EVALUATING MANAGEMENT STRATEGIES TO IMPROVE FORAGE QUALITY AND ANIMAL PERFORMANCE IN ALFALFA BERMUDAGRASS SYSTEMS IN THE COASTAL PLAINS

by

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(Under the Direction of Jennifer J. Tucker)

ABSTRACT

Interseeding alfalfa (*Medicago sativa*) into bermudagrass (*Cynodon* spp.) can offset nitrogen fertilization requirements, improve forage quality, extend bermudagrass grazing days, and increase economic returns. Previous research demonstrated an optimal harvest management strategy, cut-and-graze, produces high quality stored feed and has potential to fill a regional grazeable forage deficit during the summer to fall transition. The objectives of this research were to evaluate three- and four-year-old alfalfa-bermudagrass (ABG) mixtures under a cut-and-graze harvest management system to: 1) evaluate if preservative and inoculant application would improve ABG baleage nutritive value, 2) evaluate stocker calf and forage performance during the summer to fall forage transition when rotationally grazing two bermudagrass cultivars (Russell and Tifton-85) interseeded with alfalfa as part of a strategic management system, 3) evaluate *in vitro* mixed ruminal microorganisms fermentation parameters when a microbial-based forage preservative was applied at mowing in ABG mixtures harvested as baleage. Forage preservative and inoculant application did not improve forage nutritive value; however, the ABG baleage had over 200 $g \cdot kg^{-1}$ crude protein and 630 $g \cdot kg^{-1}$ total digestible nutrients. ABG mixtures were grazed for 57 and 62 days during the summer to fall forage transition (September to November) and supported stocker calf average daily gains from 0.4 to 1.2 $kg \cdot hd^{-1} \cdot day^{-1}$. Higher overall animal and system performance attained during grazing in 2023 suggests that targeting 28 to 35 days of rest between the previous baleage harvest and grazing initiation would increase forage quality and animal gains. The addition of microbial-based forage preservative at mowing did not improve ruminal fermentation for ABG mixtures when evaluated *in vitro*. Ruminal fermentation parameters primarily differed between forage type (sampled pre-ensiling or post-ensiling). These evaluations demonstrate that ABG mixtures are productive into the third and fourth year after alfalfa establishment. Harvesting ABG as baleage allows producers to harvest their own stored feed resource, but the application of forage preservatives and/or inoculants may not improve nutritive value or ruminal fermentation. Additionally, ABG mixtures, under cut-and-graze management, have potential to fill the summer to fall forage transition with a high-quality grazeable forage.

INDEX WORDS: Alfalfa; Bermudagrass; Grazing; Forage; Southeastern US; Coastal Plains; Legume; Pasture; Cattle; Livestock; alfalfa-bermudagrass mixtures; forage preservative; forage inoculant; ruminal fermentation; baleage;

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DEDICATION

To my Granny Pearl – you've always been one of my biggest cheerleaders! From betting quarters in a rummy card game to just sitting on the back porch, being able to visit you more often has made my time in Georgia sweeter!

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CHAPTER 1

INTRODUCTION

Nearly year-round forage production can be achieved in the Coastal Plains of Georgia (Ball et al., 2015). The forage base in the Coastal Plains is primarily warm season perennial grasses, predominately bermudagrass (*Cynodon* spp.) and bahiagrass (*Paspalum notatum*). While these grasses can be highly productive during the summer months, May to September, forage nutritive value is moderate at best, and alternative feeds must be utilized when warm season grasses are dormant from October to April. Historically, cattleman have recognized these forage deficits that occur in the winter, and the spring and fall forage transition periods (Permanent Pastures in the South and How to Make them Good, 1893). Producers mitigate forage deficits with a variety of practices depending on their operation, including overseeding cool-season annual forages onto dormant bermudagrass stands, stockpiling bermudagrass, harvesting stored forage as hay or baleage to feed later, or purchasing by-product feeds for supplementation (Ball et al., 2015).

Improved, regionally adapted alfalfa (*Medicago sativa*) varieties became available in Georgia in the early 2000's and can be successfully established into bermudagrass pastures (Bouton et al., 1997; Beck et al., 2017; Burt et al., 2022; Rushing et al., 2022; Burt et al., 2024). Incorporation of improved alfalfa varieties into bermudagrass stands offers a new solution for producers to have a perennial legume forage option that can extend the production season of bermudagrass stands, as well as decrease reliance on synthetic nitrogen fertilizer (Beck et al., 2017; Burt et al., 2022; Rushing et al., 2022). Previous research identified that a dual-use cut-and-graze management system could optimize alfalfa-bermudagrass (ABG) use because it offers producers the ability to harvest a stored feed when other high-quality forages for grazing are abundant and allows for grazing during the spring to summer and summer to fall forage transition periods (Burt et al., 2024). Additionally, cutting the mixture as baleage in the summer months increases alfalfa persistence in the stand as compared to grazing in the same time period, which could increase the useful life of the alfalfa in the stand (Burt et al., 2024). The objective of the research included in this dissertation was to further refine the cut and graze management system, while answering production and research questions that have been raised in the southeast.

Specifically, chapter three evaluates the efficacy of preservatives and inoculants in ABG mixtures when harvested as baleage. With continuing climatic challenges, southern producers are increasing their consideration of adoption and use of baleage technology. Producers in the region have also looked at the adoption and use of preservatives and inoculants within their forage production systems. While forage preservatives and inoculants have been extensively studied in cool-season silage production, less information is available for warm-season mixtures preserved as baleage. Chapter 3 attempted to address the efficacy of these technologies and evaluated forage mass, botanical composition, and impact of forage preservatives and inoculants on field dry down time and nutritive value of ABG when harvested as baleage.

Following previous research reported from this lab, Burt et al. (2024) documented the first use of an ABG mixture to fill the summer to fall forage transition via deferred grazing; however, stocker calf average daily gain was less than the one kg day^{-1} gain threshold that was previously identified as the economic optimum for southeastern production systems (Rankins and Prevatt, 2013). Therefore, the hypothesis was developed that economically optimum gains could be achieved by strategically targeting the summer to fall transition with grazing initiation in September instead of October to better utilize the active alfalfa growth rather than the ability to stockpile the mixture. The project discussed in chapter 4 evaluated animal and forage performance when rotationally grazing stocker calves on two bermudagrass cultivars interseeded with alfalfa during the summer to fall transition using a four-day grazing rotation.

Previous work evaluating the ruminal fermentation parameters of alfalfa bermudagrass is limited; therefore, there are gaps in understanding how incorporating alfalfa into southeastern cattle diets could affect ruminal fermentation. Having no strong basis of where to start, the project discussed in chapter 5 is a pilot study evaluating the impact of forage preservatives on ruminal fermentation parameters in vitro for ABG at harvest compared to mini laboratory silos to represent baleage stored for 8 weeks and 6 months post-harvest.

Overall, the work included herein serves as a resource for producers in the Coastal Plains that are interested in incorporating alfalfa into bermudagrass based livestock systems. While this work focuses specifically on beef cattle systems, utilization of ABG mixtures could have applications to other livestock species in the southeast. This work documented forage mass, botanical composition, and forage nutritive value for alfalfa interseeded into two different bermudagrass bases, 'Tifton-85' and 'Russell', which represent common hybrid bermudagrass varieties grown throughout the southeast. Additionally, this work evaluated ABG systems through their fourth production year, whereas previous research documented ABG mixtures in years one to three only. Finally, this research adds evidence for producers and researchers that ABG mixtures can be maintained and continue to produce high-quality forage for both grazing and stored feed in the Coastal Plains when research-based best management practices are followed.

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CHAPTER 2

LITERATURE REVIEW

Introduction

Alfalfa (M*edicago sativa*) is the world's oldest cultivated forage crop (Barnes et al, 1988; Bolton et al., 1972). Alfalfa use precedes recorded history; however, it is theorized that alfalfa originates from the Asian continent (Bolton et al., 1972). Turkish brick tablets from 1400 – 1200 B.C. reference that alfalfa was fed to animals through the winter season, and Greek writers (440-322 B.C.) also referenced alfalfa's history and importance. The earliest recorded presence of alfalfa in Georgia occurred in 1736 and was likely introduced with colonists arriving through the Savannah, GA port (Bolton et al., 1972). Colonists had difficulty establishing alfalfa in the eastern United States, probably due to the acid soils and humid environment; however, from the 1850's to today alfalfa presence is documented in Georgia, with various people promoting its use over time (Crawford, 1854; Burton, 1976; Tucker et al., 2021). Thus, while most alfalfa grown before the 1900's was grown west of the Mississippi river (Bolton et al., 1972), alfalfa seems to have had a presence in Georgia much earlier than most give credit for.

Advocates for forages and alfalfa can be found widely through digitally archived historical newspapers in Georgia (Georgia Historic Newspapers). J. Crawford wrote to the Savannah Georgian in 1854 noting that he had started an alfalfa patch in Blakey, GA and that it was growing well in his garden (Crawford, 1854). The Albany News and Advertiser offered $25 to the person in Southwest Georgia that had the best alfalfa hay yield in the year 1888 ("$25 For the Best Acre of Alfalfa," 1888). Alfalfa was primarily grown in monoculture for hay production in Georgia until the 1950's when G.W. Burton researched alfalfa's ability to grow in mixtures with bermudagrass (*Cynodon spp.*; Burton, 1976). Additionally, many early reporters commented on alfalfa's general inability to perform well under a grazing environment (Crawford, 1854; Smith and Bouton, 1993). Thus, when Dr. Joe Bouton started his career in Georgia, and developed the grazing tolerant alfalfa cultivar "Alfagraze", it was a major milestone to further alfalfa use in Georgia (Bouton et al., 1991; Smith and Bouton, 1993). Together with Dr. Burton's work on breeding improved bermudagrass varieties, and Dr. Bouton's work on breeding improved alfalfa varieties, these forage pioneers increased the likelihood of success when growing grass legume mixtures in the challenging humid southeast environment. While many producers have established improved bermudagrass varieties, there are fewer producers who have established alfalfa in the south (Silva et al., 2021).

Producers utilizing bermudagrass have mainly grown the forage in monoculture because it can tolerate heavy grazing and, prior to the 1950's, nitrogen fertilizer was inexpensive. Bermudagrass can produce high yields when recommended applications of N, P, and K are utilized and there are many pesticide options for producers to utilize for weed and insect management (Baxter et al., 2023). Recently, increasing fertilizer prices- especially since 2020- are making producers evaluate adding legumes to their stands in order to offset or eliminate nitrogen fertilizer costs. While bermudagrass and alfalfa share similar preferences for phosphorus and potassium fertility, as well as preference for well drained soils, alfalfa requires a higher soil pH between 6.5 and 7. This often means investment in lime is necessary for southeastern producers as most soils in the southeast are naturally acidic (Hancock et al., 2015). When alfalfa is incorporated into a grass stand, herbicide options for weed control become very limited, and alfalfa cannot stand the heavy grazing that bermudagrass tolerates. Interseeding alfalfa into existing bermudagrass stands increases crop diversity, which has many benefits over a monoculture grass or legume stand. These benefits include protection against legume root heaving, faster drying time as compared to pure legume mixtures, increased forage quality as compared to pure grass stands, reduced bloat potential as compared to pure legume stands, and reduced grass tetany risk as compared to pure grass stands (Hall and Vough, 2007).

Mixed Alfalfa Bermudagrass Stands

In the 1950's, Dr. G.W. Burton successfully seeded one of the first documented research trials evaluating interseeding legumes, including 'Kansas' alfalfa, into 'Coastal' bermudagrass in Tifton, GA (Burton, 1976). While the stand was successful and Dr. Burton continued his work with alfalfa-bermudagrass (ABG) mixtures, his conclusion from the first experiment was that crimson clover (*Trifolium incarnatum*) was the best legume option for seeding into bermudagrass at that time (Burton, 1976). While there are many benefits to interseeding alfalfa into bermudagrass, there is increased skill and labor involved with utilizing legumes in warm season grass mixtures, and many producers may be unwilling or unable to manage the stand successfully (Burton, 1976). Currently, southeastern producers are hesitant to grow alfalfa due to concerns over establishment cost, stand longevity, and weather conditions (Silva et al., 2021); however, it is an economical consideration to offset nitrogen fertilizer requirements and can help lengthen the growing season (Hendricks et al., 2020; Burt et al., 2022; Rushing et al., 2022). Increasing grazing days in any pasture-based beef production system can decrease the stored forage feeding days, which generally can increase profitability (Ball et al., 2015).

When improved alfalfa cultivars are interseeded into bermudagrass, these mixtures can be utilized for a dual purpose, grazing and stored forage production, in the same year (Hendricks et al., 2020; Burt et al., 2022; Burt et al., 2024a). Bermudagrass generally breaks dormancy in May, is highly productive through August, and enters dormancy by late September in the Coastal Plains. Alfalfa can be harvested as early as March in South Georgia and can continue growing through December as long as air temperature, soil temperature, and precipitation is conducive to growth (Hendricks et al., 2020; Burt et al., 2022; Burt et al., 2024a). Historically, Georgia cattle producers recognized the limitation of the bermudagrass forage production calendar being that the stand only produces forage for 5 to 6 months out of the year (Permanent Pastures in the South and How to Make them Good, 1893). Overseeding annuals in the fall for spring grazing became a common practice in the Coastal Plains (Permanent Pastures in the South and How to Make them Good, 1893; Mullenix and Rouquette Jr., 2018). Annuals generally are not available for grazing until December, at the earliest, and usually are most productive during the spring to summer forage transition from January to May (Mullenix and Rouquette Jr., 2018). Therefore, the summer to fall forage transition period, September to November, is still difficult for producers to fill with a grazeable forage option (Martin et al., 2015). While bermudagrass can be stockpiled for grazing, it is often low-quality forage that needs to be supplemented (Bivens et al., 2017). Feeding stored forages or supplemental feeds during forage deficits is an option, but it costs more to feed calves supplements than if they were grazing high quality forage (Rankins Jr. and Prevatt, 2013). Utilization of alfalfa interseeded into bermudagrass would give producers a perennial forage option that could fill both the summer and fall forage transition periods with a high-quality grazeable forage that would be suitable for cow-calf and/or stocker production.

Managing Alfalfa Bermudagrass Mixtures

Alfalfa's ability to replace N fertilizer in bermudagrass for grazing and baleage was previously evaluated in field scale trials (Beck et al., 2017a,b,c,d; Hendricks et al., 2020; Burt et al., 2022; Burt et al., 2024a). Alfalfa persistence is favored when rotational grazing and rest periods are implemented in ABG mixtures (Beck 2017d; Burt 2022). Rotationally grazing ABG in year one after establishment allowed alfalfa to become established and maintain a larger stand percentage at the end of the first growing season as compared to continuous grazing (Beck 2017d). Additionally, in year two, continuously grazing pastures resulted in alfalfa prevalence declining below the critical 30% threshold. In year three, both rotationally and continuously grazed pastures had less than 30% alfalfa, but rotationally grazed ABG maintained 12% higher alfalfa as compared to the continuously grazed system (Beck 2017d).

Incorporating alfalfa into BG can extend the grazing season and allow for grazing earlier in the spring and later into the fall as compared to bermudagrass monoculture pastures (Beck et al., 2017a, Burt et al., 2022). During the cooler months (April, May, October), ABG mixtures have higher carrying capacity and forage mass as compared to BG monoculture, but there is usually no difference during the summer months as the alfalfa goes into summer dormancy (June, July, August; Beck et al., 2017a,c). ABG mixtures provide higher crude protein throughout the season than bermudagrass monocultures that are not fertilized with N; however, when N fertilizer is supplied to BG, ABG mixtures usually provide similar levels of crude protein to BG fertilized with 112 to 224 kg N ha^{-1} (Beck et al., 2017b, Hendricks et al., 2020, Burt et al., 2022). Hendricks et al. (2020) showed ABG mixtures generally have higher TDN as compared to BG once alfalfa becomes established, but Beck et al. (2017b) showed that BG fertilized with 112 kg N ha^{-1} had higher TDN across the season. While TDN can be a limiting factor to growing cattle, ABG mixtures have produced increased gain as compared to BG monocultures (Cassida et al., 2006, Beck et al., 2017c, Burt et al., 2022).

Hendricks et al. (2020) compared the yield and nutritive value of baleage produced from Tifton-85 bermudagrass fertilized with nitrogen split applied with a seasonal total of 336 N ha^{-1}

yr^{-1} (T85) or as a mixture interseeded with alfalfa (T85+A). Interseeding alfalfa into bermudagrass increased seasonal dry matter forage accumulation by 13,000 kg DM ha^{-1} in the second year as compared to BG fertilized with 84 kg N ha^{-1} after each cutting (Hendricks et al., 2020). Additionally, ABG mixtures were harvested 6 to 8 times from March to November, as compared to bermudagrass which was harvested only 4 times each year (Hendricks et al., 2020). After the establishment year, ABG nutritive value ranges were 180 to 247 g kg^{-1} crude protein and 662 to 726 g kg^{-1} total digestible nutrients. Differences in forage quality were mainly influenced by changing seasonal botanical composition and forage maturity at harvest (Hendricks et al., 2020).

Burt et al. (2022) compared Tifton 85 bermudagrass with no fertilizer (BG), bermudagrass receiving nitrogen fertilizer (BG+N), and bermudagrass interseeded with alfalfa (BG+A) when rotationally grazed by stocker calves. Incorporation of alfalfa into the stand improved nutritive value, maintained yields equivalent to BG+N (90 kg N per year), and increased forage mass compared to BG. Additionally, BG+A had increased seasonal average daily gain, gain per hectare, and stocking rates when compared to BG and BG+N. Enterprise budgets were developed based on the required inputs and expected profit from calf gain for all three treatments. While the cost of alfalfa establishment increased the overall cost of forage production for the BG+A system, the BG+A system had higher estimated revenue due to increased seasonal stocking rate and gain per hectare that the BG+A system supported. Thus, the BG+A system had the highest estimated net return ($ ha^{-1}) as compared to BG or the BG+N system. Additionally, the BG+N system had a negative estimated net return, due to the expense of N fertilizer which did not improve seasonal stocking rate or gain per hectare over the BG system in this study (Burt et al. 2022).

Similar to Burt et al. (2022), Rushing et al. (2022) compared BG, BG+N, and BG+A in Mississippi. This study utilized a common bermudagrass pasture interseeded with Bulldog 505 alfalfa. BG+A pastures had higher forage mass, average daily gain, and gain per hectare as compared to BG or BG+N. Crude protein and total digestible nutrients for BG+A and BG+N were not different; however, both treatments maintained higher nutritive value than BG. Additionally, total production costs for BG+A was higher than either BG treatment, but BG+A also had the highest estimated revenue. In this study, net returns were not significantly different, but were positive for BG+A, while BG+N had a negative net return (Rushing et al., 2022).

Burt et al. (2024a) compared two bermudagrass varieties interseeded with alfalfa under contrasting harvest management strategies. These strategies included the mixture harvested for baleage (Cut), stocker calf grazing (Graze), or a dual use system (Cut and Graze). Alfalfa cultivars that tolerate both grazing and hay management systems have been available since the 1990's (Bouton et al., 1997); however, research comparing management strategies when these improved cultivars are interseeded into hybrid bermudagrass had not been previously documented (Burt et al., 2024a). A dual use system would allow producers the opportunity to cut and preserve forage during part of the growing season and graze the stand during the other parts of the year. The graze only system had the lowest system performance, as measured by total calf weight gain. While the cut only system had the highest predicted gain, the authors concluded that the cut and graze system would be the optimal use of an alfalfa bermudagrass mixture as it allows for both grazing during forage transition periods, and harvesting of stored forage during the summer, when alfalfa needs rest from grazing pressure to remain productive.

Most of the previously mentioned grazing research had seven day rotational grazing periods with 21 days of rest (Beck et al., 2017a, 2017b, 2017c; Burt et al. 2022; Burt et al., 2024a); however, Beck et al. (2017d) utilized a 3-day graze and 21-day rest period. Burt et al. (2022) noted that 21-day rest periods were not sufficient for alfalfa regrowth in their study, which was also complicated by drought conditions. In a report from an agricultural research station in Australia, sheep grazed alfalfa in a 4 or 8 paddock rotation (Peart, 1968). The 8-paddock system had a 5-day grazing period and a 35-day rest period. The 4-paddock system had a 12-day grazing period and a 36-day rest period. The 8-paddock rotation had a 6% alfalfa loss (6.6 to 6.2 plants/m^2) as compared to a 17% loss in the 4-paddock rotation (6.5 to 5.4 plants/m^2). Overall, the author concluded that the 8-paddock rotation allowed sheep to maintain bodyweight better during stress periods with more consistent feed being provided in the five day moves (Peart, 1968).

While grazing ABG mixtures in the spring and summer was evaluated across the southeast, the ability of the mixture to provide grazing during the summer to fall forage transition, is only mentioned by Burt et al. (2024a). In their study, a set stocking rate was used after forage accumulated for 6 to 8 weeks, and temporary fencing was moved to allocate new forage every 2 to 3 days from October to November/December. Animal performance was lower than spring grazing, likely due to mature forage and decreased forage quality (Burt et al., 2024a). It has been suggested that for stockering calves to be economical, calves should gain 1 kg hd^{-1} day^{-1} (Rankins Jr. and Prevatt, 2012). Additionally, it has been documented that in several Alabama experiment station evaluations, stocker calves are fed supplemental feeds for an average of 61 days after purchase in October while cool season annual forages are growing to a grazeable height (Rankins Jr. and Prevatt, 2012). While Burt et al. (2024a) documented that ABG can be deferred graze for 48 to 65 days in the fall, the ADG of stocker calves (0.41-0.60 kg/day) was below the suggested economical threshold of 1 kg day^{-1}. This was most likely due to mature forage that was low in energy (61% TDN) because a hard freeze did not occur to encourage dormancy and successfully stockpile the forage. Thus, winter stockpiling is not a consistent strategy that can be utilized in the Deep South because hard freezes are not guaranteed. Instead, fall grazing of alfalfa-bermudagrass mixtures while alfalfa is in active growth, is worthy of further exploration if the mixtures could provide the gain (1 kg/head/day) and days (60+ days) of grazing needed to bridge the summer to fall forage transition.

Baleage in the Southeast

While many southeastern beef producers utilize hay as their primary stored forage option, there are an increasing number of producers who are harvesting forage as baleage (NASS, 2022, Mullenix, 2023). Utilizing baleage allows producers a shorter cut-to-bale time frame, increases the forage quality produced because the increased moisture at baling allows for higher leaf retention, and the plastic wrapped bales can be stored outside with limited losses from weathering (Coblentz and Akins, 2017; Tucker et al., 2020). Historically, dairy cattleman preserved corn and alfalfa as silage stored in bag, bunker, and tower silos, which requires a dedicated fleet of silage equipment (Coblentz and Akins, 2017). Recent technological advances make baleage more accessible to producers, as it can be made with a specialized round baler, which can handle both dry hay and baleage production. Additionally, increasing options of round bale wrappers from economical singe bale wrappers to more expensive in-line bale wrappers allow producers to select what works best for their operation (Coblentz and Akins, 2017).

Benefits of feeding baleage go beyond the shorter cut to bale time frame, as research in the southeast has demonstrated the potential of baleage to increase cattle' dry matter intake and nutrient digestibility over dry hay (Henson et al., 2024). A metabolism study with fistulated steers at Auburn University reported alfalfa baleage had the highest dry matter intake (8.0 kg hd^{-1}

day $^{-1}$) as compared to ABG baleage (3.7 kg DM hd $^{-1}$ day $^{-1}$) or bermudagrass hay (2.9 kg DM hd^{-1} day $^{-1}$). While ABG baleage was not more digestible than bermudagrass hay in this study, the authors noted that the ABG baleage could have been inadequately fermented. Alfalfa baleage which had undergone adequate fermentation, had increased dry matter digestibility, crude protein digestibility, and fiber digestibility over bermudagrass hay. Additionally, rumen fluid was measured for metabolome diversity (Blinson et al, 2024). Alfalfa baleage had higher alpha diversity as compared to bermudagrass hay. Beta diversity was also different between hay and baleage samples. The authors concluded that baleage preservation can help mitigate losses in high quality forages and increase the gut microbiome diversity of ruminant animals. Future research on ABG baleage best management practices is warranted to increase success of adequate fermentation in ABG baleage, as well as to identify if proper fermentation of ABG mixtures increases the digestibility when compared to ABG hay or baleage.

Currently, it is recommended that baleage be fed within nine months of harvest; however, producers often want to store and feed baleage for longer than nine months (Tucker et al., 2020; Burt et al., 2024b). In a baleage storage study in Tifton, GA, Tifton-85 BG and ABG mixtures were harvested as baleage and tested for nutritive value at 6 weeks (initial), 9 months (recommended), 12 months (maximum), and 24 months (extended) post-harvest. For both BG and ABG baleage, ADF concentrations increased from initial to maximum storage time, while protein and TDN were unchanged. In baleage stored for an extended time, crude protein, digestibility, and TDN decreased, while ADF increased. Thus, the authors concluded that baleage could be stored and fed up to 12 months post-harvest, but an extended storage period, longer than 12 months before feeding, was not recommended.

Silage Additives

Silage additive development by both academic and corporate entities is on-going, and dairy cattleman have been utilizing these for many decades; however, research has not always documented an increase in forage quality (Muck et al., 2018; Kung et al., 2003). As beef producers have started utilizing baleage technology, they are asking University specialists if they should be utilizing silage additives. Research on forage preservatives and inoculants in baleage is more limited, as compared to traditional silage storage methods (Coblentz and Akins, 2017).

Forage Preservatives

Forage preservatives are usually chemical additives that are in an acid or salt form (Muck et al., 2018). The most common organic acids include formic, sorbic, benzoic, propionic, and acetic acids. Common salts include sodium benzoate, potassium sorbate, ammonium propionate, calcium propionate, sodium propionate, and sodium acetate (Muck et al. 2018). Generally, chemical additives are fermentation inhibitors, which restrict microbes that could negatively affect silage fermentation or aerobic stability (Kung et al., 2003). Propionic acid is a widely utilized acid because it is the most effective antimycotic acid of the short chain fatty acids (Woolford, 1975). Unfortunately, it is also corrosive, so most propionic acid included in forage preservative products today include it in a buffered form (Muck et al., 2018; Kung et al., 2003; Woolford, 1975).

Propionic acid can limit heating and improve aerobic stability in dry hay, fermented forages, and high moisture corn in storage because it has fungicidal activity (Britt and Huber, 1976; Stallings et al., 1981; Huber and Soejono, 1976; Kung et al., 2003). Propionic acid's fungicidal activity is pH dependent, as pH decreases from 6.5 to 4.0 the undissociated form increases (Woolford 1975). The undissociated form of acids is fungicidal, whereas the dissociated form is generally not considered to be fungistatic (Lambert & Stratford, 1999). Huber and Soejono (1976) reported that propionic acid effectively stabilized high dry matter (45%) corn silage and decreased silage temperature during fermentation and feed out. Coblentz and Akins (2020) evaluated the use of propionic acid-based preservative, applied at increasing rates at baling, for two moisture concentrations (43.6 and 51.6 %) of an alfalfa-orchardgrass mixed stand harvested as baleage. Initial pH was lower for preservative applied treatments, but differences in water soluble carbohydrates and buffering capacity were not consistently different across treatments. There were no differences in nutritive value of baleage at baling or after 242 days in storage post-harvest, regardless of preservative application rate. Fermentation may have been mildly reduced by application of preservatives in the study, as shown by the overall decrease in total fermentation acids in the preservative treatment; however, the authors reported a tendency for the preservative treatment to have improved lactic to acetic acid ratio. Increased 2,3-butanediol was found in higher concentrations in preservative treated baleage as compared to the control and increased as preservative application increased. Previously, increased 2,3 butanediol was found in increasing concentrations where clostridia is present, when baleage is inoculated with *Lactobacillus buchneri* (*L. buchneri*), and in silages containing alfalfa (Siemerink et al., 2011; Muck and O'Kiely, 1992; Gomes et al., 2019).

Additional research conducted by Coblentz et al. (2021) evaluated the use of propionic acid applied at baling, storage in stretch plastic film, or both on the storage characteristics of alfalfa-orchardgrass forage harvested as high moisture hay (25.8% moisture). This study also found decreased pH and increased buffering capacity of the initial baled forage when preservative was applied. The authors concluded that the use of the stretch film in this study was effective at preventing storage losses and maintaining nutritive value, regardless of preservative treatment before wrapping, and the use of plastic was better than preservative application alone. Additionally, preservative application did not impact baleage nutritive value pre- or post-storage. Due to the low moisture content, the bales experienced limited fermentation, but the preservation of nutrients was most likely due to the exclusion of oxygen.

In a mini silo experiment by Stallings et al. (1981) an alfalfa grass mixture was packed into glass jars with different preservative treatments, including propionic acid. Similar to previous studies, lower pH was documented in propionic acid treated jars. Ammonia concentration was decreased in propionic treatments, which indicates that proteolysis was decreased in haylage treated with propionic acid. Additionally, propionic acid decreased mold growth in the mini silo jars when compared to the control (no preservatives added) at 4, 14, and 56 days of ensiling while being exposed to air. This study demonstrated that adding propionic acid during silo filling was effective at decreasing pH and mold as compared to untreated alfalfa-grass silage.

Buckmaster and Heinrichs (1993) evaluated the use of two different propionic acids applied at baling in alfalfa hay harvested at varying moisture contents (11 to 38%) and its impact on storage losses and nutritive value. The authors found that pure propionic acid helped reduce storage losses for around 60 days, but buffered propionic acid treatments were not effective at reducing storage losses. While harvesting at higher moisture contents usually results in increased leaf retention and overall high-quality hay, in this study the authors found that the increased moisture resulted in more storage loss of digestible nutrients. Overall, regardless of propionic acid treatment, the authors concluded that the potential benefit in harvesting slightly wetter hay is not enough to offset the storage losses that occur.

In addition to utilizing organic acids as forage preservatives, some companies are marketing products that contain fermentation by-products from selected strains of bacteria, such as *Lactobacillus acidophilus* (Moon et al., 1981; Griffin et al., 2018). Limited information is available on these commercially available products; however, two studies evaluating different forms of *Lactobacillus acidophilus* have been conducted with one study noting that inoculation increased lactic acid concentration in corn silage, but not alfalfa silage (Moon et al., 1981). An additional study in Alabama, evaluated Promote® HayDefender™ (Cargill Animal Nutrition; Minneapolis, MN), a *Lactobacillus acidophilus* fermentation product, and noted that dry matter recovery in ryegrass baleage tended to increase with inoculation, but did not improve other fermentation parameters as compared to the control (Griffin et al., 2018). Some southeastern producers had shared with Extension specialists that they were utilizing these products to decrease dry down time in bermudagrass hay production, but to date, replicated research trials to confirm this claim have not been published (Stefancik et al., 2024).

Forage Inoculants

Silage inoculants have been researched and marketed in the United States for decades. Silage inoculants can be broken down into different groups. Muck et al. (1991) identified four different silage inoculant groups: homofermentative lactic acid bacteria (LAB), obligate heterofermentative LAB, combination inoculants containing obligate heterofermentative LAB plus homofermentative or facultative heterofermentative LAB, and other non-LAB species inoculants. Homofermentative LAB are the oldest and most commonly used LAB inoculants for silage; however many of these species have been reclassified as facultative heterofermentative LAB species. Facultative heterofermentative bacteria can ferment pentoses to produce lactic and acetic acid, whereas obligate homofermentative species can only utilize glucose and produce mainly lactic acid. Combination inoculants can utilize facultative and obligate LAB to combine the benefits of each species to improve silage fermentation. Facultative heterofermentative LAB increase the initial pH decline during the early stages of silage fermentation, and the obligate heterofermentative species slowly converting lactic acids to acetic acids, which can improve aerobic stability (Muck et al., 1991). Research on various ensiled forages have shown that combination inoculants containing *L. buchneri* and *Pediococcus pentosaceus* (*P. pentosaceus*) can help silage have a rapid decline in pH, as well as increased aerobic stability (Driehuis et al., 2001; Arriola et al., 2015; Reich and Kung, 2010).

In a meta-analysis of silages treated with *L. buchneri*, Kleinschmit and Kung (2006) found that in corn silage, inclusion of *L. buchneri* had greater pH, lower lactic acid, higher acetic acid, and lower DM recovery as compared to untreated corn silages. While lower DM recovery and lactic acid concentrations are not desirable, the aerobic stability corn silage treated with > 100,000 CFU *L. buchneri*/g of fresh forage was almost 500 hours as compared to 25 hours for the untreated control. Similar findings were reported for small grain and grass silage in this review. Higher levels of propionic acid production were reported for small grain and grass silages than corn silage in this review, and both low and high levels of *L. buchneri* inoculation increased propionic acid production. Aerobic stability was higher for the untreated grass and small grain silage as compared to the untreated corn silage control; however, inoculation still improved the aerobic stability by almost 40 hours for the high level of inoculation over the control (Kleinschmit and Kung, 2006).

Driehuis et al. (2001) compared the use of *L. buchneri, L. buchneri* plus *P. pentosaceus* and *L. plantarum, P. pentosaceus* and *L. plantarum*, or no inoculant in farm scale silos and laboratory silos using cool season grasses. Treatments that contained *P. pentosaceus* and L.

plantarum had a faster pH decline and lower final pH than the other treatments. The addition of *L. buchneri* with *P.pentosaceus* and *L. plantarum* resulted in a higher dry matter loss as compared to *P.pentosaceus* and *L. plantarum* without *L. buchneri*, The dry matter loss was still lower than the untreated silage. Silage inoculation treatments containing *L. buchneri* remained aerobically stable throughout the measurement period, which was 20 days. Increased acetic acid was found in silage inoculated with *L. buchneri* alone, as compared to the other treatments (Driehuis et al. 2001). The increased acetic acid improves aerobic stability due to its inhibition of yeasts and molds (Woolford, 1975).

Schmidt et al., (2009) compared alfalfa silage receiving no inoculation, *L. buchneri*, or *L. buchneri* plus *P. pentosaceus*. Silage receiving either inoculant had higher acetic acid concentrations and higher pH from 45 to 180 days of ensiling as compared to the control. Addition of *P. pentosaceus* with *L. Buchneri* increased fermentation rates in the early stages of ensiling. Additionally, higher concentration of 1,2- propanediol was found for the combination inoculant as compared to *L. buchneri* or the control.

Arriola et al. (2015) compared 4 different commercially available inoculants applied to Tifton-85 bermudagrass preserved as baleage. Two inoculants that contained *P. pentosaceus* had a more rapid pH decline in the first 30 days as compared to the other treatments, including the control. However, by day 112 all inoculant treatments had lower pH than the untreated control. Two inoculants that contained, 1) *P. pentosaceus* plus *L. buchneri* and 2) *P. pentosaceus* plus *Propionibacteria freudenreichii*, had increased lactic acid and lactic to acetic acid ratio. All inoculants that contained homolactic bacteria improved aerobic stability; however, the inoculant that contained both *L. buchneri* and *P. pentosaceus* had the greatest increase in aerobic stability over the control treatment.

Overall, limited work is published evaluating inoculants in baleage production in the southeastern United States. Hendricks et al. (2021) evaluated ferulic acid esterase-producing (**FAE**) microbial inoculants in a mini-silo study in Tifton, GA. The inoculants evaluated included a *L. plantarum* and *L. buchneri* inoculant (no FAE activity), *L. plantarum* plus *L. buchneri* LN4017 strain which does produce FAE, and an untreated control. After 60 days in the experimental silos, no differences in forage nutritive value were observed. In an in-vitro experiment utilizing these inoculated treatments as substrate, the FAE microbial inoculant had slightly elevated propionic acid for alfalfa baleage (0.7 % DM) as compared to the control (0.3 % DM). In ABG baleage, the FAE inoculant treated baleage had decreased lactic acid and increased acetic acid, and decreased propionic acid as compared to the control. Overall, the authors concluded that these results were inconclusive, as the FAE inoculant did not improve forage nutritive value over the non-FAE inoculant (Hendricks et al., 2021).

While both forage preservatives and inoculants have been studied extensively in corn silage production, less information is available for alfalfa silages, and only a few studies have evaluated silages that contain a warm season grass with or without alfalfa. The use of microbial inoculants containing *L. buchneri* consistently increased aerobic stability in numerous studies; however, the nutritive value of inoculated silages is generally the same as un-inoculated silages. Combination inoculant products have shown mixed results but generally help improve silage fermentation by contributing to initial pH decline and increasing acetic acid production during storage controls molds and yeasts, which increases aerobic stability. While one previous study has evaluated forage inoculants in alfalfa bermudagrass baleage (Hendricks et al., 2021), there are currently no studies that have evaluated both forage preservatives and inoculants simultaneously in alfalfa bermudagrass baleage.

Summary and Objectives

Utilizing alfalfa-bermudagrass mixtures is a worthy consideration for southeastern producers that have well drained soils, and who are willing to take extra management steps to produce high quality forage. Previous research has documented that a dual use, cut and graze, system is an optimal strategy for producers that allows for stored forage production, alfalfa to rest in the summer, and grazing during forage transition periods. Fall grazing ABG mixtures would bridge the summer to fall forage transition period, when high quality grazeable forage is limited in the Coastal Plains; however, there is no data currently reported in the literature on optimal stocking rates, expected liveweight gain per hectare, forage quality, or forage mass when grazing during active growth in this timeframe. Additionally, limited research exists to demonstrate potential benefits of including forage preservatives or inoculants when harvesting ABG mixtures as baleage.

Therefore, the objectives of this research were to:

1) evaluate forage mass, botanical composition, and the use of forage preservatives and inoculants on field dry down time and nutritive value of ABG harvested as baleage in a dual use, cut-and-graze, system
2) evaluate stocker calf and forage performance when rotationally grazing two bermudagrass cultivars interseeded with alfalfa in the fall as part of a dual use, cut and graze, management system
3) evaluate the impact of forage preservatives on ruminal fermentation parameters *in vitro* for ABG at harvest and two storage lengths

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CHAPTER 3

EFFECTS OF FORAGE PRESERVATIVES AND COMBINATION INOCULANT ON ALFALFA-BERMUDAGRASS BALEAGE NUTRITIVE VALUE

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Abstract

While southeastern producers may consider incorporation of alfalfa into their production systems and harvesting the forage as baleage, there is limited research evaluating forage preservatives and inoculants in baleage. The objective of this study was to evaluate if forage preservatives applied at mowing and/or *L. Buchneri* plus *P. Pentosaceus* combination forage inoculant applied at baling would improve forage nutritive value. This study was conducted in 2022 and 2023 utilizing an established alfalfa-bermudagrass mixture in Tifton, GA. The experiment was organized in a split-plot design with whole plots arranged in a completely randomized design. Preservative treatment (2022: Promote® HayDefender™; 2023: Green-Gard Hay Preservative) was applied to the whole plot. Inoculant treatment was the split plot factor. Treatments evaluated were Preservative (P+), Inoculant (I+), Both (P+, I+), or neither (NP, NI). Forage cores were collected from two bale packages for nutritive value analysis: (1) individually wrapped round bales sampled at 6 months post-harvest and (2) mini silos sampled at harvest, 8 weeks, and 6 months post-harvest. Nutritive values were not different based on forage preservative or inoculant treatment ($P > 0.05$). Crude protein was greater for mini silos than large round bales ($P < 0.01$); however, all forage samples had greater than 200 g kg^{-1} crude protein. Forage TDN was greater in mini silos than large round bales, and TDN ranged from 631 to 714 g kg^{-1}. Overall, silage additives did not improve forage nutritive value in this study; however, ABG baleage produced was a high-quality feed that could support moderate gain in any class of beef cattle.

Introduction

While many southeastern beef producers utilize hay as their primary stored forage option, there are an increasing number of producers who are harvesting forage as baleage (NASS, 2022, Mullenix, 2023). Baleage allows producers a shorter cut-to-bale time frame, may increase the forage quality produced because baling at increased moisture allows for higher leaf retention, and plastic wrapped bales can be stored outside with limited losses from weathering (Coblentz and Akins, 2017; Tucker et al., 2020). Historically, dairy operations preserved corn (*Zea mays*) and alfalfa (*Medicago sativa*) as silage stored in bag, bunker, and tower silos, which requires a dedicated fleet of silage equipment (Coblentz and Akins, 2017). Recent technological advances make baleage more accessible to beef cattle producers, as it can be made with a specialized round baler, which can handle both dry hay and baleage production. Additionally, increasing round bale wrapper options from economical single bale wrappers to more expensive in-line bale wrappers allow producers to select what works best for their operation (Coblentz and Akins, 2017).

Silage additives have been available to producers for decades; however, there is limited research evaluating forage preservatives and inoculants in baleage, which is stored at a different moisture range than silage (Coblentz and Akins, 2017). Forage preservatives are usually chemical additives that are in an acid or salt form (Muck et al., 2018). Generally, chemical additives are fermentation inhibitors, which restrict microbes that could negatively affect silage fermentation or aerobic stability (Kung et al., 2003). Propionic acid is one of the most commonly utilized forage preservatives as it can limit heating and improve aerobic stability in dry hay, fermented forages, and high moisture corn in storage because it has fungicidal activity (Britt and Huber, 1976; Huber and Soejono, 1976; Stallings et al., 1981; Kung et al., 2003).

In addition to utilizing organic acids as forage preservatives, some companies are marketing products that contain fermentation by-products from selected strains of bacteria, such as *Lactobacillus acidophilus* (Moon et al., 1981; Griffin et al., 2018). Limited information is available on these commercially available products; however, two studies evaluating different forms of *Lactobacillus acidophilus* have been conducted with one study noting that inoculation increased lactic acid concentration in corn silage, but not alfalfa silage (Moon et al., 1981). An additional study in Alabama, evaluated Promote® HayDefender™ (Cargill Animal Nutrition; Minneapolis, MN), a *Lactobacillus acidophilus* fermentation product, and noted that dry matter recovery in ryegrass (*Lolium multiflorum*) baleage tended to increase with inoculation, but did not change other fermentation parameters as compared to the control (Griffin et al., 2018). Some southeastern producers had shared with Extension specialists that they were utilizing these products to decrease dry down time in bermudagrass (*Cynodon* spp.) hay production, but to date, replicated research trials to confirm this claim have not been published (Stefancik et al., 2024).

Combination forage inoculants utilize facultative and obligate lactic acid bacteria (LAB) with the goal of combining the reported benefits of each species to improve silage fermentation (Muck et al., 2017). Facultative heterofermentative LAB increase the initial pH decline during the early stages of silage fermentation, and the obligate heterofermentative species slowly converting lactic acids to acetic acids, which can improve aerobic stability (Muck et al., 2017). One of the most commonly used combination inoculants reported in previous research contains *L. Buchneri* and *P. pentosaceus* (Muck et al., 2017). This combination is documented to support a more rapid initial pH decline and increased aerobic stability (Driehuis et al., 2001; Reich and Kung, 2010; Arriola et al., 2015; Muck et al., 2017).

Hendricks et al. (2021) evaluated ferulic acid esterase-producing microbial inoculants applied to alfalfa-bermudagrass mixtures (**ABG**) in a mini-silo study in Tifton, GA, but the authors reported that these results were inconclusive, as the FAE inoculant did not improve forage nutritive value over the non-FAE inoculant (Hendricks et al., 2021). Limited research exists to demonstrate potential benefits of including forage preservatives and/or inoculants when harvesting ABG mixtures as baleage in the southeastern United States. Thus, the objective of this study was to evaluate if forage preservative application could decrease field dry down time and if preservative and inoculant application would improve ABG baleage nutritive value.

Materials and Methods

Experimental location

This research was conducted at the University of Georgia Blackshank Farm (Tifton, GA; 31° 30' N 83° 32' W; 100 m elevation) during 2022 and 2023 on previously established alfalfa-bermudagrass pastures that consisted of 'Bulldog 805' alfalfa interseeded into either Tifton-85 (**T85+A**) or Russell (**RUS+A**) bermudagrass. Establishment information can be found in Burt et al. (2024). Soils were classified as Tifton loamy sand (2 to 5% slope; fine-loamy, kaolinitic, thermic Plinthic Kandiudults) or Fuquay loamy sand (0 to 5% slope; Loamy, kaolinitic, thermic Arenic Plinthic Kandiudults; Soil Survey Staff, 2025). Daily cumulative rainfall and air temperatures were recorded from January 1 to December 31 during the experimental years from automated weather stations (Figure 2.1.; UGA-AEMN, 2024).

Forage management

A dual use, cut and graze, management system was implemented in each year (Burt et al., 2024). Each paddock (1 ha.) was harvested as baleage during the spring and summer and grazed in the fall each year. Soil sampling occurred every year in January, and maintenance fertilizer was applied according to UGA recommendations (Tucker et al., 2021). Briefly, in both years, potassium fertilizer in the form of muriate of potash was split applied (135 K_2O kg ha^{-1} per application) after the clean-off cut, mid-season, and prior to fall grazing initiation. In 2023, 54 kg

P_2O_5 ha^{-1}, 13 kg S ha^{-1}, and three kg B ha^{-1} were applied after the clean-off cut. Soil pH was adjusted prior to alfalfa establishment, and additional lime was not required during this study. Paddocks were scouted weekly for pests, and pesticides were applied as needed to control insect and weed pressure. Pendimethalin (Prowl H2O [N-(1-ethylpropyl)-3,4- dimethyl-2,6-dinitrobenzenamine], BASF Ag Products) at the rate of 4.3 kg a.i. ha^{-1} was applied after the clean-off cut, mid-season (2022 only), and prior to grazing to control annual grass and broadleaf weeds. Zeta-cypermethrin (Mustang Maxx [zeta-cypermethrin*S-cyano(3-phenoxyphenyl)methyl (+) cis/trans 3-(2,2-dichloro-ethenyl)-2,2, dimethylcyclopropane carboxylate], FMC Corporation) at the rate of 24.5 g a.i. ha^{-1} was applied mid-season (2022 only) for bermudagrass stem maggot [Atherigona reversura Villeneuve] control and prior to grazing with chlorantraniliprole (2022: Prevathon at 100 g a.i. ha^{-1}; 2023: Vantacor at 87 g a.i. ha^{-1} [3-Bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole5-carboxamide], FMC Corporation) to control fall armyworm [Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae)].

Harvest management and treatment application

Forage harvests occurred in June and July 2022, and April and May 2023, with target harvest intervals of 28 to 25 days. Botanical composition was evaluated by hand-harvesting eight randomly placed 0.09 m^2 quadrats within each paddock to a 10 cm height the day before mowing began. All samples were hand separated to identify alfalfa (**A**), bermudagrass (**BG**), and other (**O**) components (other included any forage or weed species). Samples were placed in a forced-air dryer at 55°C for 4 days, then weighed to determine botanical composition. Component dry weights from each quadrat were summed to determine forage mass (**FM**).

Forage mowing (New Holland Discbine 313; New Holland Agriculture, New Holland, PA) started at approximately 1:00 PM for each harvest. During mowing of the paddocks assigned to receive preservative treatment, a mower-mounted sprayer applied the preservative solution to forage immediately following ejection from the conditioning flails. In 2022, the preservative solution was a 3.4% liquid Lactobacillus acidophilus fermentation product (Promote® HayDefenderTM (Cargill Animal Nutrition; Minneapolis, MN) applied at mowing and 28.4 liters of solution was applied per paddock. In 2023, the preservative solution was 4.3% propionic acid (Green-Gard Hay Preservative, John Deere, Moline, IL) and 28.4 liters of solution was applied per paddock.

Forage moisture testing began the morning after mowing occurred and when the dew had dried (usually 10 AM) using the microwave method (Ball et al., 2015). Moisture testing per treatment occurred every hour until the forage reached target moisture. When the forage reached 65% moisture, forage was randomly collected at multiple representative points in each treatment paddock, and was hand compressed into a 114 L plastic tote (one per paddock) until the tote was full. The totes were transported to a climate-controlled building where the forage was randomly sampled from the tote and assigned to inoculant or no inoculant treatment. A spray bottle with inoculant solution [3.4×10^6 cfu/ gram; Certillus Buchneri 200T WS 2.0 Silage Inoculant, Waukesha, WI] was utilized to apply the treatment to forage from each paddock that was assigned to the inoculant treatment. Three subsamples from each treatment were placed in paper bags to determine “initial” nutritive value. Six additional subsamples were sealed in individual gallon sized plastic bags as “mini silos”, placed into a larger vacuum sealed bag, and stored at room temperature to undergo fermentation. Three mini silos were sampled at 8 weeks, and three sampled at 6 months to determine nutritive value at two time points post-fermentation.

After forage was collected for mini silo preparation, raking began in field to create large round bales with the same treatments as the mini silos. Inoculant solution [3.4×10^6 cfu/ gram; Certillus Buchneri 200T WS 2.0 Silage Inoculant, Waukesha, WI] was sprayed from an ATV mounted sprayer onto forage immediately before it was fed into the baler (Kubota BV5160, Osaka, Japan). One bale per paddock was inoculated and labeled with spray paint immediately after ejection from the baler. One untreated bale was labeled in each paddock and served as the non-inoculated control. Any additional bales produced during harvest were stored separately and not included in nutritive value analysis. Bales (16 per harvest) were individually wrapped using an Anderson RB-200 single bale wrapper (Groupe Anderson Inc.) immediately after all paddocks were harvested with 6 layers of pre-stretch (55%) polypropylene baleage wrap (Sunfilm Stretch Wrap, TAMA Group, Dubuque, IA), identified with spray paint, and stored for 6 months. Three bale cores were taken (Penn State Probe, Nasco Corporation) from each treatment bale perpendicular from the radius of each bale using a drill-driven uni-forage sampler with spiral assist (Star Quality Samplers; Irricana, AB, Canada) for nutritive value analysis.

Forage analysis

After mini silo and bale core samples were collected, samples were placed in a forced-air dryer at 55°C for 4 days and ground for nutritive value analysis. Samples were ground to pass a 1-mm Wiley Mill sieve (Thomas-Wiley Laboratory Mill; Thomas Scientific). Samples were split equally to create two subsamples, one for wet chemistry and one for near infrared reflectance spectroscopy (**NIRS**) analysis. A subset (20%) of samples were randomly selected to represent each treatment for validation of nutritive value parameters using wet chemistry techniques. The wet chemistry analysis included: CP (AAOC 990.03); NDF and ADF (Van Soest et al., 1991);

IVTD48 (Ankom IVTD Method 3). NIRS samples were further ground to pass a 1 mm sieve utilizing a cyclone mill (Foss CT293; Foss Analytical, Hillerød, Denmark).

Forage samples were analyzed for crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and 48 hour *in vitro* dry matter digestibility (**IVTD48**) usings the 2023 Mixed Haylage calibration equations, as provided by the NIRS Forage and Feed Testing Consortium (**NIRSC**, Berea, KY). Samples were analyzed using a Foss DS2500 NIR spectrometer (Foss Analytical, Hillerød, Denmark) that was standardized to the NIRSC master instrument to ensure prediction accuracy. Total digestible nutrients (**TDN**) were calculated using the NIRS output and the following equation (Undersander and Moore, 2002):

$$TDN = (NFC \times 0.98) + (CP \times 0.93) + (FA \times 0.97 \times 2.25) + \left[NDFn \times \left(\frac{NDFD}{100}\right)\right] - 7$$

Where:

- NFC = non fibrous carbohydrate (% DM) = 100 – (NDFn + CP + Fat + ash)
- CP = crude protein (% DM)
- FA = fatty acids = ether extract – 1 (% DM)
- NDFn = nitrogen free NDF = NDF*.93
- NDFD = 48-hour *in vitro* NDF digestibility (% NDF)

Experimental design and statistical analysis

The experiment was organized in a split plot design with whole plots arranged in a completely randomized design. Paddock served as the whole plot factor with bermudagrass variety and preservative treatment applied to the whole plot and inoculant treatment was the split plot factor. Data were analyzed by restricted maximum likelihood using PROC MIXED in SAS v 9.4 (SAS Institute Inc., 2013, Littell et al., 2006) with an autoregressive (1) covariance structure, selected based on the lowest Bayesian Information Criterion (Littell et al., 2006).

Forage mass and botanical composition models fixed effects included harvest date (**HD**), bermudagrass variety (**BG**), and their interaction (**HD** × **BG**). Nutritive value models fixed effects included BG, HD, sample time (**ST**; Initial; mini silos: eight weeks or six months post-harvest; baleage six months post-harvest), preservative treatment, inoculant treatment, and all interactions. Data were analyzed by year for all models. The random statement subject was paddock. The repeated measure subject was paddock by inoculant treatment. A Kenwood-Rogers adjustment was applied to correct the denominator degrees of freedom and ensure appropriate standard errors and F-statistics. Means were compared using the LSMEANS procedure with Tukey-Kramer adjustment. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Dry down time

All paddocks reached target moisture at the same time, as there were no numerical differences in dry down time to target moisture between preservative and non-preservative paddocks. Statistical analysis for dry down time was not conducted because all paddocks reached moisture at the same time. Therefore, forage preservative application at mowing was not shown to decrease dry down time in this study.

Botanical composition and forage mass

There were no HD × BG interactions in forage mass for either year (Table 2.1; $P > 0.51$). In both years, there were differences in forage mass between HD ($P < 0.01$). Forage mass differed between BG in 2022 ($P < 0.05$), but not 2023 ($P = 0.69$). There was greater ($P < 0.01$) forage mass in the second harvest each year, as compared to the first harvest. Forage mass for 2022 is lower than April to July values reported in Hendricks et al. (2020) and Beck et al. (2017) for their third year stands which were, 2977 to 4156 kg ha^{-1} and 2695 to 3417 kg ha^{-1}, respectively. Forage mass in 2023 for this study was similar to the April to July values for Hendricks et al. (2020) and Beck et al. (2017) and greater than Burt et al. (2022), who reported average ABG forage mass of 1966 kg ha^{-1}. Differences in forage mass between BG for 2022 and 2023 are most likely caused by the timing of harvest in each year. In 2022, harvests occurred in June and July, where there was greater bermudagrass prevalence, as compared to 2023, which occurred in April and May before bermudagrass had fully come out of winter dormancy.

In 2022, alfalfa and bermudagrass prevalence differed between HD and BG (Figure 2.2; $P < 0.02$), but there were no HD × BG interactions ($P > 0.13$). In 2023, there was a HD × BG interaction for both alfalfa and bermudagrass prevalence ($P < 0.03$). In 2023, alfalfa prevalence differed between HD for T85+A ($P < 0.01$), but not RUS+A ($P = 0.51$). In 2023, bermudagrass contribution did not differ ($P = 0.78$) in first HD between BG, but T85+A had increased BG as compared to RUS+A in the second HD ($P < 0.01$). In both years, the first harvest had greater (P < 0.01) alfalfa (2022 = 89% and 2023 = 85%) than the second harvest (2022 = 64% and 2023= 75%). Additionally, RUS+A had greater alfalfa and less bermudagrass prevalence in both years as compared to T85+A ($P < 0.05$). Other prevalence did not differ between HD, BG, or their interaction in 2022 ($P > 0.07$). There was no HD x BG interaction for other prevalence in 2023 ($P = 0.44$), but prevalence differed between HD and BG ($P < 0.04$). In 2023, T85+A had a greater other prevalence than RUS+A ($P = 0.04$). Additionally, in 2023, there was increased other prevalence in the first HD ($P < 0.01$). The other component reported for 2023 was primarily annual ryegrass (*Lolium multiflorum*). Botanical composition fluctuations throughout the growing season have been documented previously for ABG mixtures (Hendricks et al., 2020; Burt et al., 2022; Rushing et al., 2022). The alfalfa composition in this study is slightly higher than the mentioned studies; however, the stand exhibited the common ebb and flow relationship of ABG mixtures where alfalfa contribution is greatest March to June, and bermudagrass contribution is greatest July to September (Hendricks et al., 2020; Whatley, 2023).

Nutritive value

Nutritive value did not differ based on the main effect of bermudagrass cultivar, forage preservative or inoculant treatment ($P > 0.05$). There were three models that had a statistical difference between preservative or inoculant treatment, but consistent results were not found between years and harvest dates. Inoculated forage (232 g kg^{-1} CP and 676 g kg^{-1} TDN) in 2023 had increased CP and TDN, as compared to non-inoculated forage (226 g kg^{-1} CP and 669 g kg^{-1} TDN; $P < 0.01$). Silage treated with *Lb. Buchneri* is reported to have increased aerobic stability (Muck et al. 2017), thus the authors believe the increase in crude protein and TDN for the model is likely due to differences in botanical composition. Bermudagrass and alfalfa composition fluctuates throughout seasons (Hendricks et al., 2020) and paddocks, thus achieving equal proportions of bermudagrass and alfalfa in field scale trials was not possible in this study. Nutritive value is reported by HD and ST because nutritive value parameters differed between HD, ST, and their interaction within each year (Table 2.2).

Crude protein differed between HD, ST, and HD × ST interactions in both years ($P < 0.01$). Crude protein was higher for mini silos as compared to large round bales ($P < 0.01$); however, all forage samples had greater than 200 g kg^{-1} crude protein, which would meet the needs of all classes of beef cattle (Ball et al. 2015). Crude protein reported in this study is greater than reported by Burt et al. (2022) and Hendricks et al. (2021), 182 g kg^{-1} and 144 g kg^{-1}, respectively, but was similar to the highest crude protein reported in a fall established first-year ABG stand harvested in May (219 g kg^{-1}) by Whatley (2023). Hendricks et al. (2020) reported nutritive value by month for

3 years of ABG harvests, and the crude protein in this study is similar to their reported values from April to July of a three-year-old stand (196 to 232 g kg^{-1}).

NDF differed between HD, ST, and HD × ST interactions in both years ($P < 0.01$). In both years, NDF was highest for 6MB ($P < 0.01$), and 8W had lower NDF than 6M ($P < 0.01$). NDF differed between HD in both years, where the second harvest date each year had greater NDF ($P < 0.01$). ADF differed between ST, and HD × ST interactions in both years ($P < 0.01$); however, HD differed in ADF for 2023 ($P < 0.01$), but not 2022 ($P = 0.39$). In 2022, initial and 8W did not differ in ADF ($P = 0.12$), but ADF increased ($P < 0.01$) during storage for 6M and 6MB, where 6MB had the greatest ADF ($P < 0.01$). In 2023, ADF increased during the storage period and was greatest for 6MB ($P < 0.01$). ADF did not differ between HD in 2022 ($P = 0.39$), but in 2023, there was increased ADF for the second HD ($P < 0.01$). Increased NDF and ADF in the large round baleage could be related to losses associated with normal field harvesting procedures, as some leaf loss is expected during raking and baling. Another possible explanation is that since baleage is a high moisture forage product, as fermentation occurred, readily fermentable carbohydrates were oxidized and the percent increase in fiber would be due to loss of those nutrients, as opposed to a true increase in fiber (Miller et al., 1967; Rotz and Muck, 1994).

NDF and ADF ranges reported by Hendricks et al. (2020), 345 to 467 g kg^{-1} NDF and 249 to 315 g kg^{-1} ADF, for second and third year ABG stands harvested monthly are similar to the current study. This study's June (371 g kg^{-1}) and July (392 g kg^{-1}) harvests had lower NDF than Whatley (2023) harvested at a similar time (June: 549 g kg^{-1} and July: 506 g kg^{-1}). NDF reported in this study is lower than reported by Hendricks et al. (2021) and Burt et al. (2022), which were 593 and 487 g kg^{-1}, respectively. ADF reported in this study is similar to May values (289 g kg^{-1}

and 209 g kg^{-1}) reported by Whatley (2023) and Burt et al. (2022), respectively, and less than reported (379 g kg^{-1}) by Hendricks et al. (2021).

There were differences in IVDMD between HD and ST in both years ($P < 0.01$), and a HD × ST interaction occurred in 2023 ($P < 0.01$). In both years, 8W and 6M had greater ($P < 0.01$) IVDMD as compared to initial and 6MB, which also differed ($P < 0.01$). In both years, there was a difference between HD, where the second HD in 2022, and the first HD in 2023 had greater IVDMD ($P < 0.01$). The highest IVDMD (854 g kg^{-1}) was from the first HD in 2023, where there was also the greatest "other" component (13%), which was primarily annual ryegrass. The highest IVDMD in this study is similar to Hendricks et al. (2020) for a two year old stand harvested in March (850 g kg^{-1}). Our range of reported IVDMD is similar to Whatley (2023) who reported 726 to 808 g kg^{-1} for ABG harvested monthly and Burt et al. (2022) who reported ABG IVDMD averaged 757 g kg^{-1} in their grazing study.

There were differences in TDN between HD and ST in both years ($P < 0.01$), and a HD × ST interaction occurred in 2023 ($P < 0.01$). In 2022, Initial, 8W, and 6M did not differ in TDN ($P > 0.06$), but 6MB had the lowest TDN ($P < 0.01$). The second HD in 2022 had greater TDN than the first ($P < 0.01$). In 2023 during the first HD, 8W and 6M did not differ ($P = 0.65$), but had greater TDN than Initial and 6MB ($P < 0.01$), which also differed ($P < 0.01$). For the second HD in 2023, all ST differed ($P < 0.01$). Additionally in 2023, the first HD had greater TDN than the second HD ($P < 0.01$). TDN in this study is greater than reported (520 g kg^{-1}) by Burt et al. (2022) and similar to the range reported (656 to 726 g kg^{-1}) by Hendricks et al. (2020).

Lower nutritive values were generally found in 6M baleage cores as compared to the initial and mini silo forages. This could be due to the sampling method used where forage collected for initial and mini silos were hand harvested after mowing; whereas the forage for the 6M cores was raked and baled in field with regular harvesting equipment. Forage quality losses from leaf shattering is expected when forage is harvested with normal hay equipment (Ball et al., 2015). Burt et al. (2024b) evaluated baleage harvested and stored for various time points and reported that quality was maintained with some increase in fiber concentrations through 12 months post-harvest. This study differed in that initial forage was sampled from the field prior to raking beginning, while Burt et al. (2024b) evaluated forage quality on material collected directly from the bales, which were harvested with normal field operations, and the initial sample was collected after 6 weeks of fermentation.

Previous research in Georgia evaluating ferulic acid esterase producing microbial inoculants also reported that inoculation did not improve forage nutritive value (Hendricks et al. 2021). The authors reported inoculants did not improve fermentation in alfalfa; however, both inoculants tended to increase acetic acid in ABG (Hendricks et al. (2021). Application of *Lb. buchneri* is expected to increase acetic acid concentrations in silage (Muck et al. 2017). As pH declines, *Lb. Buchneri* becomes more active and converts lactic acid to acetic acid, which usually occurs during the later stages of fermentation and through the storage period (Muck et al. 2017). Forage mass and nutritive values in this study primarily differed between storage method, with differences in harvest season and botanical composition of the stand also playing a role. In 2022, harvests occurred in June and July; whereas, in 2023, harvests occurred in April and May. Botanical composition fluctuates as ABG stands transition from spring to summer, which influences the nutritive value due to changing legume-grass ratios.

Conclusion

Overall, utilizing forage preservatives and inoculants did not impact forage nutritive value in this study; however, harvesting ABG mixtures as baleage allowed for timely harvests, and some increases in nutritive value for ABG preserved as baleage in mini silos were documented. Additionally, harvested forage was high quality and should support beef cows at all stages of production, or to meet the needs for moderate weight gain in growing animals. Full silage fermentation profiles were not completed in this experiment. Thus, future research evaluating aerobic stability and initial silo pH decline would indicate if application of preservatives or inoculants improve forage storage parameters, other than nutritive value. While previous research has documented utilizing ABG for grazing and baleage in years one to three, studies have not been published on the mixture past year three. Forage mass, botanical composition, and forage quality were documented for a three- and four-year-old ABG stand in Tifton, GA. Alfalfa prevalence was maintained into year four in this study, which shows promise to producers and researchers for the longevity of ABG stands in the Deep South.

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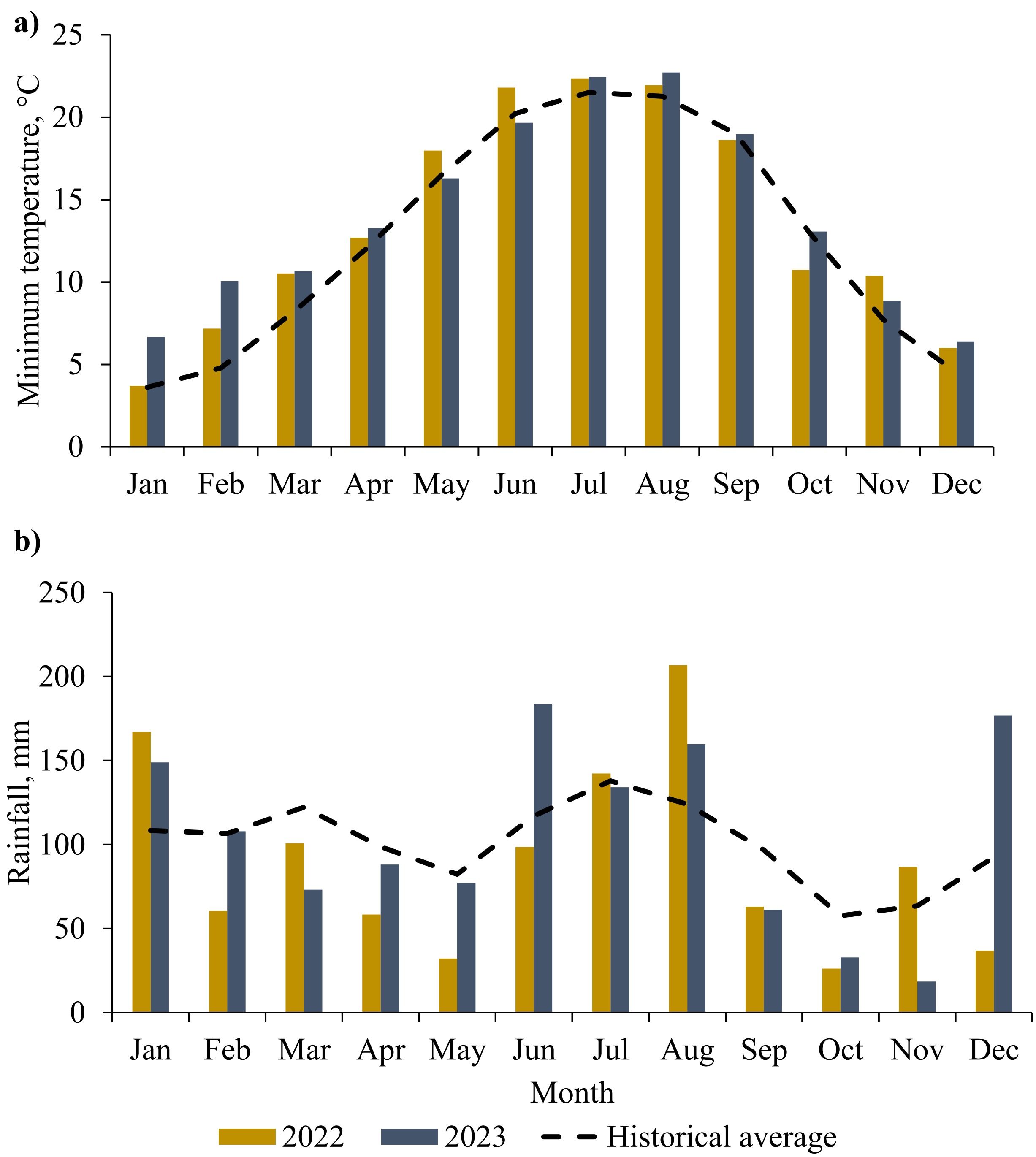

Figure 3.1 a) Average minimum temperatures and b) total monthly rainfall during 2022, 2023, and the 100-year average for Tifton, GA. Data were collected from University of Georgia Automated Environmental Monitoring Network (UGA-AEMN, 2020)

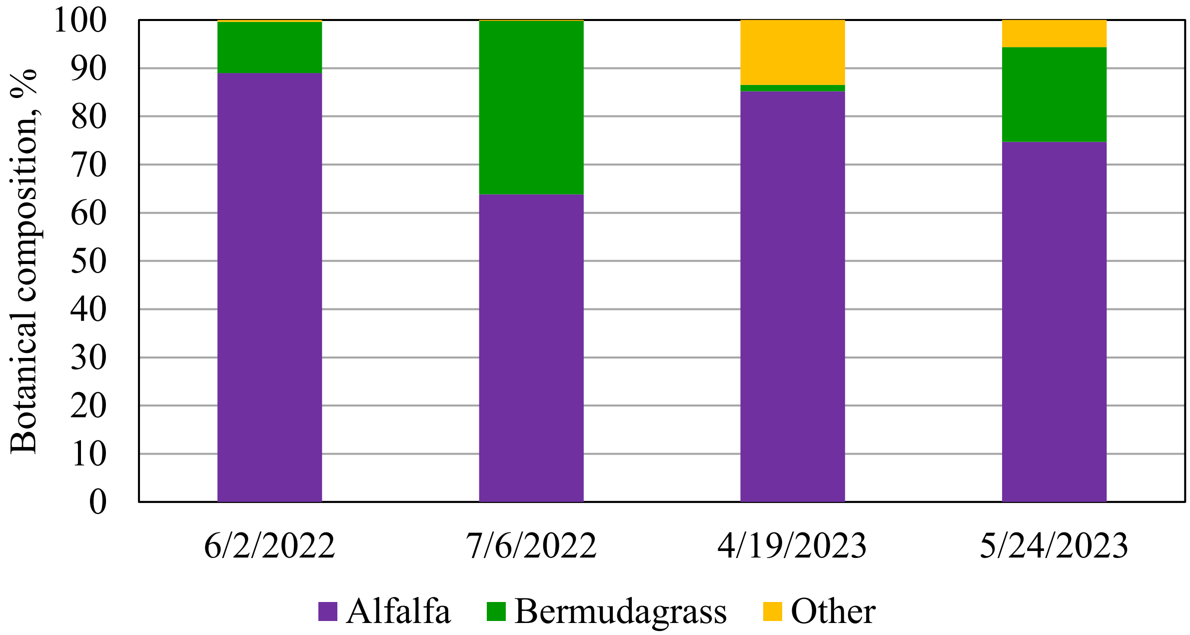

Figure 3.2 Botanical composition (alfalfa, bermudagrass, and other [any other forage or weed]) for Russell (Rus+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with 'Bulldog 805' alfalfa and harvested as baleage during 2022 and 2023 in Tifton, GA

Table 3.1. Forage mass (kg·ha^{-1}) for Russell (Rus+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with 'Bulldog 805' alfalfa and harvested as baleage during 2022 and 2023 in Tifton, GA

	Harvest 1		Harvest 2		SEM[1]	*P* – value[2]		
Year	T85+A	RUS+A	T85+A	RUS+A		BG	HD	HD×BG
2022	1265^{bc}	1085^{c}	1640^{a}	1410^{ab}	94.6	0.05	<0.01	0.78
2023	2770^{b}	2992^{b}	3589^{a}	3558^{a}	213.4	0.69	<0.01	0.51

[1]SEM: Standard Error of the Mean.
[2]BG: Bermudagrass cultivar; HD: Harvest date.
[a-d]Letters denote differences within year at $P < 0.05$.

Table 3.2 Alfalfa bermudagrass nutritive value using near-infrared spectroscopy analyses when fermented in mini silos or large round baleage and sampled at four time points (initial; mini silos: 8 weeks [8W] or 6 months [6M] post-harvest; baleage 6 months post-harvest[6MB]) across two harvest dates in 2022 (June and July) and 2023 (April and May) in Tifton, GA

Item[3], g · kg^{-1}	June				July				SEM[1]	*P*-value[2]		
2022	Initial	8W	6M	6MB	Initial	8W	6M	6MB		HD	ST	HD × ST
CP	227^{b}	239^{a}	241^{a}	216^{d}	224^{bc}	219^{bc}	217^{cd}	217^{d}	3.0	<0.01	<0.01	<0.01
NDF	371^{b}	355^{a}	369^{b}	413^{d}	392^{c}	402^{cd}	413^{d}	411^{d}	7.0	<0.01	<0.01	<0.01
ADF	263^{bc}	255^{ab}	263^{bc}	303^{e}	249^{a}	270^{cd}	279^{d}	277^{d}	4.9	0.47	<0.01	<0.01
IVTDMD48	775^{c}	797^{a}	795^{a}	761^{d}	791^{ab}	802^{a}	800^{a}	782^{bc}	4.3	<0.01	<0.01	0.11
TDN	674^{c}	688^{a}	675^{bc}	656^{d}	696^{a}	696^{a}	694^{a}	685^{ab}	4.8	<0.01	<0.01	0.07
2023	April				May							
CP	251^{a}	252^{a}	253^{a}	217^{bc}	216^{c}	217^{bc}	220^{b}	205^{d}	2.0	<0.01	<0.01	<0.01
NDF	364^{b}	349^{a}	369^{b}	431de	427^{d}	418^{c}	439^{e}	454^{f}	3.9	<0.01	<0.01	<0.01
ADF	245^{a}	276^{b}	290^{c}	311^{e}	306^{d}	329^{e}	344^{f}	345^{f}	2.4	<0.01	<0.01	<0.01
IVTDMD48	838^{b}	854^{a}	848^{a}	803^{c}	770^{e}	787^{d}	787^{d}	758^{f}	2.6	<0.01	<0.01	<0.01
TDN	694^{b}	714^{a}	712^{a}	664^{d}	648^{e}	665^{d}	655^{d}	631^{f}	2.3	<0.01	<0.01	<0.01

1. Standard error of the mean.
2. *P* – values represent harvest date (HD), sample time (ST), and their interaction (HD×ST).
3. Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; IVTDMD48, *in-vitro* true dry matter digestibility after 48 hours; TDN, total digestible nutrients.

$^{a-d}$ Letters denote differences within a row at $P < 0.05$.

CHAPTER 4

FILLING THE SPRING TO FALL FORAGE TRANSITION WITH ALFALFA INTERSEEDED INTO TWO BERMUDAGRASS BASES AND THE ASSOCIATED IMPACTS ON PLANT AND STOCKER CALF PERFORMANCE

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Abstract

Interseeding alfalfa into bermudagrass can offset nitrogen fertilization requirements, improve forage quality, extend forage grazing days, and increase economic returns. Previous research identified a cut-and-graze harvest management strategy had potential to fill a regional forage deficit during the summer to fall transition. The objective of this study was to evaluate stocker calf and forage performance during the summer to fall forage transition when rotationally grazing two bermudagrass cultivars interseeded with alfalfa as part of a strategic, dual use cut-and-graze management system. A two-year evaluation was conducted from September to November of 2022 and 2023 on a three- and four-year-old stand of Bulldog 805 Alfalfa interseeded into two bermudagrass bases. Eight (1 ha.) paddocks were arranged in a completely randomized design with four replications per treatment. The treatments evaluated were alfalfa interseeded into Tifton 85 (T85+A) or Russell (R+A) bermudagrass. Paddocks were harvested as baleage from spring into August each year, followed by grazing initiation in early September. Vegetation was collected pre- and post- grazing to calculate forage mass (kg ha^{-1}) and forage allowance (kg DM kg^{-1} liveweight). Stocking rate (kg bodyweight acre^{-1}) was adjusted using the put and take method and average daily gain (kg day^{-1}) was calculated from tester steer weights measured at study initiation and conclusion using the double weight method. In both years, T85+A had higher FM (4473 and 3109 kg ha^{-1}) than RUS+A (3323 and 2713 kg ha^{-1}) during grazing cycle one, respectively (P<0.01), with no differences between treatments during grazing cycle two either year (P>0.51). The same result occurred for stocking rates, where T85+A (2022: 2931 kg ha^{-1}; 2023: 2490 kg ha^{-1}) supported greater (P< 0.01) stocking rates in grazing cycle one as compared to RUS+A (2022: 1727 kg ha^{-1}; 2023: 2091 kg ha^{-1}). Nutritive values of both treatments supported an average daily gain of 0.6 kg hd^{-1} day^{-1} in 2022, and 1.0 kg hd^{-1} day^{-1} in 2023. In 2023, a shorter re-growth period at grazing

initiation resulted in increased forage nutritive value, which increased average daily gain, and gain per hectare (2022: 125 kg ha^{-1}; 2023: 271 kg ha^{-1}) as compared to 2022. Grazing alfalfa bermudagrass mixtures is a viable option for southeastern forage producers seeking a high-quality forage option for the summer to fall transition. Strategically targeting grazing initiation in the fall was demonstrated to be crucial as delaying grazing initiation past 35 days of re-growth resulted in decreased forage nutritive value and animal gain per hectare in 2022.

Introduction

Beef cattle operations in the Deep South are primarily cow-calf operations that sell calves after weaning (Rankins and Prevatt, 2013; Ball et al., 2015). Stockering calves after weaning could increase producer revenue; however, the economic viability of this option depends on availability of low-cost feeds that meet growing calves' nutritional requirements (Rankins and Prevatt, 2013). High quality, grazeable forage mixtures for stockering calves are more economical per pound of gain when legumes are included with grasses rather than being fertilized with nitrogen (Ball and Prevatt, 2009).

In the 2000s, adapted, dual use, grazing tolerant alfalfa (*Medicago sativa* L.) varieties became available to southeastern producers (Bouton and Gates, 2003). Improved alfalfa cultivars can be interseeded into bermudagrass (*Cynodon spp*.) and these mixtures (**ABG**) can be utilized for grazing and stored forage production in the same year (Hendricks et al., 2020; Burt et al., 2021; Burt et al., 2022; Burt et al., 2024). Interseeding alfalfa into bermudagrass mixtures can offset nitrogen fertilization requirements, improve forage quality, extend bermudagrass grazing days, and increase economic returns per hectare for producers (Beck et al., 2017abc; Burt et al., 2022; Rushing et al., 2022). Researchers have successfully interseeded alfalfa into bermudagrass stands since the 1950's (Burton, 1976); however, many southeastern producers are hesitant to grow alfalfa due to concerns over establishment cost, stand longevity, and weather conditions (Silva et al., 2021).

Previous ABG evaluations in the Southeast United States focused on using the mixture for stocker calves grazing in the spring and summer (Beck et al., 2017abcd; Burt et al., 2022; Rushing et al., 2022); however, many southeastern producers overseed cool-season annuals on bermudagrass pastures to provide spring grazing before bermudagrass breaks dormancy for summer grazing (Mullenix and Rouquette, 2017). Consequently, utilizing ABG mixtures for spring and summer grazing may not be as crucial to producers in the region who need a high-quality grazeable forage option in the fall (Ball et al., 2015; Mullenix and Rouquette, 2017). Burt et al. (2024) demonstrated that a dual-purpose cut-and-graze harvest management strategy allowed ABG mixtures to be grazed during the summer to fall forage transition period, which would fill a producer identified forage gap in the region (J.J. Tucker, personal communication) For summer to fall grazing to be successful, the ABG mixture should be harvested as stored forage during the summer, instead of grazing, to allow alfalfa to rest during its most stressful growth period (Burt et al., 2024). Additionally, previous research suggested rest periods between grazing events should be longer than 21 days to maintain alfalfa persistence (Burt et al., 2022) and a shorter grazing period can help maintain alfalfa plant density and seasonal yield (Peart, 1968; Constable et al., 1977; Jennings and Loftin, 2021).

While Burt et al. (2024) documented ABG mixtures can be grazed in the fall, there is currently no research published that evaluates if the cut-and-graze management system can be modified to target grazing ABG mixtures during active growth in the summer to fall transition, when a local seasonal forage deficit occurs. Understanding calf and forage performance expectations for varying bermudagrass bases interseeded with alfalfa would allow producers to decide if utilizing ABG mixtures for stockering calves is an economical decision for their operation. Therefore, the objective of this study was to evaluate stocker calf and forage performance during the summer to fall transition when rotationally grazing two bermudagrass cultivars interseeded with alfalfa as part of a dual-use cut-and-graze management system.

Materials and Methods

This project was approved by the University of Georgia IACUC Committee (IACUC AUP #: A2024 03-006-Y1-A0) for the use of animals in research.

Experiment location

This research was conducted at the University of Georgia Tifton Campus “Better Grazing Program” (Tifton, GA; 31° 30' N 83° 32' W; 100 m elevation) during fall 2022 and 2023 on previously established ABG mixtures (Burt et al., 2024). Soils were classified as Tifton loamy sand (2 to 5% slope; fine-loamy, kaolinitic, thermic Plinthic Kandiudults) or Fuquay loamy sand (0 to 5% slope; Loamy, kaolinitic, thermic Arenic Plinthic Kandiudults; Soil Survey Staff, 2025). Daily cumulative rainfall and air temperatures were recorded each year from January 1 to December 31 by automated weather stations (UGA-AEMN, 2024). Treatments evaluated were ‘Bulldog 805’ alfalfa interseeded into ‘Tifton-85’ (**T85+A**) or ‘Russell’ (**RUS+A**) bermudagrass.

Forage management

A dual-use cut-and-graze harvest management system was implemented each year (Burt et al., 2024). In both years, paddocks (*N*=8; 1 ha.) were harvested as baleage from April to July and grazed from September to November. Treatment paddocks were divided into eight subsections using temporary electric fencing and rotationally grazed with calves being moved to a new subsection every four days. Grazing occurred from 8 Sept to 9 Nov (62 days) in 2022 and 10 Sept to 6 Nov (57 days) in 2023 of the evaluation. The study was terminated each year when the FM was less than 1,120 kg DM ha^{-1}. Each subsection was grazed for 4 days, which allowed for 28 days of rest between grazing events. A grazing cycle (**GC**) was defined as one full rotation through all 8 subsections (32 days total).

Soil sampling was conducted in January each year. Soil pH was adjusted with agricultural lime application prior to stand establishment, and no additional lime was required during this study. No fertilizers or pesticides were applied during the grazing evaluation; however, field management for the spring and summer baleage harvests were outlined in Chapter 2.

Forage responses and nutritive value analysis

Forage was hand-harvested to a 10 cm height pre- and post-grazing from five randomly placed 0.1 m^2 quadrats within each paddock subsection. Samples were hand separated to identify alfalfa, bermudagrass, and other components (other included any other forage or weed species present). Other components were primarily bahiagrass (*Paspalum notatum*), vaseygrass (*Paspalum urvillei*), crabgrass (*Digitaria spp.),* and pigweed (*Amaranthus hybridus* L.). Samples were dried in a forced-air drying oven at 55°C for four days and weighed to determine botanical composition. Component dry weights from pre-graze quadrat samples were summed to determine forage mass (**FM**).

Dried botanical components from each quadrat were composited and ground for nutritive value analysis. All samples (*N*=1240) were ground to pass a 1-mm Wiley Mill sieve (Thomas-Wiley Laboratory Mill: Thomas Scientific). Samples (*n*=128) containing greater than 25 g were split equally to create two subsamples, one for wet chemistry and one for near infrared reflectance spectroscopy (**NIRS**) analysis (McIntosh et al., 2022). A subset (10%) of samples were randomly selected to represent each treatment for validation of nutritive value parameters using wet chemistry techniques. The wet chemistry analysis included: CP (AAOC 990.03); NDF and ADF (Van Soest et al., 1991); IVTD48 (Ankom IVTD Method 3). NIRS samples were further ground to pass a 1 mm sieve utilizing a cyclone mill (Foss CT293; Foss Analytical, Hillerød, Denmark). Samples (*n* = 1,112) less than 25 g were used for NIRS analysis only. Samples for NIRS were further ground to pass a 1 mm sieve using a cyclone mill (Foss CT293; Foss Analytical).

Forage samples were analyzed for crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and 48 hour *in vitro* dry matter digestibility (**IVTD48**) using the 2024 Mixed Hay calibration equations, provided by the NIRS Forage and Feed Testing Consortium (**NIRSC**, Berea, KY). Samples were analyzed using a Foss DS2500 NIR spectrometer that was standardized to the NIRSC master instrument to ensure prediction accuracy. Total digestible nutrients (**TDN**) were calculated using the NIRS output and the following equation (Undersander and Moore, 2002):

$$TDN = (NFC \times 0.98) + (CP \times 0.93) + (FA \times 0.97 \times 2.25) + \left[NDFn \times \left(\frac{NDFD}{100}\right)\right] - 7$$

Where:

- NFC= non fibrous carbohydrate (% DM) = 100 – (NDFn + CP + Fat + ash)
- CP = crude protein (% DM)
- FA = fatty acids = ether extract – 1 (% DM)
- NDFn = nitrogen free NDF = NDF*.93
- NDFD = 48-hour *in vitro* NDF digestibility (% NDF)

Grazing management

Yearling crossbred (*Bos taurus* × *Bos indicus*) stocker cattle (Year 1: *N*=73; Year 2: *N*=87) were selected from the UGA Alapaha Research Station (Alapaha, GA) based on temperament and body weight (**BW**). Before study initiation, stockers were trained to temporary fencing while backgrounded on warm season mixed pastures including bermudagrass, bahiagrass, and crabgrass with minimal legume content at the University of Georgia Animal Science Farm (Tifton, GA).

Throughout the experiment, calves had *ad-libitum* access to water and shade. Mineral was given to meet the intake recommended on the label (**2022**: AMPT A RU, ADM Animal Nutrition, Quincy, IL; Monensin = 1.8 g kg^{-1}; Ca = minimum 138 g kg^{-1} and maximum 164 g kg^{-1}; P = minimum 40 g kg^{-1} ; NaCl = minimum 192 g kg^{-1} and maximum 228 g kg^{-1}; Mg = minimum g kg^{-1}; K = none added; Co = minimum 0.15 g kg^{-1}; Mn = minimum 3.6 g kg^{-1}; Zn = minimum 4.2 g kg^{-1}; Cu = minimum 1.2 g kg^{-1}; Se = minimum 25 g kg^{-1}; I = minimum 0.2 g kg^{-1}; Vitamin A = minimum 90,718 IU kg^{-1}; Vitamin D3 = minimum 2268 IU kg^{-1}; Vitamin E = minimum 45 IU kg^{-1}; **2023**: Moormans Minerals; ADM Animal Nutrition, Quincy, IL; Lasalocid= 3.1 g kg^{-1}; Ca = minimum 155 g kg^{-1} and maximum 230 g kg^{-1}; P = minimum 80 g kg^{-1}; NaCl =minimum 127.5 g kg^{-1} and maximum 153 g kg^{-1}; Mg = minimum 20 g kg^{-1}; K= none added; Co = minimum 300 ppm; Cu = minimum 2,000 ppm; I = minimum 150 ppm; Mn = minimum 6,000 ppm; Se = minimum 39 ppm; Zn = minimum 8,000 ppm; Vitamin A = minimum 90,718 IU kg^{-1}; Vitamin D3 = minimum 9072 IU kg^{-1}; Vitamin E = 91 IU kg^{-1}).

Stocking rate was adjusted utilizing the put and take stocking method (Mott and Lucas, 1952). Two tester steers (*N*=32; BW: year 1 = 291 ± 27 kg and year 2 = 282 ± 34 kg) were stratified by body weight and randomly assigned to each pasture and remained there throughout the trial. Tester steers were implanted (Ralgro, 36 mg zeranol; Merck, Rahway, NJ) the day before study initiation. Grazers (steers and heifers) were allocated as needed to target a 1 kg DM to 1 kg BW forage allowance. Stocking decisions were made the day before the calves were moved to a new subsection by estimating forage mass with a pasture ruler. Forage allowance

(**FA**) was calculated as the average forage mass collected pre- and post- grazing divided by the total animal weight for each subsection. The stocking rate (**SR**) was determined by dividing the total weight from testers plus grazers on a subsection by the total area of the subsection. Total forage allowance and stocking rate reported for each paddock was determined by averaging the values from each subsection.

Cattle responses

Tester steers were weighed following the double weight method (Mott and Lucas, 1952) at study initiation and termination to determine average daily gain (**ADG**). All calves were weighed at study midpoint, which occurred at the end of grazing cycle one, to calculate stocking rate during grazing cycle two. Grazers were weighed before moving into and upon leaving a treatment paddock. Average daily gain was calculated by dividing the total weight gained by the tester animals by the total days on trial. Liveweight gain (**LWG**) per hectare was calculated by summing the weight gained by testers and grazers on a paddock and dividing by the specific paddock area.

Experimental design and statistical analyses

All analyses were performed using paddock as the experimental unit. The experiment was organized in a completely randomized design with repeated measures. Paddock within year served as the repeated measure subject. All models were analyzed by year. Fixed effects included bermudagrass cultivar (**BC**), GC, and their interaction (**BC × GC**). Models were analyzed by restricted maximum likelihood using PROC MIXED in SAS v 9.4 (SAS Institute Inc., 2013, Littell et al., 2006) with an autoregressive (1) covariance structure, selected based on the lowest Bayesian Information Criterion (Littell et al., 2006). A Kenwood-Rogers adjustment was applied to correct the denominator degrees of freedom and ensure appropriate standard errors and F-statistics. Means were compared using the LSMEANS procedure with Tukey-Kramer adjustment. Differences were considered significant at $P \leq 0.10$.

Results

Average temperature followed the trend of the 100-year average in both years; however, less rainfall fell during the experimental period in both years as compared to the 100-year average (Figure 3.1a,b). Rainfall reported during November 2022 was primarily from a single rainfall event, during Hurricane Nicole, which led to the study conclusion.

Forage mass and botanical composition

There was a forage mass BC × GC interaction ($P < 0.01$) in that T85+A had increased FM as compared to RUS+A during GC1 in 2022 (Table 3.1; P <0.01). There was no BC x GC interaction in 2023 ($P = 0.11$), but there were BC and GC main effects ($P < 0.01$). The T85+A treatment had increased forage mass in GC1; however, there were no differences in forage mass between treatments during GC2 in either year ($P > 0.51$).

In both years, there were BC × GC interactions ($P < 0.05$) for alfalfa prevalence where RUS+A had greater ($P < 0.01$) alfalfa as compared to T85+A in both GC, and GC2 had greater alfalfa than GC1 (Figure 3.2; $P < 0.01$). There was a BC × GC interaction ($P < 0.04$) for bermudagrass prevalence in both years. The T85+A treatment had greater bermudagrass than RUS+A in both GC ($P < 0.01$), and GC1 had greater bermudagrass as compared to GC2 ($P < 0.01$). There were no interactions or main effect differences for other prevalence in 2022, as other was < 1 % for all treatments. In 2023, there was no BC × GC interaction ($P = 0.80$), but there were BC and GC main effects ($P < 0.01$). There was greater other prevalence for GC1 and T85+A treatments, as compared to GC2 or RUS+A treatments ($P < 0.01$).

Forage nutritive value

There was a BC × GC interaction for all forage nutritive value parameters ($P < 0.06$), except CP and ADF in 2023 ($P > 0.13$; Table 3.2). The RUS+A treatment had greater CP than T85+A during all grazing cycles ($P < 0.01$), except in 2022 GC2, where treatments did not differ ($P = 0.59$). The T85+A treatment had greater ADF and NDF during all grazing cycles ($P < 0.01$) except GC2 in 2022, where treatments did not differ ($P > 0.12$). In both years, IVTD48 did not differ ($P > 0.20$) during GC1; however, during GC2, T85+A had greater IVTD48 in 2022 and less IVTD48 in 2023 than RUS+A ($P < 0.01$). There was no difference ($P = 0.97$) between treatment TDN during GC1 2022, but T85+A had greater TDN than RUS+A during GC2 in 2022 and GC1 in 2023 ($P < 0.01$). In contrast, RUS+A had greater TDN than T85+A during GC2 in 2023 ($P < 0.01$).

Cattle responses

There were BC × GC interactions for all cattle responses in both years ($P < 0.03$), except for ADG and GPH in 2022 ($P > 0.22$; Table 3.3). In 2022, ADG was not different ($P > 0.24$) between treatments in either GC; however, in 2023, RUS+A had decreased ADG as compared to T85+A in GC2 ($P < 0.01$), but ADG did not differ in GC1(P > 0.10). During GC1 in both years, GPH was not different between treatments ($P > 0.21$); however, during GC2, T85+A had more GPH than RUS+A in both years ($P < 0.06$). Stocking rates were greater ($P < 0.01$) for T85+A during GC1 in both years, but SR was not different during GC2 in both years ($P > 0.15$). Forage allowance was greater for RUS+A as compared to T85+A during GC1 in 2022 ($P < 0.01$) but was not different between treatments during GC2 in 2023 and GC1 in 2022 ($P > 0.52$). Forage allowance was greater for T85+A calves during GC2 in 2023 as compared to RUS+A ($P < 0.01$).

Discussion

Forage mass and botanical composition

Forage mass reported in this study (3109 to 4473 kg ha^{-1}) for T85+A during GC1 is greater when compared to September forage mass, 2021 and 2224 kg ha^{-1}, reported by Hendricks et al. (2020) and Beck et al. (2017d), respectively. Forage mass for T85+A during 2022 GC1 was similar to forage mass (4845 kg ha^{-1}) reported by Rushing et al. (2022), who utilized a 7.6 cm cutting height to estimate forage mass and the current study utilized a 10 cm cutting height. Forage mass during GC2 (1857 kg ha^{-1}) is similar to that reported by Hendricks et al. (2020) for a three-year-old stand in October (1600 kg ha^{-1}) and reported by Vasco et al. (2023) for a first-year stand harvested in September after 6 weeks of regrowth (1732 kg ha^{-1}).

Botanical composition reported for GC1 in this study is similar to September and October botanical composition reported in Hendricks et al. (2020) and Burt et al. (2025); however, this study reported increased alfalfa prevalence for GC2 (46 to 75%) as compared to October (30 to 45%) values reported by Hendricks et al. (2020) and deferred grazing alfalfa prevalence (18 to 36%) by Burt et al. (2025). Fall planted alfalfa has higher alfalfa establishment success, as measured by alfalfa prevalence, when compared to spring planted alfalfa in the southeast United States (Whatley, 2023). In the current study, alfalfa was planted in the fall; whereas, Hendricks et al. (2020), planted alfalfa in the spring. Burt et al. (2025) planted alfalfa in the fall, but paddocks had been grazed during the spring and deferred grazing occurred after 6 weeks of regrowth, which is longer than the regrowth period allowed in the current study. Additionally, the ebb and flow relationship of alfalfa and bermudagrass within a season for ABG mixtures (Hendricks et al., 2020; Burt et al., 2022; Whatley, 2023), makes comparison of stand composition across studies in different months difficult. This study started with lower alfalfa prevalence as compared to starting alfalfa prevalence (80%) reported by Burt et al. (2022); however, grazing for the current study started in September and Burt et al. (2022) began grazing in May. This study began with higher alfalfa percentage as compared to the last grazing cycle (August to September; < 20% alfalfa) reported in Burt et al. (2022); however, the authors reported their forage mass became limiting due to drought and shorter rest periods between grazing events. Differences between botanical composition in the mentioned studies is most likely due to the differences in grazing management, sampling month, and stand age. Burt et al. (2022) used a seven-to-ten-day rotation with fourteen to twenty-one days of rest, while this study utilized a four-day rotation with twenty-eight days of rest in a dual-use management strategy. This study demonstrated that alfalfa percentage in a three- and four- year old ABG stand was maintained over 30%, showing that alfalfa can persist for more than three years in Coastal Plains ABG mixtures with rotational grazing management, and baleage harvests that allow alfalfa to rest during the summer.

Forage nutritive value

Crude protein, TDN, and IVTDMD48 during GC1 were lower, and ADF and NDF higher, than reported by Hendricks et al. (2020) in September of a three-year-old ABG stand. This is likely due to increased forage maturity at grazing initiation, as well as increased bermudagrass in the stand as compared to Hendricks et al. (2020). Forage CP and TDN reported by Burt et al. (2025) for deferred grazing is similar to GC1 of 2022 in the current study. Grazing in the current study and for Burt et al. (2025) deferred grazing occurred beyond the recommended harvest interval of 28 to 35 days for ABG mixtures (Tucker et al., 2021). Forage quality declines rapidly with stand maturity (Ball et al., 2015). When the calves reached the last subsection of the paddocks during GC1 forage had accumulated for 66 (2022) and 59 (2023) days. The authors recognize that timing the previous baleage harvest to target grazing initiation for each subsection to begin between 28 and 32 days of re-growth during GC1 would enhance system performance overall; however, due to land limitations within the research acreage, this was unattainable.

During GC2, all forage in all subsections had accumulated for 28 days when grazing began. As a result, forage nutritive value in GC2 was similar to October harvest values reported by Hendricks et al. (2020), but greater than reported for deferred grazing by Burt et al. (2025). TDN values reported by Burt et al. (2022), Rushing et al. (2022), and Beck et al. (2017d) are less than reported in this study; however, crude protein is similar between all studies. NDF and ADF are similar to values reported by Burt et al. (2022) and Beck et al. (2017d). IVTD48 in this study is similar to Burt et al. (2022). Fluctuations in alfalfa and bermudagrass composition in the stand, environmental differences, as well as forage maturity at the time of harvest, can explain much of the variation when evaluating nutritive value differences between the mentioned studies. Additionally, this study reported that ABG mixtures maintained forage nutritive value during fall grazing on a four year old stand that is similar to previous studies who reported on ABG mixtures during spring and summer grazing of stands one to three years old, indicating the potential for ABG mixtures to be a worthy consideration for producers who want a perennial, high-quality, grazeable forage option for the summer to fall transition.

Cattle responses

Average daily gain reported during 2022 in this study is similar to deferred grazing gains reported by Burt et al. (2024) in 2022, but the current study had greater ADG in GC1 in 2023. Burt et al. (2022) and Rushing et al. (2022) had higher ADG during summer grazing on ABG mixtures than reported for 2022; however, this study had increased ADG during GC1 of 2023. Increased ADG was mostly likely due to increased forage TDN in this study. Gain per hectare during GC1 in 2022 was similar to Burt et al. (2022), but this study had lower GPH than reported by Rushing et al. (2022). The greatest GPH occurred during GC1 of 2023 and was greater than previously reported GPH (Beck et al., 2017d; Burt et al., 2022; Rushing et al., 2022). This study has demonstrated with data from 2023 GC1 that GPH and ADG can be increased when ABG mixtures are grazed with less days of regrowth and during active growth in the fall, as opposed to deferred grazing (Burt et al., 2025), due to increased forage nutritive value.

In this evaluation, stocking rates were higher than previously reported (Burt et al., 2022; Rushing et al., 2022). Forage allowance dropped below the target of 1 kg DM per kg BW during GC2 of 2022 and GC1 of 2023 due to difficulties associated with closely estimating available DM in forage mass of grass-legume mixtures with a pasture ruler, which has been previously documented in ABG mixtures (Burt et al., 2022b). Generally, forage allowances reported in this study were similar to the range of forage allowances reported monthly by Beck et al. (2017d). Burt et al. (2024) maintained higher forage allowance in their cut-and-graze treatment throughout the season, and in their deferred grazing period (Burt et al., 2025). Previously, Bates et al. (2013) evaluated pure alfalfa stands in Tifton, GA, stocked to target a low, medium, or high forage allowance. The low forage allowance (1 kg DM per kg BW) treatment resulted in decreased ADG, but increased stocking rates, grazing days, and gain per acre in years 2 and 3 after establishment (Bates et al., 2013); however, the authors noted that alfalfa stand density decreased and weed pressure increased under the low forage allowance treatment. This highlights the trade-off between individual animal performance as measured by average daily gain or the system's overall performance by finding the optimal stocking rates and gain per hectare for each producer, depending on their economic and production goals.

Conclusion

Overall, ABG research in the Deep South has documented the mixture provides forage for grazing and stored forage production from March to November. The mixture may not be the right choice for every producer, as alfalfa requires a higher soil pH, increased management, and is less tolerant of continuous grazing than other perennial forages commonly used in the area. Previous studies have demonstrated that alfalfa can be an economical choice, and this study demonstrated that ABG mixtures under a dual-use management strategy provided high quality forages that were grazed from September into November during a regional drought, a time when there were few other grazeable forage options. Thus, this research documented ABG mixtures, when managed as a dual-use cut-and-graze system can fill the summer to fall forage transition period that is challenging for producers in the region. Future research looking at timing of grazing initiation after a baleage harvest to pinpoint the optimum trade-off between forage maturity and quality and the associative impacts on stocking rates and animal performance is warranted. Higher overall animal and system performance during 2023 suggests that targeting 28 to 35 days of rest between a previous harvest and grazing would increase forage quality and animal gains. Additionally, further studies refining prediction of dry matter yield by either a pasture ruler or other non-destructive tool would help future researchers and producers in better achieving desired forage allowance in-field, as current prediction equations did not accurately calculate desired forage allowance during this study. Finally, increased GPH and ADG during 2023, when FA was 0.8, would suggest future studies should evaluate forage allowance, animal performance, and system performance to determine optimal stocking strategies on an economic basis when utilizing improved alfalfa and bermudagrass varieties.

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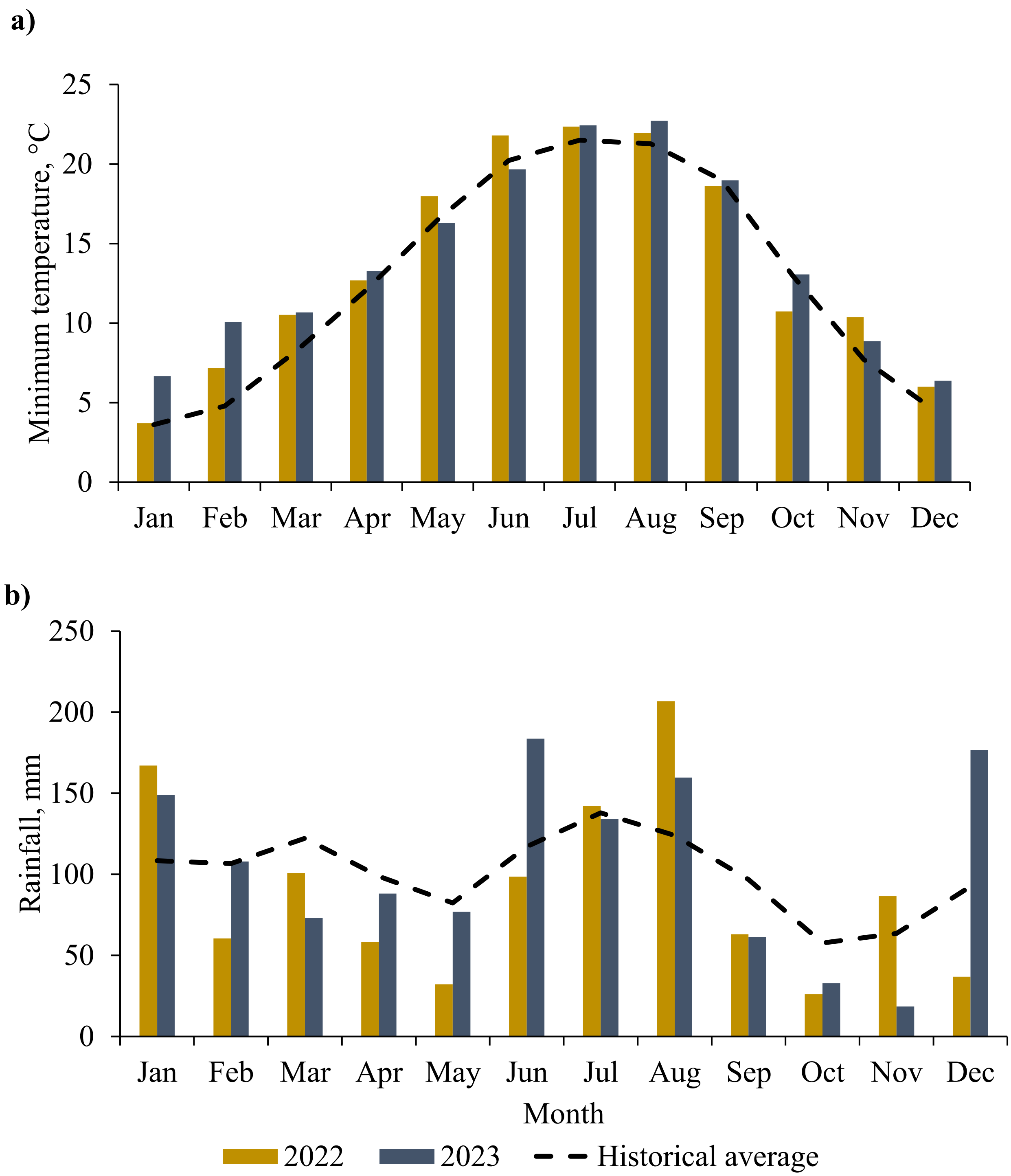

Figure 4.1 a) Average minimum temperatures and b) total monthly rainfall during 2022, 2023, and the 100-year average for Tifton, GA. Data were collected from University of Georgia Automated Environmental Monitoring Network (UGA-AEMN, 2020)

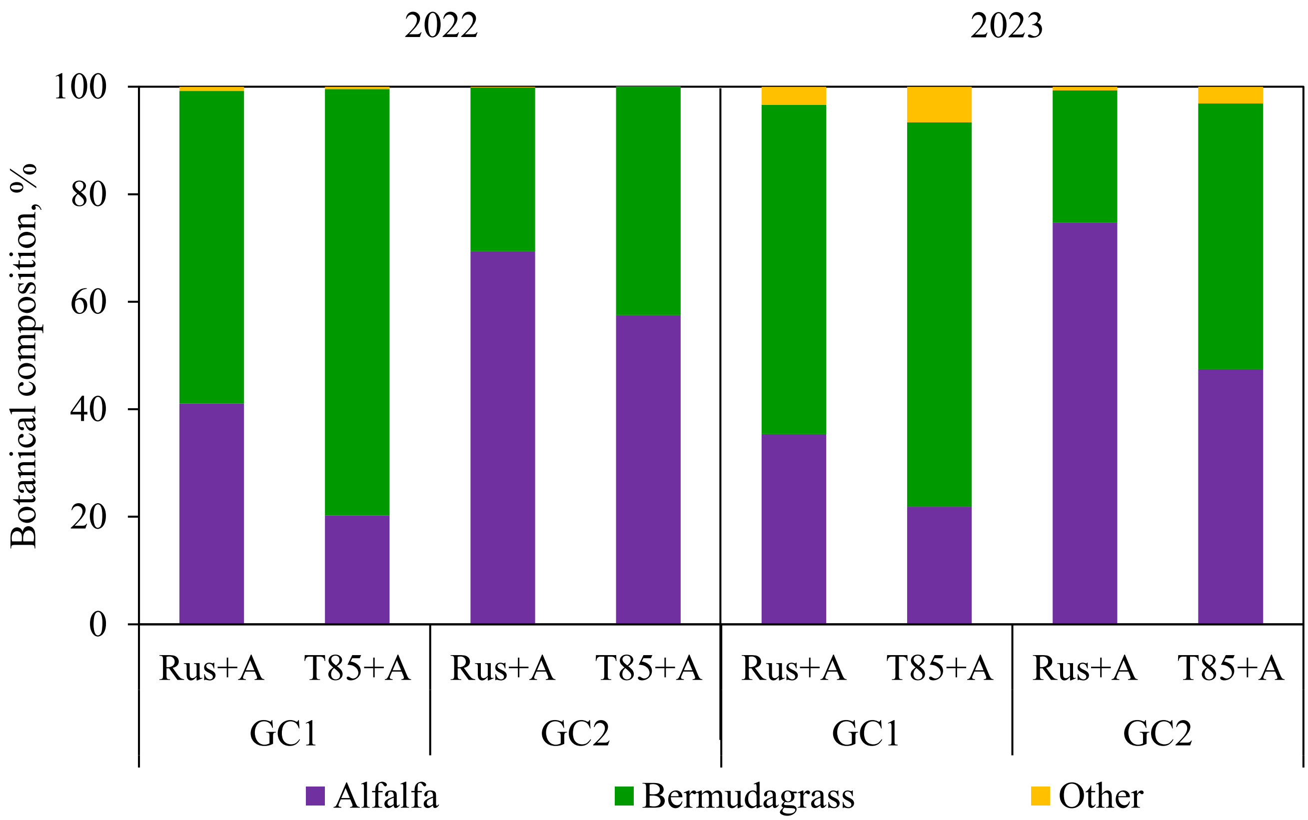

Figure 4.2 Botanical composition (alfalfa, bermudagrass, and other [any other forage or weed]) for Russell (Rus+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with ‘Bulldog 805’ alfalfa and rotationally grazed in two grazing cycles (GC) during fall 2022 and 2023 in Tifton, GA

Table 4.1 Forage mass for three- and four-year-old Russell (RUS+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with 'Bulldog 805' alfalfa and rotationally grazed for two grazing cycles (GC) in fall 2022 and 2023 in Tifton, GA

Forage mass, kg · ha^{-1}	Grazing cycle 1		Grazing cycle 2		SEM[1]	P-value[2]		
	T85+A	RUS+A	T85+A	RUS+A		BC	GC	BC×GC
2022	4473[a]	3323[b]	1872[c]	1900[c]	99.3	<0.01	<0.01	<0.01
2023	3109[a]	2713[b]	1813[c]	1901[c]	93.8	<0.01	<0.01	0.11

[1]Standard error of the mean.
[2] BC: bermudagrass cultivar; GC: grazing cycle.
[a-c]Letters denote differences within a row at $P < 0.10$.

Table 4.2 Seasonal pregraze nutritive value using near-infrared spectroscopy (NIRS) analyses of three- and four-year-old Russell (Rus+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with 'Bulldog 805' alfalfa and rotationally grazed for two grazing cycles (GC) in fall 2022 and 2023 in Tifton, GA

	Grazing cycle 1		Grazing cycle 2		SEM[1]	*P*-value[3]		
Item[2], g · kg^{-1}	Rus+A	T85+A	Rus+A	T85+A		BC	GC	BC × GC
2022								
CP	153[a]	119[b]	213[c]	210[c]	4.0	<0.01	<0.01	<0.01
ADF	368[a]	402[b]	309[c]	306[c]	3.7	<0.01	<0.01	<0.01
NDF	566[a]	663[b]	436[c]	455[c]	8.8	<0.01	<0.01	<0.01
IVTD48	688[a]	681[a]	749[b]	768[c]	5.3	0.26	<0.01	0.01
TDN	599[a]	599[a]	633[b]	653[c]	3.7	0.01	<0.01	0.01
2023								
CP	167[a]	137[b]	243[c]	201[d]	4.0	<0.01	<0.01	0.13
ADF	332[a]	357[b]	271[c]	303[d]	3.7	<0.01	<0.01	0.30
NDF	531[a]	602[b]	375[c]	479[d]	9.1	<0.01	<0.01	0.06
IVTD48	714[a]	723[a]	796[b]	768[c]	5.2	0.05	<0.01	<0.01
TDN	627[a]	642[b]	675[c]	658[d]	3.8	0.91	<0.01	<0.01

[1]Standard error of the mean.
[2]Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; IVDMD48, *in-vitro* true dry matter digestibility after 48 hours; TDN, total digestible nutrients.
[3]BC: bermudagrass cultivar; GC: grazing cycle.
[a-d] Letters denote differences within a row at $P < 0.10$.

Table 4.3 Average daily gain (ADG), seasonal gain per hectare (GPH), seasonal stocking rate (SR), and seasonal forage allowance (FA) of stocker calves rotationally grazing three- and four-year-old Russell (Rus+A) or Tifton-85 (T85+A) bermudagrass pastures interseeded with 'Bulldog 805' alfalfa for two grazing cycles (GC) in the fall 2022 and 2023 in Tifton, GA

	Grazing cycle 1		Grazing cycle 2		SEM[1]	P-value[2]		
Item	Rus+A	T85+A	Rus+A	T85+A		BC	GC	BC × GC
2022								
ADG, $kg \cdot d^{-1}$	0.7	0.6	0.5	0.6	0.27	0.76	0.24	0.22
GPH, $kg \cdot ha^{-1}$	111[a]	138[a]	55[c]	97[ab]	14.5	0.06	0.01	0.60
SR, $kg \cdot ha^{-1}$	1727[a]	2931[b]	1459[c]	1631[ac]	85.4	< 0.01	<0.01	<0.01
FA, $kg \cdot kg^{-1}$	1.3[a]	1.1[b]	0.8[c]	0.8[c]	0.06	0.05	<0.01	0.03
2023								
ADG, $kg \cdot d^{-1}$	1.2[a]	1.0[a]	0.4[b]	1.1[a]	0.23	0.01	0.01	<0.01
GPH, $kg \cdot ha^{-1}$	272[a]	269[a]	34[c]	121[b]	16.7	0.09	<0.01	<0.01
SR, $kg \cdot ha^{-1}$	2091[a]	2490[b]	1237[c]	1133[c]	90.3	0.01	<0.01	0.02
FA, $kg \cdot kg^{-1}$	0.7[a]	0.8[a]	0.9[a]	1.2[b]	0.07	< 0.01	<0.01	0.03

[1]Standard error of the mean
[2] BC: bermudagrass cultivar; GC: grazing cycle
[a-c] Letters denote differences within a row at $P < 0.10$

CHAPTER 5

EFFECTS OF *LACTOBACILLUS ACIDOPHILUS* FERMENTATION PRODUCT PRESERVATIVE APPLICATION AT MOWING ON MIXED RUMINAL MICROORGANISM *IN VITRO* FERMENTATION OF ALFALFA BERMUDAGRASS BALEAGE

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Abstract

In vitro fermentation techniques allow for evaluation of ruminal degradation of forages on a small scale to guide decisions on which treatments justify further labor-intensive grazing or feeding trials. Southeastern producers are interested in utilizing forage preservatives; however, to date, ruminal degradation of preservative treated forages has not been widely evaluated. Thus, an *in vitro* ruminal fermentation study was conducted in April 2024 utilizing forage preserved in individual mini-silos from a July 2022 cutting of 'Bulldog 805' alfalfa interseeded into 'Tifton-85' bermudagrass (ABG) in Tifton, GA. The objectives of the study were to evaluate in-vitro ruminal fermentation parameters for 1) ABG sampled at different time points post-harvest and 2) ABG receiving forage preservative application. The experimental design was a completely randomized design with treatments arranged in a 2 x 3 factorial (preservative treatment: forage type). The treatments evaluated were ABG receiving Promote® HayDefender™ (Cargill Animal Nutrition; Minneapolis, MN) forage preservative (P+) or no preservative (NP) at mowing and then sampled at harvest (Initial), 8 weeks post-harvest (8W), and 6 months post-harvest (6M). Forage was lyophilized and ground to pass a 1 mm screen on a Wiley grinder. Samples were fermented in individual anaerobic mixed ruminal microorganism fermentations in bottles sealed with butyl rubber stoppers and aluminum crimps in triplicate at 4 time points: 2, 4, 24, and 48 hours of fermentation. Total gas production was greater for Initial (59 mL) as compared to 8W (52 mL) and 6M (47 mL), respectively, after 48 hours of fermentation ($P < 0.04$). Methane production at 24 hours was greater for P+ (7.1 mmol) as compared to NP (6.6 mmol; $P < 0.01$), but did not differ between treatments at 48 hours ($P > 0.20$). IVDMD at 48 hours differed by forage type ($P = 0.02$), but not preservative treatment ($P = 0.39$), in that Initial (755 g kg^{-1}) forage had greater IVDMD ($P < 0.01$) than 6M (719 g kg^{-1}) with 8W (740 g kg^{-1}) not different from Initial or 6M ($P > 0.06$). This

preliminary data suggests that application of preservatives to forages does not improve the characteristics of the *in vitro* mixed ruminal microorganism fermentation; however, future research examining feeding cattle ABG baleage is warranted to expand upon this introductory experiment.

Introduction

Forage preservatives have been widely evaluated for their use at baling in hay and silage production; however, less research has explored utilizing preservative application in baleage (Kung et al., 2003; Muck et al., 2017). Southeastern bermudagrass hay producers reported that they were applying a *Lactobacillus acidophilus* fermentation product forage preservative (Promote® HayDefenderTM, Cargill Animal Nutrition; Minneapolis, MN) at mowing in bermudagrass (*Cynodon spp.*) pastures to decrease dry down time (Stefancik et al., 2024); however, replicated research trials to evaluate this claim have not been published.

Limited information is published on commercially available microbial-based forage preservatives products. Previously, two studies that have specifically evaluated different forms of *Lactobacillus acidophilus* as silage additives. Moon et al. (1981) found that inoculation increased lactic acid concentration in corn silage, but not alfalfa silage (Moon et al., 1981). An additional study in Alabama, evaluated Promote® HayDefenderTM (Cargill Animal Nutrition; Minneapolis, MN), a *Lactobacillus acidophilus* fermentation product, and noted that dry matter recovery in ryegrass baleage tended to increase with inoculation, but inoculation did not change other fermentation parameters as compared to the control (Griffin et al., 2018).

While previous research evaluating ruminal fermentation of alfalfa silage diets with and without additives is fairly extensive, there are fewer studies that evaluated ruminal fermentation of alfalfa bermudagrass (**ABG**) mixtures harvested as baleage (Muck et al., 2017; Hendricks et al. 2021; Henson et al., 2024). Many southeastern beef producers utilize hay as their primary stored forage option; however, there are an increasing number of producers who utilize baleage to preserve surplus forage (NASS, 2022; Mullenix, 2023). Baleage allows producers a shorter cut-to-bale time frame, increases the forage quality produced because the increased moisture at baling allows for higher leaf retention, and plastic wrapped bales can be stored outside with limited weathering losses (Coblentz and Akins, 2017; Tucker et al., 2020). No studies have evaluated the *in vitro* mixed ruminal microorganisms fermentation of ABG baleage receiving forage preservative application. Thus, the objective of this study was to evaluate *in vitro* mixed ruminal microorganisms fermentation parameters when a microbial-based forage preservative was applied at mowing in ABG mixtures harvested as baleage.

Materials and Methods

All procedures involving animals were approved by the University of Georgia's Office of Animal Use (AUP #: A2022 03-013-Y3-A0).

Experimental location and design

This research was conducted at the University of Georgia Tifton Campus "Better Grazing Program" (Tifton, GA; 31° 30' N 83° 32' W; 100 m elevation) on previously established ABG mixtures (Burt et al., 2024). Treatments evaluated included ABG forage harvested as baleage with or without forage preservative application at mowing, and sampled at harvest (initial), 8 weeks post-harvest, or 6 months post-harvest baleage fermented in mini silos.

Forage management and sampling

In July 2022, 'Tifton-85' bermudagrass pastures interseeded with 'Bulldog 805' alfalfa were harvested with whole paddocks assigned to a preservative (**P+**) or no preservative (**NP**) treatment. Detailed field and sample management information can be found in Chapter 2. Briefly, for the P+ treatment, the preservative solution was a 3.4% liquid *Lactobacillus acidophilus* fermentation product (Promote® HayDefenderTM (Cargill Animal Nutrition; Minneapolis, MN) applied at mowing and 28.4 liters of solution was applied per paddock. When forage reached 65% moisture and before raking began, forage was representatively hand-collected from two 1.0-hectare paddocks. Botanical composition for sampled paddocks are listed in Table 4.1. Forage was taken into a climate-controlled building and mixed in individual totes assigned to each paddock. Forage samples to represent the crop at baling were immediately placed into the freezer (initial). Additional forage samples were randomly taken from the tote and placed into individual resealable plastic storage bags to create mini silos, which were then placed into larger vacuum sealed plastic bags.

Subsequent sampling of the mini silos occurred at 8-weeks (**8W**) and 6-months (**6M**) post-harvest. At the sampling time, forage was equally split from the mini silo and half was dried at 55°C for 4 days, and the other half was frozen. Frozen initial, 8-week, and 6-month samples were lyophilized (Freezone 6L Bulk Tray Freeze Dryer; Labconco, Kansas City, MO) and ground using a Wiley Mill to pass a 1 mm screen.

In vitro experimental procedures

Samples (N=6) were evaluated in triplicate at 4 time points: 2, 4, 24, and 48 hours of fermentation in mixed ruminal organisms (Callaway et al., 1997). Approximately 0.5 g of each treatment were weighed into acetone-rinsed and heat-sealed nylon bags (F57 Ankom Fiber Filter Bag; Ankom Technology, Macedon, NY). Two blank samples were evaluated at each time point, where empty acetone-rinsed and heat-sealed nylon bags were used for blank bag correction factors. Fiber bags were placed into individual 120 ml glass serum bottles and filled with 60 ml of mixed ruminal media. Fiber bags were submerged into the mixed ruminal fluid in the bottle. Bottles were sealed with butyl rubber stopper and metal crimp. The mixed rumen microorganisms fluid was obtained in the morning from beef steers grazing annual ryegrass mixed pastures at the University of Georgia Animal Science Farm (Tifton, GA). The steers had ad libitum access to water and mineral and received no concentrate feeds. Ruminal fluid was strained through eight layers of cheesecloth during in-field collection and transferred in a warmed thermos to the laboratory. Supernatant fluid containing mixed ruminal bacteria and small forage particles was transferred anaerobically to a medium containing (per liter): 292 mg $K_2HPO_4 \cdot 3H_2O$, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2 \cdot 2\ H_2O$, 600 mg of cysteine hydrochloride, and 4 g of Na_2CO_3. The final ruminal fluid concentration was 33% (vol/vol), and pH was 6.5. Bottles were placed in an oscillating incubator (Thermo Scientific MaxQ 6000, Thermo Fisher Scientific, Waltham, MA) at 39°C for the allotted time (2, 4, 24, or 48 hours) at 60 RPM.

Following the incubation time, total gas production was measured using a lubricated syringe. Total gas production was calculated by subtracting total gas produced by the blank jars from the total gas measured from the sample jars. Total gas production was too low for measurement at the 2-hour sampling time. Thus, only 4-, 24- and 48-hour measurements for total gas and methane production are reported. A five mL subsample of this gas was withdrawn using a gas sealed syringe and analyzed for CH_4 using a gas chromatograph (**GC**; Thermo Fisher Scientific Trace 1310 Gas Chromatograph, Thermo Fisher Scientific, Waltham, MA). Methane was measured using a flame ionization detector and a capillary column (Porapak-Q GC Column, part no. 1518282 Thermo Fisher Scientific, Waltham, MA). Gas flow (N_2) was 5 mL/min, column oven was set at 60°C, and detector temperature was 250°C.

After gas sample collection, bottles were opened, fiber bags were removed and rinsed to stop fermentation, and incubation liquid pH was measured. Fifty mL of incubation liquid was frozen for subsequent VFA and NH_3-N analysis.

Post in vitro fermentation analyses

Following the fermentation, fiber bags were dried at 60°C for 24 hours. Duplicates that did not undergo fermentation were also dried and included in fiber analysis to determine initial (Table 4.1) dry matter (**DM;** AAOC 967.03), crude protein, neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**). Crude protein was analyzed using a LECO FP628 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI; AAOC 990.03). All NDF and ADF concentrations were measured using an Ankom Fiber Analyzer (Model A2000, Ankom Technology, Macedon, NY; Van Soest et al., 1991). After drying, samples were weighed to determine *in vitro* dry matter digestibility (**IVDMD**). NDF and ADF disappearance was calculated by subtracting the final post-digestion NDF from initial NDF and dividing by the initial NDF.

Frozen samples were thawed before analysis for VFA and NH_3-N. Samples for NH_3-N analysis were analyzed using colorimetric determination (Chaney and Marbach, 1962). Samples were incubated at 39°C for 20 minutes, and absorbance was measured at 630 nm using a GENESYS 30 Visible Spectrophotometer (Thermo Scientific, Chadds Ford Township, PA). Samples for VFA analysis were prepared by centrifuging the thawed sample at 5,000 × *g* for 10 minutes at 4°C. Duplicate 2.0 ml aliquots of supernatant were taken and 0.5 ml of ice cold 25% (wt/vol) of metaphosphoric acid solution containing 2-ethylbutyrate as an internal standard was added to the sample. Samples were vortexed, then refrigerated for 30 minutes at 4°C. Samples were centrifuged at 10,000 × *g* for 15 minutes at 4°C. The resulting supernatant was pipetted, transferred to a GC vial, and analyzed by GC equipped with autosampler (Thermo Fisher Scientific Trace 1310 Gas Chromatograph, Thermo Fisher Trace A1 1310 Autosampler) and with a 30-m TG-WAXMS column with internal diameter of 0.25 mm and a film thickness of 0.25µm; using a temperature gradient for analysis. At the beginning of the program, the column temperature was 110°C and was maintained for 1.4 minutes, then column temperature was increased to 190°C at a rate of 24°C per minute. Peak detection was by a flame ionization detector that used a H_2 carrier gas and air flame. Peak identification was determined by relative retention time using quantification by comparison with an external standard of known VFA concentration.

Statistical analysis

All analyses were performed using the fermentation bottle as the experimental unit. The experiment was analyzed as a completely randomized design with each parameter analyzed by incubation time. Data were analyzed by restricted maximum likelihood using PROC MIXED in SAS v 9.4 (SAS Institute Inc., 2013, Littell et al., 2006) with an autoregressive (1) covariance structure, selected based on the lowest Bayesian Information Criterion (Littell et al., 2006). The fixed effects included preservative treatment (**PT**), forage sampling type (**FT**; initial, 8W, 6M), and their interactions (**PT × FT**). The subject of the random statement was the bottle replicate. Denominator degrees of freedom were adjusted using the Kenward-Roger approximation (Kenward and Roger 2009). Means separation was by Tukey's significant difference test, with differences considered significant at $P < 0.05$.

Results and Discussion

Gas production

Total gas production did not differ between FT, PT, or their interaction at 4 hours (Table 4.2; $P > 0.11$). At 24 hours, there were no differences between FT or PT × FT interactions ($P > 0.21$); however, total gas production differed by PT where P+ treatment produced more total gas than NP treatment ($P < 0.02$). After 48 hours, there were no differences between PT or PT × FT interactions ($P > 0.13$), but all FT differed ($P < 0.01$). Gas production at 24 hours was lower than previously reported for a 50:50 alfalfa ryegrass mixture (91.92 ml g^{-1} DM; Garrett et al., 2021).

Previously, Xue et al. (2019) found no difference in total gas production at 48 hours between alfalfa-orchardgrass harvested as hay or silage; however, gas production was greater for a 75% alfalfa-orchardgrass hay treatment (124 to 128 ml g^{-1} DM) than in the present study. These previous studies utilized automated gas measuring equipment which could explain some of the differences in gas production when compared to this study, which utilized sealed bottles and a single gas measurement via syringe. Additionally, different substrates and rumen fluid donors were used.

Methane production was not different between FT, PT, or their interaction at 4 or 48 hours (Table 4.2; $P > 0.26$). At 24 hours, methane concentrations were different between PT ($P < 0.01$), but not different between FT or FT × PT interactions ($P > 0.13$). At 24 hours, NP forage had lower methane production than P+ forage ($P < 0.01$). Increased methane production from P+ forage fermentations could be due to the increased BG prevalence in P+ paddocks, and the subsequent increase in NDF for P+ forage at initial and 8W time points (Shibata and Terada, 2010). Methane concentrations reported at 24 hours in this study are similar to those of Tifton-85 bermudagrass methane concentrations reported at 24 hours (6.2 mmol; Hines et al., 2024), and were lower than reported for Coastal bermudagrass (14.5 mM) at 48 hours (Martin and Nisbet, 1989).

pH

There was a FT × PT interaction at 4 hours for pH ($P < 0.01$), but there were no interactions at any other time (Table 4.2; $P > 0.79$). pH was lower for 6M P+ forage at 4 hours, but there were no differences between any other treatments. After 48 hours, there was a difference ($P < 0.04$) in pH between forage types where initial samples had lower pH than 8W or 6M ($P < 0.04$), which did not differ ($P > 0.68$). While there were minor differences between pH within hour, ruminal pH stayed within the expected range for forage based diets (Kaufmann et al., 1980). pH reported in this study is similar to pH values reported by Hines (2024), 6.5 to 6.8, for Tifton-85 bermudagrass hay and similar to pH reported at 48 hours, 6.3, for Coastal bermudagrass (Martin and Nisbet, 1989).

Ammonia

Ammonia was not different between FT, PT, or their interaction at 2, 24, or 48 hours (Table 4.2; $P > 0.23$). After 4 hours NH_3-N differed ($P < 0.01$) by FT and PT, but there was no FT × PT interaction ($P = 0.29$). Initial samples had lower ($P < 0.01$) NH_3-N as compared to 8-week or 6-month samples, which did not differ ($P = 0.73$). Additionally, NP forage had higher NH_3-N as compared to P+ forage ($P < 0.01$). Increased alfalfa composition in the NP forage could increase NH_3-N, as Xue et al. (2019) found that increasing alfalfa percentage from 0 to 100 % in their alfalfa-orchardgrass *in vitro* experiment increased NH_3-N. Hines (2024) reported peak ammonia production (8 mg/100mL) for Tifton-85 bermudagrass occurred 3 hours after feeding, then ammonia decreased in their *in vivo* study. Ammonia production reported in this study at 4 hours (6 to 8 mg/100mL) is similar to Hines (2024), but in this study ammonia continued to increase up to the 48 hours sampling point due to the lack of end product removal in the *in vitro* system. Ammonia production of coastal bermudagrass *in vitro* at 48 hours was previously reported at 237.8 mg/L (Martin and Nisbet, 1989), whereas in this study the maximum ammonia concentration was 129 mg/L after 48 hours.

In vitro dry matter, ADF, and NDF digestibility

IVDMD differed by FT and PT at 2 and 4 hours but did not differ after 24 hours (Table 4.3; $P < 0.01$). There was a FT × PT interaction at 2 hours where mini silo fermented NP forage had higher IVDMD as compared to all other samples ($P < 0.02$); however, there were no FT ×

PT interactions for 4, 24, or 48 hours (*P* > .15). After 48 hours of fermentation, FT differed where initial samples had greater (*P* < 0.01) IVDMD than 6M. The 8W treatment did not differ from initial or 6M (*P* > 0.07). Digestibility in this study is higher than reported for Tifton-85 bermudagrass hay (58.4%) by Hines (2024); however, increased DMD from inclusion of alfalfa into bermudagrass has been previously documented (Henson et al., 2024). Previously, it was reported that alfalfa-orchardgrass mixtures when preserved as silage had greater IVDMD as compared to hay when fermented *in vitro* for 48 hours (Xue et al., 2019). In this study, baleage had higher IVDMD at 2 and 4 hours, as compared to initial, which would be comparable to hay. However, in contrast to Xue et al. (2019), after 48 hours, Initial had increased IVDMD as compared to baleage. Thus, this data documents that forage preserved as baleage is digested more rapidly immediately after feeding, whereas hay may have higher total digestibility if rumen retention time allows for hay to be fermented over 24 hours.

There was a FT × PT interaction for NDF disappearance at 4 hours, and FT differed at 2, 4, and 48 hours (Table 4.3, *P* < 0.01). There was a PT main effect at 2 and 4 hours (*P* < 0.01) in that NP forage had greater NDF disappearance, but PT did not differ after 24 hours (*P* > 0.37). The 6M P+ did not have measurable NDF disappearance until 24 hours of fermentation, while all other samples had measurable disappearance starting at 2 hours. At 4 hours, the FT × PT interaction was caused by 6M P+ not having measurable NDF disappearance as compared to all other samples (*P* < 0.01), which did not differ (*P* > 0.21). At 2 hours, all FT differed (*P* < 0.01), where 8W had increased NDF disappearance as compared to Initial and 6M (*P* < 0.04). NP forage had greater disappearance after 2 hours (*P* < 0.01). At 48 hours, Initial and 8W did not differ in NDF disappearance (*P* = 0.14), but had greater disappearance than 6M samples (*P* < 0.01).

There was an FT × PT interaction for ADF disappearance after 2 and 4 hours of fermentation (Table 4.4; $P \leq 0.01$), where preservative application did not affect ADF disappearance for Initial or 8W ($P > 0.43$), but preservative application decreased ($P < 0.01$) ADF disappearance in 6M baleage. There were no differences in ADF disappearance at 24 hours ($P > 0.18$). After 48 hours, Initial and 8W did not differ ($P = 0.30$), but had greater ADF disappearance than 6M ($P < 0.05$). The ADF and NDF disappearance in this study is less than that of Coastal bermudagrass digested for 48 hours, where ADF and NDF digestibility was 59.9 and 60.5%, respectively (Martin and Nisbet, 1989). Disappearance of ADF (55.8%) and NDF (57.7%) reported by Henson et al. (2024) for ABG baleage is greater than reported in the current study. Decreased NDF and ADF digestibility could be explained by the high percentage of alfalfa in the mixture, as many studies have evaluated increasing legume contents of both cool and warm season grass-based mixtures and found that NDF digestibility decreased as legume content increased (Xue et al., 2019; Bhatti et al., 2007; Bowman and Asplund, 1987). This is because legumes have an increased undigestible portion of NDF as compared to grasses, which have a higher potentially degradable NDF fraction (Buxton and Redfearn, 1997; Bhatti et al., 2007). Finally, this experiment utilized Ankom F57 bags during incubation time, where previous experiments mixed forage directly into the rumen fluid solution and filtered then dried and determined NDF; differences in NDF digestibility could be due to microbial exclusion of the Ankom F57 bags (Schlau et al., 2020; Valentine et al., 2018).

VFA production

Total VFA production was not different between FT, PT, or FT × PT at 2, 4, or 24 hours (Table 4.4; $P > 0.11$). Total VFA production differed between FT and PT, but there was no FT × PT interaction at 48 hours ($P < 0.01$). NP had more total VFA production than P+ ($P = 0.02$).

All FT had different ($P \leq 0.05$) total VFA after 48 hours, where Initial produced more total VFA as compared to 8W and 6M samples. Xue et al. (2019) found that increasing alfalfa in a grass-legume mixture increased total VFA concentrations but found no difference between forage preservation method. Total VFAs reported in this study are lower than those of Xue et al. (2019), 110 to 120 mM/L, but were similar to total VFA (66.8 mM) sampled from ruminally-fistulated heifers on a Tifton-85 bermudagrass diet (Hines, 2024). Similar to our results after 48 hours, Martin et al. (2015) reported that bermudagrass hay (95.8 mM/L) produced higher total VFA concentrations as compared to bermudagrass baleage (78.9 mM/L).

Acetate and butyrate production were not different between FT, PT, or their interaction after 2, 4, or 24 hours of fermentation ($P > 0.14$). Acetate production differed after 48 hours for FT ($P < 0.01$), PT ($P = 0.01$), but there was no interaction ($P = 0.29$). Initial and 8W samples did not differ ($P = 0.10$), but both had higher acetate production than 6M samples ($P < 0.03$). NP had higher acetate production as compared to P+ ($P < 0.02$). Initial, 8W, and 6M forage differed (P < 0.02) in butyrate production after 48 hours. NP forage had higher butyrate production than P+ forage after 48 hours ($P < 0.01$). Acetate production in the current study after 24 hours is less than reported by Hines (2024) at 24 hours (48.1 mM) after feeding; however, our 48 hour acetate production (46.5 mM) similar to their 24 hour measurement. Butyrate concentrations reported in this study (5.5 to 8.1 mM) were similar to previously reported from 2 to 24 hours (4 to 6.5 mM; Hines, 2024) and to 48 hour butyrate (8 mM) reported by Martin and Nisbet (1989), but lower than reported at 48 hours (9 to 10 mM) by Xue et al. (2020)

There was a FT × PT interaction for propionate production after 2 hours, where initial NP samples had lower propionate production as compared to all other samples ($P < 0.01$). There were no differences in propionate production between FT, PT, or their interaction after 4 or 24 hours ($P > 0.40$). After 48 hours, propionate production differed by FT and PT ($P \leq 0.01$), but there was no FT × PT interaction ($P = 0.22$). At 48 hours, Initial forage produced more propionate as compared to 8W or 6M ($P < 0.03$), and NP forage produced more propionate than P+ samples ($P = 0.01$). Propionate production in this study was similar to *in vivo* production from 0 to 24 hours post-feeding as reported by Hines (2024) for Tifton-85 bermudagrass (8 to 11 mMol/L), and lower than propionate concentrations (17.2 to 19.5 mMol/L; 20.08 mMol/L) reported by Xue et al. (2020) and Martin and Nisbet (1989), respectively. Xue et al. (2020) found no differences in propionate production as alfalfa proportion increased, and in contrast to the current study, found no differences between silage or hay.

Conclusions

Overall, the addition of microbial-based forage preservative at mowing did not improve *in vitro* mixed ruminal microorganism fermentation for ABG mixtures when evaluated *in vitro*. Ruminal fermentation parameters mainly differed between forage sampling time, pre-ensiling or post-ensiling. The ABG preserved in mini silos had increased digestibility in the early stages of fermentation; however, after 48 hours, the initial samples had greater total digestibility suggesting that ABG mixtures preserved as hay may be more digestible than baleage if rumen retention time exceeds 24 hours. Total VFA production was also increased for initial as compared to mini silos after 48 hours. Additional research evaluating rumen retention time, VFA production, and digestibility of ABG mixtures preserved at varying levels of alfalfa inclusion and preserved as hay or silage would help further explain results documented in this pilot study.

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Table 5.1 Nutritive value and field botanical composition of Bulldog 805 alfalfa interseeded into Tifton-85 bermudagrass harvested, treated with (P+) or without (NP) *Lactobacillus acidophilus* based forage preservative at mowing, and packed into mini silos (n=6) that were sampled at three time points (at harvest [initial], 8-weeks, or 6-months post-harvest).

	Initial		8week		6month	
Item, %	NP	P+	NP	P+	NP	P+
Mini Silo dry matter	42	41	-	-	42	35
Lab dry matter	95.8	95.3	92.8	93.8	92.2	94.2
Crude Protein	21.5	20.7	22.3	20.5	23.2	21.8
NDF	43.8	46.0	43.5	45.7	42.5	42.4
ADF	25.6	28.9	26.9	28.0	29.4	28.7
Alfalfa	72	65	-	-	-	-
Bermudagrass	28	35	-	-	-	-

Table 5.2 Total gas, methane, pH, and ammonia produced by alfalfa bermudagrass sampled at three time points (at harvest [initial], 8-weeks, or 6-months post-harvest) and treated with (P+) or without (NP) *Lactobacillus acidophilus* based forage preservative at mowing and 0.5g forage fermented *in vitro* with mixed ruminal microorganisms(n = 72) for 2, 4, 24, and 48 hours

	Initial		8week		6month		SEM[1]	*P* - value[2]		
Item	NP	P+	NP	P+	NP	P+		FT	PT	FT×PT
Total Gas[3], mL										
4h	9	13	9	10	8	8	1.2	0.17	0.12	0.38
24h	44[abc]	49[ab]	43[bc]	49[a]	42[c]	44[abc]	2.8	0.21	0.02	0.49
48h	58[a]	60[a]	54[ab]	49[bc]	46[c]	49[bc]	2.0	<0.01	0.95	0.13
Methane, mM										
4h	1.5	1.4	1.4	1.4	1.5	1.6	0.08	0.27	0.91	0.74
24h	6.4[a]	6.9[ab]	6.5[a]	7.4[b]	7.0[ab]	7.2[b]	0.24	0.13	0.01	0.32
48h	7.3	7.0	6.8	7.3	6.8	7.1	0.26	0.72	0.47	0.30
pH										
2h	6.76	6.78	6.76	6.73	6.76	6.73	0.029	0.51	0.32	0.98
4h	6.71[a]	6.73[a]	6.73[a]	6.73[a]	6.71[a]	6.64[b]	0.012	<0.01	0.09	<0.01
24h	6.49	6.49	6.50	6.49	6.49	6.48	0.013	0.81	0.54	0.79
48h	6.43[ab]	6.43[a]	6.51[b]	6.48[ab]	6.50[b]	6.47[ab]	0.026	0.04	0.26	0.88
Ammonia, mM										
2h	3.80	4.09	3.47	3.79	3.77	3.95	0.253	0.46	0.23	0.96
4h	3.93[cd]	3.74[d]	4.55[a]	4.04[cd]	4.45[ab]	4.22[bc]	0.103	<0.01	<0.01	0.29
24h	8.16	7.74	7.62	8.35	8.36	8.23	0.413	0.65	0.86	0.38
48h	7.36	7.59	7.62	7.24	7.60	7.23	0.294	0.98	0.48	0.52

[1]SEM: Standard error of the mean.
[2]FT: Forage type; PT: Preservative treatment.
[3]Gas production was not measurable at 2 hours, thus no data for gas or methane is reported.
[a-d]Letters denote differences within rows at $P < 0.05$.

Table 5.3 *In vitro* dry matter degradation of alfalfa bermudagrass sampled at harvest [initial], 8-weeks, or 6-months post-harvest and treated with (P+) or without (NP) *Lactobacillus acidophilus* based forage preservative at mowing and 0.5g forage fermented *in vitro* with mixed ruminal microorganisms (n = 72) for 2, 4, 24, and 48 hours

	Initial		8week		6month		SEM[1]	*P* – value[2]		
Hours	NP	P+	NP	P+	NP	P+		FT	PT	FT×PT
2	57.0[c]	54.4[d]	57.7[ab]	55.3[c]	58.0[a]	57.3[bc]	0.19	<0.01	<0.01	<0.01
4	58.0[a]	55.4[b]	58.2[a]	55.8[b]	58.9[c]	57.2[d]	0.23	<0.01	<0.01	0.19
24	68.7	66.2	67.7	66.7	69.1	67.2	1.50	0.66	0.06	0.77
48	77.0[a]	73.9[ab]	74.4[ab]	73.6[b]	71.1[b]	72.6[b]	1.49	0.02	0.39	0.15

[1] SEM: Standard Error of Mean.
[2] *P* – values represent forage type (FT), preservative treatment (PT) and their interaction.
[a-d] Letters represent differences within rows at $P < 0.05$.

Table 5.4 ADF and NDF disappearance of alfalfa bermudagrass mixtures sampled at harvest [initial], 8-weeks, or 6-months post-harvest and treated with (P+) or without (NP) *Lactobacillus acidophilus* based forage preservative at mowing and 0.5g forage fermented *in vitro* with mixed ruminal microorganisms (n = 72) for 2, 4, 24, and 48 hours

Item	Initial		8week		6month		SEM[1]	*P* – value[2]		
	NP	P+	NP	P+	NP	P+		FT	PT	FT×PT
NDF disappearance[3], %										
2h	2.0^{abc}	0.9^{c}	2.8^{a}	2.2^{ab}	1.3^{bc}	-0.7^{d}	0.45	<0.01	<0.01	0.31
4h	4.1^{a}	3.0^{a}	3.8^{a}	3.3^{a}	3.3^{a}	-0.9^{b}	0.53	<0.01	<0.01	<0.01
24h	28.7	26.6	25.6	27.1	27.3	22.8	2.38	0.56	0.39	0.48
48h	47.5^{a}	43.3^{a}	41.1^{ab}	42.2^{ab}	32.1^{c}	35.5^{bc}	1.19	<0.01	0.95	0.30
ADF disappearance, %										
2h	3.1^{bc}	2.5^{c}	3.9^{bc}	4.3^{ab}	5.5^{a}	2.6^{c}	0.52	0.04	0.03	0.02
4h	3.6^{b}	2.4^{bc}	3.6^{b}	3.8^{b}	7.0^{a}	0.9^{c}	0.59	0.28	<0.01	<0.01
24h	27.7	26.1	27.4	26.9	31.6	24.7	2.57	0.88	0.18	0.44
48h	47.9^{a}	44.5^{ab}	43.5^{ab}	43.7^{ab}	37.5^{b}	39.0^{b}	2.42	0.02	0.72	0.58

[1]Standard error of the mean.
[2] *P* – values represent forage type (FT), preservative treatment (PT) and their interaction.
[3] Disappearance represents the difference between initial and final fiber at each time point.
[a-d] Letters represent differences within a row at $P < 0.05$.

Table 5.5 Volatile Fatty Acid concentration of alfalfa bermudagrass mixtures sampled at harvest [initial], 8-weeks, or 6-months post-harvest and treated with (P+) or without (NP) *Lactobacillus acidophilus* based forage preservative at mowing and 0.5g forage fermented *in vitro* in mixed ruminal microorganisms (n = 72) for 2, 4, 24, and 48 hours

Item	Treatment						SEM	*P* - value		
	Initial		8week		6month					
	NP	P+	NP	P+	NP	P+		FT	PT	FT×PT
Total VFA, mM										
2h	45.3	49.7	49.9	50.7	50.0	50.0	1.90	0.11	0.15	0.26
4h	47.2	39.9	44.7	44.5	41.1	43.9	4.63	0.82	0.57	0.31
24h	58.6	63.5	61.6	67.6	66.7	59.7	6.49	0.74	0.73	0.33
48h	74.4^{a}	68.9^{ab}	66.9^{ab}	62.8^{b}	63.8^{b}	49.5^{c}	4.46	<0.01	<0.01	0.25
Acetic Acid, mM										
2h	29.7	32.6	32.7	33.1	32.6	32.8	1.26	0.15	0.14	0.30
4h	30.7	25.9	28.4	28.7	26.3	28.2	2.99	0.81	0.63	0.29
24h	36.4	39.9	39.1	42.9	42.5	38.3	4.00	0.59	0.66	0.31
48h	46.5^{a}	43.1^{ab}	42.3^{ab}	40.1^{b}	40.2^{b}	31.6^{c}	2.87	<0.01	0.01	0.29
Propionic Acid, mM										
2h	8.5^{a}	9.8^{b}	9.7^{b}	9.7^{b}	9.8^{b}	9.8^{b}	0.35	0.04	0.08	0.04
4h	9.4	7.9	8.9	9.1	8.6	9.0	1.07	0.88	0.63	0.42
24h	12.7	13.4	12.7	13.5	13.7	12.0	1.42	0.96	0.99	0.41
48h	16.5^{a}	15.5^{ab}	14.2^{bc}	13.4^{c}	13.7^{bc}	10.5^{d}	0.96	<0.01	0.01	0.22
Butyric Acid, mM										
2h	5.5	5.7	5.8	6.0	5.9	5.8	0.29	0.22	0.60	0.59
4h	5.5	4.7	5.1	5.3	4.8	5.2	0.49	0.87	0.82	0.24
24h	7.1	7.6	7.1	8.1	7.6	6.8	0.78	0.72	0.55	0.30
48h	8.5^{a}	7.7^{ab}	7.5^{ab}	6.9^{b}	7.2^{b}	5.4^{c}	0.49	<0.01	<0.01	0.26

[1]Standard error of the mean.
[2] *P* – values represent forage type (FT), preservative treatment (PT) and their interaction.
[a-d] Letters represent differences within a row at $P < 0.05$.

CHAPTER 6

CONCLUSIONS AND IMPLICATIONS

While alfalfa has grown in Georgia to some degree from the 1850's to today, many producers are still hesitant to incorporate alfalfa into their production systems. The cultural belief that alfalfa doesn't grow well in Georgia stems from significant stand losses due to insect and disease pressures and the historical use of un-adapted varieties in the challenging southeastern environment; however, improved pest control options and adapted alfalfa varieties bred to tolerate the harsh climate were released in the early 2000's. Research evaluating alfalfa-bermudagrass (ABG) mixtures has demonstrated the economic and livestock performance benefits as compared to BG monocultures, yet producers are still hesitant to change from traditional warm-season grass production systems. There are producers across the southeast who are successfully incorporating improved alfalfa varieties into their production systems and many of these producers are sharing the benefits of alfalfa production in the Coastal Plains with their peers.

Previous research on ABG mixtures in the Deep South has documented forage production contributions from March to November in the calendar year. More recently work in Tifton identified that a cut-and-graze harvest management strategy allows for both stored forage production and strategic grazing to better maximize on the production system. The spring and summer baleage harvests associated with this system provided a high-quality stored feed option for feeding at a later time. However, the deferred fall grazing in that work had less than economical stocker calf gains, most likely due to increased forage maturity that resulted in lower forage quality. Additionally, while research across the United States has evaluated silage preservatives and inoculants applied to ensiled cool-season forages, there is less information available on utilizing these additives in warm-season and mixed stands when harvested as silage or baleage.

In the current study, utilizing forage preservatives and inoculants did not improve forage nutritive value; however, harvesting ABG mixtures as baleage allowed for timely harvests, strategic rest periods, and some increases in nutritive value for ABG preserved as baleage in mini silos were documented. Additionally, harvested forage was high quality and should support beef cows at all stages of production, or to meet the needs for moderate weight gain in growing animals. Forage mass, botanical composition, and forage quality were documented for three- and four-year-old stands in Tifton, GA. Alfalfa prevalence was maintained into year four in this study, which shows promise to producers and researchers to be able to manage for the longevity of ABG stands in the Deep South.

While ABG mixtures can provide high quality stored feed, there is a producer identified grazable forage deficit during the summer to fall forage transition. Previous studies demonstrated that stockpiling or deferred grazing ABG mixtures is challenging because the Deep South does not always experience a hard freeze between September and December. The current study demonstrated that ABG mixtures could be grazed during alfalfa's active growth from September to November, potentially providing grazing for 60 days while maintaining stocker calf gains at 1 kg head^{-1} day^{-1}. Thus, when managed as a dual-use cut-and-graze system, ABG can fill the summer to fall forage transition period that is challenging for producers in the region.

As technological advances make baleage production more accessible to beef producers, there is increasing interest in additive products that may improve baleage fermentation. Research evaluating how silage additives affect ruminal fermentation will guide producers and researchers in their search for products that are beneficial to beef production. This study evaluated if the addition of microbial-based forage preservative at mowing could improve ruminal fermentation for ABG mixtures when evaluated *in vitro*. Ruminal fermentation parameters mainly differed between forage sampling time, pre-ensiling or post-ensiling, in that ABG sampled post-ensiling had increased digestibility in the early stages of fermentation. However, after 48 hours, the pre-ensiling forage had greater total digestibility suggesting that ABG mixtures preserved as hay may be more digestible if rumen retention time exceeds 24 hours.

Overall, ABG mixtures produce a high-quality feed from March to November, while offering producers flexibility in management as they choose whether to graze or harvest the material for stored feed. Use of rotational grazing, summer rest periods, and following best management practices during harvesting of stored feeds, are critical to creating high quality feed while maintaining alfalfa persistence beyond three years in ABG mixtures. Future research should evaluate if aerobic stability or ruminal fermentation parameters are improved by utilizing forage preservatives or inoculants. Determining the optimum timing of grazing initiation after a baleage harvest to identify the trade-offs between forage maturity and quality and the associative impacts on stocking rates, animal performance, and economic viability is warranted. Finally, research evaluating rumen retention time, VFA production, and digestibility of ABG mixtures preserved at varying levels of alfalfa inclusion and preserved as hay or silage would help determine optimal alfalfa incorporation levels in beef diets.