Suitability of Secondary Metabolite-Producing Forbs for Southeastern Beef Cattle Production Systems

By

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ABSTRACT

Seasonal fluctuations in forage availability and nutritive value limit productivity in forage-based beef cattle systems in the southeastern United States. Although forbs have the potential to improve forage diversity, nutritional quality, and pasture resilience, they remain largely underappreciated and underutilized in U.S. grazing systems. Integrating forbs into grass-dominated pastures may improve forage quality and animal performance; however, adoption remains limited due to variability in establishment, productivity, and the presence of plant secondary metabolites that may influence nutrient utilization and rumen fermentation. The objectives of this research were to (1) evaluate the establishment, yield potential, and nutritive value of cool- and warm-season forage forb species across Alabama, and (2) determine how the best-performing warm-season forbs species from the first study would influence nutrient utilization, rumen fermentation characteristics, and methane (CH_4) emissions in beef cattle. To achieve the first objective, a randomized complete block design with three field replications per location was conducted at three sites across Alabama and repeated under greenhouse conditions. A total of 24 cool-season forage species, and 12 warm-season species were planted at each location in both years. Differences among species were observed for dry matter yield (DMY; $P < 0.01$), neutral detergent fiber (NDF; $P < 0.01$), acid detergent fiber (ADF), and acid detergent lignin (ADL; $P < 0.01$) among species in the field in both seasons. Crude protein concentration (CP) also differed among species ($P < 0.01$), with hairy vetch (27%) in the cool season and cowpea (20%) in the warm season exhibiting high protein concentrations. Among cool-season species, hairy vetch and mustard produced the greatest yields (7750 and 6453 kg DM/ha, respectively), whereas sunn hemp (31421.7 kg DM/ha) and cowpea (20189.6 kg DM/ha) were most productive during the warm season. Sunn hemp (*Crotalaria juncea* L.; SUN), lablab (*Lablab purpureus* [L.] Sweet; LAB), and soybean (*Glycine max* [L.] Merr; SOY) were supplemented with bahiagrass (*Paspalum notatum* Flueggé) in an in vivo metabolism experiment arranged as a 4 × 4 Latin square design. Columns represented experimental periods, and rows represented ruminally-fistulated steers. Dietary treatments consisted of a 70:30 bahiagrass-to-forb ratio, with a grass-only treatment serving as the control (CON). The forb ratio in the diet differed among treatments ($P = 0.06$) and remained below the targeted inclusion ratio of 30%. While apparent dry matter digestibility (DMD; 56.6–63.1%; $P = 0.18$), apparent acid detergent lignin digestibility (ADLD; $P \geq 0.18$) were not different among diets, apparent neutral detergent fiber digestibility (NDFD; $P = 0.03$) and apparent acid detergent fiber digestibility (ADFD; $P = 0.04$) were influenced by diet. Apparently absorbed nitrogen and apparentily retained nitrogen (AAN and ARN, respectively) did not differ among diets ($P \geq 0.39$). Differences in vivo dry matter digestibility were expected to follow a similar pattern, as the diet was mostly dominated by bahiagrass. However, there was an effect ($P = 0.02$) on in vitro dry matter digestibility (IVDMD) of the bahiagrass control substrate and no effect ($P = 0.92$) of diet for the bahiagrass-forb incubations. There was an interaction of diet and substrate ($P = 0.04$) for potential activity for methane production (PAMP). Supplementing bahiagrass hay with leguminous forbs improved ruminal fermentation profiles ($P \leq 0.01$). Results from both studies indicated considerable variation among species in both yield and nutritional composition, highlighting several forb species with strong potential for integration into southeastern grazing systems. Overall, forb species can contribute to improved forage diversity and nutritional value in southeastern pasture systems while influencing rumen fermentation and CH_4 production.

ARTIFICIAL INTELLIGENCE (AI) USE DISCLOSURE

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Grammarly and ChatGPT. Grammarly was used for spelling, grammar, and language clarity. ChatGPT was used as a writing support tool to improve sentence structure, rephrase text for clarity, and adjust the academic tone. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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LIST OF ABBREVIATIONS AND SYMBOLS

The author has made all efforts to observe the accepted abbreviations for *Journal of Animal Science* and *Applied Animal Science* as these are potential outlets for publication. The following list provides the abbreviations accepted by these journals as well as additional acronyms or abbreviations used throughout this document:

Abbreviation	**Definition**
°C	degree(s) Celsius
A:P	Acetate- to- propionate ratio
ADF	acid detergent fiber
ADFD	acid detergent fiber digestibility
ADIA	acid detergent insoluble ash
ADL	acid detergent lignin
ANOVA	analys(es) of variance
AOAC	Association of Official Analytical Chemists
BUN	blood urea nitrogen
BW	body weight
CON	control diet
CP	crude protein, calculated as nitrogen times 6.25
Cr (III)- EDTA	chromium (III) ethylenediaminetetraacetic acid
d	day(s)
DE	digestible energy

DM	dry matter
DMI	dry matter intake
doi	digital object identifier (used with citations)
g	gram(s)
GE	gross energy
h	hour(s)
IVDMD	in vitro dry matter digestibility
LAB	lablab diet
M	molar (mol/L)
ME	metabolizable energy
min	minute(s)
N	nitrogen
n	sample size
NDF	neutral detergent fiber, assayed inclusive of α-amylase (unless otherwise stated), exclusive of sodium sulfite (unless otherwise stated), and expressed inclusive of residual ash
NDFD	neutral detergent fiber digestibility
NE	net energy
NEFA	non-esterified fatty acid
OM	organic matter
PSM	plant secondary metabolite(s)
SAS	SAS Institute, Inc. (formerly known as Statistical Analysis System)
SEM	standard error of the mean

SM	secondary metabolite(s)
SOY	soybean diet
SUN	sunn hemp diet
TiO2	titanium dioxide
US	United States
USDA	United States Department of Agriculture
VFA	volatile fatty acid(s)

CHAPTER I

INTRODUCTION

Background

Forbs have historically been an integral part of diverse native grassland ecosystems, but modern land management practices have greatly reduced their presence, resulting in simplified, grass-dominated pastures. Over the years, their incorporation into these grass monoculture pastures have offered several distinct advantages over grasses, which contribute to the overall health, productivity, and sustainability of a grazing system (Prigge et al., 2024). Considering their great forage quality, with CP values ranging from 10.5 to 18.9% (Prigge et al., 2024), forbs have gradually been reintroduced into modern production systems with the hope that they can supplement the diet when grass quality declines. In their study on management of beef cattle pastures, Rouquette et al, (2020) emphasized that, if well managed, forbs could bridge the seasonal gap during the late fall, winter, and early spring, a period when warm-season grasses are dormant, thereby reducing the need for stored feed like hay and supplemental feeds.

While most studies have shown great advantages when incorporated into grazing systems, establishing and maintaining productive stands of forbs and legumes in the southeastern U.S. presents significant challenges that require intensive and knowledgeable management (Rouquette et al., 2020). The aggressive nature of dominant warm-season grasses, accompanied by climatic pressures, soil management, species variability, and complex management requirements, makes the establishment and long-term persistence of legumes a difficult task (Dinkins et al., 2021).

Statement of the Problem

Most forb species remain underutilized in today's grazing and pasture systems (Muir et al., 2011). Producers have had limited exposure to the wide range of species available, making it difficult to identify those best suited to their environments, nutritional goals, and production systems. Additionally, there is limited research on optimal diet ratios, palatability, and CH_4 reduction potential, and little understanding of the ruminal metabolism of species such as sunn hemp (*Crotolaria juncea* L.), soybean (*Glycine max* [L.] Merr), and lablab (*Lablab purpureus* [L.] Sweet), which further limit their effective use in sustainable livestock systems.

Research Objectives

The primary objective of this study was to identify forb species suitable for incorporation into existing southern grass pastures to mitigate enteric methane emissions in beef production systems without compromising animal performance.

Specific Objectives

1. To determine the effects of species and location on yield potential, nutritive value, and plant secondary metabolite (PSM) expression of forage forb species.
2. To determine digestive and metabolic parameters in beef cattle exposed to grass/forb systems, including naturally occurring plant secondary metabolites such as condensed tannins, isoflavones, saponins, and glucosinolates.

Style and Form

This manuscript was prepared according to "Instructions to Authors (revised 2017)" from *Journal of Animal Science* (ASAS, 2017). All attempts were made to adhere to this style, except in cases where divergence was needed to adhere to the policies of the Auburn University or to increase clarity in the document.

CHAPTER II

REVIEW OF LITERATURE

Forbs as Forages

Forbs are herbaceous (non-woody) plants that are not grasses, sedges, or rushes. They are typically flowering plants, which distinguishes them from grass-like plants (Muir et al., 2011). Forbs include a wide variety of native wild species, cover-crop legumes, and annual herbs that historically received little attention in forage research. According to Oregon State University, (2016), although all legumes are forbs, forbs may be annuals, biennials, or perennials and may exhibit C_3 or C_4 physiology depending on their seasonal adaptation. These distinctions are crucial when selecting forbs for forage systems, as they determine grazing windows, stand persistence, and management requirements.

While legumes, mostly clovers, are increasingly studied, the integration of diverse forbs, both native and commercial, into these baseline systems is underexplored. Recent grazing trials with native-warm season grass pastures interseeded with a mixture of forb species, including *Rudbeckia hirta* L. (black-eyed Susan), *Echinacea purpurea* (L.) Moench (purple coneflower), and *Desmodium canadense* (L.) DC. (tick trefoil) improved forage quality leading to better cattle performance as compared to grass monocultures stands (Prigge et al., 2024). Interseeded plots exhibited more stable CP levels, lower fiber concentrations in late summer (31.8% ADF, 58.2% NDF), and a 13% increase in season-long average daily gain (0.77 kg/d vs. 0.68 kg/d) for steers grazing the forb-enhanced plots compared to grass monocultures (Prigge et al., 2024).

Legumes as forages uniquely fix atmospheric nitrogen through their symbiotic relationship with rhizobia bacteria, enriching soil nitrogen pools (Ohyama, 2017). This reduces or eliminates the need for synthetic nitrogen fertilizer inputs, thus decreasing costs and making forage systems more environmentally sustainable, enhancing the soil fertility and organic matter, and providing a long-term benefit to pasture ecosystems (Muir et al., 2011). Interseeding alfalfa (*Medicago sativa* L.) into bermudagrass (*Cynodon dactylon* [L.] Pers) pasture provided organic nitrogen through fixation and cattle excreta, reducing the need for synthetic fertilizers (Burt et al., 2022). This approach improves forage quality, boosts cattle gains, supports vigorous grass regrowth, and lowers input costs, therefore enhancing both productivity and long-term sustainability for southeastern grazing systems (Burt et al., 2022).

Forbs often develop deep taproots that allow them to access soil moisture and nutrients beyond the reach of grasses, fix atmospheric nitrogen, enriching soil fertility and enhancing plant diversity and biomass in low-input systems. However, compared to grasses, forbs typically have slower recovery after grazing, produce fewer seeds, and their seedlings often display reduced vigor and reduced germination percentages (20 to 25% vs. 30 to 45% (Fay and Schultz, 2009; Muir et al., 2011). The incorporation of tannin-containing forbs, especially of CT in legumes like sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.), and hydrolyzable tannins in forbs like small burnet (*Sanguisorba minor* Scop.*)*, has been shown to reduce enteric CH_4 emissions from ruminants (MacAdam et al., 2025). The relationship, however, is influenced by the type of tannin, plant species, impacts on intake, and diet composition. Other studies have shown that non-bloating forbs, such as cicer milkvetch (*Astragalus cicer* L.), not only alter rumen fermentation pathways to favor less CH_4 production but also enhance nitrogen retention, decreasing environmentally problematic urinary nitrogen excretion and promoting more efficient nutrient use (MacAdam et al., 2022). This advantage lowers the environmental footprint of beef cattle production and promotes sustainability in the system.

Sunn Hemp

Sunn hemp (*Crotalaria juncea* L.*)* is a fast-growing, warm-season annual legume native to India, now cultivated globally as a green manure cover crop, forage, non-wood fiber, and in bioenergy production. It is valued for its rapid biomass accumulation, soil-enriching properties, pest suppression, and adaptability to marginal soils (Cook and White, 1996). Sunn hemp produces biomass yields averaging 7.6 Mg DM/ha across the two-year study period (Balkcom and Reeves, 2005), providing effective ground cover, weed and erosion control, raising organic carbon levels, and keeping soils cooler during hot periods. There is growing interest in its use as livestock forage, particularly in tropical and subtropical regions, due to its promising nutritional value (Balkcom and Reeves, 2005).

Jaramillo et al. (2020) confirmed that sunn hemp serves as extremely productive summer forage, maintaining productivity during periods when alternative annual legume options are limited. Its fast growth and capacity to yield high-quality forage in as little as 60 d provide livestock producers with an extra supply of forage during seasonal transition periods, thereby reducing dependence on supplementation (Eberle and Shortnacy, 2021). At 60 d after seeding, CP concentrations can range from 11.0 to 15.5%, with ‘AU Golden’ having the greatest concentration (155 g/kg DM at 60 d and 162 g/kg DM at flowering; Garzon et al., 2021). This concentration is sufficient to cover the nutritional needs of most beef cattle systems. In vitro digestible organic matter (IVDOM) was likewise reported to be greatest in early harvests, with AU Golden and ‘Crescent Sunn’ maintaining greater digestibility than other genotypes (Garzon et al., 2021). Although leaves and stems both contribute to total biomass, leaves contain lower concentrations of NDF and lignin, which increases the rate of ruminal digestion and allows for greater voluntary intake. Conversely, stems provide the bulk of the ADF and structural lignin necessary for plant height but at the cost of slower digestibility in ruminants (Lepcha and Naumann, 2021). Some of the downsides of using sunn hemp as a forage are management responsibilities. Different harvest intervals significantly impact the herbage accumulation rates, which affect forage quality (Shekinah and Stute, 2018; Jaramillo et al., 2020c). Like any other forage, quality decreases with increasing maturity as lignification takes place (Buxton, 1996).

Lablab

Lablab bean (*Lablab purpureus* [L.] Sweet) is an annual vining legume that has been evaluated and utilized as a forage crop due to its ability to improve the CP content of silage and provide other nutritive benefits in livestock diets, particularly in mixed forage systems such as intercropping with corn (*Zea mays* L.; Armstrong and Albrecht, 2008). It is adaptable and drought-tolerant, which makes it suitable for diverse climates and mixed cropping systems (Athmaselvi et al., 2020). Beyond its resilience, lablab provides a significant biomass contribution, often yielding 4,000–8,000 kg DM/ha. In Puerto Rico, lablab cv. ‘Rongai’ produced about 6,000 kg DM/ha annually when used as a cover crop (Tirado-Corbalá et al., 2018). The leaves of lablab contain 21 to 38% CP, and the seeds contain 20 to 28% CP, making it a nutritious option for both grazing and hay production. It is considered one of the most palatable legumes for livestock. The leaves, which make excellent hay for cattle and goats, are highly palatable, while the stems are less preferred (Athmaselvi et al., 2020). Foster et al. (2017) state that most lablab cultivars have had relatively elevated nitrogen content, which is beneficial for soil fertility and can reduce fertilizer requirements when used in crop rotations or as a green manure. Selection efforts have resulted in improved types like 'Rio Verde,' is notable for its ability to withstand frequent grazing defoliation while maintaining productivity (Rouquette et al., 2020).

Lablab exhibits moderate fiber content, making it a suitable forage option for ruminants. The neutral detergent fiber (NDF) content is 48.7%, and the acid detergent fiber (ADF) content is 37.1% (Mokoboki et al., 2002). These fiber concentrations fall within the range in which NDF is still expected to be digestible and, therefore, would not negatively impact the bioavailability of crude protein. Lablab contains a relatively high level of CT compared to what is typically considered nutritionally safe for ruminant diets (MacAdam and Villalba, 2015) The extracted CT content in lablab is 33 g/kg DM, which falls within or slightly above the ideal CT concentration range of 20 to 40 g/kg DM suggested for cattle consumption (Mokoboki et al., 2002).

Soybean

Soybean (*Glycine max* [L.] Merr) originated in central China and was domesticated 9,000 years ago from the wild soybean (*Glycine soja* [L.] Merr; Sedivy et al., 2017). It was introduced to the USA in the mid-1800s and was originally promoted as a forage crop (Sheaffer et al., 2001), then used for hay, silage, pasture, or as a green manure crop to improve soil fertility. As a forage, soybeans can be grown alone as a monoculture or intercropped into other plants, such as corn or grasses, to increase crop yield or CP. Like any other forage, harvest maturity, row spacing, cultivar, and plant density will affect the yield of the soybean forage (Hintz et al., 1992). Thus, for optimal forage yield and acceptable quality, soybeans should be grown using narrow rows and harvested at the R6–R7 stage, using locally adapted cultivars and remembering that a plant density beyond approximately 250,000 to 300,000 plants/ha does not further enhance yields (Hintz et al., 1992).

The nutritional composition of soybean forage can be appealing to ruminant diets. Foster et al. (2009) reported CP of 14% in soybean hay, which is significantly greater than bahiagrass hay (8% CP) and like other legume hay tested, such as cowpea and peanut hays. Forage soybeans have greater fiber content in their total herbage than grain soybeans, mainly because they are harvested at less mature stages with fewer pods and more stems and leaves. As the soybean matures and pod content increases, total fiber concentration decreases (Sheaffer et al., 2001). Thompson et al. (2023) reported crude protein concentrations ranging from 12.4 to 17.9%, with NDF and ADF values ranging from 37.5 to 44.9% and 28.2%, respectively, depending on cultivar and clipping height. Soybean forage contains a diverse profile of secondary metabolites, primarily isoflavones (such as genistein and daidzein) and saponins, with smaller amounts of flavonoids, phenolic acids, and low levels of CT (Bragagnolo et al., 2021). These compounds can influence plant defense, forage quality and intake, and livestock health, but their impact on ruminant performance is generally minor due to rumen microbial degradation (Ku et al., 2020). Isoflavones in soybean, particularly daidzein, can be metabolized by rumen microbes into equol, a compound with greater estrogenic activity, although reproductive effects are typically minimal in cattle under practical feeding conditions (Adams, 1995; Grgic et al., 2021). In addition, isoflavones such as biochanin A have been reported to modulate ruminal nitrogen metabolism by inhibiting hyper-ammonia-producing bacteria, thereby potentially improving nitrogen utilization efficiency (Harlow et al., 2020; Flythe and Harlow, 2023).

Plant Secondary Metabolites

Plant secondary metabolites are a group of organic compounds that are critical for plant defense against herbivores, pathogens, and environmental stresses. Secondary metabolites act as attractants for pollinators and seed-dispersing animals and give plants their flavors and pigmentation (Crozier et al., 2006). These compounds include polyphenols (isoflavones and tannins), saponins, and glucosinolates. In forage systems, secondary metabolites play a key role in defense against grazing animals. While these compounds can offer health benefits to livestock at certain levels, their concentrations can change in response to grazing pressure and environmental conditions (Bennett and Wallsgrove, 1994). As a result, forbs that are safe and nutritious under some management strategies may become toxic under others, thus the importance of grazing management.

Tannins

Tannins are a diverse group of high-molecular-weight, water-soluble polyphenolic compounds found widely in the plant kingdom (Das et al., 2020). They are characterized by their ability to bind and precipitate proteins and various other organic compounds, such as amino acids, alkaloids, and polysaccharides, forming insoluble complexes (Bhattacharya, 2019). According to Khanbabaee and Ree, (2001), tannins can be classified into two main categories based on their chemical structure: CT, or proanthocyanidins, and hydrolysable tannins.

Role in plants

Plants primarily use tannins to defend themselves against herbivores, pathogens, and environmental stress (Mueller-Harvey et al., 2019). Tannins create complexes with proteins, polysaccharides, and enzymes, which make plant tissues unpalatable and indigestible to herbivores while altering feeding behavior and pathogen activity (Crozier et al., 2006). The protein-binding ability of tannins helps control plant growth (Mora et al., 2022) while simultaneously affecting allelopathic relationships between plants through modifications of rhizosphere microbial communities and nutrient cycling patterns (Bennett and Wallsgrove, 1994).

In addition, tannin concentrations in different plant structures change according to seasonal patterns and external triggers such as insect attacks and abiotic stress (Bennett and Wallsgrove, 1994), which demonstrates their active role in plant defense and adaptation. Tannins function as defensive compounds, but recent research shows they perform multiple overlapping roles, including ultraviolet protection and wound response, and signaling functions, which prove their essential role in plant-environment interactions and survival strategies (Bennett and Wallsgrove, 1994; Mora et al., 2022).

Role in animals

In animals, especially herbivores, tannins have a significant impact on nutrition, digestion, and health of the animal. They tend to quickly form bonds with dietary proteins and digestive enzymes after consumption, which decreases protein breakdown in the rumen and directs protein absorption toward the small intestine (Barry and McNabb, 1999; Lorenz et al., 2014). The protein-sparing effect of moderate tannin consumption occurs through the formation of reversible tannin–protein complexes in the rumen, which reduce microbial degradation of dietary protein and limit excessive ammonia production. As a result, a greater proportion of dietary protein escapes ruminal breakdown and is digested and absorbed in the small intestine as amino acids (MacAdam and Villalba, 2015). This increases the supply of essential amino acids to the animal, improves nitrogen utilization efficiency, and reduces nitrogen losses in urine. Collectively, these mechanisms contribute to improved growth rates, milk production, wool growth, and reproductive performance in ruminants (Barry and McNabb, 1999).

In contrast, high CT consumption (> 5% DM) leads to decreased feed consumption and reduced nutrient absorption and animal performance because tannins strongly bind to proteins and block microbial fermentation processes (Villalba et al., 2011; Lorenz et al., 2014). In addition, high CT concentrations inhibit cellulolytic bacteria and fibrolytic enzymes, which significantly slows the fermentation of structural carbohydrates and reduces organic matter digestibility by as much as 20 to 28% (Tedeschi et al., 2014; Norris et al., 2020). Consequently, the benefits of ruminal protein protection are negated because the tannin-protein complexes become too stable to dissociate in the lower digestive tract, resulting in reduced nutrient absorption and increased fecal nitrogen excretion (Norris et al., 2020). The antiparasitic and antimicrobial properties of CT described by Mueller-Harvey et al. (2019) help grazing animals reduce their gastrointestinal nematode loads and fight pathogenic bacteria. Shimada (2006) demonstrated that browsers and herbivores have developed proline-rich salivary proteins to neutralize ingested tannins, which reduces their antinutritional effects or even excessive concentration of tannins. When it comes to grazing systems, the strategic use of tannin-rich forages under proper management leads to important advantages for ruminant agriculture and health and environmental conservation (Lorenz et al., 2014).

Research has extensively examined CT present in forage legumes and trees because they show potential to reduce CH_4 emissions from ruminant livestock. Their effectiveness is largely attributed to their ability to bind to proteins and interact with rumen microbial populations, leading to modified fermentation patterns and a reduction in methanogenesis (Hassanat and Benchaar, 2013). Studies conducted in vitro showed that both CT from sources such as acacia and quebracho and hydrolysable tannins from chestnut reduced CH_4 production when included in diets at concentrations of 50 g/kg DM or higher, achieving reductions of up to 40% compared to control diets (Hassanat & Benchaar, 2013). In vivo research corroborates these findings, demonstrating that grazing cattle on increased condensed tannin legumes such as sainfoin and birdsfoot trefoil led to a 25–63% decrease in CH_4 emissions compared to those grazing grass pastures (MacAdam et al., 2025).

Saponins

Saponins are naturally-occurring glycosides widely distributed in plants, especially in legumes, such as alfalfa, soybean, and yucca (*Yucca schidigera* Roezl ex Ortgies), and are characterized by their amphipathic structure (Desai et al.2009). Saponins are present in various plant tissues, including seeds, leaves, roots, and stems, with composition and content varying by species, cultivar, and environmental conditions (Francis et al., 2002). The amphipathic nature of saponins allows them to disrupt microbial membranes by targeting cholesterol in rumen protozoa, causing cell lysis and a reduction in protozoal populations known as defaunation (Wina et al., 2005). This reduction decreases methane production by eliminating the habitat for methanogenic archaea, while their surfactant properties alter the surface tension of rumen fluid, influencing the risk of frothy bloat (Francis et al., 2002; Patra and Saxena, 2009).

Role in plants

Saponins function as natural surfactants due to their amphipathic structure, which enables them to facilitate cellular transport processes, activate chemical defenses, and distribute signaling compounds throughout plant tissues (Desai et al., 2009). The defense systems of plants and forages heavily depend on these compounds. The compounds demonstrate strong antifungal, antibacterial, anti-insect, and antiparasitic properties, protecting plants from microbial pathogens and herbivores (Augustin et al., 2011).

Research has shown that saponins possess allelopathic properties influencing competition between plant species and affecting seed germination and seedling development of neighboring plants (Augustin et al., 2011). This characteristic becomes a disadvantage in forage systems where yield is of great importance. Saponin biosynthesis and concentrations change across different tissues and developmental stages, and under environmental conditions such as drought and pest attacks, which indicates their adaptive function in plant stress responses (Mugford and Osbourn, 2012). The greatest levels of saponins occur in young tissues and reproductive organs because these stages need maximum protection during their critical development.

Role in animals

The presence of saponins in alfalfa negatively affects animal productivity mainly through reduced nutrient absorption and decreased feed intake and growth rates by 11% in monogastric species and poultry (Pecetti et al., 2006). In ruminants, the rumen microbial populations experience cell membrane disruption from saponins, which specifically target protozoa cells, thus lowering their numbers. The decrease in protozoal numbers reduces CH_4 production because protozoa form associations with methanogenic archaea (Crozier et al., 2006). The presence of saponins at reduced levels (1 to 2% of daily DMI) in diverse diets with tannins leads to health advantages through reduced parasitism and cholesterol levels, but greater concentrations may cause bloat and decrease feed intake (Lisonbee et al., 2009). Preference studies have shown that lambs receiving saponin infusions directly into the rumen at 1.5% body weight (BW) developed a strong aversion to alfalfa, indicating their ability to associate post-ingestive negative effects with saponin consumption (Villalba et al., 2011). The effects of saponins on ruminants are dose-dependent; moderate concentrations (0.5 to 3% DM) can enhance nitrogen utilization, whereas excessive intake may pose health risks to animals (Crozier et al., 2006). These findings highlight the importance of appropriate grazing management strategies or the interseeding of saponin-containing forbs within perennial grass systems to optimize animal performance while minimizing potential adverse effects.

Isoflavones

Isoflavones are a subgroup of flavonoids mostly found in leguminous plants such as soybean, red clover (*Trifolium pratense* L.), and alfalfa. Structurally, isoflavones possess a C6-C3-C6 skeleton, and their primary representatives in the diet include genistein, daidzein, formononetin, and biochanin A, which are often found in conjugated glycoside forms in plants (Yang and Wang, 2024). These forms are hydrolyzed to the active aglycones during digestion in animals or humans (Crozier et al., 2006).

Role in plants

Isoflavones serve essential functions in plant and forage systems. The plant produces phytoalexins as compounds that defend plants against biotic stresses, including fungal and bacterial attacks, and abiotic stresses, including UV-B exposure. The plant increases its resistance through the elevation of isoflavone levels when it faces pathogenic threats or environmental stressors (Wyse et al., 2022a). The phytoestrogen compounds function as key signaling molecules to facilitate the symbiotic connection between legumes and nitrogen-fixing rhizobia (Dinkins et al., 2021) thus the advantage of forbs in fixing their own nitrogen, unlike grasses.

Furthermore, genistein and daidzein released by root tissues activate nod gene expression in suitable rhizobia, leading to root nodule development and nitrogen fixation (Wyse et al., 2022).

These compounds function in allelopathic interactions by influencing nearby plant development and by suppressing microbial competition in the rhizosphere (Dinkins et al., 2021). Dinkins et al. (2021) used CRISPR/Cas9 to knock out isoflavone synthase in red clover and found that nodulation with rhizobia remained unchanged, implying that isoflavones are not essential for symbiosis; however, the elevated expression of defense-related genes in the mutants suggests a compensatory response triggered by reduced isoflavone levels.

Role in animals

When isoflavones are ingested, they undergo biotransformation in the rumen and gastrointestinal tract to aglycones or further metabolites (Wyse et al., 2022a). These metabolites can bind to estrogen receptors, exerting estrogenic or antiestrogenic effects in mammals, thus classifying isoflavones as phytoestrogens (Crozier et al., 2006). This type of plant metabolite disrupts reproductive function in ruminants by binding to estrogen receptors and altering endocrine signaling within the hypothalamic–pituitary–gonadal axis. Ruminal conversion of daidzein to equol, a more potent estrogenic metabolite, may interfere with ovulation, estrous cyclicity, and conception, leading to temporary or prolonged infertility (Crozier et al., 2006; Grgic et al., 2021; Wyse et al., 2022a). Isoflavones, such as biochanin A and formononetin from red clover, act as natural growth promoters that reduce enteric methane by 13.5%–26% by inhibiting methanogens and shifting fermentation toward propionate. These compounds enhance nitrogen utilization by reducing ammonia concentrations up to 37% and inhibiting hyper-ammonia-producing bacteria, ultimately improving average daily gain by 15%–29% and enhancing fiber digestibility (Adams, 1995; Harlow et al., 2020).

Glucosinolates

Glucosinolates originate from amino acid precursors, including methionine, tryptophan and phenylalanine, before undergoing chain elongation, hydroxylation, and methoxylation (Tripathi and Mishra, 2007; Crozier et al., 2006). Plants maintain glucosinolates in separate storage compartments from myrosinase to prevent their mixture. The two substances combine after tissue damage through chewing or mechanical stress, triggering myrosinase to break down glucosinolates into protective compounds like isothiocyanates and nitriles that deter animals and pathogens (Bennett and Wallsgrove, 1994).

Role in plants

Glucosinolates are a stress response in plants, induced by abiotic and biotic stresses, including pathogen and insect attack, wounding, and hormonal signals (Bennett and Wallsgrove, 1994). Some insect herbivores are actually attracted to glucosinolate-containing plants and have evolved mechanisms to tolerate or even utilize these compounds in their own defense. These compounds are of importance in plants as they have allelopathic effects, thus participating in competition and plant community structure (Crozier et al., 2006). This might be an advantage or disadvantage in forage systems, depending on the interseeding and weed control preferences. According to Crozier et al. (2006), these compounds are used by plants as sulfur reserves, and under sulfur-deficient conditions, sulfur can be mobilized from glucosinolates to meet metabolic needs.

Role in animals

When it comes to ruminant animals, glucosinolates have both positive and negative effects. Reduced amounts of glucosinolates from forage brassicas can improve digestibility, increase milk urea nitrogen levels, and improve nitrogen utilization and animal performance as long as intake remains moderate (0.10 to 0.21% DM; Gao et al., 2025). Brassica forages with glucosinolate concentrations DM have been shown to reduce enteric CH_4 emissions per unit of DM intake in ruminants compared with grass-based diets (Sun, 2020; Gao et al., 2025). The reduction of CH_4 emissions through glucosinolate intake is believed to occur through changes in rumen microbial activity and fermentation product ratios that increase propionate and butyrate while decreasing acetate and enhancing digesta passage rate and thyroid hormone stimulation (Dillard et al., 2018). There have been multiple meta-analyses that confirm that, while glucosinolates can enhance certain aspects of nutrient use and suppress greenhouse gas production, their effects on milk yield, milk composition, and animal performance are variable and depend on dose, animal species, and dietary context (Gao et al., 2025). Sun et al. (2015) demonstrated that feeding forage rape (*Brassica napus* L.) to lambs significantly and persistently reduced their CH_4 emissions due to diet-driven shifts in rumen fermentation patterns and methanogenic archaea, particularly favoring propionate over acetate production. Tripathi and Mishra (2007) stated that ruminants are relatively more tolerant to dietary glucosinolates than monogastric, because rumen microbes can process this compound. In addition, glucosinolates can reduce palatability, intake, and animal performance when consumed in levels exceeding 10% (Tripathi and Mishra, 2007; Gao et al., 2025).

Methane

Environmental Impact

Methane is a potent greenhouse gas (GHG), approximately 28 times more effective at trapping heat in the atmosphere than CO_2 (Hill et al., 2016). Livestock agriculture, especially ruminant production, is recognized as a principal source of CH_4 emissions. For instance, 40% of anthropogenic livestock emissions are CH_4, and within this figure, enteric fermentation from livestock is a primary contributor originating from grazing ruminants such as cattle (77%), buffalos (13%), and small ruminants (10%; IPPC, 2019). As the global population grows, these numbers are expected to increase by at least 30% by 2050 (Mrutu et al., 2025) to meet the food demands. In addition, enteric CH_4 contributes to both climate warming and atmospheric pollution, especially through the formation of tropospheric ozone, making its reduction or mitigation of great importance. Because CH_4 persists in the atmosphere for only about 10 to 12 years (Mrutu et al., 2025), cutting emissions can quickly slow near-term warming, making ruminant CH_4 a high-impact target for climate action.

Methane loss is not only an environmental concern but also represents an energy loss from ruminant diets; typically, about 5.5 to 6.5% of gross energy consumed is lost as CH_4 (Johnson and Ward, 1996), reducing overall feed conversion efficiency. Strategies that reduce enteric CH_4 emissions can therefore improve the efficiency of energy utilization by redirecting dietary energy toward animal productivity (Cuervo et al., 2025). Forage species containing bioactive compounds, such as tannins, saponins, and glucosinolates, have been shown to influence rumen fermentation patterns and reduce CH_4 production, potentially enhancing metabolizable energy availability to the animal.

Ruminal Processes and Pathways

The rumen functions as a specialized anaerobic fermentation space, which allows ruminants to digest fibrous plant materials that monogastric animals cannot digest efficiently (Church, 1993). The rumen contains microbes that transform complex carbohydrates into volatile fatty acids (VFA), including acetate, propionate, and butyrate, which serve as the primary energy source for the animals (Hungate et al., 1970). The fermentation process produces hydrogen (H_2) and carbon dioxide (CO_2) in addition to its main products. The accumulation of H_2 can inhibit rumen fermentation; therefore, methanogenic archaea play a critical role by removing excess hydrogen through the hydrogenotrophic pathway ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$), resulting in CH_4 production. The removal of hydrogen by these microorganisms maintains low concentrations H_2, which enables efficient fermentation to continue (Wolin et al., 1997). Other pathways for using hydrogen, such as making acetate or propionate, exist in the rumen, but methanogenesis is more common because it is more energy-efficient under normal conditions (Attwood and McSweeney, 2008). According to Li et al. (2022), this does not happen to all ruminant animals; the diet and forage type play a crucial role in the fermentation process, as they can shift the microbial populations. Diets rich in structural carbohydrates tend to produce greater CH_4 emissions due to increased acetate and H_2 formation, while high-concentrate diets promote propionate production, a H_2 sink that competes with methanogenesis, and thus may reduce CH_4 emissions (Volmer et al., 2023). According to Pedreira et al.(2013), increasing the dietary concentrate for beef cattle from 5 to 65% resulted in a 19.8% reduction in CH_4 emissions per unit of DMI, with CH_4 intensity decreasing from 21.7 to 17.4 g/kg DMI. The selection of forage species, particularly legumes with greater non-fiber carbohydrate concentrations and lesser fiber content, has been shown to reduce CH_4 emissions per unit of feed intake compared with grass-based systems (MacAdam et al., 2025).

Methanogens

Methanogenic archaea make up only about 0.1–4% of rumen microbes, yet they are essential for CH_4 production (Volmer et al., 2023). The most common genera are *Methanobrevibacter*, *Methanosphaera*, and *Methanomicrobium*, though recent metagenomic studies have revealed much greater diversity, including little-known or uncultured groups like *Methanomassiliicoccales*, some of which can use alternative methylated substrates (Mi et al., 2024). Other rumen microbes, such as hydrogen-producing bacteria, fiber-digesting fungi, and ciliate protozoa promote methanogenesis by influencing hydrogen availability, while microbes that use propionate or nitrate compete for hydrogen and reduce CH_4 formation (Shinkai et al., 2024). According to Henderson et al. (2015), the composition of microbiome depends heavily on dietary factors because forage-based diets with greater fiber content promote fibrolytic and H_2-producing bacteria resulting in elevated CH_4 emissions, while propionate producers thrive on high-concentrate or lipid-supplemented diets to inhibit methanogens.

CHAPTER III

GROWTH POTENTIAL AND NUTRITIVE VALUE OF COOL- AND WARM-SEASON FORB SPECIES ACROSS ALABAMA

Synopsis

In the southeastern United States, forage-based livestock operations face productivity and nutritional limitations during seasonal transition. These gaps in forage availability affect animal performance and overall production efficiency. Although integrating forbs into these systems may help alleviate seasonal gaps, adoption has been limited by inconsistent establishment, variable growth performance, sensitivity to environmental stress, and concerns about anti-nutritional effects associated with plant secondary metabolites (PSM). The objective of this study was to evaluate the establishment, production potential, and nutritive value of cool- and warm-season forb species across Alabama. An experiment using a randomized complete block design with three field replications per location was conducted at three sites across Alabama. The same experiment was repeated under greenhouse conditions. A total of 24 cool-season and 12 warm-season species were planted at each location in both years. Differences among species were observed for dry matter yield (DMY; $P < 0.01$), NDF ($P < 0.01$), ADF, and ADL ($P < 0.01$) among species in the field in both seasons. Crude protein concentration also differed among species ($P < 0.01$), with hairy vetch (27%) in the cool season and cowpea (*Vigna unguiculata*; 20%) in the warm season exhibiting greater CP concentrations. Among cool-season species, hairy vetch and mustard (*Brassica rapa*) produced the greatest yields (7,750 and 6,453 kg DM/ha, respectively), whereas sunn hemp (31,421.7 kg DM/ha) and cowpea (20,189.6 kg DM/ha) were most productive during the warm season. Greenhouse DMY exceeded field DMY in both seasons, likely reflecting reduced

environmental stress under controlled conditions. Species with reduced DMY generally exhibited greater fiber concentrations. The results indicated that selected forage forbs possess the productive capacity and nutritive quality to help mitigate seasonal forage shortages; however, species selection and management strategies are essential to optimize their effectiveness in grazing systems.

Introduction

Forb species represent a diverse and often underappreciated group of pasture plants with considerable potential. Unlike grasses, many forbs provide superior forage quality, including greater CP concentrations, lesser fiber fractions, and greater digestibility (Muir et al., 2014). Sunn hemp (*Crotalaria juncea*), for example, can produce CP concentrations between 17.6 to 18.8% and in vitro digestibility values of 53 to 56%, both of which are greater than those of typical warm-season perennial grasses (Jaramillo et al., 2020c). Their diversity in production and nutritive value provides opportunities for extending the grazing season, improving animal performance, effective nitrogen cycling, and supporting more resilient forage systems (Dubeux Jr et al., 2024). Muir et al. (2014) revealed that most of these species are well adapted to countries across the world, as they provide benefits beyond nutrition. Moreover, certain forbs contain PSM, such as tannins, flavonoids, and saponins, that have been associated with reduced enteric CH_4 emissions and improved nitrogen retention in ruminants (Pecetti et al., 2006; Dubeux Jr et al., 2024). Despite their potential, forbs remain underutilized in forage systems of the southeastern United States. Research focusing on identifying forb species that can adapt and perform well under the region's diverse soil and climatic conditions is still limited (Muir et al., 2011). Expanding this knowledge base is crucial for improving forage diversity and resilience in beef production systems since different environmental influences can play a major role in the adaptation and establishment of these species. Thus, the objective of this study was to evaluate environmental influences, such as location, on yield, nutritional value, and PSM expressions on both native and commercially available forb species in Alabama.

Materials and Methods

Field Sites

A two-year study was conducted at three Auburn University Research and Extension Centers across Alabama: Chilton Research and Extension Center, Clanton, AL (CREC); Swine Research and Education Center, Auburn, AL (SREC); and Wiregrass Research and Extension Center, Headland, AL (WREC). These sites represented central and southern Alabama's diverse soil and climatic conditions. The CREC site was dominated by Ruston fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults); SREC by Pacolet sandy loam (fine, kaolinitic, thermic Typic Kanhapludults); and WREC by Dothan fine sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults). Fertilization and liming recommendations were made based on soil tests conducted by the Auburn University Soil Testing laboratory in 2022 (Table III-1).

Temperature and Precipitation

During experimental period, monthly average air temperature and precipitation varied by location and season, influencing establishment and production of the forb species. Across all locations, warm-season temperatures ranged from 20 to 29°C (Table III -2), supporting active growth of warm-season species, whereas cool-season temperatures ranged from 4 to 18°C and were more favorable for cool-season forage development. Precipitation varied among years, with

2024 receiving greater total rainfall compared to 2023 across all sites (Table III -3). Despite 2024 being a relatively wet year, precipitation distribution was uneven. Notably, the establishment period for cool-season forages in October 2024 saw a severe moisture deficit, receiving no recorded rainfall in CREC.

Experimental Design

This experiment was arranged as a replicated randomized complete block design (RCBD) consisting of three field blocks per location. Each field plot measured 1.5 × 1.5m (2.25-m^2), with 1.5m tilled or herbicide-treated alleys separating plots within and between blocks to minimize edge interference and lateral root competition. A 1.5m border was maintained on all outer plot edges to reduce border effects.

A total of 24 cool-season and 12 warm-season species were planted at each location in both years (Table III -5). Perennial species were not replanted during the warm season to evaluate their capacity for natural reestablishment following initial establishment. Seeding rates followed the Auburn University Extension recommendations for each species to ensure agronomic relevance and stand uniformity (Dillard, 2023; Table III -5).

Establishment and management

Fertilizer applications were standardized to provide adequate nitrogen levels at planting and during early growth stages (331 kg/ha at each location). Throughout both growing seasons, weeds within plots were controlled by hand removal to minimize plant–weed competition. Weeds in alleys were managed using a nonselective, systemic herbicide, Roundup$^{@}$ containing 50.2% glyphosate (N-(phosphonomethyl)glycine). The herbicide was applied as a solution at a concentration of 4ml/L using a knapsack sprayer. This provided broad-spectrum control of grasses and broadleaf weeds through foliar uptake and translocation to roots.

Sampling

The first forage harvest was conducted when individual plant species reached either 25% flowering (for warm-season species) or an average canopy height of 30 cm (for cool-season species; Mullenix and Johnson, 2018). Subsequent harvests were performed on 28-d intervals for cool-season species or at 25% flowering for warm-season species, continuing until vegetative growth ceased, resulting in four harvest cycles per growing season. For each harvest, total aboveground biomass was recorded in a 1-m^2 area per plot. A subsample of fresh material was collected and transported to the laboratory for the determination of dry matter (DM), organic matter (OM), NDF, ADF, and CP concentration.

Greenhouse Trial

In addition to the three field sites, a fourth site was added under controlled environment conditions (greenhouse) at the Plant Science Research Center, Auburn, AL, (PSRC). Plants were grown in 6.05-L plastic pots filled with a commercial peat-based substrate (Pro-Mix BX Premier Tech, PA, USA). The substrate consisted of a blend of 75 to 85% sphagnum peat moss, horticultural-grade perlite, and vermiculite, amended with limestone to adjust pH and containing a proprietary mycorrhizal inoculant (*Glomus intraradices*). In the cool-season, a total of 24 pots were used per block, representing 24 cool-season species, resulting in 72 pots. For the warm-season species, 12 pots were used per block with a total of 36 pots. At establishment, each pot was seeded with 1g (Table III -5) of its respective forb species. Pots were watered to saturation on the day of seeding, and moisture was maintained using a drip irrigation system that operated daily throughout the period. Defoliation events occurred according to the same triggering benchmarks as in the field. Each pot was harvested to a stubble height of 15cm, and subsequent harvests followed the same protocol used at the field sites.

Analytical Procedures

Samples from each experiment were dried at 55°C in a forced-air oven for approximately 72 h. Samples were ground to pass through a 1-mm screen using an Eberbach E3500 series Mill (Eberbach Corporation, Van Buren Charter Township, MI, USA). Composite samples were made to create a single sample per plot per location, such that there were 72 samples per location in the cool season and 36 samples per location in the warm season.

Dry matter and OM were determined using procedures from the Association of Official Agricultural Chemists (AOAC, 2000). Neutral detergent fiber (NDF) and ADF were analyzed using an ANKOM $^{\text{Delta}}$ and ANKOM ^{2000} Fiber Analyzers (Ankom Technologies, Macedon, NY, USA) following the procedures of Vogel et al. (1999). Acid detergent lignin (ADL) was determined using the sulfuric acid method (AOAC, 2000) with an ANKOM Daisy $^{\text{II}}$ incubator (Ankom Technologies, Macedon, NY, USA). Forage samples were evaluated for CP using the Dumas combustion method and calculated as N × 6.25 (AOAC, 2000) at Cumberland Valley Analytical Services (Waynesboro, PA).

Samples were sent to the Texas A&M AgriLife Research laboratory in Stephenville, TX, where CT analyses were conducted. Condensed tannin concentration was determined using a protein-binding assay according to Naumann et al. (2014). Ground forage samples were analyzed for their ability to bind protein, and tannin–protein complexes were quantified spectrophotometrically at 510 nm. The assay estimates biologically active CT, expressed as mg CT bound per g of plant material. All samples were analyzed in triplicate and averaged across subsamples for each plot.

Statistical Analysis

Data were evaluated using PROC GLIMMIX in SAS 9.4 (SAS Institute, Inc., Cary, NC). A generalized linear mixed model was fit for each response variable. Response variables were assumed to follow a Gaussian distribution. Models were fitted using restricted maximum likelihood (REML) estimation. Forage species were treated as the sole fixed effect. Year, location, and block were included as random effects to account for spatial variability within locations, and the plot served as the experimental unit. Degrees of freedom for fixed effects were calculated using the second-order Kenward–Roger method (Kenward and Roger, 2009), and standard errors were adjusted accordingly. Least squares means were separated using Tukey–Kramer multiple comparison adjustments (Kramer, 1956), and statistical significance was declared at $P \leq 0.05$.

Results and Discussion

Cool-Season Species

There were differences in DMY among species in the field ($P < 0.01$; Table III-6). Hairy vetch produced the greatest DMY ($P < 0.01$; 7,750 kg DM/ha), followed by mustard (6,453 kg DM/ha), turnip (5,146 kg DM/ha), and the turnip–rape hybrid (4,619 kg DM/ha). Birdsfoot trefoil and cicer milkvetch produced the least DMY, and swede failed to establish. The same patterns were observed in the greenhouse experiment; there was a difference among species ($P < 0.01$; Table III-7) for DMY. The greatest DMY were observed for ball clover (21,617 kg DM/ha), kale

(18,473 kg DM/ha), white sweetclover (17,736 kg DM/ha), berseem clover (17,331 kg DM/ha), mustard (17,258 kg DM/ha), red clover (1,758.7 kg DM/ha), and white clover (15,772 kg DM/ha). Hairy vetch also produced moderate DMY (15,052 kg DM/ha), while the least DMY was observed for sainfoin, Texas bluebonnet, and cicer milkvetch. White lupin and swede failed to establish and were excluded from greenhouse DMY comparisons.

The satisfactory productivity of hairy vetch in this study exceeded the averages reported by Cook et al. (2010), who observed yields of approximately 4,500 kg DM/ha in late-spring harvests. Our results align more closely with Steven et al. (2017), who noted that hairy vetch can produce between 7,600 and 9,000 kg DM/ha when well managed. The performance of ball, red, and berseem clovers in the greenhouse suggests high genetic potential for biomass, though their field performance is often moderated by environmental stressors (Araújo et al., 2015). The relatively less DMY of birdsfoot trefoil and cicer milkvetch are characteristic of many perennial legumes, which prioritize root development and carbohydrate storage over biomass during the seedling year (Zimdahl, 2018). Brassica species produced comparatively greater yields, consistent with previous reports where brassicas produced over 2,000 kg DM/ha within 45 d under southeastern U.S. conditions (Dillard, 2024). Furthermore, mustard yields in the greenhouse (17258 kg DM/ha) align with research by Watt et al. (2021) and Karydogianni et al. (2022) which demonstrated that brassica genotypes can match or exceed traditional forage crops like oats (*Avena sativa* L.) in terms of biomass across different types of environments. The reduction in DMY in field plots compared to greenhouse containers highlights the impact of environmental variability. While greenhouse conditions provided uniform moisture, optimal temperatures, and a lack of interspecific competition, field plots were subjected to soil heterogeneity and weather variability. The intensive management of small plots, including biweekly weeding, likely mitigated the competitive exclusion typically seen in mixed-species pastures, allowing a clearer expression of each species' yield potential (Smith & Doran, 2021).

Unlike DMY, there were no differences in OM concentrations among species in either the field ($P < 0.01$) or the greenhouse ($P < 0.01$; Table III-6). However, NDF differed among species ($P < 0.01$). In the field, the greatest NDF concentrations were observed in crimson clover (48%), radish (46%), and hairy vetch (45%), while rape (24%) and kale (25%) were least. Greenhouse results followed a similar pattern; berseem clover (41%) and white sweetclover (41%) exhibited the greatest NDF, whereas rape (20%) and turnip (21%) remained the least fibrous. Acid detergent fiber and lignin also showed significant variation ($P < 0.01$). Radish (34%) and crimson clover (33%) had the greatest field ADF, while cicer milkvetch (16%) and rape (16%) had lesser concentrations. For ADL, sainfoin exhibited the greatest concentration in the field (13%), followed closely by chicory (12%). In the GH, chicory (11%) and sainfoin (10%) remained the most lignified, while brassica species (rape, turnip, kale) consistently reported ADL values below 2%. Crude protein concentration was affected by species ($P < 0.01$; Table III-6). In the field, hairy vetch (27%) had the most CP, followed by birdsfoot trefoil (24%) and red clover (24%). The least field CP was found in cicer milkvetch (12%). In the GH, hairy vetch (29%) and white sweetclover (28%) were the greatest protein sources, while rape forage (16%) and turnip (16%) had lesser values.

Detergent fiber system described by Van Soest et al. (1991) emphasizes that forage utilization by ruminants is largely governed by cell wall composition, particularly NDF and ADF. Organic matter concentration did not differ among species, suggesting that ash was not a major driver of nutritional differences. There were fiber fraction differences among species, with a clear separation between brassicas (rape, kale, turnip–rape hybrid) and many of the clovers and other forb species. This was expected as brassicas tend to have an increased leaf-to-stem ratio as compared to other forbs (Buxton, 1996). Brassica species exhibited the least NDF (e.g., rape 24%, kale 25%), whereas legumes such as crimson clover, hairy vetch, and berseem clover, as well as radish, had NDF concentrations ≥ 40%. This pattern closely aligns with existing studies that demonstrated that brassica forages usually have 18 to 24% NDF which is less than perennial ryegrass (*Lolium perenne* L.; 53%) or most legumes (Barry, 2013a). The relatively increased NDF and ADF in crimson clover, hairy vetch, and some other legumes are also consistent with the broader literature, where leguminous forbs and clovers generally exhibit greater fiber concentrations than brassicas, particularly at more advanced phenological stages (Waghorn, 2008; Sollenberger and Dubeux, 2022). Increased NDF values in legumes are often associated with increased stem development and greater structural carbohydrate deposition as plants mature (Buxton and Redfearn, 1997). In contrast, brassicas such as rape and kale have less NDF concentrations, consistent with their minimal structural tissue and high proportion of soluble cell contents (Barry, 2013b). These NDF values suggest a greater potential for voluntary intake when brassicas are incorporated into ruminant diets which can be further studied in preference and grazing studies.

Similar patterns were observed with ADF; radish (*Raphanus sativus* L.*)*, crimson clover (*Trifolium incarnatum* L.*)*, hairy vetch (*Vicia villosa* Roth.*)*, and chicory (*Cichorium intybus* L.*),* showing greater ADF concentrations, indicating a greater proportion of less digestible fiber fractions. In contrast, brassicas maintained lesser ADF concentrations, supporting previous findings that brassica forages are highly digestible despite moderate biomass yields (Ball et al., 2007). Collectively, these results reinforce the importance of evaluating fiber composition alongside yield when selecting forage species for grazing or conserved feed systems.

Sainfoin, chicory, and berseem clover had the greatest ADL concentrations, whereas brassicas such as rape and kale had the least. The increased lignin content observed in sainfoin and chicory is consistent with their classification as tannin-containing forbs, in which lignified tissues contribute to structural integrity and are often associated with condensed tannin presence (Huyen et al., 2016; Butkutė et al., 2018). Elevated lignin typically reduces fiber digestibility; however, sainfoin's tannins can also confer anti-bloat and protein-protective effects that may offset some negative impacts on overall feeding value (Waghorn, 2008; Huyen et al., 2016).

Crude protein is an important forage factor in ruminant nutrition. Hairy vetch, red clover, and alfalfa contained greater CP than the rest of the species. In a Tunisian study of rainfed vetch–triticale mixture, mean CP concentrations of about 18% were reported (BenYoussef et al.), which is almost in the same range as the current study. These results are supported by most literature that demonstrates increased concentration of CP in legumes compared to grasses (Phelan et al., 2015). Yildiz et al. (2022) reported CP concentration ranging from 14 to 15% of turnip cultivars harvested at full flowering. As cultivar maturity increased, a drop to a 9% CP concentration was reported. This is to be expected with increased plant maturity (Van Soest, 1967). In addition, Greveniotis et al. (2025) in Greece had 18 to 20% CP concentrations in a red clover cultivar evaluation. Reduced CP concentrations (12 to 16%) observed in species such as burr medic, Texas bluebonnet, and cicer milkvetch may be attributed to slower establishment, reduced biomass accumulation, or greater structural development at harvest. Although these results could be considered low in the current study, CP is above typical legume CP concentrations (15 to 20%) and well above those of warm season perennial grasses (5 to 12 %; Riaz et al., 2025). In the current study, patterns in nutritive value, particularly NDF, ADF, and CP concentrations, were largely consistent between greenhouse and field studies, suggesting that inherent species characteristics play a dominant role in determining forage quality.

Warm-Season Species

Warm-season forb species differed in DMY in both fields (*P* < 0.01; Table III-6) and GH (*P* < 0.01; Table III-7*)*. In the field, sunn hemp produced the greatest DMY (31,421.7 kg DM/ha), exceeding cowpea (20,189.6 kg DM/ha), lablab (16,787.2 kg DM/ha), hairy indigo (10,228.6 kg DM/ha), sericea lespedeza (9,313.6 kg DM/ha), and panicled leaf tick trefoil (5,560.1 kg DM/ha). Maximilian sunflower and tall bush clover had the least DMY (< 400 kg DM/ha). In the GH, cowpea (388,180 kg DM/ha) and sunn hemp (316,620 kg DM/ha) remained the most productive species in terms of DMY, consistent with the field. While species such as lablab (240,500 kg DM/ha) and hairy indigo (144,050 kg DM/ha) were intermediate, several species produced less DMY including maximilian sunflower (5,192.3 kg DM/ha), and tall bush clover (16,875 kg DM/ha). Organic matter differed in the field (*P* < 0.01; Table III-6), ranging from 84% (lablab), to 94% (sericea lespedeza), but no differences were observed in the GH (*P* = 0.88).

Sunn hemp produced the greatest DMY in both environments. These findings are supported by Jaramillo et al. (2020b). Warm-season forbs are well adapted to the South and establish readily because they are tolerant of warm climate conditions (Curto et al., 2015). Cantrell et al. (2010) described sunn hemp as a fast-growing, high-biomass-yielding tropical legume. The report stated a biomass of 10,700 kg DM/ha in just 12 weeks of growth. Cowpea and lablab also produced greater yields, supporting their classification as productive warm-season legumes capable of substantial biomass accumulation under favorable conditions. These results were further supported by Foster et al. (2017) who reported biomass production of 8,650 kg DM/ha from sunn hemp, 4,700 kg DM/ha for cowpea, and 4,000 kg DM/ha for lablab. In contrast, species such as fenugreek, Illinois bundleflower, slickseed fuzzybean, tall bush clover, and Maximilian sunflower exhibited poor establishment or limited biomass production, suggesting weaker adaptation to the environment or slower early growth rates (Sollenberger and Dubeux, 2022). Also, poor weed management in the first year (2023) affected DMY for species such as Maximilian sunflower.

There were differences in species when evaluating NDF and ADF ($P < 0.01$; Table III-6). In the field, fenugreek had the greatest NDF (69%) and ADF (55%), while Maximilian sunflower (37%) and Illinois bundleflower (39%) were the least n NDF. In the GH, the greatest fiber concentrations were found in sunn hemp (55% NDF; 44% ADF), while lablab exhibited the lowest NDF (38%). Lignin concentrations varied among ($P < 0.01$) species. Sericea lespedeza and panicled leaf tick trefoil had the greatest field ADL (18% and 18%, respectively). Sunn hemp maintained less lignin levels in both the field (6%) and GH (6%) despite its high biomass and fiber. Crude protein differed among species ($P < 0.01$). In the field, cowpea (20%), sunn hemp (20%), and lablab (19%) were the greatest protein sources while Maximilian sunflower had the least field CP (5%). In the GH, fenugreek (22%) and slickseed fuzzybean (21%) achieved the greatest CP concentrations.

Fiber fraction and lignin concentration varied widely and are critical for interpreting potential intake and digestibility. Warm-season annuals such as sunn hemp provide a robust yield increase during the summertime quality gap when perennial grasses' quality declines (Sollenberger and Dubeux, 2022). Warm-season forage species showed broadly similar patterns in fiber and CP concentrations both in the field and GH; however, these responses differed between environments. Fiber fractions concentrations were greater under greenhouse conditions for several species, including sunn hemp, sericea lespedeza, and panicled leaf tick trefoil. This suggests that enhanced growth under controlled conditions was accompanied by increased structural development, likely due to uninterrupted vegetative growth and reduced environmental stress (Ball et al., 2007). In contrast, species such as lablab maintained less NDF and ADF concentrations in both environments, indicating more stable forage nutritive value parameters regardless of growing conditions. Comparisons between the field and GH environments indicate that fiber composition and CP were in part influenced by growing conditions.

In the field, the variations in fiber and CP concentrations observed among warm-season forage species reflect inherent differences in plant morphology, growth habit, and maturity at harvest (Buxton, 1996; Foster et al., 2017). Fenugreek exhibited the greatest NDF and ADF concentrations, indicating a more fibrous and structurally developed canopy (Van Soest et al., 1991). Studies by Jaramillo et al. (2020) in Florida reported CP concentrations of 18 to 19% in sunn hemp. This was similar to the current study's findings on sunn hemp CP of 19%. Fenugreek had the greatest CP concentration of 22% in the GH. These findings align with a Canadian study by Mir (1997), which reported a 22% CP concentration for fenugreek grown under both greenhouse and irrigated field conditions. By establishing a consistent nutritional profile across different environments, this data provides a strong foundation for future research into the adaptability and integration of forbs within diverse pasture systems.

Condensed Tannins

Condensed tannin concentrations across the evaluated species revealed distinct patterns between seasons. In the cool-season, CT levels were generally low or undetectable ($P = 0.10$; Table III-6), with red clover (20.5 mg/g DM) and white lupin (18.3 mg/g DM) exhibiting the greatest concentrations, although there were no differences among species ($P = 0.10$). In contrast, there were differences among species in warm-season (*P* = 0.03). Illinois bundleflower (44.5 mg/g DM) stood out with the highest concentration, followed by Hairy Indigo (29.5 mg/g DM) and Sericea lespedeza (27.1 mg/g DM).

This seasonal difference in CT concentration aligns with (Bennett and Wallsgrove, 1994), who suggest that CT concentrations fluctuate as a defensive adaptation to external triggers and abiotic stress. The elevated CT in warm-season species likely serves as a survival strategy against the increased herbivory and heat stress characteristic of summer environments. These species fall within the moderate range (under 50 mg/g DM) described by (Lisonbee et al., 2009; Villalba et al., 2011; Lorenz et al., 2014). According to (MacAdam and Villalba, 2015), these concentrations are ideal for creating reversible tannin-protein complexes that prevent excessive ruminal ammonia production and increase the flow of bypass protein to the small intestine. This suggests that while cool season species like hairy vetch offer more CP (27.3%; Table III-6), their lack of CT may lead to less efficient nitrogen utilization compared to warm season species like illinois bundleflower.

Furthermore, the increased CT concentration in forb species presents significant environmental advantages (Hassanat and Benchaar, 2013) noted that CT concentrations near 50 mg/kg DM can reduce methane production by up to 40%. Illinois bundleflower, at 44.5 mg/g DM, closely approaches this range, indicating its high potential for methane mitigation and modified fermentation patterns. However, it also comes with a fiber fraction limitation; sericea lespedeza and panicled leaf tick trefoil also exhibited the greatest ADL (18.3 and 17.8%, respectively). Condensed tannin levels, when combined with high lignification, may slow DMI and reduce overall digestibility (MacAdam and Villalba, 2015).

Conclusions

Results from this study indicate that both cool- and warm-season forage forbs can be successfully established and produce meaningful DMY under southeastern U.S. conditions when appropriate planting timing, seeding rates, and weed control are implemented. These species function primarily as annual forage components within pasture systems and should be viewed as strategic, short-term interventions rather than replacements for perennial forage bases. Consequently, grassland managers must consider establishment costs, timing, and integration with existing pasture systems when adopting these species.

Distinct differences were observed between cool- and warm-season forbs that have important management implications. Cool-season species generally provided higher CP concentrations but lower biomass production, making them well suited to address forage quality limitations during winter months when perennial grasses are less productive. In contrast, warm-season forbs such as sunn hemp, cowpea, and lablab produced greater biomass, offering an effective solution to summer forage shortages, although nutritive value may decline with advancing maturity due to increased fiber and lignin concentrations.

From a practical standpoint, forage systems in the South are often limited by forage quantity during cooler months and by forage quality during warmer months. Therefore, integrating both cool- and warm-season forbs provides a complementary strategy to address these seasonal constraints. Leguminous forbs offer additional benefits through biological nitrogen fixation and high CP concentrations, which may reduce reliance on supplemental nitrogen inputs and improve overall system efficiency. The successful use of these species will depend on timely grazing or harvest management to prevent declines in digestibility associated with maturity, as well as consideration of species persistence. Recommendations on species should also depend on the needs of the pastures; forage availability or forage quality supplementation. Overall, these findings support the use of diverse, seasonally complementary forage forbs to enhance pasture productivity, improve forage nutritive value, and increase resilience of ruminant production systems in the South. Future studies should evaluate grazing-based systems to determine how these species perform under continuous or rotational grazing and their long-term impacts on soil fertility and pasture persistence.

Table III-1 Soil test report, including soil nutrient levels (P, K, Ca, and Mg) and recommendations for application (limestone), across three Alabama field sites in which forage forb species were planted**.**

			Soil Nutrients2 kg/ha				
Site[1]	**County**	**pH**	**P, kg/ha**	**K, kg/ha**	**Ca, kg/ha**	**Mg, kg/ha**	**Limestone, kg/ha**
CREC	Chilton	5.8	72	76	906	80	2,240
SREC	Lee	6.8	3,716	174	10,645	1,003	0
WREC	Henry	6.1	132	221	1,143	108	0

[1] CREC = Chilton Research and Extension Center; SREC = Swine Research and Education Center; WREC = Wiregrass Research and Extension Center

[2] P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium

Table III-2 Monthly average air temperature (°C) at SREC, CREC, and WREC in Alabama from April 2023 through April 2025

	Location[1]								
	SREC			CREC			WREC		
Month	2023	2024	2025	2023	2024	2025	2023	2024	2025
January		7	5		6	4.6		10	4
February		12	13		11.2	11.6		13	12
March		15	14		14.2	14.5		17	15
April	17	19	20	17	18	19	20	20	18
May	20	23		21	23		23	25	
June	24	26		24	26		27	28	
July	28	27		27	27		29	29	
August	27	28		28	27		30	29	
September	24	24		24	24		26	26	
October	18	19		18	19		20	21	
November	13	17		12	16		15	18	
December	10	10		9	9		12	12	

[1] SREC = Swine Research and Education Center; CREC = Chilton Research and Extension Center; WREC = Wiregrass Research and Extension Center

Table III-3 Monthly average precipitation (mm) at SREC, CREC, and WREC in Alabama from April 2023 through April 2025

	Location[1]								
	SREC			CREC			WREC		
Month	2023	2024	2025	2023	2024	2025	2023	2024	2025
January		201	80		270	87		153	71
February		181	120		191	158		105	72
March		185	113		175	156		152	109
April	128	75	175	98	95	114	153	86	69
May	70	239		67	161		82	164	
June	222	74		108	74		126	94	
July	120	188		128	199		112	110	
August	137	8		66	75		63	24	
September	63	240		18	152		66	266	
October	103	4		41	0		29	2	
November	78	100		55	57		102	77	
December	79	130		58	66		121	60	

[1] SREC = Swine Research and Education Center; CREC = Chilton Research and Extension Center; WREC = Wiregrass Research and Extension Center

Table III-4 Planting dates across three Alabama field sites and a controlled-climate environment in which forage forb species were evaluated.

Season	**Year**	**CREC[1]**	**SREC**	**WREC**	**PSRC**
Cool season	2023	02 Nov	27 Oct	24 Oct	27 Oct
	2024	14 Oct	29 Oct	08 Nov	16 Oct
Warm season	2023	03 Apr	04 Apr	06 Apr	04 Apr
	2024	10 Apr	12 Apr	17 Apr	12 Apr

[1] CREC = Chilton Research and Extension Center, Prattville, AL; SREC = Swine Research and Education Center, Auburn, AL; WREC = Wiregrass Research and Extension Center, Headland, AL; PSRC=Plant Science Research Center (greenhouse), Auburn, AL.

Table III-5 Descriptions of forage forb species evaluated across three Alabama field sites and a controlled-climate

Common name	Scientific name	Season of growth	Cultivar[1]	Seeding rate, g/plot
Cicer milkvetch	*Astragalus cicer*	Cool	VNS	2.53
Turnip	*Brassica campestris*	Cool	Purple Top	0.72
Rape	*Brassica napus*	Cool	Bonar	4.33
Swede	*Brassica napus*	Cool	Major Winton	0.63
Turnip-rape hybrid	*Brassica napus*	Cool	T-Raptor	1.45
Kale	*Brassica oleracea*	Cool	Maris Kestrel	1.17
Mustard	*Brassica rapa*	Cool	Florida Broadleaf	4.64
Chicory	*Cichorium intybus*	Cool	Forage Feast	1.81
Birdsfoot trefoil	*Lotus corniculatus*	Cool	VNS	2.22
White lupin	*Lupinus albus*	Cool	AU Alpha	43.43
Texas bluebonnet	*Lupinus texensis*	Cool	VNS	8.74
Burr medic	*Medicago polymorpha*	Cool	Cavalier	10.41
Alfalfa	*Medicago sativa*	Cool	Bulldog 805	7.07
White sweetclover	*Melilotus alba*	Cool	Silver River	11.02
Sainfoin	*Onobrychis viciifolia*	Cool	VNS	10.53
Radish	*Raphanus sativus*	Cool	Driller	4.07
Berseem clover	*Trifolium alexandrinum*	Cool	Frosty	7.24
Crimson clover	*Trifolium incarnatum*	Cool	AU Robin	9.20
Ball clover	*Trifolium nigrenscens*	Cool	AU Don	2.22
Red clover	*Trifolium pratense*	Cool	AU Red Ace	6.51
White clover	*Trifolium repens*	Cool	Neches	1.09
Subterranean clover	*Trifolium subterraneum*	Cool	Dalkeith	9.37
Arrowleaf clover	*Trifolium vesiculosum*	Cool	Apache	7.07
Hairy vetch	*Vicia villosa*	Cool	AU Merit	14.48
Sunn hemp	*Crotalaria juncea*	Warm	VNS	12.51
Illinois bundleflower	*Desmanthus illinoensis*	Warm	Reno	1.02
Panicled-leaf tick-trefoil	*Desmodium paniculatum*	Warm	VNS	0.09

Soybean	*Glycine max*	Warm	Laredo	17.73
Lablab	*Lablab purpureus*	Warm	Rongai	8.31
Sericea lespedeza	*Lespedeza cuneata*	Warm	AU Grazer	9.38
Tall bush-clover	*Lespedeza stuevei*	Warm	VNS	12.71
Slickseed fuzzybean	*Strophostyles leiosperma*	Warm	VNS	3.25
Fenugreek	*Trigonella foenum-graecum*	Warm	VNS	9.70
Cowpea	*Vigna unguiculata*	Warm	Iron & Clay	34.72

[1] VNS = variety not stated

Table III-6 Yield, tannins, and nutritive value of cool and warm-season forage forb species evaluated across three Alabama field sites.

				Nutritive value component[4], %				
Species	**Season[1]**	**DMY[2], Kg/ha**	**CT[3], mg/g DM**	**OM**	**NDF**	**ADF**	**ADL**	**CP**
Alfalfa	CS	1072 ± 193.89^{CDE}	0	73.0 ± 0.10	32.7 ± 0.03 ^{ABCD}	21.2 ± 0.03	5.0 ± 0.01 ^{B}	23.3 ± 0.03 ^{AB}
Arrowleaf clover	CS	1204 ± 193.89 ^{BCDE}	0	77.1 ± 0.09	30.2 ± 0.03 ^{ABCD}	21.1 ± 0.02	4.1 ± 0.01 ^{B}	22.9 ± 0.02 ^{AB}
Ball clover	CS	1395 ± 193.89 ^{BCDE}	0	75.2 ± 0.10	28.7 ± 0.03 ^{BCD}	19.2 ± 0.03	5.4 ± 0.01 ^{B}	20.1 ± 0.02 ^{B}
Berseem clover	CS	1753 ± 193.89 ^{BCDE}	11.5 ± 10.11	83.0 ± 0.10	42.3 ± 0.03 ^{A}	27.4 ± 0.02	10.7 ± 0.01 ^{AB}	22.2 ± 0.02 ^{AB}
Birdsfoot trefoil	CS	105 ± 193.89 ^{E}	0	84.8 ± 0.12	31.5 ± 0.04 ^{ABCD}	22.0 ± 0.04	5.3 ± 0.02 ^{B}	23.6 ± 0.03 ^{AB}
Burr medic	CS	2008 ± 193.89 ^{BCDE}	10.5 ± 10.81	85.7 ± 0.09	40.6 ± 0.03 ^{A}	27.9 ± 0.03	6.7 ± 0.01 ^{B}	16.6± 0.02 ^{B}
Chicory	CS	1852 ± 193.89 ^{BCDE}	0	74.2 ± 0.09	40.3 ± 0.03 ^{A}	31.9 ± 0.02	11.6 ± 0.01 ^{AB}	21.1 ± 0.02 ^{B}
Cicer milkvetch	CS	113 ± 193.89 ^{E}	0	93.7 ± 0.27	29.6 ± 0.06 ^{ABCD}	15.5 ± 0.06	2.7 ± 0.03 ^{B}	12.2 ± 0.04 ^{B}
Crimson clover	CS	2024 ± 193.89 ^{BCDE}	0	71.5 ± 0.09	48.2 ± 0.03 ^{A}	32.8 ± 0.02	6.5 ± 0.01 ^{B}	17.5 ± 0.02 ^{B}
Hairy vetch	CS	7750 ± 193.89 ^{A}	0	83.4 ± 0.09	44.7 ± 0.03 ^{A}	32.7 ± 0.03	8.4 ± 0.01 ^{B}	27.3 ± 0.02 ^{A}
Kale	CS	2275 ± 193.89 ^{BCDE}	6 ± 10.28	63.0 ± 0.10	25.2 ± 0.03 ^{CD}	16.4 ±0.02	2.9 ± 0.01 ^{B}	23.4 ± 0.03 ^{AB}
Mustard	CS	6453 ± 193.89 ^{A}	15.1 ± 9.58	88.3 ± 0.09	36.3 ± 0.02 ^{AB}	25.1 ± 0.02	5.4 ± 0.01 ^{B}	19.7 ± 0.02 ^{B}

Radish	CS	4474 ± 193.89 ^{ABC}	15.3 ± 9.81	86.3 ± 0.09	45.9 ± 0.03 ^{A}	33.8 ± 0.02	6.8 ± 0.01 ^{B}	17.7 ± 0.02 ^{B}
Rape	CS	681 ± 193.89 ^{CDE}	13.7 ± 10.28	72.6 ± 0.10	23.5 ± 0.03 ^{D}	16.0 ± 0.02	2.9 ±0.01 ^{B}	22.0 ± 0.02 ^{AB}
Red clover	CS	1080 ± 193.89 ^{BCDE}	20.5 ± 9.8	86.4 ± 0.09	37.5 ± 0.03 ^{AB}	23.5 ± 0.02	8.9 ± 0.01 ^{B}	23.6 ± 0.02 ^{AB}
Sainfoin	CS	1272 ± 193.89 ^{BCDE}	0	90.9 ± 0.11	34.5 ± 0.03 ^{ABC}	23.8 ± 0.02	12.9 ±0.01 ^{A}	20.1 ± 0.02 ^{B}
Subterranean clover	CS	2061 ± 193.89 ^{BCDE}	11.8 ± 10.5	72.0 ± 0.10	42.2 ± 0.03 ^{A}	30.2 ± 0.03	7.4 ± 0.01 ^{B}	17.9 ± 0.02 ^{B}
Texas bluebonnet	CS	479 ± 193.89 ^{DE}	0	74.3 ± 0.10	39.0 ± 0.03 ^{AB}	28.5 ±0.03	4.2 ± 0.01 ^{B}	16.3 ± 0.03 ^{B}
Turnip	CS	5146 ± 193.89 ^{AB}	0	70.6 ± 0.09	30.2 ± 0.03 ^{ABCD}	20.2 ± 0.02	4.1 ± 0.01 ^{B}	22.9 ± 0.02 ^{AB}
Turnip-rape hybrid	CS	4619 ± 193.89 ^{AB}	12.9 ± 9.7	85.4 ± 0.08	32.6 ± 0.03 ^{ABCD}	20.7 ± 0.02	4.1 ± 0.01 ^{B}	23.8 ± 0.02 ^{AB}
White clover	CS	1524 ± 193.89 ^{BCDE}	0	70.3 ± 0.09	34.2 ± 0.03 ^{ABC}	23.2 ± 0.02	5.3 ± 0.01 ^{B}	22.5 ± 0.02 ^{AB}
White lupin	CS	3793 ± 193.89 ^{ABCDE}	18.3 ± 9.81	72.9 ± 0.09	39.2 ± 0.03 ^{A}	26.1 ± 0.02	5.4 ± 0.01 ^{B}	19.7 ± 0.02 ^{B}
White sweetclover	CS	1308 ± 193.89 ^{BCDE}	0	68.2 ± 0.08	32.5 ± 0.03 ^{ABCD}	19.7 ± 0.02	5.0 ± 0.01 ^{B}	21.8 ± 0.02 ^{B}
Cowpea	WS	20189.6 ± 359.11 ^{B}	11.2 ± 7.65 ^{AB}	89.9 ± 0.02 ^{AB}	43.2 ± 0.04	32.0 ± 0.02 ^{A}	7.8 ± 0.01 ^{C}	20.4 ± 0.02 ^{A}
Fenugreek	WS	820.125 ± 359.11 ^{DE}	0	93.9 ± 0.07 ^{AB}	68.8 ± 0.09	54.5 ± 0.06 ^{A}	14.1 ± 0.03 ^{ABC}	6.7 ± 0.03 ^{DE}
Hairy Indigo	WS	10228.6 ± 359.11 ^{CD}	29.5 ± 9.51 ^{AB}	90.1 ± 0.02 ^{AB}	42.2 ± 0.05	32.9 ± 0.02 ^{A}	10.8 ± 0.01 ^{C}	19.3 ± 0.02 ^{ABC}
Illinois bundleflower	WS	2457.0 ± 359.11 ^{DE}	44.5 ± 8.43 ^{AB}	92.0 ± 0.02 ^{AB}	39.3 ± 0.05	24.7 ± 0.02 ^{B}	10.3 ± 0.01 ^{C}	16.1 ± 0.02 ^{ABCD}

Lablab	WS	16787.2 ± 359.11 ^{BC}	0	83.9 ± 0.02 ^{B}	45.7 ± 0.05	32.7 ± 0.02 ^{A}	7.9 ± 0.01^{C}	19.4 ± 0.02 ^{AB}
Maximilian Sunflower	WS	163.8 ± 359.11 ^{E}	0	91.2 ± 0.07 ^{AB}	37.1 ± 0.09	29.2 ± 0.06 ^{AB}	8.2 ± 0.03 ^{C}	4.8 ± 0.0.02 ^{E}
Panicled leaf tick trefoil	WS	5560.1 ± 359.11 ^{DE}	26.7 ± 9.51 ^{AB}	93.9 ± 0.02 ^{A}	50.2 ± 0.05	39.1 ± 0.02 ^{A}	17.8 ± 0.01 ^{AB}	14.1 ± 0.02 ^{BCD}
Sericea lespedeza	WS	9313.6 ± 359.11 ^{CDE}	27.1 ± 8.15 ^{AB}	94.1 ± 0.02 ^{A}	48.2 ± 0.04	37.6 ± 0.02 ^{A}	18.3 ± 0.01 ^{A}	16.1 ± 0.02 ^{ABCD}
Slickseed fuzzybean	WS	2219.8 ± 359.11 ^{DE}	24.4 ± 11.16 ^{AB}	93.1 ± 0.03 ^{AB}	48.8 ± 0.05	34.9 ± 0.03 ^{A}	9.9 ± 0.01 ^{C}	12.1 ± 0.02 ^{CDE}
Soybean	WS	5490.2 ± 359.11 ^{DE}	12.2 ± 11.16 ^{AB}	90.3 ± 0.02 ^{AB}	47.6 ± 0.05	34.3 ± 0.3 ^{A}	8.9 ± 0.01 ^{C}	14.7 ± 0.02 ^{ABCD}
Sunn hemp	WS	31421.7 ± 359.11 ^{A}	12.3 ± 11.16 ^{AB}	92.4 ± 0.02 ^{AB}	48.5 ±0.05	35.8 ± 0.03 ^{A}	6.3 ± 0.01^{C}	19.6 ± 0.02 ^{AB}
Tall bush clover	WS	342.50 ± 359.11 ^{E}	0	92.6 ± 0.04 ^{AB}	42.3 ± 0.06	30.6 ± 0.04 ^{AB}	11.9 ± 0.02 ^{BC}	10.3 ± 0.02 ^{DE}
P-value	CS	< 0.01	0.1	0.8	< 0.01	< 0.01	<0.01	< 0.01
P-value	WS	< 0.01	0.03	0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1] CS = Cool season; WS= Warm season

[2] DMY = dry matter yield

[3] CT = Condensed tannin

[4] OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

$^{A\text{-}I}$Means within a column different letters indicate significant differences ($P < 0.05$).

Values are presented as Mean ± Standard Error of the Mean (SEM)

Table III-7 Yield, tannins, and nutritive value of cool and warm-season forage forb species evaluated in the greenhouse

				Nutritive value component[4], %				
Species	**Season[1]**	**DMY[2], kg DM /ha**	**CT[3], mg/g DM**	**OM**	**NDF**	**ADF**	**ADL**	**CP**
Alfalfa	CS	12349 ± 921.02 ^{ABC}	0	88.1 ± 5.07	36.3 ± 1.96 ^{ABCD}	23.0 ± 1.27 ^{ABCD}	5.4 ± 1.39 ^{CDEFGH}	26.2 ± 2.92 ^{AB}
Arrowleaf clover	CS	10515 ± 921.02 ^{ABC}	0	88.2 ± 5.07	33.2 ± 1.96 ^{ABCDE}	19.8 ± 1.27 ^{CDEF}	3.6 ± 1.39 ^{EFGHI}	20.3 ± 2.92 ^{BCDEF}
Ball clover	CS	21617 ± 921.02 ^{A}	7.1 ± 6.45	87.7 ± 5.07	37.0 ± 1.96 ^{ABCD}	22.7 ± 1.27 ^{ABCD}	4.9 ± 1.39 ^{CDEFGHI}	24.4 ^{ABCD}
Berseem clover	CS	17331 ± 921.02 ^{AB}	0	85.3 ± 5.07	40.8 ± 1.96 ^{A}	27.7 ± 1.27 ^{A}	7.7 ± 1.39 ^{ABCD}	23.7 ± 2.92 ^{ABCDE}
Birdsfoot trefoil	CS	9205 ± 921.02 ^{ABC}	0	89 ± 5.07	30.6 ± 1.96 ^{BCDEF}	20.4 ^{BCDE}	5.7 ± 1.39 ^{CDEFG}	25.4 ± 2.92 ^{ABC}
Burr medic	CS	6386 ± 921.02 ^{ABC}	0	80.9 ± 5.07	38.0 ± 1.96 ^{ABC}	24.8 ± 1.27 ^{ABCD}	5.1 ± 1.39 ^{CDEFGH}	22.8 ± 2.92 ^{ABCDEF}
Chicory	CS	12667 ± 921.02 ^{ABC}	0	89.6 ± 5.07	34.3 ± 1.96 ^{ABCDE}	24.6 ± 1.27 ^{ABCD}	10.8 ± 1.39 ^{A}	21.2 ± 2.92 ^{ABCDEF}
Cicer milkvetch	CS	3645 ± 921.02 ^{BC}	0	88.3 ± 5.07	28.8 ± 1.96 ^{CDEFG}	21.9 ± 1.27 ^{ABCDE}	3.5 ± 1.39 ^{EFGHI}	23.2 ± 2.92 ^{ABCDEF}
Crimson clover	CS	9060 ± 921.02 ^{ABC}	0	88.4 ± 5.07	39.7 ± 1.96 ^{AB}	21.7 A ± 1.27 ^{BCDE}	3.5 ± 1.39 ^{EFGHI}	23.4 ± 2.92 ^{ABCDE}
Hairy vetch	CS	15052 ± 921.02 ^{ABC}	0	88.9 ± 5.07	39.3 ± 1.96 ^{AB}	27.9 ± 1.27 ^{A}	5.9 ± 1.39 ^{CDEF}	28.5 ± 2.92 ^{A}
Kale	CS	18473 ± 921.02 ^{AB}	0	86.4 ± 5.07	23.5 ± 1.96 ^{FG}	14.1 ± 1.27 ^{FGH}	1.8 ± 1.39 ^{HI}	18.3 ± 2.92 ^{CDEF}
Mustard	CS	17258 ± 921.02 ^{AB}	0	87 ± 5.07	22.5 ± 1.96 ^{FG}	15.5 ± 1.27 ^{EFGH}	1.9 ± 1.39 ^{GHI}	17.7 ± 2.92 ^{DEF}
Radish	CS	9253 ± 921.02 ^{ABC}	14.5 ± 6.45	87.9 ± 5.07	27.4 ± 1.96 ^{DEFG}	19.3 ± 1.27 ^{DEFG}	4.2 ± 1.39 ^{DEFGHI}	19.5 ± 2.92 ^{BCDEF}
Rape	CS	14934 ± 921.02 ^{ABC}	13.8 ± 6.45	86.3 ± 5.07	19.9 ± 1.96 ^{G}	12.6 ± 1.27 ^{H}	1.2 ± 1.39 ^{I}	15.5 ± 2.92 ^{F}

Red clover	CS	17587 ± 921.02 ^{AB}	18.9 ± 6.45	89.7 ± 5.07	38.0 ± 1.96 ^{ABC}	26.3 ± 1.27 ^{AB}	8.1 ± 1.39 ^{ABC}	23.9 ± 2.92 ^{ABCDE}
Sainfoin	CS	5592 ± 921.02 ^{BC}	0	89.8 ± 5.07	33.4 ± 1.96 ^{ABCDE}	25.5 ± 1.27 ^{ABC}	10.4 ± 1.39 ^{AB}	24.3 ± 2.92 ^{ABCD}
Subterranean clover	CS	11703 ± 921.02 ^{ABC}	0	86.7 ± 5.07	35.8 ± 1.96 ^{ABCD}	21.4 ± 1.27 ^{BCDE}	3.9 ± 1.39 ^{DEFGHI}	25.2 ± 2.92 ^{ABC}
Texas bluebonnet	CS	398.0 ± 921.02 ^{BC}	0	86.8 ± 5.07	31.8 ± 1.96 ^{ABCDEF}	20.8 ± 1.27 ^{BCDE}	4.1 ± 1.39 ^{DEFGHI}	16.5 ± 2.92 ^{EF}
Turnip	CS	10576 ± 921.02 ^{ABC}	0	71.7 ± 5.07	20.8 ± 1.96 ^{G}	13.2 ± 1.27 ^{GH}	1.2 ± 1.39 ^{I}	16.0 ± 2.92 ^{F}
Turnip-rape hybrid	CS	6122 ± 921.02 ^{BC}	14.4 ± 6.45	85.1 ± 5.07	25.1 ± 1.96 ^{EFG}	15.6 ± 1.27 ^{EFGH}	2.1 ± 1.39 ^{FGHI}	15.8 ± 2.92 ^{F}
White clover	CS	15772 ± 921.02 ^{AB}	0	88.2 ± 5.07	36.0 ± 1.96 ^{ABCD}	21.9 ± 1.27 ^{ABCDE}	4.9 ± 1.39 ^{CDEFGHI}	24.4 ± 2.92 ^{ABCD}
White sweetclover	CS	17736 ± 921.02 ^{AB}	0	71.6 ± 5.07	40.7 ± 1.96 ^{A}	26.3 ± 1.27 ^{AB}	6.7 ± 1.39 ^{BCDE}	28.3 ± 2.92 ^{A}
Cowpea	WS	388180 ± 7488 ^{A}	0	86.1 ± 0.06	43.6 ± 0.02 ^{AB}	32.2 ± 0.02 ^{BCD}	6.1 ± 0.02 ^{BC}	15.6 ± 0.03 ^{AB}
Fenugreek	WS	10024 ± 7488 ^{C}	0	93.3 ± 0.14	40.1 ± 0.04 ^{AB}	27.6 ± 0.03 ^{CD}	5.7 ± 0.03 ^{BC}	21.6 ± 0.04 ^{A}
Hairy Indigo	WS	144050 ± 7488 ^{BC}	18.8 ± 9.55	89.9 ± 0.06	45.2 ± 0.02 ^{AB}	35.5 ± 0.02 ^{BC}	7.6 ± 0.02 ^{B}	16.1 ± 0.03 ^{AB}
Illinois bundleflower	WS	34954.3 ± 7488 ^{C}	22 ± 11.32	93.6 ± 0.08	45.9 ± 0.02 ^{AB}	31.4 ± 0.02 ^{BCD}	7.6 ± 0.02 ^{BC}	17.4 ± 0.04 ^{AB}
Lablab	WS	240500 ± 7488 ^{AB}	13.8 ± 9.55	92.7 ± 0.06	37.6 ± 0.02 ^{B}	27.1 ± 0.02 ^{D}	4.7 ±0.02 ^{C}	10.7 ± 0.03 ^{B}
Maximilian Sunflower	WS	5192.3 ± 7488 ^{C}	0	-	41.6 ± 0.04 ^{AB}	29.8 ± 0.03 ^{BCD}	7.1 ± 0.03 ^{BC}	-
Panicled leaf tick trefoil	WS	122810 ± 7488 ^{BC}	32 ± 12.74	96.7 ± 0.09	53.9 ± 0.03 ^{A}	41.4 ± 0.02 ^{AB}	9.5 ± 0.02 ^{B}	14.3 ± 0.03 ^{AB}

Sericea lespedeza	WS	56502.4 ± 7488 ^{C}	0	80.1 ± 0.06	53.4 ± 0.02 ^{A}	41.1 ± 0.02 ^{AB}	13.1 ± 0.02 ^{A}	13.9 ± 0.03 ^{AB}
Slickseed fuzzybean	WS	19038.5 ± 7488 ^{C}	0	93.5 ± 0.09	46.6 ± 0.03 ^{AB}	31.1 ± 0.02 ^{BCD}	6.1 ± 0.02 ^{BC}	20.4 ± 0.03 ^{A}
Soybean	WS	138100 ± 7488 ^{BC}	0	94.4 ± 0.09	45.8 ± 0.03 ^{AB}	32.5 ± 0.02 ^{ACD}	7.1 ± 0.02 ^{BC}	16.3 ± 0.03 ^{AB}
Sunn hemp	WS	316620 ± 7488 ^{A}	0	92.9 ± 0.06	55.3 ± 0.02 ^{A}	43.7 ± 0.02 ^{A}	5.9 ± 0.02 ^{BC}	15.7 ± ^{AB}
Tall bush clover	WS	16875.0 ± 7488 ^{C}	0	93.7 ± 0.09	45.6 ± 0.03 ^{AB}	33.7 ± 0.02 ^{BCD}	7.6 ± 0.02 ^{BC}	14.3 ± 0.03 ^{AB}
P-value	CS	< 0.01	0.22	0.38	< 0.01	< 0.01	< 0.01	< 0.01
P-value	WS	< 0.01	0.49	0.88	< 0.01	< 0.01	< 0.01	< 0.01

[1] CS = Cool season; WS = Warm season

[2] DMY = dry matter yield [2] DMY = dry matter yield

[3] CT = condensed tannins

[4] OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

$^{A-I}$Means within a column different letters indicate significant differences ($P < 0.05$

Values are presented as Mean ± Standard Error of the Mean (SEM)

CHAPTER IV

EFFECT OF FORB INCLUSION ON DIGESTION AND METABOLISM OF WARM-SEASON FORAGE DIETS IN BEEF CATTLE

Synopsis

Warm-season forbs have gained attention in grazing systems due to their high productivity, favorable nutritive value, and potential to reduce enteric CH_4 emissions in beef cattle. Many of these species contain naturally-occurring plant secondary metabolites, including CT, isoflavones, saponins, and glucosinolates, which may alter rumen fermentation and methane (CH_4) production. The objective of this study was to evaluate the intake and acceptance of selected warm-season forage forbs and determine the influence of their PSM on nutrient utilization, rumen fermentation parameters, and CH_4 emission rates in beef cattle. A 4 × 4 Latin square in vivo metabolism experiment was conducted, with columns corresponding to experimental periods and rows to ruminally-fistulated steers. Dietary treatments consisted of a 70:30 ratio of 70% bahiagrass (*Paspalum notatum* Flueggé.) with 30% sunn hemp (*Crotalaria juncea* L.; SUN), lablab (*Lablab purpureus* [L.] Sweet; LAB), or soybean (*Glycine max* [L.] Merr.; SOY). A grass-only treatment served as the control (CON). The forb dry matter intake (DMI) in the diet differed among treatments ($P = 0.06$) and remained below the targeted inclusion ratio of 30%. While apparent dry matter digestibility (DMD; $P = 0.18$) and apparent acid detergent lignin digestibility (ADLD; $P \geq 0.18$) were not different among diets, apparent acid detergent fiber digestibility (ADFD; $P = 0.04$) and apparent neutral detergent fiber digestibility (NDFD; $P = 0.03$) was influenced by diet. Nitrogen utilization reported as apparently absorbed nitrogen and apparently retained nitrogen (AAN and ARN, respectively) did not differ among diets ($P \geq 0.39$). Differences in vivo

digestibility were expected to follow a similar pattern, as the diet was mostly dominated by bahiagrass. However, there was an effect of bahiagrass control substrate ($P = 0.02$) on *in vitro* dry matter digestibility (IVDMD) and no effect of diet for the bahiagrass-forb incubations ($P = 0.92$). There was an interaction of diet and substrate ($P = 0.04$) for potential activity for methane production (PAMP). Lower CH_4 production in the SUN was likely driven by CT concentrations in the sunn hemp, supported by a corresponding decline in H_2 output. Supplementing bahiagrass hay with leguminous forbs improved ruminal fermentation profiles ($P \leq 0.01$). Forb species have the potential to reduce CH_4 due to the presence of PSM however, there is need to improve DMI to achieve optimum efficiency.

Introduction

Over the years, reaching a grazing period of 300 to 360 d has been a major goal for most beef cattle producers in the southeastern USA (Silva et al., 2021). Warm-season forbs such as sunn hemp, lablab, and soybean have gained popularity in this region due to their productivity and ability to bridge seasonal gaps when perennial grasses are less productive. Muir (2002) reported DMY over 9,000 kg DM/ha from lablab in Texas, while Jaramillo et al. (2020) reported a production of approximately 1,764 kg DM/ha of sunn hemp in Florida. Warm-season forbs are also valued for their increased nutritive value. The elevated CP concentration can effectively supplement grass-based diets and improve overall forage quality, especially given the high expenses associated with supplemental feeds (Eberle and Shortnacy, 2021). Despite these advantages, forbs have naturally occurring PSM that may reduce palatability or pose potential toxicity risks to grazing animals (Robbins et al., 1987). There is limited research on the ideal diet inclusion rates, palatability, and ruminal metabolism of most warm-season forage forbs under grazing conditions. Therefore, the objective of this study was to investigate how forbs with naturally-occurring PSM affect nutrient absorption, rumen parameters, and CH_4 emission rates in beef cattle. The overall goal was to identify forb species that can be integrated into Southeastern beef cattle production systems without compromising animal productivity.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2024-5515.

Experimental Design

This metabolism experiment was conducted as a 4 × 4 Latin square design. Columns corresponded to the experimental periods (n = 4) as presented in Table IV 1. Each period was 22 d in duration, and there was a 10-d animal-rest period between each period. Rows were the ruminally-fistulated steers; (630 ± 14 kg body weight [BW]).

Hay Treatments

There were four dietary treatments in this experiment: bahiagrass hay with no supplemental forbs (CON); bahiagrass hay supplemented with 30% lablab (LAB); bahiagrass hay supplemented with 30% soybean (SOY); and bahiagrass hay supplemented with 30% sunn hemp (SUN). Bahiagrass hay was sourced from Beasley Land and Cattle, LLC (Notasulga, AL) in August 2024. Sunn hemp and lablab were established at the Wiregrass Research and Extension Center (31° 21′ 34.15″ N, 85° 19′ 9.38″ W) on April 29, 2024, and were harvested on July 13 and July 24, 2024, respectively. Soybean was planted at the same location and first harvested on July 30, 2024. To facilitate forage handling, sunn hemp was harvested and subsequently mowed using a John Deere rotary cutter (John Deere, Moline, IL). Initial baling was performed with a mini-round baler (CAEB International srl, Via Botta Sassa, Italy); however, due to equipment failure, the remaining forage was compressed into large round bales using a John Deere 460M Round Baler (John Deere, Moline, IL). Lablab harvest was delayed by heavy precipitation. Forage was initially baled at an elevated moisture content and subsequently unrolled for further field curing before re-baling. Soybean was harvested following exposure to two rainfall events during the drying period. These harvesting and curing conditions deviated from standard haymaking practices, potentially resulting in increased leaf shatter and reduced leaf retention. Treatments were randomly assigned to steers in each period such that each steer received each diet once throughout the experiment. The resulting experimental design is presented in Figure IV-1.

Experimental Timeline

Each experiment had a duration of 22 d consisting of a 14-d adaptation phase in which steers were fed the treatment diets, a 5-d total fecal and urinary collection phase, and a 3-d ruminal sampling phase. On Day 1, steers were weighed and moved into the individual 9.29-m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn, AL. On Day 15, the last day of the adaptation phase, steers were weighed and moved into 2.97-m^2 metabolism pens, where urine and fecal samples were collected. On Day 19, steers were returned to the individual 9.29-m^2 for a 3-d rumen sampling phase. In vitro digestibility and in vitro CH_4 experiments were also conducted on day 19. On Day 23, the steers were moved to resident pastures for a 10-d rest period before the next experimental period.

Experimental Procedures

Feeding and sampling

Steers were fed their respective diets once daily in the morning at 2.5% DM of BW on the first day of each period, and thereafter feed offered was adjusted daily based on the previous day's intake, calculated from refusals, to provide approximately 110% of the prior day's DMI throughout the period. Feed remained available throughout the day. A representative sample of each diet was collected at feeding. Feed refusals (orts) were collected and weighed prior to each morning feeding, and subsamples were taken for analysis. Steers had *ad libitum* access to fresh water throughout the experiment. All feed and refusal samples were dried at 55°C in a forced-air oven for 72 h before further analysis.

Blood collection

Blood samples were collected on Day 1 and 15 of every experimental period via the jaguar venipuncture. Serum and samples were collected by centrifugation at 15,000 × g for 10 min and subsequently stored at -20°C until further analysis. Body weights were also recorded on the same days.

Total fecal and urinary collections

On Day 15, steers were moved to the metabolism room and housed individually in stalls for total urine and total fecal collection. Each stall was equipped with a grated floor, a plastic tray, and a mesh screen that allowed urine to pass into a collection tray beneath while preventing fecal contamination. Fecal samples were collected every 2 to 4 h until the following morning. All fecal samples were weighed, thoroughly mixed using a concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), subsampled, and dried at 55°C. Urine was collected in plastic trays, weighed, and subsampled for storage at −20°C. To prevent nitrogen volatilization, 6N HCl was added to each urine tray daily (Chizzotti et al., 2008).

Rumen fluid collections

On Day 20, rumen fluid samples were collected through the rumen cannula at 0, 0.5, 1, 2, 3, 4, 8, and 12 h relative to feeding. At each point, approximately 50 mL of rumen fluid was collected by straining through four layers of cheesecloth. The pH was immediately measured, and two 1,000 µL aliquots were combined with 200 µL of metaphosphoric acid solution (125 mL/L) containing 2-ethylbutyric acid as an internal standard for volatile fatty acid (VFA) analysis.

Passage and dilution rate

On Day 21, steers were infused with chromium EDTA (Cr(III)-EDTA) before feeding to determine the liquid dilution rate (Teeter and Owens, 1983). The solution was prepared following the method described by Van Soest and Hall (2020). A total of 1,000 mL of the solution containing 5 g of Cr was infused through the rumen cannula, and five minutes later, a 300mL rumen fluid sample was collected by straining through two layers of cheesecloth. Subsequent samples were collected at 0, 4, 8, and 12 h relative to feeding.

On Day 22, the steer's rumen was evacuated at 0, 6, and 12 h relative to feeding. After each rumen evacuation, the samples were weighed, and a subsample was stored at -20°C for further solid passage rate analysis (Waldo et al., 1972).

Analytical Procedures

Nutritive value

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA).

Dry matter and OM were determined using procedures from the AOAC (2000). Neutral detergent fiber and ADF were analyzed using an ANKOM $^{\text{Delta}}$ and the ANKOM ^{2000} Fiber Analyzer (Ankom Technologies, Macedon, NY, USA) following the procedures of Vogel et al 1999). Acid detergent lignin was determined using the sulfuric acid method (AOAC, 2000) with an ANKOM Daisy $^{\text{II}}$ incubator (Ankom Technologies, Macedon, NY, USA) following the procedure of Goering and Van Soest (1970). Forage samples were sent to Cumberland Valley Analytical Services (Waynesboro, PA) for evaluation of CP using the Dumas combustion method and calculated as N × 6.25 (AOAC, 2000). Total nitrogen from urine samples was determined according to the Kjeldahl procedure (AOAC, 2000). The chemical composition of forage is presented in (Table IV-1).

Energetics

Gross energy (GE) of the hay, orts, and fecal samples was determined via direct combustion using a bomb calorimeter (LECO AC600; Leco Corporation, St. Joseph, MO) according to established protocols (AOAC, 2000). Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500

Series Mill (Eberbach Corporation, Charter Township, MI, USA). The instrument was calibrated using benzoic acid standards. Gross energy intake was calculated as the difference between the total energy of hay offered and the energy contained in the orts. Digestible energy (DE) was subsequently calculated as the difference between GE intake and fecal GE output.

Rumen fluid dynamics

Rumen fluid aliquots collected during the in vivo experiment were analyzed for volatile fatty acids (VFA) following the procedures described by Akins et al. (2009). Concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, 4-methylvalerate, and caproate were determined using gas chromatography (Agilent 8890; Agilent Technologies, Santa Clara, CA, USA). Nitrogen was used as the carrier gas according to Zou (2018; Agilent Application Note #5991-9223EN). Separation was performed using an Agilent J&W DB-FATWAX Ultra Inert (UI) column (30 m × 0.25 mm × 0.25 µm) with an operating temperature range of 20 to 250°C. To minimize sample carryover and contamination, concentrated methanol was used to clean the column before each analytical run and after every five samples.

The passage rate for rumen evacuation samples was determined using acid detergent insoluble ash (ADIA) content (Waldo et al., 1972). Frozen Cr-EDTA rumen samples were shipped to the University of Georgia Feed and Environmental Water Laboratory (Athens, GA, USA) and analyzed for Cr concentration via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; AOAC, 2000).

Blood parameters

Blood urea nitrogen (BUN) was analyzed using the ThermoFisher Urea Nitrogen colorimetric detection kit (Pub. No. MAN0025406). The serum samples were left to thaw at room temperature and transferred to a 96-well plate for assay to minimize nitrogen degradation. The standard curve was prepared with eight decreasing concentrations from 10 to 0.3135 mg/dL, while the serum samples were diluted 1:10 with deionized water. The assay procedure involved adding 50 µL of diluted samples or standards to each well, followed by 75 µL of color reagents A and B, and a 30-minute incubation at room temperature. The standard curve enabled BUN concentration determination through linear regression analysis of absorbance readings at 480 nm.

Serum non-esterified fatty acid (NEFA) concentrations were analyzed using the RayBio® NEFA/FFA Detection Assay Kit (Catalog # MA-NEFA-2; RayBiotech Inc., Peachtree Corners, GA) according to the manufacturer's instructions. Samples were diluted 1:2 in Sample Buffer and analyzed in duplicate. Absorbance was measured at 550 nm using a microplate reader, and concentrations were calculated from a standard curve (0–500 µM). The minimum detectable concentration was 3.9 µM.

In vitro assays

In vitro digestibility

The IVDMD experiment was conducted as a hierarchical addition to the *in vivo* Latin square design. Rumen fluid collected from steers in the *in vivo* ruminal fluid collection procedure was used to incubate duplicated flasks containing either bahiagrass or the 70/30 combination of bahiagrass and the assigned forb of that experimental period.

On d 20 of each *in vivo* period at the 4-h sampling point, a larger volume (approximately 1,000 mL) of whole rumen contents (Goering and Soest, 1970) was collected for *in vitro* digestibility calculations and for PAMP calculations (Anderson et al., 2006). Rumen fluid and solid contents were collected into pre-warmed (39°C) thermos containers for transport to the laboratory. On arrival at the laboratory, rumen inoculum was homogenized and used for *in vitro* digestion following the Tilley and Terry (1963) procedure.

Potential activity of methane production (PAMP)

Samples of the bahiagrass and forbs from the *in vivo* experiment were collected from bales being used during that experimental period. A sample of alfalfa was included as a standardized reference substrate sample for the PAMP experiment. Samples were dried at 50°C in a forced-air oven until weight loss ceased. Dried samples were ground to pass through a 2-mm screen using an Eberbach E3500 series Mill (Eberbach Corporation, Van Buren Charter Township, MI, USA). On arrival at the laboratory, rumen inoculum was homogenized and used for *in vitro* digestion following the Anderson et al. (2006) procedure.

Statistical Analysis

Data were analyzed as a 4 × 4 Latin square design using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Intake, hay nutritive value, digestibility, IVDMD and metabolic response variables were analyzed using generalized linear mixed models assuming a Gaussian distribution. Animals and periods were random effects. Dietary treatments were fixed effects, and denominator degrees of freedom were adjusted using the 2nd -order Kenward–Roger approximation (Kenward and Roger, 2009). In addition, PAMP data were analyzed separately using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). For H_2 and CH_4, dietary treatment, substrate, and the diet × substrate interaction were included as fixed effects, while steer and period were included as random effects. Least-squares means were separated using Tukey–Kramer multiple-comparison adjustments (Kramer, 1956). Differences among responses were declared significant at $P \leq 0.05$.

Results and Discussion

Intake and Digestibility

Forb ratio in the diet differed among treatments ($P = 0.06$*;* Table IV 2). The forb intake remained below the targeted inclusion ratio of 30%, with the SOY diet reaching the greatest forb intake of 8%, more than double that of LAB (4%) or SUN (3%). Total DMI averaged 9.6–10 kg/d and did not differ among treatments ($P = 0.90$). Similarly, DM excretion (3.5 to 4.2 kg/d; $P = 0.34$) and apparent DM digestibility (56.6–63.1%; $P = 0.18$) were not affected by dietary treatment, supporting the notion that steers primarily consumed the bahiagrass component of the diet. When it comes to NDF, intake ($P = 0.79$; Table IV 2) and excretion ($P = 0.33$) were similar across the diets. However, NDF digestibility (NDFD) was influenced by diet ($P = 0.03$). Control diet had the greatest NDFD (75.7%), followed by SOY (67.4%), while LAB (72.2%) and SUN (70.8%) were intermediate. There were no differences in ADF digestibility (ADFD; $P = 0.04$) among diets. Acid detergent lignin intake, excretion, and apparent ADLD ($P \geq 0.18$) were not affected. Nitrogen utilization, measured as apparently absorbed nitrogen (AAN) and apparently retained nitrogen (ARN), did not differ among diets ($P \geq 0.39$*;* Table IV 2).

Forage forbs have long been recognized for their great nutritive value, and their ability to meet the nutritional requirements of cattle, and their inclusion in perennial grass pastures represents a sustainable approach to improving forage quality and dietary diversity (Waghorn, 2008; Muir et al., 2014; Sollenberger and Junior, 2022). However, voluntary intake remains the primary determinant of their practical contributions to animal performance. In the present study, the forb proportion reached only 8% compared with the targeted 30%, indicating a substantial limitation in diet selection. Total DMI in beef heifers decreased from 1.6 to 1.4 and 1.2% of BW as sunn hemp hay inclusion increased to 0, 50, and 100% of the forage portion, respectively. Although sunn hemp exhibited good nutritive value, higher inclusion levels appeared to limit voluntary intake (Garzon et al., 2021b). In addition, the current results may also have been influenced by the leaf-to-stem ratio of the forbs at harvest. The harvesting and baling methods used likely caused leaf loss, reducing the proportion of highly digestible material (Van Soest, 1994). Because the forages were offered without being chopped, the material remained relatively stemmy, which may have limited the steer's ability to selectively consume preferred leafy fractions.

Despite differences in forage species composition among treatments in this study, total DMI and DMD did not differ, likely reflecting the low level of forb intake. Apparent DM digestibility ranged from 56.6 to 63.1%, aligning with the 56.2% reported by Valencia and Rivera (2023) for lambs fed soybean hay (cv. Hinson) and exceeding the 49.6% reported for lablab. The most notable responses were observed in NDFD. Legume forbs, such as soybean, often possess a greater proportion of stem tissue and more lignified cell walls as they advance in maturity, which can lower NDFD and ADFD (Buxton & Redfearn, 1997). In the current study, SOY was harvested later due to excessive rainfall, resulting in increased forage maturity.

Changes in rumen fermentation dynamics associated with increased soluble protein or nonstructural carbohydrate from soybean forage might have altered the ruminal environment, reducing fibrolytic activity (Mertens, 1994). Soybean forages contain isoflavones (Ku et al., 2020), which influence rumen microbial populations and may have contributed to changes in fiber digestion (Grgic et al., 2021). Lablab studies characterize it as highly digestible with moderate fiber concentrations when harvested at vegetative to early reproductive stages (Sollenberger & Collins, 2017; Cline, 2010; Valencia and Rivera, 2023), which could explain its ability to maintain NDFD and ADFD throughout the current study. Sunn hemp, showing intermediate fiber digestibility, may reflect its known tendency to develop coarser stems and increased lignin concentrations as it matures (Jaramillo et al., 2020c). Differences among treatments likely reflect variation in protein supply, fiber characteristics, and secondary metabolites among species, which can influence rumen microbial activity even at low inclusion levels. Thus, species-specific interactions with rumen fibrolytic microbes may occur. Nitrogen utilization was not affected by dietary treatment, which was expected given that bahiagrass was the predominant diet component across diets, and forb inclusion was less than 8%. Although SOY had an increased intake (8%), the protein provided was sufficiently digested and absorbed, thus maintaining the nitrogen balance.

Blood Constituents

There was a difference of day in BUN ($P < 0.01$; Table IV-3). Concentration was greater on d 1 (52 mg/dL) compared to d 15 (36 mg/dL). The BUN levels observed between the two days, exceed the standard physiological range of 10 to 25 mg/d reported for beef cattle (Byers and Moxon, 1980). Elevated BUN is a primary indicator of an imbalance in the nitrogen-to-energy ratio in the rumen, where ruminal ammonia production from degradable intake protein exceeds the capacity of rumen microbes to utilize it for microbial protein synthesis (Hammond, 1998). Although Foster et al. (2009) suggested that the increased protein content of tropical legumes like lablab and sunn hemp rapidly increased the pool of ruminal ammonia, the nitrogen intake in my study was relatively stable and not affected by treatment (Table IV-2). The elevated BUN concentrations on d 1 likely reflect the increased nitrogen intake from the preceding Bermudagrass pastures the steers were consuming. Previous studies by Hammond (1998) reported that BUN levels equilibrate to nitrogen intake; the transition from fresh pasture to a hay diet explains the decline in BUN observed by d 15. Interestingly, forb intake ratios were highest in SOY (8%). The increased BUN in SUN on d 15 might reflect the effect of tannins that may have been bound to proteins in the rumen, which are more concentrated in sunn hemp and not soybean (Fagundes et al., 2020) and not entirely based on the intake of nitrogen. It is also important to note that sunn hemp forb had the least CP compared to the other diets (Table IV-1).

Serum non-esterified fatty acid (NEFA) concentrations were not affected by diet ($P = 0.15$), d ($P = 0.07$), or the diet × day interaction ($P = 0.28$). These results can be attributed to the stable energy source from all the diets that prevented major mobilization of the adipose tissue. This can be further supported by a review by Adewuyi et al. (2005) on NEFA in dairy cattle that reported that elevated concentrations of NEFA indicate that an animal is breaking down body fat stores to meet maintenance energy requirements.

Energetics and Ruminal Parameters

Gross energy (GE) and DE of diets were not affected by dietary treatment ($P = 0.26$ and $P = 0.75$, respectively; Table IV-4). Gross energy values were similar among the four diets, averaging about 4.2 Mcal/kg. The GE was used to characterize the chemical energy content of each diet (NRC, 2016), and similar GE values indicate comparable total chemical energy among treatments. The proportion of GE recovered as DE was similar, suggesting comparable energy utilization across diets.

Nutrient absorption and availability in cattle are mostly determined by ruminal pH, as low pH tends to damage ruminal epithelia, thus reducing VFA absorption (Nagaraja and Lechtenberg, 2007; Dijkstra et al., 2012). In the current study, rumen pH was greater for SOY and SUN diets (6.8) compared to CON and LAB (6.7; Table IV-5, $P < 0.01$). All diets maintained an ideal range of pH 5.8-6.8 for forage-based diets (Van Soest, 1994), which would favor the growth and activity of cellulolytic bacteria. These results are supported by previous studies evaluating sunn hemp inclusion, where rumen pH was not markedly altered across increasing inclusion (Wanapat et al., 2021). Although sunn hemp contains alkaloids and CT, their concentrations may not have been sufficient to meaningfully influence rumen pH under the conditions of the present study.

There was no effect of treatment ($P = 0.14$; Table IV-5) for dilution rate. The lack of treatment effect on dilution rate suggests that rumen liquid turnover was not substantially altered by forage species inclusion. Dilution rate is influenced primarily by intake level, rumen fill, and fermentation characteristics (Kammes and Allen, 2012), and the similarity observed among treatments is consistent with the comparable dry matter intake and predominance of bahiagrass in the diet. Previous studies have reported that forage-based diets with similar intake levels typically result in comparable rumen liquid dynamics (Allen, 1997).

Dietary treatments in this study altered the ruminal VFA profile (Figure IV-2), with a significant diet effect observed for propionate, butyrate, and caproate ($P < 0.01$; Table IV-6). The SOY diet produced the greatest propionate concentration (13.5 mM), while LAB and SUN were intermediate and remained significantly greater than CON. Soybean (9.6 mM) and LAB (8.7 mM) produced most butyrate concentrations, whereas SUN and CON levels were less. All forb treatments increased caproate levels relative to the control, with SOY again providing the greatest production. In contrast, there were no diet, hour or interaction differences on isobutyrate ($P \geq 0.32$;

Table IV -4). There were diet × hour interactions for acetate (*P* < 0.01; Figure IV-3) and valerate (*P* < 0.01; Figure IV-5). Soybean and SUN generally maintained more acetate concentrations than CON, with SOY reaching a sustained peak of 38 mM by h 12. Similarly, the A:P ratio fluctuated over time (*P* < 0.01), with CON peaking sharply at h 4, while LAB achieved the least overall ratio by hour 3. Valerate production peaked early for LAB (h 0.5) but was most sustained in the SOY, while there were hourly differences on isovalerate production (*P* < 0.01), peaking early before declining toward hour 12 (Figure IV-5).

Rumen fermentation is primarily driven by diet type, quality, and digesta passage rate, which collectively determines the fermentation rate and the resulting VFA profile (Allen and Mertens, 1988). The shift toward higher propionate concentrations in the SOY, LAB, and SUN groups compared to CON suggests that the inclusion of these legumes provided a greater proportion of readily fermentable components than the bahiagrass hay alone. Traditionally, diets rich in rapidly degradable carbohydrates favor propionate pathways, whereas fibrous, forage-based diets favor acetate (Hungate, 1975; Van Soest, 1994). The increased propionate and lowered A:P ratio in the LAB and SOY treatments indicate a more energetically efficient fermentation process, as propionate is a primary glucogenic pathway for the ruminant (Bergman, 1990)

Acetate production and increased VFA concentrations in the SOY likely reflect a combination of great-quality soluble nutrients and fermentable fiber. While some literature suggests that secondary metabolites like saponins in soybeans can reduce digestibility and microbial activity (Hess et al., 2003), the elevated VFA levels here suggest that the nutritional benefits of the SOY forage outweighed any potential antinutritional effects. In contrast, the sharp peak in acetate for SUN at h 3 followed by a rapid decline may indicate a highly soluble but quickly exhausted fiber fraction, whereas the lower overall VFA production in SUN might be attributed to its increased lignin or tannin concentration, which can limit microbial access and slow the rate of fermentation (Naumann et al., 2017).

The diet × hour interaction for valerate, a marker for the fermentation of branched-chain amino acids (proline and leucine) might highlight differences in protein solubility between the forbs. The rapid initial spike in LAB at 0.5 hours suggests a highly soluble protein fraction that is immediately available to the microbial population (Apajalahti et al., 2019). The more prolonged valerate production in SOY suggests a slower, more sustained degradation of its protein content. These peaks, generally occurring 3 to 6 h post-feeding, align with standard forage-based fermentation models where the slow degradation of structural carbohydrates leads to sustained activity rather than the volatile spikes seen in grain-heavy diets (Church, 1993). The subsequent decline across all treatments by hour 12 likely reflects the dilution effects of saliva production and the natural turnover of rumen fluid (Van Soest, 1994).

In Vitro Digestibility

In vitro dry matter digestibility differed between the bahiagrass substrates (*P* = 0.02) and the 30:70 substrate *(P*= 0.92). For the bahiagrass substrate, dietary treatment had a effect on IVDMD (Table IV 7), CON had the greatest digestibility of 52.1%, greater than the SUN diet (41.0%). LAB and SOY were intermediate (47.5 and 49.0%, respectively). In contrast, for the 30:70 substrate, there was no difference observed among the dietary treatments *(P* = 0.92).

The CON diet should have increased digestibility among the diets, taking into context that the forb intake ratio from the *in vivo* experiment was low, hence the microbes were well adapted to the bahiagrass fermentation. The reduced IVDMD of SUN compared to the CON and other forbs on the bahiagrass substrate could be attributed to structural and biochemical characteristics.

These findings are also supported by studies that increased levels of fiber fractions in sunn hemp stems, and their decreased digestibility as the plant lignifies (Lepcha et al., 2019). Leaf fractions are easily digested, yet as plants mature, stem mass becomes the dominant part of the total forage. Lignification and a lower leaf:stem ratio might have reduced the total fermentation of the SUN and might have decreased the effectiveness of digestibility.

In addition to fiber, tropical legume PSM can influence rumen fermentation. Many warm-season legumes contain CT or saponins that can bind dietary protein, inhibit fibrolytic microbes, or reduce protozoa and methanogens (Goel and Makkar, 2012; Mueller-Harvey et al., 2019; Ku-Vera et al., 2020). While this study did not measure detailed profiles for sunn hemp, lablab, and forage soybean, tropical legumes often have tannins and saponins at levels that can change microbial activity (Hess et al., 2003; Frutos et al., 2004; Naumann et al., 2013). In contrast to our study, lablab and forage soybean hays typically show moderate to high DMD (around 50 to 56% in vivo) and elevated voluntary intake when fed as sole forages (Valencia and Rivera, 2023). Soybean results also contradict the results reported by Baral et al. (2025) when soybean was harvested at different stages. The lack of treatment differences in the 30:70 substrate can be attributed to the overall adaptation of the microbial population to the bahiagrass fermentation in the *in vivo* study.

Potential Activity of Methane Production

Hydrogen (H_2) production was influenced by the interaction between diet and substrate ($P < 0.01$; Table IV 8). Alfalfa substrate, CON, LAB, and SOY supported similar H_2 production (0.17, 0.11, and 0.14 g/kg, respectively), while SUN resulted in the least H_2 production (0.06 g/kg). For bahiagrass substrate, however, SOY and SUN had 0.18 and 0.20 g/kg, respectively, compared to

CON and LAB (0.11 and 0.08 g/kg, respectively). There was a diet × substrate effect on CH_4 production. The greatest CH_4 production was in CON, followed by LAB and SOY while SUN had the least production.

Our results are similar to other research showing that the impact of dietary treatments on rumen H_2 and CH_4 output depends strongly on the fermentability and chemical composition of the forage (Sun, 2020). In the present study, alfalfa and bahiagrass provided contrasting fermentation environments, which modified how the forb supplements altered ruminal fermentation and gas production. In the alfalfa substrate, CON, LAB, and SOY produced similar H_2, whereas SUN reduced H_2 production. This pattern was likely because the inclusion of sunn hemp may have shifted fermentation away from pathways that generate excess reducing equivalents, possibly by favoring propionate production or other alternative hydrogen sinks, thereby decreasing the availability of H_2 for methanogenesis (Sun, 2020). With bahiagrass as the substrate, SOY and SUN produced greater H_2 than CON and LAB, but SUN still had the lowest CH_4 yield. This might be because hydrogen production was not reduced but instead redirected toward other fermentation pathways. A similar result was reported in lambs fed forage rape, highlighting how changes in microbial communities can alter fermentation pathways without necessarily reducing hydrogen generation (Sun et al., 2015). The diet × substrate effect on CH_4 further demonstrates that interactions among substrate quality, microbial ecology, and plant organic compounds drive methanogenesis. The Archaea in the rumen use H_2 and CO_2 to produce CH_4 through hydrogenotrophic and methylotrophic pathways, and the relative abundance and activity of these pathways vary with diet (Danielsson et al., 2017). While most forage-based diets favor hydrogen production, some forb forages can alter rumen pH, fermentation rate, or hydrogen production, thus shifting the microbial community composition and reducing CH_4 production. The more H_2 is produced, the more CH_4 the animal produces as methanogenic microbes use H_2 (Hungate, 1975). The reduced CH_4 production observed with SUN, especially with alfalfa, is consistent with the recognized antimethanogenic potential of legumes rich in CT (Frutos et al., 2004; Villalba et al., 2011). Legumes like soybean, alfalfa, and forb species accumulate substantial levels of isoflavones and other flavonoids through the phenylpropanoid pathway. Studies by Messina (1999), Francis et al. (2002), and Hess et al. (2003) reported that these compounds can modulate rumen microbial populations, including methanogens and protozoa, thereby altering hydrogen turnover and methanogenesis.

Conclusions

Although forage forbs often improve nutritive value and augment protein contribution relative to warm-season grasses, our results indicate that their practical impact depends largely on ruminant voluntary intake. While forb inclusion has the potential to influence digestion and metabolism, in our study low consumption limited their effective contribution to the diet and likely masked any measurable advantages. Physical characteristics and palatability appeared to be primary determinants of intake, suggesting that improved hay processing or strategies to enhance leaf retention may increase utilization. Observed differences *in vivo* and IVDMD may reflect species-specific interactions with rumen fibrolytic microorganisms. Ruminant forage forb selection and incorporation into grass-based pastures require complementary management strategies to enhance voluntary intake and optimize the effectiveness of plant secondary metabolites.

Table IV-1 Nutritive value parameters (% DM basis) in beef cattle offered bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

	Treatment[2]			
Item[1]	**CON**	**LAB**	**SOY**	**SUN**
DM, %	94	94	94	94
NDF, %	76	64	61	71
ADF, %	39	48	43	57
ADL, %	5	10	10	12
CP, %	12	14	15	9

[1] DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

[2] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30% soybean; SUN = bahiagrass supplemented with 30% sunn hemp

Table IV-2 Forage intake and digestibility parameters in beef cattle offered bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

Item[1]	**Treatment**[2]					
	CON	**LAB**	**SOY**	**SUN**	**SEM**[3]	***P*-value**
Forb intake, % DMI	0 ^{B}	3.7 ^{AB}	7.6 ^{A}	3.1 ^{AB}	1.84	0.06
		Dry matter				
Intake, kg/d	9.6	9.8	9.8	9.9	0.87	0.90
Excretion, kg/d	3.5	3.9	4.2	4.0	0.55	0.34
Digestibility, %	63.1	59.9	56.6	59.9	4.21	0.18
		Neutral detergent fiber				
Intake, kg/d	7.5	7.4	7.1	7.5	0.68	0.79
Excretion, kg/d	1.8	2.0	2.3	2.2	0.27	0.33
Digestibility, %	75.7 ^{A}	72.2 ^{AB}	67.4 ^{B}	70.8 ^{AB}	2.49	0.03
		Acid detergent fiber				
Intake, kg/d	3.8	3.9	3.6	3.8	0.35	0.63
Excretion, kg/d	1.0	1.0	1.2	1.2	0.2	0.15
Digestibility, %	74.1	74.3	66.7	70.4	2.88	0.04
		Acid detergent lignin				
Intake, kg/d	0.5	0.6	0.5	0.7	0.10	0.41
Excretion, kg/d	0.1	0.2	0.2	0.2	0.03	0.20
Digestibility, %	81.4	74.9	66.4	71.1	4.57	0.18
		Nitrogen utilization				
AAN, %	57.5	52.7	57.8	51.0	7.41	0.53
ARN, %	28.5	24.7	31.4	14.1	7.33	0.39

[1] AAN = apparently absorbed nitrogen, % N; ARN = apparently retained nitrogen, % N

[2] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30% soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[3] SEM = standard error of the mean

$^{A, B}$Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-3 Blood urea nitrogen and non-esterified fatty acids from beef cattle ingesting bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

Item[1]	DAY 1	DAY 15	SEM[2]	*P*-value
BUN, mg/dL	51.5 ^{A}	36.3 ^{B}	3.92	0.01
NEFA, µmol/L	318.9	379.5	25.2	0.07

[1] BUN = blood urea nitrogen; NEFA =non-esterified fatty acids

[2] SEM = standard error of the mean

$^{A, B,}$ Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-4 Energy partitioning of bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species offered to ruminally-fistulated beef cattle

	Treatment[1]					
Item	**CON**	**LAB**	**SOY**	**SUN**	**SEM[2]**	***P*-value**
GE, Mcal/kg	4.2	4.2	4.2	4.1	0.04	0.26
DE, Mcal/kg	2.6	2.4	2.3	2.4	0.24	0.75

[1] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30% soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[2] SEM = standard error of the mean

[A, B] Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-5 Ruminal dynamics from beef cattle offered bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

	Treatment[2]					
Item[1]	**CON**	**LAB**	**SOY**	**SUN**	**SEM[3]**	***P*-value**
Dilution, %	11.7	9.8	11.6	9.6	1.27	0.14
pH	6.7 ^{B}	6.7 ^{B}	6.8 ^{A}	6.8 ^{A}	0.08	0.01

[1] GE = gross energy; DE = digestible energy

[2] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30 % soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[3] SEM = standard error of the mean

$^{A,\ B}$Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-6 Volatile fatty acid production from beef cattle offered bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

	Treatment[1]					
VFA, (mM)	**CON**	**LAB**	**SOY**	**SUN**	**SEM**[2]	***P*-value**
Propionate	11.0 ^{C}	12.4 ^{B}	13.5 ^{A}	12.4 ^{B}	1.76	< 0.01
Butyrate	5.8 ^{B}	8.7 ^{A}	9.6 ^{A}	5.2 ^{B}	0.97	< 0.01
Caproate	0.4 ^{C}	0.5 ^{B}	0.6 ^{A}	0.5 ^{B}	0.14	< 0.01
Isobutyrate	1.7	1.4	1.7	1.5	0.18	≥ 0.32

[1] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30 % soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[2] SEM = standard error of the mean

^{A,B,C}Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-7 *In vitro* digestibility of bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

	Treatment[1]					
Substrate	**CON**	**LAB**	**SOY**	**SUN**	**SEM[2]**	***P*-value**
Bahiagrass, %	52.1^{A}	47.5^{AB}	49.0^{AB}	41.0^{B}	3.28	0.02
Bahiagrass + Forb, %	-	43.0	41.3	42.5	5.47	0.92

[1] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30% soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[2] SEM = standard error of the mean

$^{A,\ B}$Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

Table IV-8 Potential activity of hydrogen and methane production from beef cattle ingesting bahiagrass hay with or without the inclusion of plant secondary metabolite-producing forb species

	Treatment[1]					
Substrate	**CON**	**LAB**	**SOY**	**SUN**	**SEM[2]**	***P*-value**
		Hydrogen, g/kg				
Alfalfa	0.17 ^{ABC}	0.11 ^{ABC}	0.14 ^{ABC}	0.06 ^{C}	0.06	< 0.01
Bahiagrass	0.11^{ABC}	0.08 ^{ABC}	0.18 ^{AB}	0.20 ^{A}		
		Methane, g/kg				
Alfalfa	1.80 ^{BC}	1.50 ^{BC}	0.84 ^{C}	0.19 ^{C}	1.32	0.04
Bahiagrass	5.06 ^{A}	4.29 ^{AB}	6.14 ^{A}	1.63 ^{BC}		

[1] CON = bahiagrass hay with no supplemental forbs; LAB = bahiagrass hay supplemented with 30% lablab; SOY = bahiagrass hay supplemented with 30% soybean; SUN = bahiagrass supplemented with 30% sunn hemp

[2] SEM = standard error of the mean

$^{A, B, C}$Means sharing the same superscript letter do not differ ($P > 0.05$) according to Tukey-Kramer adjustment

	Period 1	**Period 2**	**Period 3**	**Period 4**
Steer 1	CON	SUN	SOY	LAB
Steer 2	LAB	CON	SUN	SOY
Steer 3	SOY	LAB	CON	SUN
Steer 4	SUN	SOY	LAB	CON

Figure IV-1 Latin square design for in vivo evaluation for steers fed a control diet of bahiagrass hay only (CON), bahiagrass supplemented with lablab (LAB), bahiagrass supplemented with soybean (SOY), or bahiagrass supplemented with sunn hemp (SUN).

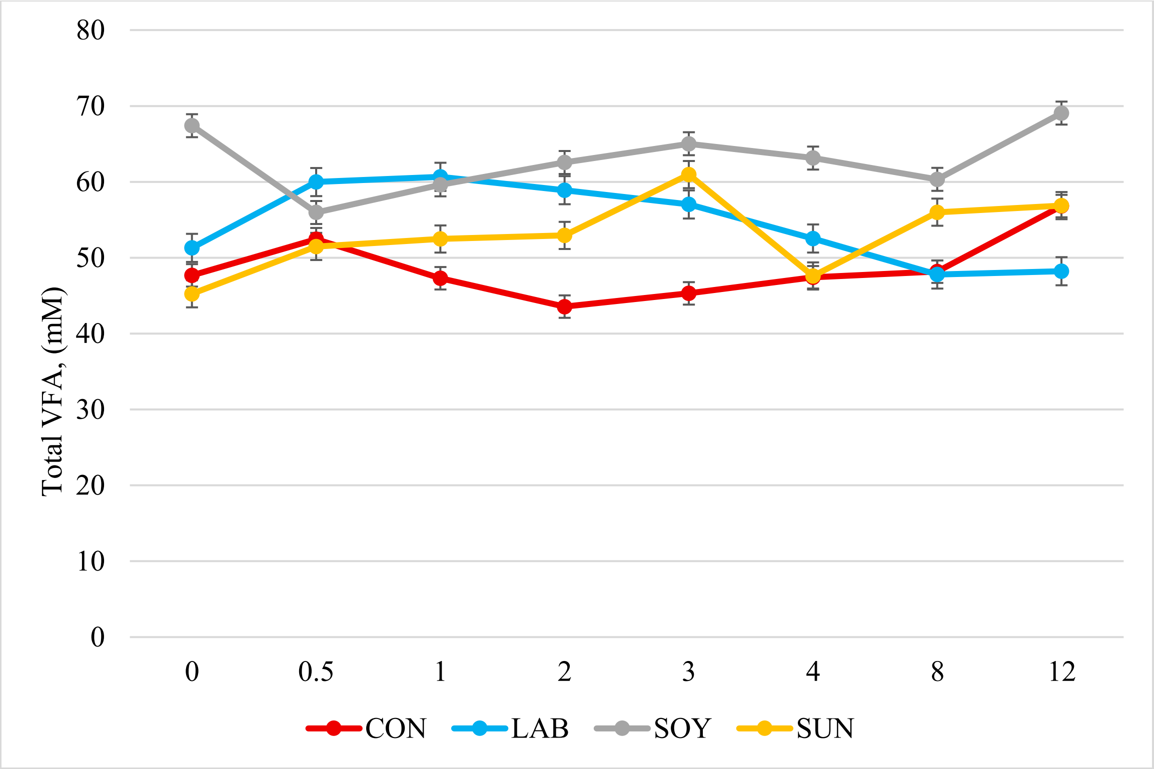

Figure IV-2 Total volatile fatty acid production for steers fed a control diet of bahiagrass hay only (CON), bahiagrass supplemented with lablab (LAB), bahiagrass supplemented with soybean (SOY), or bahiagrass supplemented with sunn hemp (SUN).

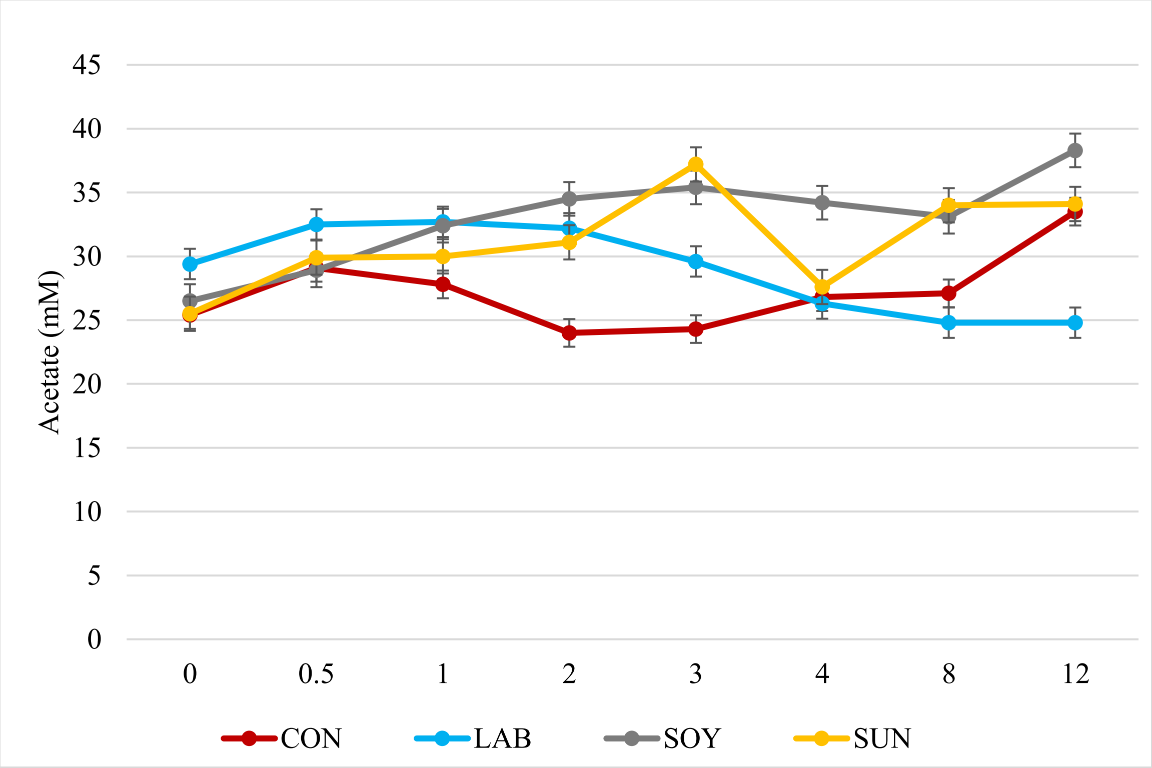

Figure IV-3 Rumen acetate concentration (molar concentration) for steers fed a control diet of bahiagrass hay only (CON); bahiagrass supplemented with lablab (LAB); bahiagrass supplemented with soybean (SOY); bahiagrass supplemented with sunn hemp (SUN). Diet × Hour interaction ($P < 0.01$) according to Tukey-Kramer adjustment

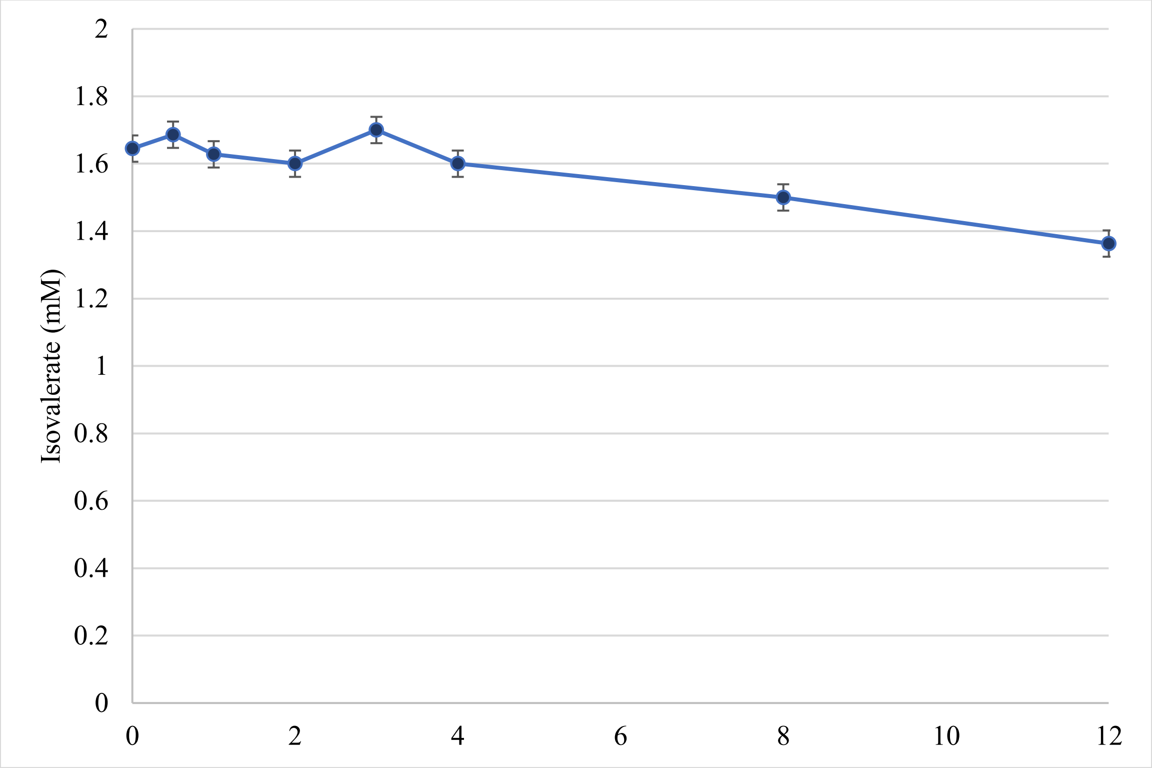

Figure IV-4 Rumen isovalerate concentration (molar) for steers fed a control diet of bahiagrass hay only (CON); bahiagrass supplemented with lablab (LAB), bahiagrass supplemented with soybean (SOY), bahiagrass supplemented with sunn hemp (SUN). Hour differences ($P < 0.01$) according to Tukey-Kramer adjustment.

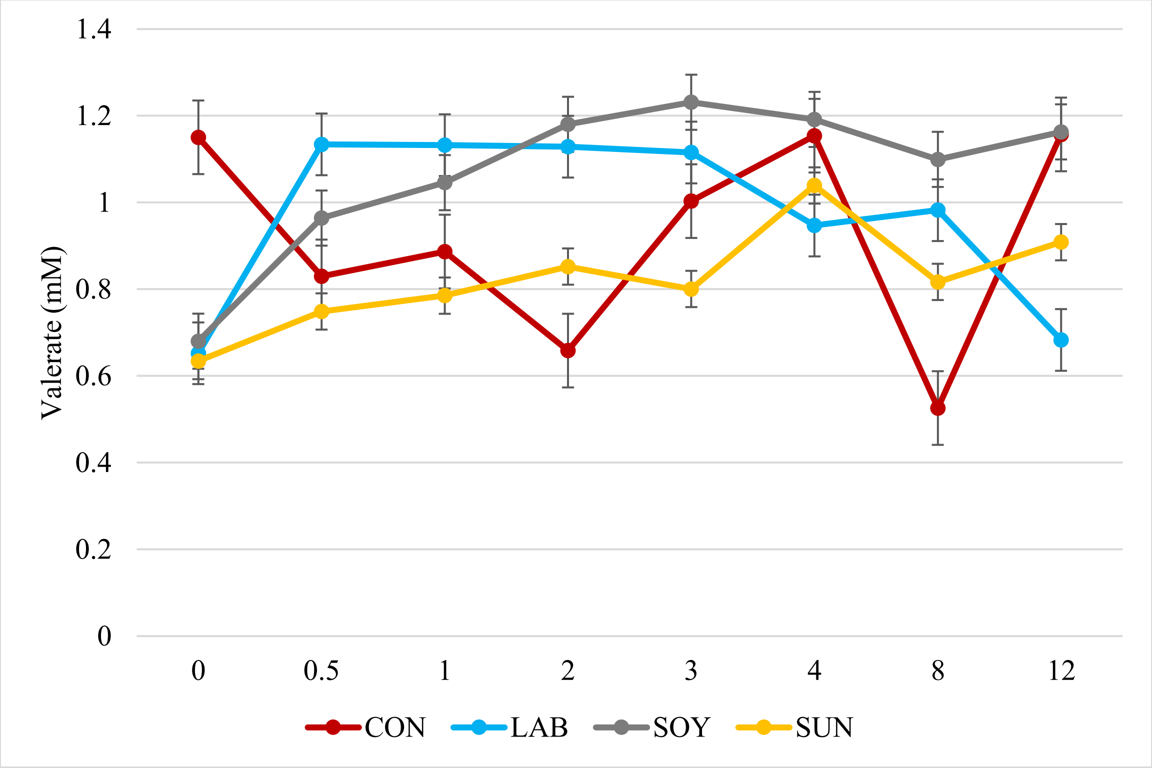

Figure IV-5 Rumen valerate (molar concentration) for steers fed a control diet of bahiagrass hay only (CON), bahiagrass supplemented with lablab (LAB), bahiagrass supplemented with soybean (SOY), or bahiagrass supplemented with sunn hemp (SUN). Diet × Hour interaction ($P < 0.01$) according to Tukey-Kramer adjustment

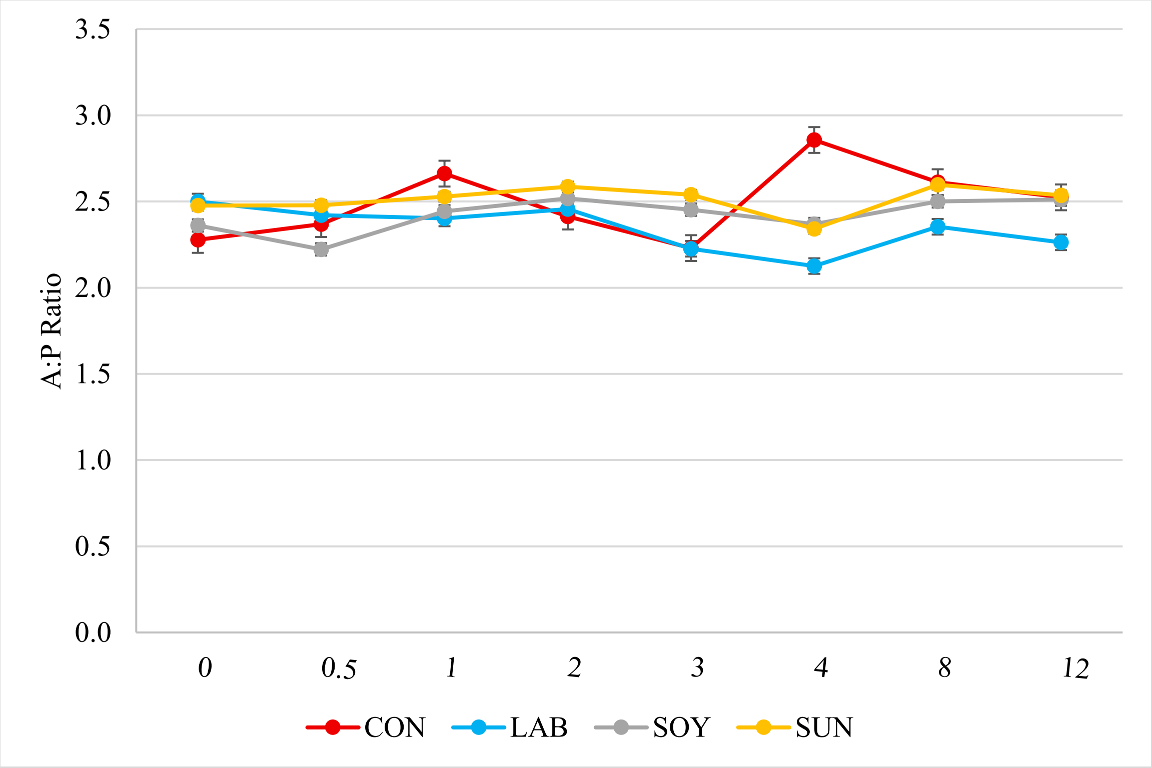

Figure IV-6 Acetate to propionate ratio for steers fed a control diet of bahiagrass hay only (CON), bahiagrass supplemented with lablab (LAB), bahiagrass supplemented with soybean (SOY), or bahiagrass supplemented with sunn hemp (SUN). Diet × Hour interaction ($P < 0.01$) according to Tukey-Kramer adjustment

Conclusions

Forb species evaluated in our research demonstrated strong productive potential and favorable nutritive characteristics, highlighting their capacity to enhance grazing systems in the Southern US. However, their successful integration depends on the appropriate timing of establishment, seasonal adaptation, environmental conditions, and management practices. The diversity of available forb species provides producers with flexible options to address forage gaps, improve nutritional supplementation of warm-season grasses, and potentially support extended or year-round grazing systems. Despite this potential, results from the *in vivo* evaluation indicated that species selection and agronomic suitability alone are not sufficient to guarantee functional benefits. Although several forbs exhibited desirable nutritive and metabolic attributes, reduced voluntary intake limited their effective dietary inclusion and reduced the impact of associated secondary metabolites.

From a grassland management perspective, these findings emphasize that the sustainability and regenerative potential of forb inclusion depend on strategies that enhance both plant persistence and animal utilization. Managers can improve pasture sustainability by incorporating forbs into diverse, mixed-species rather than monocultures, thereby increasing plant functional diversity, improving soil structure, and enhancing nutrient cycling. Rotational or adaptive grazing systems can be used to control grazing pressure and encourage more uniform forage utilization, particularly by increasing the likelihood of animals consuming less-preferred forb species, thus giving more rest period to the forbs that are constantly overgrazed and slow to recover. Practices such as higher stocking density for short grazing periods, followed by adequate rest, may improve intake while supporting plant recovery and long-term pasture resilience.

Additionally, strategic placement of water, minerals, or supplements can influence grazing behavior and promote more even distribution of grazing across pasture landscapes, preventing selective overgrazing of forbs. Establishing forbs during optimal seasonal windows, when there is less competition and selecting species adapted to local soil and climatic conditions can further improve establishment success and persistence. From a regenerative standpoint, the inclusion of forbs may reduce reliance on external inputs such as protein supplements and synthetic fertilizers by enhancing biological nitrogen cycling and improving overall forage quality. However, achieving these benefits requires management approaches that prioritize palatability, intake, and proper grazing timing to ensure that the nutritional and ecological advantages of forbs are fully realized within the production system.

Overall, this research demonstrated that while forage forbs show promise for Southern grazing systems, their ability to reduce enteric CH_4 without sacrificing performance depends on management strategies that enhance palatability, increase effective intake, and optimize utilization. Future efforts should focus on grazing trials of these forbs to further evaluate real life effects forbs on animal performance.

REFERENCES

Adams, N. R. 1995. Detection of the effects of phytoestrogens on sheep and cattle. J. Anim. Sci. 73:1509–1515. doi:10.2527/1995.7351509x.

Adewuyi, A. A., E. Gruys, and F. J. C. M. Van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet. Q. 27:117–126. doi:10.1080/01652176.2005.9695192.

Akins, M. S., E. B. Kegley, K. P. Coffey, J. D. Caldwell, K. S. Lusby, J. C. Moore, and W. K. Coblentz. 2009. Comparison of bloat potential between a variety of soft-red versus a variety of hard-red winter wheat forage1. J. Anim. Sci. 87:3278–3287. doi:10.2527/jas.2008-1664.

Allen, M. S., and D. R. Mertens. 1988. Evaluating Constraints on Fiber Digestion by Rumen Microbes. J. Nutr. 118:261–270. doi:10.1093/jn/118.2.261.

Anderson, R. C., G. E. Carstens, R. K. Miller, T. R. Callaway, C. L. Schultz, T. S. Edrington, R. B. Harvey, and D. J. Nisbet. 2006. Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen. Bioresour. Technol. 97:2421–2426. doi:10.1016/j.biortech.2005.10.013.

Apajalahti, J., K. Vienola, K. Raatikainen, V. Holder, and C. A. Moran. 2019. Conversion of Branched-Chain Amino Acids to Corresponding Isoacids - An in vitro Tool for Estimating Ruminal Protein Degradability. Front. Vet. Sci. 6. doi:10.3389/fvets.2019.00311.

Araújo, S. S., S. Beebe, M. Crespi, B. Delbreil, E. M. González, V. Gruber, I. Lejeune-Henaut, W. Link, M. J. Monteros, E. Prats, I. Rao, V. Vadez, and M. C. V. Patto. 2015. Abiotic Stress Responses in Legumes: Strategies Used to Cope with Environmental Challenges. Crit. Rev. Plant Sci. 34:237–280. doi:10.1080/07352689.2014.898450.

Armstrong, K. L., and K. A. Albrecht. 2008. Effect of Plant Density on Forage Yield and Quality of Intercropped Corn and Lablab Bean. Crop Sci. 48:814–822. doi:10.2135/cropsci2007.08.0487.

Athmaselvi, K. A., A. Sukumar, and S. Bhokarikar. 2020. Hyacinth Beans. A. Manickavasagan and P. Thirunathan, editors. Pulses: Processing and Product Development. 119–128. doi:10.1007/978-3-030-41376-7_7.

Attwood, G., and C. McSweeney. 2008. Methanogen genomics to discover targets for methane mitigation technologies and options for alternative H2 utilisation in the rumen. Aust. J. Exp. Agric. 48:28. doi:10.1071/EA07203.

Augustin, J. M., V. Kuzina, S. B. Andersen, and S. Bak. 2011. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry. 72:435–457. doi:10.1016/j.phytochem.2011.01.015.

Balkcom, K., and D. Reeves. 2005. Sunn-hemp utilized as a legume cover crop for corn production. Agron. J. 97:26–31. doi:10.2134/agronj2005.0026.

Baral, R., J. Kim, B. Bhattarai, H. Koirala, I. Massigoge, E. Denson, C. Guareschi, S. Cominelli, J. Rud, J. de Oliveira, P. Helguera, I. Ciampitti, C. Rice, and D. Min. 2025. Cropping potential of forage soybean as a summer forage in Midwest US rainfed systems. Front. Agron. 7. doi:10.3389/fagro.2025.1570567.

Barry, T. N. 2013a. The feeding value of forage brassica plants for grazing ruminant livestock. Anim. Feed Sci. Technol. 181:15–25. doi:10.1016/j.anifeedsci.2013.01.012.

Barry, T. N. 2013b. The feeding value of forage brassica plants for grazing ruminant livestock. Anim. Feed Sci. Technol. 181:15–25. doi:10.1016/j.anifeedsci.2013.01.012.

Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81:263–272. doi:10.1017/S0007114599000501.

Bennett, R. N., and R. M. Wallsgrove. 1994. Secondary metabolites in plant defence mechanisms. New Phytol. 127:617–633. doi:10.1111/j.1469-8137.1994.tb02968.x.

BenYoussef, S., S. S. Kachout, S. Abidi, B. Saddem, J. Ismail, and H. B. Salem. Effect of Different Levels of Nitrogen Fertilization on Forage Yields and Quality of Hairy Vetch (Vicia villosa, Roth) Triticale (Xtritcosecale, Witmack) Mixtures. doi:10.2174/1874331501913010090. Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567–590. doi:10.1152/physrev.1990.70.2.567.

Bhattacharya, A. 2019. High-Temperature Stress and Metabolism of Secondary Metabolites in Plants. In: Effect of High Temperature on Crop Productivity and Metabolism of Macro Molecules. Elsevier. p. 391–484.

Bragagnolo, F. S., C. S. Funari, E. Ibáñez, and A. Cifuentes. 2021. Metabolomics as a Tool to Study Underused Soy Parts: In Search of Bioactive Compounds. Foods. 10:1308. doi:10.3390/foods10061308.

Burt, J. C., L. L. Baxter, C. G. Prevatt, M. Kimberly Mullenix, R. L. Stewart Jr., and J. J. Tucker. 2022. Improving bermudagrass in the Southeastern United States with alfalfa as an alternative nitrogen source in grazing systems. Grassl. Res. 1:280–289. doi:10.1002/glr2.12038.

Butkutė, B., A. Padarauskas, J. Cesevičienė, L. Taujenis, and E. Norkevičienė. 2018. Phytochemical composition of temperate perennial legumes. Crop Pasture Sci. 69:1020–1030. doi:10.1071/CP18206.

Buxton, D. R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. 59:37–49. doi:10.1016/0377-8401(95)00885-3.

Buxton, D. R., and D. D. Redfearn. 1997. Plant Limitations to Fiber Digestion and Utilization1. J. Nutr. 127:814S-818S. doi:10.1093/jn/127.5.814S.

Byers, F. M., and A. L. Moxon. 1980. Protein and Selenium Levels for Growing and Finishing Beef Cattle. J. Anim. Sci. 50:1136–1144. doi:10.2527/jas1980.5061136x.

Chizzotti, M. L., S. de C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113:218–225. doi:10.1016/j.livsci.2007.03.013.

Church, D. C. 1993. The Ruminant Animal: Digestive Physiology and Nutrition. Waveland Press.

Cook, C., and G. White. 1996. CROTALARIA JUNCEA: A POTENTIAL MULTI-PURPOSE FIBER CROP. CROTALARIA JUNCEA POTENTIAL MULTI-Purp. FIBER CROP. https://www.ars.usda.gov/research/publications/publication/?seqNo115=65245

Cook, J. C., R. S. Gallagher, J. P. Kaye, J. Lynch, and B. Bradley. 2010. Optimizing Vetch Nitrogen Production and Corn Nitrogen Accumulation under No-Till Management. Agron. J. 102:1491–1499. doi:10.2134/agronj2010.0165.

Crozier, A., M. N. Clifford, and H. Ashihara, eds. 2006. Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet. 1st ed. Wiley. https://onlinelibrary.wiley.com/doi/book/10.1002/9780470988558

Cuervo, W., C. Gomez-Lopez, and N. DiLorenzo. 2025. Methane Synthesis as a Source of Energy Loss Impacting Microbial Protein Synthesis in Beef Cattle—A Review. Methane. 4:10. doi:10.3390/methane4020010.

Curto, G., E. Dallavalle, R. Santi, N. Casadei, L. D'Avino, and L. Lazzeri. 2015. The potential of Crotalaria juncea L. as a summer green manure crop in comparison to Brassicaceae catch crops for management of Meloidogyne incognita in the Mediterranean area. Eur. J. Plant Pathol. 142:829–841. doi:10.1007/s10658-015-0655-2.

Danielsson, R., M. Ramin, J. Bertilsson, P. Lund, and P. Huhtanen. 2017. Evaluation of a gas in vitro system for predicting methane production in vivo. J. Dairy Sci. 100:8881–8894. doi:10.3168/jds.2017-12675.

Das, A., M. N. Islam, Md. O. Faruk, M. Ashaduzzaman, and R. Dungani. 2020. Review on tannins: Extraction processes, applications and possibilities. South Afr. J. Bot. 135:58–70. doi:10.1016/j.sajb.2020.08.008.

Desai, S. D., D. G. Desaib, and H. Kaurc. Saponins and their Biological Activities.

Dijkstra, J., J. L. Ellis, E. Kebreab, A. B. Strathe, S. López, J. France, and A. Bannink. 2012. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed Sci. Technol. 172:22–33. doi:10.1016/j.anifeedsci.2011.12.005.

Dillard, S. 2024. Cool-season Annual Legumes and Forbs for Grazing and Cover Crop Systems. IGC Proc. 1985-2023. https://uknowledge.uky.edu/igc/XXV_IGC_2023/Livestock/7

Dillard, S. L. 2023. Cool-Season Annual Legumes and Forbs for Grazing and Cover Crop Systems. In: XXV International Grassland Congress (IGC 2023). International Grassland Congress 2023, Covington, KY USA. p. 1100–1103. http://www.proceedings.com/071171-0266.html

Dillard, S. L., A. I. Roca-Fernández, M. D. Rubano, K. R. Elkin, and K. J. Soder. 2018. Enteric methane production and ruminal fermentation of forage brassica diets fed in continuous culture1. J. Anim. Sci. 96:1362–1374. doi:10.1093/jas/sky030.

Dinkins, R. D., J. Hancock, B. L. Coe, J. B. May, J. P. Goodman, W. T. Bass, J. Liu, Y. Fan, Q. Zheng, and H. Zhu. 2021. Isoflavone levels, nodulation and gene expression profiles of a CRISPR/Cas9 deletion mutant in the isoflavone synthase gene of red clover. Plant Cell Rep. 40:517–528. doi:10.1007/s00299-020-02647-4.

Dubeux Jr, J. C. B., D. M. Jaramillo, E. R. S. Santos, L. Garcia, L. M. D. Queiroz, I. L. Bretas, C. H. L. de Souza, and K. R. Trumpp. 2024. Sustainable intensification of livestock systems using forage legumes in the Anthropocene. Grass Forage Sci. 79:481–498. doi:10.1111/gfs.12696.

Eberle, C., and L. Shortnacy. 2021. Sunn hemp planting date effect on growth, biomass accumulation, and nutritive value in southeastern Wyoming. Crop Sci. 61. doi:10.1002/csc2.20614.

Fay, P. A., and M. J. Schultz. 2009. Germination, survival, and growth of grass and forb seedlings: Effects of soil moisture variability. Acta Oecologica. 35:679–684. doi:10.1016/j.actao.2009.06.007.

Flythe, M. D., and B. Harlow. 2023. Use of Plant Secondary Metabolites and Impact on Beef Cattle Production. J. Anim. Sci. 101:45–45. doi:10.1093/jas/skad068.052.

Foster, J., A. Adesogan, J. Carter, A. Blount, R. Myer, and S. Phatak. 2009. Intake, digestibility, and nitrogen retention by sheep supplemented with warm-season legume hays or soybean meal. J. Anim. Sci. 87:2891–2898. doi:10.2527/jas.2008-1637.

Foster, J. L., J. P. Muir, J. R. Bow, and E. Valencia. 2017. Biomass and nitrogen content of fifteen annual warm-season legumes grown in a semi-arid environment. Biomass Bioenergy. 106:38–42. doi:10.1016/j.biombioe.2017.08.016.

Foster, J., J. Muir, J. Bow, and E. Valencia. 2017. Biomass and nitrogen content of fifteen annual warm-season legumes grown in a semi-arid environment. BIOMASS BIOENERGY. 106:38–42. doi:10.1016/j.biombioe.2017.08.016.

Francis, G., Z. Kerem, H. P. S. Makkar, and K. Becker. 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88:587–605. doi:10.1079/BJN2002725.

Frutos, P., G. Hervás, F. J. Giráldez, and A. R. Mantecón. 2004. Review. Tannins and ruminant nutrition. Span. J. Agric. Res. 2:191–202. doi:10.5424/sjar/2004022-73.

Gao, M., A. Irawan, M. El-Sherbiny, M. Szumacher-Strabel, A. Cieślak, M. A. Setiawan, H. Jallal, I. Fusaro, A. Jayanegara, Y. R. Yanza, and Y. Liu. 2025. Meta-Analysis of Incorporating

Glucosinolates into Diets and Their Effects on Ruminant Performance, Ruminal Fermentation, Methane Emissions, Milk Composition, and Metabolic Biochemical Attributes. Animals. 15:1480. doi:10.3390/ani15101480.

Garzon, J., J. M. B. Vendramini, M. L. Silveira, P. Moriel, H. M. S. da Silva, J. C. B. Dubeux Jr., M. Kaneko, C. C. Carnelos, and P. A. Mamede. 2021a. Harvest management and genotype effects on sunn hemp forage characteristics. Agron. J. 113:298–307. doi:10.1002/agj2.20465.

Garzon, J., J. M. B. Vendramini, M. L. Silveira, P. Moriel, H. M. S. da Silva, J. C. B. Dubeux Jr., M. Kaneko, C. C. Carnelos, and P. A. Mamede. 2021b. Harvest management and genotype effects on sunn hemp forage characteristics. Agron. J. 113:298–307. doi:10.1002/agj2.20465.

Goel, G., and H. P. S. Makkar. 2012. Methane mitigation from ruminants using tannins and saponins. Trop. Anim. Health Prod. 44:729–739. doi:10.1007/s11250-011-9966-2.

Goering, H. K., and P. J. V. Soest. 1970. Forage Fiber Analyses (apparatus, Reagents, Procedures, and Some Applications). U.S. Agricultural Research Service.

Greveniotis, V., E. Bouloumpasi, A. Skendi, A. Korkovelos, D. Kantas, and C. G. Ipsilandis. 2025. Evaluation and Stability of Red and White Trifolium Species for Nutritional Quality in a Mediterranean Environment. Agriculture. 15:391. doi:10.3390/agriculture15040391.

Grgic, D., E. Varga, B. Novak, A. Müller, and D. Marko. 2021. Isoflavones in Animals: Metabolism and Effects in Livestock and Occurrence in Feed. Toxins. 13:836. doi:10.3390/toxins13120836.

Hammond, A. C. 1998. Use of BUN and MUN as guides for protein and energy supplementation in cattle.

Harlow, B. E., M. D. Flythe, I. A. Kagan, J. P. Goodman, J. L. Klotz, and G. E. Aiken. 2020. Isoflavone supplementation, via red clover hay, alters the rumen microbial community and promotes weight gain of steers grazing mixed grass pastures. PLOS ONE. 15:e0229200. doi:10.1371/journal.pone.0229200.

Hassanat, F., and C. Benchaar. 2013. Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production in vitro. J. Sci. Food Agric. 93:332–339. doi:10.1002/jsfa.5763.

Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young, and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5:14567. doi:10.1038/srep14567.

Hess, H. D., M. Kreuzer, T. E. Díaz, C. E. Lascano, J. E. Carulla, C. R. Soliva, and A. Machmüller. 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Anim. Feed Sci. Technol. 109:79–94. doi:10.1016/S0377-8401(03)00212-8.

Hill, J., C. McSweeney, A.-D. G. Wright, G. Bishop-Hurley, and K. Kalantar-zadeh. 2016. Measuring Methane Production from Ruminants. Trends Biotechnol. 34:26–35. doi:10.1016/j.tibtech.2015.10.004.

Hintz, R. W., K. A. Albrecht, and E. S. Oplinger. 1992. Yield and Quality of Soybean Forage as Affected by Cultivar and Management Practices. Agron. J. 84:795–798. doi:10.2134/agronj1992.00021962008400050007x.

Hungate, R. E. 1975. The Rumen Microbial Ecosystem. Annu. Rev. Ecol. Syst. 6:39–66.

Hungate, R. E., W. Smith, T. Bauchop, I. Yu, and J. C. Rabinowitz. 1970. Formate as an Intermediate in the Bovine Rumen Fermentation. J. Bacteriol. 102:389–397. doi:10.1128/jb.102.2.389-397.1970.

Huyen, N. T., O. Desrues, S. J. J. Alferink, T. Zandstra, M. W. A. Verstegen, W. H. Hendriks, and W. F. Pellikaan. 2016. Inclusion of sainfoin (*Onobrychis viciifolia*) silage in dairy cow rations affects nutrient digestibility, nitrogen utilization, energy balance, and methane emissions. J. Dairy Sci. 99:3566–3577. doi:10.3168/jds.2015-10583.

IPPC. 2019. Tackling Climate Change through Livestock: A global assessment of emissions and mitigation opportunities. https://openknowledge.fao.org/server/api/core/bitstreams/3b36953e-5689-480b-9280-71e4ab73646a/content/i3437e03.pdf

Jaramillo, D. M., J. C. B. Dubeux Jr, J. M. B. Vendramini, L. M. D. Queiroz, E. R. S. Santos, M. Ruiz-Moreno, L. Garcia, D. S. de Abreu, L. R. de Miranda, and M. C. F. de Siqueira. 2020a. Establishment techniques affect productivity, nutritive value and atmospheric N2 fixation of two sunn hemp cultivars. Grass Forage Sci. 75:153–158. doi:10.1111/gfs.12472.

Jaramillo, D. M., J. C. B. Dubeux Jr, J. M. B. Vendramini, L. M. D. Queiroz, E. R. S. Santos, M. Ruiz-Moreno, L. Garcia, D. S. de Abreu, L. R. de Miranda, and M. C. F. de Siqueira. 2020b. Establishment techniques affect productivity, nutritive value and atmospheric N2 fixation of two sunn hemp cultivars. Grass Forage Sci. 75:153–158. doi:10.1111/gfs.12472.

Jaramillo, D. M., J. C. B. Dubeux Jr, J. M. B. Vendramini, L. M. D. Queiroz, E. R. S. Santos, M. Ruiz-Moreno, L. Garcia, D. S. de Abreu, L. R. de Miranda, and M. C. F. de Siqueira. 2020c.

Establishment techniques affect productivity, nutritive value and atmospheric N2 fixation of two sunn hemp cultivars. Grass Forage Sci. 75:153–158. doi:10.1111/gfs.12472.

Johnson, D. E., and G. M. Ward. 1996. Estimates of animal methane emissions. Environ. Monit. Assess. 42:133–141. doi:10.1007/BF00394046.

Kammes, K. L., and M. S. Allen. 2012. Rates of particle size reduction and passage are faster for legume compared with cool-season grass, resulting in lower rumen fill and less effective fiber. J. Dairy Sci. 95:3288–3297. doi:10.3168/jds.2011-5022.

Karydogianni, S., I. Roussis, A. Mavroeidis, I. Kakabouki, E. Tigka, D. Beslemes, P. Stavropoulos, N. Katsenios, E. Tsiplakou, and D. Bilalis. 2022. The Influence of Fertilization and Plant Density on the Dry Matter Yield and Quality of Black Mustard [Brassica nigra (L.) Koch]: An Alternative Forage Crop. Plants. 11:2683. doi:10.3390/plants11202683.

Kenward, M. G., and J. H. Roger. 2009. An improved approximation to the precision of fixed effects from restricted maximum likelihood. Comput. Stat. Data Anal. 53:2583–2595. doi:10.1016/j.csda.2008.12.013.

Khanbabaee, K., and T. van Ree. 2001. Tannins: Classification and Definition. Nat. Prod. Rep. 18:641–649. doi:10.1039/B101061L.

Kramer, C. Y. 1956. Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. Biometrics. 12:307–310. doi:10.2307/3001469.

Ku, Y.-S., C. A. Contador, M.-S. Ng, J. Yu, G. Chung, and H.-M. Lam. 2020. The Effects of Domestication on Secondary Metabolite Composition in Legumes. Front. Genet. 11. doi:10.3389/fgene.2020.581357.

Ku-Vera, J. C., R. Jiménez-Ocampo, S. S. Valencia-Salazar, M. D. Montoya-Flores, I. C. Molina-Botero, J. Arango, C. A. Gómez-Bravo, C. F. Aguilar-Pérez, and F. J. Solorio-Sánchez. 2020. Role of Secondary Plant Metabolites on Enteric Methane Mitigation in Ruminants. Front. Vet. Sci. 7. doi:10.3389/fvets.2020.00584.

Lepcha, I., and H. D. Naumann. 2021. Partitioning of Forage Mass and Nutritive Value in Sunn Hemp Leaf and Stem Components. Int. J. Agron. 2021. doi:10.1155/2021/5547120.

Li, Q. S., R. Wang, Z. Y. Ma, X. M. Zhang, J. Z. Jiao, Z. G. Zhang, E. M. Ungerfeld, K. Le Yi, B. Z. Zhang, L. Long, Y. Long, Y. Tao, T. Huang, C. Greening, Z. L. Tan, and M. Wang. 2022. Dietary selection of metabolically distinct microorganisms drives hydrogen metabolism in ruminants. ISME J. 16:2535–2546. doi:10.1038/s41396-022-01294-9.

Lisonbee, L., J. Villalba, and F. Provenza. 2009. Effects of tannin on selection by sheep of forages containing alkaloids, tannins and saponins. J. Sci. FOOD Agric. 89:2668–2677. doi:10.1002/jsfa.3772.

Lorenz, M. M., L. Alkhafadji, E. Stringano, S. Nilsson, I. Mueller-Harvey, and P. Udén. 2014. Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. J. Sci. Food Agric. 94:963–968. doi:10.1002/jsfa.6344.

MacAdam, J. W., L. R. Pitcher, A. I. Bolletta, R. D. Guevara Ballesteros, K. A. Beauchemin, X. Dai, and J. J. Villalba. 2022. Increased Nitrogen Retention and Reduced Methane Emissions of Beef Cattle Grazing Legume vs. Grass Irrigated Pastures in the Mountain West USA. Agronomy. 12:304. doi:10.3390/agronomy12020304.

MacAdam, J. W., and J. J. Villalba. 2015. Beneficial Effects of Temperate Forage Legumes that Contain Condensed Tannins. Agriculture. 5:475–491. doi:10.3390/agriculture5030475.

MacAdam, J. W., J. J. Villalba, S. Lagrange, E. K. Stewart, L. R. Pitcher, K. A. Slebodnik, J. M. Norton, J. R. Reeve, Y. Zhang, A. I. Bolletta, J. F. Legako, R. G. Christensen, and S. R. Hunt. 2025. In Vivo Reductions in Methane and Urinary Nitrogen by Perennial Non-Bloating Temperate Legume and Forb Functional Forages Produced in the Mountain West United States. Grass Forage Sci. 80:1–13. doi:10.1111/gfs.12719.

Messina, M. J. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. Am. J. Clin. Nutr. 70:439S-450S. doi:10.1093/ajcn/70.3.439s.

Mi, J., X. Jing, C. Ma, F. Shi, Z. Cao, X. Yang, Y. Yang, A. Kakade, W. Wang, and R. Long. 2024. A metagenomic catalogue of the ruminant gut archaeome. Nat. Commun. 15:9609. doi:10.1038/s41467-024-54025-3.

Mir, Z. 1997. Nutrient composition, in vitro gas production and digestibility of fenugreek (Trigonella foenum-graecum) and alfalfa forages. Nutr. Compos. Vitro Gas Prod. Dig. Fenugreek Trigonella Foenum-Graecum Alfalfa Forages. doi:10.4141/A96-061.

Mokoboki, H. K., L. R. Ndlovu, and K. K. Ayisi. 2002. Chemical and physical parameters of forage legume species introduced in the Capricorn region of Limpopo Province, South Africa. South Afr. J. Anim. Sci.

Mora, J., D. Pott, S. Osorio, and J. Vallarino. 2022. Regulation of Plant Tannin Synthesis in Crop Species. Front. Genet. 13. doi:10.3389/fgene.2022.870976.

Mrutu, R. I., A. M. Abdussamad, K. M. Umar, A. Abdulhamid, and N. G. Farny. 2025. Mitigating methane emissions and promoting acetogenesis in ruminant livestock. Front. Anim. Sci. 6. doi:10.3389/fanim.2025.1489212.

Mueller-Harvey, I., G. Bee, F. Dohme-Meier, H. Hoste, M. Karonen, R. Kölliker, A. Lüscher, V. Niderkorn, W. Pellikaan, J. Salminen, L. Skot, L. Smith, S. Thamsborg, P. Totterdell, I. Wilkinson, A. Williams, B. Azuhnwi, N. Baert, A. Brinkhaus, G. Copani, O. Desrues, C. Drake, M. Engstrom, C. Fryganas, M. Girard, N. Huyen, K. Kempf, C. Malisch, M. Mora-Ortiz, J. Quijada, A. Ramsay, H. Ropiak, and G. Waghorn. 2019. Benefits of Condensed Tannins in Forage Legumes Fed to Ruminants: Importance of Structure, Concentration, and Diet Composition. CROP Sci. 59:861–885. doi:10.2135/cropsci2017.06.0369.

Mugford, S. T., and A. Osbourn. 2012. Saponin Synthesis and Function. In: T. J. Bach and M. Rohmer, editors. Isoprenoid Synthesis in Plants and Microorganisms. Springer New York, New York, NY. p. 405–424.

Muir, J., J. Dubeux, M. Dos Santos, I. Maposse, W. Pitman, and T. Butler. 2014. Challenges to domesticating native forage legumes. Trop. Grassl.-FORRAJES Trop. 2:94–96. doi:10.17138/TGFT(2)94-96.

Muir, J. P. 2002. Hand-Plucked Forage Yield and Quality and Seed Production from Annual and Short-Lived Perennial Warm-Season Legumes Fertilized with Composted Manure. Crop Sci. 42:897–904. doi:10.2135/cropsci2002.8970.

Muir, J. P., W. D. Pitman, and J. L. Foster. 2011. Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci. 66:301–315. doi:10.1111/j.1365-2494.2011.00806.x.

Mullenix, K., and J. Johnson. 2018. Collecting Forage Samples for Laboratory Analysis. Ala. Coop. Ext. Syst. https://www.aces.edu/blog/topics/counties-statewide/collecting-forage-samples-for-laboratory-analysis/

Nagaraja, T. G., and K. F. Lechtenberg. 2007. Acidosis in Feedlot Cattle. Vet. Clin. North Am. Food Anim. Pract. 23:333–350. doi:10.1016/j.cvfa.2007.04.002.

Naumann, H. D., A. E. Hagerman, B. D. Lambert, J. P. Muir, L. O. Tedeschi, and M. M. Kothmann. 2014. Molecular weight and protein-precipitating ability of condensed tannins from warm-season perennial legumes. J. Plant Interact. 9:212–219. doi:10.1080/17429145.2013.811547.

Naumann, H. D., L. O. Tedeschi, W. E. Zeller, and N. F. Huntley. 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. Rev. Bras. Zootec. 46:929–949. doi:https://doi.org/10.1590/S1806-92902017001200009.

Naumann, H., L. Tedeschi, J. Muir, B. Lambert, and M. Kothmann. 2013. Effect of molecular weight of condensed tannins from warm-season perennial legumes on ruminal methane production in vitro. Biochem. Syst. Ecol. 50:154–162. doi:10.1016/j.bse.2013.03.050.

Ohyama, T. 2017. The Role of Legume-Rhizobium Symbiosis in Sustainable Agriculture. In: S. Sulieman and L.-S. P. Tran, editors. Legume Nitrogen Fixation in Soils with Low Phosphorus Availability: Adaptation and Regulatory Implication. Springer International Publishing, Cham. p. 1–20. https://doi.org/10.1007/978-3-319-55729-8_1

Oregon State University. 2016. Forbs | Forage Information System | Oregon State University. Species Sel. Inf. Syst. FORBS.

Patra, A. K., and J. Saxena. 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr. Res. Rev. 22:204–219. doi:10.1017/S0954422409990163.

Pecetti, L., A. Tava, A. Romani, M. De Benedetto, and P. Corsi. 2006. Variety and environment effects on the dynamics of saponins in lucerne (Medicago sativa L.). Eur. J. Agron. 25:187–192. doi:10.1016/j.eja.2006.04.013.

Pedreira, M., S. Oliveira, O. Primavesi, M. Lima, R. Frighetto, and T. Berchielli. 2013. Methane emissions and estimates of ruminal fermentation parameters in beef cattle fed different dietary concentrate levels. Rev. Bras. Zootec. 42:592–598. doi:10.1590/S1516-35982013000800009.

Phelan, P., A. P. Moloney, E. J. McGeough, J. Humphreys, J. Bertilsson, E. G. O’Riordan, and P. O’Kiely. 2015. Forage Legumes for Grazing and Conserving in Ruminant Production Systems. Crit. Rev. Plant Sci. 34:281–326. doi:10.1080/07352689.2014.898455.

Prigge, J. L., E. Bisangwa, J. D. Richwine, V. R. Sykes, J. L. Z. Ivey, and P. D. Keyser. 2024. Native Forbs Provide Pollinator Resources and Improve Forage Nutrient Composition, Animal Performance, and Pasture Productivity. Agronomy. 14:2184. doi:10.3390/agronomy14102184.

Riaz, R., R. M. Bilal, M. U. Hassan, M. Todaro, R. Gannuscio, F. Inal, M. N. U. Haque, and M. N. Tahir. 2025. Crude Protein Degradation Kinetics of Selected Tropical Forages in Buffalo Using NorFor In Situ Standards. Animals. 15:585. doi:10.3390/ani15040585.

Robbins, C. T., S. Mole, A. E. Hagerman, and T. A. Hanley. 1987. Role of Tannins in Defending Plants Against Ruminants: Reduction in Dry Matter Digestion? Ecology. 68:1606–1615. doi:10.2307/1939852.

Rouquette, M., V. Corriher-Olson, and G. R. Smith. 2020a. Management strategies for pastures and beef cattle in the Middle-South: The I-20 Corridor. In: Management Strategies for Sustainable Cattle Production in Southern Pastures. Elsevier. p. 123–187.

Rouquette, M., V. Corriher-Olson, and G. R. Smith. 2020b. Management strategies for pastures and beef cattle in the Middle-South: The I-20 Corridor. In: Management Strategies for Sustainable Cattle Production in Southern Pastures. Elsevier. p. 123–187.

Sedivy, E. J., F. Wu, and Y. Hanzawa. 2017. Soybean domestication: the origin, genetic architecture and molecular bases. New Phytol. 214:539–553. doi:10.1111/nph.14418.

Sheaffer, C. C., J. H. Orf, T. E. Devine, and J. G. Jewett. 2001. Yield and Quality of Forage Soybean. Agron. J. 93:99–106. doi:10.2134/agronj2001.93199x.

Sheaffer, C., J. Orf, T. Devine, and J. Jewett. 2001. Yield and quality of forage soybean. Agron. J. 93:99–106. doi:10.2134/agronj2001.93199x.

Shekinah, D. E., and J. K. Stute. 2018. Sunn Hemp: A Legume Cover Crop with Potential for the Midwest? Sustain. Agric. Res. doi:10.22004/ag.econ.301841.

Shimada, T. 2006. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 32:1149–1163. doi:10.1007/s10886-006-9077-0.

Shinkai, T., S. Takizawa, O. Enishi, K. Higuchi, H. Ohmori, and M. Mitsumori. 2024. Characteristics of rumen microbiota and Prevotella isolates found in high propionate and low methane-producing dairy cows. Front. Microbiol. 15. doi:10.3389/fmicb.2024.1404991.

Silva, T. B. P., T. A. Del Valle, L. G. Ghizzi, G. G. Silva, L. S. Gheller, J. A. Marques, M. S. S. Dias, A. T. Nunes, N. T. S. Grigoletto, C. S. Takiya, and F. P. Rennó. 2021. Partial replacement of corn silage with whole-plant soybean and black oat silages for dairy cows. J. Dairy Sci. 104:9842–9852. doi:10.3168/jds.2021-20200.

Sollenberger, L. E., and J. C. B. Dubeux. 2022. Warm-climate, legume-grass forage mixtures versus grass-only swards: An ecosystem services comparison. Rev. Bras. Zootec. 51:e20210198. doi:10.37496/rbz5120210198.

Sollenberger, L. E., and J. C. B. D. Junior. 2022. Warm-climate, legume-grass forage mixtures versus grass-only swards: An ecosystem services comparison. R Bras Zootec. 51. doi:10.37496/rbz5120210198.

Steven, M., V. Ackroyd, S. Cordeau, W. Curran, M. Hashemi, C. Reberg-Horton, M. Ryan, and J. Spargo. 2017. Hairy vetch biomass across the eastern United States: Effects of latitude, seeding rate and date, and termination timing. https://www.ars.usda.gov/research/publications/publication/?seqNo115=333080

Sun, X. 2020. Invited Review: Glucosinolates Might Result in Low Methane Emissions From Ruminants Fed Brassica Forages. Front. Vet. Sci. 7. doi:10.3389/fvets.2020.588051.

Sun, X., G. Henderson, F. Cox, G. Molano, S. J. Harrison, D. Luo, P. H. Janssen, and D. Pacheco. 2015. Lambs Fed Fresh Winter Forage Rape (Brassica napus L.) Emit Less Methane than Those Fed Perennial Ryegrass (Lolium perenne L.), and Possible Mechanisms behind the Difference. PLoS ONE. 10:1–16. doi:10.1371/journal.pone.0119697.

Teeter, R. G., and F. N. Owens. 1983. Characteristics of Water Soluble Markers for Measuring Rumen Liquid Volume and Dilution Rate. J. Anim. Sci. 56:717–728. doi:10.2527/jas1983.563717x.

Thompson, S. J., J. Koebernick, L. S. Silva, M. K. Mullenix, C. Heaton, R. C. Carrell, and S. L. Dillard. 2023. Forage Mass and Nutritive Value of Grain- and Forage-Type Soybean Cultivars Managed under Different Row Spacings and Clipping Heights. Agronomy. 13:487. doi:10.3390/agronomy13020487.

Tilley, J. M. A., and R. A. Terry. 1963. A Two-Stage Technique for the in Vitro Digestion of Forage Crops. Grass Forage Sci. 18:104–111. doi:10.1111/j.1365-2494.1963.tb00335.x.

Tirado-Corbalá, R., L. Y. López-Ramos, E. Valencia-Chin, and E. Román-Paoli. 2018. Yield, Decomposition, Mineralization and Nitrification of Annual Legumes in an Oxisol. Agronomy. 8:244. doi:10.3390/agronomy8110244.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132:1–27. doi:10.1016/j.anifeedsci.2006.03.003.

Valencia, E., and F. Rivera. 2023. Voluntary intake and digestibility of lambs fed hay of forage soybeans cv. ‘Rongai’ [Lablab purpureus (L.) Sweet]. J. Agric. Univ. P. R. 107:1–10. doi:10.46429/jaupr.v107i1.21219.

Van Soest, P. J. 1967. Development of a Comprehensive System of Feed Analyses and its Application to Forages. J. Anim. Sci. 26:119–128. doi:10.2527/jas1967.261119x.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. Cornell University Press.

Van Soest, P. J., and M. B. Hall. 2020. Cobalt (III)-EDTA dissociates and chromium (III)-EDTA is slightly more stable under in vitro reducing conditions comparable to those in the rumen. J. Dairy Sci. 103:10152–10160. doi:10.3168/jds.2020-18945.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Villalba, J., F. Provenza, A. Clemensen, R. Larsen, and J. Juhnke. 2011. Preference for diverse pastures by sheep in response to intraruminal administrations of tannins, saponins and alkaloids. GRASS FORAGE Sci. 66:224–236. doi:10.1111/j.1365-2494.2010.00779.x.

Vogel, K. P., J. F. Pedersen, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a Filter Bag System for NDF, ADF, and IVDMD Forage Analysis. Crop Sci. 39:cropsci1999.0011183X003900010042x. doi:10.2135/cropsci1999.0011183X003900010042x.

Volmer, J. G., H. McRae, and M. Morrison. 2023. The evolving role of methanogenic archaea in mammalian microbiomes. Front. Microbiol. 14. doi:10.3389/fmicb.2023.1268451.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Anim. Feed Sci. Technol. 147:116–139. doi:10.1016/j.anifeedsci.2007.09.013.

Waldo, D. R., L. W. Smith, and E. L. Cox. 1972. Model of Cellulose Disappearance from the Rumen. J. Dairy Sci. 55:125–129. doi:10.3168/jds.S0022-0302(72)85442-0.

Wanapat, M., P. Totakul, B. Viennasay, and M. Matra. 2021. Sunnhemp (Crotalaria juncea, L.) silage can enrich rumen fermentation process, microbial protein synthesis, and nitrogen utilization efficiency in beef cattle crossbreds. Trop Anim Health Prod. 53:187. doi:10.1007/s11250-021-02628-z.

Watt, L. J., L. W. Bell, B. D. Cocks, A. D. Swan, R. S. Stutz, A. Toovey, and J. De Faveri. 2021. Productivity of diverse forage brassica genotypes exceeds that of oats across multiple environments within Australia's mixed farming zone. Crop Pasture Sci. 72:393–406. doi:10.1071/CP21034.

Wina, E., S. Muetzel, and K. Becker. 2005. The Impact of Saponins or Saponin-Containing Plant Materials on Ruminant ProductionA Review. J. Agric. Food Chem. 53:8093–8105. doi:10.1021/jf048053d.

Wolin, M. J., T. L. Miller, and C. S. Stewart. 1997. Microbe-microbe interactions. In: P. N. Hobson and C. S. Stewart, editors. The Rumen Microbial Ecosystem. Springer Netherlands, Dordrecht. p. 467–491. http://link.springer.com/10.1007/978-94-009-1453-7_11

Wyse, J., S. Latif, S. Gurusinghe, J. McCormick, L. A. Weston, and C. P. Stephen. 2022a. Phytoestrogens: A Review of Their Impacts on Reproductive Physiology and Other Effects upon Grazing Livestock. Anim. 2076-2615. 12:2709. doi:10.3390/ani12192709.

Wyse, J., S. Latif, S. Gurusinghe, J. McCormick, L. A. Weston, and C. P. Stephen. 2022b. Phytoestrogens: A Review of Their Impacts on Reproductive Physiology and Other Effects upon Grazing Livestock. Animals. 12:2709. doi:10.3390/ani12192709.

Yang, Q., and G. Wang. 2024. Isoflavonoid metabolism in leguminous plants: an update and perspectives. Front. Plant Sci. 15:1368870. doi:10.3389/fpls.2024.1368870.

YILDIZ, S., S. DENİZ, F. ÖZKAN, and Ç. KALE. 2022. Forage turnip (Brassica rapa) harvested in different phases of vegetative stage andensiled with the additives of molasses and barley and the effects of additives on silagequality, in vitro digestibility, and energy content. Turk. J. Vet. Anim. Sci. 46:475–482. doi:10.55730/1300-0128.4218.

Zimdahl, R. L. 2018. Weed Classification. In: Fundamentals of Weed Science. Elsevier. p. 47–60. https://linkinghub.elsevier.com/retrieve/pii/B9780128111437000032