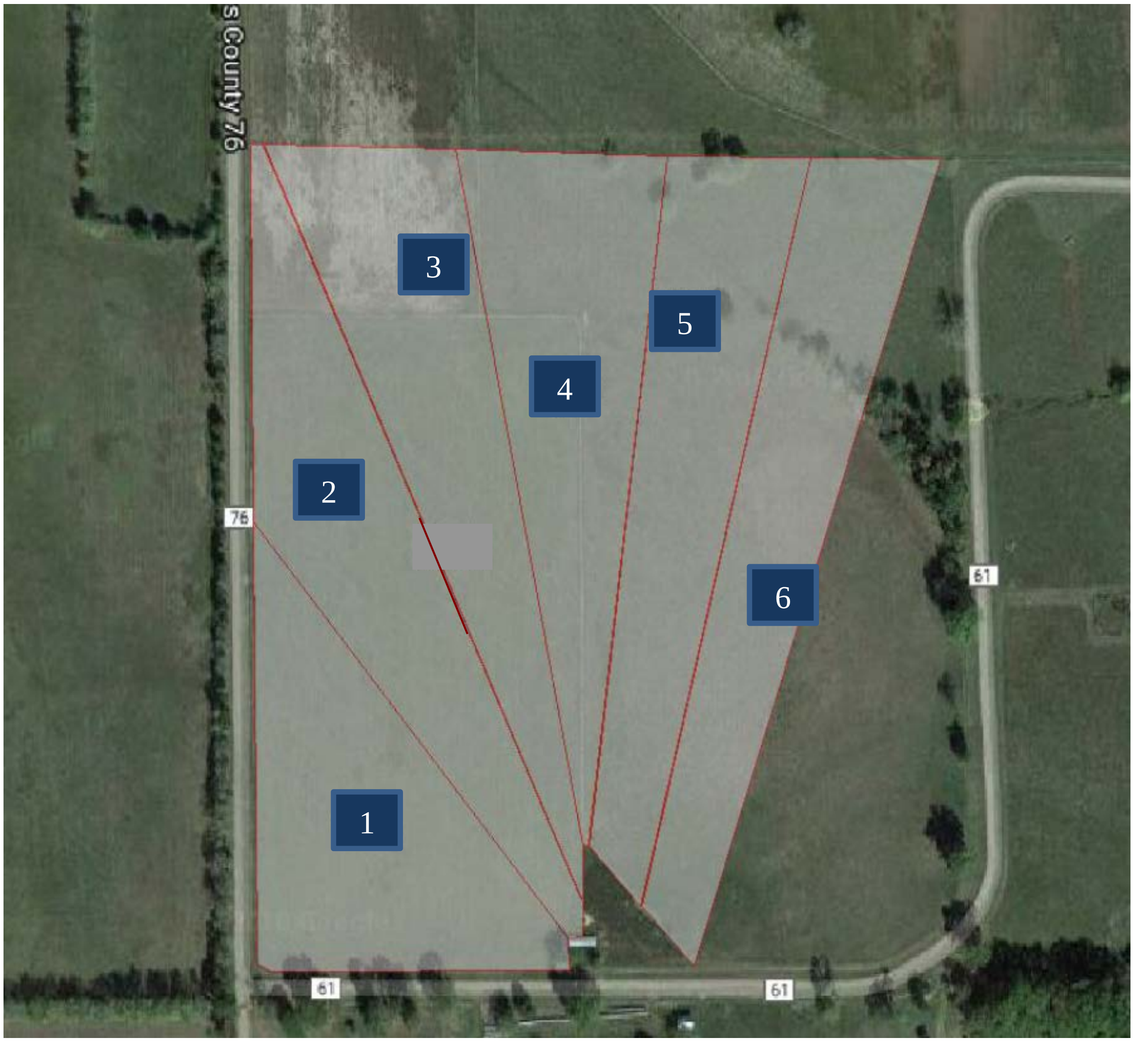

Figure 4. Layout of experimental plots for NWSG paddocks.

For bahiagrass, two plots (0.8-ha each) were identified the previous growing season (2017) for the project (Figure 5). The paddocks were comprised of two-year old ‘Pensacola’ bahiagrass stand. Plots were mowed during the 2017 growing season twice throughout the summer, and grazing was utilized to manage the forage to a target height of 15 cm. Soil tests were performed in February 2017, and soil amendments were made according to the Auburn University Soil Testing Laboratory results.

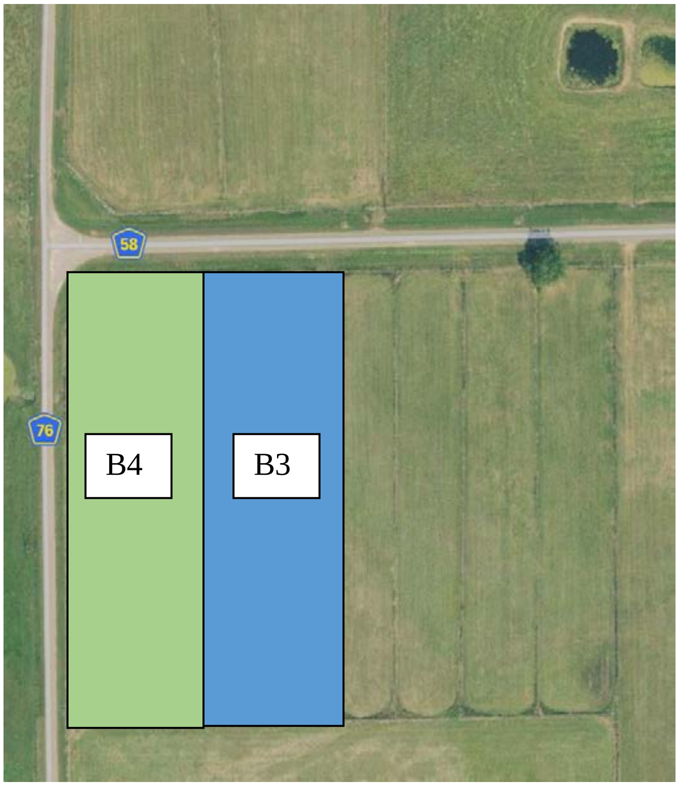

Figure 5. Layout of experimental plots for ‘Pensacola’ bahiagrass paddocks

For ‘KY-31’ tall fescue, two plots (0.8-ha each) were identified the previous growing season (2017) for the project (Figure 6). The paddocks comprised existing (45-year old stand) ‘KY-31’ tall fescue. Plots were mowed during the 2017 and 2018 growing season twice throughout the winter and spring, and grazing was utilized to manage the forage to a target height of 15 cm. Soil tests were performed in February 2017 and soil amendments were made according to the Auburn University Soil Testing Laboratory results.

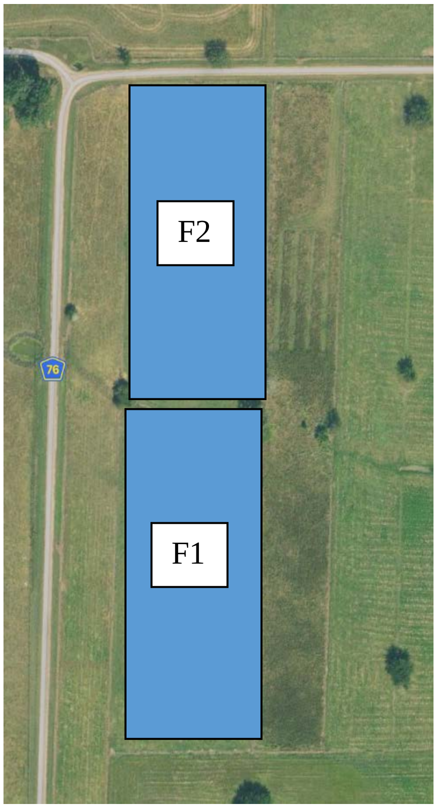

Figure 6. Layout of experimental plots for tall fescue paddocks.

Experimental Design

Thirty-six fall born Angus × Simmental beef heifers (initial BW 288 kg) were selected and randomly assigned to one of four grazing treatments in each year of the study: 1) tall fescue, 2) NWSG with N, 3) NWSG no N, or 4) bahiagrass. Heifers were born between September 11, 2017 and October 18, 2017 in Year 1 and September 3, 2018 and October 11, 2018 in Year 2. Heifers were weaned according to the standard operating procedure of the Black Belt Research and Extension Center (PRN 2016-2990) in May 2018 and May 2019, respectively. From weaning until the start of the study, heifers were placed in a dry lot setting with annual ryegrass hay and supplemented with 50:50 pelleted corn gluten and soybean hulls at 1% of their BW hand-fed daily. Initial test weights for beef heifers were collected on June 4, 2018, and May 29, 2019, respectively, and four test heifers were randomly assigned per paddock to NWSG, and three test heifers per paddock were assigned to tall fescue and bahiagrass pastures based on forage allowance.

Forage Management

Nitrogen (67 kg N/ha) fertilization was applied on April 19, 2018 and April 15, 2019, respectively on NWSG-N and bahiagrass paddocks. No fertilizer was applied to the tall fescue or NWSG-No, to simulate common management practices, paddocks in 2018 or 2019. Weed control was applied on June 29, 2018 to NWSG and bahiagrass paddocks. No herbicide was applied to tall fescue paddocks in 2018 or 2019. No herbicide was applied in 2019. Weed control decisions were made when visual canopy contributions of undesirable species exceeded 30% to reflect best management practices for desirable forage stands. Grazing was initiated on June 4, 2018 and May 29, 2019 when the NWSG plots reached an average height of 60 cm, and bahiagrass and tall fescue plots reached an average height of 20 cm. Stocking rates were adjusted using put-and-take heifers and cows to maintain forages in a vegetative state to a target height of 30 to 38 cm, or 2,800 kg DM/ha for NWSG, and a target height of 7 to 12 cm, or 2,200 kg DM/ha for bahiagrass and tall fescue. Grazing was discontinued when forage canopy height decreased to ≤ 22 cm (NWSG) and ≤ 6 cm (bahiagrass and tall fescue), or 1,960 kg DM/ha for the test heifers.

Forages were continuously stocked throughout the growing season and managed to maintain a target forage DM availability of 2,800 kg DM/ha (NWSG) and 2,200 kg DM ha (bahiagrass and tall fescue). Stocking rate adjustments were made using put-and-take heifers and cows based on the calculation of forage availability and animal utilization at the time of sampling. Animal utilization since the previous sampling date was estimated using an assumed dry matter intake of 2% of mean BW plus an additional 40% waste due to trampling. Stocking rate adjustments were determined based on amount of predicted consumption by cattle within a plot over a 2-week period. If the amount of projected available forage was estimated to be over desired target forage availability, stocking rate adjustments were made using put-and-take heifers or mature cows. During the early months of the grazing season, stocking densities required to maintain forage to the target canopy height, mature cows (approximately 545 kg) were used in addition to put-and-take heifers due to limited animal availability.

Response Variables

Forage Mass and Nutritive Value

Forage mass and nutritive value was determined by clipping 0.10-m^2 quadrats (10 per paddock for NWSG and 5 per paddock for bahiagrass and tall fescue) at the initiation of the trial and every 2 weeks during the study. Forage within quadrats was clipped to leave an aboveground stubble height of 5 cm for forage mass. Hand-plucked forage nutritive value samples were collected for each forage treatment by clipping 10 random locations per plot to a 25-cm stubble height for NWSG and a 15 cm stubble height for bahiagrass and tall fescue. Fresh-cut forage was then placed into cloth bags for transportation back to the Auburn University Ruminant Nutrition Laboratory. Samples from each paddock were oven-dried at 60°C for 72 hr, air-equilibrated, and weighed. Forage mass was calculated for each paddock based on dry-weight data multiplied by the area of the paddock. Dried, air-equilibrated forage samples were ground in a Wiley mill to pass a 1-mm screen. Concentration of DM was determined by drying samples to constant weight at 100°C according to procedures of AOAC (1995). Forage concentration of N was determined by the Kjeldahl procedure (AOAC, 1995), from which CP was calculated. Forage *in vitro* true digestibility (IVTD) was determined according to the Van Soest (1991) modification of the Tilley and Terry procedure (1963) using the Daisy II incubator system (Ankom Technology^{TM}, Macedon, NY). Ruminal fluid was collected from a cannulated Holstein cow at the Auburn University College of Veterinary Medicine. The collected rumen fluid was stored in thermos containers to maintain a temperature supportive of the microbial population and transported to the Auburn University Ruminant Nutrition laboratory.

Animal Performance During the Grazing Season

Thirty-six beef heifers were weaned for two weeks prior to the start of the grazing trial. When paddocks reached the target grazing height, heifers were weighed, body condition scored, and randomly assigned to paddocks. Heifer weights and BCS were taken again every 28 days until the completion of the study. Following the first weigh period in each year of the study, heifers had less than 0.22 kg/d gain. Heifers were supplemented with 50:50 soyhulls and corn gluten feed to target 0.5 kg/d gain as needed during each year of the experiment based on forage nutritive value to keep heifers growing at a rate to support a projected early winter breeding window.

Pre-Breeding Performance and Conception Rate

Heifer management post-grazing included being fed free-choice annual ryegrass hay and a 50:50 soyhull/corn gluten feed mixture at 1% of BW per day to achieve a target gain of 1 kg/heifer/day. Approximately 30 days before the start of their first breeding season, heifers were evaluated for body condition score [BCS; scale of 1-9 with 1 = emaciated and 9 = obese; (Wagner et al., 1988)] and assessed for RTS [RTS; scale of 1-5; 1 = pre-pubertal, 5 = pubertal, luteal phase; (Anderson et al., 1991)] as well as pelvic area [cm^2, using a Rice Pelvimeter;

(Johnson et al., 1988)] by a single, experienced veterinarian. At approximately 15 months of age, treatment heifers underwent estrous synchronization for AI utilizing the Select Synch + CIDR protocol to begin their first breeding season. Heifers received an injection of GnRH (i.m.; 100 μg; Cystorelin®; Merial, Duluth, GA) and insertion of a controlled internal drug release (CIDR) device (intravaginal insert; 1.38 g progesterone; Eazi-Breed® CIDR®; Zoetis Inc., Kalamazoo, MI, USA). Each CIDR® was removed following 7 days, and an intramuscular injection of 25 mg of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI) was administered at the same time. Heifers were then detected for estrus (AM/PM) and artificially inseminated 12-h post estrus detection with a single straw of semen originating from a single Angus bull source. Non-estrus detected heifers were all bred 72 h post injection of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI). Two professionals in random rotation were responsible for AI procedures at each experimental station for each year.

Fourteen days after AI, heifers were exposed to 1 fertile bull for natural breeding for the remainder of the breeding season. An experienced veterinarian performed initial pregnancy evaluation by transrectal palpation on day 62 to 89 post AI, followed by final pregnancy evaluation on day 85 to 140 post insemination. Presence or absence of a conceptus, alongside morphological features indicating fetal age were recorded, and heifers were classified as ‘pregnant to AI’ (Preg AI), ‘pregnant to natural service’ (Preg NS), or ‘not pregnant’ (Not Preg).

Statistical Analysis

Forage mass, nutritive value, and animal performance variables were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC) for a completely randomized design. Independent variables included harvest date, forage treatment, and their interactions. Year was considered a random variable. The CORR procedure in SAS 9.4 (SAS Institute, Cary, NC) was utilized for analyses of reproductive performance parameter relationships. Treatment means were separated using the PDIFF option of the LSMEANS procedure (SAS Institute, Cary, NC) and were determined to be significant when $\alpha = 0.05$; tendencies were described when $\alpha \geq 0.05$ and ≤ 0.10.

Weather Data

Weather instruments operated by AWIS Weather Services, Inc. collected daily minimum and maximum ambient temperatures and daily total precipitation data throughout the experimental period. Weather instruments were located in Marion Junction, AL. Temperature data and total precipitation are reported in Table 13 and 14.

Results and Discussion

Temperature and Precipitation

In Yr 1, mean temperatures (Table 8) for May and August were 4 and 9% greater than the 10-yr average, respectively and mean temperatures for June and July were 2 and 4% less, respectively. In Yr 2, mean temperatures for May and August were 5 and 17% greater than the 10-yr average, respectively and mean temperatures for June and July were 3 and 1% less, respectively. In Yr 1, monthly total precipitation (Table 9) was 75, 20, and 3% greater than the 10-yr average in May, July, and August, respectively and 31% less in July. In Yr 2, monthly total precipitation was 9 and 78% less than the 10-yr average in May and July, respectively and 45 and 34% greater than 10-yr average in June and August, respectively. As daytime temperatures increase throughout the summer cattle tend to graze less during the day and seek shade, which may reduce their daily forage intake (Blackshaw and Blackshaw, 1994).

Table 8. Monthly mean air temperatures (˚C) for Yr 1, Yr 2, and 10-yr averages for Marion Junction, AL.

Item		Year 1†			Year 2†		
Month	Avg. High, ˚C†	Avg. Low, ˚C	Mean, ˚C	Avg. High, ˚C†	Avg. Low, ˚C	Mean, ˚C	10-yr Avg., ˚C
May	30.1	17.8	23.5	30.3	17.6	23.9	22.7
June	32.4	21.4	26.3	31.9	20.3	26.1	26.8
July	32.5	22.3	26.6	33.2	21.4	27.3	27.7
August	31.7	21.7	25.7	33.8	21.7	27.7	23.6

†Data was collected from AWIS Weather Services, Inc.

†Year 1 = 2018 and Year 2 = 2019 growing seasons.

Table 9. Monthly total precipitation for Yr 1, Yr 2, and 30-yr averages and differences from 10-yr averages for Marion Junction, AL.

Item	Total Precipitation, mm†			Differences, mm	
Month	Yr 1†	Yr 2	10-yr Avg.	Yr 1†	Yr 2
May	175	91	100	+75	-9
June	61	128	88	-27	+40
July	120	22	100	+20	-78
August	105	137	102	+3	+35

†Data was collected from AWIS Weather Services, Inc.

†Year 1 = 2018 and Year 2 = 2019 growing seasons.

Forage Mass

There were no differences (*P* > 0.0500) in forage mass between PB (4,338 kg/DM ha), ETF (5,165 kg DM/ha) and NWSG-No application (4,388 kg DM/ha). Native warm-season grasses without N application exhibited less production (3,620 kg DM/ha) than the ETF (*P* = 0.0134), but did not differ (*P* = 0.1026) from the NWSG with N application over the two-year study under continuous stocking. Tall fescue forage mass in the present study was greater than values reported by McKee et al. (2017) where a 2-yr average of tall fescue was 3,087 kg DM/ha under continuous grazing from March until late June. Seasonal forage mass values for PB in this study was greater than those reported by Utley et al. (1974) at the Coastal Plain Experiment Station, Tifton, GA under similar levels of fertility and pasture stocking strategies (3,362 kg DM/ha). Mason et al. (2019) measured forage mass of Eastern gamagrass grown in the Black Belt region of Alabama following overseeding with cool-season annuals. The study reported lower forage mass of Eastern gamagrass monocultures for May through September (2,954 kg DM/ ha) compared to NWSG mixtures evaluated in the present study when fertilized with 0 or 68 kg N/ha. Backus et al. (2017) evaluated management options for switchgrass, Eastern gamagrass, and a mixture of big bluestem and indiangrass under two grazing strategies to produce beef cattle and biomass at two different Tennessee locations. During the first 30-days of the grazing season, which occurred in May, mixtures of big bluestem and indiangrass at the two locations had a mean yield of 3,655 kg DM/ ha, which is similar to the observed NWSG forage mass in this study.

Nutritive Value

Mean forage *in vitro* true digestibility (IVTD) was not different (*P* = 0.2815) between NWSG-N (67%) and NWSG-No (69%) and not different (*P* = 0.6168) between PB (74%) and

ETF (73%) during the grazing season. Native warm-season grasses without N application (69%) tended (*P* = 0.0786) to be less than ETF (73%) and was less (*P* = 0.0278) than PB (74%). There was no date × treatment interaction for forage IVTD observed during the trial (*P* = 0.8280).

Mean forage CP percentage was greatest for ETF (11.0%) followed by PB (7.9%), than N-fertilized NWSG mixtures (7.1%), followed by NWSG mixtures that did not receive fertilizer (6.1%; *P* < 0.0001). A date × treatment interaction (*P* = 0.0252) was observed for forage CP concentration (Table 10). For NWSG-N and NWSG-No, CP concentration decreased as the season progressed, whereas TF and PB increased in CP concentration mid-season followed by a decrease in CP late-season.

Percentage CP for NWSG mixtures in the present study was low compared with data reported by Rushing et al. (2019) for big bluestem, little bluestem, and indiangrass grown in Mississippi and managed under clipping, having a two-year mean of 9.7% for each component, respectively. Percentage CP was also low compared with values reported for other commonly used warm-season perennials under similar management in the region. Lower CP percentages could be attributed to forage maturity and N application. Venuto et al. (2003) reported a mean CP value of 12% (DM basis) under continuous stocking for common dallisgrass grown in southern Louisiana and southeastern Texas in a three-year evaluation. Percentage IVTD was greater than that reported by Rushing et al. (2019) for NWSG. The authors reported a two-year mean of 58.1%, 57.9%, and 57.9% for big bluestem, little bluestem, and indiangrass grown in Mississippi, respectively. Percentage IVTD values reported at the beginning of the NWSG growing season could meet requirements for growing beef heifers (NRC 2016), but would likely not meet the target requirements of 0.5 kg/d gain by the end of the season. In general, forage CP values reported throughout the season for NWSG-N, NWSG-No fertility, and PB would not meet requirements for growing beef heifers for these gain targets. The TF system met the heifers nutrient requirements for CP and IVTD except for the end of the grazing season in August, which could be partially attributed to dallisgrass and clover mixed into the TF stand. Although there is an extensive range of literature related to TF management and nutritive value during the active growing seasonor in stockpiling systems, few trials report forage quality data through the summer slump time period for comparison purposes. Billman and Phillips (2016) reported greater mean percent CP (2014 – 20%; 2015 – 18%) and mean IVTD (2014 – 82%; 2015 – 79%) for grazed TF in Kentucky over 2 years (2014 and 2015) when samples were collected in June, July and September.

The nutritive value of C_4 grasses can be excellent early in the growing season, but because of their rapid growth and maturity, nutritive value can decrease significantly as the growing season progresses (Coleman et al., 2004). The C_4 mechanism of photosynthesis allows high CO_2 fixation at relatively low leaf-N concentrations and low concentrations of rubisco (Moore et al., 2004), which results in plants with smaller CP concentrations than C_3 species. Vendramini et al. (2010) compared herbage accumulation and nutritive value of nine species of warm-season grasses in South Florida and found differences among species and cultivars of the same species. Several factors may contribute to the differences in nutritive value in C_4 grasses, including species, cultivars within species, maturity, fertilization, conservation practices, etc.

Table 10. Effect of forage treatment and harvest date on concentration of crude protein (% DM basis).

Item	NWSG-No fertility	NWSG-N (67 kg N/ha)	Tall Fescue	Bahiagrass
Date*	------------------------%, DM basis-------------------			
1	$7.0^{a,e}$	$9.3^{a,e}$	$11.6^{b,f}$	$8.5^{b,e}$
2	$6.5^{a,e}$	$8.2^{b,f}$	$14.4^{a,g}$	$10.5^{a,h}$
3	$6.5^{a,e}$	$7.1^{c,e}$	$9.9^{b,c,f}$	$8.5^{b,f}$
4	$6.4^{a,e}$	$6.5^{c,e}$	$10.2^{b,c,f}$	$6.4^{b,c,e}$
5	$5.3^{b,e}$	$6.7^{c,e}$	$11.6^{b,g}$	$8.3^{b,c,f}$
6	$5.0^{b,e}$	$5.2^{d,e}$	$9.9^{b,c,g}$	$7.1^{b,c,f}$
7	-	-	$8.8^{b,c,e}$	$5.8^{d,f}$
Mean	6.1	7.2	11.0	7.9

$^{a-d}$ Within a column, least squares means with differing superscripts differ ($P < 0.05$), SEM=0.40.

$^{e-h}$ Within a row, least squares means with differing superscripts differ ($P < 0.05$), SEM=0.35.

* Harvest date 1 = June 4, 2018 and May 28, 2019; 2 = June 20, 2018 and June 30, 2019; 3 = July 2, 2018 and June 25, 2019; 4 = July 18, 2018 and July 9, 2019; 5 = July 30, 2018 and July 22, 2019; 6 = August 13 and 16, 2018 and August 5, 2019.

Animal Performance

No differences were observed among forage systems for initial or final heifer BW (P = 0.1260 and P = 0.2652, respectively), ADG (P = 0.4089) or BCS (P = 0.1976) during the summer grazing season (Table 11). Length of the grazing season differed among forage treatments ($P < 0.0001$) such that heifers grazed PB (85 d) and ETF (85 d) longer than NWSG-N (76 d) and NWSG-No (72 d) (Table 11). Grazing days for ETF were similar to those reported by McKee et al. (2017) where tall fescue × white clover were grazed for 75 and 84 days over a 2-yr project in Alabama from March until mid-June. Overall, mean heifer ADG was low across all treatments in the 2-yr study with less than 0.5 kg/d. While heifer gains were low in this study, beef heifers maintained a BCS of 5 or greater during the summer grazing season. Several grazing trials have been conducted with NWSG monocultures in the Midwest to Western US with reported stocker gains of 0.7 kg/d or greater (Anderson et al., 1988; Karges et al., 1992; Mitchell et al., 2005). Few studies have evaluated animal performance on NWSG mixtures in the Southeast US. A study by Keyser (2011) in Tennessee reported gains of 0.96 kg/d for stocker steers weighing 272 kg that were grazing a big bluestem and indiangrass mixture which was slighter greater gains than heifers exhibited on the NWSG mixture. Sollenberger et al. (1988) evaluated animal performance with yearling steers in Florida continuously grazing ‘Pensacola’ bahiagrass for 168-d. Steers in this study gained 0.4 kg/d over a 2-yr grazing study, which was greater than the gains observed in the current study. In these studies, steers had gone through an initial backgrounding phase, ranging from 30 to 60 days in length, prior to the grazing study initiation. In the present study, heifers were weaned for two weeks prior to direct initiation of the grazing trial, and some performance differences may be in part attributed to carryover effects of weaning into the experimental period.

Reproductive data presented represent a relatively small number (n=54) of breeding animals, and thus values presented represent characteristics of subsequent heifer performance post-summer grazing through breeding with limited animal numbers. There were no differences ($P > 0.10$) observed in heifer RTS, breeding time (48 h, 60 h, 72 h, or 84 h post-prostaglandin F2α), days pregnant, percentage of AI pregnant, or total percentage of heifers pregnant after last pregnancy check when heifers from each respective forage system treatment were tracked through breeding following the summer grazing season (Table 12). Weight at breeding (P = 0.0112) differed among forage system treatments and there was a tendency for differences in BCS at breeding (P = 0.0624). Heifers that had grazed ETF during the summer weighed less at the time of breeding than NWSG heifers; however, there were no differences in weight at breeding between heifers developed on TF or PB. Heifer BCS tended to differ (P = 0.0624) between grazing systems where heifers grazing NWSG-N and NWSG-No had a greater BCS than heifers grazing PB or TF, but no difference was observed between heifers grazing PB and TF. Phillips et al. (2018) observed no differences in phenotypic parameters (BCS, RTS, BW at time of breeding, age at breeding) for beef heifers (n=20) across 3 different locations in Alabama which were similar to findings in the present study except for BW at breeding for heifers grazing ETF was less than those grazing NWSG-N and NWSG-No.

Table 11. Beef heifer performance and grazing season length for forage systems during the summer grazing season in 2018 and 2019.

Item	NWSG-No	NWSG-N*	Tall Fescue	Bahiagrass	SE
Initial BW (kg)	290	286	291	281	8.8
Final BW (kg)	310	315	313	304	5.6
ADG (kg/d)†	0.29	0.38	0.27	0.28	0.16
BCS‡	5.7	5.7	5.5	5.6	0.34
Grazing Season Length (d)§	72	76	85	85	4.8

$^{a\text{-}c}$ Within a row, least squares means with differing superscripts differ ($P < 0.05$).
*Fertilized with 67 kg N/ha.
†Average daily gain (kg/heifer/day).
‡ Mean body condition score of heifers.
§ Grazing season length for forage systems (days per season) for 2018 and 2019.

Table 12. Effect of forage treatment on heifer growth and reproductive performance.

Item	NWSG-No	NWSG-N*	Tall Fescue	Bahiagrass	SE
Pregnant (d)†	102^{a}	96^{a}	85^{a}	94^{a}	12.4
AI Pregnant (%)‡	39^{a}	35^{a}	25^{a}	33^{a}	12.4
Total Pregnant (%)§	90^{a}	86^{a}	82^{a}	82^{a}	7.8
BCS at Breeding	6.4^{a}	6.4^{ab}	6.1^{bc}	6.2^{c}	0.1
RTS	3.7^{a}	4.7^{a}	1.9^{a}	3.4^{a}	1.7
BW at Breeding (kg)	369^{ab}	370^{ab}	340^{c}	355^{bc}	15.3
Breeding Time (h) #	60.1	64.1	65.2	67.0	4.7

a-c Within a row, least squares means with differing superscripts differ ($P < 0.05$).
*Fertilized with 67 kg N/ha.
†Days pregnant represents pregnancy detection 140 days post AI.
‡ AI pregnant represents total percentage of heifers conceiving to first service AI.
§ Total pregnant represents first service AI and natural service percent conception.
# Breeding Time represents hours post prostaglandin F2α injection in which breeding (AI) occurred.

Conclusions

Over the 2 years of this study, heifer gain responses and conception rates were similar across various forage systems, demonstrating the potential for using warm-season forages as an alternative to tall fescue in the Alabama Black Belt soil region without repercussion of forage impact on ability to conceive to first service AI. Evaluating this alternative grazing system is beneficial for producers who are trying to reduce input costs and alleviate the reproductive effects of fescue toxicosis in their heifer development programs during the summer months. This study provides information on potential perennial forage systems options that are adapted for growth in the Black Belt soil region of Alabama and possibly across the Southeast USA.

CHAPTER 5

SAND MOUNTAIN ELITE HEIFER DEVELOPMENT PROGRAM[3]

Introduction

The Sand Mountain Elite Heifer Development Program was established to demonstrate recommended best practices for replacement heifer development including, the use of artificial insemination (AI) to Northeast Alabama commercial cattle producers using adapted forage-based systems options. Heifer development is an essential step in the longevity of a cow-calf enterprise due to replacement rates averaging 10 to 20% annually for cows. Critical attention to the management of heifers during development can have a sustained impact on long-term efficiency. Due to reduced reproductive efficiency of young beef cows, development of replacement females to fit specific production environments is crucial for efficient cow-calf production (Meek et al., 1999).

Developing heifers to a target weight has been the standard in the past (Patterson et al., 1992), but with cost of production increasing, cattle producers are searching for alternative methods of developing heifers. Utilizing production practices that minimize input costs and increase the selection for reproductively sound heifers may provide a viable opportunity for increased long-term efficiency of beef cattle production systems. Heifer grazing behavior and management practices that expose heifers to their production environment during development may influence future performance. Research has shown that previous metabolic status during certain physiological events (maturation of reproductive tract includes ovaries and ovarian follicles; maturation of hypothalamus; frequency of release of luteal hormone pulses; oestradiol

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[3] Target Journal Submission to *Applied Animal Science* as a Case Study

concentrations to stimulate behavioral estrus) influences their ability to reproductively respond later in life (Roche et al., 2005; Chagas et al., 2006).

This program was initiated as a demonstration project to educate cattle producers on the concepts of developing heifers from yearling to conception on cool-season annual and perennial forages. The program is a partnership between the Alabama Cooperative Extension System (ACES) and the Alabama Agriculture & Experiment Station (AAES).

Materials and Methods

All animals sourced in this study belonged to AAES or private consigners. All procedures with animals were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee (PRN 2017-3007).

The program is hosted at the Sand Mountain Research and Extension Center (SMREC) in Crossville, AL using rotational stocking of 0.81-ha paddocks (n = 17). This program was initiated in fall 2015 and is currently ongoing. Data presented are from 4 consecutive years of forage and heifer response variables from January through June 2016 to 2019. This program is designed to develop replacement heifers in a forage-based system, utilizing proper animal husbandry and current technologies. Consignors agree that heifers in this program may be used in Extension programs for demonstration purposes during educational programs, as well as 4-H and FFA contests. A follow-up meeting/field day is held to summarize the project results to the consigners and all interested cattle producers.

Heifers are nominated for the program and a selection committee, consisting of livestock Extension agents and specialists, screen the heifers for physical and behavioral parameters essential for success in the program. The heifers are nominated in November each year and selected by December for an early January arrival to the SMREC. Heifers must meet the following eligibility requirements for selection: birth month/year, with actual birthdate preferred (must be born prior to February 15); sire and dam (breed composition) of heifers should be known, with registration numbers of sires provided; heifers should weigh a minimum of 238 kg at delivery.

Health is an extremely important aspect of pre-conditioning cattle during the weaning process and guidance is given to consigners, along with their herd veterinarian, to ensure cattle arrive and stay healthy during the development process. Consigners are asked to dehorn cattle if needed, deworm with an anthelmintic, wean cattle a minimum of 45-d prior to arrival at the research station, and vaccinate and booster for IBR/BVD/PI3/BRSV, 7-Way Blackleg; 5-Way Leptosporosis, and Vibriosis (*Campylobacter fetus*). After arrival at the SMREC, heifers are tested for persistently infected – BVD, and any heifers testing positive are removed from the program.

Nutritional Development Systems Management

Heifers are developed primarily on cool-season forages (annual ryegrass, cereal rye, white and black oats, triticale, wheat, brassicas, wheat×crimson clover×oats×triticale×rape, tall fescue (‘Martin II’), or ryegrass×crimson clover planted in the fall utilizing a no-till drill into existing sod or prepared seedbeds at a seeding rate based on ANR-0149 Alabama Planting Guide for Forage Grasses (Dillard et al., 2019) and ANR-0150 Alabama Planting Guide for Planting Forage Legumes (Mason et al., 2019). Soil tests were conducted annually. Phosphorus and potassium were applied based on soil test recommendations and N was applied at 56 kg/ha after seedling emergence. Supplementation with a least cost commodity feed blend (2/3 soy hull pellets and 1/3 dried distillers grains with solubles) is provided as needed to maintain 0.68 kg ADG.

During establishment of cool-season annual grasses and stockpiling of tall fescue, 56 kg N/ha was applied to each of the paddocks. In March, an additional 33.6 kg N /ha was applied to each of the paddocks. Paddocks were rotationally stocked ensuring each heifer group grazed each forage type the same number of days. Heifers were allowed continuous access to water and mineral supplementation (Purina Wind & Rain Storm Hi-Mag 4 Complete Beef Cattle Mineral: Calcium (Ca) (Min)12.15% Calcium (Ca) (Max) 14.58% Phosphorus (P) (Min) 4.00% Salt (NaCl) (Min) 15.75% Salt (NaCl) (Max) 18.90% Magnesium (Mg) (Min) 10.00% Potassium (K) (Min) 0.1% Manganese (Mn) (Min) 3600 ppm Cobalt (Co) (Min) 12 ppm Copper (Cu) (Min) 1200 ppm Iodine (I) (Min) 60 ppm Selenium (Se) (Min) 27 ppm Zinc (Zn) (Min) 3600 ppm Vitamin A (Min) 75000 IU/LB Vitamin D3 (Min) 7500 IU/LB Vitamin E (Min) 75IU/LB). Forage height was taken when heifers were turned into each paddock using a grazing stick and converted to kg DM by multiplying kg of forage per centimeter by centimeter of forage height for each forage type. Heifers were moved to a new paddock when 50% forage utilization had occurred (range 1 to 10 days) as determined by height of the forage. During years of decreased forage growth, heifers were managed as a single group in a DL system where they are fed hay and supplement to gain 0.68 kg/d. Once forages reach a threshold of 2,017 kg DM/ha heifers were returned to the paddocks for grazing and rotated when 50% of the aboveground forage mass has been removed, and 50% of the entering stubble height remains.

Cost and Care

Daily care and management was provided by SMREC staff and ACES personnel. Consignors whose heifers need veterinary care and/or treatment beyond what is provided to all animals was billed as additional costs. The SMREC has a local herd veterinarian who was used according to IACUC (PRN 2017-3007) procedures. The development fee was $400 per head where half was paid at delivery and the remaining balance was collected when heifers were returned to consigners. The development fee includes: feeding, breeding, routine health, carcass ultrasound, and pregnancy diagnosis.

Performance Characteristics

Once heifers arrived on the station, they were co-mingled with other consigned heifers and weighed for BW, hip-height measured by a trained technician, and ear-tagged for heifer identification. Once heifers were processed, they were divided into groups based on consigner and BW to prevent social dominance among heifers and to better manage forage utilization in the paddocks. Therefore, groups cannot be considered random as they had differing mean BW. The number of groups was dependent on the total number of consigned heifers and available forage per paddock.

Growth and Performance

Weights and hip heights were taken at 28-day intervals to ensure acceptable growth and BW gain was achieved. In early March, carcass ultrasound was performed by a certified technician to evaluate carcass merit, and results were reported back to the consigners.

Reproduction

Approximately 30 days before the start of breeding season, heifers were evaluated for body condition score (BCS) [scale of 1-9 with 1 = emaciated and 9 = obese; (Wagner et al., 1988)] and assessed for RTS [scale of 1-5; 1 = pre-pubertal, 5 = pubertal, luteal phase; (Anderson et al., 1991)] as well as pelvic area [cm^2 using a Rice Pelvimeter; (Johnson et al., 1988)] by a single, experienced veterinarian. The first week of April, heifers undergo estrous synchronization for artificial insemination utilizing the Select Synch + CIDR protocol to begin their first breeding. Heifers receive an injection of GnRH (i.m.; 100 μg; Cystorelin®; Merial, Duluth, GA) and insertion of a CIDR (intravaginal insert; 1.38 g progesterone; Eazi-Breed® CIDR®; Zoetis Inc., Kalamazoo, MI) on day -9, followed by CIDR removal and an injection of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI) on day -2. All heifers then received a second GnRH injection (i.m.; 100 µg; Cystorelin®; Merial, Duluth, GA) and are inseminated with a dose of semen of proven fertility 12 hours after estrus detection or 74-h after injection of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI). Two professionals in random rotation were responsible for AI procedures for each year.

Ten days after AI, heifers were exposed to 1 fertile bull (calving-ease clean-up bull, leased from Auburn University Beef Teaching Center) for natural breeding. An experienced veterinarian performed initial pregnancy evaluation by transrectal palpation on day 62 to 89 post AI. Presence or absence of a conceptus, alongside morphological features indicating fetal age were recorded, and heifers were classified as ‘pregnant to AI’ (Preg AI), ‘pregnant to natural service’ (Preg NS), or ‘not pregnant’ (Not Preg).

Statistical Analysis

Forage yield and growth characteristics were analyzed using the GLM procedure and reproductive characteristics were analyzed using the GLIMMEX procedure in SAS 9.4 (SAS Institute, Cary NC) for a completely randomized design. Independent variables for grazing period, forage type, year, and the interaction of grazing period × forage type, weight gain, hip height, age, cumulative weight gain, ADG, weight per day of age (WDA), frame and the interactions. Treatment means were separated using the PDIFF option of the LSMEANS procedure and were determined to be significant when $\alpha = 0.05$. Tendencies were determined to be significant at $0.1 \geq P > 0.05$.

Results and Discussion

Characterization of Program

Over the course of the Sand Mountain Elite Heifer Development Program, 155 heifers (2016 – 48 hd; 2017 – 32 hd; 2018 – 50 hd; 2019 – 25 hd) were consigned from12 different consigners and 7 different Alabama counties in North Alabama. Ten of these consigners were within a 50-mile radius of the SMREC, and the furthest consigner was located 124 miles from the research station. Since 2016, heifers were sired by 13 different sire breeds (Angus, Angus-Brahman, Beefmaster, Brangus, Charolais, Hereford, Hereford-Brahman, Red Angus, Red Poll, Santa Gertrudis, Simmental, Sim-Angus and Ultrablack).

Forage Yield

In 2016, 2018 and 2019, grazing was available within 30 days of arrival following a planting date of cool-season forages in October. In 2017, winter grazing was not available until 56 days after arrival to SMREC, and heifers were fed a 2/3 soyhull pellets and 1/3 dried distillers grains with solubles until forage was available. During this year, cool-season forages were planted late into the growing season (December, February, and March, respectively) as a response to drought the previous fall. Grazing in 2017 did not persist past late-May due to elevated daytime temperatures in May and June.

When diet changes occurred in the early part of the management season from a concentrate-based receiving diet (Diets prior to arrival at SMREC) to grazed cool-season forages, cumulative ADG was observed to be lowest during the first 28 days of each year, except 2017. This decrease in heifer ADG as cattle moved from a hay and supplemental feed-based system to grazing high-quality cool-season forages may be partially related to the transition time associated with a shift in diet composition. Fernando et al . (2010) reported that it takes two to three weeks for rumen microbes to transition to effectively digest a new diet. In most cases, heifers received a concentrate-based diet prior to arrival rather than coming from a grazing-based system. In 2017, the heifers did not experience a drop in ADG until after day 56 when forage was available for grazing.

Table 13 contains forage growth and persistence by year. Annual ryegrass is the only common forage planted across years. Overall forage mass ranged from 607 to 1838 kg DM/ha, depending on year of planting and forage species used. Grazing days were affected by the number of hectares planted of each forage, which were not equal. Forage consumed was estimated by assuming the heifers ate half of the forage that disappeared during the rotation (Greene and Brazee, 2012). The other half disappeared due to defecation, urination and trampling.

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Table 13. Forage Production and Grazing Days by Species and Year.

	Simple Means		Least Squares Means[1]	
Forage Type	No. Grazing Days	Stocking Density (hd/ha)[2]	Forage Mass (kg DM/ha)	Forage Consumed (kg/day/heifer)
Year = 2016				
Tall Fescue[3]	84	4.8	940^{a}	5.9^{a}
Rye	71	8.1	607^{b}	6.5^{a}
Annual Ryegrass	143	6.4	1039^{a}	8.6^{b}
Year = 2017				
Annual Ryegrass	70	6.1	1658	11.2
Year = 2018				
Rye	25	10.5	789^{a}	4.4^{a}
Rye and Crimson Clover	44	4.5	918^{a}	5.1^{a}
Annual Ryegrass	11	3.9	1298^{a}	5.2^{a}
Annual Ryegrass & Crimson Clover	51	8.8	1838^{b}	10.8^{b}
Black Oats	3	10.4	602^{a}	4.1^{a}
Black Oats & Crimson Clover	21	4.26	1072^{a}	6.6^{a}
White Oats	11	2.2	988^{a}	1.5^{a}
White Oats & Crimson Clover	25	7.7	1056^{a}	6.7^{a}
Year = 2019				
Tall Fescue[3]	13	1.3	1449^{ab}	9.3
Annual Ryegrass	30	1.6	1072^{b}	14.5
Annaul Ryegrass & Crimson Clover	171	1.7	1475^{a}	11.3
Small Grains[4]	126	2.4	1074^{b}	10.2

[1] Differing subscripts within column and year are significantly different ($P < 0.05$).

[2] Represents number of heifers per paddock (0.8-ha each) managed under rotational stocking to a target of 50% forage utilization.

[3] In 2016 the variety of tall fescue used was ‘Texoma’ which died during the drought of 2017. In 2019, the variety of tall fescue used was ‘Martin II’. Both varieties were stockpiled prior to grazing.

[4] The small grain mixture consisted of oats, wheat, triticale, rape and crimson clover.

Growth and Performance

Average daily gain change between weigh periods and across years is presented in Table 14. For ADG in every year except 2017, ADG increased from 28 d to 56 d on the project, which was most likely due to compensatory gain from previous management to grazing cool-season forages. In 2017, drought impacted the forage establishment time window, and heifers did not start grazing forages until after the second weigh period. Each year except 2016, where there was an extended spring with cooler than average temperatures, ADG decreased from 81 d to 166 d during the grazing season, most likely due to cool-season forages becoming overly mature and less nutritious. The mean ADG across years is similar for each year, with the exception of 2017.

Regardless of year, all heifers reached a minimum of 60% of their projected mature weight based on frame score. When heifers were weighed in mid-June, most heifers needed to continue to gain 0.13 to 0.45 kg/d until calving to reach 85% of their projected mature body weight. This rate of growth should be achievable with continued adequate forage availability on warm-season perennial pastures and fall growth of tall fescue.

Table 14. ADG (kg/d) of heifers among years and across (28 d) weigh periods.

	Heifer weigh periods (every 28 days)				
Date	28-d	56-d	81-d	166-d	Mean
2016	$0.49^{a,e}$	$0.83^{a,f}$	$0.91^{a,f}$	$0.83^{a,f}$	0.77^{a}
2017	$0.76^{b,e,f}$	$0.85^{a,e}$	$0.71^{b,f}$	$0.54^{b,g}$	0.72^{a}
2018	$0.36^{a,e}$	$0.45^{b,f}$	$0.78^{a,b,g}$	$0.85^{a,h}$	0.61^{b}
2019	$0.50^{a,e}$	$0.68^{c,f}$	$1.07^{c,g}$	$0.82^{a,h}$	0.77^{a}

$^{a-d}$ Within a column, means differ ($P < 0.05$, SEM = 0.06, n = 4).
$^{e-h}$Within a row, means differ ($P < 0.05$, SEM = 0.09, n = 4).

Body weight gain between weigh periods and across years is presented in Table 15. For BW gain, heifers increased BW between each weigh period across all years. Mean BW gain during the grazing season was similar across years, except for 2018 in which mean BW gain was less. In 2018, heifers gained more slowly through the 56-d weigh period, but compensated with positive comparable gains to other years in the last two weigh periods.

Table 15. Weight gain (kg) of heifers among year and across (28 d) weigh periods.

	Heifer weigh periods (every 28 days)				
Date	28-d	56-d	81-d	166-d	Mean
2016	13.08^{a,e}	48.01^{a,f}	82.12^{a,g}	130.04^{a,h}	68.33^{a}
2017	21.46^{a,e}	46.57^{a,f}	57.75^{b,g}	86.62^{b,h}	52.01^{b}
2018	9.03^{b,e}	24.57^{b,f}	62.41^{c,g}	103.96^{c,h}	50.00^{b}
2019	14.67^{a,e}	36.60^{c,f}	83.71^{a,g}	130.92^{a,h}	61.94^{a}

$^{a-d}$ Within a column, means differ ($P < 0.05$, SEM = 5.91, n = 4).
$^{e-h}$Within a row, means differ ($P < 0.05$, SEM = 3.98, n = 4).

Frame score (1-9) change on heifers among year and across weigh periods is presented in Table 16. With frame score, the expectation is for the heifers to grow in height over time during the development phase across weigh periods. In all years except 2017, heifers frame score increased across weigh periods. The average frame score range was 5.0 – 6.3. The mean frame score was similar in 2016 and 2018, as well as 2017 and 2019.

Table 16. Frame scores (1-9) of heifers among years and across (28-d) weigh periods.

	Heifer frame score (every 28 days)				
Date	28-d	56-d	81-d	166-d	Mean
2016	$5.6^{a,e}$	$5.8^{a,f}$	$6.0^{a,g}$	$6.0^{a,g}$	5.9^{a}
2017	$5.2^{b,f}$	$5.4^{b,e,f}$	$5.5^{b,e}$	$5.0^{b,g}$	5.3^{b}
2018	$5.7^{a,e}$	$5.8^{a,e}$	$5.9^{a,f}$	$6.3^{c,g}$	5.9^{a}
2019	$5.1^{b,e}$	$5.3^{b,f}$	$5.5^{b,f}$	$5.7^{d,g}$	5.4^{b}

$^{a-d}$ Within a column, means differ ($P < 0.05$, SEM = 0.08, n = 4).
$^{e-h}$Within a row, means differ ($P < 0.05$, SEM = 0.11, n = 4).

Reproduction

Reproductive tract score and hour bred (48-h, 60-h, 65-h, 72-h, 84-h post prostaglandin F2α injection) were not significant sources of variation for pregnancy outcome. However, method bred (estrus-detected vs. non-detected) tended (*P* = 0.0921) to improve conception rate to AI where 35% of heifers exhibiting behavioral estrus and 5 % of heifers time-bred conceived to first service AI across years of the program. Across years, 40% of heifers conceived to first service AI, 42% conceived to clean-up bull, and 18% of heifers remained open after last pregnancy check. Overall mean conception across the 4 years was 78% total conception rate (2016 – 70%; 2017 – 82%; 2018 – 74%; 2019 – 84%).

Cost and Care

Table 17 contains the actual costs of the various aspects of developing heifers from weaning to breeding. In 2017, the heifers were fed for the first 56 days of the program. Breeding costs included the cost of timed AI, lease of a clean-up bull and veterinarian costs for pregnancy checking the heifers. Since 2016, only one heifer has passed away due to health issues, The Auburn University Diagnostic Lab, Auburn, AL determined the cause of death to be hardware's disease resulting from a 10 cm long piece of wire that was recovered from her intestinal tract. One heifer was removed from the program due to being pregnant on arrival to SMREC.

Table 17. Cost per Hd of Heifer Development by Year.

Item	2016	2017	2018	2019
Forage (establishment & fertility)	$99.50	$68.34	$91.13	$89.89
Minerals	$21.08	$4.69	$4.69	$23.45
Feed	$0.00	$152.70	$0.00	$16.15
Health	$14.15	$12.05	$10.09	$13.77
Carcass Ultrasound	$14.42	$19.88	$19.88	$20.44
Breeding	$63.37	$67.04	$67.04	$63.26
Total	$212.52	$324.70	$192.83	$226.96

Summary and Conclusions

Heifer development is one of the most important aspects of cattle production and the cow-calf sector. Critical attention to management of replacement heifers during development can have a sustained impact on long-term efficiency. Being able to make informed selection decisions based on phenotypic and performance parameters in an environment similar to where the heifer will have to be a productive cow is important to the sustainability of a cattle business. Developing heifers on cool-season forages is an option that demonstrates steady growth and performance with minimized cost of gain. Developing heifers at a central location or by a 3rd party can reduce the need for producers to maintain multiple herd sires and facilities or paddocks to develop heifers separate from the main herd, it also supports producer education on heifer development practices. Developing herd management goals for selecting and replacing females with performance data collected in university-based heifer development programs enhances private-farm beef herd potential over time.

CHAPTER 6

CONCLUSIONS AND IMPLICATIONS

Heifer development is one of the most important aspects of beef cattle production and the cow-calf sector. Being able to make informed selection decisions based on phenotypic and performance parameters in an environment similar to where a heifer will ultimately become a productive cow is important to the sustainability of a cattle business. Developing heifers on forages is an option that demonstrates steady growth and performance at a reduced cost compared to drylot options. Developing heifers at a central location with multiple locations reduces the need for producers to maintain multiple herd sires and facilities or paddocks to develop heifers separate from the main herd.

While recommended planting time for alfalfa into bermudagrass sod is in the fall, drought years and/or unseasonably warm winters, or previous herbicide treatment can delay or affect successful stand establishment. Adjusting stand establishment to late winter/early spring can be an option, but as observed in this experiment, may decrease alfalfa persistence and increase contribution from undesirable grass and weed species. New technology may offer potential solutions to weed control such as Round-Up Ready® alfalfa varieties or time release herbicide technology. Information obtained from this project aims to move the cattle industry in the southeastern region towards a year-round grazing management system, through effective utilization of higher quality forage combinations and decreased dependence on costly fertilizer and feed inputs. Understanding the growth patterns and persistence of alfalfa- bermudagrass mixtures is an important step in producing a better quality forage in warm-season systems with less N inputs. Additional research with fall planting, more grazing tolerant varieties, and additional pest control could allow more success and eventual adoption of interseed alfalfa into bermudagrass stands.

Endophyte-infected TF is the dominant forage in the Blackbelt and North Alabama region. Even though TF can provide adequate nutritive quality, the fescue toxicosis symptoms reduce intake and cause physiological changes effecting overall health and well-being. Over the 2 years of this study (one year for metabolomics), heifer gain responses and conception rates were similar across various forage systems, demonstrating the potential for using warm-season forages as an alternative to tall fescue in the Alabama Black Belt soil region without repercussion of forage impact on ability to conceive to first service AI. Evaluating this alternative grazing system is beneficial for producers who are trying to reduce input costs and alleviate the reproductive effects of fescue toxicosis in their heifer development programs during the summer months. Forage quantity and nutritive value are the main factors influencing C_4 grasses quality, however, additional variables, such as temperature, humidity, canopy structure, and management practices may affect C_4 forage quality in subtropical areas. Cattle producers can make alternative forage system plans without animal performance based on information garnered from this study.

Future grazing and animal performance studies may be beneficial in demonstrating and evaluating the intensive management practices required by native grasses, such as rotational stocking and prescribed burning. Other alternative summer grazing systems for the region should also be evaluated in comparison to NWSG blends, PB and ETF. Evaluating this alternative grazing system is beneficial for producers who are trying to reduce input costs and alleviate the reproductive effects of fescue toxicosis in their heifer development programs during the summer months. This study provides information on alternatives to endophyte-infected tall fescue and perennial forage systems options that would perform well in the Black Belt soil region of Alabama and possibly across the Southeast USA.

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Appendix

(Preliminarily data related to metabolomics associated with the project in chapter 4)

Investigating Plasma Metabolomic Profiles at the Time of Artificial Insemination, Based on Pregnancy Outcomes in Beef Heifers

Introduction

Biomarker development for the identification of infertile or fertile heifers has the potential to improve the efficiency of cow-calf production. Unexplained infertility remains a significant source of inefficiency and being able to identify those heifers with increased reproductive potential or decreased reproductive potential could assist with heifer recruitment in the herd. Currently, selection efficiency remains limited due to the low heritability of reproductive performance, which results in the recommendation to select approximately 25% more heifers than required (Kuhn et al., 2006). Overall pregnancy rates in heifers range from 70-90% utilizing AI and natural breeding programs (Schatz et al., 2008). Poor fertility accounts for the majority of cows culled and remains largely unmanageable due to a lack of informative biomarkers for fertility (Wathes et al., 2008). The development of an early detection assay utilizing biomarkers will minimize costs associated with over-selecting or under-selecting heifers.

Metabolomics, or metabolomics profiling, involves the quantitative measurement of the global set of low-molecular-weight metabolites in a biological fluid (Goodacre et al., 2004). Metabolite levels can be compared among different phenotypic states and can potentially be used as health indicators. Mass spectrometry-based metabolite analysis has been applied in the development of many informative biomarkers to help identify hard-to-diagnose disorders (van der Kloet et al., 2012; Günther, 2015; Jafarzadeh et al., 2015; Zhou et al., 2007). Recent studies have utilized metabolomics analysis in assessing embryo and oocyte quality (Singh et al., 2007; Nagy et al., 2009; Revelli et al., 2009). Within cattle, metabolomics analysis of follicular fluid has been used to potentially explain differences in fertility between heifers and lactating cows (Bender et al., 2010). Authors in this study discovered metabolites significantly differed in the blood serum between heifers and cows (Bender et al., 2010).

In this limited number study, metabolomics profiling was utilized to identify metabolites in the blood plasma that may be useful in identifying heifers conceiving to first service AI, natural service, or remaining open.

Materials and Methods

Animal Use

All animals sourced in this study belonged to AAES. All procedures with animals were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee (PRN 2019-3591).

Research Site

A summer grazing trial was conducted at the Black Belt Research and Extension Center (BBREC) in Marion Junction, AL (32º28’50.29”N latitude, 87º15’26.61”W longitude) during the 2018 and 2019 growing seasons to evaluate forage production, persistence, nutritive value and beef heifer performance through breeding using 1) a native-warm season grass (NWSG) mixture of big bluestem (‘KY ecotype’ Andropogon gerardii), little bluestem (‘Cimarron’ Schizachyrium scoparium), and indiangrass (‘Boone’ Sorghastrum nutans) with no nitrogen (N) fertilization, 2) NWSG fertilized with 56 kg N/ha, 3), endophyte-infected ‘KY-31’ tall fescue or 4) bahiagrass under continuous stocking.

For NWSG, six plots (2 ha each) comprising Houston clay were demarcated in early 2018 for the project (Figure 4). The NWSG mixture was established in April 2016 using a seeding rate of 11 kg/ha (pure live seed) for the tertiary blend, consisting of 6 kg/ha big bluestem, 4 kg/ha indiangrass, and 1 kg/ha of little bluestem. Paddocks were sampled on a 2-ha grid basis in mid-April 2016 during the establishment year and amended with variable rates of P and K in July 2017 according to soil test recommendations of the Auburn University Soil Testing Laboratory. During the 2016 and 2017 growing seasons, both mowing and mob grazing were utilized to manage the forage to a target residual height of 25 cm. A prescribed burn was performed in late February 2018 and 2019 to reduce the amount of thatch present before the growing season initiated in May during the years of the grazing trial.

For bahiagrass, two plots (0.8-ha each) were identified the previous growing season (2017) for the project (Figure 5). The paddocks were comprised of two-year old ‘Pensacola’ bahiagrass stand. Plots were mowed during the 2017 growing season twice throughout the summer, and grazing was utilized to manage the forage to a target height of 15 cm. Soil tests were performed in February 2017, and soil amendments were made according to the Auburn University Soil Testing Laboratory results.

For ‘KY-31’ tall fescue, two plots (0.8-ha each) were identified the previous growing season (2017) for the project (Figure 6). The paddocks comprised existing (45-year old stand) ‘KY-31’ tall fescue. Plots were mowed during the 2017 and 2018 growing season twice throughout the winter and spring, and grazing was utilized to manage the forage to a target height of 15 cm. Soil tests were performed in February 2017 and soil amendments were made according to the Auburn University Soil Testing Laboratory results.

Experimental Design

Thirty-six fall born Angus × Simmental beef heifers (initial BW 288 kg) were selected and randomly assigned to one of four grazing treatments in each year of the study: 1) tall fescue, 2) NWSG with N, 3) NWSG no N, or 4) bahiagrass. Heifers were born between September 11, 2017 and October 18, 2017 in Year 1 and September 3, 2018 and October 11, 2018 in Year 2. Heifers were weaned according to the standard operating procedure of the Black Belt Research and Extension Center (PRN 2016-2990) in May 2018 and May 2019, respectively. From weaning until the start of the study, heifers were placed in a dry lot setting with annual ryegrass hay and supplemented with 50:50 pelleted corn gluten and soybean hulls at 1% of their BW hand-fed daily. Initial test weights for beef heifers were collected on June 4, 2018, and May 29, 2019, respectively, and four test heifers were randomly assigned per paddock to NWSG, and three test heifers per paddock were assigned to tall fescue and bahiagrass pastures based on forage allowance.

Animal Performance During the Grazing Season

Thirty-six beef heifers were weaned for two weeks prior to the start of the grazing trial. When paddocks reached the target grazing height, heifers were weighed, body condition scored, and randomly assigned to paddocks. Heifer weights and BCS were taken again every 28 days until the completion of the study. Following the first weigh period in each year of the study, heifers had less than 0.22 kg/d gain. Heifers were supplemented with 50:50 soyhulls and corn gluten feed to target 0.5 kg/d gain as needed during each year of the experiment based on forage nutritive value to keep heifers growing at a rate to support a projected early winter breeding window.

Pre-Breeding Performance and Conception Rate

Heifer management post-grazing included being fed free-choice annual ryegrass hay and a 50:50 soyhull/corn gluten feed mixture at 1% of BW per day to achieve a target gain of 1 kg/heifer/day. Approximately 30 days before the start of their first breeding season, heifers were evaluated for body condition score [BCS; scale of 1-9 with 1 = emaciated and 9 = obese; (Wagner et al., 1988)] and assessed for RTS [RTS; scale of 1-5; 1 = pre-pubertal, 5 = pubertal, luteal phase; (Anderson et al., 1991)] as well as pelvic area [cm^2, using a Rice Pelvimeter; (Johnson et al., 1988)] by a single, experienced veterinarian. At approximately 15 months of age, treatment heifers underwent estrous synchronization for AI utilizing the Select Synch + CIDR protocol to begin their first breeding season. Heifers received an injection of GnRH (i.m.; 100 µg; Cystorelin®; Merial, Duluth, GA) and insertion of a controlled internal drug release (CIDR) device (intravaginal insert; 1.38 g progesterone; Eazi-Breed® CIDR®; Zoetis Inc., Kalamazoo, MI, USA). Each CIDR® was removed following 7 days, and an intramuscular injection of 25 mg of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI) was administered at the same time. Heifers were then detected for estrus (AM/PM) and artificially inseminated 12-h post estrus detection with a single straw of semen originating from a single Angus bull source. Non-estrus detected heifers were all bred 72 h post injection of prostaglandin F2α (PGF; i.m.; 25 mg; Lutalyse®; Zoetis Inc., Kalamazoo, MI). Two professionals in random rotation were responsible for AI procedures at each experimental station for each year.

Fourteen days after AI, heifers were exposed to 1 fertile bull for natural breeding for the remainder of the breeding season. An experienced veterinarian performed initial pregnancy evaluation by transrectal palpation on day 62 to 89 post AI, followed by final pregnancy evaluation on day 85 to 176 post insemination. Presence or absence of a conceptus, alongside morphological features indicating fetal age were recorded, and heifers were classified as ‘pregnant to AI’ (Preg AI), ‘pregnant to natural service’ (Preg NS), or ‘not pregnant’ (Not Preg).

Blood Collection and Processing

At the time of artificial insemination, 10 mL of blood was collected via tail (coccygeal) vein of each heifer using an 18G needle into an EDTA blood collection tube (BD Vacutainer). The sample was immediately inverted 10 times, immersed on ice, and transported to the Reproductive Biology and Development Laboratory at the Center for Advanced Science, Innovation, and Commerce in Auburn, AL. Once in the laboratory, the blood tubes were sprayed with 70% ETOH to ensure elimination of contamination from the onsite farm location. Samples were centrifuged at 2,000 x g for 15 minutes at 4 degrees Celsius. Two 500-μl samples of blood plasma were removed and stored at -80 degrees Celsius for metabolomics data analysis. Samples were stored at -80 degrees Celsius until further processing.

Metabolomics Data Collection

Blood plasma samples from 27 animals collected at the time of AI (n = 13 pregnant by AI) and (n = 14 pregnant by clean-up bull) were used to identify metabolites at different levels. Samples had metabolomic profiles generated via untargeted profiling of primary metabolism by automatic linear exchange/cold injection at the West Coast Metabolomics Center (Davis, California, U.S.A.). An Agilent 6890 GC equipped with a Gerstel automatic liner exchange system (ALEX) that includes a multipurpose sample (MPS2) dual rail, and a Gerstel CIS cold injection system (Gerstel, Muehlheim, Germany) was used to collect GC-TOF. Temperature program was as follows: 50°C to 275°C final temperature at a rate of 12 °C/s and hold for 3 minutes. Injection volume is 0.5 μl with 10 μl/s injection speed on a splitless injector with purge time of 25 seconds. Liner (Gerstel #011711-010-00) is changed after every 10 samples (using the

Maestro1 Gerstel software vs. 1.1.4.18). Before and after each injection, the 10-µl injection syringe is washed three times with 10 µl ethyl acetate. Data were acquired with the following chromatographic parameters: column used Rtx-5Sil MS (30 m X 0.25 mm diameter Restek corp.) with a 0.25-µm 95% dimethyl/5% diphenylpolysiloxane film; mobile phase Helium with a 1 mL/min flow rate; injection volume 0.5 µL [18] . The oven temperature is held constant at 50°C for 1 min and then ramped at 20°C/min to 330°C at which it is held constant for 5 min. A Leco Pegasus IV time of flight mass spectrometer is controlled by the Leco ChromaTOF software vs. 2.32 (St. Joseph, MI). The transfer line temperature between gas chromatograph and mass spectrometer is set to 280°C. Electron impact ionization at 70V is employed with an ion-source temperature of 250°C. Acquisition rate is 17 spectra/second, with a scan mass range of 85-500 Da. Raw data files were preprocessed directly using ChromaTOF vs. 2.32 without smoothing, 3-s peak width baseline subtraction just above the noise level, and automatic mass spectral deconvolution and peak detection at signal to noise levels of 5:1. Absolute spectra intensities were further processed by a filtering algorithm implemented in the metabolomics BinBase database. The BinBase algorithm used the following settings: validity of chromatogram (< 10 peaks with intensity >10^7 counts/s), unbiased retention index marker detection (MS similarity > 800, validity of intensity range for high m/z marker ions), retention index calculation by 5th-order polynomial regression. Spectra are cut to 5% base peak abundance and matched to database entries from most to least abundant spectra using the following matching filters: retention index window ± 2,000 units (equivalent to about ±2 s retention time), validation of unique ions and apex masses (unique ion must be included in apexing masses and present at >3% of base peak abundance), mass spectrum similarity must fit criteria dependent on peak purity and signal/noise ratios and a final isomer filter. Failed spectra are automatically entered as new database entries if s/n >25, purity < 1.0 and presence in the biological study design class was >80%. All thresholds reflect settings for ChromaTOF v. 2.32. Quantification is reported as peak height using the unique ion as default, unless a different quantification ion is manually set in the BinBase administration software BinView. A quantification report table is produced for all database entries that are positively detected in more than 10% of the samples of a study design class (as defined in the miniX database) for unidentified metabolites. The data were then prepared as peak heights for the quantification ion at the specific retention index. Binned data were normalized and scaled to remove potential bias arising due to sample handling and variability. Normalization by sum was performed followed by scaling (mean-centering and division by the square root of standard deviation of each variable), to give all variables equal weight regardless of their absolute value.

Pregnancy Detection

Pregnancy was determined at 65 days post AI via transrectal palpation by a trained veterinarian. Heifers were identified as pregnant (AI), pregnant (Bull) or nonpregnant (Open) based on the size of the conceptus or lack thereof. All heifers were analyzed for metabolite concentrations.

Statistical Analysis

Univariate Statistical Analysis

Univariate analysis was applied to a total of 122 metabolites from 14 natural bred (Pregnant by clean-up bull) and 13 AI (Pregnant by AI) heifer plasma samples. Data were normalized by sum in order to minimize concentration differences. Following normalization, scaling (mean-centering and division by the square root of standard deviation of each variable) was performed to equally weight each variable regardless of absolute value. T-tests were performed with an FDR cutoff of 0.05. Metabolites were considered at significantly different levels when $P \leq 0.05$ Data are presented as mean ± standard deviation of the mean (Figure 7).

Multivariate statistical analysis

Multivariate analysis was applied to a total of 122 metabolites from 10 natural bred (Pregnant by clean-up bull) and 10 AI (Pregnant by AI) heifer's plasma samples. Data were normalized by sum in order to minimize concentration differences. Following normalization, scaling (mean-centering and division by the square root of standard deviation of each variable) was performed to equally weight each variable regardless of absolute value. Partial Least Squares Discriminant Analysis (PLS-DA) was then performed using MetaboAnalyst [accessible at http://metaboanalyst.com (Sabatine et al., 2005)] using functions from the R and Bioconductor packages (Zhang et al., 2012) in order to maximize class discrimination (Figure 8).

Results and Discussion

Metabolome assessment and analysis based upon pregnancy outcome

Univariate t-test analysis found 0 differently expressed metabolite levels between the naturally bred heifers and AI heifers (n=27) plasma samples. PLS-DA (Partial Least Squares Discriminant Analysis) displayed a non-significant separation between naturally bred heifers (green – 2) and AI heifers (red – 1) ($P > 0.05$). Phillips et al. (2018) reported analysis of metabolomic profiles in beef heifers at the time of AI revealed 15 metabolites at significantly different levels (T-test $P \leq 0.05$), with seven metabolites having a greater than 2-fold difference (T-test $P \leq 0.05$, fold change ≥ 2, ROC-AUC ≥ 0.80) between infertile and fertile heifers. Following filtering for metabolites with at least a 2-fold change between samples, tryptophan, cystine, histidine, ornithine, asparagine, glutamine, and lysine were identified as significantly different ($P < 0.05$, FDR 0.05) between the fertile and infertile groups (Phillips et al., 2018).

Metabolome assessment and analysis based upon forage treatment

Multi-group analysis, one-way analysis of variance (ANOVA) found 0 differently expressed metabolite levels between the 4 different forage treatments (NWSG-N; NWSG-No; TF; PB). PLS-DA (Partial Least Squares Discriminant Analysis) displayed a non-significant separation between NWSG-No (red – 1), PB (green – 2), TF (purple – 3) and NWSG-N (blue – 4) ($P > 0.05$).

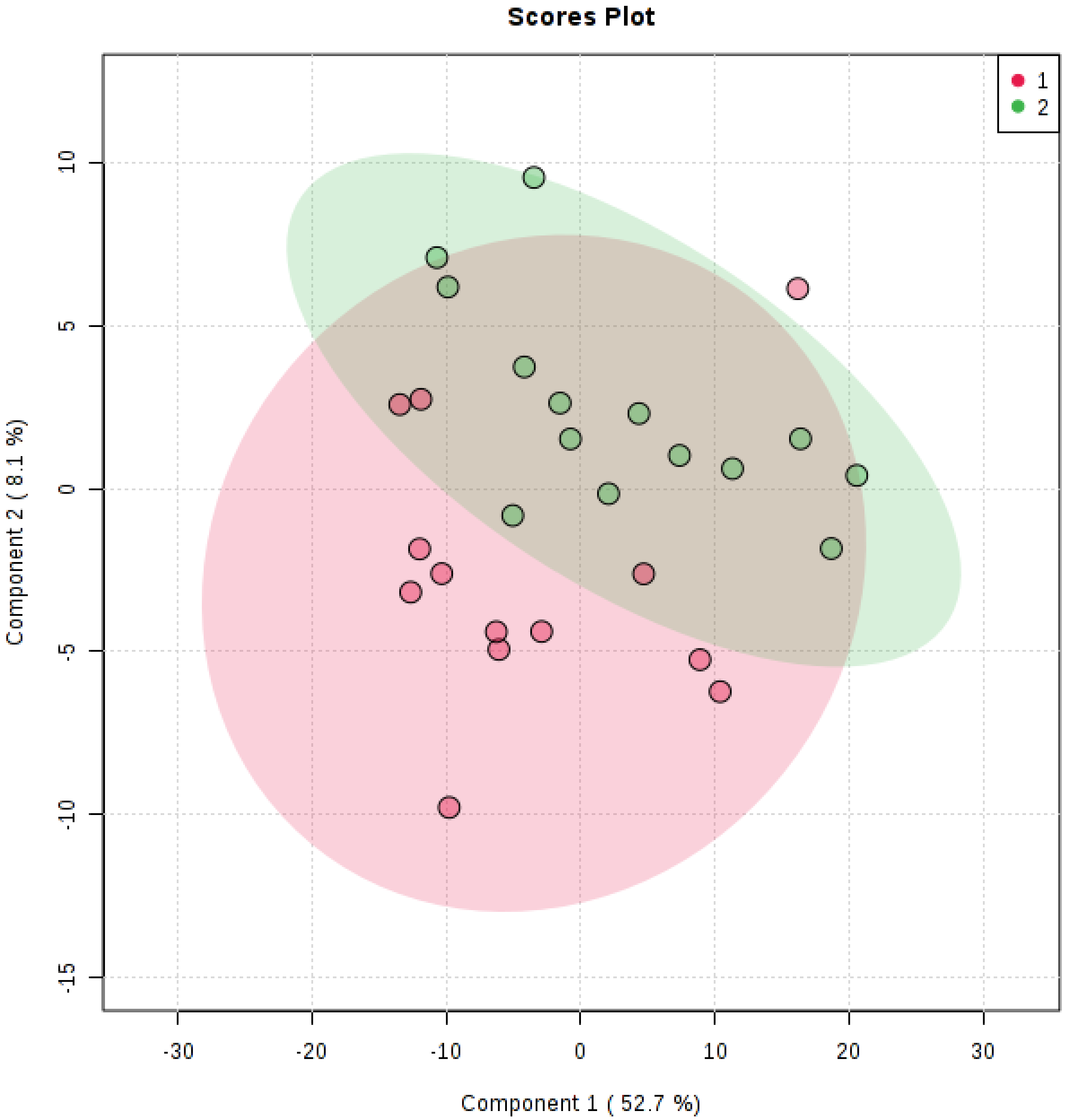

Figure 7. PLS-DA scores plot displaying a non-significant separation between naturally bred heifers (green – 2) and AI heifers (red – 1).

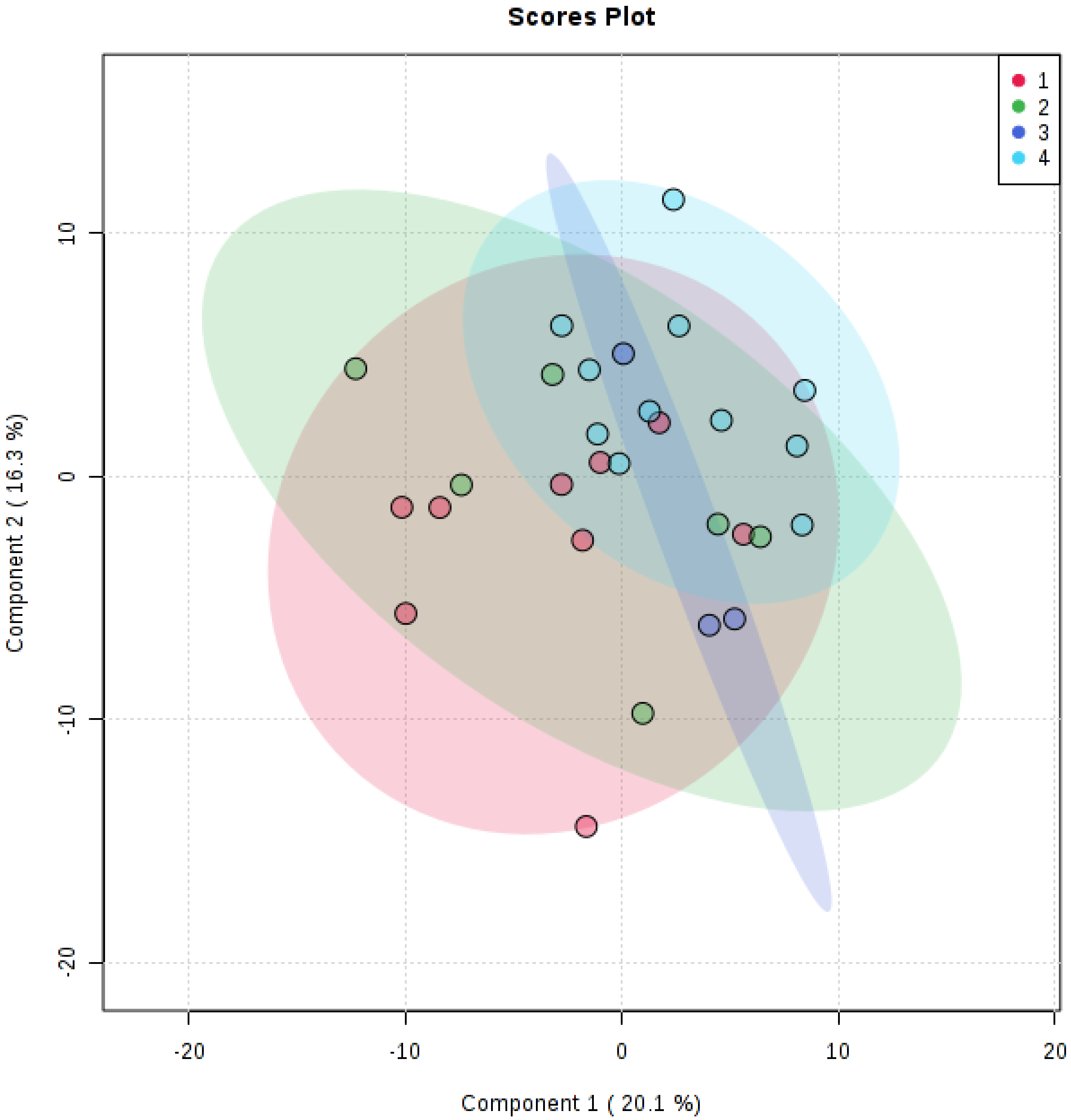

Figure 8. PLS-DA (Partial Least Squares Discriminant Analysis) displayed a non-significant separation between NWSG-No (red – 1), Pensacola bahiagrass (green – 2), tall fescue (purple – 3) and NWSG-N (blue – 4).

The main goal of our study was to generate comparative metabolomics profiles at the time of AI between heifers conceiving first service AI, natural service or open. With the limited number of observations (n=27) only 2 heifers remained open after the conclusion of the breeding season not allowing for a comparison of open heifers to AI or natural service. Therefore, heifers conceiving to first service AI or natural service were compared.

Implications of metabolomics

The ability to detect heifers unable to conceive to AI or first clean-up bull service would provide a mechanism to remove inefficient females from the herd prior to investing time and money. Biomarkers able to detect heifers with fertility problems or diets causing reproductive failure would be highly utilized by cow-calf producers to minimize costs associated with heifer production, development and forage variety selection.

Previous studies have shown that differential levels of metabolites are able to detect various difficult-to-diagnose ailments including diabetic kidney disease (Kloet et al., 2012) Parkinson's disease (Bogdanov et al., 2008), myocardial ischemia (Sabatine et al., 2005), ovarian cancer (Zhang et al., 2012), and endometriosis (Dutta et al., 2012). Moreover, within the agriculture sector, metabolite concentrations in follicular fluid and blood were used to potentially explain differences in fertility between heifers and lactating cows (Bender et al., 2010). Phillips et al., (2018) utilized metabolomic profiling to identify metabolites in the blood plasma that may be useful in identifying infertile heifers at the time of artificial insemination (AI). Prior to AI, phenotypic parameters including body condition, weight, and reproductive organ measurements were collected. These were determined not effective at differentiating between fertile and infertile heifers in this study. Analysis of the resulting metabolomic profiles revealed 15 metabolites at significantly different levels, with seven metabolites having a greater than 2-fold difference (t-test $P \leq 0.05$, fold change ≥2, ROC-AUC ≥ 0.80) between infertile and fertile heifers. This study was approached to detect metabolite differences in the blood plasma of heifers managed under different forage treatments during the summer months, heifers were returned grazing tall fescue and supplemented with 50:50 corn gluten and soyhull pellet mix at 1.5% of BW until breeding (~98-d). Current management practices utilize phenotypic methods to attempt to minimize heifers remaining open following the breeding season, therefore, we compared common phenotypic parameters between the heifers included in our analysis mentioned previously.