PRODUCTION, MANAGEMENT, AND STORAGE OF ALFALFA-BERMUDAGRASS BALEAGE

by

TAYLOR J. HENDRICKS

(Under the Direction of Dennis Hancock and Jennifer Tucker)

ABSTRACT

Alfalfa-bermudagrass baleage technology may be used to improve forage nutritive value, minimize the need for livestock supplementation, and minimize risks associated with weather, but producing and maintaining high quality baleage poses a concern for many producers. The objectives of this research were to: 1) compare the forage yield and quality of 'Tifton 85' bermudagrass fertilized with N (T85) and 'Tifton 85' bermudagrass interseeded with 'Bulldog 805' alfalfa (T85+Alf), 2) determine the effect of storage length on nutritive value, 3) evaluate commercially available microbial inoculants for improved fermentation, and 4) determine the potential of a ferulic acid esterase (FAE)-producing microbial inoculant for improved fermentation and fiber digestibility in alfalfa-bermudagrass harvested and stored as baleage in the Southeast. Studies were conducted on alfalfa-bermudagrass mixtures in Tifton and Watkinsville, GA between 2016 and 2018. T85+Alf produced greater cumulative yield as well as increased CP, IVDMD, and TDN compared with T85. Further, during storage, CP, TDN, and IVDMD of T85+Alf did not decrease beyond the 9-month time point and in T85 parameters decreased between harvest and 6-weeks, but not thereafter. Therefore, these

data suggest that forage can be stored longer than the current feeding recommendations without decreasing nutritive value. Upon treatment with one of five commercially available inoculants, fermentation characteristics were affected by the ensiling period, however fermentation was not affected by inoculant treatment. Further, fermentation characteristics, digestibility, gas production, and ruminal pH were not impacted by the addition of a microbial inoculant containing an FAE-producing bacteria.

INDEX WORDS: Alfalfa, Bermudagrass, Baleage, Microbial inoculants

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DEDICATION

This work is dedicated to my wonderful family for never letting me think I dreamt too big. It is also for all the little girls and grown women who decided not to accept being told that they couldn't do something.

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CHAPTER 1

INTRODUCTION

Livestock producers across the United States are constantly looking for the most sustainable production methods. In the Southeast, livestock producers have the advantage of a mild climate that allows for forage growth nearly year-round. With the mild temperatures, however, come a myriad of other challenges. Pest and disease pressure can limit the species and varieties that thrive, while high humidity and frequent summer storms make harvesting and storing forage as hay difficult.

Beef cattle producers in the Southeast take advantage of the long growing season, utilizing perennial warm-season forages and winter annuals, but there is still a forage gap that needs to be filled during the spring and fall. Bermudagrass (*Cynodon dactylon* L. Pers.) is the primary warm-season forage used in the region. Bermudagrass is often referred to as the "King of Forages" because of its high yields, although it has, at best, moderate quality that often requires additional supplementation during certain stages of production (i.e. lactation). Additionally, to maintain these high yields, bermudagrass requires high levels of nitrogen (N) fertilization, which require time and money from the producer.

Incorporating a forage legume into a grass-based system has been shown to improve the forage quality of a given stand while also reducing the need for additional N fertilization (Beck et al., 2017; Stringer et al., 1996). Many of the forage legumes available for use in the Southeast are cool-season annual species, making them suitable

for incorporation with winter annual grasses. Although there are fewer warm-season legume choices, alfalfa is one option worth considering.

Alfalfa is the third most valuable crop in the United States, responsible for $9.3 billion cash receipts in 2017 (NASS, 2017). As a legume, it fixes N and reduces the need for N fertilizer in a stand. It is a high-quality forage that is a staple in the dairy and equine industries because of its high concentrations of crude protein (180 to 220 g kg^{-1}), total digestible nutrients (640 to 670 g kg^{-1}), and relative forage quality (125 to 160; Lacefield at al., 2009). Additionally, unlike many forage legume options, alfalfa is high-yielding, meaning there will be little to no yield drag when incorporated with a perennial grass. Alfalfa is widely used in the Northern and Western states and has recently seen a resurgence in the southeastern U.S. (Lacefield et al., 2009). This increase in acreage can be attributed to the development of alfalfa varieties more suited to the region's climate. Southern-adapted varieties, such as 'Bulldog' and 'Alfagraze,' have been bred with pest and disease resistance, as well as lower fall dormancy ratings. This lengthens the growing season, making alfalfa an appealing option for many producers. While we know that alfalfa can grow in the Southeast, the published research using a southern-adapted alfalfa variety in a mixed-grass legume system is minimal.

Even with a long growing season, forage gaps can still exist. When forage availability is low, producers often turn to stored forages such as dry hay. However, during the peak growing season in the southeastern U.S., there is significant risk associated with traditional hay production. Hay curing in the region generally lasts three to five days during the peak growing season due to high relative humidity, although finding a long enough rain-free period is difficult. To minimize the risk of weather

associated losses, producers might opt to sacrifice yield, or more likely, quality by delaying harvests. It has been widely noted that forage quality is directly related to plant maturity and delaying harvests can lead to overmature forage and a drastic reduction in feed value (Hancock et al., 2014).

Baled silage, or baleage, is a stored forage production method that can help minimize some of the weather risks associated with forage harvesting in the southeastern U.S. Baleage is produced at a higher forage moisture, 40-60%, compared with the 10-18% desirable in traditional dry hay. This makes the drying time for baleage much shorter than dry hay. Most baleage can be baled within 24 hours of cutting, compared with the 3 to 5 days necessary for dry hay. In addition to the higher moisture, baleage is wrapped in plastic to create an anaerobic environment where the water soluble carbohydrates in the forage are fermented. This creates a highly palatable livestock feed (Collins and Owens, 2003).

One concern associated with baleage is its relatively short "shelf life" compared with dry hay. Current feeding recommendations state that baleage should be fed within 9 months post-harvest to prevent quality losses or spoilage (Hancock et al., 2019). However, this recommended feed out type may not align with the nutrient requirements of the animals in the system or the forage gaps that necessitate forage. Feeding baleage, which is normally extraordinarily high quality, to animals with lower nutrient requirements, or when adequate forage is available for grazing, makes the system economically wasteful. There has been no research examining the change in nutritive value over an extended storage period.

In addition to storage considerations, producers incorporating baleage technology must consider how to produce baleage to promote fermentation and optimize aerobic stability. Microbial inoculants for baleage production are homofermentative, heterofermentative, or combination products. Homofermentative microbial products produce exclusively lactic acid and promote initial forage fermentation while heterofermentative microbial inoculants produce lactic and acetic acids that increases the aerobic stability, or longevity of baleage when fed, and combination products contain both homo- and heterofermentative bacterial species. Although the effect of each type of bacterial strain has been investigated, there has been little work to investigate the effectiveness of these bacterial strains as commercially available products on a production scale.

Further, new heterofermentative microbial inoculants contain strains of *L.buchneri* that produce a ferulic acid esterase (FAE)-producing enzyme. The FAE enzyme is associated with the degradation of fiber when applied to ensiled forage. If the fiber content of the forage can be degraded with the application of a microbial inoculant, it is possible that forage digestibility, and subsequently animal performance, may be improved. The information associated with FAE-producing inoculants is limited, but some previous work has shown promise to improve feed efficiency and animal gains in alfalfa.

The high buffering capacity of alfalfa makes the use of a silage/baleage inoculant advantageous to promote proper fermentation and prevent spoilage. There are many commercially available products, but there has been little research on their efficacy in the Southeast, especially in large round bales.

Therefore, the objectives of this research are to:

1) evaluate and compare the forage quality and yield of Bermudagrass with an alfalfa-bermudagrass mixture when harvested as baleage;
2) determine if the length of storage affects the feed value of alfalfa-bermudagrass baleage;
3) determine the efficacy of five commercially available microbial inoculants for improving fermentation characteristics of alfalfa-bermudagrass baleage; and
4) determine the impact of treatment with an FAE-enhanced microbial inoculant on silage fermentation characteristics, nutritive value, and dry matter digestibility when produced from either pure-stand alfalfa or an alfalfa-bermudagrass mixture.

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CHAPTER 2

REVIEW OF LITERATURE

Introduction to Forage Systems

Forages in the Southeast

Forages are a critical component for any livestock production system and are particularly important for ruminant species. Ruminants are able to convert the fiber fractions of a forage species into usable energy, making forages an economical source of feed for the animals (Ball et al., 2015). In the Southeast U.S., the climate allows near year-round forage growth, making forage production for livestock more economical than in some other regions. The majority of beef cattle operations in the Southeast are cow-calf, meaning a permanent cow herd is kept to produce an annual calf crop to sell, and therefore nutrient requirements of the animals change within the herd throughout the year, although these nutrient requirements are rarely as high as those seen in the feedlot sector or the dairy industry (Hoveland, 1986). Nutrient requirements in the cow-calf sector range from 7 to 12% crude protein (CP) and 50 to 60% total digestible nutrients (TDN) for a dry or lactating beef cow, respectively (Ball et al., 2015; NRC, 2017).

Forages are often defined in one of three ways: grass and legume; annual and perennial; or warm-season and cool-season species. In the Deep South, warm-season perennial forages, such as bermudagrass (*Cynodon dactylon* L. Pers.) and bahiagrass (*Paspalum notatum* Flüggé), make up the basis of most forage systems, growing on an estimated 24 million hectares across the region, accounting for almost 75 percent of

pasture (Ball et al., 2015). Perennial forages are moderate to high-yielding, moderate quality, and relatively inexpensive to maintain. Because they do not need to be replanted each year, once established, perennial forage maintenance costs consist of the fertilizer, pest management, and labor to maintain them. However, while both bermudagrass and bahiagrass are high-yielding, they may not produce sufficient levels of digestible energy or protein to sustain animals with higher nutrient requirements (e.g. lactating beef cows). Additionally, these species do not grow year-round, leading to the need for supplementation via stored forages (e.g. hay), other forage species, or grain and byproduct supplement. Ultimately, the goal of livestock producers is to optimize their profitability – by balancing high production while minimizing input costs. This can be achieved by providing high-quality grazing for as much of the year as possible to reduce the costs of supplementation and hay or stored forage production.

Bermudagrass

Although both bermudagrass and bahiagrass are key warm-season species grown in the region, bermudagrass is the primary warm-season perennial forage in the Southeast; it is grown on an estimated 8.1 million hectares (Redfearn and Nelson, 2003). It is used for both grazing and stored forage operations, producing harvestable forage between May and October (Ball et al., 2015). Bermudagrass is native to Southeastern Africa and is thought to have been introduced in the southern U.S. during the 1600s (Ball et al., 2015) and to Georgia in 1751 (Hancock et al., 2013). Originally, bermudagrass was considered to be and treated as a weed, but breeding efforts beginning in the 1930s by USDA-ARS plant geneticist, Dr. Glen Burton, led to the development and release of the

first hybrid variety, Coastal bermudagrass. This variety was the first that was bred for drought and frost-tolerance as well as high yields.

Since the late 1930s, there have been over 10 additional hybrid bermudagrass varieties released, including ‘Alicia’, ‘Coastcross I’, ‘Tifton-44’, ‘Tifton-68’, ‘Tifton-78’, ‘Tifton-85’, and ‘Russell’ (Hancock et al., 2013). Hybrid bermudagrasses produce little if any viable seed. Rather, they reproduce via vegetative propagation from tops or stolons. Hybrid bermudagrass varieties must be established through sprigging, which can be expensive and somewhat risky. However, once established, hybrid varieties are high-yielding (producing 9 to 13 Mg ha^{-1} annually, when provided with adequate fertility) and long-lived compared with seeded bermudagrass varieties, making them a preferred choice (Hancock et al., 2015).

Hybrid varieties should be established during March, April, or May. These varieties are established using vegetative sprigs that can be planted into a prepared seedbed either in rows (at a rate of 10 bu A^{-1}) or through broadcasting (at a rate of 25 to 40 bu A^{-1}) at 5 to 7.6-cm depth to ensure root coverage and establishment (Ball et al., 2015). Following planting, the area should be treated with a preemergence herbicide to minimize competition from annual weeds and nitrogen should be applied 3 to 4 weeks after at a rate of 45 to 67 kg ha^{-1}.

Bermudagrass yield is highly responsive to N fertilization. Burton et al. (1963) found that increasing N fertilizer rates up to 1008 kg ha^{-1} could increase dry matter production from ‘Coastal’ bermudagrass up to 15.82 Mg ha^{-1}. This was supported by the findings of Power (1980), who observed a positive correlation between applied N fertilizer and forage yields up to 224 kg N ha^{-1}. Further, Osborne et al. (1999) found that

even in rainfed systems, bermudagrass yields could be doubled at high rates of N (>672 kg N ha^{-1}). Generally, bermudagrass yield increases linearly with N fertilization up to 448 kg N ha^{-1} (Stringer et al., 1994).

N fertilization is not solely responsible for yield, however. Studies have shown that providing N fertilization to bermudagrass stands can also improve the nutritive value of harvested forage. Increases in CP in response to higher rates of N fertilization were observed as early as the 1950s (Prine and Burton, 1956; Burton et al., 1963), where increasing N fertilization up to 1008 kg N ha^{-1} increased the CP in bermudagrass to 18 g CP kg^{-1}. These results were supported by Johnson et al. (2001) and Rao et al. (2007) who found a positive linear relationship between N fertilization and CP. However, Beck et al. (2017b) found that CP response to increased N fertilization was inconsistent. The difference was attributed to application timing and the fact that Beck et al. (2017b) analyzed a grazed stand while previous work was conducted in harvested plots simulating hay harvests.

Both Beck et al. (2017b) and Rao et al. (2007) found that forage digestibility was also improved with increased N rates. Total digestible nutrients (TDN) increased as N fertilization increased in pre-grazed rotationally stocked pastures throughout the growing season. Total digestible nutrients maintained at least 600 g kg^{-1} DM in all pre-grazed pastures and was only a limiting nutritional factor in late-summer (August and September) in pastures receiving no N fertilization (Beck et al, 2017*b*). This concentration of TDN is adequate to maintain steers with an average daily gain (ADG) of 0.9 kg day^{-1} or a lactating beef cow, indicating that N fertilization could help to provide

high quality bermudagrass (NRC, 2017). Finally, Stringer et al. (1996) observed a decrease in both neutral detergent fiber (NDF) and acid detergent fiber (ADF) by increasing N fertilization from just 0 to 112 kg N ha^{-1}; decreased fiber fractions may lead to greater forage intake and improve forage degradability.

In addition to high levels of N for yield, bermudagrass also requires high levels of potassium fertilization for stand longevity. Potassium (K) is used for plant disease resistance and the production of rhizomes (Ball et al., 2015). Rhizome production has been shown to increase up to 30% between 0 and 112 kg K ha^{-1}, leading to improved winter hardiness of the bermudagrass stand (Keisling et al., 1979). Additionally, low potassium (K) may be linked to leaf spot and poor or slow regrowth in grazed or cut bermudagrass (Keisling et al., 1979).

High forage yield is not the only appealing aspect of bermudagrass production. Compared with other warm-season forages, bermudagrass has moderately high forage quality, making it unnecessary to supplement animals during the optimum growing season. When harvested on the recommended 4-week interval, producers should expect bermudagrass to be 100 to 120 g kg^{-1} DM CP, 330 to 380 g kg^{-1} DM ADF, 630 to 680 g kg^{-1} DM NDF, and 520 to 580 g kg^{-1} DM TDN, which is adequate to support a dry cow (Ball et al., 2015; NRC, 2017). These values will vary throughout the growing season and with variety. For example, Hill et al. (1993) reported CP ranging from 119 to 154 and 114 to 156 g kg^{-1} DM CP in Tifton-78 and Tifton-85 bermudagrass, respectively. Burns and Fisher (2007) compared three bermudagrass varieties – ‘Coastal’, ‘Tifton-44’, and ‘Tifton-85’ – and observed Coastal had the least digestible DM and NDF components while Tifton-85 had the greatest digestible components. Additionally, Coastal

bermudagrass had the greatest DM intake, while Tifton-85 had greater DM and fiber digestion (Burns and Fisher, 2007). These differences may be attributed to structural differences between the two varieties. Tifton-85 has a higher ester:ether ferulic acid ratio compared with Coastal bermudagrass, making the lignin component of the Tifton-85 more digestible and leading to a greater NDF concentration (Mandebvu et al., 1999). This may be because ethers can form a secondary cross-linkage with lignin, reducing the activity of ferulic acid esterase in the rumen and decreasing fiber digestion (Jung et al., 2011).

It is important to note that forage quality of bermudagrass can be difficult to maintain when not harvested appropriately. Literature states that forage quality can rapidly decrease as a response to forage maturity. This occurs as the plant cells become more fibrous as they mature, making them more difficult to digest (Hancock et al., 2014b). For example, increasing the harvest interval for bermudagrass from 4-weeks to 6-weeks can reduce CP by 20 to 40 g kg^{-1} and TDN from 62 to 51 g kg^{-1}. Further increasing the interval to 8-weeks could lead to only 60 to 80 g kg^{-1} CP and 450 to 500 g kg^{-1} TDN (Hancock et al., 2014b). While increasing the interval from 4 to 6-weeks may still produce adequate quality forage for some animal classes, an 8-week harvest interval would require supplementation to maintain even a dry cow.

It is possible to produce a large amount of high-quality livestock feed from bermudagrass, if it is managed correctly. This requires high levels of fertilization and a proper harvest interval. But, bermudagrass alone is not enough to maintain a cow-calf herd in the Southeast year-round. Bermudagrass production is largely dependent on high temperature and adequate daylight, making its peak production from May to October.

Therefore, it would still be necessary to incorporate a secondary species to extend the growing season and minimize the number of days that producers would be required to provide stored forage or supplementation to their animals.

Alfalfa

Alfalfa (*Medicago sativa* L.) is the third most valuable crop in the United States generating $9.3 billion dollars in cash receipts (NASS, 2017). Alfalfa originated in Iran and was first introduced in the United States in 1736 in Savannah, GA, although it was not successfully established (Ball et al., 2015). In the early 1900s, alfalfa acreage increased across the United States, but southern alfalfa populations were decimated by the alfalfa weevil [(*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)] (Lacefield et al., 2009).

Throughout the United States, alfalfa remains a staple forage in livestock production systems. It is used as a basis for diets in the beef, dairy, and equine industries because of its high protein and digestible energy. In the western and northern U.S. where alfalfa remains popular, it is traditionally produced as hay. These climates have low humidity making drying conditions optimal for producing high quality hay (Lacefield et al., 2009).

Efforts to increase alfalfa acreage in the Southeast has centered on plant breeding efforts which began at the University of Georgia in the late 1970s under Dr. Joe Bouton (Ball et al., 2015). Breeders have focused on four main traits: disease resistance, pest resistance, grazing tolerance, and semi- and non-dormancy. The first grazing tolerant alfalfa variety adapted to southern regions was released in 1990. Since that time, several

varieties of alfalfa adapted for the Southeast have been released, including ‘Alfagraze 600’, ‘Bulldog 805’, and ‘Bulldog 505’. These semi- and non-dormant varieties can produce 6 to 8 alfalfa harvests annually, with yields ranging from 9 to 18 Mg ha^{-1} depending on irrigation and growing conditions (Lacefield et al., 2009).

Alfalfa must be established on well-drained soils with a neutral pH. Soils with a low pH can release aluminum (Al) ions into the soil solution, especially in the clay soils typically found in the Southern Piedmont. This Al can reach levels toxic to an alfalfa taproot, stunting root development and ultimately leading to lower forage yields (Hancock et al., 2015). Therefore, it is critical that land should be prepared appropriately, including the application of lime to raise the pH to 6.5 or greater at the surface and at least 5.5 in the subsoil.

To establish alfalfa as a pure stand in the Deep South, it should be planted into a prepared seedbed during the fall of the year (mid-October to mid-November) at a seeding rate of 16.8 to 22.4 kg ha^{-1}. Fall establishment of alfalfa is recommended to minimize competition from weed and insect pests however, an early spring establishment (approximately February) is also possible if conditions do not allow for a fall planting (Ball et al., 2015).

Although Southeastern varieties are bred for disease and pest resistance, they are still at risk. The most common alfalfa diseases are: Anthracnose (*Colletotrichum spp.*), Aphanomyces root rot (*Aphanomyces euteiches*), crown and root rot complex (*Phytophthora spp.*), leaf spot complex (*Pseudomonas spp.*), and sclerotinia crown and stem rot (*Sclerotinia sclerotiorum*). Most alfalfa diseases can be controlled through proper management, including selecting tolerant varieties, maintaining stand fertility, and

harvesting on schedule (Ball et al., 2015; Hancock et al., 2015). Additionally, insect pests can pose a challenge to alfalfa producers. Alfalfa weevil [(*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)], potato leafhopper [*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae)], three-cornered alfalfa hopper [*Spissistilus festinus* (Say) (Hemiptera: Membracidae)] and fall armyworm [*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)] are common alfalfa pests in the Southeast that can be scouted for and managed through chemical control.

Alfalfa is a legume, which is allows for the fixation of atmospheric N, and therefore N fertilizer poses no additional advantage. While N fertilization may not affect yield, phosphorus (P) and potassium (K) have been shown to significantly increase alfalfa yield. Berg et al. (2005) examined the effect of P and K fertilizer on alfalfa yield components – measured through plants area^{-1}, shoots plants^{-1}, and mass shoot^{-1} – and stand persistence. Previous work demonstrated a 50% decline in stand density in fertilized plots, and a 73% decline in stand density in unfertilized alfalfa plots over a two-year period (Collins et al., 1986). While K fertilization is associated with stand persistence of alfalfa, plant populations were not affected by increasing rates. When P fertilization increased from 29 to 59 kg P ha^{-1}, plants area^{-1} declined. This decline could be attributed to the inverse relationship between plant density and shoots plant^{-1} and mass shoot^{-1} (Berg et al., 2005). Finally, long-term studies concluded that increasing rates of P and K were required to maintain forage yields and stand performance in older stands (Berg et al., 2007).

Alfalfa is popular in livestock diets because of its exceptional forage quality. Alfalfa is generally harvested at either bud or early (10%) bloom stage to optimize forage

quality and yield. Bud stage alfalfa typically has 220 to 260 g kg^{-1} CP, 380 to 470 g kg^{-1} NDF, 280 to 320 g kg^{-1} ADF, and 640 to 670 g kg^{-1} TDN which is enough to support a growing steer or for use in a dairy ration. Typically, beef cattle operations harvest alfalfa at a later maturity (10% bloom or greater) to match increased yield potential with lower nutrient requirements of a cow-calf operation. At the 10% bloom growth stage, alfalfa CP content is typically 180 to 220 g kg^{-1}, 420 to 500 g kg^{-1} NDF, 320 to 360 g kg^{-1} ADF, and 640 to 670 g kg^{-1} TDN (Lacefield et al., 2009).

Compared with most legumes, alfalfa has a high concentration of lignin. Lignin, which is indigestible to ruminants, rapidly increases as plants mature. Lignin remains in the rumen for a long period of time compared with other fiber fractions, reducing the digestibility of the forage and dry matter intake and performance of the animal. Because the lignin content increases with forage maturity, proper management of alfalfa is critical. For hay, alfalfa should be harvested on a 28- to 35-day interval at a 7.6-cm stubble height (Ball et al., 2015). In a grazed system, alfalfa should follow the same pattern and be rotationally stocked on a 20 to 35-day rest period (Popp et al., 1999). This interval optimizes yield and quality while promoting root carbohydrate reserves for stand longevity.

Studies have been conducted to observe the effects of including alfalfa in the diet on animal performance. Hoffman et al. (1998) reported greater DMI and increased milk production in dairy cows fed alfalfa silage compared with those fed a perennial ryegrass (*Lolium perenne* spp. perenne L.). Increased milk production was observed despite higher DM, CP, and ADF digestibility of the perennial ryegrass, likely due to an increased rumen retention time of perennial ryegrass compared with alfalfa. Finally, there was no

difference in fermentation characteristics that could have accounted for palatability differences. These findings were supported by Broderick et al. (2002) who also observed greater feed intake, milk production, and body weight (BW) gains in Holstein cows fed alfalfa silage compared with annual ryegrass silage. Supplementing beef cattle with alfalfa, whether harvested at early or late bloom (approximately high or low quality), showed increased intake, digestibility, and improved cow body weight and body condition when compared with animals on grass hay alone (Weder et al., 1999). Thus, producers may be able to eliminate or reduce additional supplementation to their herds by including alfalfa, subsequently lowering feed costs and improving profitability of their system.

Nitrogen Fixation

Biological N fixation is a process carried out by legume species under N limiting conditions. When N is limited, roots of a legume plant secrete chemical attractants (e.g. isoflavonoids, betaines) to attract rhizobial bacteria. Rhizobial nodulation genes cause rhizobial bacteria to congregate near the root until bacterial populations reach the threshold for infection (Ball et al., 2015). Lipochitin oligosaccharides (*nod factors*) code for enzymes that synthesize calcium oscillations in the root that are recognized by a calmodulin-dependent protein kinase and initiate the nodule organogenesis phase (Wedin and Russelle, 2007; Taiz and Zeiger, 2010).

During infection, rhizobia attach to root hairs and release nod factors that cause a curling growth in the root where rhizobia begin to multiply. The root cell wall degrades

as a chemostatic response to nod factors and allows bacteria to access the plant plasma membrane. Once in the plasma membrane, an infection thread is formed, fusing with the Golgi vesicles of the root cell. Cortical cells in the root divide to form a nodule primordium which, once rhizobia enter and the infection thread fuses with the plasma membrane, forms a nodule (Wedin and Russelle, 2007; Taiz and Zeiger, 2010).

Rhizobial bacterial specialize to form N-fixing bacteroides that synthesize the nitrogenase enzyme and catalyze the reduction of dinitrogen (N_2) to ammonia (NH_3). The reduction reaction occurs when N_2 diffuses into the nodule and attaches to the nitrogenase. Ferrodoxin acts as an electron donor to the Fe protein, which then hydrolyzes ATP and reduces a MoFe protein. The reduced MoFe protein reduces N_2 to N_2H_2, which is further reduced to N_2H_4. This is subsequently split to form two molecules of ammonia. The ammonia is then bound to glutamate to form amino acids for transport to plant leaves before use in photosynthesis (Taiz and Zeiger, 2010).

Because biological N fixation occurs only when N is limiting, providing legumes with synthetic N fertilization will prevent the biological process from occurring. Legumes will readily take up N through the roots using the same mechanism as grasses if N is not limited in the soil. Therefore, if the stand has been fertilized with inorganic N, nodulation and N fixation will be much lower, if it occurs at all and minimal, if any, yield response would be observed. Legumes receiving N fertilization will be less efficient, ultimately increasing input costs for producers without providing a similar increase in productivity.

Environmental and soil factors are largely responsible for the success or failure of biological N fixation. Because the process is based on microbial activity, soil conditions

such as moisture and temperature affect the process. *Rhizobium* are most active in soil temperatures ranging from 25 to 29°C and moist, but not saturated soils. Additionally, the soil pH should be at least 5.8 to ensure adequate bacterial populations. The soil pH will also affect the availability of nutrients critical to nodule formation and N fixation. When macronutrients Ca and K or micronutrients Mo and B are deficient, N fixation will decrease or halt (Ball et al., 2015; Taiz and Zeiger, 2010).

Because biological N fixation occurs in the root system, it is highly affected by defoliation due to haying or grazing. When a plant is cut or grazed, it causes the roots to die back to provide carbohydrates for regrowth of the aboveground material. Because the underground carbohydrate stores are depleted, the rhizobial bacteria and nodules are deprived of energy sources and will slough off, releasing N into the soil N pool that becomes available for other plants (grasses) in the system. While the amount of N fixation decreases when plants are defoliated, as a plant recovers its carbohydrate stores and leaf area, new nodules will form, and the process will repeat itself.

Alfalfa - Bermudagrass Mixtures

Nitrogen Fixation and Transfer in Mixed Stands

In a mixed stand of alfalfa-bermudagrass, the general process of nodule formation and biological N fixation is the same as a pure-stand legume. Adding legumes to a grass monoculture system is known to reduce the need for N fertilization; however, the amount of nitrogen transferred to the grass crop is not well known. This information is critical for understanding the benefits of using a legume as part of a forage mixture.

Alfalfa can be expected to produce 112 to 224 kg N ha^{-1} yr^{-1} through biological N fixation (Ledgard and Steele, 1992). This number is supported previous research which found biological N fixation ranging from 50 to 168 kg N ha^{-1} yr^{-1} (Ta and Faris, 1987) to 112-280 kg N ha^{-1} yr^{-1} (Hardarson et al., 1988). Since the Ledgard and Steele study in 1992, Haby et al. (2006) has also supported this range of N fixation, finding approximately 224 kg N ha^{-1} yr^{-1} to be typical for a healthy stand of alfalfa. However, not all the N produced by the alfalfa is transferred to the grass component of the stand.

Haby et al. (2006) explored the N fixation capacity of alfalfa and its potential to transfer and provide N to the grass component of a stand when interseeded. Atmospheric N fixation contributed 42 to 91% of the total plant N of the alfalfa in the alfalfa-bermudagrass mixture. Atmospheric N fixation was affected by alfalfa row spacing; the 23-cm row space consistently fixed the least atmospheric N. This could be due, in part, to competition between the rhizobia bacteria when plants are in close proximity.

The fixed nitrogen yield of the alfalfa in the alfalfa-bermudagrass stand ranged from 80 to 222 kg N ha^{-1} yr^{-1}, however the transferred N yield was only 2 to 17 kg N ha^{-1} yr^{-1}. This suggests that little of the atmospheric nitrogen fixed by the alfalfa was transferred to the bermudagrass although sandy soil conditions and early spring regrowth of alfalfa may have limited the soil N availability and reduced bermudagrass N needs from lower composition of bermudagrass in the stand (Haby et al., 2006).

Belowground N transfer via root excretion, sloughing of nodules, or microbial decomposition from alfalfa is expected be 2-37% of the biological N fixation (Ledgard and Steele, 1992), or 2 to 67 kg N ha^{-1} yr^{-1}. This number has been revised by Haby et al.

(2006) to 2 to 18 kg N ha^{-1} yr^{-1}, although he notes that the low value of N transfer is likely due, in part, to a low presence of bermudagrass.

On the other hand, Beck et al. (2017a) observed that both the alfalfa-bermudagrass mixture and the clover-bermudagrass mixtures led to similar carryover N, herbage mass, and pasture carrying capacity as the pastures fertilized with either 56 or 112 kg N ha^{-1} during the growing season. This research coincides with observations made in Knight (1990) and Morris et al. (1990) that N transfer from legumes to grasses occurs during the early stages of the following growing season, rather than during the same season (Beck et al., 2017a).

Finally, it is believed that in alfalfa-bermudagrass stands, N transfer from the legume to the grass increases in the second and third years in the stand's life compared to other perennial legume species such as white clover. Louarn et al. (2015) found that alfalfa was twice as effective as white clover at productivity and biological N fixation during years 2 and 3, although clover root residues provided more N to other species upon senescence.

Botanical Composition of a Mixture

The botanical composition of a mixed grass-legume stand is imperative for determining the effectiveness of the stand to produce its own nitrogen. Extension publications suggest that for a mixed stand to produce enough nitrogen to support the stand, it needs to be at least 30% legume. While a greater proportion of legumes in the stand will increase the amount of N in the system, it may actually lead to decreased N transfer, as observed in Haby et al. (2006). This may be explained because of the

changing species composition of the stand. As the proportion of alfalfa in the stand increases, the grass component decreases thus, fewer plants are taking up soil available N and belowground transfer is decreased.

There are several factors that could affect the botanical composition of the grass-legume stand. A study conducted at the University of Georgia Plant Sciences Farm showed that after the first growing season, alfalfa made up approximately 50% of an alfalfa-bermudagrass mixture (Brown and Byrd, 1990). Increases in alfalfa stand density may have been caused by a variety of factors, including: a lower optimum growing temperature, horizontal leaf display, and drought tolerance. The lower optimum growing temperature provides a competitive advantage to the alfalfa because it begins growing as early as March (20°C), while the bermudagrass does not begin its peak growth until later in the season when the temperature is approximately 30°C. The increased growth rate earlier in the growing season lends itself to an increased alfalfa stand because of the increased leaf area and taller alfalfa plants and subsequent shade intolerance of the bermudagrass (Brown and Byrd, 1990).

Coupled with the earlier seasonal growth, alfalfa's horizontal leaves both increase its photosynthetic potential and stunt the growth of the grass portion of the stand. Alfalfa has wide, horizontal leaves which increases the surface area that is exposed to light. Bermudagrass has narrow, erect leaves that have less photosynthetic capability. Because the alfalfa is already taller and can shade the bermudagrass when it reaches its optimum growing temperature, it leads to a higher percentage of alfalfa than bermudagrass in a mixed stand after the alfalfa's establishment year (Brown and Byrd, 1990).

Finally, alfalfa has a large tap root that can extend several feet through the soil if favorable conditions exist. Because the tap root of alfalfa extends deeper into the soil profile than the branching bermudagrass roots, it can better withstand drought conditions. This allows for growth to continue longer in the absence of adequate rainfall (Brown and Byrd, 1990).

These hypotheses are supported by the observations from Stringer et al. (1994) in which bermudagrass suffered stand loss and productivity due to competition from alfalfa. Stringer et al. (1994) hypothesized that alfalfa's deep roots provided it an advantage under drought conditions and winter carryover of soil water. Additionally, studies have shown that bermudagrass root and shoot growth as well as nonstructural carbohydrate reserves are depressed by shading.

Although during peak production (years 2-4 of the stand) alfalfa may outcompete the bermudagrass, alfalfa stand longevity is a concern in the Deep South. Depletion may occur for a variety of reasons, most commonly, alfalfa's crown growth. As an alfalfa plant is repeatedly cut (either mechanically, or grazed), the crown is damaged, and regrowth slows. This leads to a decline in the percent alfalfa within a stand over time. Alfalfa stand depletion was observed in a Beck et al. (2017a) study where the mixed stands of either alfalfa-bermudagrass or white clover, red clover, and bermudagrass both decreased in the percent legume from 34.5 to 15% and 58.4 to 18%, respectively after the fourth year growing system. Additionally, after the establishment year grazing treatment, the alfalfa had to be re-established after falling below the critical stand density level (Beck et al., 2017a).

Therefore, incorporating alfalfa into a pre-existing bermudagrass stand may alleviate concerns about stand losses in pure alfalfa, because the bermudagrass component will increase and persist as alfalfa density decreases over time. Further, replacing an alfalfa stand can be challenging because of its autotoxic tendencies. Mature alfalfa plants exude a chemical signal that prevents alfalfa seeds from germinating and seedlings from successfully establishing. Currently, recommendations state that a field should be rested for at least one year before re-planting alfalfa. It has been suggested that in a mixed alfalfa-grass stand, this rest period may be shorter, though there is little work to support these claims at present.

Row Spacing in a Mixed Stand

Due to the shade intolerance of bermudagrass, row spacing is a critical factor in the success or failure of an alfalfa-bermudagrass mixture. Row spacing will ultimately impact stand composition, N transfer, forage yield, and forage quality of a mixed stand. It is important to balance the alfalfa row spacing so that rows are wide enough to allow bermudagrass sufficient sunlight to grow, but also narrow enough that bermudagrass is in proximity to receive N fixed by the alfalfa plants. At narrow row spacing (17-cm) there is an increase in the measurable N transfer to the soil and grass species. At a wide row spacing, the alfalfa may struggle to produce enough N to support the increased bermudagrass population, or transfer the N produced over long distances. Additionally, at wide row spacing, alfalfa may not contribute a great enough proportion to positively effect yield or quality of the sward, making the additional input cost hard to justify (Stringer et al., 1996).

The effect of row spacing on factors such as stand composition, yield, and quality was evaluated in a series of studies conducted by Stringer et al. (1994; 1996) and Brown and Byrd (1990). Stringer et al. (1994) evaluated the yield and botanical composition of a 'Tifton-44' bermudagrass stand at increasing rates of N fertilization with and without alfalfa interseeded at increasing row spacings. The authors determined that plots that received no additional N fertilization but were interseeded with alfalfa at every row spacing (20-, 40-, and 60-cm) produced the same or greater forage yield than the bermudagrass monoculture receiving 224 kg N ha^{-1} yr^{-1}. Additionally, there was no difference in the mean stand yield between the three row spacing treatments. Therefore, there is no yield advantage to either a narrow (20 cm) or extremely wide (60 cm) row spacing. A wide (60 cm) row spacing did show a slight linear yield response to N fertilization while narrower row spacings did not. Therefore, a 40-cm row spacing is the most practical to produce high forage yield with minimal fertilization costs.

A main concern of using an alfalfa-bermudagrass mixture is possible damage to the existing bermudagrass stand. While this was observed in Stringer et al. (1994), previous work did not observe bermudagrass stand loss. It is possible that this decline in bermudagrass may have been the result of either the earlier growing season of alfalfa or an already thinning stand of bermudagrass. Therefore, the intermediate row spacing (40 cm) is again the most practical for a mixed alfalfa-bermudagrass stand; narrow row spacing led to over 40% loss of the bermudagrass component, but adopting a wide row spacing led to depressed yields (Stringer et al., 1994).

Forage Yield of Mixed Stands

Studies agree that the addition of a legume, such as alfalfa, to a grass monoculture can improve forage yields similarly to N fertilization. Brown and Byrd (1990) showed interseeding alfalfa into bermudagrass with a 30-cm row spacing can produce mean dry matter yields equivalent to a bermudagrass monoculture receiving 200 kg N ha^{-1} throughout the growing season (11.2 Mg ha^{-1} for each treatment). The alfalfa-bermudagrass mixture produced significantly greater yields than a bermudagrass monoculture fertilized with only 100 kg N ha^{-1} (11.2 Mg ha^{-1} and 8.6 Mg ha^{-1}, respectively).

In a separate study, alfalfa interseeded into bermudagrass (20-cm row spacing) had higher herbage yields than a grass monoculture receiving 224 kg N ha^{-1} at every location and growing season (Stringer et al., 1994). New studies agree that the addition of a legume to bermudagrass increased the herbage mass and pasture carrying capacity to the same level as a bermudagrass stand receiving 56 kg N ha^{-1}; in the early growing season herbage mass and carrying capacity was similar to a stand receiving 112 kg N ha^{-1} (Beck et al., 2017a).

This is to be expected in the Southeast with the introduction of semi- and non-dormant alfalfa varieties. The varieties suited for the region have a long growing season, lasting from late March through October. Under good conditions, producers can expect an average of six cuttings annually, with producers in the Deep South regularly harvesting eight times each year (Hancock et al., 2015). Comparatively, a bermudagrass monoculture will average just four cuttings annually, with its primary growth between May and September (Ball et al., 2015).

Forage Quality and Animal Performance of Mixed Stands

Crude protein concentrations have shown a response to increased rates of nitrogen fertilization, and therefore it is possible that the crude protein (or total N) of the grass crop may also increase in response to the addition of legumes into the stand as nitrogen uptake increased. In a trial conducted on Edisto Island, SC, the CP of 'Tifton 44' bermudagrass alone, fertilized with synthetic N fertilizer, and interseeded with 'Cimarron' alfalfa at 20, 40, or 60-cm rows, was measured. The CP concentration of alfalfa was not affected, but CP concentrations in the bermudagrass CP decreased with increased row spacing. The bermudagrass CP in a monoculture ranged from 102 g kg^{-1} with 0 N fertilization to 146 g kg^{-1} following 448 kg N ha^{-1} (Stringer et al., 1996). However, when alfalfa was interseeded, CP of the bermudagrass component ranged from 130 to 170 g kg^{-1}. While the highest bermudagrass CP (170 g kg^{-1}) occurred in bermudagrass that was interseeded with alfalfa on a 20-cm row spacing and fertilized with 448 kg N ha^{-1}, the bermudagrass interseeded with alfalfa (at any row spacing or N fertilization) had greater CP content than the bermudagrass monoculture at any fertilization rate. Brown and Byrd (1990) observed similar results; monoculture bermudagrass had a lower mean N concentration in harvested forage compared with the mixture, regardless of N fertilization rate. Concentrations of N between the mixed stand and pure alfalfa were not different.

Additionally, at all fertilization rates, the CP content decreased as the row spacing of the alfalfa increased. This suggests that the nitrogen produced by the legume species was more available than the synthetic N fertilizer. Additionally, a narrower row spacing might also increase the amount of N available to the grass species due to the proximity of

the grass roots to the roots/nodules of the legume species. Similar responses to row spacing and N fertilization were observed in other studies involving alfalfa-bermudagrass mixtures and orchardgrass (*Dactylis glomerate* L.) mixed with white clover (Stringer et al., 1996). In Beck et al. (2017a), the white clover stand density in the clover-bermudagrass mixture declined in the presence of N fertilization. These results suggest that nitrogen uptake is increased in grass species when they are interseeded in close proximity to a legume.

The lack of CP response in alfalfa was likely due, in part, to reduced nodulation in alfalfa that was exposed to increased levels of N fertilization. This is because in the presence of adequate or luxury concentrations of soil N, rhizobia activity, and subsequently biological N fixation, is reduced (Stringer et al., 1996).

Fiber components of forage showed only a slight response to interseeding. For example, the ADF of the bermudagrass monoculture was slightly higher than the grass component of an interseeded stand during the establishment year, although the trend did not continue. Ultimately, fiber fractions had a greater response to N fertilization than row spacing or the presence of alfalfa. However, these decreases in NDF and ADF concentration would not be great enough to justify the extra cost of N fertilization in a mixed grass-legume stand (Stringer et al., 1996).

Newer studies in grazing systems have supported the early findings by Stringer et al. (1994; 1996) and Brown and Byrd (1990). Beck et al. (2017b) concluded that bermudagrass monocultures did have improved CP and TDN with increasing N fertilization. Interseeding alfalfa or a mixture of white and red clover (*Trifolium repens* L. and *Trifolium pretense* L., respectively) produced the same or larger increases in nutritive

value as 112 kg N ha^{-1} without sacrificing forage yield. Even during the late season, when bermudagrass is likely to outcompete the legume species, both mixtures maintained the same level of forage quality as the bermudagrass receiving 56 kg N ha^{-1}.

Further, animals on the legume mixtures in the Beck trial had daily live weight (LW) gains comparable to pastures fertilized with 56 kg N ha^{-1} (Beck et al., 2017b). Additionally, the use of the mixtures increased the number of grazing days available for the animals by approximately 300 days over a five-year period (Beck et al., 2017c). This ultimately led to total LW gains greater than animals maintained on a bermudagrass pasture fertilized with 112 kg N ha^{-1}, where animals gained 69, 71, and 72 kg $steer^{-1}$ in the 112 g N kg^{-1} bermudagrass, bermudagrass-alfalfa, and bermudagrass-clover treatments, respectively (Beck et al., 2017c). Although gains were slightly greater in the stands interseeded with clover compared with alfalfa, the longevity of alfalfa makes it a good option for southeastern producers.

Management of an Alfalfa-Bermudagrass Mixture

The management of use of alfalfa and bermudagrass in the Southeast can be very similar; recommendations for both include high potassium fertility with multiple applications throughout the growing season, and timely cutting intervals of approximately 4 weeks. Growing these two species simultaneously can provide producers with dual benefit as interseeding alfalfa into bermudagrass can reduce or eliminate the need for N fertilization, increase the relative forage quality, and decrease the need for additional supplementation when fed to livestock, as compared to monoculture bermudagrass.

Baleage in the Southeast

Overview of Baleage Production

While grazing is the most cost-effective method of forage harvest, year-round grazing is not an option in most parts of the country. To mitigate the need for a supplemental forage source, forage conservation is used to supplement the animal feed sources. However, the production of high-quality hay can be a challenge in the Southeast due to humid weather conditions, high levels of forage loss, and the need for increased forage quality in rations. Baleage, or baled silage, is an option that could balance the need for stored forages while minimizing some of the issues associated with traditional dry hay production. Baleage is a forage preservation method based on the principles used in traditional silage. It is characterized by baling harvested forage at a larger particle size than silage and higher moisture (50-65% moisture) than is acceptable for dry hay (target 15-18%) (McCormick, 2013 and Ball et al., 2015).

Fermentation converts the plant available sugars into products including lactic acid, acetic acid, butyric acid, propionic acid, carbon dioxide, and heat. Lactic and acetic acids are the two most desirable products because they are the strongest acids produced and lead to greatest pH drop. A lower pH prevents the growth of yeasts and molds associated with spoilage, and in some cases, toxicity to livestock (Dunière et al., 2013).

Forage stored as baleage goes through four phases of fermentation: the aerobic phase, lag phase, fermentation phase, and stable phase. The aerobic phase occurs in the first 24-48 hours after forage is baled and wrapped. During this time, the pH of the forage remains at its pre-harvest level and the oxygen level within the bale begins to drop as microbial respiration occurs and no new oxygen is introduced. After oxygen is excluded,

the forage enters a lag phase where the pH remains high. During this time, plant cells begin to breakdown and the population of anaerobic microbes increases with the increase in plant available sugars (Collins and Owens, 2003).

The third phase of the forage cycle is the fermentation phase. This phase begins in as little as 48 hours post-harvest. During this phase, bacteria convert plant available sugars that have been released during the plant cell breakdown of the lag phase into organic acids and gases. Most commonly, the bacteria are homofermentative or homolactic, meaning they produce exclusively lactic acid. This is the phase when the pH of the stored forage will drop as lactic and acetic acids are produced. Desirable baleage products include 2-8% lactic acid production and 0.5-3% acetic acid production; other products are desirable in quantities under 1% to minimize the potential for spoilage during storage (Collins and Owens, 2003).

The final phase of baleage is the stable phase where the population of acid-producing bacteria drops as sugars become a limiting factor for the bacterial populations. After the sugars have been fermented, the pH of the forage is at its lowest point and, if no oxygen is reintroduced, will remain approximately the same until bales are opened. While the final pH is largely dependent on type of forage harvested, it should fall between 3.8 and 5. A pH of 5 or less is critical to minimize yeast and mold production (Collins and Owens, 2003).

Advantages of Baled Silage

A major concern in dry hay production is nutrient losses as a result of rainfall or weather, especially in the humid southeast. Early work focused on leaching losses when

cut forage was exposed to rain during the drying process. Overall, losses due to rainfall during the wilting process contributes 4-16% nutrient loss (McGechan, 1989). Rain damage that negatively impacts hay production throughout the southeast can be largely eliminated because of the differences in dry matter at baling between the two systems (Hancock and Collins, 2006). Dry hay is routinely baled at 8 to18% moisture which takes, depending on evaporative demand, hours to days of field wilting to achieve. Hay that is produced above 15% moisture is considered "at risk" for spontaneous heating, which converts plant sugars to carbon dioxide, water, and heat (Martinson, 2011). Baled silage, on the other hand, has an optimum moisture of 40-60% which can often be reached in under 24 hours in good drying conditions.

By eliminating the extended drying time needed for dry hay production, baled silage is able to minimize losses in forage quality. Studies have shown that increasing the harvest interval of both warm- and cool-season forages can decrease the nutritive value of the harvested forage. Increasing the harvest interval of hybrid bermudagrass from 4 weeks (recommended) to 8 weeks, CP can be reduced by 6 percent and TDN by up to 17%. The same trends were apparent in tall fescue where CP and TDN declined up to 6 and 20%, respectively, when harvest was delayed from the late boot to dough stage (Hancock, 2014b).

Large decreases in nutritive value will increase the need for livestock supplementation to maintain production. Therefore, reducing the necessary wilting time can make harvest at the recommended cutting schedule a possibility during humid summer months. Additionally, in studies using cool-season annuals, the shorter wilting time allowed annual ryegrass being preserved as baleage to be harvested up to two weeks

earlier than the same forage harvested as dry hay; leading to higher milk yields for baleage-fed cattle (McCormick et. al, 2007; Borreani et al., 2007). Similarly, the use of baleage to harvest native grass pastures in an alpine system enabled harvest at an earlier maturity, which improved the CP and net energy for lactation and reduced the need for feed supplementation in dairy cattle (Borreani et al., 2007).

Differences in production losses can vary considerably between dry hay and baleage production. Mechanical losses occur at all stages of production, from mowing to tedding to baling. Losses are often categorized into one of two processes: 1) true shatter loss or 2) pick up losses (i.e. losses from windrowing and baling) (McGechan, 1989). These losses largely occur as fragments of the crop become detached and either shatter, fall, or are blown away. Losses may also accumulate in the system during the tedding and pickup stages. Losses due to baled and raked systems (using a variety of mower and mower conditioner systems) may account for total mechanical losses of up to 35% (McGechan, 1989). Baling at higher moisture, however, reduces the brittleness of the forage, therefore reducing the amount of shatter due to mechanical processes.

Production Considerations

To produce high quality baleage, there are a few important considerations. The ability to bale and wrap forage in a timely manner; appropriate forage moisture; bale density; and storage. The ability to wrap forage and quickly exclude oxygen is critical for fermentation and forage preservation. Vough et al. (2006) studied the effect of wrapping delay on bale temperature over time. Immediate exclusion of oxygen is key to minimizing bale heating, which can be especially problematic in the higher moisture

forage. With no wrapping delay, baleage increased from 32.7° to its maximum temperature (35°C) within 2 days from wrapping. However, bales that were wrapped after a 96-hr delay reached 63.8°C at wrapping and remained above 43°C for 5 days post-wrapping. This temperature was comparable to bales that were never wrapped.

Forage moisture is critical to good baleage fermentation. Baleage should be harvested at 40-60% moisture. However, baling at a moisture that is too high (>60%) or too low (<40%) can lead to spoilage from either clostridial mold or poor fermentation (Müller et al., 2007). In studies exploring the effects of baleage moisture, alfalfa silage pH reached 4.8 when baled at 50.2% moisture but when baled at only 37.4% moisture, the pH only reached 4.98. The lowest pH was 4.6, in the forage baled at 61.3% moisture. The high moisture baleage also had the greatest lactic acid production, 4.6% (Hancock and Collins, 2006). The bales harvested at lower moisture had an elevated pH which is a sign of low lactic acid production and subsequently poor fermentation.

Bale density is also an important consideration for baleage. When bales are produced at a higher density, less oxygen remains in the bale, further promoting fermentation (Han et al., 2014). It is for this reason that silage bunks are repeatedly packed using heavy machinery. Han et al. (2004) evaluated the effects of different bale densities and moistures on fermentation. They found that when the pH in bales was lower, lactic acid content was greater in bales produced at higher density (200 kg m^{-3}), regardless of moisture (Han et al., 2004).

Storage

Losses during production are not the only concern for producers. Storage losses can be quite high, especially in dry hay when stored outside. However, baleage often has lower associated losses if an anaerobic environment is maintained. Previous studies on the effect of storage method showed that outdoor storage increases the moisture of the baled forage, even when baled under the 15-18% target moisture, due to weathering (McCormick et al., 2011). Crude protein did not differ between indoor and outdoor storage of dry hay, although the acid detergent fiber (ADF) and neutral detergent fiber (NDF) of hay stored was 5.5% greater for hay stored outdoors than hay stored indoors (McCormick et al., 2011). Energy and dry matter losses in outdoor-stored hay were 12.8% greater than dry hay stored in a barn or as baleage (McCormick et al., 2011). Baleage had losses of less than 5% which were comparable to the losses of indoor-stored dry hay (McCormick et al., 2011). These differences resulted in a depression of the net energy for lactation when compared to either the hay stored indoors or the baled silage (McCormick et. al, 2011). In total, the baled silage incurred only 25 percent of the losses seen in hay stored outdoors.

Storage losses can be minimized through proper management of plastic materials. Bisaglia et al. (2011) observed no differences in fermentation characteristics or DM loss based on bale tying method, number of plastic layers, storage position. However, each of these factors did have an effect on mold coverage. Storage position of the individual bales correlated to surface mold coverage. Bales that were stored on their flat ends, rather than round sides had lower percentages of surface mold, likely due to extra overlap in the plastic on these ends which provided greater protection. Additionally, bales covered with

6 layers of plastic had decreased mold compared with 4 layers (4.9% vs 8.0% mold, respectively). Modifying storage techniques to minimize mold coverage can further decrease forage losses to baleage producers compared with dry hay.

Current recommendations for producers state that baleage should be fed within 9 months of harvest (Hancock et al., 2019). This recommendation is based on the tendency of bales to squat and become difficult to handle over time. Additionally, degradation of the plastic wrap used to store bales can lead to spoilage if it tears or leaks oxygen or moisture into the forage (Han et al., 2014). This 9-month feeding window can be a concern for producers. Because baleage is more expensive to produce than dry hay, it is often reserved for high quality forages. Thus, baleage is often reserved for feeding high nutrient requirement animals, such as lactating cows. A nine-month storage window may not provide enough time to economically feed the baleage produced in a season, especially if it is a high yielding year where grazeable forage is not limiting. There is nothing to suggest that baleage would continue to ferment or degrade once it enters the stable phase of fermentation. However, there has been no published research evaluating changes in the nutritive value of forages produced as baleage and stored long term.

Animal Performance

The use of baleage as a feed source can improve forage palatability and animal performance compared with dry hay. Bernard et al. (2010) concluded that bermudagrass harvested as baleage could be included with a corn silage ration at up to 15% of the dry matter in the diet before milk yields are depressed. Hancock and Collins (2006) concluded that cows consumed more dry matter as well as a larger proportion of the dry

matter provided to them when it was in the form of baled silage rather than dry hay. These findings were supported by previous work by Han et al. (2004) which found that baleage had lower NDF, ADF, and acid detergent lignin (ADL) compared with dry hay. This led to increased forage digestibility and palatability for the animals. In addition to greater dry matter intake, feed efficiency was improved in cattle consuming baleage compared with either indoor or outdoor-stored hay, although no direct connection to milk production or body weight gains were observed among the three forage preservation methods (McCormick et al., 2011).

Managing Baleage for Fermentation and Stability

Stored forage production comes with the additional challenge of conserving that forage and minimizing losses until it is fed. Previous work has demonstrated that proper long-term storage can minimize dry matter loss in both hay and baleage. This can be accomplished through barn storage of hay, or proper plastic application and maintenance in baleage (Bisaglia et al., 2011; McCormick et al., 2011). The use of preservatives to minimize mold growth and reduce hay heating have been used for many years.

Undesirable Byproducts of Fermentation

There are several byproducts that are undesirable in baleage which can be characterized as either chemical compounds (e.g. butyric acid, ethanol) or living species (e.g. mold, yeast). The presence of any of these byproducts can lead to dry matter loss, spoilage, low animal intake and performance, or even toxicity. Therefore, it is necessary to maintain bales to prevent the concentration or reproduction of undesirable products.

Butyric acid production often occurs during secondary fermentation and goes hand-in-hand with clostridial spore growth. Clostridial, or secondary, fermentation occurs when baleage is produced at a high moisture and after lactic acid bacteria are actively growing (Muck et al., 2010). Clostridial bacteria, (e.g. *Clostridium butyricum, Clostridum perfinagen*) ferment the lactic acid produced in the baleage to butyric acid or amino acids to VFAs, NH_3, and CO_2.

The presence of butyric acid (i.e. secondary fermentation) is problematic because it can lead to high levels of DM loss, up to 51% in some cases. Additionally, the presence of butyric acid and clostridium can lead to depressed performance and lactating animals that consume forages with high levels of butyric acid (> 5 g kg^{-1}) are more susceptible to ketosis (Muck et al., 2010) because butyric acid is more ketogenic than either acetic or propionic acids. High levels of ethanol in fermented forage can also signify problems, because it is a byproduct of yeast production.

Molds and yeasts can also present a challenge baleage management. Mold and yeast are often the main product leading to spoilage and loss at feeding. Both species are aerobic, although there are some yeast species that can reproduce anaerobically. While the bales are stored, mold and yeast spores remain dormant, but when oxygen is reintroduced to the system, they break dormancy and begin to reproduce. Yeasts are tolerant of low pH and use lactic acid as a substrate for growth (Dunière et al., 2013). Therefore, the presence of yeast at opening can raise the pH of the baleage as lactic acid is converted to ethanol, carbon dioxide, and heat. As the pH increases, molds are able to reproduce as well. Molds are particularly concerning because some produce mycotoxins,

which can cause severe health risks, including immune system impairment and metabolic imbalances even when fed at low levels (Dunière et al., 2013).

Chemical Additives

Chemical forage preservatives are often produced from organic acids (e.g. propionic acid) or as salt-based additives and applied to hay that was baled at an elevated moisture (Coblentz et al., 2013; Muck et al., 2018). The use of salt-based additives has been shown to reduce clostridial growth as well as butyric-acid production in baleage when compared to an untreated forage (Muck et al., 2018). However, animal performance responses vary by the type of product and forage species to which it is applied. For example, in a study by Agnew and Carson (2000), salt-based additives increased DMI of animals compared to an untreated silage, however as additive rates increased, DMI decreased. Other studies suggest there was no observed effect on intake or milk yield in dairy cows fed a treated forage (Muck et al., 2018).

Propionic-based preservatives have also been explored. In some trials, these preservatives demonstrated the ability to limit hay heating, although DM losses were not improved (Coblentz et al., 2013). While effective in dry hay, propionic-based preservatives will likely be less effective in baleage, where good fermentation would produce greater concentrations of organic acids than a propionic acid-based preservative could provide (Arriola et al., 2015). The application of an organic acid-based product (e.g. propionic or formic acid) to legume baleage or silage decreased the production of lactic and acetic acid concentrations (Muck et al., 2018). While in some cases the use of acid-based products has been shown to reduce DM losses and mold or yeast growth, the

use of these acids also reduces fermentation and protein degradation in the forage. These changes may improve animal intake and performance; however, their observed effects have not been consistently replicated (Muck et al., 2018).

The Use of Microbial Inoculants

A key component to the production and stability of high-quality alfalfa baleage is good fermentation. Like many legumes, alfalfa has naturally lower concentrations of carbohydrates when compared with other crops commonly used for silage (i.e. corn), which may limit fermentation of alfalfa. Two major elements for good fermentation are: creating an anaerobic environment – by producing a high density bale and quickly applying and maintaining silage wrap – and promoting bacterial fermentation (Muck et al., 2010).

To enhance proper bacterial fermentation in the alfalfa silage, commercially available microbial inoculants can be applied to the forage. Inoculants act similarly to a probiotic, by providing high quantities of beneficial bacteria to the forage. The use of inoculants has demonstrated several benefits including: decreased pH, decreased dry matter and energy losses, and improved stability of the ensiled forage. Additionally, using microbial inoculants will minimize the dry matter losses of silage because the pH is reduced more rapidly with improved fermentation.

Evidence suggests that using microbial inoculants may increase the stable storage time before feeding, further reducing losses (Adesogan et al., 2004). Studies by Arriola et al. (2015) and Adesogan et al. (2004) both demonstrated a significant drop in pH as well as in mold counts in the forages treated with a commercial inoculant when compared with

an untreated control. The decrease in pH to below 5.0 indicates good fermentation conditions as well as a high lactic to acetic acid ratio.

Beneficial bacteria can be characterized as either obligate homofermentative, obligate heterofermentative, or facultative heterofermentative bacteria, referring to the amount and type of products they generate. Homofermentative bacteria produce only lactic acid while the heterofermentative variety produce a combination of lactic acid, ethanol, acetic acid, and carbon dioxide.

Homofermentative Fermentation and Inoculants

Homofermentative bacteria ferment only hexose sugars (e.g. glucose) and produce lactic acid in excess of 90%. These bacteria are acid tolerant and under optimal conditions can decrease the forage pH as low as 3.8. Common species of homofermentative bacteria include: *Lactobacillus plantarum, L. casei, Enterococcus faecium*, and *Pediococcus spp.* These species rarely occur naturally in the field, so increasing their population requires application of a microbial inoculant. Homofermentative species have a high lactic:acetic acid ratio, which may increase bale heating initially, as well as decrease the aerobic stability. However, because homofermentative bacteria produce lactic acid exclusively, silage or baleage treated with a homofermentative product will generally have lower pH and DM loss and greater lactic acid concentration. A review of over 200 inoculant trials showed that DM losses were reduced in 38% of trials, with an average improvement of 6% (Muck et al., 2010). Therefore, forage treated with a homofermentative product will have less quality loss and be comparable to the forage at harvest. Unfortunately, if baleage is not managed correctly

the lactic acid produced by homofermentative bacteria may go through secondary fermentation, leading to spoilage of the forage.

Previous research has investigated the effects of homofermentative bacteria (most frequently *L. plantarum*) on fermentation characteristics and aerobic stability. Arriola et al. (2015) noted decreased pH and increased lactic acid, acetic acid, and lactic: acetic acid ratio in each of the homofermentative inoculant treatments compared with untreated bermudagrass haylage. Yeast counts between homofermentative and untreated bales were not different, but treated bales showed a tendency ($P = 0.06$) for lower mold counts compared with untreated bales (2.73 and 4.47 cfu g^{-1} for treated and untreated, respectively). These results were supported by Guo et al. (2013) where the pH was lowest and concentrations of lactic acid, total acid, and lactic: acetic acids were greatest in tall fescue (*Festuca arundinaceum* Schreb.) treated with *L. plantarum* prior to ensiling. Ranjit and Kung (2000) also observed numerically lower yeast and mold counts and ammonia concentrations in forage treated with *L. plantarum* alone, although improvements were not great enough to justify the use of a homofermentative bacteria alone.

While some improvements in animal performance from inoculated silage or baleage have been observed, results have been inconsistent (Muck et al., 2018). A meta-analysis of inoculation effects on dairy production have shown that there was an overall increase in milk yield of animals fed a homofermentative-treated product, although DMI and feed efficiency of those animals did not change based on forage treatment (Oliveira et al., 2017). Other studies observed a difference in animal production, although no associated differences in fermentation were found (Muck et al., 2018).

Heterofermentative Fermentation and Inoculants

Heterofermentative bacteria are able to ferment either hexose or pentose plant sugars. Their main products are lactic and acetic acid, but they also form ethanol and carbon dioxide during fermentation. Initially, heterofermentors convert hexose sugars to lactic acid, which decreases the forage pH. Acetic acid (and 1, 2-propanediol) will be produced more slowly via conversion of lactic acid, in a process that can take as long as 45 to 60 days (Muck et al., 2010; Muck et al., 2018). Several studies have supported these findings, suggesting that increases in acetic acid concentrations did not begin until 28 days post-treatment (Schmidt et al., 2009; Muck et al., 2018). Heterofermentative bacteria may also convert 1,2-propanediol to propionate, although the mechanism is not well researched or understood (Kung et al., 2003; Muck et al., 2018).

Heterofermentative bacteria belong to the *Lactobacillaceae* family, with *L. buchneri* as the primary bacteria evaluated for use in silage inoculants. The use of heterofermentative bacteria as a silage inoculant has been explored, largely to improve the aerobic stability of baleage when exposed to oxygen at feeding. Although the original homofermentative products improve stability under anaerobic conditions, their water-soluble carbohydrate (WSC) production can lead to rapid mold and yeast growth when the system becomes aerobic. Rather than apply a second preservative (i.e. propionic acid) to minimize yeast growth, a product that can both improve fermentation and aerobic stability is advantageous (Kung et al., 2003).

The crop and rate of application have been shown to have an effect on the fermentation results in *L. buchneri*. For example, Ranjit and Kung (2000) reported that lactate and lactic: acetic acid was greater, and acetic acid was lower in corn silage treated

with an intermediate rate (1 x 10^5 cfu g^{-1}) of *L. buchneri* compared with a high rate (1 x 10^6 cfu g^{-1}). Acetic acid concentrations in corn silage treated at a high rate of inoculant was double that of the un-inoculated silage (3.60% versus 1.82% in high rate and untreated, respectively). The pH of the intermediate rate silage was also numerically greater, and not different from that of the untreated control. Additionally, the high rate of inoculant decreased yeast counts from 6.05 $\log_{10}$ cfu g^{-1} in the untreated control to 2.01 $\log_{10}$ cfu g^{-1}; mold counts were not statistically different, but numerically less in the high rate of *L. buchneri* (Ranjit and Kung, 2003). Silage treated with the high rate of inoculant remained stable for a 912 hour monitoring period, compared with the 33 hour stable period of *L. plantarum*-treated silage or the 26.5 hour stable period of the untreated control (Ranjit and Kung, 2003).

Further studies have not agreed that the rate of inoculation impacts fermentation, except in extreme cases. Kung et al. (2003) observed an increase in acetic acid in all alfalfa silage, regardless of inoculation rate. Treatment of alfalfa silage with *L. buchneri* did decrease the lactic:acetic ratio of forage, and no yeasts were detected in any alfalfa silage sample.

Concerns about heterofermentative inoculants generally focus on (1) the potential for greater DM loss due to greater CO_2 production compared with a homofermentative product, and (2) the potential negative impact on animal performance. However, these concerns appear to be unsubstantiated (Muck et al., 2018). Although additional DM loss has been observed in corn silage and barley to varying degrees, alfalfa silage had a greater DM recovery when treated with a moderate (5 x 10^5 cfu g^{-1}) rate of *L. buchneri* and all rates of *L. buchneri* application were numerically greater than untreated alfalfa

silage (97.6 %, 93.6%, and 86.8%, for moderate rate, mean treated silage, and untreated silage, respectively).

Animal performance concerns also appear to be unfounded. In a meta-analysis of ruminant performance studies, dry matter intake (DMI) of animals fed a variety of *L.buchneri* treated crops (e.g. corn, sugarcane, alfalfa, barley, etc.) was analyzed. No differences in DMI were observed in any trial; in fact, the *L. buchneri* treated forage had numerically greater DMI in every study (Muck et al., 2018). Moreover, dairy cattle fed alfalfa silage treated with *L. buchneri* had greater milk production compared with cattle fed untreated silage (40.7 versus 39.9 kg d^{-1}), and numerically greater DMI, and milk fat percent (Kung et al., 2003).

Combination Inoculant Products

Because neither a homofermentative nor heterofermentative product can provide excellent initial fermentation and aerobic stability, research into the potential for "combination" inoculants has steadily increased. Combination products are microbial inoculants that combine both types of bacterial fermentation to maximize the efficiency of silage or baleage while minimizing the risks associated with its storage and feeding.

In a combination product, homofermentative bacteria (e.g. *L. plantarum*) rapidly consume the WSCs to produce lactic acid and rapidly lower pH. After the initial active fermentation, *L. buchneri* (i.e. heterofermentative bacteria) would consume the lactic acid and convert some of it to acetic acid. This would slightly raise the pH albeit providing additional protection against yeast and mold growth at opening (Muck et al., 2010; Muck et al., 2018).

Studies evaluating commercially available combination inoculants or the combined usage of a homo- and heterofermentative inoculant followed this trend to varying degrees. It should be noted, however, that most studies evaluating the use of combination products were conducted at the laboratory scale on miniature silos rather than on large round baleage bales, with the exception of Arriola et al. (2015). Therefore, a limitation of the research is the evaluation of these products at the same production scale at which they would be used.

Arriola et al. (2015) observed the lowest forage pH and greatest lactic and acetic acid concentrations as well as greatest lactic: acetic ratio. This was somewhat unexpected, as research has suggested that the concentration of lactic acid should decrease in combination products compared with homofermentative-only options (Adesogan et al., 2004). Mold and yeast counts were not significantly different, although they were numerically lower in the combination product.

The combination product also performed poorly in research by Adesogan et al. (2004) that evaluated an inoculant mixture containing *P. pentosaceous* and *L. buchneri* on fermentation characteristics in bermudagrass for a 60-d ensiling period. At 2, 4, 7, and 30-d post-ensiling, the combination inoculant had a significantly lower pH than the untreated control, however the pH was greater than other forage preservation treatments (namely molasses or an inoculant + molasses treatment). After ensiling for 60 days, the combination inoculant had the lowest pH of any treatment and the greater DM recovery than the untreated forage. Additionally, this inoculant produced the highest quantity of acetic acid, and lowest butyric acid but it was the least aerobically stable of any treatment, with a 6.9-d period of stability, compared with 27.8-d in the untreated forage.

Finally, its mold and yeast counts were numerically, although not statistically greater than any other treatment.

Guo et al. (2013) observed results that were more expected for a product containing both inoculant types. The silage pH and acetic acid concentrations were greater in the combination product compared with a homofermentative product but were still improved compared with the untreated forage. Results were confirmed with the ensiling of a second cutting of the same forage. Additionally, the aerobic stability of the forage increased with either inoculant treatment, however the combination product increased the aerobic stability by 44% compared with untreated forage (587-h versus 417-h for combination and untreated forage, respectively).

The research into the use of combination products does not reveal a cohesive picture, making it difficult to determine the viability of these products. Microbial inoculant trials are largely conducted at laboratory scale only, making it difficult to infer how these products will perform in the field. Further, ensiling time is not constant among studies, often ranging from 60 to 120 days. Because the combination products generally have slower fermentation from the *L. buchneri* bacteria, a shorter ensiling period may provide a poor representation of the product efficacy. Additionally, bacterial species and inoculation rates are determined by the manufacturer, making it even more difficult to compare products to one another. It is possible that certain combinations of bacteria do not perform as well as others due to competition between populations, or do not perform as well below a given population threshold (Muck et al., 2018).

Finally, there have been few studies gauging the effects of animal performance by treatment with these products. Although research has concluded that neither

homofermentative nor heterofermentative products negatively affect animal performance (Muck et al., 2018), the interaction of these type of products is virtually unknown. Therefore, research on animal intakes and performance from these products should be explored further.

Ferulic Acid Esterase

The strains of *L. buchneri* used in commercial inoculants are being explored not only for their improvements to aerobic stability, but also for the capability to produce a ferulic acid esterase (FAE; Muck et al., 2013; Muck et al., 2018). This enzyme may help degrade lignin within the harvested forage.

Numerous studies have shown that the type of carbohydrate-lignin linkages is critical to determining the degradability of a forage and rate of digestion (Cornu et al., 1994; Jung et al., 2011). Ferulic acid and p-coumaric acid are etherified to form lignin as ferulate esters are incorporated to lignin cross-linkages. The presence of either ferulic acid esters or ethers interfere with digestion, although to varying degrees.

Most ferulic acid-lignin linkages are formed with ethers, rather than esters. While some bacteria (e.g. *F. succinogenes*) can synthesize the esterases required to break ester linkages, ether linkages are undegradable. When ethers are bound to lignin, it not only prevents access to the polysaccharides, but also blocks existing ester cross-linkages from being degraded Cornu et al., 1994). If phenolic acids are converted to, or bound up in, lignin with esters through esterification, they can be hydrolyzed, and the bound polysaccharides are available to the animal in the rumen. Therefore, as ferulate acid ethers increase, forage digestibility decreases, but if linkages are ester-based, they may be

degraded in the presences of the appropriate esterase enzyme (Cornu et al., 1994; Jung et al., 2011). The variation in linkage types account for some of the observed differences in digestibility between bermudagrass varieties (Mandebvu et al., 1999).

Research exploring the effects of ferulic acid esterase-producing inoculants on animal performance of forage digestibility have been inconclusive. Lynch et al. (2014) observed that FAE-containing products provided no positive response on fermentation or forage nutritive value of harvested alfalfa, even when the FAE product was combined with additional fibrolytic enzymes. Aboagye et al. (2015) observed improvements in aerobic stability of hay treated with a FAE-product compared with untreated forage. However, these results were to be expected between an inoculated and uninoculated bale. Although there were no observed improvements in fiber fractions when the inoculant was applied to forage at baling (NDF, ADF, cellulose, and lignin were similar), animal performance, measured as ADG and feed:gain were improved in sheep fed the forage treated with the FAE-containing product. These findings were supported by Addah et al. (2011) who concluded that the use of a FAE-producing microbial inoculant may improve aerobic stability of forage and feed efficiency in feedlot steers.

While the research is promising, more work needs to be conducted. There are few commercially available inoculants or bacterial strains capable of producing the FAE enzyme. Therefore, widespread work needs to be done to validate the usefulness of these products on digestibility and animal performance before they should be recommended for producer use.

Conclusions

The benefits of incorporating a grass-legume mixture have been well-documented. Mixtures are able to provide quality improvements, reduce the need for N fixation, and in some cases extend the growing season to fill forage gaps. In the Southeast, many of these mixtures include clovers, rather than alfalfa. But recently, new varieties that are well-suited to the region have made the use of alfalfa-grass mixtures more popular. However, much of the research examining the use of alfalfa-bermudagrass mixtures is based on the establishment and use of older varieties.

As producers strive to become more efficient, the use of baleage has also been on the rise. This storage technology combines the principals of silage with the convenience of large round bales for feeding. Baleage can provide increased animal performance coupled with increased producer flexibility to harvest more independently of weather patterns in the humid Southeast. The majority of research has focused on the initial production of baleage, however there has been little research into appropriate storage length and long-term management. Work investigating the viability of microbial inoculants has largely focused on their effects in miniature-silos, rather than production scale systems, and the majority of studies have focused on the use of specific bacterial strains rather than commercially available products. Further, the research exploring the viability of products including ferulic acid esterase have been inconclusive. Therefore, the objectives of the following studies are to evaluate the use of alfalfa-bermudagrass mixtures when harvested as baleage on the basis of forage production and quality as well as fermentation characteristics and long-term storage.

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CHAPTER 3

TIFTON-85 BERMUDAGRASS AND TIFTON-85 BERMUDAGRASS-ALFALFA MIXTURES DIFFER IN YIELD AND NUTRITIVE VALUE WHEN HARVESTED AS BALEAGE[1]

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Abstract

Interseeding a legume, such as alfalfa (*Medicago sativa* L.), into bermudagrass (*Cynodon dactylon* L. Pers.) for baleage production can be an effective way to both improve the forage quality and reduce the need for additional supplementation. The objective of this research is to compare the nutritive value and yield of bermudagrass with and without interseeded alfalfa when harvested as baleage. In an established field of 'Tifton 85' (T85) bermudagrass at the University of Georgia Coastal Plains Experiment Station in Tifton, GA, 0.2-ha plots were assigned in a randomized complete block design to either T85 or to be T85 interseeded with 'Bulldog 805' alfalfa (T85+Alf) on 19 February 2016. T85 received 84 kg N ha^{-1} four times throughout the growing season. Plots were harvested at early bloom stage every 28 to 35 days throughout the growing season, baled at 40-60% moisture, and individually wrapped. At each harvest, plots were evaluated for botanical composition and forage yield, and bales were sampled prior to wrapping for nutritive value analysis. Although T85 yields were greater during year 1, alfalfa-bermudagrass plots produced additional harvests each season, leading to greater ($P < 0.01$) cumulative yield in the alfalfa-bermudagrass treatment (33,230 vs. 23,430 kg ha^{-1}, respectively) over the study period. Analyses of nutritive value show that CP and in-vitro true digestibility (IVTDMD48) were greater ($P < 0.01$ and $P = 0.03$, respectively) in the T85+Alf than T85 treatment (183.6 vs 119.2 g kg^{-1} CP and 797.6 vs. 731.8 g kg^{-1} IVTDMD, respectively). Therefore, interseeding alfalfa into a bermudagrass system may improve both forage yield and nutritive value for producers.

Keywords: Alfalfa, Bermudagrass, Grass-Legume Mixtures

Introduction

The goal of a forage-based livestock producer is to find a year-round forage system that is both high-yielding and high-quality. In many parts of the United States, a year-round grazing system is not possible, and forages are harvested as dry hay to be fed when forage availability is low. In the Southeast, many producers utilize a warm-season perennial forage base. However, these warm-season perennial forages can be high in fiber and lower in digestible energy, making it necessary for producers to provide additional supplementation to their animals, especially in conserved forage systems. Bermudagrass (*Cynodon dactylon* L. Pers.) is the primary warm-season perennial forage in the Southeast because of its extremely high yields and moderate forage quality. Additionally, bermudagrass requires high levels of nitrogen to achieve desired yields. This need for high N fertilization can be problematic, and the rising cost of synthetic N fertilizers has led producers to explore alternative methods (Beck et al., 2017; Rouquette and Smith, 2010).

In the northern and western United States, alfalfa has been widely used for livestock diets, including in the dairy and equine industries, because of its high crude protein and digestible energy concentrations (Lacefield et al., 2009; Ball et al., 2015). Although there are many benefits to including alfalfa in a forage program, disease and pest pressures have limited the adoption of alfalfa in southeastern states (Lacefield et al., 2009). This trend has begun to change, however, 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).

It has been widely accepted that the addition of a legume, such as alfalfa, to a grass monoculture can provide similar forage yields as a grass monoculture fertilized with N (Brown and Byrd, 1990; Stringer et al., 1994). Further, the addition of any legume to a grass monoculture has demonstrated the ability to improve forage quality of that stand. Beck et al. (2017b) found that the inclusion of alfalfa and/or clover to a bermudagrass monoculture increased crude protein (CP) and total digestible nutrients (TDN), especially during the early season.

'Tifton-85' bermudagrass is a popular choice for producers across the South because of its extremely high yields. Tifton-85 generally produces yields of 18 to 25,000 kg ha^{-1}, which are 135% of the yields expected from Coastal, and the greatest of the commercially available hybrid bermudagrass varieties (Hancock et al., 2013; Ball et al., 2015). 'Tifton-85' is a hybrid bermudagrass developed from 'Tifton 68' bermudagrass and PI290884, a bermudagrass (*Cyondon dactylon* L. Pers.) variety from South Africa (Burton et al., 1993). 'Tifton-85' is taller and has thick stems and broad leaves compared with other hybrid bermudagrass options.

Initial studies of 'Tifton-85' demonstrated a 26% increase in dry matter yields and 11% increase in digestibility compared with Coastal bermudagrass (Burton et al., 1993). A study by Mandebvu et al. (1999) not only found that Tifton-85 produced 7.1% greater DM yields than Coastal bermudagrass (4082 vs 3810 kg ha^{-1}, respectively), but also greater *in-vitro* dry matter digestibility (587 vs 584 g kg^{-1}). Further, compared with 'Tifton-78', Tifton-85 bermudagrass produced 47% more live weight gains (LWG) over a three-year period.

Traditionally, alfalfa mixtures have been harvested and stored as dry hay. However, in the Southeast this can be challenging because of the humid climate and frequent and relatively unpredictable rainfall in the region. Therefore, alternate methods of forage harvesting, such as the use of baled silage, may be advantageous to minimize risk of forage loss resulting from weather. Additionally, baling alfalfa at the low moisture necessary for dry hay can result in significant quality loss due to leaf shatter and damage.

It is understood that incorporating a legume, such as alfalfa, into a forage system has the potential to increase the nutritive value of a forage and produce similar yields without additional N fertilization. However, there is little information on how new varieties of alfalfa will perform and behave when mixed with a bermudagrass monoculture in the Southeast or harvested for baleage rather than dry hay. Because Tifton-85 is an extremely high yielding, high nutritive value bermudagrass variety, incorporating alfalfa into a Tifton-85 monoculture may improve the forage yield and quality of a stand compared with interseeding into other bermudagrass options. Therefore, the objective of this study is to evaluate and compare the forage yield and quality of 'Tifton-85' bermudagrass with and without alfalfa interseeded when harvested as baleage in the Southeast.

Materials and Methods

Forage Treatments and Plot Management

Forage treatments of 'Tifton-85' bermudagrass (Burton et al., 1993; T85) and a mixture of 'Tifton-85' bermudagrass and 'Bulldog 805' alfalfa (Bouton et al., 1997; T85+Alf) were evaluated during the summers of 2016, 2017, and 2018 at the University

of Georgia Coastal Plain Experiment Station (Tifton, GA). In February 2016, ten 0.2-ha plots were established in an existing Tifton-85 bermudagrass hayfield using a randomized complete block design with five blocks and two treatments per block. Plots designated to the T85+Alf treatment were planted with 'Bulldog 805' alfalfa at a seeding rate of 22.4 kg ha^{-1} at a depth of 1.27 cm using a no-till drill (Tye Pasture Pleaser, 2007; AGCO Inc., Duluth, GA) on a 35-cm row spacing.

Soil tests were conducted in the fall of 2015 prior to plot establishment. Average soil pH of the field was 6.7, soil phosphorus (P) was 45.8 mg kg^{-1}, potassium (K) was 65.3 mg kg^{-1}, calcium (Ca) was 1322.5 mg kg^{-1}, and magnesium (Mg) was 154.3 mg kg^{-1}. Following plot establishment, samples were collected from individual plots during February of each year and soil test results are presented in Table 3.1. Additionally, plant tissue samples were taken from T85+Alf plots prior to the August cutting each year to determine possible nutrient deficiencies and macro- and micronutrient applications were determined as needed.

Each year, calcium ammonium nitrate was applied to the T85 plots three times throughout the growing season (prior to the first harvest and last harvests and immediately following the second and fourth harvests) at a rate of 84 kg N ha^{-1}. Muriate of potash was applied to all plots three times throughout the growing season (immediately following the first and fourth harvests and approximately three weeks prior to the final harvest of the season) at 112 kg K_20 ha^{-1}. Boron (10% liquid solution) was applied July 2017 at 2.24 kg B $ha^{-1}$and molybdenum was applied during October 2017 at 0.23 kg Mo ha^{-1}.

Throughout the growing season, plots were scouted weekly for insect pests, including alfalfa weevil [(*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)], and potato leafhopper [*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae)], three-cornered alfalfa hopper [*Spissistilus festinus* (Say) (Hemiptera: Membracidae)], fall armyworm [*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)], and bermudagrass stem maggot [*Atherigona reversua* (Villaneuve) (Diptera: Muscidae)]. In July, August, and September 2016, T85+Alf plots were sprayed with lambda cyhalothrin (Lambda Star; Nufarm Americas Inc., Burr Ridge, IL) at a rate of 34 g a.i. ha^{-1} to control potato leafhopper. Additionally, spinosad (Blackhawk; Corteva AgriSciences, Wilmington, DE) was applied to T85 plots during July and August 2016 at 55 g a.i. ha^{-1} to control fall armyworm. During 2017 and 2018 zeta-cypermethrin (Mustang Maxx; FMC Corporation, Philadelphia, PA) was applied to both T85 and T85+Alf plots during March (2017 only), May (2018 only), July, August, and September (2017 only) at a rate of 28 g a.i. ha^{-1} to control three-cornered alfalfa hopper. Chlorantraniliprole (Prevathon; Corteva Agriscience, Wilmington, DE) was also applied during July, August, and September at 100 g a.i. ha^{-1} to control fall armyworm. In 2018, lambda cyhalothrin (Lambda-Cy; Nufarm Americas Inc., Burr Ridge, IL) was applied in February at 34 g a.i. ha^{-1} for alfalfa weevil. Finally, malathion (Malathion 5EC; Drexel Chemical, Memphis, TN) was applied July 2018 to control fall armyworm at 1.4 kg a.i. ha^{-1}.

In 2016, 2017, and 2018, pendimethalin (Prowl H2O; BASF Ag Products, Floram Park, NJ) was applied at the beginning, middle, and end of each season (approximately April, August, and October) for pre-emergent control of annual grass weeds following harvest at a rate of 1.1 kg a.i. ha^{-1}. In June 2017, aminopyralid + metsulfuron (Chaparral

DF; Corteva Agriscience, Wilmington, DE) was applied to T85 plots at 155 g a.i. ha^{-1} to control bahiagrass infestation. In June 2018, metsulfuron-methyl (Osprey; Bayer CropScience LP, Research Triangle Park, NC) was applied at 112 g a.i. ha^{-1} in a mixture with 840 g ha^{-1} spray grade ammonium sulfate and 37.8 L H_2O to control annual grass weeds.

Irrigation

Although irrigation was not a component of the study, in 2016 the Coastal Plain Experiment Station experienced moderate to extreme drought conditions and rescue irrigation measures were taken to prevent stand loss (Table 3.2). Plots received ca. 2.5 cm of irrigation weekly using a traveling gun irrigation system from June 15 through July 21 and again from September 9 through November 7. Soil moisture conditions were monitored throughout 2017 and 2018, though no irrigation was deemed necessary.

Forage Harvest Management

Forage harvests were delayed one month during the establishment year (2016) and began May 2016 when the T85+Alf plots reached 25 percent bloom to allow for adequate development of root carbohydrate stores. Subsequent harvests occurred when T85+Alf reached early (10 percent) bloom stage, with growth stage determination adapted from the procedure outlined in Mueller and Fick (1989) and continued on a 28 to 35 day cutting interval throughout the growing season until growth was limited by environmental conditions (e.g. temperature or daylight). T85 plots were harvested when growth became adequate and T85 was at least 20 to 25-cm in height, beginning in June

and every 28 to 35 days thereafter through September of each year. During May 2016, T85 plots were mowed to remove annual weeds and begin the same growth interval as T85+Alf plots following harvest.

At each harvest, plots were cut beginning at ca. 1800 h using a mower-conditioner (New Holland Discbine 313; New Holland Agriculture, New Holland, PA). Beginning at ca. 0930 h the morning after cutting, grab samples from each plot were collected and forage moisture of each treatment (T85 and T85+Alf) was determined using the microwave moisture test (Ball et al., 2015). This procedure was repeated hourly until forage moisture reached 55 percent. When the target moisture was achieved, forage was raked into windrows using a one-sided wheel rake and baled using a Krone Fortima 1500 MC baler (Krone NA, Inc., Memphis, TN). During baling, a lactic acid producing forage inoculant 'hemicellulose *A. Niger*' to provide 71 billion cfu g^{-1} (Silage Supreme; Kent Nutrition Group, Muscatine, IA) was sprayed using a continuous flow spray boom in the baling chamber of the Krone baler. Forage was baled to a target bale weight of 2200 to 2640 kg. When forage growth was adequate, two bales per plot were baled and designated 'A' or 'B.'

Bales were then transported to the wrapping and storage location using a MT455B Challenger tractor (AGCO Corp., Jackson, MN). Bales were individually wrapped with six layers of pre-stretched (55%) polypropylene baleage wrap (Sunfilm Stretch Wrap; TAMA Group, Dubuque, IA) using an Anderson RB-200 individual bale wrapper (Groupe Anderson Inc., Chesterville, QC, Canada) and stored in a bermudagrass hay field on a layer of polyethylene silage wrap (Up North Plastics, Inc.; Cottage Grove, MN) that had been previously used as a bunker cover for silage. The purpose of storage on this

plastic was to minimize the risks of puncture from under the bale and for weed control around the bales. Bales were stored there for use in a subsequent baleage storage trial.

Forage Sampling and Analysis

Prior to cutting, all plots were evaluated for species composition and stand maturity. Stand composition was determined using a visual stand assessment from 0.1-m^2 quadrats at five locations per plot. The stand was characterized as either 'alfalfa,' 'bermudagrass,' or 'other' ('other' was designated as either an undesirable species or bare ground). At each quadrat, botanical composition was also measured by cutting forage and separating alfalfa, bermudagrass, and other/undesirable species. Botanical composition samples were immediately weighed and placed into a forced dry oven at 55°C for 4 days and dry samples were weighed to determine the yield component of each species/category. T85+Alf plots also measured stand maturity by evaluating percent bloom and average alfalfa height at each quadrat.

Prior to wrapping, core samples (3) were taken from each bale using a drill-driven aluminum cannister multi-forage sampler (Star Quality Samplers; Irricana, AB, Canada) to be used for nutritive value analysis. Samples were weighed, placed into a forced air dryer at 55°C for 3 days and reweighed to determine forage dry matter. After drying, core samples were ground through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for wet chemistry analysis, then ground through a 1-mm screen in a Cyclone Sample Mill (Model 3010-030; UD Corporation; Boulder, CO). Nutritive value analysis was determined by near infrared reflectance spectroscopy using an NIRSystems 6500 NIR (Foss NIRS System Inc., Laurel, MD). Metrics included neutral detergent fiber

(NDF), acid detergent fiber (ADF), crude protein (CP), in-vitro true dry matter digestibility at 48-h (IVTDMD48), lignin, ash, dry matter intake (DMI), total digestible nutrients (TDN), and relative forage quality (RFQ). Prior to scanning, samples were separated by treatment and placed in a forced air oven at 55°C for 90 minutes then packed into cells and scanned. Calibration statistics for 2018 Grass Hay NIR equations were as follows: NDF, SEC = 2.32, R^2 = 0.961; SECV = 2.391; ADF, SEC = 1.564, R^2 = 0.956; SECV = 1.650; CP, SEC = 0.917, R^2 = 0.977; SECV = 0.945, and 2018 Mixed Grass Hay NIR equations were NDF, SEC = 2.209, R^2 = 0.968; SECV = 2.318; ADF, SEC = 1.747, R^2 = 0.929; SECV = 1.826; CP, SEC = 0.827, R^2 = 0.976; SECV = 0.885, where SEC = standard error of calibration and SECV = standard error of validation, in g kg^{-1} on a DM basis. Additionally, forage quality parameters (e.g. CP, NDF, ADF, IVTDMD48, and ash) for each harvest were validated using wet chemistry techniques for crude protein, digestibility, and fiber determination using traditional laboratory techniques (Pomerleau-Lacasse et al., 2018; AOAC, 1990; Goering and Van Soest, 1970).

Statistical Analysis

All statistical analyses were conducted using PROC MIXED in SAS 9.4 (SAS Insitute Inc., Cary, NC) to determine fixed effects of treatment, harvest, and year as well as their interactions and block was considered a random effect. Means separation was conducted using Tukey's honest significant difference (HSD) test, and differences were considered significant using an alpha level of 0.05, unless otherwise specified.

Results and Discussion

Environmental Data

Monthly average maximum and minimum temperatures and monthly rainfall during the three-year trial, and historical climate data from March through November were acquired from the University of Georgia's Automated Environmental Monitoring Network (UGA-AEMN, 2018) weather station located on the University of Georgia Coastal Plain Experiment Station in Tifton, GA (Table 3.2). Monthly maximum temperatures were comparable to the 100-year average in each of the three years. However, monthly minimum temperatures were at or above normal each year of the study, especially in the later portion of the growing season.

Each of the three years (2016, 2017, and 2018) showed precipitation trends that were different (Tables 3.2 and 3.3). Conditions in 2016 were very dry, while 2017 was comparable to the 100-year average, and 2018 was extremely wet. Cumulative precipitation in 2016 was 85.4 cm. This is comparable to the 100-year average, but it is important to note that because of spring establishment, the year 1 growing season was delayed from the prior fall to a February planting date, and harvests occurred late-May through November. While March and April had above average rainfall, the cumulative precipitation during the 2016 growing season (May – Nov) was 55.9 cm. Additionally, September 2016 had well above average precipitation due to rainfall received during Hurricane Matthew, which contributed an additional 12-cm of rainfall and skewed the monthly precipitation data. Excluding precipitation before the 2016 growing season (March and April) and the effects of Hurricane Matthew, a more accurate picture of severe drought in Georgia during 2016 emerges.

An average year occurred in 2017 with cumulative and monthly precipitation similar to the 100-year average (Table 3.3). Environmental conditions deviated substantially from normal in 2018, with cumulative precipitation of 117.3 cm, making it one of the top 10 wettest years on record (NOAA, 2019). May and August had cumulative precipitations that were nearly double the 100-year average. Additionally, in 2018, the precipitation was not from a few large rainfall events, but rather from frequent, small to moderate events, making it a challenge to harvest forage on schedule (Table 3.3).

Forage Yield

There was an interaction between forage treatment and year on forage yield ($P < 0.01$) during the growing seasons. Additionally, main effects of forage treatment ($P < 0.01$), harvest ($P < 0.01$), and year ($P < 0.01$) were significant, likely results of the changes in stand composition throughout the three growing seasons (Table 3.4).

Differences ($P < 0.01$) in cumulative yield were observed during 2016 and 2018 (Figure 3.1). In 2016, T85 yield was greater than T85+Alf (9,823 vs. 7,631 kg ha^{-1}, respectively). In 2018, however the cumulative yields over the three-year period were greater in T85+Alf stands compared with T85 (33,230 vs. 23,430 kg ha^{-1}, respectively). Cumulative yields for the study were similar during 2017. The shift in cumulative yields between the two treatments were the result of a longer growing season for the alfalfa, which resulted in eight additional harvests of T85+Alf during the 3-year trial. Regardless of year, T85 yields were greatest during July and August (Table 3.4). In addition to the additional spring and fall harvests, yield of T85+Alf was greater in the early summer (June) than mid-summer harvests. These results were supported by the observations of

Brown and Byrd (1990) and Stringer et al. (1994) where the mean dry matter yield of an alfalfa-bermudagrass mixture on 15-cm row spacing was comparable to bermudagrass receiving 300 kg N ha^{-1} and greater than bermudagrass receiving 200 kg N ha^{-1} annually.

The T85+Alf produced the greatest forage yield in 2017 of any forage treatments (14,811 kg ha^{-1}) and was greater ($P = 0.02$ and $P < 0.01$) than the 2017 and 2018 T85 yields. The 2016 T85 yield was greater ($P = 0.02$ and $P < 0.01$) than T85 yields in either 2017 or 2018 (12,021 vs. 7297 vs. 6390 kg ha^{-1} for 2016, 2017, and 2018, respectively). Finally, T85+Alf forage yields were comparable between 2017 and 2018; T85+Alf was greater during 2018 ($P < 0.01$) than T85 in either 2017 or 2018.

Seasonal yield patterns changed between years, likely due to differences in stand age and weather patterns (Table 3.4). During 2016, T85 produced greater forage yield than T85+Alf in May, June, and September 2016 ($P < 0.01$, $P < 0.01$, $P = 0.01$, respectively). Finally, during the fall of the year, T85+Alf had adequate growth to warrant an additional harvest in November, after the T85 treatment had gone dormant (350 kg ha^{-1}). Even with the additional fall harvest, T85 had greater ($P = 0.02$) seasonal yield during 2016. This is typical of a first-year alfalfa stand, which generally produces lower forage yields during establishment.

In 2017, the T85+Alf produced eight cuttings of forage while T85 produced four (Table 3.4). In March, April, and May 2017, T85+Alf was harvested before the initial T85 harvest, producing 3582, 2260, and 578 kg ha^{-1} forage, respectively. Additionally, the growing season for T85+Alf extended further into the fall, producing 580 kg ha^{-1} forage during October after T85 was no longer actively growing. During June and September, T85+Alf produced greater ($P = 0.01$ and 0.03, respectively) forage yield than

T85 (1737 vs. 1199 kg ha^{-1} and 1010 vs. 724 kg ha^{-1} in June and September, respectively). However, during the middle of the growing season (July), T85 still produced greater (P = 0.03) forage yield than T85+Alf (2477 vs. 1965 kg ha^{-1}, respectively). During the August harvest, T85 produced numerically greater (P = 0.48) forage yield (2898 vs. 2829 kg ha^{-1}). This suggests that although bermudagrass fertilized with N will likely produce greater yields during the mid-summer months, seasonal yields of alfalfa mixtures will be greater because of the longer growing season and greater number of harvests.

During the third year of the study, T85+Alf again produced a greater number of harvests than T85 (7 vs. 4, respectively). T85+Alf plots produced harvestable forage in March, May, and October (3096, 2592, and 1401 kg ha^{-1}, respectively) while the bermudagrass was dormant. Additionally, T85+Alf produced greater (P < 0.01) forage yield in September (1092 vs 894 kg ha^{-1}), and numerically greater yields in June and July (P = 0.06 and 0.18, respectively). August yields were similar between forage treatments. In the Southeast, the third year of an alfalfa stand is considered its peak production year. That, coupled with frequent rainfall, contributed to the high T85+Alf yields during the 2018 season.

Species Composition

Species composition of T85 and T85+Alf forage treatments throughout the 3-year trial are shown in Table 3.5. The percent alfalfa and bermudagrass were affected by the main effects of forage treatment, harvest month, and year (P < 0.01). The percent

undesirable, or “other” species was affected by the main effects of harvest month and year ($P < 0.01$) but was not affected by forage treatment ($P = 0.29$).

Alfalfa percentage was the lowest ($P < 0.01$) during year one of the trial (2016). This was to be expected because 2016 was the establishment year for the stand. Additionally, alfalfa was planted in spring 2016, rather than the previous fall, so it would have had less time to establish before harvesting began. Alfalfa percentages remained low throughout most of the 2016 season, with August of that year having the least alfalfa ($P < 0.01$; 17.4% alfalfa). This is likely due to the extreme drought throughout the Southeast during that year which led to increased weed pressure and the need for emergency irrigation for stand survival. During 2017 and 2018, however, there was no difference in yearly alfalfa percentage. During the second two years of the trial, the greatest ($P < 0.01$) proportion of alfalfa occurred during the April (2017) and June (2018) harvests. As expected, the lowest percentages of alfalfa occurred during the late summer of 2017 and 2018, when temperatures are high, and alfalfa is not growing as rapidly as other forages (e.g. bermudagrass).

Bermudagrass was also affected by year and harvest, as well as forage treatment ($P < 0.01$). T85+Alf had overall lower ($P < 0.01$) percentage of bermudagrass, regardless of year. Additionally, the proportion of bermudagrass was lowest ($P < 0.01$) in 2018 and greatest in 2016. During 2016, stand composition of bermudagrass was lowest ($P < 0.01$) during August, regardless of treatment. This was likely due to a combination of severe drought stress and a high percentage of drought tolerant annual weeds (e.g. crabgrass) that were present because of open canopy throughout the stands.

During 2017, T85 bermudagrass was lower ($P < 0.01$) in June than July and August harvests, and numerically lower ($P = 0.07$) than the September harvest. The low presence of bermudagrass may have been because June was the first harvest of the T85 plots during 2017. Furthermore, they had been mowed as a reset during November 2016, when the T85+Alf plots were cut, which may have reduced root carbohydrate stores and slowed spring regrowth. T85 in 2018 behaved quite differently. June produced the greatest ($P < 0.01$) proportion of bermudagrass of any harvest month. Extremely wet weather, an extended stretch of cloudy days, and annual grass weeds may have contributed to depressed bermudagrass growth through the season; this trend was observed throughout the Southeast.

Finally, T85+Alf had the lowest presence of bermudagrass ($P < 0.01$) in the early harvests of March, April, and May in both 2017 and 2018. These harvests all produced less than 25% bermudagrass, which were lower than the later season cuttings. The early season cuttings in these years were predominantly alfalfa because the majority of bermudagrass had not yet broken dormancy. Changes in botanical composition throughout the growing season are typical for a mixed alfalfa stand, where alfalfa is the predominant species in the cooler harvests but bermudagrass dominates during the hot summer months while alfalfa experiences a "summer slump."

Undesirable, or "other" species, were defined as any species that was not alfalfa or bermudagrass. 2016 and 2017 had similar presence of weeds, and 2018 had the greatest ($P < 0.01$) proportion of other species. In 2016, August had the greatest proportion of weeds (37.2 and 55.9% for T85 and T85+Alf, respectively) due to the extreme drought conditions and lower percentages of alfalfa in T85+Alf stands. During

2017, the first cutting of either treatment had the greatest percentage of other species. T85 plots had 32% "other" species in June, compared with 12.6, 15.1, and 19.6% in July, August, and September, respectively. The March 2017 cutting of T85+Alf also had the greatest ($P < 0.01$) proportion of "other" species, with 45.0%. May 2017 had similar weed pressure issues, with 41.4% of the stand characterized as an "other" species. Regardless of treatment, June had the lowest ($P < 0.01$) percentage of weeds during 2018 (12.2 and 7.6% for T85 and T85+Alf, respectively).

While "other" species were not individually measured, primary weed species observed throughout the trial consisted of annual ryegrass (*Lolium multiflorum* Lam.) during the early spring, and crabgrass (*Digitaria* L.) and vaseygrass (*Paspalum urvillei* Steud.) during the middle of the growing season.

Nutritive Value

Nutritive value parameters were affected by multiple interactions as well as the main effects of year, forage treatment, and harvest. Therefore, mean nutritive value parameters for each year are presented by harvest and treatment (Tables 3.6-3.8). Additionally, seasonal averages are presented by treatment in Table 3.9.

During each of the harvest seasons, NDF and ADF were greater ($P < 0.01$) in T85 than T85+Alf (Table 3.9). CP was greater ($P < 0.01$) in T85+Alf compared with T85 (139 vs. 108 kg ha^{-1}, 216 vs. 136 kg ha^{-1}, and 199 vs. 125 kg ha^{-1}, for T85+Alf and T85 in 2016, 2017, and 2018, respectively). T85+Alf also had greater ($P < 0.01$) TDN, IVTDMD48, and RFQ compared with T85 in each of the three growing seasons. With the exception of TDN and RFQ ($P = 0.23$ and 0.11, respectively), each of the parameters

displayed an interaction between forage treatment and year, where T85+Alf had improved values in each of the nutritive value parameters (lower NDF and ADF; greater CP, IVTDMD48, TDN, and RFQ). Seasonal averages of quality parameters (e.g. CP, TDN, IVTDMD48, and RFQ) in T85+Alf were at or above the levels needed to support a lactating beef cow in each of the three growing seasons (NRC, 2017).

Weather patterns between the three years influenced forage growth and harvests, which in turn affected nutritive value parameters. T85 nutritive value parameters were not different among the three years. All nutritive value parameters of T85+Alf were greatest ($P < 0.01$) during the 2017 growing season. Measurements were lower in 2016 due to low percentages of alfalfa in the T85+Alf stand following spring establishment and drought stress. Harvest delays because of frequent rainfall during 2018 decreased nutritive value in what is generally considered the best production year for alfalfa. T85+Alf stands had both high percentages of alfalfa and good growing and harvest conditions during year 2, leading to the greatest nutritive value.

The T85 treatment had the greatest CP, IVTDMD48, TDN, and RFQ during the June harvest in 2016 and 2018 (Tables 3.6 and 3.8). In 2017, the CP and IVTDMD48 were greatest in July (Table 3.7). During 2017 and 2018, ADF and NDF were greatest during the mid- to late-summer harvests (July and August). CP and TDN were greatest ($P = 0.01$ and $P < 0.01$) during June of both 2017 and 2018. CP, IVTDMD48, TDN, and RFQ were likely lower in July 2018 because of harvest delays due to weather during that month. The CP of T85 was comparable to T85 harvested as dry hay by both Hill et al. (1993) and Burns and Fisher (2007). However, forage concentration of NDF was lower and IVTDMD was greater in this study than in either of the previous studies. These

results were to be expected as baleage typically has lower NDF, ADF, and ADL compared with dry hay (Hancock and Collins, 2006).

Although nutritive value parameters were different among the three growing seasons, they followed the same trends after alfalfa was established. All 2016 nutritive value parameters were greatest ($P < 0.01$) during the November harvest once alfalfa had fully established. The NDF and ADF of T85+Alf was also greatest ($P = 0.02$ and 0.04) during May 2016, likely because of the presence of mature winter weeds within the plots prior to the initial harvest. In 2016, IVTDMD, TDN, and RFQ were all lowest during August when plants were drought stressed and there was a high proportion of weeds in each stand. In 2017, T85+Alf had the greatest ($P < 0.01$) CP, IVTDMD48, TDN, and RFQ in June, July and August harvests. During the early spring and early fall harvests of 2017 and 2018, nutritive value parameters were greatest because of the high proportions of alfalfa during these times of year.

Increases in the nutritive value of a sward with the addition of a legume has been well-documented. Beck et al. (2017a) also observed increases in CP, TDN, and digestibility with the addition of either an alfalfa or clover species to bermudagrass. In this study, changes in nutritive value between harvests and year within each treatment are due to differences in weather which affected the forage maturity at harvest; forage maturity from delayed harvests are major contributors to decreases in forage quality and nutritive value. Although some harvests during the 3-year trial were delayed due to weather, the shortened drying time associated with baleage technology made such delays minimal and promoted nutritive value.

Conclusions

In this study, the alfalfa-bermudagrass system produced increased forage yield and nutritive value when compared with bermudagrass fertilized with nitrogen alone. Although forage yields were lower during the establishment year, this is to be expected with alfalfa's first season. Despite decreased first-year yields, the extended growing season and additional fall and spring harvests ultimately led to greater cumulative yields over the three-year study. The addition of alfalfa not only increased yields, but also improved nutritive value. Alfalfa-bermudagrass mixed stands had improved nutritive value compared with the bermudagrass-only stands, regardless of weather and harvest influences. Because forage maturity has a direct influence on the nutritive value of harvested material, incorporating baleage technology could further improve forage quality of an alfalfa-bermudagrass stand by allowing producers to harvest more independently of the weather. Therefore, incorporating alfalfa into a bermudagrass system may not only improve nutritive value, but also increase forage yields, making it an ideal high-quality forage option for livestock producers.

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Table 3.1. Soil pH, phosphorus (mg kg^{-1}), potassium (mg kg^{-1}), calcium (mg kg^{-1}), and magnesium (mg kg^{-1}) of alfalfa-bermudagrass plots taken February 2017 and 2018 in Tifton, GA and analyzed by the University of Georgia Soil, Plant, and Water laboratory (SPW) in Athens, GA.

	Year	Plot									
		101	**102**	**201**	**202**	**301**	**302**	**401**	**402**	**501**	**502**
pH	**2017**	6.8	6.8	6.9	7.0	7.0	7.0	7.1	6.9	6.8	6.5
	2018	6.8	6.9	7.1	7.3	7.2	7.3	7.3	7.2	6.9	7.1
Phosphorus **(mg kg^{-1})**	**2017**	45.0	42.0	26.0	25.5	17.5	19.5	22.0	22.5	29.0	33.5
	2018	56.8	35.2	33.7	44.5	24.7	24.1	13.2	29.5	33.6	36.3
Potassium **(mg kg^{-1})**	**2017**	44.5	56.0	42.5	31.0	31.5	30.0	34.5	36.5	47.5	66.0
	2018	46.5	26.8	33.4	57.3	31.0	50.0	34.3	23.4	77.3	37.6
Calcium **(mg kg^{-1})**	**2017**	730	825	718	835	855	888	845	785	667	548
	2018	884	960	1060	1395	1074	1070	887	1004	749	1283
Magnesium **(mg kg^{-1})**	**2017**	114	124	104	120	117	129	120	106	92	74
	2018	133	137	135	196	167	157	124	149	110	151

Table 3.2. Monthly rainfall (cm) and average maximum and minimum monthly temperature (°C), in comparison to 100-year average for March through November 2016-2018 at the University of Georgia Coastal Plain Experiment Station near Tifton, Georgia.

Month	Rainfall				Avg. Max Temperature				Avg. Min Temperature			
	2016	2017	2018	100-yr avg	2016	2017	2018	100-yr avg	2016	2017	2018	100-yr avg
	--------cm----------				---------°C----------				---------°C----------			
March	13.4	3.8	8.6	12.2	22.6	26.5	19.9	21.2	11.2	13.0	7.5	8.2
April	16.1	9.7	7.0	9.9	24.4	27.2	23.4	25.4	12.9	13.8	10.9	12.1
May	3.7	6.7	17.6	8.2	28.7	28.9	30.0	29.3	16.3	16.3	18.9	16.5
June	10.0	13.0	15.0	11.7	32.3	29.9	32.2	32.0	20.9	20.4	21.7	20.2
July	8.6	12.4	14.9	13.8	34.0	32.3	31.8	32.8	22.2	22.4	22.4	21.5
August	16.0	13.5	24.2	12.4	32.7	32.5	32.4	32.7	22.2	22.3	22.1	21.3
September	15.6	9.4	6.1	9.7	31.1	30.1	32.9	30.7	19.9	18.9	22.1	19.0
October	0.2	5.3	7.0	5.8	28.1	28.0	27.5	26.3	13.9	17.4	16.7	13.0
November	1.8	1.7	16.9	6.4	23.8	22.0	23.2	21.2	9.0	9.2	15.1	7.7

Table 3.3. Average precipitation per rainfall event and number of rainy days March through November during 2016, 2017, and 2018 at the Coastal Plain Experiment Station in Tifton, GA.

Month	Avg. Rainfall per Event			No. Rainfall Events		
	2016	2017	2018	2016	2017	2018
	----- cm -----			----- days -----		
March	0.4	0.2	0.5	13	6	7
April	0.6	1.0	0.4	11	4	8
May	0.2	0.3	0.5	8	8	14
June	0.4	0.2	0.4	9	22	16
July	0.4	0.3	0.3	9	15	20
August	0.3	0.6	0.4	19	9	22
September	0.9	0.5	0.2	7	7	13
October	0.03	0.2	0.6	2	11	5
November	0.4	0.1	0.4	2	6	16

Table 3.4. Forage yield (kg ha^{-1}) of Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) harvested on a 28-35-day harvest interval in 2016, 2017, and 2018 at the University of Georgia Coastal Plain Experiment Station in Tifton, GA.

		MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	Seasonal Yield[2]	No. Cuttings
2016	T85	--	--	--	2477^{a}	3150	3169	1027^{a}	--	--	9823^{b}	4
	T85+Alf	--	--	888	1239^{b}	2586	2746	711^{b}	--	350	8519^{bc}	6
	SEM[1]	--	--	616	132	435	260	64	--	85	418	--
2017	T85	--	--	--	1199^{b}	2477^{a}	2898	724^{b}	--	--	7297^{cd}	4
	T85+Alf	3582	2260	578	1737^{a}	1965^{b}	2829	1010^{a}	580	--	14811^{a}	8
	SEM[1]	145	169	112	128	151	90	86	62	--	372	--
2018	T85	--	--	--	2397	1359	1660	894^{b}	--	--	6390^{d}	4
	T85+Alf	3096	--	2592	3018	1779	1662	1092^{a}	1401	--	14640^{a}	7
	SEM[1]	195	--	51	243	259	70	34	159	--	800	--

[1]SEM values are calculated between forage treatments at each harvest within year and and differences in means are represented using different superscripts ($P < 0.05$).

[2] Differences in means between forage treatment and year are represented using different superscripts ($P < 0.05$).

Table 3.5. Composition of alfalfa, bermudagrass, and other (%) components in Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) at each harvest in 2016, 2017, and 2018 at the University of Georgia Coastal Plain Experiments Station in Tifton, GA.

			MAR	APR	MAY	JUN	JULY	AUG	SEPT	OCT	NOV
2016	Alfalfa	T85	--	--	--	--	--	--	--	--	--
		T85+Alf	--	--	22.2	24.4	22.7	17.4	34.4	--	35.6
		SEM	--	--	4.13	2.63	2.55	5.20	3.41	--	1.91
	Bermuda	T85	--	--	--	98.6[a]	78.6[a]	62.8[a]	82.9[a]	--	--
		T85+Alf	--	--	51.3	64.1[b]	48.6[b]	26.6[b]	48.3[b]	--	44.8
		SEM	--	--	3.46	2.88	5.63	7.03	5.59	--	2.79
	Other	T85	--	--	--	1.4[b]	23.5	37.2	20.7	--	--
		T85+Alf	--	--	25.8	11.5[a]	28.7	56.0	19.5	--	19.6
		SEM	--	--	3.59	3.58	4.03	10.38	7.60	--	5.22
2017	Alfalfa	T85	--	--	--	--	--	--	--	--	--
		T85+Alf	54.6	60.6	34.6	42.0	43.4	44.2	35.5	45.6	--
		SEM	4.11	5.54	2.79	8.70	4.38	2.71	2.18	3.14	--
	Bermuda	T85	--	--	--	68.0[a]	87.4[a]	87.4[a]	80.4[a]	--	--
		T85+Alf	0.2	14.0	24.0	29.2[b]	35.2[b]	43.8[b]	46.6[b]	36.5	--
		SEM	0.2	2.41	4.56	6.24	5.03	6.82	4.79	3.08	--
	Other	T85	--	--	--	32.2	12.6	15.1	19.6	--	--
		T85+Alf	45.2	26.2	41.4	28.8	21.4	13.5	17.9	17.6	--
		SEM	4.11	3.66	3.82	6.94	3.92	3.34	3.19	1.50	--
2018	Alfalfa	T85	--	--	--	--	--	--	--	--	--
		T85+Alf	41.0	--	45.8	63.6	38.2	39.4	35.6	31.4	--
		SEM	3.77	--	5.07	3.14	4.46	2.18	1.57	1.25	--
	Bermuda	T85	--	--	--	87.8[a]	73.2[a]	43.8[a]	55.8[a]	--	--
		T85+Alf	1.0	--	5.4	27.0[b]	29.4[b]	21.4[b]	25.6[b]	27.2	--
		SEM	1.0	--	0.81	2.96	3.64	7.33	4.68	8.28	--
	Other	T85	--	--	--	12.2	26.8	56.2	44.2	--	--
		T85+Alf	58.1	--	53.6	7.6	32.4	39.2	38.8	41.4	--
		SEM	3.85	--	9.23	3.39	6.74	8.55	4.79	8.05	--

[1]SEM values are calculated between forage treatments at each harvest within year. Differences are represented by different superscripts.

Table 3.6. Chemical composition (g kg^{-1}) of Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) at each harvest during 2016 at the University of Georgia Coastal Plain Experiment Station in Tifton, GA.

		MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV
CP (g kg^{-1})	T85	--	--	--	150	120	93^{b}	113^{b}	--	--
	T85+Alf	--	--	82	152	119	120^{a}	151^{a}	--	211
	SEM[1]	--	--	4.92	4.63	3.29	4.38	4.02	--	8.49
NDF (g kg^{-1})	T85	--	--	--	579^{a}	637^{a}	620^{a}	621^{a}	--	--
	T85+Alf	--	--	633	485^{b}	556^{b}	551^{b}	492^{b}	--	347
	SEM[1]	--	--	8.10	33.0	9.45	3.31	7.56	--	11.3
ADF (g kg^{-1})	T85	--	--	--	296^{a}	337	336	321	--	--
	T85+Alf	--	--	389	280^{b}	335	333	308	--	243
	SEM[1]	--	--	4.29	4.42	9.4	2.20	5.43	--	2.32
TDN[2] (g kg^{-1})	T85	--	--	--	584^{b}	562^{b}	563^{b}	557^{b}	--	--
	T85+Alf	--	--	495	625^{a}	600^{a}	599^{a}	606^{a}	--	633
	SEM[1]	--	--	2.80	3.82	7.58	3.25	3.34	--	9.63
IVTDMD48 (g kg^{-1})	T85	--	--	--	775^{b}	737^{b}	724^{b}	727^{b}	--	--
	T85+Alf	--	--	689	816^{a}	772^{a}	784^{a}	782^{a}	--	809
	SEM[1]	--	--	6.63	4.49	8.12	4.15	4.12	--	2.10
RFQ[3] (g kg^{-1})	T85	--	--	--	94^{b}	83^{b}	82^{b}	80^{b}	--	--
	T85+Alf	--	--	59	118^{a}	101^{a}	101^{a}	109^{a}	--	154
	SEM[1]	--	--	1.51	4.23	3.42	1.10	1.29	--	7.60
ASH (g kg^{-1})	T85	--	--	--	88	79	76	77	--	--
	T85+Alf	--	--	61	85	79	82	81	--	110
	SEM[1]	--	--	2.03	2.92	1.77	2.36	1.97	--	4.90

[1]SEM values are calculated between forage treatments at each harvest within year and differences in means are represented using different superscripts ($P < 0.05$).

[2]TDN: Predicted total digestible nutrients = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDF_n x ($NDFD_p$ /100)] – 10.

[3] RFQ: Estimated relative forage quality = DMI (% BW) x TDN (% DM) / 1.23

Table 3.7. Chemical composition (g kg^{-1}) of Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) at each harvest during 2017 at the University of Georgia Coastal Plain Experiment Station in Tifton, GA.

		MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV
	T85	--	--	--	114^{b}	190^{b}	125^{b}	120^{b}	NR	--
CP	T85+Alf	191	243	227	209^{a}	222^{a}	202^{a}	222^{a}	NR	--
(g kg^{-1})	SEM[1]	6.27	1.00	2.81	10.48	3.91	8.52	11.11	--	--
	T85	--	--	--	598^{a}	567^{a}	630^{a}	600^{a}	NR	--
NDF	T85+Alf	350	340	329	397^{b}	447^{b}	459^{b}	380^{b}	NR	--
(g kg^{-1})	SEM[1]	7.46	2.10	4.24	14.86	5.82	12.73	19.42	--	--
	T85	--	--	--	307	336^{a}	357^{a}	319^{a}	NR	--
ADF	T85+Alf	256	256	253	267	278^{b}	291^{b}	248^{b}	NR	--
(g kg^{-1})	SEM[1]	4.05	2.94	3.42	12.42	9.21	13.89	12.04	--	--
	T85	--	--	--	576^{b}	537^{b}	527^{b}	551^{b}	NR	--
TDN[2]	T85+Alf	651	644	636	626^{a}	608^{a}	601^{a}	637^{a}	NR	--
(g kg^{-1})	SEM[1]	2.66	2.68	0.45	9.61	5.05	9.36	11.61	--	--
	T85	--	--	--	738^{b}	754^{b}	702^{b}	710^{b}	NR	--
IVTDMD48	T85+Alf	850	846	813	825^{a}	822^{a}	800^{a}	845^{a}	NR	--
(g kg^{-1})	SEM[1]	4.59	1.66	2.14	15.49	2.46	5.12	15.92	--	--
	T85	--	--	--	90^{b}	82^{b}	72^{b}	76^{b}	NR	--
RFQ[3]	T85+Alf	160	162	162	138^{a}	119^{a}	114^{a}	145^{a}	NR	--
	SEM[1]	4.04	1.71	2.29	7.48	2.29	4.03	9.12	--	--
	T85	--	--	--	76^{b}	107	80^{b}	73	NR	--
ASH	T85+Alf	96	103	106	101^{a}	93	96^{a}	94	NR	--
(g kg^{-1})	SEM[1]	1.47	1.88	2.34	2.47	2.79	5.66	3.62	--	--

‡ NR: Samples not reported via NIR procedures

[1]SEM values are calculated between forage treatments at each harvest within year and differences in means are represented using different superscripts ($P < 0.05$).

[2] TDN: Predicted total digestible nutrients = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDF_n x ($NDFD_p$ /100)] – 10.

[3] RFQ: Estimated relative forage quality = DMI (% BW) x TDN (% DM) / 1.23

Table 3.8. Chemical composition (g kg^{-1}) of Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) at each harvest during 2018 at the University of Georgia Coastal Plain Experiment Station in Tifton, GA.

		MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV
CP (g kg^{-1})	T85	--	--	--	158^{b}	102^{b}	129^{b}	112^{b}	--	--
	T85+Alf	227	--	226	221^{a}	184^{a}	173^{a}	179^{a}	183	--
	SEM[1]	2.48	--	1.88	4.54	7.43	4.24	3.76	6.08	--
NDF (g kg^{-1})	T85	--	--	--	598^{a}	671^{a}	592^{a}	614^{a}	--	--
	T85+Alf	353	--	353	427^{b}	441^{b}	442^{b}	453^{b}	448	--
	SEM[1]	3.75	--	3.41	14.0	7.44	8.10	5.70	9.24	--
ADF (g kg^{-1})	T85	--	--	--	308	337	322	337^{a}	--	--
	T85+Alf	265	--	252	317	332	314	308^{b}	316	--
	SEM[1]	3.33	--	3.18	9.11	7.89	4.60	5.46	4.55	--
TDN[2] (g kg^{-1})	T85	--	--	--	566^{b}	553^{b}	574^{b}	556^{b}	--	--
	T85+Alf	631	--	645	588^{a}	601^{a}	599^{a}	600^{a}	585	--
	SEM[1]	2.80	--	1.74	6.76	4.34	2.00	1.61	3.85	--
IVTDMD48 (g kg^{-1})	T85	--	--	--	772	704^{b}	782	731^{b}	--	--
	T85+Alf	821	--	835	785	782^{a}	787	787^{a}	758	--
	SEM[1]	3.82	--	2.79	11.75	9.77	5.01	6.03	3.63	--
RFQ[3]	T85	--	--	--	91^{b}	77^{b}	93^{b}	82^{b}	--	--
	T85+Alf	151	--	156	114^{a}	115^{a}	115^{a}	113^{a}	109	--
	SEM[1]	2.37	--	2.12	23.5	2.05	1.97	1.55	2.11	--
ASH (g kg^{-1})	T85	--	--	--	107	75^{b}	100	88^{b}	--	--
	T85+Alf	108	--	104	106	92^{a}	97	94^{a}	99	--
	SEM[1]	1.56	--	0.82	3.21	3.02	2.61	1.64	1.89	--

[1]SEM values are calculated between forage treatments at each harvest within year and differences in means are represented using different superscripts ($P < 0.05$).

[2] TDN: Predicted total digestible nutrients = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDF_n x ($NDFD_p$ /100)] – 10.

[3] RFQ: Estimated relative forage quality = DMI (% BW) x TDN (% DM) / 1.23

Table 3.9. Average seasonal chemical composition (g kg^{-1}) of Tifton-85 bermudagrass-only (T85) and 'Tifton-85' interseeded with 'Bulldog 805' alfalfa (T85+Alf) during 2016, 2017, and 2018 at the University of Georgia Coastal Plain Experiment Station in Tifton, GA.

		2016	2017	2018
CP (g kg^{-1})	T85	108^{c}	136^{bc}	125^{bc}
	T85+Alf	139^{b}	216^{a}	199^{a}
	SEM[1]	5.27	4.74	3.47
NDF (g kg^{-1})	T85	635^{a}	599^{a}	619^{a}
	T85+Alf	499^{b}	386^{c}	417^{c}
	SEM[1]	10.6	11.6	5.96
ADF (g kg^{-1})	T85	339^{a}	329^{a}	326^{ab}
	T85+Alf	315^{ab}	264^{c}	301^{b}
	SEM[1]	6.43	3.73	3.69
TDN[2] (g kg^{-1})	T85	552^{c}	549^{c}	562^{c}
	T85+Alf	604^{b}	629^{a}	607^{b}
	SEM[1]	4.17	3.83	2.79
IVTDMD48 (g kg^{-1})	T85	717^{d}	729^{cd}	747^{cd}
	T85+Alf	775^{bc}	829^{a}	793^{b}
	SEM[1]	7.38	4.33	4.29
RFQ[3]	T85	80^{d}	80^{d}	86^{d}
	T85+Alf	110^{c}	143^{a}	125^{b}
	SEM[1]	2.64	4.22	2.36
ASH (g kg^{-1})	T85	77^{b}	80^{b}	92^{a}
	T85+Alf	81^{b}	99^{a}	100^{a}
	SEM[1]	1.70	1.42	1.39

[1]SEM values are calculated between forage treatments and year and differences in means are represented using different superscripts ($P < 0.05$).

[2]TDN: Predicted total digestible nutrients = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDFn x (NDFDp /100)] – 10.

[3]RFQ: Estimated relative forage quality = DMI (% BW) x TDN (% DM) / 1.23

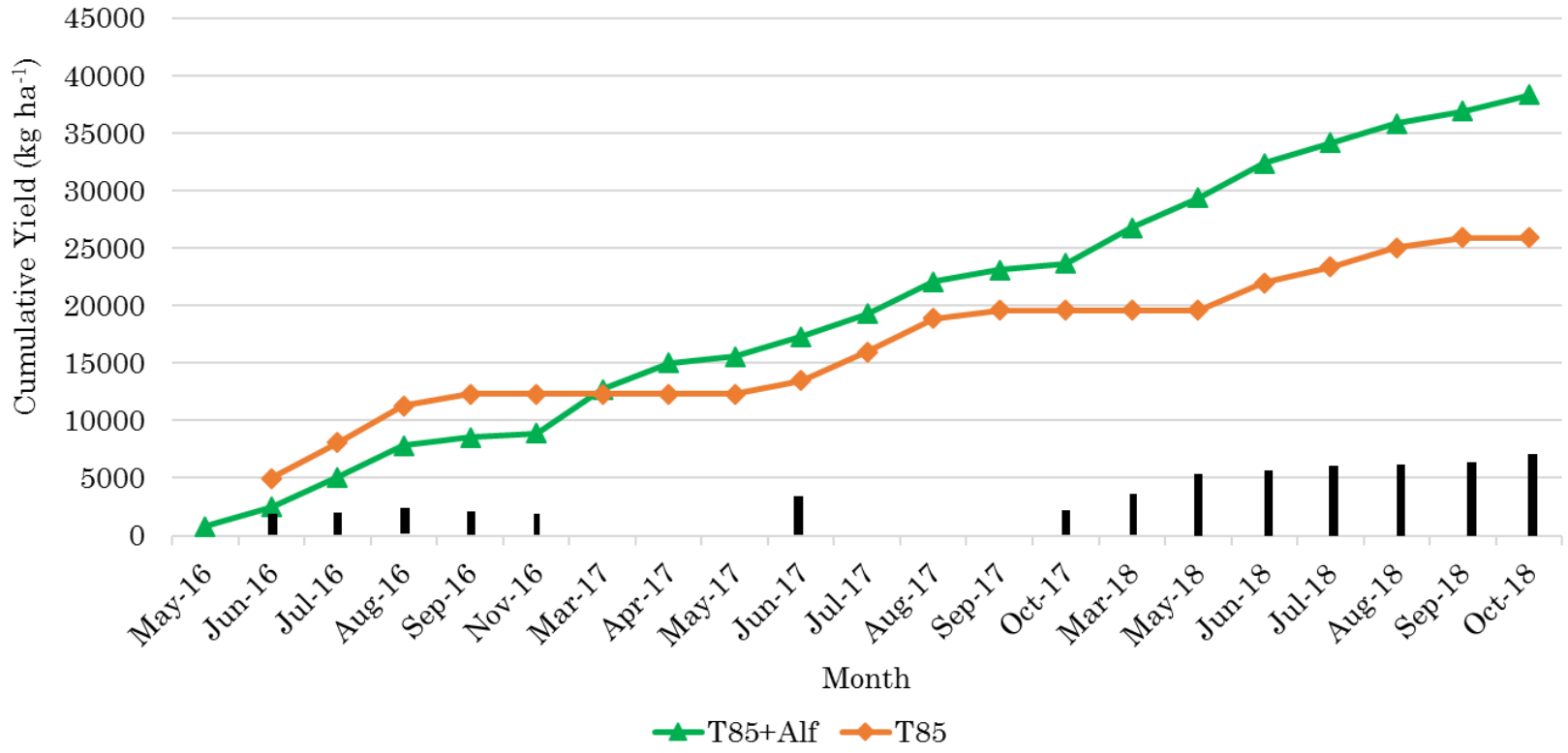

Figure 3.1. Cumulative yield (kg ha^{-1}) of 'Tifton-85' bermudagrass-only and 'Tifton-85' bermuagrass interseeded with 'Bulldog 805' alfalfa at each harvest interval in 2016, 2017, and 2018 at the Coastal Plain Experiment Station in Tifton, GA. Black bars represent the least significant difference (LSD) at $P < 0.05$ and are present when differences occurred.

CHAPTER 4

THE EFFECT OF STORAGE LENGTH ON THE NUTRITIVE VALUE OF T85-BERMUDAGRASS OR T85 BERMUDAGRASS-ALFALFA BALEAGE[2]

[2] Hendricks, T.J., J.J. Tucker, D.W. Hancock, J.R. Segers, and R.L. Stewart, Jr. To be submitted to *Journal of Animal Science*

Abstract

The use of baleage technology as a stored forage method can improve forage quality and minimize risks associated with weather. However, current recommendations state that baleage should be fed within 9-months post-harvest to minimize bale deformation, reduce DM losses, and improve ease of transportation and feed out. This storage period may not provide producers enough flexibility to match their highest quality forages with the animal nutrient requirements. Therefore, the objective of this research was to determine the effect of storage length on nutritive value of forage harvested and stored as baleage in the Southeast. This study was conducted on forage harvested during 2016 and 2017 at the University of Georgia Coastal Plain Experiment station in Tifton, GA. Forage treatments included 'Tifton-85' bermudagrass (*Cyondon dactylon*) (T85) or 'Tifton-85' bermudagrass interseeded with 'Bulldog 805' alfalfa (*Medicago sativa*) (T85+Alf). Forage was harvested every 28-35 days when T85+Alf reached early (10%) bloom, baled at 40-60% moisture, wrapped using an individual bale wrapper, and stored for 12-months. Bales were sampled at four timepoints: at harvest prior to wrapping and at 6-weeks, 9-, and 12-months post-harvest for nutritive value. During storage, CP in the T85+Alf treatment decreased ($P < 0.01$) between harvest and fermentation (6-weeks), but not between 9- and 12-months ($P = 0.65$). IVTDMD48 of both T85 and T85+Alf decreased ($P < 0.01$) between harvest and 12-months ($P = 0.08$ and $P < 0.01$, respectively) in either forage. Finally, although measured parameters decreased to a greater extent in T85+Alf than T85 baleage, it maintained adequate nutritive value to support a lactating beef cow throughout the 12-month storage period.

Keywords: Baleage, nutritive value, stored forage

Introduction

The goal of most forage-based livestock systems is to produce a year-round supply of high-quality forage. Harvesting, storing, and feeding additional forage throughout the year contributes to the largest percentage of input costs for a typical beef cattle producer, costs which could be eliminated with a year-round forage supply. Because it is nearly impossible to produce the quantity and quality of forage that would eliminate the need for additional feed, it is important for producers to harvest and store forage as efficiently as possible to minimize additional costs.

Traditionally, forages are stored as dry hay; however, in some regions of the United States, the use of baleage as a stored forage method is increasing. It is well-understood that forage quality is directly related to forage maturity (Hancock et al., 2014; Ball et al., 2015), therefore it is important to maintain a timely harvest interval as any subsequent delays can result in decreases in nutritive value and forage digestibility. The higher moisture level of baleage may be reached in as few as 4 hours under optimal drying conditions. This truncated drying time is appealing to producers in the Southeast who wish to optimize forage quality and quantity but struggle to find an adequate window of drying time for hay production.

The higher moisture at baling can also be advantageous for alfalfa forage systems by reducing quality losses associated with leaf shatter. In alfalfa, the leaves have the highest concentrations of water soluble carbohydrates (WSCs) and least amount of undigestible fiber and lignin, making them highest in nutritive value. However, the leaves are also the most susceptible to shattering during the harvesting process and may account for up to 35% of the total mechanical losses (McGechan, 1989). Baling at a higher

moisture can reduce the brittleness of the leaves and reduce the amount of shatter loss during production.

Currently, it is generally recommended that producers should feed baleage within 9 months of harvest to minimize the loss of forage quality and the risk of spoilage (Hancock et al., 2019). These recommendations stem from the need to store baleage anaerobically to minimize mold and yeast growth. It may be difficult for producers to maintain the integrity of the plastic used to store baleage in order to keep the forage in an anaerobic environment and prevent spoilage. Additionally, because baleage is produced at a higher moisture, they may begin to "squat," or change shape, as they are stored, making it difficult to handle and move bales at feeding (Hancock and Collins, 2006).

These feeding recommendations may not always be practical for producers in the Southeast. Baleage production is most economical when fed to animals with higher nutrient requirements than a dry cow. Therefore, producers generally try to match baleage feeding with their high nutrient requiring animals, such as lactating beef cows or backgrounding systems. A nine-month feeding window may not allow producers to utilize their forage during their calving seasons, making it less economical. Additionally, in growing seasons with high forage production, there may be adequate forage in the field, making it unnecessary to provide stored forage.

There is little research into how the nutritive value of baleage may change over time, however it is reasonable to believe that quality losses should be minimal as long as the forage is protected and maintained in anaerobic conditions. Thus, the objective of this research is to determine how the length of storage affects the nutritive value of forage harvested and stored as baleage.

Materials and Methods

Forage Treatments and Plot Management

Baleage treatments of 'Tifton-85' bermudagrass (Reg. No. CV-20, PI 562699; Burton et al., 1993; T85) and a mixture of 'Tifton-85' bermudagrass and 'Bulldog 805' alfalfa (T85+Alf) were evaluated during a 3 year trial (2016-2018) at the University of Georgia Coastal Plain Experiment Station (Tifton, GA). Baleage was produced from ten 0.2-ha plots in a previously established 'Tifton-85' bermudagrass hayfield where plots designated to the T85+Alf treatment were interseeded with 'Bulldog 805' alfalfa (Reg. no. CV-194, P1 594913; Bouton et al., 1997) at 22.4 kg ha^{-1} during February 2016.

Each year, calcium ammonium was applied to the T85 plots three times throughout the growing season at a rate of 84 kg N ha^{-1}. Muriate of potash was applied to all plots in the study three times throughout the growing season at 112 kg K_2O ha^{-1}. Boron (10% liquid solution) was applied July 2017 at 2.24 kg B $ha^{-1}$and molybdenum was applied during October 2017 at 0.23 kg Mo ha^{-1}. Soil tests were conducted during February of each year and plant tissue samples were collected prior to the August cutting to determine nutrient deficiencies and additional fertilization was applied according to these recommendations. Additionally, irrigation was provided from June 15 to July 21 and September 9 through November 7 at 2.54-cm per week to prevent stand loss during extreme drought.

Forage Harvest Management

Forage was harvested during 2016 and 2017 beginning in the spring when adequate forage growth allowed (e.g. T85 was at least 20 to 25 cm), and alfalfa reached

early (10 percent) bloom. Subsequent harvests continued on a 28 to 35 day cutting interval throughout each growing season until growth was limited by environmental conditions (e.g. temperature or daylight). The first harvests of T85+Alf were in May 2016 and March 2017; T85 plots were harvested beginning in June of each year.

At each harvest, plots were cut beginning at ca. 1800 h using a mower-conditioner (New Holland Discbine 313; New Holland Agriculture, New Holland, PA) to minimize respiration losses. Beginning at ca. 0930 h the morning after cutting, grab samples were taken from each plot to determine forage moisture of each treatment (T85 and T85+Alf) using the microwave moisture test (Ball et al., 2015) and this procedure was repeated until forage moisture reached 55 percent.

When the target moisture was achieved, forage was raked into windrows using a one-sided wheel rake and baled using a Krone Fortima 1500 MC baler (Krone NA, Inc., Memphis, TN). During baling, a lactic acid producing forage inoculant 'hemicellulose *A. Niger*' to provide 71 billion cfu g^{-1} (Silage Supreme; Kent Nutrition Group, Muscatine, IA) was applied using a continuous flow spray boom in the chamber of the baler. Forage was baled to a target bale weight of 2200 to 2640 kg. When forage growth was adequate, two bales per plot were baled and designated 'A' or 'B.'

Bale Storage and Sampling

Following baling, bales were transported to the wrapping and storage location using a MT455B Challenger tractor (AGCO Corp., Jackson, MN). Bales were wrapped with six layers of pre-stretched (55%) polyethylene baleage wrap (Sunfilm Stretch Wrap; TAMA Group, Dubuque, IA) using an Anderson RB-200 individual bale wrapper

(Groupe Anderson Inc., Chesterville, QC, Canada) and stored in a bermudagrass hay field on polyethylene silage wrap (Up North Plastics, Inc.; Cottage Grove, MN) for storage.

After wrapping, bales were painted with the harvest date and a bale identification number to denote the harvest and plot of each bale. Additionally, bales were scouted weekly and any tears or scratches were immediately patched using silage tape (Agricultural Repair Tape, Blue Lake Plastics, LLC; Sauk Centre, MN) in an "X" pattern to ensure protection against water and oxygen permeability. The border around the storage area was mowed every two weeks to prevent forage overgrowth and weeds.

Forage Sampling and Analysis

Core samples (3) were collected from each bale using a drill-driven aluminum cannister multi-forage sampler (Star Quality Samplers; Irricana, AB, Canada) at four storage periods for nutritive value analysis. Bales were sampled prior to wrapping, and at 6-weeks, 9- and 12-months post-harvest. Time periods were chosen to show potential changes in nutritive value following fermentation (6-weeks), at the current feed out recommendation (9 months), and at an extended storage period (12 months). After coring, bales were immediately sealed in an "X" pattern using silage tape (Agricultural Repair Tape, Blue Lake Plastics, LLC; Sauk Centre, MN).

Nutritive value samples were placed in a paper bag, weighed, and dried in a forced air dryer at 55°C for 3 days. After drying, samples were ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) for wet chemistry analysis, then double-ground through a 1-mm screen in a Cyclone Sample Mill (Model 3010-030; UD Corporation; Boulder, CO). Nutritive value analysis was determined by

near infrared reflectance spectroscopy using a Foss NIR (NIRSystems 6500, Foss NIRS System Inc., Laurel, MD) for metrics including neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ash, *in-vitro* true dry matter digestibility at 48-h (IVTDMD48), dry matter intake (DMI), total digestible nutrients (TDN), and relative forage quality (RFQ). Prior to scanning, samples within each harvest and plot were combined, placed in a forced air oven at 55°C for 90 minutes, then packed into cells and scanned. Predictions were made using the 2018 NIR consortium equations (Hillsboro, WI) for T85 and T85+Alf samples, respectively. NDF, SEC = 2.32, R^2 = 0.961; SECV = 2.391; ADF, SEC = 1.564, R^2 = 0.956; SECV = 1.650; CP, SEC = 0.917, R^2 = 0.977; SECV = 0.945, and 2018 Mixed Grass Hay NIR equations were NDF, SEC = 2.209, R^2 = 0.968; SECV = 2.318; ADF, SEC = 1.747, R^2 = 0.929; SECV = 1.826; CP, SEC = 0.827, R^2 = 0.976; SECV = 0.885, where SEC = standard error of calibration and SECV = standard error of validation, in g kg^{-1} on a DM basis. NIR predictions for nutritive value parameters (e.g. ash, ADF, NDF, CP, and IVTDMD48) were validated for crude protein, digestibility, and fiber using traditional laboratory techniques (Pomerleau-Lacasse et al., 2018; AOAC, 1990; Goering and Van Soest, 1970).

Statistical Analysis

Statistical analyses were conducted using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC) to determine the main effects of forage treatment, storage length, and their interactions. Repeated measurements on the bales from 2016 and 2017 allowed the use of a repeated measures design, wherein the data analysis was conducted using the compounded symmetric covariance structure and multiple mean comparisons were

determined using the Tukey-Kramer test. Data were pooled across 2016 and 2017 by considering block, harvest, and year as random effects. The significance of main effects was declared at an alpha level of 0.05.

Results and Discussion

Nutritive Value

Nutritive value parameters were generally affected by the main effects of forage type, storage length, and their interactions, with the exception that the effect of storage length on NDF did not differ ($P = 0.54$) between forage types (Table 4.1).

Crude protein (CP) was affected by storage length, forage type, and their interactions. Throughout the study, the lowest CP was 121 g kg^{-1} (12-month T85). CP was always less ($P < 0.01$) in T85 than T85+Alf, which is typical when comparing a grass to a grass-legume mixture (Beck et al., 2017). However, both T85 and T85+Alf baleage provided adequate CP to support beef cattle production systems common in the southeastern USA, such as stocker cattle or lactating beef cows which require 100 and 110 g CP kg^{-1} in their diet, respectively (NRC, 2017). CP declined ($P < 0.01$) in T85+Alf between the harvest and the 6-week time point (181 vs 162 g kg^{-1}, respectively) and declined ($P = 0.01$) further between 6-weeks and 9-months (162 vs 156 g kg^{-1}, respectively). Although CP decreased during fermentation, it was not different between 9- and 12-months (156 vs 153 g kg^{-1}, respectively).

It has been observed that forages with high levels of nitrogen, like legumes, are more susceptible to protein hydrolysis and ammonia production during fermentation, which may account for the additional CP losses observed in these samples (Owens and

Albrect, 1999). Papdopoulus and McKersie (1983) reported that among six forages cut for silage, alfalfa consistently had the highest concentration of soluble non-protein nitrogen (SNPN) and subsequently, the highest protein hydrolysis. Further, the high initial CP concentrations of at harvest T85+Alf meant that this decrease would not impact the ability to feed this forage to animals with high nutrient requirements without additional supplementation. In contrast, the CP levels in the T85 treatment did not decline ($P = 0.18$) between harvest and 12-months (127 vs 121 g kg^{-1}, respectively), indicating that if grass baleage is stored properly, the decline in CP should be minimal, if any.

NDF ($P < 0.01$) and ADF ($P = 0.03$) were both affected by forage type and storage length ($P < 0.01$) and ADF was affected by their interaction ($P < 0.01$). NDF and ADF were both greater in T85 than T85+Alf (594 vs 443 g kg^{-1} and 325 vs 307 g kg^{-1}, for T85 and T85+Alf in NDF and ADF, respectively). NDF of both treatments increased during the 12-month study period, although increases were less than 22 g kg^{-1} in other forage. ADF of T85 did not significantly increase following the 6-week time point (328 vs 332 g kg^{-1}, respectively), but there was an increase in the T85+Alf.

ADF of T85+Alf increased ($P < 0.01$) steadily between harvest and 12-months (280 vs 326 g kg^{-1}, respectively). However, the total increase was 46.8 g kg^{-1}, and ADF increased just 19.5 g kg^{-1} between 6-weeks and 12-months (307 vs. 326 g kg^{-1}, respectively). The initial increase in ADF content may be due to increased fermentation of nonstructural carbohydrates from the broadleaf alfalfa component. Because NDF influences the dry matter intake and ADF influences digestibility, and TDN of a forage (Saha et al., 2017), low ADF content is critical for maintaining the nutritive value of stored forage over time. Although statistically significant, the increase between 6-weeks

and 12-months was relatively small and should not significantly impact other nutritive value parameters.

The total digestible nutrients (TDN), 48-hr *in-vitro* true dry matter digestibility (IVTDMD48), and relative forage quality (RFQ) in the forages were affected by storage length, forage type, and their interactions. While TDN, IVTDMD48, and RFQ were greater ($P < 0.01$) in T85+Alf than T85 that is to be expected, as nutritive value parameters are generally improved with the addition of a legume if forage is harvested in a timely manner (Beck et al., 2017). Both T85 and T85+Alf bales exhibited a decrease ($P < 0.01$) in IVTDMD48 between harvest and 6-week samples (729 vs 684 g kg^{-1} and 807 vs 789 g kg^{-1} at harvest and 6-weeks for T85 and T85+Alf, respectively). Additionally, IVTDMD48 of both forages decreased between 6-weeks and 12-months ($P = 0.03$ and $P < 0.01$ for T85 and T85+Alf, respectively). Although statistically significant, the total decrease over the entire 12-month storage period was 10.3 and 10.9 g kg^{-1}, respectively (Table 4.1) and forage nutritive value remained above the level of digestibility required to maintain a lactating beef cow (600 g kg^{-1}) regardless of storage length (NRC, 2017).

TDN and RFQ were also affected by storage length, although the different forage types reacted differently to storage. TDN and RFQ of T85 both decreased ($P < 0.01$) between harvest and 6-weeks but did not decrease further post-fermentation ($P = 0.06$ and 0.43 for TDN and RFQ, respectively). Although there appeared to be a tendency ($P = 0.06$) for TDN to decrease, the 6-week, 9- and 12-month samples were all within 9.9 g kg^{-1} of one another, indicating changes were likely due to sample variation rather than changes in nutritive value over time.

On the other hand, the TDN and RFQ of T85+Alf bales did not significantly decrease between harvest and 6-week samplings, although at-harvest samples were greater than 9- or 12-months (Table 4.1). While there was a decline between 6-weeks and 12-months ($P = 0.03$ and $P < 0.01$ for TDN and RFQ, respectively), the nutritive value was not different between 9- and 12-months. Further, the change in TDN of T85+Alf between 6-weeks and 12-months was 8.7 g kg^{-1}, and therefore not practically significant (617 vs 608 g kg^{-1} for 6-weeks and 12-months, respectively), indicating the difference may have been due to sample variation.

Ash content increased ($P < 0.01$) between at-harvest and the 6-week time point marking the stable phase of fermentation. However, following the 6-week sampling, ash content did not change, regardless of forage (Table 4.1). Ash content of T85+Alf was greater ($P < 0.01$) than T85 at harvest (91.2 vs 82.9 g kg^{-1}, respectively), but forage type did not affect ash content at any other time point.

While these changes may affect the development of a feeding strategy for livestock with high nutrient requirements, if forage is baled at high nutritive value it will easily support most other animal classes (e.g. dry cows), despite undergoing a quality decline. Even though T85+Alf bales exhibited a decline in TDN, IVTDMD, and RFQ, their nutritive value remained higher post-storage than all T85 bales at harvest. T85+Alf baleage remained well above the nutrient requirements of a lactating beef cow while T85 bales would need additional supplementation when fed. It is important to remember that greater nutritive value at harvest will lead to greater nutritive value at feed out, even taking potential quality declines into consideration. Finally, regardless of these results,

producers should test stored forages to confirm the nutritive value of their baleage when developing a feeding strategy for their livestock.

Conclusions

Based on these results, it is reasonable to believe that baleage may be stored for longer than the recommended 9-months before feeding. Although there were declines in several nutritive value parameters between harvest and the 12-month storage length, there were no differences between 9- and 12-months. Therefore, if bales are being stored and fed according to current recommendations, a longer storage period should not be detrimental to forage quality. It is important to realize that a longer storage period before feeding would not be without its challenges. Because of the high moisture content of the forage at baling, bales will still experience squatting, which makes their transport and handling more difficult. While longer storage should not have a major impact on nutritive value, maintaining bale integrity for longer than 9 months is still a challenge. Further, extending the feed out recommendations from 9- to 12-months may not provide producers with enough flexibility to justify a change from dry hay to baleage production. Therefore, further research is needed to determine if baleage can be maintained over a greater storage length without adverse effects on nutritive value or bale stability.

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Table 4.1. Chemical composition of 'Tifton-85' bermudagrass only (T85) and 'Tifton-85' bermudagrass-'Bulldog 805' alfalfa (T85+Alf) baleage as measured by near-infrared spectroscopy at harvest, 6-week, 9-month, and 12-month time points. Samples were pooled between 2016 and 2017, and block, harvest, and year were treated as random effects.

	Forage	Storage Length				SEM[1]	P-Value		
		Harvest	6-week	9-month	12-month		Forage	Time	Forage*Time
CP (g kg^{-1})	T85	127^{a}	125^{a}	124^{a}	121^{a}	2.75	< 0.01	< 0.01	< 0.01
	T85+Alf	181^{a}	162^{b}	156^{c}	153^{c}	2.04			
NDF (g kg^{-1})	T85	584^{b}	601^{ab}	589^{ab}	601^{a}	6.61	< 0.01	< 0.01	0.54
	T85+Alf	431^{b}	445^{a}	444^{ab}	452^{a}	4.92			
ADF (g kg^{-1})	T85	315^{c}	328^{ab}	325^{b}	332^{a}	3.73	0.03	< 0.01	< 0.01
	T85+Alf	280^{d}	307^{c}	316^{b}	326^{a}	2.78			
TDN[2] (g kg^{-1})	T85	561^{a}	514^{b}	521^{b}	524^{b}	4.41	< 0.01	< 0.01	< 0.01
	T85+Alf	621^{a}	617^{ab}	612^{bc}	608^{c}	3.31			
IVTDMD48[3] (g kg^{-1})	T85	729^{a}	684^{b}	682^{bc}	673^{c}	4.19	< 0.01	< 0.01	< 0.01
	T85+Alf	807^{a}	789^{b}	784^{bc}	778^{c}	3.13			
RFQ[4]	T85	93^{a}	82^{b}	81^{b}	79^{b}	2.41	< 0.01	< 0.01	< 0.01
	T85+Alf	132^{a}	129^{ab}	125^{bc}	123^{c}	1.80			
Ash (g kg^{-1})	T85	83^{c}	103^{b}	109^{a}	105^{b}	1.47	0.97	< 0.01	< 0.01
	T85+Alf	91^{c}	101^{b}	103^{a}	105^{ab}	1.09			

[1]Standard error of means calculated within forage and differences in means are represented using different superscripts ($P < 0.05$).
[2]Total Digestible Nutrients (TDN) = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDFn x (NDFDp /100)] – 10.
[3]*In-vitro* Dry Matter Digestibility at 48-hours (IVTDMD48)
[4]Relative Forage Quality (RFQ) = (TDN x DMI) / 1.23.

CHAPTER 5

EVALUATING FIVE COMMERCIALLY AVAILABLE MICROBIAL INOCULANTS FOR IMPROVED FERMENTATION IN ALFALFA-BERMUDAGRASS BALEAGE[3]

[3] Hendricks, T.J., D.W. Hancock, J.J. Tucker, R.L. Stewart, Jr. To be submitted to *Crop, Forage, and Turfgrass* Management.

Abstract

Microbial inoculants are widely used for preservation of silage, yet there is limited information about their value in baleage systems. The objective of this research is to determine the efficacy of five commercially available microbial inoculants for improving fermentation characteristics of alfalfa-bermudagrass baleage. The study was conducted on three 3.8 to 7-ha fields of a previously established 'Tifton-44' bermudagrass and 'Bulldog 505' alfalfa mixture at the J. Phil Campbell Research and Extension Center in Watkinsville, GA. Fields were harvested at the 10% bloom stage at a target moisture of 55 to 60% during the 2017 and 2018 growing seasons. Bales were randomly assigned to one of 6 treatments: 1) Pioneer 1174 (DuPont Pioneer Johnston, IA) at 11 ppm; 2) Pioneer 11H50 (DuPont Pioneer Johnston, IA) at 11 ppm; 3) Pioneer 11G22 (DuPont Pioneer Johnston, IA) at 11 ppm; 4) SiloKing (AgriKing Inc.) at 7.9 ppm; 5) SiloSolve MC (CHR Hansen, Horsholm, Denmark) at 1.1 ppm; and 6) untreated control. Inoculants were applied at the labeled rate using a tractor-mounted applicator. Bales were weighed prior to wrapping, and sampled prior to wrapping (pre-storage), at 7, 14, 21, and 60-days post-harvest; and at 4-months post-harvest (post-storage). Pre-storage samples were analyzed for nutritive value while 7, 14, 21, and 60-day samples were analyzed for fermentation characteristics. Post-storage samples were also analyzed for fermentation and mold counts. Concentrations of total volatile fatty acids (VFA), lactic acid, and acetic acid increased ($P < 0.01$) while pH decreased ($P < 0.01$) during fermentation, regardless of treatment. However, no consistent effect on total VFA, lactic acid, acetic acid, or pH was observed in response to treatment.

Keywords: Baled Silage, Baleage, Microbial Inoculants, Fermentation

Introduction

Baled silage, or baleage, is a conservation method wherein forage is harvested and baled at a higher moisture (typically 40 to 60%), compared with traditional hay (15 to 18%). Since the time between when the crop is cut and baled is shorter than for making hay, conservation as baleage is less susceptible to weather-related risks. This makes baleage an appealing stored forage option for producers in high humidity, high rainfall climates. The higher moisture of baleage, however, makes good fermentation critical to maintaining forage quality and preventing spoilage. Preventing spoilage in baleage is key for producers to provide high-quality livestock feed and reduce input costs (Muck et al., 2018).

Alfalfa is a forage legume that is widely used in livestock diets because of its high protein and digestibility. Conservation of alfalfa as silage is beneficial because its leaves are susceptible to loss through shatter when harvested at higher moisture compared to when it is conserved at the low moisture needed for dry hay (McCormick, 2013). Additionally, it has thick stems that require longer drying time, which can be difficult to achieve in a high humidity climate. Using a high moisture forage conservation method, such as baleage, can minimize some of the issues associated with harvesting alfalfa (McGechan, 1989). However, alfalfa has lower levels of fermentable carbohydrates when compared with other crops typically used for silage. Moreover, alfalfa's high calcium content and high buffering capacity making fermentation more difficult and prevents more of a pH drop.

Microbial inoculants can be applied to forage to promote initial fermentation, rapidly drop pH, and improve aerobic stability by preventing mold and yeast growth

when bales are exposed to oxygen (Guo et al., 2013; Muck et al., 2018). Many microbial inoculants, such as those containing *Lactobacillus plantarum* or *Enterococcus faecium*, contain primarily obligate homofermentative bacteria that produce lactic acid and thrive in the low pH of fermented forages (Muck et al., 2018). Low forage pH is critical to prevent spoilage and mold/yeast growth. However, baleage treated with homofermentative inoculants alone can be susceptible to spoilage when opened (Adesogan et al., 2004).

Heterofermentative inoculants containing *L. buchneri* can produce high quantities of both lactic and acetic acid and have been shown to improve the aerobic stability of forage (Adesogan et al., 2004; Guo et al., 2013). However, fermentation generally occurs more slowly with these products and, consequently, DM losses can be greater than those treated with homofermentative inoculants alone (Ranjit and Kung, 2000). To promote initial fermentation and pH while also improving long-term baleage stability, some microbial products combine both homofermentative and heterofermentative bacteria (Arriola et al., 2015).

Most microbial inoculant studies have been conducted on forage ensiled in mini-silos, rather than large round bales. Arriola et al. (2015) assessed the effect of microbial inoculants on fermentation characteristics in large round bale silage, but this work was conducted on a bermudagrass monoculture rather than mixtures containing alfalfa. The objective of this study was to evaluate the efficacy of five commercially available microbial inoculants to improve fermentation characteristics and reduce mold and yeast growth in alfalfa-bermudagrass baleage in the Southeast.

Materials and Methods

Study Site and Plot Management

Three previously established mixed stands of 'Bulldog 505' alfalfa and 'Tifton-44' bermudagrass at the University of Georgia J. Phil Campbell Research and Extension Center (JPC-REC; Watkinsville, GA) were utilized during the 2017 and 2018 growing seasons. These three fields named GY, W10, and CF are 3.96, 3.88, and 6.82 ha, respectively. Soil tests were conducted annually, and the mixtures were fertilized based on soil test recommendations (Table 5.1). In May 2017, gypsum was applied to GY and W10 at a rate of 2.8 Mg ha^{-1}. At this time, each field also received muriate of potash at a rate of 161 kg K_2O ha^{-1}. Finally, W10 was fertilized with 15.7 kg N ha^{-1}, 40.3 kg P ha^{-1}, and 80.6 kg K ha^{-1} in both October 2017 and May 2018, and boron (0.47 L ha^{-1}) and molybdenum (0.23 L ha^{-1}) in May 2018. Baleage for this trial was harvested May 2017 (GY; W10), July 2017 (CF), and April and June 2018 (W10). When forage was not harvested for this project, locations were harvested as hay or haylage, or grazed by the research station's livestock.

Plots were scouted periodically for insect pests, such as alfalfa weevil [*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)], potato leafhopper [*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae)], three-cornered alfalfa hopper [*Spissistilus festinus* (Say) (Hemiptera: Membracidae)], fall armyworm [*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)], and bermudagrass stem maggot [*Atherigona reversua* (Villaneuve) (Diptera: Muscidae)] throughout the season. In April 2018, lambda cyhalothrin (Lambda Star; Nufarm Americas Inc., Burr Ridge, IL) was applied in February at 28 g a.i. ha^{-1} to control potato leafhopper.

Pre-Harvest Sampling and Preparation

Three to five days prior to trial harvests, botanical composition was determined at ten locations throughout each field using a 0.1-m^2 quadrat. At each quadrat, forage was cut and separated into alfalfa, bermudagrass, other grass, and other broadleaves. Botanical composition samples were immediately weighed and placed into a forced dry oven at 55°C for 3 days and dry samples were weighed to determine the yield component of each species/category.

Forage Harvest

Forage was cut from the aforementioned fields using a John Deere 835 Rotary Mo-Co mower-conditioner (John Deere Corp., Moline, IL) and leaving a 7-cm stubble height beginning at approximately 3 p.m. when alfalfa reached 10 percent bloom. The morning of baling, forage moisture was tested using a windrow hay moisture tester (Farmcomp Oy; Tuusula, Finland) in the field. When the forage reached a target moisture of 55%, forage was raked into windrows using a wheel rake (Frontier WR1010, John Deere Corp.; Moline, IL) and bales were made using a round baler (Krone Fortima V1800, Krone NA, Inc.; Memphis, TN). Forage moisture samples (approximately 0.5 kg) were collected from three random locations within the windrows prior to baling, immediately weighed, then placed into a forced dry oven at 55°C for 4 days and reweighed to calculate forage moisture at the start of baling.

Inoculant Treatments and Application

The trial included five different commercially available inoculant treatments, and an untreated water control (CON). The five inoculant treatments were applied according to manufacturer recommendations and included: 1) Pioneer 1174 (DuPont Pioneer Johnston, IA) at 11 mg kg^{-1} to supply 1.25 x 10^{11} cfu g^{-1} of *L. plantarum and Enterococcus faecium* (P1174); 2) Pioneer 11H50 (DuPont Pioneer Johnston, IA) at 11 mg kg^{-1} to supply 1.25 x 10^{11} cfu g^{-1} of *L.s plantarum* (P11H50); 3) Pioneer 11G22 (DuPont Pioneer Johnston, IA) at 11 mg kg^{-1} (P11G22) to supply 1.1 x 10^{11} cfu g^{-1} of *L. plantarum,* and *L. buchneri* ; 4) SiloKing (AgriKing Inc.) at 7.9 mg kg^{-1} to supply 1.0 x 10^5 cfu g^{-1} of a mixture of *L. plantarum*, *E. faecium*, and *Pediococcus pentosaceus* (SK); and 5) SiloSolve MC (CHR Hansen, Horsholm, Denmark) at 1.1 mg kg^{-1} to supply 1.5 x 10^4 cfu g^{-1} of *L. plantarum*, *E. faecium* and *L. lactis* (SS). Each of the inoculants contained homofermentative bacteria while 11G22 contained both homo- and heterofermentative species. The SiloKing product is the sole dry inoculant used in the experiment and was chosen based on work by Arriola et al. (2015) wherein the fermentation profile indicated significantly less secondary fermentation and resulted in lower clostridia counts, lower non-protein N concentrations, and superior IVDMD compared to other inoculant treatments. Treatments were assigned a letter (A-F) randomly to minimize bias. Prior to baling, the application order was determined using a random number generator (where A=1, B=2, etc.). Treatments were randomized within a block based on the moisture gradient of starting at a higher moisture and ending at a lower moisture as a result of crop wilting in the field. This resulted in a randomized complete block design (RCBD) and minimized the random effects of moisture variation

in forage moisture or field conditions throughout the baling process by nesting moisture in the block effect.

During baling, inoculants were sprayed using a tractor-mounted tank and continuous flow boom sprayer that had been fitted with 3.8-L polypropylene screw top bottles (Thermo Scientific Nalgene Labware; Waltham, MA) that were specific to each treatment and interchangeable on the sprayer system. Each of the liquid inoculants were added to their respective bottles in the amount needed to treat a single bale and mixed with 3.8-L deionized water to ensure each inoculant was sprayed at the same rate. The sprayer was mounted to the pickup of the baler and oriented to spray the entire windrow just before the forage entered the baling chamber. The SiloKing (SK) dry inoculant was applied using a pneumatic applicator with a deflector that spread the material across the entire windrow just before the forage entered the baling chamber. After each inoculated bale, a "flush" bale was formed while being sprayed with deionized water in the spray tank. This served to clean the sprayer system and baling chamber. Each treated bale was spray painted immediately following baling with an identification number including the treatment letter and repetition number (i.e. A1, A2, etc.). Flush bales were left blank.

Forage Sampling and Analysis

After baling, bales were transported to the wrapping site, weighed (MTI-500, MTI Weight Systems, Inc.; N. Kingston, RI) and measured on both the horizontal and vertical axes to quantify the cylindrical variability and deformation of the bale. Core samples (5) were collected from each bale using a Star Hay Probe (Star Spiral Assist, Star Quality Samplers; Irricana, AB, Canada) to be used for nutritive value analysis (Cumberland

Valley Analytical Services; Waynesboro, PA). Bales were wrapped with six layers of pre-stretched (55%) polypropylene baleage wrap (Sunfilm Stretch Wrap; TAMA Group, CITY) using an in-line wrapper (Frontier LW1166, John Deere Corp.; Moline, IL) with a "flush," or untreated bale placed between each treatment bale to minimize possible cross-contamination. The plastic was spray-painted with the bale identification number as wrapping occurred.

Immediately following wrapping, temperature sensors encased in aluminum piping were inserted approximately 45-cm into the center of 12 bales (2 bales per treatment) per harvest and bale temperature was recorded via datalogger (WatchDog 1000 series, Spectrum Technologies, Inc.; Aurora, IL) every 30 minutes for 60 days post-harvest.

Core samples (5) were collected from bales at days 7, 14, 21, and 60 post-harvest using a Star Hay Probe (Star Spiral Assist, Star Quality Samplers; Irricana, AB, Canada). Additionally, "post-storage" samples were taken from each bale (6 cores) at 4 months (approximately 120 days) post-harvest. After coring, bales were immediately sealed using silage tape (Agricultural Repair Tape, Blue Lake Plastics, LLC; Sauk Centre, MN) in an "X" pattern to ensure protection against water and oxygen permeability. Bales were scouted periodically and re-taped as necessary.

At the "post-storage" bale sampling, forage samples were split into two quart-sized freezer bags. One set of bags was labeled, placed on ice, and immediately shipped to a commercial forage laboratory (Cumberland Valley Analytical Services, CVAS; Waynesboro, PA) for yeast and mold count analyses. The second set of post-storage samples was placed in a quart-sized freezer bag, deposited in a cooler for transport, then

immediately frozen until the completion of all sampling dates. All frozen samples (days 0, 7, 14, 21, 60, and "post-harvest") were sent to CVAS for further analysis. Day 0 samples were analyzed for nutritive value using near-infrared reflectance spectroscopy (NIR); days 7 and 14 were analyzed for fermentation characteristics; and day 60 and "post-storage" samples were analyzed for nutritive value and fermentation, in addition to the previously detailed mold and yeast counts. Nutritive value analysis was determined by near infrared reflectance spectroscopy using a Foss 5000 NIR (Foss NIRS System Inc., Laurel, MD) for metrics including analysis of dry matter, moisture, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), starch, and ash. Calibration statistics for NIR haylage equations were as follows: NDF, SEC = 0.811, R^2 = 0.993; SECV = 0.826; ADF, SEC = 0.770, R^2 = 0.972; SECV = 0.794; CP, SEC = 0.519, R^2 = 0.988; SECV = 0.529; where SEC = standard error of calibration and SECV = standard error of validation, in g kg^{-1} on a DM basis. The fermentation profile included analysis of total volatile fatty acids (VFAs), lactic acid, acetic acid, propionic acid, butyric acid, iso-butyric acid, pH, and ammonia-N.

Statistical Analysis

Statistical analyses were conducted using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC) to determine the main effects of inoculant treatment, harvest, day, and their interactions. Repeated measures were conducted using the compounded symmetric covariance structure and multiple mean comparisons were determined using the Tukey-Kramer test. Field, block, and year were considered random effects. Significance of main effects was declared at an alpha level of 0.05 and tendencies were

reported at $0.05 \leq P \leq 0.10$. Mold and yeast counts were analyzed using nonparametric methods, specifically a left-tailed Fischer's exact test with Bonferroni adjustment and significance was declared at $P < 0.05$.

Results and Discussion

Nutritive Value

Forage nutritive value was analyzed at both pre- and post-storage (ca. 120 days post-harvest) for each harvest date to determine changes in nutritive value over time. Forage quality parameters prior to wrapping are presented in Table 5.2. Crop moisture, CP, starch, and calcium (Ca) were similar among all treatments and harvests and were not affected by any interactions. Several parameters were affected by treatment, but these were generally not affected by harvest or an interaction of harvest and treatment. Most notably, NDF, ADF, and TDN were affected by treatment ($P < 0.03$). This is likely the result of happenstance where certain bales where more influenced by large patches of Johnsongrass (*Sorghum halepense* L. Pers.) which was randomly distributed within the harvested fields. This also resulted in minor but significant differences in DM density within the bales, with the CON treatment having a higher mean DM density (211 kg m^{-3}) than the SK treatment (182 kg m^{-3}), and the other treatments' bales being intermediate (Appendix Table A.6).

Post-storage forage nutritive values are presented in Table 5.3. Crop moisture, CP, NDF, ADF, TDN, starch, and calcium (Ca) were similar among all treatments and harvests and were not affected by any interactions. Only ESC was affected by treatment

($P < 0.02$) and it is intriguing that the ESC concentrations in the 11G22 treatment (41.9 g kg^{-1}) was considerably lower than in the other treatments.

Fermentation Characteristics

The effect on length of ensiling on fermentation characteristics is presented in Table 5.4. Fermentation characteristics were not affected by inoculant treatment, but all parameters were affected by the sampling date ($P < 0.01$). Regardless of treatment, pH decreased throughout storage which is reflected through the associated increase in VFA. However, pH did not change until after the 21-day sampling. These results are supported by the findings in several studies, where pH of ensiled forage decreases regardless of treatment with an inoculant (Muck et al., 2010; Arriola et al., 2015; Adesogan et al., 2014). Further, pH was not affected by in interaction of treatment and day, meaning that no inoculant caused pH to drop more rapidly than another.

Total VFA, lactic acid, and acetic acid concentrations increased throughout the entire storage period so that 7-day sampling was least, and 60-day or post-storage samples were greatest, regardless of inoculant treatment or harvest. The Lactic:VFA ratio and lactic and acetic acid concentrations were highest following the 21-day sampling time point, but post-storage and 60-day sampling were not different. This is consistent with conclusions by Muck et al. (2010) who found that acetic acid (and 1, 2-propanediol) are produced more slowly from the conversion of lactic acid in a process that can take as long as 45 to 60 days. Several studies have subsequently confirmed this phenomenon, suggesting that increases in acetic acid concentrations did not begin to increase until 28 d after the crop has been ensiled (Schmidt et al., 2009; Muck et al., 2018), and the current

data confirms this largely occurs at least 21 days after ensiling. Again, the lack of an inoculant treatment by sampling date interaction suggests that all inoculant treatments performed similarly, despite 11G22 containing heterofermentative bacteria, *L. buchneri*.

While fermentation characteristics were affected by storage length, no consistent effect on pH, total VFAs, lactic, or acetic acid in response to treatment was observed during this trial. These results differ from several previous trials (Guo et al., 2013; Adesogan et al., 2014; Arriola et al., 2015; Muck et al., 2018) which observed differences in fermentation parameters with the use of a homofermentative, heterofermentative, or combination product.

Mold and Yeast Sampling

Yeast and mold counts from post-storage sampling are presented in Table 5.5. Mold was not affected by inoculant treatment ($P = 0.34$), and no inoculant reduced the number of bales with countable mold when compared with the untreated control. Treatment appeared to have an effect on the presence of yeast ($P = 0.03$). The control treatment had the greatest number of bales with countable yeast, while bales treated with P11G22, P1174, or SS produced no countable yeast colonies. Arriola et al. (2015) also observed that yeast counts between homofermentative and untreated bermudagrass baleage were not different.

Because of the variability in sample size (i.e. number of bales producing countable qualities), results are not definitive. Additionally, differences in surface mold coverage were observed at bale opening. Therefore, additional research of the treatment influences on mold and yeast coverage should be considered.

Bale temperature

Overall, bale temperature was not affected by inoculant treatment. Temperatures of all bales decreased slightly over the 60-day monitoring period. The greatest change in bale temperature was associated with the ambient temperature and its diurnal cycle, where bales temperatures were elevated in the late afternoon and at their lowest near dawn. At days 7 and 21 post-harvest, inoculant treatment did not significantly affect bale temperature (P = 0.65 and 0.15, respectively). However, at 14-days post-harvest, the untreated water control had the lowest bale temperature of any treatment (P = 0.01), but no temperature differences appeared to be substantively different (i.e., > 1.5 °C).

Conclusions

ADF increased during ensiling while ESCs and starch decreased, indicating the consumption of plant available sugars during fermentation. No other nutritive value parameters were affected by sampling date. Fermentation characteristics of baleage were not affected by inoculant treatment. However, all measured parameters improved as the length of ensiling increased. Further, pH was not affected by a treatment by day interaction, indicating that inoculant treatments decreased forage pH at the same rate, which was not different from the untreated control. Mold and yeast counts were inconclusive, which is consistent with previous research of this type. Although fermentation results were not affected by treatment, sample size and harvest conditions were limiting, warranting additional research into these products before producer recommendations can be developed.

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Table 5.1. Soil pH, phosphorus (mg kg^{-1}), potassium (mg kg^{-1}), calcium (mg kg^{-1}), and magnesium (mg kg^{-1}) from CF, W10, and GY fields taken prior to the initiation of the inoculant trial at the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA and analyzed by the University of Georgia Soil, Plant, and Water laboratory (SPW) in Athens, GA during 2017.

	Field		
	CF	W10	GY
pH (mg kg^{-1})	6.6	6.5	6.8
Phosphorus (mg kg^{-1})	36	35	28
Potassium (mg kg^{-1})	76	55	92
Calcium (mg kg^{-1})	1049	741	1178
Magnesium (mg kg^{-1})	69	43	101

Table 5.2. Pre-storage forage moisture (%) and chemical composition (g kg^{-1}) of forages treated with each microbial inoculant treatment (control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) across both harvests in 2017, as measured by Cumberland Valley Analytical Service laboratory in Waynesboro, PA.

Inoculant	Moisture (%)	Crude Protein	Neutral Detergent Fiber	Acid Detergent Fiber	Total Digestible Nutrients[2]	Starch	Ethanol Soluble Carbohydrates	Calcium
	---------- g kg^{-1} ----------							
Control	45.2	165	535[a]	362[a]	579[b]	20	75	10
Pioneer 11G22	42.1	179	487[b]	347[a]	595[a]	27	74	13
Pioneer 1174	42.1	178	496[a]	345[a]	597[a]	25	75	12
SiloSolve MC	41.8	175	489[a]	343[a]	598[a]	28	79	12
Pioneer 11H50	41.7	182	484[b]	337[b]	604[a]	26	76	12
SiloKing	42.9	175	503[a]	355[a]	585[b]	25	73	12
SEM[1]	2.24	5.23	13.6	4.96	5.75	2.08	2.91	0.69
P-value	0.31	0.13	0.02	0.03	< 0.01	0.11	0.24	0.17

[1]Standard error of means (SEM) calculated at $P < 0.05$ and means within a column without a common superscript differ.
[2]Total digestible nutrients (TDN) = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDF_n x (NDFDp /100)] – 10.

Table 5.3. Post-storage forage moisture (%) and chemical composition (g kg^{-1}) of forages treated with each microbial inoculant treatment (control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) across both harvests in 2017, as measured by Cumberland Valley Analytical Service laboratory in Waynesboro, PA.

Inoculant	Moisture (%)	Crude Protein	Neutral Detergent Fiber	Acid Detergent Fiber	Total Digestible Nutrients[2]	Starch	Ethanol Soluble Carbohydrates	Calcium
					---------- g kg^{-1} ----------			
Control	46.5	172	502	380	589	19.7	48.0[ab]	11.4
Pioneer 11G22	43.6	182	483	375	597	23.2	41.9[b]	12.3
Pioneer 1174	43.2	169	503	371	597	23.8	55.6[a]	11.4
SiloSolve MC	42.6	176	486	367	602	22.6	52.6[a]	12.0
Pioneer 11H50	43.0	178	482	369	599	23.5	53.8[a]	11.9
SiloKing	43.2	173	501	376	592	20.8	51.4[ab]	11.7
SEM[1]	1.64	5.94	5.65	5.33	5.96	2.01	3.53	0.46
P-value	0.24	0.25	0.24	0.18	0.32	0.28	<0.01	0.28

[1]Standard error of means (SEM) calculated at $P < 0.05$ and means within a column without a common superscript differ.
[2]Total digestible nutrients (TDN) = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDF_n x (NDFDp /100)] – 10.

Table 5.4. Fermentation characteristics including pH, total volatile fatty acids (VFA), lactic acid, lactic:VFA ratio, and acetic acid concentrations at 7, 14, 21, and 60-days post-harvest and post-storage samples as measured by Cumberland Valley Analytical Service lab in Waynesboro, PA.

Sample Date	pH	Total VFA (g kg $^{-1}$)	Lactic Acid (g kg $^{-1}$)	Lactic: VFA	Acetic acid (g kg $^{-1}$)
7	5.63^{a}	11.11^{d}	3.92^{c}	32.02^{b}	7.39^{c}
14	5.57^{a}	16.33^{c}	5.87^{b}	34.36^{b}	10.66^{b}
21	5.55^{a}	17.66^{c}	7.13^{b}	38.78^{ab}	10.69^{b}
60	5.3^{b}	21.88^{b}	10.22^{a}	44.47^{a}	11.83^{ab}
Post-Store	5.13^{b}	24.49^{a}	11.52^{a}	45.45^{a}	13.13^{a}
SEM[1]	0.08	1.78	2.02	8.12	0.84
P-Value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1]Standard error of the mean (SEM) calculated at $P < 0.05$ and means within a column without a common superscript differ.

Table 5.5. Number of bales containing countable populations (cfus) of mold and yeast at "post-storage" bale sampling as measured by Cumberland Valley Analytical Services laboratory in Waynesboro, PA.

Treatment	Mold		Yeast	
	No. Bales <1000 cfu	No. Bales[1] 1000+ cfu	No. Bales <1000 cfu	No. Bales[1] 1000+ cfu
Control	5	1	3	3
Pioneer 11G22	3	4	7	0
Pioneer 1174	6	1	7	0
SiloSolve MC	5	2	7	0
Pioneer 11H50	5	2	5	2
SiloKing	7	0	6	1

[1]Number of bales in each treatment (6 per treatment) that produced a countable number (>1000 cfu) of mold or yeast at sampling.

CHAPTER 6

FERULIC ACID ESTERASE-PRODUCING MICROBIAL INOCULANTS IMPACTS ON FERMENTATION, NUTRITIVE VALUE, AND DIGESTIBILITY OF ENSILED ALFALFA AND ALFALFA-BERMUDAGRASS MIXTURES[4]

[4] Hendricks, T.J., D.W. Hancock, J.J. Tucker, F. Maia, and J.M. Lourenco. To be submitted to *Journal of Crop, Forage, and Turfgrass Management.*

Abstract

New silage inoculants contain a bacterial strain that produces ferulic acid esterase (FAE) which may facilitate lignin break down, which may increase the digestibility of the ensiled forage. The objective of this study was to evaluate the efficacy of an FAE-producing microbial inoculant for improving fermentation characteristics, nutritive value, and digestibility of alfalfa or alfalfa-bermudagrass mixtures as silage. This study was conducted at the Coastal Plain Experiment Station in Tifton, GA and the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA on 0.25-acres of previously established 'Bulldog 805' alfalfa (Tifton) and Russell bermudagrass interseeded with 'Bulldog 505' alfalfa (Watkinsville). Forage was harvested twice during the growing season at 10% bloom to simulate differences in lignin content due to growing conditions. Harvested forage was treated with one of three treatments: ferulic acid esterase-producing microbial inoculant (MI+FAE); a heterofermentative microbial forage inoculant (MI); or an untreated water control (CON) before packed into miniature silos to undergo a 60-day fermentation. After fermentation period, forage was analyzed for fermentation characteristics, nutritive value, and digestibility parameters. MI+FAE did not improve fermentation characteristics, nutritive value, or digestibility parameters compared with the MI inoculant, although both MI+FAE and MI generally showed an improvement in fermentation over the control.

Keywords: Silage, Microbial inoculant, ferulic-acid Esterase

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most widely grown crops in the world, grown on 11.8 million acres in the USA alone. However, while harvesting alfalfa at the appropriate maturity is critical, timing is often delayed by weather conditions that do not allow for equipment operation or sufficient wilting time. Since it has been demonstrated that forage maturity is inversely related to forage quality, delaying harvests due to weather conditions can lead to sharp quality declines (Hancock et al., 2014). High concentrations of lignin in alfalfa can decrease fiber digestibility. Harvesting as silage can be used to minimize decreases in nutritive value associated with harvest delays. Further, high quality silage has lower neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) than traditional dry hay (Hancock and Collins, 2006) which can improve palatability and animal performance.

The production of a high-quality silage product is dependent on effective bacterial fermentation. However, fermentation may be depressed in legumes, such as alfalfa, that have lower concentrations of carbohydrates than other common silage crops. To encourage rapid bacterial fermentation, commercially available microbial inoculants may be applied to the crop at harvest. These products often include *Lactobacillus plantarum*, a homofermentative bacteria that rapidly ferments plant available sugars to produce organic acids (e.g. lactic acid). Other products contain both homofermentative and heterofermentative bacteria (e.g. *L. buchneri*) to promote both rapid fermentation and aerobic stability (Muck et al., 2018; Arriola et al., 2015). The use of effective microbial inoculants can decrease the amount of forage lost to poor fermentation or to spoilage, thus reducing forage storage losses and waste.

New microbial inoculant products incorporate a bacterial strain that produces ferulic acid esterase (FAE). This enzyme can break down the ferulic acid linkages in lignin, releasing the hemicellulose-lignin cross-linkages and increasing the surface area of the hemicellulose and cellulose exposed to microbial digestion, thereby increasing forage digestibility (Cornu et al., 1994; Jung et al., 2011). Improving forage quality of silage can improve forage digestibility and animal performance and decrease the need for additional animal supplementation when fed.

Research exploring the efficacy of microbial inoculants containing ferulic acid esterase has been inconclusive thus far. Addah et al. (2011) concluded the use of an FAE-containing microbial inoculant may improve feed efficiency and aerobic stability in feedlot steers. These observations were supported by Aboagye et al. (2015), who saw enhanced animal performance in sheep fed forage treated with an FAE-containing product. However, Lynch et al. (2014) observed that FAE-containing products elicited no positive response on fermentation characteristics or nutritive value, even when combined with additional fibrolytic enzymes. Therefore, the objective of this research is to assess the impact of treatment with an FAE-enhanced microbial inoculant on fermentation characteristics, nutritive value, and dry matter digestibility when applied to alfalfa or an alfalfa-bermudagrass mixture at different harvest times throughout the growing season.

Methods and Materials

Study Sites and Plot Management

This experiment was conducted during the summer of 2018 using previously established 0.2-ha stands of pure-stand of ‘Bulldog 805’ alfalfa (*Medicago sativa* L.;

ALF) located at the Coastal Plains Experiment Station (Tifton, GA) and a mixed stand of 'Bulldog 505' alfalfa and 'Tifton-44' bermudagrass (*Cyondon dactylon* L. Pers.; ABG) located at the J. Phil Campbell Research and Education Center (JPC-REC; Watkinsville, GA). The ALF stand was planted December 2016 using 19-cm row spacing at a rate of 22.4 kg ha^{-1}. The ABG stand had been interseeded with alfalfa in December 2017 using a 35.6-cm row spacing at a seeding rate of 14 kg ha^{-1}.

In both locations, stands were mowed in early May and early July 2018 and forage residue removed in the course of their normal harvest schedule. In early June (8 and 14 June) and early August (7 and 9 August), herbage was harvested from randomly selected areas within the respective fields to a 7.5-cm stubble height when alfalfa reached the early (10%) bloom stage using a flail-type plot harvester (Swift harvester, Swift Machine and Welding, Ltd., Sask., Canada and Gravely harvester, AriensCo, Brillion, WI in Tifton and Watkinsville, respectively) to chop the forage to approximately 2-cm in length. Growth stage determination was estimated based on the procedure from Mueller and Fick (1989).

Soil test results for the ALF and ABG stands in Tifton and Watkinsville, respectively, are presented in Table 6.1. Both the ALF stand in Tifton and ABG stand in Watkinsville were fertilized during March 2018. In Tifton, the ALF stand was fertilized with 121.7 kg K_2O ha^{-1}, 78.5 kg P_2O_5 ha^{-1} (Mono Ammonium Phosphate, 12-61-0, N-P-K, %; Haifa; Haifa North America, Altamonte Spring, FL), and 3.4 kg B ha^{-1} (10% Liquid Solution; CNI Liquid, CNI AgriMinerals, Albany, GA) and in Watkinsville, ABG stand received 112 kg K_2O ha^{-1}, 44.8 kg P_2O_5 ha^{-1} phosphorus (Mono Ammonium Phosphate, 12-61-0, N-P-K, %; Haifa; Haifa North America, Altamonte Spring, FL); 44.8

kg N ha^{-1} as ammonium sulfate, and 3.36 kg B ha^{-1} (10% Liquid Solution; CNI Liquid, CNI AgriMinerals, Albany, GA).

Beginning in March, both locations were scouted weekly for insects, including: alfalfa weevil [(*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)], potato leafhopper [*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae)], three-cornered alfalfa hopper [*Spissistilus festinus* (Say) (Hemiptera: Membracidae)], fall armyworm [*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)], and bermudagrass stem maggot [*Atherigona reversua* (Villaneuve) (Diptera: Muscidae)] (Watkinsville only). In Tifton, lambda cyhalothrin (Lambda-Cy; Nufarm Americas Inc., Burr Ridge, IL) was applied in February 2018 at 34 g a.i. ha^{-1} to control alfalfa weevil. Zeta-cypermethrin (Mustang Maxx; FMC Corporation, Philadelphia, PA) was applied in May and June 2018 at a rate of 28 g a.i. ha^{-1} to control three-cornered alfalfa hopper. Finally, malathion (Malathion 5EC; Drexel Chemical, Memphis, TN) was applied July 2018 to control fall armyworm at 1.4 kg a.i. ha^{-1}. In both locations, zeta-cypermethrin (Mustang Maxx; FMC Corporation, Philadelphia, PA) was applied in July 2018 at a rate of 28 g a.i. ha^{-1} to control three-cornered alfalfa hopper. Pendimethalin (Prowl H2O; BASF Ag Products, Floram Park, NJ) was applied to control annual grass weeds following harvest in June and August at a rate of 1.1 kg a.i. ha^{-1}. No additional applications were made until after the termination of the trial.

Forage Preparation and Application of Inoculant Treatments

Harvested forage was mixed and spread onto a 6-m x 12-m tarpaulin to wilt to approximately 60% moisture. Throughout wilting, forage was mixed by hand twice to

ensure even wilting. Forage moisture was tested every 30 minutes using the microwave moisture method (Ball et al., 2015). When forage reached 58% moisture, a representative sample was collected, immediately weighed, and dried in a forced air oven at 55°C for three days to confirm forage moisture.

A subsample of forage was placed onto one of three additional tarpaulins of the same size, each corresponding to one of three inoculant treatments applied in an aqueous solution in deionized water: 1) a conventional, commercially available non-FAE-producing microbial inoculant (MI), 2) a FAE-producing microbial inoculant (MI+FAE), and 3) a similarly applied quantity of deionized water as a control (CON). The MI treatment was Pioneer 11G22 (Pioneer DuPont, Johnston, IA) to provide 1.1×10^{11} cfu g^{-1} of *L. plantarum* and *L. buchneri*. The MI+FAE treatment was Pioneer 11AFT (pure-stand alfalfa) or Pioneer 11GFT (alfalfa-bermudagrass mixture) in accordance with company recommendations for crop differences and both products provided 1.1×10^{11} cfu g^{-1} and 1.3×10^{11} cfu g^{-1} of *L. plantarum* and *L. buchneri* of the LN4017 strain which produces FAE. These application rates are consistent with the manufacturer recommended rates.

The inoculant treatments were applied using one of three 3.8-L garden sprayers (ISO 14001 Home and Garden Sprayer, Chapin; Batavia, NY) assigned to each treatment that are identical except for the contents. Pre-weighed powdered inoculant was added to 3.8 L of deionized water and thoroughly mixed. All tarpaulins and sprayer tanks were color coded and numbered to correspond with their associated treatments to prevent cross-contamination. Forage was sprayed thoroughly to ensure coverage with the liquid inoculant treatment.

Once treated, the forage was immediately packed into miniature silos for storage so that fermentation may proceed. Miniature silos were constructed from 76.2-cm polyvinyl chloride (PVC) tubing and sealed on each end using rubber end caps. The bottom of the silo was packed with a small layer (~2.5 cm) of the chopped and treated alfalfa, then covered with a small layer of plastic before the mini-silo was filled with treated alfalfa and compacted to a density of 0.20-0.24 kg DM/L (12-15 lbs DM/ft^3) until 3 cm from the top. Forage dry matter densities in each silo were held constant by weighing the same amount of forage into each miniature silo. There were differences in mass, however, between the pure-stand alfalfa and the alfalfa-bermudagrass mixture. After filling, a second layer of plastic was placed on the packed alfalfa. Before the silo was sealed an additional 2.5-cm layer of treated alfalfa was packed into the silo to prevent air leakage. To provide a consistent moisture amount within a block, a complete set of the 3 treatments (CON, MI, and MI+FAE) was treated and packed into the mini-silos before the process was replicated. Thus, the experimental design was a 2 x 3 factorial, with two forage types and three inoculant treatments in a randomized complete block design with five replications.

After packing, silos were kept outdoors in ambient air conditions but under cover. Carbon dioxide was manually released daily from every silo for the first 21 days post-harvest, and silos were monitored, and pressure released as necessary for the duration of the trial.

Forage Sampling and Analysis

After a 60-day fermentation period, the miniature silos were opened, and forage was collected for analysis. The top and bottom 20-cm of each silo was discarded, and samples were obtained from the center ca 30-cm. Forage was placed into a quart-sized bag and immediately frozen. A portion of the sample was sent to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) for nutritive value analysis including: dry matter, moisture, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, starch, and ash and a fermentation profile, including: pH, total volatile fatty acids (VFA), lactic acid, acetic acid, propionic acid, butyric acid, and Ammonia N. Calibration statistics for nutritive value NIR haylage equations were as follows: NDF, SEC = 0.811, R^2 = 0.993; SECV = 0.826; ADF, SEC = 0.770, R^2 = 0.972; SECV = 0.794; CP, SEC = 0.519, R^2 = 0.988; SECV = 0.529; where SEC = standard error of calibration and SECV = standard error of validation, in g kg^{-1} on a DM basis.

Approximately 25 g of each of the samples was freeze-dried (VirTis FreezeMobile 12ES; SP Industries, Warminster, PA) and ground to pass through a 6-mm Wiley Mill screen (Thomas Scientific, Swedesboro, NJ). The 6-mm grind size was selected due to concerns that a 1-mm screen would not detect differences in digestibility between the MI and MI+FAE products (Addah et al., 2010). Following grinding, samples were subjected to in-vitro dry matter digestibility (IVDMD) and ruminal digestion kinetics in the rumen microbiology lab at the University of Georgia. To do this, 0.6-g of each freeze-dried forage sample was weighed into a heat-sealed nylon bag in triplicate (n = 3 for each forage) (F57 Ankom Fiber Filter Bag; Ankom Technology, Macedon, NY) and placed into an *in vitro* fermentation system using mixed ruminal microorganisms

based on the procedure of Callaway et al. (1997). Fiber bags were placed into individual 125-mL serum glass bottles and 100-mL of mixed ruminal media was added to each bottle. Media was comprised of 33% ruminal fluid obtained from dairy steers at the University of Georgia Teaching Dairy (Athens, GA; AUP #: A2018 10-023-Y1-A0) and 67% anoxic media (Cotta and Russell, 1982) maintained at pH 6.5. Fiber bags were fully submerged in the mixed ruminal fluid and gas was released and measured via syringe throughout. Samples were maintained in a water bath (Blue M Constant Temperature Bath, Blue M Electric Company; Blue Island, Illinois) at 39°C for 48 hours. Following a 48-h incubation, samples were removed, placed on ice to halt fermentation, rinsed in deionized water, placed in a forced air oven at 55°C for 48 h, and weighed to determine IVDMD.

Forage analysis to determine NDF and ADF disappearance was conducted using an Ankom Fiber Analyzer (Model A2000, Ankom Technology; Macedon, NY). Additionally, immediately following the 48-h incubation, ruminal fluid was measured for pH (Accument AB150; Fisher Scientific, Waltham, MA) and an aliquot of ruminal fluid was collected for VFA and NH_3 analyses (Callaway et al., 1997). A 0.5-mL ruminal fluid subsample was analyzed for VFA by gas chromatography (Shimadzu GC-2010 Plus; Shimadzu Corp., Kyoto, Japan) using a flame ionization detector and a capillary column (Zebron ZB-FFAP GC Cap. Column 30m x 0.32 mm x 0.25 µ; Phenomenex Inc., Torrance, CA). The column was initially set to 110°C, and gradually increased to 200°C. Injector and detector temperatures were set to 250 and 350°C, respectively (Lourenco et al., 2016). Ammonia nitrogen concentrations were measured using the meta-phosphoric acid-2 ethyl butyrate method as described by Lourenco et al. (2016) using

spectrophotometry at 625 nm (GENESYS 30 Visible Spectrophotometer; ThermoFisher Scientific, Waltham, MA).

Statistical Analysis

The experiment was analyzed using the PROC MIXED model procedure in SAS 9.4 (Cary, NC). Inoculant treatment, harvest time, and their interactions were considered fixed effects within each forage type (pure-stand alfalfa or alfalfa-bermudagrass mixture) and replication was considered the random effect. Mean separation was by Tukey's honest significant difference (HSD) test, with differences considered significant at $P \leq 0.05$ and tendency at $0.05 < P < 0.10$.

Results and Discussion

Environmental Data

Monthly precipitation and average maximum and minimum temperatures during the 2018 growing season and historical climate data from March through August for both study sites were acquired from the University of Georgia's Automated Environmental Monitoring Network (UGA-AEMN, 2018) weather stations located on the University of Georgia Coastal Plain Experiment Station in Tifton, GA and the University of Georgia JPC-REC in Watkinsville, GA (Table 6.2). Monthly average maximum and minimum temperatures were slightly greater in Tifton, GA than in Watkinsville, GA, which is typical for the two locations.

Average maximum temperatures in 2018 were slightly below the 100-year average during March, April, July, and August and slightly above the 100-year average

during May and June at both study sites. During May, the monthly average minimum temperature was above the 100-year average, but otherwise temperatures were comparable. Precipitation in both locations was well above the 100-year average. In Tifton, precipitation was almost double the normal monthly average in May and August. In Watkinsville, precipitation was slightly above average in April and May, and more than double than average in June. Precipitation between the two locations was comparable throughout the study.

Nutritive Value

Chemical composition ALF and ABG forage treatments are presented in Table 6.3. Compositions of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, total digestible nutrients (TDN), ash, and calcium (Ca) were different ($P < 0.01$) between ALF and ABG forages. Concentrations of CP, ADF, lignin, and Ca were higher in ALF than ABG, which is to be expected based on differences in stand composition. Fiber concentrations (e.g. NDF, ADF, and lignin) are directly linked to the digestibility of a forage. Calcium can also affect the fermentation capacity of a forage by providing a buffering capacity. Forages with high Ca are more resistant to pH change, which may make ensiled forages more susceptible to spoilage when exposed to oxygen.

Due to the differences in stand type between ALF and ABG forages (pure-stand alfalfa vs grass-legume mix), ALF and ABG were treated with different strains of the FAE-producing inoculant based on recommendations of the manufacturer. Although the FAE-producing bacterial strain is present in both inoculants, other bacterial species that differ between the two products could inhibit the efficacy of FAE production in one

product or the other (Muck et al., 2018). Therefore, results of this study are presented separately for each forage.

Chemical composition of ALF was affected by the main effect of harvest, but not by the inoculant treatments or their interaction with the other factors. Crude protein, NDF, ADF, lignin, ethanol soluble carbohydrates (ESC), TDN, and ash were affected by harvest ($P < 0.01$), but not inoculant treatment. Crude protein and TDN concentrations were higher in the August harvest than in June while NDF, ADF, and lignin were lower. Pre-ensiling moisture and starch were not affected by inoculant, harvest, or their interaction.

Chemical composition of ABG was affected by the main effects of harvest, inoculant treatment, and their interactions. Pre-ensiling moisture, CP, lignin, ESC, starch, and Ca were all affected by harvest ($P < 0.010$) and NDF, ADF, and TDN had a tendency ($P < 0.1$) to be affected harvest. Crude protein, ADF, lignin, and Ca concentrations were all greater in the June harvest compared with August, which may indicate a greater proportion of alfalfa present in the stand during that harvest.

Ethanol-soluble carbohydrate and starch concentrations were higher ($P < 0.01$) in August-harvested ABG (24.3 and 23.7 g kg^{-1} for ESC and starch, respectively) compared with June-harvested forage (12.9 and 14.7 g kg^{-1}). Because plant available sugars are critical for fermentation, it can be inferred that forages with high levels of plant available sugars have the greatest potential for fermentation. However, since these samples were collected post-ensiling, the greater concentration of ESC and starch in August-ABG suggest that this treatment had the least fermentation occur.

Further, ADF and ESCs of the ABG forage treatment were also influenced by inoculant treatment ($P = 0.04$ and $P < 0.01$ for ADF and ESC, respectively). Acid detergent fiber concentration was higher ($P = 0.04$) in MI than CON, and MI+FAE tended to be higher than CON ($P = 0.09$); MI and MI+FAE were not different (378.6, 389.2, and 387.8 g kg^{-1} for CON, MI, and MI+FAE, respectively).

Additionally, ESC was higher ($P < 0.01$) in CON than either the MI or MI+FAE inoculant (23.1 vs 16.0 vs 16.7 g $kg^{-1}$for CON, MI, and MI+FAE, respectively). Similar to the changes observed in soluble carbohydrates based on forage type, greater ESC post-fermentation suggests MI+FAE and MI- treated forages may have undergone a more extensive degree of fermentation than the untreated control. Guo et al. (2013) observed the same trend, where grass silage treated with a homo- and heterofermentative inoculant combination had lower NSC concentrations following a 60-day ensiling period compared with the untreated forage. Addah et al. (2011) also observed lower WSC and starch in post-fermentation samples treated with an FAE inoculant than in untreated forage, however this comparison was made between untreated forage and an FAE product, therefore no conclusions can be drawn regarding the use of an FAE product and similar combination inoculant.

Fermentation Characteristics

Similarly to nutritive value parameters, fermentation characteristics were analyzed separately by forage treatment to account for possible differences in the MI+FAE formulations used for each forage. Data are presented inoculant treatments in each forage and pooled across harvest in Table 6.4.

Fermentation characteristics of ALF were not affected by harvest, treatment, or their interactions with the exception of propionic acid concentrations. Propionic acid was higher (P = 0.02) in MI+FAE than CON, with MI not different (3.0, 5.0, and 7.2 g kg^{-1} in CON, MI, and MI+FAE, respectively).

The pH and total VFA of ABG were also not influenced by harvest, inoculant, or their interactions, however the concentrations of individual acids assessed were affected. Unlike the ALF forage, propionic acid was higher (P = 0.01) in CON than MI+FAE than CON (0.6 and 0.1 g kg^{-1} for CON and MI+FAE), while being intermediate in the MI treatment (0.3 g kg^{-1}), however the extremely low values of propionic acid make it difficult to draw conclusions about the practical implications. Further, Addah et al. (2011) found no differences in propionic acid between forages treated with or without an FAE-producing microbial inoculant.

Lactic acid of ABG was higher in CON than either MI or MI+FAE (28.5 vs 15.9 vs 17.0 g kg^{-1}). The lactic:VFA ratio was also higher in the CON than MI or MI+FAE (49.8 vs 28.1 vs 27.6). Similar trends were observed by Addah et al. (2011) who found greater lactic acid production in an untreated control compared that treated with an FAE product. Further, Guo et al. (2013) also recorded a decrease in the lactic:VFA ratio in forages treated with a combination of *L. plantarum* and *L. buchneri* compared with forages not treated with an inoculant. Conversely, Lynch et al. (2014) found that FAE-treated forage was higher in lactic:VFA compared with an untreated control.

Inoculant treatments also tended to affect (P = 0.08) the concentrations of acetic acid in ABG. The MI and MI+FAE treatments produced greater acetic acid than the control (40.2 and 41.9 vs. 28.8 g kg^{-1} for MI, MI+FAE, and CON, respectively), and were

not different from one another. It should be noted that although not significant, in the ALF treatment, lactic acid concentration was higher ($P = 0.21$) and acetic acid concentration was lower ($P = 0.11$) in CON (30.0 and 31.36 g kg^{-1} for lactic and acetic acids) than MI (25.6 and 40.5 g kg^{-1}) or MI+FAE (16.6 and 38.4 g kg^{-1}). The high concentrations of acetic acid in the MI and MI+FAE are likely because of the inclusion of the heterofermentative bacteria, *L. buchneri*, which produces high levels of acetic acid (Kung et al., 2003; Adesogan et al., 2014). Because the heterofermentative bacteria use lactic acid as a substrate to produce acetic acid, the *L. buchneri* in both MI and MI+FAE are likely the cause of both the low lactic and elevated acetic acid concentrations in treated forages.

In-Vitro Dry Matter Digestibility and Gas Production

Inoculant treatment did not affect IVDMD, gas production, NDF disappearance, or ADF disappearance of either forage (Tables 6.5 and 6.6). The difference in IVDMD among the three inoculants was less than 3.5% in ALF and less than 2.5% in ABG. Additionally, neither IVDMD or gas production was influenced by harvest or the interaction of treatment and harvest. Aboagye et al. (2015) and Addah et al. (2011) reported improved animal performance and feed efficiency through the use of an FAE-containing product, but they also did not observe a significant improvement in IVDMD. Disappearance of neutral and acid detergent fibers were affected (P = 0.02 and 0.07) by harvest in ALF but not ABG.

Rumen Fluid pH and Volatile Fatty Acid Profile

Inoculant treatment did not affect ruminal pH, total VFA, individual volatile fatty acids that were measured, the acetate:propionate ratio, or ammonia production of ALF (Table 6.5). Harvest influenced acetate concentrations and ammonia production and butyrate was influenced by the interaction of inoculant and harvest. Acetate concentration was higher ($P = 0.05$) in ALF harvested in June compared with August (43.7 vs 40.1 *m*M), but NH_3 production was higher ($P < 0.01$) in August-harvested forage (50.1 vs 47.2 *m*M for August and June, respectively).

None of the response variables in ABG were affected by inoculant treatment, harvest date, or their interaction (Table 6.6). To date, no other studies have looked at the effect of an FAE-producing microbial inoculant on gas production, rumen fluid pH, or VFA or ammonia production. Therefore, additional research to determine the effects of an FAE-producing inoculant on ruminal digestion kinetics should be conducted.

Conclusions

The use of microbial inoculants to improve fermentation and reduce forage losses through spoilage is promising, although research evaluating the use of microbial inoculants that include an FAE-producing bacterial strain have been inconclusive. While some studies have shown a marked improvement in aerobic stability and animal performance from FAE-treated forages, others have not observed any positive changes with the inclusion of FAE-containing products. In this study, pure- and mixed-stand alfalfa harvested at two time points during the growing season did not have an observable change in forage nutritive value, digestibility, or ruminal fatty acid profiles based on

inoculant treatment. Rather, differences were observed based harvest date, indicating forage nutritive value and digestibility are more likely to be improved by harvesting forage at the appropriate maturity. Some changes in the fermentation profile were observed among treatments; however, the FAE-containing inoculants did not perform better than the other microbial inoculant included in the study. Based on our results, the FAE-containing inoculant appears unlikely to improve fermentation, nutritive value, or forage digestibility compared with a similar microbial inoculant product without the capacity for FAE production.

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Table 6.1. Soil pH, phosphorus (mg kg^{-1}), potassium (mg kg^{-1}), calcium (mg kg^{-1}), and magnesium (mg kg^{-1}) from topsoil of pure stand or alfalfa-bermudagrass plots harvested in Tifton and Watkinsville, GA and analyzed by the University of Georgia Soil, Plant, and Water laboratory (SPW) in Athens, GA during 2018.

	Tifton, GA	**Watkinsville, GA**
pH (mg kg^{-1})	6.9	6.7
Phosphorus (mg kg^{-1})	50.4	19.0
Potassium (mg kg^{-1})	53.3	48.5
Calcium (mg kg^{-1})	587	617
Magnesium (mg kg^{-1})	80.6	49.0

Table 6.2. Monthly rainfall (cm) and average maximum and minimum monthly temperature (°C), in comparison to 100-year average from March through November 2016-2018 at the University of Georgia Coastal Plain Experiment Station in Tifton, Georgia and the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA.

	Tifton, GA						Watkinsville, GA					
	Rainfall ---cm---		Avg. Max Temp. ---C---		Avg. Min Temp. ---C---		Rainfall ---cm---		Avg. Max Temp. ---C---		Avg. Min Temp. ---C---	
Month	**2018**	**100-yr avg**	**2018**	**100-yr avg**	**2018**	**100-yr avg**	**2018**	**100-yr avg**	**2018**	**100-yr avg**	**2018**	**100-yr avg**
March	8.6	12.2	19.9	21.2	7.5	8.2	10.8	13.4	16.3	18.2	4.7	4.6
April	7.0	9.9	23.4	25.4	10.9	12.1	14.7	10.3	20.9	23.2	7.7	8.8
May	17.6	8.2	30.0	29.3	18.9	16.5	15.2	10.3	28.3	27.1	17.0	13.5
June	15.0	11.7	32.2	32.0	21.7	20.2	21.3	9.9	31.3	30.6	20.0	17.9
July	14.9	13.8	31.8	32.8	22.4	21.5	9.3	11.4	30.9	32.0	21.0	19.9
August	24.2	12.4	32.4	32.7	22.1	21.3	10.6	10.1	30.8	31.4	20.2	19.5

Table 6.3. Forage moisture (%) and chemical compositions (g kg^{-1}) of pure-stand alfalfa (ALF) and alfalfa-bermudagrass mixture (ABG) harvested in June and August 2018 as measured by commercial laboratory following a 60-day ensile and fermentation period.

	Month	Forage Treatment		SEM[1]	P-Value		
		ALF	ABG		Forage	Harvest	Forage*Harv
Moisture (%)	June	74.0	65.3	2.08	< 0.01	< 0.01	< 0.01
	August	74.0	55.4	2.08			
CP[2] (g kg^{-1})	June	179	150	2.73	< 0.01	0.29	< 0.01
	August	192	141	2.73			
NDF (g kg^{-1})	June	517	592	7.58	< 0.01	< 0.01	< 0.01
	August	464	605	7.58			
ADF (g kg^{-1})	June	448	389	5.81	< 0.01	< 0.01	< 0.01
	August	410	382	5.81			
TDN[3] (g kg^{-1})	June	537	567	4.4	< 0.01	< 0.01	< 0.01
	August	569	572	4.39			
Lignin (g kg^{-1})	June	111	73	2.29	< 0.01	< 0.01	0.04
	August	100	69	2.29			
ESC (g kg^{-1})	June	4.5	13.9	1.73	< 0.01	< 0.01	0.03
	August	10.5	24.3	1.47			
NSC (g kg^{-1})	June	6.6	27.5	2.59	< 0.01	< 0.01	< 0.01
	August	15.5	47.9	2.43			
Starch (g kg^{-1})	June	3.25	14.7	1.37	< 0.01	< 0.01	< 0.01
	August	6.29	23.7	1.34			
Ca (g kg^{-1})	June	15.6	7.7	0.24	< 0.01	< 0.01	0.04
	August	15.2	6.6	0.24			
Ash (g kg^{-1})	June	102	87	1.54	< 0.01	0.16	< 0.01
	August	109	84	1.53			

[1]Standard error of means (SEM) calculated at $P < 0.05$.
[2]Crude Protein (CP) = 6.25 x %N
[3]Total Digestible Nutrients (TDN) = (NFC x 0.98) + (CP x 0.87) + (FA x 0.97 x 2.25) + [NDFn x (NDFDp /100)] – 10.

Table 6.4. Fermentation characteristics of pure-stand alfalfa (ALF) or alfalfa-bermudagrass mixture (ABG) harvested treated with either an untreated control (CON), microbial inoculant (MI), or microbial inoculant containing ferulic-acid esterase (MI+FAE) following a 60-day ensile and fermentation period as measured by commercial laboratory.

Forage		Inoculant Treatment			SEM[1]	P-Value
		CON	MI	MI+FAE		
ALF	**pH**	5.10	5.16	5.29	0.11	0.22
	Total VFA (g kg^{-1})	95.2	100.8	114.7	7.98	0.14
	Lactic Acid (g kg^{-1})	30.0	25.6	16.6	7.32	0.21
	Lactic:Total VFA	30.5	25.7	15.7	7.11	0.19
	Acetic Acid (g kg^{-1})	31.4	40.5	38.4	4.09	0.11
	Propionic Acid (g kg^{-1})	3.0^{b}	5.0^{ab}	7.2^{a}	0.93	0.02
ABG	**pH**	4.74	4.72	4.71	0.06	0.86
	Total VFA	57.3	56.1	58.9	4.92	0.92
	Lactic Acid (g kg^{-1})	28.5^{a}	15.9^{b}	17.0^{b}	3.63	0.02
	Lactic:Total VFA	49.8^{a}	28.1^{b}	27.6^{b}	4.33	< 0.01
	Acetic Acid (g kg^{-1})	28.8^{b}	40.2^{a}	41.9^{b}	3.87	0.08
	Propionic Acid (g kg^{-1})	0.58^{a}	0.34^{ab}	0.16^{b}	0.08	0.01

[1]Standard error of means (SEM) and means without common superscript within the same row are considered different at $P < 0.05$.

Table 6.5. *In-vitro* dry matter digestibility, rumen pH, gas production, acetate, propionate, butyrate, total volatile fatty acids (VFA), the ratio of acetate to propionate (A:P), and ammonia production as measured by gas chromatography at the University of Georgia ruminant nutrition laboratory in ALF treated with either an untreated control (CON), microbial inoculant (MI), or microbial inoculant containing ferulic-acid esterase (MI+FAE) harvested in June and August 2018 following a 60-d ensile and fermentation and subjected to a 48-hr incubation and ruminal fermentation.

	Inoculant					Harvest			
	CON	**MI**	**MI+FAE**	**SEM[1]**	**P-Value**	**June**	**August**	**SEM[1]**	**P-Value**
Digestibility[2] (%)	53.5	51.7	50.0	1.52	0.22	50.7	52.7	1.31	0.22
Ruminal pH	6.61	6.63	6.62	0.01	0.31	6.62	6.62	0.004	0.32
Gas Production (mL g aDMD^{-1})	321	313	313	6.1	0.59	322	309	4.9	0.08
Acetate (*m*M)	41.6	42.0	42.1	1.69	0.97	43.7	40.1	1.46	0.05
Propionate (*m*M)	10.0	9.9	9.7	0.43	0.86	10.3	9.5	0.37	0.09
Butyrate (*m*M)	7.3	7.4	8.0	0.28	0.08	7.5	7.6	0.26	0.92
Total VFAs (*m*M)	64.9	64.9	65.8	2.38	0.93	67.2	63.2	2.06	0.10
A:P	4.1	4.3	4.3	0.06	0.10	4.3	4.2	0.05	0.48
NH_3	48.8	48.1	49.1	0.59	0.46	47.2[b]	50.1[a]	0.48	< 0.01

[1]Standard error of means (SEM) and letters without common superscript within row represent differences at $P < 0.05$.
[2] Digestibility (%) was calculated following a 48-hr incubation and fermentation period

Table 6.6. *In-vitro* dry matter digestibility, rumen pH, gas production, acetate, propionate, butyrate, total volatile fatty acids (VFA), the ratio of acetate to propionate (A:P), and ammonia production as measured by gas chromatography at the University of Georgia ruminant nutrition laboratory in ABG treated with either an untreated control (CON), microbial inoculant (MI), or microbial inoculant containing ferulic-acid esterase (MI+FAE) harvested in June and August 2018 following a 60-d ensile and fermentation and subjected to a 48-hr incubation and ruminal fermentation.

	Inoculant					Harvest			
	CON	**MI**	**MI+FAE**	**SEM[1]**	**P-Value**	**June**	**August**	**SEM[1]**	**P-Value**
Digestibility[2] (%)	45.8	43.7	45.4	1.01	0.34	44.2	45.7	0.81	0.26
Ruminal pH	6.61	6.59	6.60	0.01	0.66	6.60	6.61	0.01	0.50
Gas Production (mL g aDMD^{-1})	362	367	367	7.4	0.84	358	372	6.1	0.11
Acetate (*m*M)	41.4	39.7	42.4	1.78	0.58	42.8	39.5	1.44	0.14
Propionate (*m*M)	10.4	10.1	10.8	0.46	0.62	10.9	9.9	0.38	0.08
Butyrate (*m*M)	7.0	6.7	7.0	0.24	0.50	7.0	6.8	0.20	0.33
Total VFAs (*m*M)	64.3	61.9	65.8	2.56	0.56	66.3	61.7	2.09	0.14
A:P	4.0	3.9	3.9	0.05	0.53	3.9	4.0	0.05	0.30
NH_3	48.1	48.6	49.9	0.70	0.27	48.8	49.0	0.52	0.87

[1]Standard error of means (SEM) and letters without common superscript within row represent differences at $P < 0.05$.
[2] Digestibility (%) was calculated following a 48-hr incubation and fermentation period

CHAPTER 7

CONCLUSIONS AND IMPLICATIONS

One key to optimizing profit in a livestock production system is providing high quality feed for minimum costs. In the Southeast, producers have the advantage of a long growing season that can sustain forage growth for much of the year. While bermudagrass (*Cyondon dactylon*) is grown throughout the region, it often requires additional supplementation to maintain livestock year-round. Incorporating a legume, such as alfalfa (*Medicago sativa*), has been proven to improve the nutritive value above the nutrient requirements of most animal classes.

Even with the long growing season in the southeastern USA, producers often rely on stored forages to provide animals with adequate feed during certain times of the year. However, producing stored forage in this climate can be challenging because of high humidity and frequent summer thunderstorms. For this reason, producers are turning to baleage technology to minimize weather-related risks and improve forage quality. Prior to this study, much of the research involving alfalfa-bermudagrass mixtures was conducted on older varieties that were not specifically adapted to the region. Furthermore, as baleage technology is relatively new, recommendations for management and use are still being refined.

It was observed that the alfalfa-bermudagrass mixture was able to produce greater cumulative yields across the three-year study period. During the establishment year, the ‘Tifton-85’ bermudagrass monoculture outproduced the ‘Tifton-85’ bermudagrass-alfalfa

mixture. However, T85 produced just four harvests each season, while the T85-alfalfa mixture produced seven harvests per season on average. While T85 out-yielded the T85-alfalfa mixture during mid-season harvests, the additional harvests each year led to greater seasonal yields in the mixed stand. Although the yield was not significantly affected by year, the weather between the three years likely played a role in the trends.

In addition to the greater yields, the nutritive value was greater in every harvest of the T85-alfalfa compared with the T85. Quality parameters fluctuated throughout the season based on the relative components of alfalfa, bermudagrass, and weeds. For example, the CP, TDN, and digestibility are greatest in the early spring and fall when alfalfa is the largest stand component. Variations in the weather between the three study seasons may have also played a role in differences. Nutritive value was at its lowest during 2016, when there was extreme drought and a high percentage of weeds. During the second year, weather was ideal for forage production, with adequate and timely rainfall; thus, forage quality was at its highest. Although the third year of stand is generally considered prime production for an alfalfa stand, quality decreased slightly because of frequent rainfall, which led to poor growing conditions and delayed harvests throughout the growing season. Nutritive value parameters were greater in both the T85 monoculture and the T85-alfalfa baleage than normally expected in traditional dry hay production of the same species.

Although the initial nutritive value of baleage is better than that of dry hay, producers are concerned with the ability to store baleage without losing the nutritive value for livestock. Under current recommendations, baleage producers are to feed forage within 9 months, but this is not always practical. In the current study, changes in baleage

nutritive value were minimal in both forage treatments. Bermudagrass-only baleage bales did have fewer decreases in nutritive value than the T85-alfalfa baleage, but this is likely due to species differences. The greater decreases in nutritive value can be attributed to the legume component which underwent a greater degree of protein hydrolysis to reduce CP. A greater proportion of plant available sugars were also devoted to fermentation from the broadleaf legume species compared to the grass baleage, reducing the TDN and digestibility more drastically in this treatment.

Most decreases in nutritive value occurred between harvest and post-fermentation (6-week) sampling; these losses are unavoidable for producers who choose to harvest forage rather than graze it. Further, any additional forage quality losses beyond the 6-week time point were minimal and occurred between 6-weeks and 9-months. Therefore, if producers plan to follow current guidelines and store baleage for 9 months, they should not observe a decline in nutritive value by storing baleage for up to one year. The limitation of a longer storage length in baleage systems is the ability to maintain an anaerobic environment to prevent spoilage of the forage. By increasing the storage potential for baleage, the economic viability of the system is also improved. Therefore, improving the flexibility of the system in which baleage can be profitably used may increase its popularity among producers.

This study has shown that it is possible to store baleage without significant quality losses; however, baleage still must undergo fermentation and be resistant to spoilage during feeding. The key to preventing spoilage is the production of organic acids (e.g. lactic and acetic acids) during fermentation. While forage will naturally undergo fermentation under correct harvest conditions, the process may be improved with the

addition of microbial inoculants. Most of the work evaluating microbial inoculants has been conducted using miniature silos, rather than large round bales on a production scale. This difference in scale may skew results. Trials involving large round bales are limited, and the only published work in the Southeast to this point was conducted on bermudagrass baleage, which has a drastically different fermentation potential than an alfalfa-grass mixture.

In this study, the goal was to evaluate commercially available microbial inoculant products in a production-scale system. Providing livestock and forage producers with unbiased information about the efficacy of these products can have significant impacts on the labor and input costs associated with their operations, ultimately impacted their profitability. In this study, we observed that inoculant treatment had little to no effect on the nutritive value and fermentation characteristics of the alfalfa-bermudagrass baleage. Rather, fermentation characteristics all behaved similarly in response to ensiling, with increases in total volatile fatty acids, lactic acid, the lactic:VFA ratio, and acetic acid, regardless of treatment.

The pH was the only fermentation parameter that was affected by inoculant treatment; it had the greatest decrease and lowest ending pH in the Pioneer 11G22 product, which contains both homo- and heterofermentative bacteria. On the other hand, the SiloKing dry inoculant had the highest pH and was no different from the untreated control. Mold and yeast counts were also inconclusive with regards to treatment. The lack of response to the commercial products was somewhat unexpected based on previous research of this type. Although harvest did not have a statistical effect, changes in harvest conditions between the two sets of bales may have contributed to the fermentation

behavior. Therefore, additional data collection and analysis is warranted before official recommendations for use can be made.

Similar results were observed in the miniature silo research trial evaluating a ferulic acid esterase (FAE)-producing inoculant with a non-FAE product. In this trial, inoculant treatment did have an effect on various fermentation characteristics. The differences observed were between the control and treated forages, not between the FAE and non-FAE producing products. Findings from previous research suggest that the use of an FAE-producing product may improve not only nutritive value parameters, but also animal performance when treated forage is fed to livestock. However, these findings cannot be confirmed by this trial. Again, additional research in this area may be warranted.

In conclusion, results from this study can be used to provide producers with preliminary guidelines for including alfalfa-bermudagrass mixtures and baleage technology into their forage programs. While interseeding alfalfa into a perennial grass is not an option for everyone, producers who have appropriate site conditions and management skills may find alfalfa provides not only increased forage quality, but also additional yield. Future studies should consider the possibility of incorporating other grass species into an alfalfa mixture. When coupled with baleage technology, high quality forages can be stored for year-round use without the concern of quality declines that would warrant additional supplementation and input costs. Despite the previous work regarding microbial inoculants, there have been a limited number of large-scale studies in forage-based systems, and therefore producers should use caution when choosing a product for their operation.

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APPENDIX A

FURTHER EVALUATION OF MICROBIAL INOCULANTS

Introduction

The use of microbial baleage inoculants has the potential to improve both the fermentation and aerobic stability of baleage. However, most inoculant products have been evaluated for use in silage, rather than baleage. In this study, five commercially available inoculants were evaluated for their potential to improve fermentation characteristics and aerobic stability of an alfalfa-bermudagrass mixture when harvested as baleage.

Methods and Materials

Baleage was harvested, treated, and sampled as described in Chapter 5 "Evaluating Five Commercially Available Microbial Inoculants for Improved Fermentation of Alfalfa-bermudagrass Baleage in the Southeast." In 2018, forage was harvested in April and June from the W10 field, producing 12 and 18 bales (28 and 40 total bales), respectively. Due to financial constraints, core samples taken following at-harvest, post-harvest, and post-storage sampling protocols were immediately frozen, divided, and stored for later analysis. Mold samples were taken during both 2017 and 2018 following protocol and immediately sent to Cumberland Valley Analytical Services laboratory (Waynesboro, PA) for analysis.

Feed-out Analysis

In both 2017 and 2018, bales from this trial were stored until forage availability dictated feeding at JPC-REC (approximately a 7-month storage period). Bales were opened weekly between January and March each year and weighed to determine dry matter loss. Additionally, the flat ends of each bale were photographed to monitor mold and yeast growth and observations were recorded. At the termination of the study, each photograph was analyzed using the unsupervised digital image classification tool in ERDAS Imagine (ERDAS Imagine 2016, Hexagon Geospatial; Madison, AL) to determine percent mold coverage.

Preliminary Results

Forage moisture and bale density were monitored during each study harvest and are presented in Table A.1 and differences based on inoculant treatment are reported in Tables A.2 and A.3. Nutritive value and fermentation analyses for 2018 bales have not yet been conducted. Changes in dry matter between harvest and feed out for each harvest are presented in Table A.4. Differences in DM changes occurred between 2017 and 2018 ($P < 0.01$) but were not affected by treatment or harvest. Bales harvested in 2017 averaged a 1.87 kg difference, while 2018 bales gained an average of 15.65 kg. Differences were likely due a combination of excessive rainfall during bale storage and greater forage moisture during 2018.

Yeast and mold counts for both 2017 and 2018 are presented in Table A.5. Yeast counts were not affected by year ($P = 0.32$), harvest ($P = 0.40$), treatment ($P = 0.54$), the interaction of year and treatment ($P = 0.68$), or the interaction of harvest and treatment (P

= 0.55). The number of bales that had countable (>1000 cfus) was greater in 2018, regardless of treatment although the average counts were numerically lower (P = 0.32). Arriola et al. (2015) also observed that yeast counts between homofermentative and untreated bermudagrass baleage were not different.

On the other hand, mold counts had a tendency (P = 0.05) to be affected by year. However, when years were analyzed separately, treatment, harvest, or their interaction did not affect average mold count (P = 0.42, 0.79, and 0.47 vs 0.67, 0.27, and 0.37 for treatment, harvest, and treatment x harvest in 2017 and 2018, respectively). While counts were not statistically different, the average mold counts of treated bales were numerically lower in treated bales compared with control bales. These findings were similar to Ranjit and Kung (2000) who observed numerically lower yeast and mold in forage treated with an *L. plantarum* inoculant. and number of bales with countable colonies was greater in 2017 than 2018, which is opposite of yeast observations.

Counts may have been affected by baleage moisture, which was lowest (P < 0.01) in the harvest 1 bales (354.6 g kg^{-1}) compared with all other harvests; these bales produced the greatest countable yeast, and second highest mold counts. Differences in mold and yeast counts were not detected among treatments, however when bales were unwrapped 7-months post-harvest, differences in surface mold were observed. Digital image analysis of percent mold coverage is ongoing.

Table A.1. Forage moisture (%) at the start of baling and bale density (kg m^{-3}) for each of the four inoculant trial harvests conducted in 2017 and 2018 at the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA.

	Harvest	Moisture (%)	Bale Density (kg m^{-3})
2017	1	39.47	215.77
	2	58.78	170.05
2018	3	50.68	171.43
	4	49.32	153.93

Table A.2. Dry matter density for each inoculant treatment (Control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) and harvest in 2017 and 2018 at the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA.

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Control	220.98	201.41	161.60	150.86
P-11G22	220.52	174.67	175.28	163.86
P-1174	209.32	173.43	183.57	156.29
SiloSolve MC	214.51	167.44	160.45	153.80
P-11H50	217.88	158.89	175.02	154.70
SiloKing	211.43	152.29	172.69	143.99
SEM[1]	6.44	10.58	13.71	7.48
P-Value	0.69	0.02	0.57	0.27

[1]Standard error of means (SEM).

Table A.3. Forage moisture (%) and bale density (kg m^{-3}) of each inoculant treatment in harvest 1 and harvest 2 of 2017 at the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA.

Treatment	Moisture (%)		Bale Density (kg m^{-3})	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Control	35.67	54.53	220.98	201.41
P-11G22	34.92	49.30	220.52	174.67
P-1174	34.63	49.58	209.32	173.43
SiloSolve MC	33.68	49.95	214.51	167.44
P-11H50	35.77	47.58	217.88	158.89
SiloKing	35.15	50.70	211.43	152.29
SEM[1]	1.73	2.36	6.44	10.58
P-Value	0.85	0.40	0.69	0.02

[1]Standard error of means (SEM).

Table A.4. pH, total volatile fatty acids (VFAs), lactic acid (g kg^{-1}), lactic:total VFA ratio, and acetic acid (g kg^{-1}) of each microbial inoculant treatment (Control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) at days 7, 14, 21, and 60-days post-harvest as measured by Cumberland Valley Analytical Services laboratory in Waynesboro, PA.

Time Period	Treatment	pH	Total VFA	Lactic Acid	Lactic:Total	Acetic
Day 7	**Control**	5.87	11.74	4.17	32.59	7.41
	P-11G22	4.98	10.46	3.55	29.17	6.80
	P-1174	5.64	11.43	4.49	36.98	6.96
	SiloSolve MC	5.74	10.95	3.58	34.97	7.16
	P-11H50	5.65	10.96	3.42	33.26	7.35
	SiloKing	5.89	11.15	3.61	25.13	9.01
	SEM[1]	0.22	2.78	1.79	7.40	2.08
	P-Value	< 0.01	0.99	0.57	0.64	0.89
Day 14	**Control**	5.68	17.68	7.06	36.52	11.0
	P-11G22	5.46	14.49	5.25	38.40	9.25
	P-1174	5.48	16.62	6.08	36.34	10.33
	SiloSolve MC	5.58	16.34	5.50	33.09	10.64
	P-11H50	5.52	16.83	5.33	32.55	11.30
	SiloKing	5.69	16.03	4.67	29.26	11.16
	SEM[1]	0.24	2.86	1.98	7.20	2.14
	P-Value	0.83	0.94	1.00	0.85	0.94
Day 21	**Control**	5.67	16.72	7.17	41.42	9.39
	P-11G22	5.33	19.25	7.04	35.47	12.01
	P-1174	5.49	21.12	9.33	42.26	11.59
	SiloSolve MC	5.57	19.74	6.79	36.22	12.75
	P-11H50	5.52	15.38	5.46	39.0	9.72
	SiloKing	5.72	13.74	4.88	38.38	8.67
	SEM[1]	0.21	2.81	1.79	7.45	2.01
	P-Value	0.46	0.06	0.99	0.92	0.26
Day 60	**Control**	5.38	26.20	13.17	47.42	12.87
	P-11G22	5.08	22.28	9.83	46.09	12.24
	P-1174	5.28	21.10	10.13	46.72	10.78
	SiloSolve MC	5.36	20.46	9.0	42.05	11.26
	P-11H50	5.28	20.50	8.63	43.84	11.67
	SiloKing	5.45	20.76	8.42	40.67	12.15
	SEM[1]	0.20	2.71	1.79	7.20	1.94
	P-Value	0.57	0.31	0.99	0.92	0.92

[1]Standard error of means (SEM).

Table A.5. Mean pH, total volatile fatty acids (VFAs), lactic acid (g kg^{-1}), lactic acid:VFA ratio, and acetic acid concentrations of each microbial inoculant (Control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) at 4-months post-harvest ("post-storage") sampling as measured by Cumberland Valley Analytical Services laboratory in Waynesboro, PA.

Treatment	pH	Total VFAs	Lactic	Lactic: VFA	Acetic
Control	5.16	26.37	14.17	51.26	12.04
P-11G22	4.98	27.93	10.88	41.17	16.85
P-1174	5.12	22.64	11.38	50.80	11.06
SiloSolve MC	5.16	23.49	9.92	39.67	13.38
P-11H50	5.08	22.26	10.04	46.17	12.01
SiloKing	5.25	24.23	10.58	43.63	13.45
SEM[1]	0.21	2.71	1.86	7.46	1.94
P-Value	0.86	0.26	0.99	0.50	0.06

[1]Standard error of means (SEM).

Table A.6. Changes in bale weight between harvest and bale feeding (at approximately 7-months post-harvest) for each inoculant treatment (Control, P11G22, P1174, SiloSolve MC, P11H50, and SiloKing) and harvest in 2017 and 2018 at the J. Phil Campbell Research and Extension Center (JPC-REC) in Watkinsville, GA.

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Control	3.38	0.32	11.47	8.29
P-11G22	7.01	-3.85	23.05	9.66
P-1174	3.63	1.03	22.37	12.53
SiloSolve MC	0.59	-1.13	10.05	18.43
P-11H50	4.0	2.39	33.49	13.29
SiloKing	-1.09	1.71	28.72	8.14
SEM[1]	6.05	3.35	11.01	6.06
P-Value	0.81	0.49	0.35	0.55

[1]Standard error of means (SEM) calculated at $P < 0.05$

Table A.7. Forage moisture (%) and number of bales containing countable populations (cfus) of mold and yeast at "post-storage" bale sampling in 2017 and 2018 as measured by Cumberland Valley Analytical Services laboratory in Waynesboro, PA.

Year	Treatment	Moisture	Mold		Yeast	
			No. Bales <1000 cfu	No. Bales 1000+ cfu	No. Bales <1000 cfu	No. Bales 1000+ cfu
2017	Control	46.3	5	1	3	3
	Pioneer 11G22	43.2	3	4	7	0
	Pioneer 1174	43.2	6	1	7	0
	SiloSolve MC	42.6	5	2	7	0
	Pioneer 11H50	42.8	5	2	5	2
	SiloKing	42.4	7	0	6	1
2018	Control	43.5	5	0	1	4
	Pioneer 11G22	44.8	4	1	4	1
	Pioneer 1174	43.8	4	0	1	3
	SiloSolve MC	45.8	5	0	3	2
	Pioneer 11H50	45.4	4	1	3	2
	SiloKing	42.8	4	1	1	2

APPENDIX B

FURTHER EVALUATION OF FERULIC ACID ESTERASE (FAE)-PRODUCING MICROBIAL INOCULANTS

Introduction

New silage inoculants contain a bacterial strain of *L. buchneri* that is capable of producing ferulic acid esterase (FAE) which may facilitate the breakdown of lignin, thus increasing forage digestibility. In this study, a commercially available microbial inoculant containing the FAE-producing strain was evaluated for its ability to improve forage nutritive value, fermentation characteristics, *in-vitro* digestibility, and ruminal kinetics compared with a comparable microbial inoculant and an untreated control.

Methods and Materials

Forage was harvested, treated and sampled as described in Chapter 6 "Ferulic Acid Esterase-Producing Microbial Inoculants Impacts on Fermentation, Nutritive Value, and Digestibility of Ensiled Alfalfa and Alfalfa-bermudagrass Mixtures." Because of manufacturer recommendations, pure-stand alfalfa (ALF) was treated with Pioneer 11AFT while the mixed stand of alfalfa-bermudagrass (ABG) was treated with Pioneer 11GFT. Although the same FAE-producing bacterial strain was present in both inoculants, differences between the two products could inhibit the effectiveness of the FAE-producing strain, necessitating that forage treatments were analyzed separately. Differences observed on the basis of forage type are presented in Tables B.1 and B.2.

Table B.1. Concentrations of pH, total volatile fatty acids (VFA), lactic acid, ratio of lactic acid to total VFAs, and acetic, propionic, and butyric acids as measured by commercial laboratory for alfalfa (ALF) and alfalfa-bermudagrass mixture (ABG) harvested in June and August 2018 following a 60-day ensile and fermentation period.

	Forage Treatment		SEM[1]	P-Value
	ALF	ABG		
pH	5.18	4.72	0.06	< 0.01
Total VFA[2]	104	58	5.18	< 0.01
Lactic Acid[2]	24.1	20.5	3.85	0.36
Lactic:Total VFA	24.0	35.2	4.14	0.01
Acetic Acid[2]	36.7	37.0	3.09	0.94
Propionic Acid[2]	5.1	0.4	0.56	< 0.01
Butyric Acid[2]	33.6	0	5.44	< 0.01

[1]Standard error of means (SEM).
[2] Concentrations of lactic, acetic, propionic, and butyric acids measured in g kg^{-1}.

Table B.2. *In-vitro* dry matter digestibility, rumen pH, gas production, acetate, propionate, butyrate, total volatile fatty acids (VFAs), and the ratio of acetate to propionate (A:P) as measured by gas chromatography at the University of Georgia ruminant nutrition laboratory in forages following a 60-d ensile and fermentation that were harvested in June and August 2018 as well as for alfalfa (ALF) and alfalfa-bermudagrass mixture (ABG) that were subjected to a 48-hr incubation and ruminal fermentation.

	Harvest				Forage Treatment			
	June	**August**	**SEM[1]**	**P-Value**	**ALF**	**ABG**	**SEM[1]**	**P-Value**
Digestibility[2] (%)	47.5	49.2	1.01	0.09	51.7	45.0	1.01	< 0.01
Ruminal pH	6.6	6.61	0.01	0.26	6.62	6.6	0.01	0.02
Gas Production (mL g $aDMD^{-1}$)	340	341	5.47	0.96	316	365	5.47	< 0.01
Acetate (*m*M)	43.2	39.8	1.38	0.02	41.9	41.2	1.38	0.59
Propionate (*m*M)	10.6	9.7	0.35	0.02	9.9	10.4	0.35	0.12
Butyrate (*m*M)	7.29	7.17	0.20	0.55	7.6	6.9	0.20	< 0.01
Total VFA (*m*M)	66.8	62.4	2.0	0.03	65.2	64.0	1.96	0.55
A:P	4.08	4.09	0.04	0.95	4.23	3.94	0.04	< 0.01

[1]Standard error of means (SEM).
[2] Digestibility (%) was calculated following a 48-hr incubation and fermentation period.