

**Evaluation of Plant Growth-Promoting Rhizobacteria on Stockpiled Bermudagrass**

by

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## Abstract

A two-year, small-plot study was conducted to evaluate plant growth-promoting rhizobacteria (PGPR) as an alternative form of N fertilization for fall-stockpiled bermudagrass. Eighteen 1-m<sup>2</sup> Coastal bermudagrass plots were mowed to a 2.5-cm stubble height prior to stockpiling. Experimental treatments included a negative control, synthetic fertilizer, DH44, DH44 + fertilizer, Blend 20, and Blend 20 + fertilizer (n = 3). Two applications of PGPR were applied at the beginning of each stockpiling season in August and again 30 d later. Ammonium sulfate was applied at a rate of 56 kg N ha<sup>-1</sup> concurrent with the first PGPR application. One-third of each plot was clipped to a height of 2.5 cm in mid-November, December, and January of each year to determine forage dry matter (DM) yield and nutritive value. Forage DM yield was greatest for Blend 20 + fertilizer, but it was not different ( $P = 0.2552$ ) from that of the synthetic fertilizer treatment. Concentration of CP was least ( $P \leq 0.0437$ ) for DH44 and Blend 20 treatments. Concentrations of NDF and ADF were similar among all treatments, except for the negative control. *In vitro* true digestibility was not different ( $P < 0.05$ ) among treatments. Yield and nutritive value parameters were greater in Year 2 than Year 1. These results indicate that PGPR are a viable option for biofertilization; however, further investigation into the effect of PGPR inoculants on a larger scale is needed.

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## List of Abbreviations

PGPR	Plant growth-promoting rhizobacteria
ISR	Induced systemic resistance
SA	Salicylic Acid
JA	Jasmonic Acid
ET	Ethylene
DM	Dry matter
ADF	Acid detergent fiber
NDF	Neutral detergent fiber
ADL	Acid detergent lignin
CP	Crude protein
IVTD	In vitro true digestibility
NUE	Nitrogen use efficiency



## I. Literature Review

### BERMUDAGRASS

#### *History and Characteristics*

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a warm-season perennial forage grown in the southeastern United States that is used extensively for pasture and as a harvested forage. It is well adapted to a wide range of soil types and variable rainfall distributions. Most varieties of bermudagrass are used in dual-purpose systems for grazing and hay production (Hill, 2001). Bermudagrass is widely used due to its exceptional agronomic characteristics, including response to N fertilization and tolerance to intensive grazing, drought, and insects. It is high-yielding and can produce 11 to 15,000 kg of dry matter per hectare with good management and ample moisture (Lee, 2017). Bermudagrass growth is initiated 30 to 45 days after the last frost, and biomass production is greatest in the late summer and early fall. Hybrids have been developed to improve characteristics such as productivity and nutritive value (Hill, 2001).

Coastal bermudagrass was the first hybrid developed for forage utilization and was released by the USDA-Agricultural Research Service in cooperation with the University of Georgia Coastal Plain Experiment Station in 1943. It is a F<sub>1</sub> hybrid cross between Tift Common bermudagrass and a South African bermudagrass (Burton, 1948). It is a light green, coarse-stemmed, tall-growing sod-forming grass. Like many hybrid bermudagrass varieties, Coastal does not produce enough viable seed and must be established from vegetative plant material.

Coastal has many advantages compared with Common bermudagrass; it can yield up to twice as much forage biomass, has decreased incidence of weeds, and is more resistant to leaf spot, thereby increasing quality (Burton, 1948). It is also resistant to root-knot nematodes and, when planted in infested soil, it can increase performance of susceptible forages (Ball, 2002). Coastal is more frost-tolerant than Common bermudagrass and has a delayed dormancy, making it a good candidate for fall stockpiling. These improved characteristics of Coastal bermudagrass lead to more extensive efforts to develop adapted bermudagrasses for the region.

### ***Establishment and Management***

Bermudagrass has greater growth rates when air temperatures are above 24°C, and growth declines when temperatures reach 18°C, at which it has with very little growth and is considered dormant (Burton and Hanna, 1995). Warm-season plants store carbohydrates as starch to survive winter dormancy; therefore, planting during dormancy is recommended due to the more optimal soil and growth conditions and greater energy reserves in late winter or early spring (Lee et al., 2017). The typical growth season is during May to September or October, depending on weather and location.

There are many different varieties of bermudagrass that have been developed for improved forage production, nutritive value, and adaptation potential. Bermudagrass is established from stolons, rhizomes, and seeds. Seeded varieties can reproduce through the seed they produce. Hybrid varieties produce very little seed and must be established through vegetative propagation from rhizomes or stolons, called sprigs (Ball, 2002). Commercial sprigging machines are used to plant sprigs into a freshly prepared seedbed. The sprigs should be planted before the rhizomes break dormancy in late February or March (Ball et al., 2015).

Fertility is extremely important in managing bermudagrass; inadequate fertilization is the most common reason for decline in a stand. Excessive defoliation affects productivity of pastures both short and long term. Limiting new stands of Coastal to light grazing or hay production for the first year helps ensure good establishment (USDA-ARS, 2016). Once established, managing animal stocking density to moderately utilize forage increases the longevity of a stand and prevents overgrazing, which leads to weed encroachment (Franzluebbers et al., 2004). Under good management, Coastal will maintain a weed-free sod longer than Common bermudagrass.

### ***Response to Fertilization***

Nitrogen fertilizer is necessary for productive, high-quality forages, and bermudagrass is highly responsive to nitrogen applications. Six forms of nitrogen sources have been evaluated on Coastal, and all forms increased forage production (Burton and Jackson, 1962). For Coastal, a linear relationship between nitrogen fertilization and biomass response was reported up to 600 kg N ha<sup>-1</sup>, and for best quality and yields it is recommended at rates greater than 400 kg N ha<sup>-1</sup>, however application at this rate is costly and may not be economically achievable (Wilkinson and Langdale, 1974). Increases in root yield were also reported with increased fertilization; however, low levels of fertilization (100 kg ha<sup>-1</sup> yr<sup>-1</sup>) met the requirements for normal root growth (Wilkinson and Langdale, 1974). As forages mature, the leaf-to-stem ratio increases and quality declines. Frequency of clipping affected nutritive value and yield more so than fertilization rate (Prine and Burton, 1956). Phosphorus and potassium also play a vital role in production and are recommended at a ratio of 4:1:2 nitrogen to phosphorus and potassium (Jackson et al., 1959).

### ***Stockpiling Management***

Stockpiling is a technique where forages are grazed or mowed at the end of the growing season, prior to dormancy, and are allowed to accumulate for later grazing (Ball et al., 2015). Stockpiled forages are often referred to as deferred grazing or standing hay because it will be used in a time of forage deficit; usually when warm-season grasses are depleted and cool-season grasses are emerging. The purpose of stockpiling is to eliminate the costs of harvesting and feeding hay. Stockpiled forages have been shown to reduce labor and winter-feeding costs by as much as 25% (Lalman et al., 2000).

In a study conducted by Holland et al. (2018), stockpiled Tifton 85 bermudagrass had sufficient nutritive quality to support lactating beef cattle without supplementation, and input costs were 66% greater for feeding hay than stockpiled bermudagrass treated with 56 kg N ha<sup>-1</sup>. Beck et al. (2016) found that forage yield from stockpiled bermudagrass was not different among varying rates of N fertilization across all sampling dates, indicating that application of 56 kg N ha<sup>-1</sup> yielded maximum amounts of DM availability and that fertilization above that rate was not necessary.

The initiation date of the stockpiling period and application of fertilization can influence forage accumulation and quality. In studies by Scarbrough et al. (2004, 2006), Common and Tifton 44 bermudagrass plots in Arkansas were examined for the effects of stockpiling initiation date and N fertilization rate on DM yield and nutritive value. Plots initiated in September yielded 40% less DM than plots initiated in early in August. Dry matter yield increased linearly in response to N fertilization and declined linearly with harvest date. A decline in nutritive value was seen between mid-October and mid-December. Concentrations of NDF and ADF decreased

29 and 10%, respectively, over harvest dates, and stands became more diluted with annual weeds.

Weather events such as frost should be considered when assessing yield and quality because it can truncate the accumulation period. Less growth can be seen in years with cooler fall temperatures and early frost dates, and high levels of precipitation can cause leaching of N in cured forages (Lalman et al., 2000). Stockpile quality is impacted due to exposure to all weather conditions. Sechler et al. (2017) found that the fiber fractions and CP in stockpiled Tifton 85 bermudagrass were more affected by temporal changes during stockpiling period than by increasing N fertilization (Sechler et al., 2017). Forage testing as stockpiled bermudagrass weathers could improve production, and allows for supplementation when necessary.

To increase utilization, stockpiled forages require more intensive management than continuous grazing. Continuous grazing causes extreme forages losses due to trampling, and is the least efficient way to utilize the stockpile. By strip grazing, the utilization can be doubled compared with continuous grazing. When strip grazing a stockpile, allocating an allowance of two to three days of forage for grazing will increase harvest efficiency as opposed to allowing unrestricted access or large areas for grazing at a time (Ball et al., 2015).

## PLANT GROWTH-PROMOTING RHIZOBACTERIA

### ***Background***

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, symbiotic bacteria that colonize the roots and seeds of plants and enhance plant growth (Kloepper and Schroth 1978; Kloepper 1993). Plant roots secrete exudates and metabolites that can be used as nutrients by bacteria (Lutenburg and Kamilova 2009). These nutrients, along with bacteria, are found in

greater densities in the rhizosphere, the layer of soil surrounding the roots (Dimkpa et al., 2009). Once PGPR are established in the roots, the bacteria stimulate growth both above and below ground. Coy et al. (2014) found that, when treated with a variety of PGPR blends developed at Auburn University, there was about a 150% greater root length compared with the non-treated control.

Plant growth-promoting bacteria are extremely versatile, can adapt to a variety of environments, and metabolize a variety of compounds (Bhattacharyya and Jha, 2012). Bacterial populations can be up to 1,000-fold greater in the rhizosphere, and a variety of microcolonies can cover up to 15% of the root surface (van Loon 2007). Because the rhizosphere is such a nutrient-rich environment, there is competition among soil microbes. Colonization is extremely competitive among bacteria, and lack of colonization limits efficiency and the plant's access to nutrients. To positively impact the plant, PGPR must be able to survive inoculation, multiply in the rhizosphere, attach to the root surface, and colonize the root system (Kloepper, 1993).

A biofertilizer is a substance that contains living microorganisms and is applied to the rhizosphere or interior of a plant to promote growth (Vessey, 2003). Only certain microorganisms are beneficial and are used as biofertilizers. For PGPR to be categorized as a biofertilizer, there must be a symbiotic relationship between the bacteria and the plant. This relationship with the host plant is characterized by where and how the bacteria colonize the host plant. There are two modes of action for colonization, entophytic (capable of living on plant surfaces) or endophytic (capable of living within the plant tissue; Vessey, 2003).

### ***Mechanisms of Action***

Increased growth and yield from biofertilization with PGPR is accomplished through both direct and indirect mechanisms. Direct enhancement is characterized by growth promotion in the

absence of plant pathogens and pests. There are many direct mechanisms that stimulate growth, and more than one mechanism can be used in the rhizosphere. Some rhizobacteria can influence the N cycle through nitrification, denitrification, and fixation (Calvo, 2013). Other bacteria decrease plant stress by decreasing ethylene (ET) levels, which are indicators of plant stress (Vessey, 2003). Phytostimulators synthesize phytohormones which stimulate growth.

Rhizoremediators survive on root exudates and degrade pollutants in the soil that could adversely affect plant growth. Certain strains of PGPR produce siderophores that convert iron into a form usable by the plant, whereas other strains solubilize phosphorus, making it available for uptake (Lugtenberg and Kamilova, 2009; Nelson, 2004).

The indirect effects of PGPR are demonstrated when they are able to reduce harmful effects of plant pathogens and pests. Rhizobacteria can suppress the phytopathogens through the production of siderophores that chelate iron, synthesis of anti-fungal metabolites, production of fungal cell wall-lysing enzymes, competition with harmful pathogens for nutrients, and induction of systemic resistance resulting in biocontrol of pathogens (Nelson et al., 2004). Signal interference, predation, and parasitism are other biocontrol mechanisms.

### ***Induced Systemic Resistance***

Induced systemic resistance (ISR) conferred by PGPR is observed as a result of the physiological and biochemical reactions along with the structural changes of the plant cells that produce defensive compounds (van Loon, 2007). These increase the plant's ability to defend itself from diseases, leading to a reduction in the rate of disease development. Plant hormones salicylic acid (SA), jasmonic acid (JA) and ET play a major role in defense signaling pathways and production of these hormones. The hormones produced vary depending on the invading

pathogen or insect (van Oosten et al., 2008). Once a plant's ISR has been triggered, it may remain protected for a considerable part of its lifetime (van Loon, 1998).

Treatment with PGPR may result in rapid structural changes within cell walls due to an increased line of defense through cell thickening, lignification, appositions, or the accumulation of phenolic compounds that act as barriers (Ramamoorthy et al., 2001). Rhizobacteria are antagonists toward pathogens by competing for nutrients, producing antibiotics, and secreting lytic enzymes that are important in the rhizosphere (van Loon, 2007). The effects of beneficial microorganisms on ISR are dependent on microbial species and plant genotype (Pineda et al., 2010). A study conducted by van Oosten et al. (2008) found that ISR was induced in *Arabidopsis* with the use of *Pseudomonas fluorescens*, which negatively affected the development of beet armyworm. Through symbiotic relationships with rhizobacteria, host-plant tolerance to herbivory and enhanced nutrient uptake allow the plant to regrow plant tissue and biomass.

### ***Performance***

Research involving PGPR in forage, pasture, and turfgrass crops is relatively new, and most previous research has been focused on agronomic and horticultural crops such as corn, soybeans, and cotton. Kloepper and Schroth (1978) were the first to use PGPR, and reported positive effects on plant growth in radishes when using specific strains of rhizobacteria. This study, along with studies that have followed, have provided a baseline for screening and selecting PGPR based on their ability to increase root growth and above-ground biomass or total plant weight.

One of the first studies to examine the effects of PGPR on grasses was Baltensperger et al. (1978). This study evaluated *Azospirillum* and *Azobacter* (nitrogen-fixing bacterial strains) on top growth and N content and the response of different genotypes of bermudagrass to



inoculation. The results of this study found that top growth from inoculation caused an increase in total N accumulation; however, there were no differences in root growth and total dry matter production. The research did observe a response among the different genotypes of bermudagrass, which lead to further research with PGPR.

The bacterial inoculants in the Auburn University's Department of Entomology and Plant Pathology collections were selected based on their ability to increase root growth, above-ground biomass, and/or plant total weight (Coy et al., 2014; Fike et al., 2017). Coy et al. (2014) evaluated various bacterial blends to determine their effects on foliar and root growth in Tifway bermudagrass, a turf-type hybrid. Two experiments were conducted, the first using growth chamber conditions, and the second in the greenhouse. The growth chamber experiment evaluated 12 bacterial strains and six blends, including Blend 20. Treatment with PGPR increased shoot weight by 236 to 345% compared with the control. The second experiment in the greenhouse evaluated eight blends which increased top growth weight by 158 to 197% compared with the control. Blend 20 increased root length by 157%, root surface area by 173%, and root volume by 186% compared with the control.

Auburn University's Department of Entomology and Plant Pathology evaluated new PGPR varieties in greenhouse trials for shoot and root biomass in turf-type bermudagrass. A few bacterial strains were noteworthy when compared with the control and Blend 20, and the highest producing strain, DH44, showed the most promise. Compared with a control, the shoot weight was 109% greater and the root weight was 364% greater. Compared with Blend 20, the shoot weight was 9% greater and the root weight was 44% greater (Held, unpublished data).

Research using forage-type bermudagrass hybrids have been conducted by Fike et al. (2017) and Gunter et al. (2018). These studies evaluated the nutritive quality of Coastal

bermudagrass hay treated with Blend 20 or fertilizer at full rate (56 kg N ha<sup>-1</sup>) and half rate (28 kg N ha<sup>-1</sup>). Fike et al. (2017) evaluated concentrations of NDF, ADF, and ADL, and the results showed that fiber fractions were not different among treatments. Gunter et al. (2018) continued this research and evaluated the CP, dry matter digestibility, and nitrogen-use efficiency (NUE). This study found that digestibility was greatest for Blend 20, intermediate for full-rate N, and least for half-rate N; and NUE was greatest for Blend 20, intermediate for half-rate N, and least for full-rate N. Blend 20 did not have any adverse effects on the fiber fractions, increased forage digestibility, and improved NUE compared with N fertilization, which warranted further investigation.

### ***Future Applications***

Fertilizers are essential in modern agriculture to produce high-quality and high-yielding crops; however, environmental concerns have increased the need for more sustainable management strategies. In the last five decades, use of N, P, and K fertilizer has increased. According to the International Fertilizer Association (2019), in 2016, the USA consumed 20.8 million tons of N, P, and K fertilizer. Because fertilizers cannot be eliminated without drastic decreases in production, there is a need for integrated nutrient management that lessens negative environmental impacts of fertilizers (Adesemoye and Kloepper 2009). Interest in biocontrol agents has increased, and it is projected that the market for biostimulants will be \$4.14 billion by 2025 (Grandview Research 2018).

Adesemoye and Kloepper (2009) found that, when PGPR inoculants were applied to tomato plants, fertilizer used reduced to 75% of the typical rate. Ker et al. (2012) concluded that there was a 40% yield increase resulting from PGPR inoculation of switchgrass seeds across a range of soils and environmental conditions. As a biocontrol agent, PGPR can be a viable

alternative; however, the efficiency and interactions with fertilizers have not been well defined in forage management. This project focuses on determining if PGPR is comparable to fertilizer in growth promotion and nutritive value in forage bermudagrass systems.

## II. Evaluation of Plant Growth-Promoting Rhizobacteria on Stockpiled Bermudagrass

### Introduction

Stockpiling is often referred to as deferred grazing or standing hay, because it is allowed to grow and accumulate for later grazing during a time of forage deficit. Stockpiling for fall and winter grazing has the potential to reduce production costs by minimizing the amount of mechanically harvested and purchased feeds (Lalman et al., 2000). Bermudagrass, a warm-season perennial, is widely used in the southeastern United States and is ideally suited for fall stockpiling. There are many advantages of hybrid bermudagrasses, including high biomass potential, drought tolerance, insect tolerance, grazing tolerance, and favorable responses to N fertilization. Coastal bermudagrass is a hybrid variety that grows best in the Coastal Plain and lower Piedmont areas. There is estimated to be approximately 6 million hectares of Coastal bermudagrass in the southern United States (Lee et al., 2017).

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, soil-inhabiting beneficial bacteria that colonize the seeds and roots of plants (Kloepper and Schroth, 1978). These bacteria benefit the host plants through increasing drought tolerance, insect resistance, nutrient uptake, and increasing top and root growth (Vessey, 2003; Nelson, 2004). Additionally, PGPR are antagonists toward pathogens through induced systemic resistance (ISR) by competing

for nutrients, producing antibiotics, and secreting lytic enzymes that are important in the rhizosphere (van Loon, 2003).

Where as most research with PGPR has been conducted in agronomic crops, there are few studies evaluating the effect of PGPR on grasses, specifically forage-type grasses. Coy et al. (2014) evaluated 16 bacterial strains on Tifway bermudagrass and reported increased root and/or top growth with Blends 19, 20, MC 2, and MC 3 (blends developed by Auburn University's Department of Entomology and Plant Pathology) as a result of inoculation. Fike et al. (2017) and Gunter et al. (2018) evaluated the nutritive quality of Coastal bermudagrass hay treated with Blend 20 or fertilizer, and concentrations of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were not different among treatments.

Management practices have been affected by increased cost of N fertilization. There is a need for alternative management practices; PGPR provides ISR for the host plant, similar DM yields, and comparable nutritive quality when compared with N fertilization (Coy et al., 2014; Gunter et al., 2018; Fike et al., 2017). The objective of this study was to evaluate PGPR as an alternative form of N delivery by determining the effects on nutritive value and forage accumulation in stockpiled Coastal bermudagrass.

## Materials and Methods

### ***Research Site and Forage Treatments***

A two-year small-plot study was initiated on an established stand of Coastal bermudagrass [*Cynodon dactylon* (L.) Pres.] located at the Alabama Agricultural Experiment Station Agricultural Land and Resource Management Facility in Auburn, AL. Eighteen 1-m<sup>2</sup> plots were demarcated and mowed on August 18, 2017 and August 13, 2018 (Year 1 and 2, respectively) to a 2.5-cm stubble height prior to stockpiling. Plots (n = 3/treatment) were randomly assigned to treatments that included a control, synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer (Figure 1).

### ***Bacterial Strains and Inoculation Preparation***

Blend 20 contains three bacterial strains (*Bacillus pumilus* AP 7, *Bacillus pumilis* AP 18, and *Bacillus sphaericus* AP 282), and DH44 is a single bacterium (*Paenibacillus sonchi*) that was selected based on the previous demonstration of growth promotion in bermudagrass by the Auburn University Department of Entomology and Plant Pathology (Coy et al., 2014; Held, unpublished data). Bacterial strains were transferred from cryovials maintained at -80°C for long-term storage to plates of tryptic soy agar (TSA). After incubation at 28°C for 48 to 72 h, bacteria were scraped from TSA plates with inoculating loops and transferred to either new TSA plates, or the bacterial growth was collected into plastic centrifuge tubes (50 ml, VWR, Radnor, PA) that contained 40 ml of sterile water, and vigorously shaken to evenly distribute bacterial cells.

Bacterial populations in the suspension were determined by serial 10-fold dilutions of each bacterial suspension into sterile-water blank tubes (20 ml Glass Culturable, VWR, Radnor, PA) to a final dilution of  $10^{-5}$ . Bacterial populations were determined by plating 50  $\mu$ l of the serially diluted bacterial suspensions onto TSA plates, incubating the plates for 24 to 48 h, then counting the number of bacterial colonies that grew on each plate. Once concentrations in the prepared suspensions of each strain were determined, the populations of all strains were used to make a bacterial stock solution. Stock solutions were prepared by the addition of bacterial suspension and distilled water to achieve a final concentration of  $1 \times 10^7$  colony forming units (CFU)  $\text{ml}^{-1}$  of each strain.

### ***Treatment Application***

On August 29, 2017 and September 4, 2018, ammonium sulfate (Profertilizer® 21-0-0, Harrell's Inc., Lakewood, FL) was applied at a rate of 56 kg N  $\text{ha}^{-1}$ , and PGPR was applied at a rate of 500 mL  $\text{m}^{-2}$ . A second PGPR application was applied 30 d later in both years. A plastic backpack sprayer was used to apply the PGPR, and each treatment had its own sprayer. The bacteria, control, and fertilizer treatments were transferred into the root zone by addition of 7.4 L of irrigation water at a rate of 0.7 cm  $\text{m}^{-2}$ .

### ***Forage Harvesting and Laboratory Analysis***

Plots were split into thirds, and each third was harvested using hand clippers on the 15<sup>th</sup> or 16<sup>th</sup> of November, December, and January in Year 1 and Year 2, respectively. Plots were clipped to a 2.5-cm stubble height. Samples of forages were placed into plastic bags and transported to Auburn University's Department of Animal Sciences Ruminant Nutrition Laboratory for laboratory analyses. Samples were weighed and then dried in a 50°C oven for 48 h. Dried, air-equilibrated samples were reweighed and ground to pass a 1-mm screen in a Wiley

Mill (Thomas Scientific, Philadelphia, PA). Forage concentrations of DM were determined according to procedures of AOAC (1990), and concentrations of NDF, ADF and ADL were determined sequentially according to procedures of Van Soest et al. (1991). Concentrations of acid insoluble ash were determined via combustion in a muffle furnace at 500°C (AOAC, 2000). Concentrations of N were analyzed according to the Kjeldhal procedure (AOAC, 1995), and CP concentrations were calculated as  $N \times 6.25$ . Forage concentration of NDF and ADF were determined using an ANKOM 2000® fiber analysis system (Ankom Technology Corporation, Fairport, NY). Forage *in vitro* true digestibility (IVTD) was determined according to the Van Soest (1994) modification of the Tilley and Terry (1963) procedure using the Daisy II® incubator system (Ankom Technology Corporation, Fairport, NY). Ruminal fluid was collected at 0800 h at the Auburn University College of Veterinary Medicine from a cannulated Holstein cow that had free access to bermudagrass hay and was limit-fed a 15% CP supplement consisting of soy hull pellets, corn gluten feed, and whole cottonseed. Ruminal fluid was stored in pre-warmed thermos containers and transported to the Ruminant Nutrition Laboratory, where it was then processed for the batch-culture IVTD procedure.

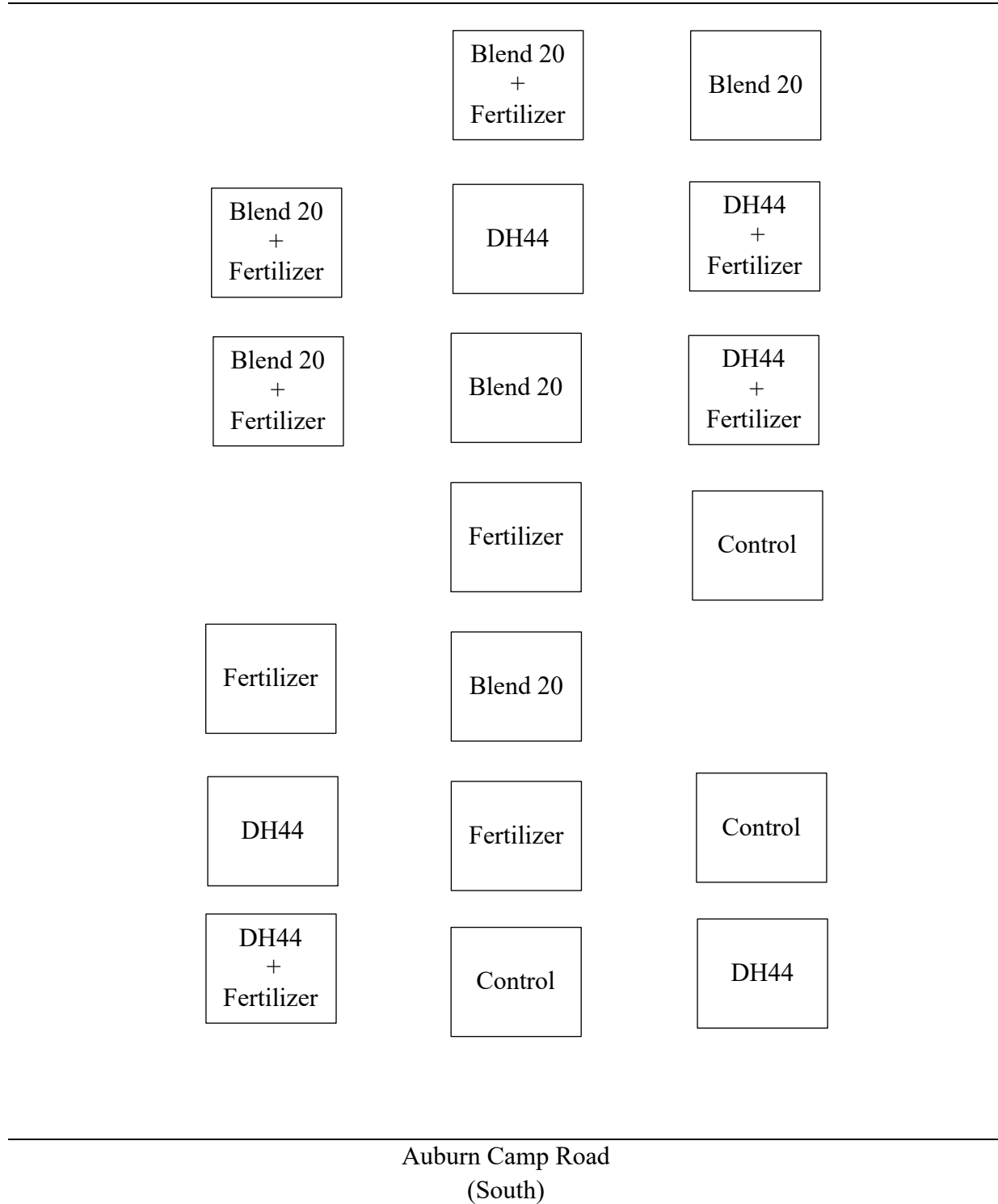
### ***Statistical Analysis***

Data were analyzed by ANOVA, for a completely randomized design, using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). The statistical model included treatment, harvest date, year, year  $\times$  harvest date, year  $\times$  treatment, and treatment  $\times$  harvest date as independent variables, and forage DM yield, and forage concentrations of CP, NDF, ADF, ADL, and percentage of IVTD as dependent variables. Treatment  $\times$  harvest date interactions were not significant for all dependent variables and are not presented. A Fisher-protected least significant



difference ( $\alpha = 0.05$ ) was used to determine significant model effects. The significance level was declared at  $P \leq 0.05$  for all yield and quality parameters.

(North)



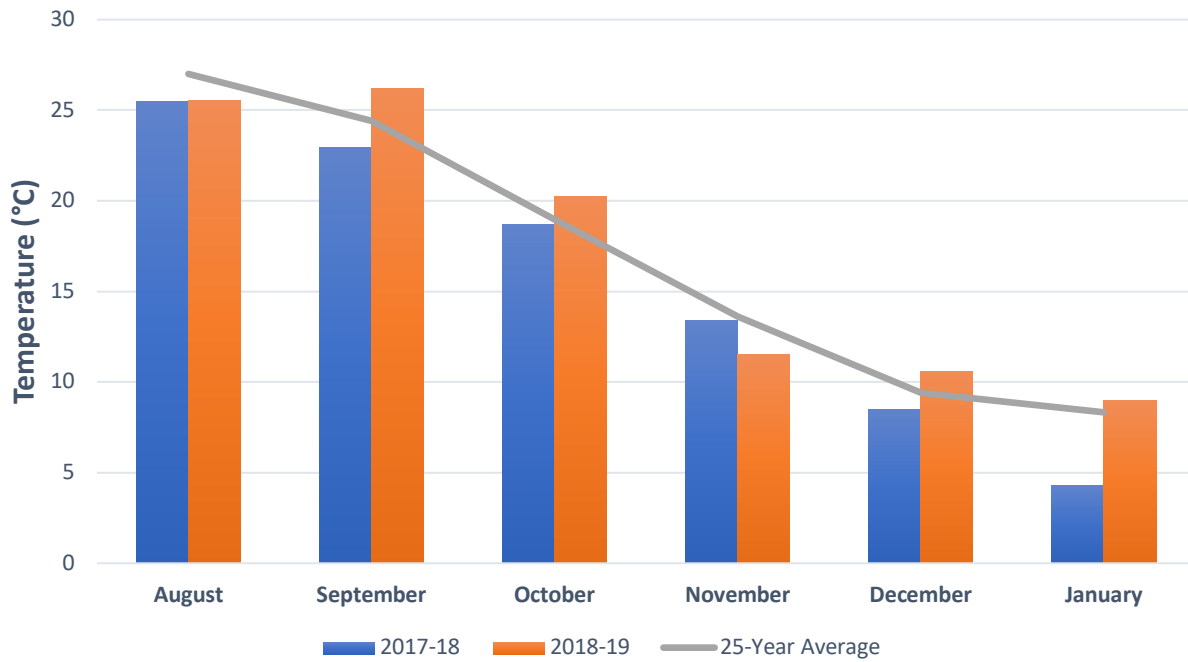
**Figure 1.** Layout of 1-m<sup>2</sup> Coastal bermudagrass plots assigned to treatments of control, synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

## Results and Discussion

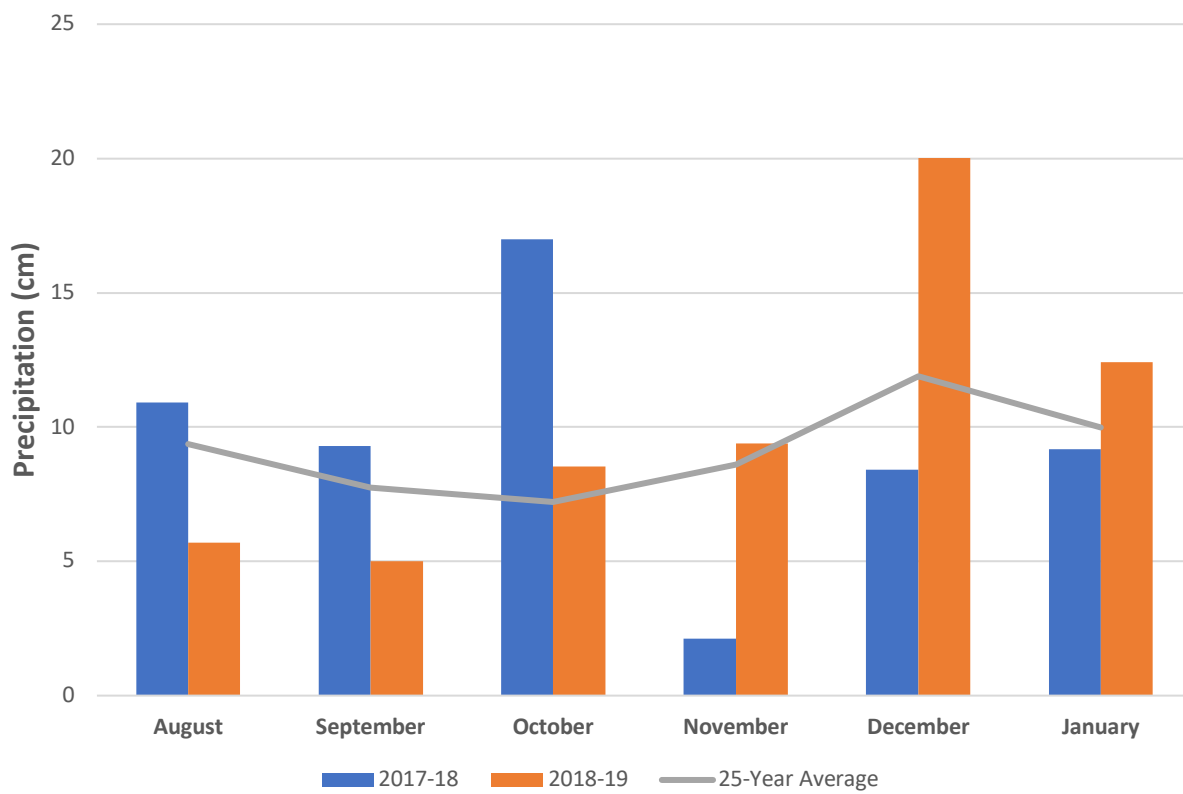
### *Temperature and Precipitation*

During the experimental period, the monthly mean air temperature varied from the 25-year average for Auburn, AL (Figure 2). Mean temperatures in Year 1 were consistently below the 25-year average. During the stockpiling season in 2017 – 2018, temperatures in August and September were 6% below average, whereas in December and January they were 9 and 48% lower, respectively. In 2018 – 2019, temperatures in August and November were 5% and 15% below average, respectively. However, in September, October, December, and January, they were at least 7, 6, 12%, and 8% above average, respectively.

The total monthly precipitation for both years varied from the 25-year average in Auburn, AL (Figure 3). In Year 1, precipitation in August, September, and October was 16, 20, and 135% above average, respectively; whereas in November, December, and January, it was 75, 30, and 9% below average, respectively. In Year 2, a different pattern was observed in which August and September precipitation was 39 and 35% below average, respectively; whereas October, November, December, and January precipitation was 18, 9, 68, and 24% above average, respectively. The timing of precipitation and warmer conditions in Year 2 created favorable responses to N and PGPR application. Year 1 had more precipitation early in the stockpiling season; however, it was colder than the 25-year average, which likely negatively affected forage response to fertilization treatments.



**Figure 2.** Mean monthly temperatures (°C) in Year 1 (2017 – 2018), Year 2 (2018 – 2019), and the 25-year average during the bermudagrass stockpiling season in Auburn, AL.



**Figure 3.** Total monthly precipitation (cm) for Year 1 (2017 – 2018), Year 2 (2018 – 2019), and the 25-year average during the bermudagrass stockpiling season in Auburn, AL.

### ***Dry Matter (DM) Yield***

Mean forage DM yield was affected ( $P \leq 0.05$ ) by treatment and year, and a harvest  $\times$  year interaction was observed. The control and DH44 treatments had the lowest ( $P \leq 0.0371$ ) yields compared with all other treatments. Blend 20 + fertilizer produced greater ( $P = 0.0051$ ) yield than DH44 + synthetic fertilizer; however, it was not different ( $P = 0.2552$ ) from synthetic fertilizer alone. Blend 20 was greater ( $P = 0.0061$ ) than the control, but was not different ( $P = 0.3006$ ) from synthetic fertilizer (Table 1). In Year 1, there was no difference ( $P \geq 0.4001$ ) among harvest dates. In Year 2, December had the lowest ( $P \leq 0.0349$ ) yield; however, November and January were similar ( $P = 0.3511$ ). The second year had 15% greater ( $P = 0.0041$ ) production compared with the first year (Table 2).

Bermudagrass is extremely responsive to N fertilization, and an increase in herbage yield was expected with N application (Ball et al., 2015). All DM yield values in this study were comparable to values that Scarbrough et al. (2004) reported for stockpiled Common and Tifton 44 bermudagrass in Fayetteville, AK; however, they were less than those reported by Holland et al. (2018) for stockpiled Tifton 85 bermudagrass in Headland, AL. Scarbrough et al. (2004) reported values from 1,036 to 2,526 kg ha<sup>-1</sup> and Holland et al. (2018) reported values from 2,800 to 6,600 kg DM ha<sup>-1</sup>. Weather conditions differed between years and likely contributed to greater forage accumulation in Year 2. Hart et al. (1969) concluded that forages in a less mature state had greater deterioration from weathering during winter dormancy. Year 1 had below average temperatures, which could explain the lower DM yield.

The Blend 20 treatment produced greater DM yield than the control, and was similar to synthetic fertilizer. These results differ from Fike et al. (2017), who found that PGPR-treated bermudagrass hay did not differ in biomass production from the untreated control. Adesemoye et

al. (2008) concluded that PGPR could reduce conventional fertilizer use and, because Blend 20 + synthetic fertilizer had the greatest DM yield at a full rate of N application, there could be opportunity to use a combination of PGPR and a reduced rate of N.

**Table 1.** Dry matter yield (kg ha<sup>-1</sup>) from stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	1,272 <sup>a</sup>	1,170	1,375
Synthetic fertilizer	1,768 <sup>bc</sup>	1,617	1,919
Blend 20	1,634 <sup>b</sup>	1,563	1,705
Blend 20 + synthetic fertilizer	1,914 <sup>c</sup>	1,650	2,179
DH44	1,271 <sup>a</sup>	1,377	1,165
DH44 + synthetic fertilizer	1,544 <sup>b</sup>	1,370	1,718
SE	90.5	128.0	128.0

<sup>a,b,c</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).



**Table 2.** Dry matter yield (kg ha<sup>-1</sup>) from stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	1,560	1,392	1,728 <sup>a</sup>
December	1,477	1,500	1,453 <sup>b</sup>
January	1,665	1,482	1,849 <sup>a</sup>
Mean		1,458 <sup>x</sup>	1,677 <sup>y</sup>
SE	64.0	128.0	128.0

<sup>a,b</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).

### ***Crude Protein (CP)***

Mean concentrations of CP were affected ( $P \leq 0.05$ ) by treatment, harvest date and year. The DH44 and Blend 20 treatments were lowest ( $P \leq 0.0437$ ) in CP concentration, whereas the control, fertilizer, and the Blend 20 + synthetic fertilizer treatments were not different ( $P \geq 0.0814$ ). Synthetic fertilizer was greater ( $P = 0.0173$ ) than DH44 + synthetic fertilizer; however, DH44 + synthetic fertilizer were similar ( $P \geq 0.2355$ ) to the control and Blend 20 + synthetic fertilizer treatments (Table 3). Across both years, the December harvest date was lower ( $P = 0.0001$ ) than November and January. A difference between years was also observed, with Year 2 being 18% greater ( $P < 0.0001$ ) in CP concentration than Year 1 (Table 4).

The lower CP concentration in Year 1 than in Year 2 is likely a result of greater precipitation during the early portion of the stockpiling season. Lalman et al. (2000) concluded that highly soluble N in standing forages is more susceptible to leaching during extended periods of high amounts of precipitation. All forage CP values reported in this study were comparable to values reported by Beck et al. (2016) for stockpiled bermudagrass in southwestern AK and Holland et al. (2018) for stockpiled Tifton 85 bermudagrass in Headland, AL. In the study conducted by Beck et al. (2016), CP concentrations for stockpiled bermudagrass harvested in November, December, and January were 125, 105, and 112 g kg<sup>-1</sup>, respectively; whereas Holland et al. (2018) reported CP concentrations in November, December and January of 108, 107, and 95 g kg<sup>-1</sup>, respectively.

**Table 3.** Concentration of crude protein (g kg<sup>-1</sup>, DM basis) in stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	105 <sup>ab</sup>	97	112
Synthetic fertilizer	109 <sup>a</sup>	103	116
Blend 20	92 <sup>c</sup>	84	101
Blend 20 + synthetic fertilizer	103 <sup>ab</sup>	92	113
DH44	90 <sup>c</sup>	81	99
DH44 + synthetic fertilizer	100 <sup>b</sup>	90	110
SE	0.3	0.4	0.4

<sup>a,b,c</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

**Table 4.** Concentration of crude protein (g kg<sup>-1</sup>, DM basis) in stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	103 <sup>a</sup>	95	112
December	93 <sup>b</sup>	86	99
January	103 <sup>a</sup>	93	114
Mean		91 <sup>x</sup>	108 <sup>y</sup>
SE	0.2	0.3	0.3

<sup>a,b</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).

### ***Neutral Detergent Fiber (NDF)***

Mean concentrations of NDF were affected ( $P \leq 0.05$ ) by treatment, harvest date, and year. The control had the lowest NDF value; however, it was not different ( $P = 0.1092$ ) from synthetic fertilizer. The Blend 20, DH44, Blend 20 + synthetic fertilizer, and DH44 + synthetic fertilizer treatments were not different ( $P \geq 0.1087$ ) from synthetic fertilizer (Table 5). A significant ( $P < 0.0001$ ) harvest effect was observed such that the December harvest date was greater ( $P = 0.0002$ ) than November and January. There was a difference between years, with Year 2 having a lower ( $P < 0.0001$ ) NDF concentration (Table 6).

Neutral detergent fiber represents total cell wall constituents in forages and is negatively correlated with forage voluntary intake (Ball et al., 2015). Throughout the stockpiling season, forages mature, which increases fiber content. The NDF values reported in this study were comparable to values by Beck et al. (2016) for stockpiled bermudagrass in southwestern AK and Fike et al. (2017) for Coastal bermudagrass hay treated with fertilizer or PGPR. In the study conducted by Beck et al. (2016), NDF values for stockpiled bermudagrass harvested in November, December, and January were 678, 742, and 765 g kg<sup>-1</sup>, respectively, whereas Fike et al., (2017) reported NDF values that ranged from 737 to 808 g kg<sup>-1</sup>. Furthermore, Mandebvu et al. (1999) reported NDF values to be within 689 (7 weeks) to 655 g kg<sup>-1</sup> (3 weeks) for Coastal bermudagrass.

**Table 5.** Concentration of neutral detergent fiber ( $\text{g kg}^{-1}$ , DM basis) in stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	703.1 <sup>a</sup>	724.4	681.9
Synthetic fertilizer	718.1 <sup>ab</sup>	753.4	682.9
Blend 20	736.0 <sup>b</sup>	764.8	707.2
Blend 20 + synthetic fertilizer	731.0 <sup>b</sup>	765.8	682.9
DH44	730.2 <sup>b</sup>	755.5	704.9
DH44 + synthetic fertilizer	733.1 <sup>b</sup>	750.8	715.4
SE	0.7	0.9	0.9

<sup>a,b</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

**Table 6.** Concentration of neutral detergent fiber (g kg<sup>-1</sup>, DM basis) in stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	719.6 <sup>a</sup>	744.3	695.0
December	745.4 <sup>b</sup>	775.3	715.4
January	710.9 <sup>a</sup>	737.7	684.0
Mean		752.4 <sup>x</sup>	698.1 <sup>y</sup>
SE	0.5	0.7	0.7

<sup>a,b</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).

### ***Acid Detergent Fiber (ADF)***

Mean concentrations of ADF were affected ( $P \leq 0.05$ ) by treatment, harvest date, and year, and there was a harvest  $\times$  year interaction. The control had the lowest ( $P \leq 0.0525$ ) ADF value, whereas all other treatments were not different ( $P \geq 0.1613$ ) (Table 7). The ADF concentration increased ( $P = 0.0060$ ) throughout the season and all harvest dates differed (Table 8). In Year 1, the November harvest had the lowest ( $P \leq 0.0001$ ) ADF concentration, and December and January were not different ( $P = 0.3361$ ). In Year 2, November and January harvest were not different ( $P = 0.9822$ ), and the December harvest was highest ( $P \leq 0.0033$ ). There was also a year effect such that the second year was lower ( $P = 0.0020$ ) in ADF concentration (Table 8) than Year 1.

Acid detergent fiber represents the lignocellulose fraction of the plant cell wall, and is negatively correlated with digestibility (Ball et al., 2015). In this study, forage ADF values were comparable to those of Fike et al. (2017) who reported values that ranged from 315 to 345 g kg<sup>-1</sup> ADF for Coastal bermudagrass hay treated with PGPR or fertilizer. Mandebvu et al. (1999) reported ADF values to be within 269 (7 weeks) to 461 g kg<sup>-1</sup> (2 weeks) for Coastal bermudagrass. Furthermore, Beck et al. (2016) reported ADF values for stockpiled bermudagrass in November, December, and January that were 370, 449, and 463 g kg<sup>-1</sup> for stockpiled bermudagrass, respectively. Scarbrough et al. (2001) reported increases in fibrous fractions (NDF and ADF) from October to December; however, there was less change from December to January.



**Table 7.** Concentration of acid detergent fiber (g kg<sup>-1</sup>, DM basis) in stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	342.8 <sup>a</sup>	343.3	342.3
Synthetic fertilizer	355.9 <sup>b</sup>	364.3	347.5
Blend 20	362.1 <sup>b</sup>	370.6	353.7
Blend 20 + synthetic fertilizer	362.8 <sup>b</sup>	367.8	357.9
DH44	354.5 <sup>b</sup>	356.7	352.2
DH44 + synthetic fertilizer	354.8 <sup>b</sup>	363.1	346.4
SE	0.4	0.6	0.6

<sup>a,b</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

**Table 8.** Concentration of acid detergent fiber ( $\text{g kg}^{-1}$ , DM basis) in stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	343.5 <sup>a</sup>	343.0 <sup>a</sup>	343.9 <sup>a</sup>
December	367.4 <sup>b</sup>	372.8 <sup>b</sup>	362.0 <sup>b</sup>
January	355.6 <sup>c</sup>	367.1 <sup>b</sup>	344.1 <sup>a</sup>
Mean		361.0 <sup>x</sup>	350.0 <sup>y</sup>
SE	0.3	0.4	0.4

<sup>a,b,c</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).

### ***Acid Detergent Lignin (ADL)***

Mean concentrations of ADL were affected ( $P \leq 0.05$ ) by treatment, harvest date, and year, and there was a harvest  $\times$  year interaction. The DH44 was not different ( $P = 0.2152$ ) from DH44 + fertilizer; however, it was less ( $P \leq 0.0446$ ) than all other treatments (Table 9). Concentration of ADL increased ( $P < 0.0001$ ) throughout the stockpiling season. A harvest  $\times$  year interaction was observed such that harvests were different ( $P \leq 0.0236$ ) from each other in Year 1, whereas in Year 2, November and December were not different ( $P = 0.1282$ ) from each other but were less ( $P < 0.0001$ ) than January. There was also a year effect such that forage ADL concentration in the first year was less ( $P = 0.0246$ ) than in the second year (Table 10).

Lignin is a structural and analytical component of ADF and has a negative relationship to digestibility, because highly lignified forage causes a decrease in DM intake due to longer retention time in the rumen and decreased digestion (Ball et al., 2015). In this study, ADL concentration was greater than reported values. Mandebvu et al. (1999) reported values between 43 to 47 g kg<sup>-1</sup> ADL for Coastal bermudagrass hay, whereas Fike et al. (2017) reported values from 40 to 59 g kg<sup>-1</sup> ADL for Coastal bermudagrass hay treated with fertilizer or PGPR. Concentrations of lignin increased considerably throughout the stockpiling season and between year one and year two as a result of weathering and the variation in temperature and precipitation between years.

**Table 9.** Concentration of acid detergent lignin ( $\text{g kg}^{-1}$ , DM basis) in stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	79.7 <sup>a</sup>	75.2	84.1
Synthetic fertilizer	82.6 <sup>a</sup>	81.7	83.6
Blend 20	80.6 <sup>a</sup>	80.0	81.3
Blend 20 + synthetic fertilizer	83.0 <sup>a</sup>	81.0	85.0
DH44	77.0 <sup>ab</sup>	72.4	81.6
DH44 + synthetic fertilizer	73.0 <sup>b</sup>	72.8	73.2
SE	0.2	0.3	0.3

<sup>a,b,c,d</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

**Table 10.** Concentration of acid detergent lignin ( $\text{g kg}^{-1}$ , DM basis) in stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	67.0 <sup>a</sup>	63.8 <sup>a</sup>	70.2 <sup>a</sup>
December	77.6 <sup>b</sup>	80.2 <sup>b</sup>	75.1 <sup>a</sup>
January	93.4 <sup>c</sup>	87.6 <sup>c</sup>	99.1 <sup>b</sup>
Mean		77.2 <sup>x</sup>	81.5 <sup>y</sup>
SE	0.2	0.2	0.2

<sup>a,b,c</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).

### ***In Vitro True Digestibility (IVTD)***

Mean IVTD was affected ( $P \leq 0.05$ ) by harvest date and year, and there was harvest  $\times$  year and treatment  $\times$  year interactions. In Year 1, the control was significantly different ( $P \leq 0.0484$ ) from Blend 20 and Blend 20 + synthetic fertilizer. In Year 2, Blend 20 and Blend 20 + synthetic fertilizer were different ( $P = 0.0025$ ), however they were not different ( $P \geq 0.1006$ ) from the control (Table 11). Harvest date impacted digestibility, and throughout the season the digestibility decreased ( $P = 0.0323$ ). In Year 1, the November harvest differed ( $P < 0.0001$ ) from December and January. In Year 2, the digestibility decreased ( $P \leq 0.0016$ ) throughout the season. The year effect was significant, and year two had a 24% greater ( $P < 0.0001$ ) digestibility than year one (Table 12).

In vitro true digestibility simulates the digestive process in a ruminant animal and gives a good approximation of digestibility in vitro (Ball et al., 2015). The concentrations of IVTD from this study were slightly lower than reported values. Mandebvu et al. (1999) reported IVTD values from 481 to 555 g kg<sup>-1</sup> (48 and 96 h digestion, respectively) in Coastal bermudagrass, and Hill et al. (1997b) reported average IVTD at 488 g kg<sup>-1</sup> in Coastal bermudagrass hay. Mandebvu et al. (1999) observed in situ disappearances and potentially digestible fractions of DM decreased with increased harvest age. Hill et al. (2001) indicated that Coastal bermudagrass has higher concentrations of ether-linked ferulic acid and is the cause of lower digestibility.

**Table 11.** *In vitro* true digestibility (g kg<sup>-1</sup>) in stockpiled Coastal bermudagrass treated with synthetic fertilizer, Blend 20, Blend 20 + synthetic fertilizer, DH44, and DH44 + synthetic fertilizer.

<i>Treatment</i>	<i>Mean</i>	<i>Year</i>	
		1	2
Control	477.2	445.4 <sup>a</sup>	509.0 <sup>ab</sup>
Synthetic fertilizer	475.8	412.6 <sup>ab</sup>	538.9 <sup>b</sup>
Blend 20	438.5	403.1 <sup>b</sup>	474.9 <sup>a</sup>
Blend 20 + synthetic fertilizer	462.2	384.7 <sup>b</sup>	539.8 <sup>b</sup>
DH44	454.5	411.8 <sup>ab</sup>	497.3 <sup>a</sup>
DH44 + synthetic fertilizer	470.2	419.8 <sup>ab</sup>	520.5 <sup>ab</sup>
SE	1.1	1.5	1.5

<sup>a,b</sup> Within a column and a row, means without a common superscript differ ( $P \leq 0.05$ ).

**Table 12.** *In vitro* true digestibility (g kg<sup>-1</sup>) in stockpiled Coastal bermudagrass harvested in November, December, and January.

<i>Harvest</i>	<i>Mean</i>	<i>Year</i>	
		1	2
November	517.5 <sup>a</sup>	460.7 <sup>a</sup>	574.3 <sup>a</sup>
December	447.3 <sup>b</sup>	387.5 <sup>b</sup>	507.2 <sup>b</sup>
January	424.4 <sup>c</sup>	390.5 <sup>b</sup>	458.3 <sup>c</sup>
Mean		412.9 <sup>x</sup>	513.3 <sup>y</sup>
SE	0.7	1.1	1.1

<sup>a,b,c</sup> Within a column, means without a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ ).



## Conclusions

The results of this study indicate that PGPR are a viable option for biofertilization that supports forage DM yield from stockpiled Coastal bermudagrass similar to that from synthetic fertilizer without materially affecting nutritive value. The nutritive value parameters reported from the current study were within ranges reported in literature. Adesemoye et al. (2009) reported that PGPR alone did not substitute completely for synthetic fertilizer; however, it could reduce conventional fertilizer use. Because Blend + synthetic fertilizer had the greatest DM yield compared with other treatments, there could be an opportunity to use a combination of PGPR and a reduced rate of N.

Temperature and precipitation likely contributed to forage performance. Forage accumulation in stockpiling systems is highly dependent on moisture and precipitation, temperature, and available soil N. Year 2 was warmer and wetter throughout the season, which caused a favorable response in forage mass and nutritive value relative to Year 1.

Further investigation into the effect of PGPR inoculants on nutritive quality, DM yield, and ISR are needed on a larger scale. Future research should include evaluating PGPR in a grazing system to determine the effects on animal performance and forage palatability, and work with other bermudagrass hybrids and perennial grasses. Studies should also investigate PGPR + synthetic fertilizer combinations that would reduce the N rate without adversely affecting plant performance.

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