FEDERAL RURAL UNIVERSITY OF PERNAMBUCO
GRADUATE PROGRAM IN ANIMAL SCIENCE

JOSE DIOGENES PEREIRA NETO

SUSTAINABLE FEEDING STRATEGIES IN BEEF CATTLE SYSTEMS

RECIFE-PE

2023

JOSE DIOGENES PEREIRA NETO

SUSTAINABLE FEEDING STRATEGIES IN BEEF CATTLE SYSTEMS

Doctorate

PPGZ

2023

JOSE DIOGENES PEREIRA NETO

SUSTAINABLE FEEDING STRATEGIES IN BEEF CATTLE SYSTEMS

Dissertation presented to the Graduate Program in Animal Science of Federal Rural University of Pernambuco, required to obtain, the Doctoral degree in Animal Science.

Concentration area: Animal Science

Committee:

Advisor: Prof. PhD. Jose Carlos Batista Dubeux Junior

Co-advisor: Prof. PhD. Mercia Virginia Ferreira dos Santos

RECIFE-PE

2023

Dados Internacionais de Catalogação na Publicação
Universidade Federal Rural de Pernambuco
Sistema Integrado de Bibliotecas
Gerada automaticamente, mediante os dados fornecidos pelo(a) autor(a)

P436s Pereira Neto, Jose Diogenes Pereira Neto
SUSTAINABLE FEEDING STRATEGIES IN BEEF CATTLE SYSTEMS / Jose Diogenes Pereira Neto Pereira Neto. - 2023.
97 f.

Orientador: Jose Carlos Batista Dubeux Junior.
Coorientadora: Mercia Virginia Ferreira dos Santos.
Inclui referências.

Tese (Doutorado) - Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Zootecnia, Recife, 2023.

1. Animal performance . 2. Cattle. 3. Concentrate. 4. Intake. 5. Protein. I. Junior, Jose Carlos Batista Dubeux, orient. II. Santos, Mercia Virginia Ferreira dos, coorient. III. Título

CDD 636

FEDERAL RURAL UNIVERSITY OF PERNAMBUCO
ANIMAL SCIENCE GRADUATE PROGRAM

SUSTAINABLE FEEDING STRATEGIES IN BEEF CATTLE SYSTEMS

Thesis elaborated by

JOSE DIOGENES PEREIRA NETO

Approved in..01/.10/.2023..

COMMITTEE

Jose Carlos Batista Dubeux Junior, Professor Dr.University of Florida, U.S.(Chair)

Documento assinado digitalmente
gov.br
VALDSON JOSE DA SILVA
Data: 12/01/2023 22:01:56-0300
Verifique em https://verificador.iti.br

Valdson Jose da Silva, Professor Dr. (UFRPE)

Nicolas DiLorenzo
Digitally signed by Nicolas DiLorenzo
DN: cn=Nicolas DiLorenzo, o=IFAS-NFREC, ou=University of Florida, email=ndilorenzo@ufl.edu, c=US
Date: 2023.01.10 16:59:56 -06'00'

Nicolas Dilorenzo, Professor Dr. University of Florida, U. S.

Documento assinado digitalmente
gov.br
ERICK RODRIGO DA SILVA SANTOS
Data: 11/01/2023 16:22:47-0300
Verifique em https://verificador.iti.br

Erick Rodrigo da Silva Santos Dr. University of Alberta, Canada

Gleise M. da Silva

Gleise Medeiros da Silva, Professor Dr. University of Alberta, Canada

BIOGRAPHY

JOSE DIOGENES PEREIRA NETO, son of Carlos Fernando Pereira and Suely Ildefonso da Silva was born on November 4th 1987, in Recife-PE. Jose started his elementary school in Colegio Santa Helena in 1994, Recife. He moved to Paulista-PE and started the middle school at Escola Menino Jesus in 2002, and concluded high school, in 2006. In 2007 started college of Marketing at Faculdades Integradas Barros Melo, in Olinda-PE. In 2009, Jose changed to Animal Science course, at the Universidade Federal Rural de Pernambuco in Recife, campus SEDE. During the college, since the beginning, Jose was a volunteer in experiments on theanimal science graduate program, always supervised by Professor Dr. Francisco Fernando Ramos de Carvalho, in small ruminant projects. In 2010, he was involved in an extension project, with a scholarship of one year supervised by Prof. Dr. Ricardo Alexandre Silva Pessoa. In 2012 started in the research assistant program (PIBIC), which was for three years supervised by Prof. Dr. Francisco Fernando Ramos de Carvalho. In the first semester of 2015, Jose graduated in Animal Science, and in January started an international internship (six months) at University of Florida in Marianna-FL, supervised by Prof. Dr. Jose Carlos Batista Dubeux Junior. In August 2015, Jose started a Master program at the Universidade Federal Rural de Pernambuco in Recife-PE under the mentorship of Prof. Dr. Jose Carlos Batista Dubeux Junior and was investigating how to trace back the diet of small ruminants using stable carbon isotope, which in July of 2017 obtained a master’s degree in animal science. In March of 2019, Jose started the Doctorate in Animal Science also with mentorship of Prof. Dr. Jose Carlos Batista Dubeux Junior.

Dedico ao meu saudoso pai Carlos, pois aquele
adeus nao pude dar, por estar concluindo este trabalho.

ACKNOWLEDGMENT

I thank GOD for everything.

I would like to thank my family Bruna, and Lucas. My parents, my mother Suely. In memory of my father, Carlos, which died in September of 2022, you will be here, in this document, forever. Thank you for the patience.

I would like to thank my advisor Prof. Jose Carlos B. Dubeux Junior, great professor and an incredible a ~~a~~ man, thanks Professor for the support and friendship, I will never forget.

Thank as well as to all professors in my graduated program at UFRPE, specially to my co-advisor Prof. Mercia Virginia Ferreira dos Santos for the support during all these years.

All my friends such as Erick Santos, Gleise Medeiros, Carol Vasco, Marina Almeida, Michelle Siqueira, Hiran Siqueira, Luana Dantas, David Jaramillo, Liza Garcia, Kenneth Oduor, Priscila Cruz, and Igor Bretas. Thank you, to all the graduate students, interns, faculty (specially Prof. Nicolas DiLorenzo), and all staff of the NFREC – Marianna (specially Martin, Tessa, Tina and Gina). Additionally, Carlos Garcia, Mateus Landi and Daniele Loures for help in the tr~~a~~ial.

Universidade Federal Rural de Pernambuco and University of Florida. CAPES for the scholarship, thank you.

That’s it! Thank you everyone.

ABSTRACT

The inclusion of legumes into grasslands is positive to reduce nitrogen fertilizer inputs, improve forage productivity, and enhance forage quality for warm and cool seasons. However, there is a forage scarcity period between the warm and cool season transition, which is a challenge faced by farmers in this region. Limpograss is a warm season forage present in Florida, that has the potential to fill the gorage gap between the warm and cool seasons. The use of limpograss as silage can be a new alternative and associated with protein supplementation may improve animal performance. The objective of this dissertation was to assess herbage and animal responses of steers in grass-legume grazing systems in North Florida, and ii. to evaluate the effect of protein supplementation on forage intake, digestibility, and animal performance of heifers fed on limpograss silage-based diet. Two experiments were performed. In *experiment 1*, treatments consisted of three grazing systems replicated three times in a randomized complete block design. Treatments included GR+N that consisted of N-fertilized ‘Argentine’ bahiagrass (*Paspalum notatum Flugge*) in the warm season (112 kg N ha^{-1} yr^{-1}) and overseeded with a N-fertilized mixture (112 kg N ha^{-1} yr^{-1}) of ryegrass (*Lolium multiflorum* Lam.) and oat (*Avena sativa* L.) in the cool season. The treatment GR+CL consisted of unfertilized bahiagrass in the warm season, overseeded by a mixture of ryegrass+oat+clover (*Trifolium* sp.) fertilized with 34 kg N ha^{-1} in the cool season. The treatment GR+CL+RP consisted of the mixture of bahiagrass with ‘Ecoturf’ rhizoma peanut (RP) (*Arachis glabrata* Benth.) during the warm season, and overseeded with ryegrass+oat+clover mixture plus 34 kg N ha^{-1} in the cool season. *In experiment 2*, 24 heifers crossbred Angus and Brahman (330 ± 16 kg live weight) were blocked by initial weight and then housed in a single pen and submitted to four different treatments such as: 1) control, no supplementation and *ad libitum* access to silage of ‘Gibtuck’ limpograss; 2) 1.4 kg of a commercial 32% CP and 68% TDN (cube) supplementation and *ad libitum* access to silage of limpograss; 3) 2.8 kg of cube and *ad libitum* access to silage of limpograss; 4) 4.2 kg of cube and *ad libitum* access to silage of limpograss. In the grazing trial, the N fertilizer did not affect the crude protein concentration (CP) and in vitro digestible organic matter (IVDOM). The herbage mass did not differ among treatments in the cool-season and warm-season. The herbage accumulation in the cool season had evaluation x treatment interaction. The herbage accumulation in the warm season did not differ among treatments. The clover nitrogen derived from atmosphere (%Ndfa) was 69% and biological nitrogen fixation (BNF) was 33 kg N ha^{-1} season^{-1}. Rhizoma peanut %Ndfa was 64% and the BNF 63 kg N ha^{-1} season^{-1}. In the cool season, the average daily gain (ADG), gain per area (GPA), stocking rate, and herbage allowance did not differ across treatments. In the warm season, the inclusion of rhizome peanut improved the ADG in relation to the GR+N treatment (0.34 kg d^{-1} vs 0.12 kg d^{-1}) and the GPA (257 kg ha^{-1} vs 101 kg ha^{-1}). Overall, all the treatments presented the same ADG in the annual animal performance, however the GR+CL+RP presented a superior compared to other treatments. Integration of legumes into grazing systems, in warm and cool seasons, contributes to the development of a sustainable livestock production system, reducing 85% of the N fertilizer need. In the feeding trial, silage intake was reduced with the inclusion of 2.8 kg of Cube, but total intake increased with supplementation level. The supplement improved the organic matter digestibility, which was 574 g kg^{-1} in non-supplemented to 638 g kg^{-1} for diets supplemented with 4.2 kg of Cube. The neutral detergent fiber digestibility was affected by the treatment and reduced from 620 g kg^{-1} to 604 g kg^{-1} in the greater supplement level. The supplement promoted an increase in the intake, but reduced NDF digestibility. The inclusion of supplement improved the ADG of growing heifers, increasing from 0.04 kg d^{-1} to 0.6 kg d^{-1} with the inclusion of 4.2 kg of cube. The use of cube supplementation increased animal performance of developing heifers fed on limpograss silage.

Keywords: *Animal performance, cattle, concentrate, forage, intake, protein.*

LIST OF TABLES

CHAPTER 2

Pag

CHAPTER 3

LIST OF ABBREVIATIONS

ADG - Average daily gain
BC - Botanical composition
BNF - Biological nitrogen fixation
BW - Body weight
CP - Crude protein
DM - Dry matter
DMI - Dry matter intake
DOM: CP - Digestible organic matter: crude protein ratio
G:F: Gain to feed
GPA - Gain per area
HA - Herbage allowance
HAR - Herbage accumulation rate
HM - Herbage mass
iNDF - Indigestible neutral detergent fiber
IVDMD – in vitro dry matter digestibility
IVDOM- In vitro digestible organic matter
LW - Live weight
NDF - Neutral detergent fiber
Ndfa - Nitrogen derived from atmospheric
NDFD - Neutral detergent fiber digestibility
OM - Organic matter
PSPS - Penn State particle separation
RBC - Red blood cells
RM - Ruminal microorganisms
SSF - Super Smartfeed
TDN - Total digestible nutrients

LIST OF FIGURES

CHAPTER 2

Pag

CHAPTER 3

SUMMARY

Overview

The beef cattle operation in the Southeast United States is characterized by cow-calf systems, which typically sell calves to feedlots in other regions or retain calves in a high-quality pasture to grow and sell (BALL et al., 2015) and warm-season perennial grasses are the basis for cow–calf operations (VENDRAMINI et al., 2010). They are used by producers in livestock systems because these forages are very efficient in water use and light conversion (VENDRAMINI AND MORIEL, 2020), since this region has the potential to provide a significant growth of this kind of forages (ROUQUETTE et al., 2020).

The forages mostly planted in this region are bermudagrass (*Cynodon dactylon L.*) and bahiagrass (*Paspalum notatum* Flügge), which are warm season perennial grasses (ROUQUETTE et al., 2017). Forage legumes are not as planted as warm-season perennial grasses, but rhizoma peanut (*Arachis glabrata* Benth.) is an option for Florida and Southern Gulf Coast (JARAMILLO et al., 2018). Because of that, these forages can provide a great amount of herbage mass in the warm season, which is very important to livestock systems of Southeast U. S..

Cattle production systems relying on warm season forages, and to increase forage productivity and nutritive value, application of nitrogen (N) fertilizer is necessary (BUTLER et al., 2012). However, the high level of N application may not be economical and might cause environmental problems, including nitrate leaching and emission of fossil fuel during the manufacturing process of the fertilizer (DUBEUX et al., 2017a). The reduction of nitrogen application has showed some environmental benefits, while improving the economic return (AGYIN-BIRIKORANG et al., 2012).

The incorporation of legumes into grass pastures may provide N via biological nitrogen fixation (BNF), and this is an efficient option (SANTOS et al., 2018). The N transference is not a lonely benefit, however some legumes can improve forage nutritive value as a result of greater crude protein and digestibility (DUBEUX ; SOLLENBERGER, 2020; MULLENIX et al., 2016).

In Florida, bahiagrass has been the most planted warm-season perennial grass. In some instances, rhizoma peanut, a forage legume, has been integrated into bahiagrass systems to reduce the amount of N applied and increase forage nutritive value (GARCIA et al., 2021; JARAMILLO et al., 2021; SANTOS et al., 2018). The reduction of N fertilizer application by integrating forage legumes is an important strategy to develop sustainable livestock systems. In the cool season, clovers could add N to grazing systems. With a mild climatic condition in the winter, the southeastern region has suitable conditions to grow cool-season forages for establishment of grazing systems to beef cattle production (MULLENIX; ROUQUETTE, 2018). The mixtures between cool-season grass and cool-season legumes are a common practice in this region, and this practice have the potential to replace N fertilizers in annual cool season pastures (BUTLER et al., 2012). During the cool season, the mixtures of small grains and annual ryegrass can extend the grazing period (DUBEUX et al., 2016). The mixtures of ryegrass and clovers are positive and can provide an adequate stocking condition passing late-January to mid-February but can extend the grazing through May (MULLENIX; ROUQUETTE, 2018), which is very important to producers, since the warm-season forages are regrowing after the fall dormancy period.

Seasonality of forage production is a challenge that most of the producers face (ABREU et al., 2022), especially in late-October, which is the transition from warm to cool season in the Southeast U. S. This transition is characterized by forage seasonality, in other words, forage scarcity. This situation occurs because warm-season forages are in a dormant stage, and the cool-season forages were recently overseeded and are not ready for grazing (BLOUNT et al., 2020). Because of that, producers must find alternatives to feed the herd, and any options are used by farmers such as hay, concentrate, stockpiling, and silage (WALLAU et al., 2015). In Florida, there is a warm-season perennial grass that has the potential to bridge the Fall gap, limpograss. In late maturity the digestibility of limpograss is considerable, because decrease slowly, as well as present a great herbage production during the summer and because of that has been used as stockpiling in Florida (VENDRAMINI; ARTHINGTON, 2010). Thus, limpograss surplus during the summer can be used as silage or hay additionally of stockpilling. However, limpograss has a crude protein limitation and the supplementation is necessary to meet animal requirement (SOLLENBERGER et al., 1988).

Overall, the global objective of this study is to assess strategies to improve the livestock feeding management, reducing cost and environmental impacts by N fertilizer uses in grazing systems. Also incorporating other forages species into beef cattle operations associated with supplementation, which has the potential to warranty animal performance and providing sustainable feeding solutions throughout seasons and critical period of the southeast United States.

CHAPTER 1

LITERATURE REVIEW

1. **Beef cattle production in the Southeast United States**

In the United States, beef production is characterized by three major components cow-calf, stocker, and feedlot sectors (GALYEAN et al., 2011). The amount of cattle and calves in 2022 are 98.8 million head, where 30.4 million are consisted of beef cows and heifers that have calved (USDA, 2022) and the southeastern region is responsible for approximately 20 % of that (DROUILLARD, 2018). There are over 12 million beef cows in the humid South, including eastern Texas and Oklahoma (BALL et al., 2015). The beef cattle production in the southeast is based on cow-calf operation, and most beef calves are shipped to western states for stocker grazing and eventual feedlot system, and the remaining calves are used for replacement (VENDRAMINI; MORIEL, 2020). Four of the 10 largest cow-calf operations in the U.S. are in Florida, where the beef industry relies on pasture system, which can be native or planted and receive minimal or no feed supplements (SILVEIRA et al., 2011).

The calving season in the southeast U.S. is between fall and winter. During the fall, the cow nutritional demands are high, while the forage availability is low as well as the nutritive value (VENDRAMINI; MORIEL, 2020). Because of that, the ideal calving period can vary in the U. S. Southern region and can be determined by forage availability, since the calving period must meet the pasture dynamic (BALL et al., 2015). In subtropical regions of the world, including the extreme southeastern U.S., fall and winter is the period of seasonality or shortage forage, which is critical for livestock operations (GATES et al., 2001). This period is characterized by a transition between warm-season and cool-season, in which the warm season forages are dormant and the cool season forages has not grown yet (ROUQUETTE, 2020).

Cool season forage species are an integral component of many forage-livestock systems in the southeastern U. S. (BRINK et al., 2001). For the cool season, producers in the southeast are familiar with the practice of mixing cool-season grasses with cool-season legumes to obtain some benefits. Cool-season legumes are a feasible alternative to replace the N fertilizer in annual cool-season grass pastures, resulting in returns to producers (BUTLER et al., 2012). During the winter, the most used cool-season annual grasses in beef cattle operations of FL are oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), triticale (*Triticosecale* L.), and rye (*Secale cereale* L.) in mixtures with annual ryegrass (*Lolium multiflorum* Lam.), and the most annual legumes in the cool season include crimson (*Trifolium incarnatum* L.), white (*Trifolium repens* L.), red (*Trifolium pretense* L.), and ball (*Trifolium nigrescens* Viv.) clovers (VENDRAMINI; MORIEL, 2020).

In the southeast U.S., the pasture systems are based on warm-season perennial grasses (MULLENIX et al., 2014). The pastures are most widely composed by warm-season grasses, which because of the water use efficiency and response under full sunlight in tropical and subtropical climates results in high production compared to cool-season grasses; however, warm-season grasses tend to have a poor nutritive value (Vendramini; Moriel, 2020) and demand N fertilization (EVERS, 1985). In the summer, the most warm-season forages used in the southeastern region, are bermudagrass, bahiagrass, dallisgrass (*Paspalum dilatatum* Poir.), limpograss [*Hermarthria altissima* (Poir.) Stapf & C.E Hubb.], and stargrass *(Cynodon* spp.), with rhizoma peanut being a promising perennial forage legume (BUTLER et al., 2012). Animal performance in the warm season in the Southern region tend to decrease with the progress of the season by the reduced nutritive value (JARAMILLO et al., 2021). In bahiagrass pastures in Florida, Stewart et al. (2007) reported a greater average daily gain of heifers grazing in low intensity (0.34 kg d^{-1}) compared with high intensity (0.28 kg d^{-1}), however, gain per area increased from low to high intensity from 101 to 252 kg ha^{-1}, respectively.

1. Annual Ryegrass

Annual ryegrass, also called Italian ryegrass, is originally from southern Europe and was introduced in the United States during the colonial period, and was spread on southeast around 1930 (EVERS, 1995; LEMUS et al., 2021). Ryegrass is characterized by the morphology in which the upper surface of the leaf is ribbed, and the lower surface is shiny and has a distinct midrib (CASLER; KELLEBACH, 2020). This annual grass is an impressive present a high nutritive value that provides a great source of feed and is used worldwide for grazing, hay, and silage (EVERS, 1997; HAVILAH, 2002; LEMUS et al., 2021). It is an essential component for forage-based livestock production systems in the southern United States (LEMUS et al., 2021), and it is popularly used because it is adapted to a wide range of soil types, simple establishment, requires minimum management, presents a high forage quality and tolerates heavy grazing (EVERS, 1995; EVERS et al., 1997).

Annual ryegrass is planted in fall and grazed from fall through late spring, at which time plants head and die (BALL et al., 2015). In the southeast U.S., annual ryegrass is overseeded in warm-season perennial grass pastures, either alone or in mixtures with rye and/or clovers (ROUQUETTE et al., 2020). Mixtures of small grains such as oat, wheat, triticale, and rye with annual ryegrass provide forages during late fall as well as during winter (VENDRAMINI AND MORIEL, 2020). Annual ryegrass is responsive to N fertilization (BALL et al., 2015) and high levels of N are applied to ensure maximum biomass production (LEMUS ET AL., 2021), but is recommended to split into two to four applications during the growing season (EVERS, 1997). However, the use of N in the mixtures has some peculiarity since N increases annual ryegrass growth but can be different for clover (EVERS, 2011a). Nitrogen fertilization in clover-ryegrass mixtures are complex due to the different N requirements of the two forages (ROUQUETTE et al., 2020). Overseeding ryegrass over warm-season perennial grasses has the potential to extend the grazing season with high-quality forage, therefore reducing the extra forage and supplementation used over the winter (EVERS, 1995). This is a great advantage of this cool -season annual grass, that is used in grazing systems around U. S.

Annual ryegrass has a crude protein (CP) concentration averaging 150 to 250 g kg^{-1}, and the in vitro digestible dry matter (IVDDM) concentration ranging from 600 to 850 g kg $^{-1}$ depending on fertilization, maturity, and season of the year (VENDRAMINI; MORIEL, 2020). According to EVERS (1995), annual ryegrass has a good potential for animal performance. Mullenix et al. (2014) assessed during a 3-yr grazing trial three cool-season grasses in the southeast U.S. They reported annual ryegrass forage mass of 1,500 kg DM ha^{-1}, herbage allowance of 1.35 kg DM kg LW^{-1}, and steer average daily gain (ADG) of 1.5 kg hd^{-1} d^{-1}. Dubeux et al., (2016) reported that ryegrass mixed with oats provide a greater ADG than rye and ryegrass mixture in growing Angus steers. Mullenix et al. (2012) presented steer ADG in oat-ryegrass of 1.39 kg hd^{-1} d^{-1} and rye-ryegrass 1.13 kg hd^{-1} d^{-1} during the cool season in the southeast U. S. The association of annual ryegrass and clover blend is an excellent option and can increase forage production (LEMUS et al., 2021).

2. Clovers

Clovers are cool-season legumes, that belong to the genus *Trifolium*, which includes a total of about 250 diverse species (TAYLOR, 1985). They are an important component of pasture systems throughout the world (PEDERSON; QUESENBERRY, 1998). In the southeast U. S., the most used clovers are crimson, white, red, and ball (VENDRAMINI; MORIEL, 2020). In this region, annual clovers are seeded in the fall and mature in late spring or early summer depending on species and cultivars (EVERS, 1985). Generally, clovers can flower and produce seeds better under medium soil fertility, medium pH levels, good drainage, and soils with moderate to heavy texture (TAYLOR, 1980). Clovers also have a great nutritive value and can be used as hay or grazing pastures (BALL et al., 2015), however, it is widely used in grazing systems.

In grazing systems, clovers grow well in a mixture of cool-season grasses in southeast U. S. Annual clovers are grown with rye, wheat, or annual ryegrass and overseeded on warm-season perennial grass pastures, such as bahiagrass and bermudagrass, which are in a dormant phase (VAN KEUREN; HOVELAND, 1985). The overseeding practice can provide valuable and critical winter forage production when the warm season is not available because is dormant (ROUQUETTE, 2020).

Growing clovers and grass together is a feasible alternative because clovers can fix N and transfer a portion fixed to the grasses, reducing the need for N fertilizer inputs as a result (MORRIS et al., 1990). Clovers can also increase forage nutritive value and extend the grazing season (GEORGE et al., 2015). Clover incorporated into pastures has the potential to fix between 20 and 60 kg N ha^{-1} yr^{-1} (MORRIS et al., 1990). Ledgard et al. (2001) reported the average estimates of fixed N in white clover herbage ranged between 91 and 23 kg N ha^{-1} yr^{-1} for the 0 N and 400 N$_{\text{low stockrate}}$ treatments, respectively. Schils et al. (2000) reported a 176 kg N ha^{-1} yr^{-1} BNF of white clover in a grass mixture pasture.

The most important annual clover in U. S. agriculture systems is crimson (ROUQUETTE, 2020). Crimson clover has a scarlet flower, which is the most visible characteristic, and is sown from the middle of September until November (VAN KEUREN; HOVELAND, 1985) and it has rapid growth in the fall and early spring (KNIGHT, 1985). This clover provides the earliest forage production and excellent regrowth after defoliation (EVERS; NEWMAN, 2008). Crimson clovers IVDOM and CP concentrations from the vegetative to the bud stage range from 770 to 830 and from 158 to 207 g kg^{-1}, respectively (LLOVERAS; IGLESIAS, 2001).

Another important annual clover for southeast U.S. agriculture is ball clover, which is also used in grazing systems (BALL et al., 2015). It has smooth, egg-shaped leaflets, and small to yellowish-white flowers (SHEAFFER; EVERS, 2007). This clover makes little growth early in the season when forage yield is generally critical. Ball clover can produce herbage in a short period in late spring when typically, the crimson clover is decreasing (HOVELAND, 1960). Ball clover produces most of its growth of about a month later in spring than crimson clover, and generally produces less total forage (HANCOCK, 2008). Although ball clover presents a low yielding, it is used in grazing systems, and it is an excellent reseeder, due to its ability to flower and produce seeds (SHEAFFER; EVERS, 2007). Red clovers are a perennial forage legume, with a high herbage production fresh or conserved herbage, but cannot tolerate severe grazing (BOLLER et al., 2010; BALL et al., 2015). Typically, red clover grows well into a mixture with cool season grasses (SMITH et al., 1985). The most important advantage of clover is the mixture with cool season grasses, due to the ability to fix N_2. However, the mixture of clovers with ryegrass is used by small producers is because they can overcome the machinery limitation and use ryegrass as a carrier to plant clover (EVERS; SMITH; HOVELAND, 1997).

3. Bahiagrass

Originally from South America, bahiagrass is planted to over 1 million ha in Florida and grows in a zone from North and South Carolina to eastern Texas, occupying an estimated 2.03 million ha (HUGHES et al., 2010; NEWMAN et al., 2010; STEWART et al., 2007; WALLAU et al., 2019). In the U.S., most part of the land planted with bahiagrass is used for extensive pastures for cow-calf production (GATES et al. 2004). Bahiagrass is well adapted to sandy soils and can tolerate low soil fertility and low pH (ROUQUETT et al., 2020; VENDRAMINI; MORIEL, 2020). The propagation is by seed, but it also spreads vegetatively by rhizomes (NEWMAN et al., 2010). Bahiagrass is one of the most popular forages for livestock production in Florida (WALLAU et al., 2019), and the most planted varieties are “Argentine” and “Pensacola” (VENDRAMINI et al., 2013). It is a very reliable feed source for low-input beef cattle production systems (GATES et al., 2004). In grazing scenarios, bahiagrass could be acceptable for mature beef cows demands, but growing cattle would require supplementation (ROUQUETTE et al., 2020).

Bahiagrass provides adequate nutrition for ruminants when it is actively growing and it is very persistent when managed under continuous high grazing management (GATES et al., 2004; SOLLENBERGER et al., 1988). Bahiagrass decreases the production in early spring and autumn and decreases nutritive value during the mid-late summer (VENDRAMINI; MORIEL, 2020). According with Rouquette et al. (2020), bahiagrass becomes dominant under low-input management strategies, and when grown on low-N soils, forage protein is usually bellow 60 g kg^{-1}. Bahiagrass cultivars Argentine and Pensacola, fertilized with 60 kg N ha^{-1} yr^{-1}, present CP 132 and 129 g kg^{-1} and in vitro organic matter digestibility of 538 g kg^{-1} and 541 g kg^{-1}, respectively, as reported by (VENDRAMINI et al., 2013). The high grazing intensity improves nutritive value of bahiagrass, because eliminates the amount of dead leaf material that accumulates under low grazing intensity (ROUQUETTE et al., 2020). Under a low management intensity, Stewart Jr. et al. (2007) reported 99 g kg^{-1} of CP and 459 g kg^{-1} IVDOM for bahiagrass, with different values obtained when was applied a high intensity (140 g kg^{-1} of CP and 505 g kg^{-1} of IVDOM). As a warm season perennial grass, bahiagrass has a low quality, and N application have been used to improve yield and enhance nutritive value, but the use of a legume that could increase N in the soil pasture could be an alternative (SANTOS et al., 2018).

4. Rhizoma peanut

Rhizoma peanut is originally from South America, and it is an excellent warm-season perennial forage legume that can be used as hay and pasture (BALL et al., 2015). Rhizoma peanut is well adapted to the southern U. S., because of the high-quality, and can provide forage for grazing animals (DUBEUX et al., 2018). It is vegetatively propagated by rhizomes (Rice et al., 1995) and for this reason is called rhizoma peanut. However, its potential is limited by the slow establishment, where two years are necessary to establish a stand (BALL et al., 2015). As a warm season forage, rhizoma peanut becomes dormant during the cool season (SANTOS et al., 2021). Rhizoma peanut has the potential to be used in association with warm season grasses in the southern U. S. (JARAMILLO et al., 2018), specially with bahiagrass. Mixing grasses and legumes resulted in greater forage yield during the cool season (SANTOS et al., 2021). Overseeding rhizoma peanut during the cool season is a feasible alternative to increase forage yield per unit of land area throughout the year (SANTOS et al., 2021). Rhizoma peanut can present a CP concentration range from 130 to 180 g kg^{-1} and digestibility of leaf 600 and stem 500 g kg^{-1}, depending on maturity and leaf-to-stem ratio (NEWMAN et al., 2010). Dubeux et al. (2017) evaluated different cultivars of rhizoma peanut and observed N concentration ranging from 19.2 to 36 g kg^{-1} and in vitro organic matter digestibility from 645 to 750 g kg^{-1}. The literature provides also values of CP concentration and IVOMD such as 143 and 737 g kg^{-1} respectively by Santos et. al (2021) and a range of 177 g kg^{-1} and 705 g kg^{-1} for CP and IVOMD, respectively (GARAY et al., 2004).

Rhizoma peanut has the potential to be integrated into pasture-based livestock systems in the U.S. Gulf Coast region (MULLENIX et al., 2014) where it may provide forage yield and nutrients. This legume is well adapted to Florida and can be an excellent component in a mixture with bahiagrass. Because of the difference between them in nutritive value, rhizoma peanut can improve cattle performance (DUBEUX et al., 2018). A considerable way to establish rhizoma peanut is by establishing strips into bahiagrass pastures, that has the potential to increase forage nutritive value, decrease N fertilizer use, and improve N cycling (CASTILLO et al., 2015). Rhizoma peanut grows laterally by rhizomes, helping to establish into a grass pasture resulting in a mixed pasture (MULLENIX et al., 2014). This strategy is recommended in order to decrease the cost of establishment and facilitate the weed control (DUBEUX et al., 2018).

5. Limpograss

Limpograss is a warm-season perennial grass native from southern Africa and cultivated in tropical and subtropical regions (VENDRAMINI et al., 2019). Four limpograss accessions were introduced in Florida in 1964 because its value as livestock forage (QUESENBERRY et al., 2004; QUESENBERRY et al., 2018). This C4 plant has small and narrow leaves, and the stolons give rise to erect-growing stems that may reach heights of 1.5 m (SOLLENBERGER et al., 2020). It is vegetatively propagated, because the seed production is low, thereby the mature stems root is widely used to propagate it with a similar procedure for bermudagrass; diverse methods for mechanically harvesting, transporting, and spreading mature limpograss stems have been evaluated (QUESENBERRY et al., 2004; SOLLENBERGER et al., 2020; VENDRAMINI; MORIEL, 2020). In Florida the best period to plant limpograss is during the rainy season (June-August) (NEWMAN et al., 2009). This stoloniferous tropical grass has superior cool season growth compared to other C_4 grasses, persistence under grazing on poorly drained soils, and relatively high forage digestibility that declines at a slower rate with increasing maturity than most C_4 grasses (QUESENBERRY et al., 2004). The limpograss leaves becomes reddish or purple with maturity, but this phenomenon can be also related with cold stress (SOLLENBERGER et al., 2020). This grass tolerates light frost and has the potential to growth quickly after very low temperatures and can survive at temperatures of -10 °C and do not last more than -13°C (QUESENBERRY et al., 2004; NEWMAN, 2008).

Limpograss has been used for grazing in livestock production systems in Florida, especially as stockpiled forage for autumn and winter grazing. Because of the thick stems, the use as hay is limited (QUESENBERRY at al., 2004; SOLLENBERGER et al., 2020). Limpograss is a good option and commonly used for stockpiled forage, and variation in forage characteristics during the stockpiling period may affect the supplementation program (VENDRAMINI et al., 2019; WALLAU et al., 2020). Limpograss can be used as silage, but it is not frequent (QUESENBERRY at al., 2004). However, limpograss has been successfully conserved as silage by local producers in Florida that appreciate the excellent silage results, which is characterized by a ‘sweet’ fragrance that remains for long time, with a good storage (NEWMAN et al., 2009).

Limpograss present low CP concentration and can present approximately 100 g kg^{-1} at 5-week regrowth interval (VENDRAMINI; MORIEL, 2020). The CP concentration can vary during the season, which in spring and fall are adequate (to no animal with low nutritional requirement), otherwise in summer can drop below 70 g kg$^{–1}$ (NEWMAN et al., 2008). The CP concentration of limpograss can be observed around 34 g kg$^{–1}$, 40 g kg$^{–1}$ as well as 111 g kg$^{–1}$ and 125 g kg$^{–1}$ (SOLLENBERGER et al., 1987; VENDRAMINI et al., 2010; AGUIAR et al., 2015; WALLAU et al., 2015). Limpograss has a characteristic of slow rate of decline in digestibility with increasing of maturity (QUESENBERRY et al., 2018). It can present IVDOM concentration range around 519 to 594 g kg^{-1} in different accesses (WALLAU et al., 2015). The cultivar “Gibtuck” presented 524 g kg^{-1}, “Kenhy” 598 g kg^{-1}, and “Floralta” 544 g kg^{-1} during 8 wk of stockpiling (WALLAU et al., 2020). The cultivar “Floralta” presented 601 g kg^{-1} of in vitro true digestibility, which was used in a silage study (VENDRAMINI et al., 2010). Regardless of the use of limpograss, most of the time protein supplementation is required (NEWMAN et al., 2009).

6. Benefits of grass and legume association in grazing systems

In livestock systems, grass and legume pastures represent the most important source of forage for animal production (ANTHONY; HARRIS, 1976). In beef cattle operations, the fertilization of perennial grasses pastures is one of the most expensive practices. Mixtures of grass and legume can reduce the demand for N fertilizer and enhance the herbage productivity (Jaramillo et al., 2018), once N supply is essential to keep the sustainability of the plant production (CARLSSON; HUSS-DANELL, 2003). According to Muir et al. (2011), grass-legume mixtures are successful when both temperate grasses and legumes are adapted. The combination of two forages can be compatible, complete, or allelopathic between them (SPRINGER et al., 2007). The incorporation of legumes in grass pastures may provide several ecosystem services (DUBEUX et al., 2018) including increase in protein content (Evers, 2011b) of forages (BURTON, 1976), reduction of protein supplementation (SLEUGH et al., 2000), increase forage yield, as well as improve animal performance (CUOMO et al., 1999), both for milk production and average daily gain (MUIR et al. 2011). Also, mixing grass and legume is a lower cost practice and results in lesser N pollution in the environment compared with high N fertilizer application rates (BARNEZE et al., 2020). Therefore, besides reducing N inputs from fertilizer, grass-legume mixtures can also improve forage nutritive value (SANTOS et al., 2018), which is very important for livestock production.

The inclusion of legumes in grasses pastures can improve the in vitro dry matter digestibility (IVDMD), CP, neutral detergent fiber (NDF), and seasonal distribution of forage yield (SLEUGH et al., 2000). It contributes to increase forage nutritive value (LÜSCHER et al., 2014), and in the bahiagrass pastures, for example, the incorporation of legume may improve forage nutritive value of the pasture system (SANTOS et al., 2018). Generally, legumes are greater in CP and digestibility than grasses, and, in a mixture, the most important legume contribution to ruminant nutrition is CP (MUIR et al., 2011). Legumes are an indispensable component in agriculture that can provide 25-35 % of the worldwide protein intake (VANCE, 1998). Commonly, legumes are rich in protein, which is available for ruminants as well as soluble carbohydrates, consequently, increasing the digestibility of grasses when they are consumed in legume-grass mixtures (MUIR et al., 2014). The inclusion of Kura clover (*Trifolium ambiguum* M. Bieb.) into a 112 kg N ha^{-1} Kentucky bluegrass (*Poa pratensis* L.) pasture reduced the neutral detergent fiber from 550 g kg^{-1} to 399 g kg^{-1}, and increase the CP from 143 g kg^{-1} to 194 g kg^{-1} and this mixture reflected on nutritive value and animal performance (ZEMENCHIK et al., 2002). Seo et al. (1997) observed an increment in forage nutritive value and animal performance of legume-grass mixtures, which were perennial ryegrass associated with orchardgrass (*Dactylis glomerata L.*) and ladino clover (*Trifolium repens L.*). The grass-legume mixture reached 232 g kg^{-1} and perennial ryegrass alone 185 g kg^{-1} of CP and 518 and 551 g kg^{-1} of NDF. They also found that the average daily gain of the heifers was twice in the mixture than in the perennial ryegrass monoculture.

Grass and legume combination could be considered a viable alternative to commercial N fertilizers (ADJESIWOR et al., 2017), once warm and cool season grasslands are N limited and demands N fertilizers to reach high productivity (SLEUGH et al., 2000; KOHMANN et al., 2018). In fact, take decision on N management is a complex task for producers, and for a long-time researcher and extensionists have been working hard to provide to farmers a useful strategy to manage N in their properties (RODRIGUEZ et al., 2019). The association of perennial legumes into grass pastures represent the most logical option to reduce N commercial dependence and enhances nutrient cycling in grasslands (DUBEUX et al. 2017; GARCIA et al., 2021). Grass monocultures depend upon N fertilizer to improve the performance (SHEPARD et al., 2022). Bahiagrass requires N fertilization to increase the pasture production, and the association with legume may reduce the N fertilizer inputs (SANTOS et al., 2021). COX et al. (2017) evaluated seasonal production of grass-legume mixture and grass monoculture fertilized at 134 kg N ha $^{-1}$ in the Intermountain Region of the western U. S. The authors did not observe a decline of forage yield with grass-forage mixtures, but they found values equivalent or superior to the ones observed in fertilized grass monocultures.

According to CRÈME et al. (2016), the incorporation of legumes in forage systems has the potential to replace N fertilizers inputs because of the BNF played by legumes. So, the utilization of grass-legume mixtures could be considered beneficial with the reduction of N inputs, which according to Byrnes (1990), contributes to reduce N losses to the environment. The action of N-fixing species in the system reduces the N fertilizers and promote soil benefits (VANCE, 1998). The N fixation is an important process, it is linked to soil organic matter formation, and it is much less susceptible to losses such as volatilization, denitrification, and leaching (VANCE, 1998; GRAHAM; VANCE, 2000). Thus, BNF in agriculture systems, promoted by legume incorporation, can be responsible to reduce commercial N uses and mitigate the N losses.

7. Biological nitrogen fixation

The BNF is the process that converts atmospheric nitrogen (N_2) into ammonia (NH_3) and other nitrogenous compounds, where it is directly accessed by the plants, and it is an extremely important mechanism to sustain life on earth (SANTOS et al., 2018; DE BRUIJIN et al., 2022). Legumes plants has evolved a symbiotic relationship with specific soil bacteria and once the symbiosis is established the rhizobia fix N_2 (FERGUSON et al., 2010). This occurs into the soil and involves different hosts and microsymbionts between legumes and rhizobia belonging to the genera *Rhizobium, Bradyhizobium,* and *Azorhizobium* (BORDELEAU; PREVOST, 1994; VANCE, 1998). The rhizobia invade the roots of compatible legume plants, leading to the development of specialized root structure called nodules (FERGUSON et al., 2010). These bacteria present in the root nodules is responsible for the capture of N_2 and this process promotes the growth of the host independent of the N source from the soil or inorganic fertilizers (COOPER; SCHERER, 2012; FERGUSON et al., 2010). The nodules provide the ideal environment for BNF, whereby the rhizobia perform for their host plant in exchange for carbohydrates (FERGUSON et al., 2019). In the nodules, the bacteria differentiate into bacteroid and catalyze the reduction (FERGUSON et al., 2010). This reduction is mediated by nitrogenase, a complex bacterial enzyme that catalyzes the ATP-dependent reduction of N_2 to NH_3 (OWEN; TEZCAN, 2018). Therefore, this operation is the ability to capture an inexhaustible source of N_2 by low cost (JUWARKAR et al., 2004), and is one alternative to N fertilizers (FERGUSON et al., 2010).

Most part of the N incorporated by perennial forage legumes is from fixation, especially mixed with grasses (CARLSSON; HUSS-DANELL, 2003). The legume N fixation by legumes in mixture pastures is influenced by three factors: legume persistence and production, soil N status, and competition with grass incorporation (LEDGARD; STEELE, 1992). The increase in forage yield will increase the demand for N and the potential for N_2 fixation (EVERS, 2011). Perennial legumes have a longer growing season and have a elevate N_2 fixation than annual legumes, which are limited in N_2 fixation because of the short active growing period (EVERS et al., 2011). The N_2 fixation of legume-grass pastures throughout the world was summarized and range from 13 to 682 kg N ha^{-1} yr $^{-1}$, while the range for grazed pastures, which have been assessed for white clover pastures only, is 55 to 296 kg N ha^{-1} yr $^{-1}$ (LEDGARD; STEELE, 1992). The BNF in cultivar of rhizoma peanut represented >80% of herbage N and average 200 kg N ha^{-1} yr $^{-1}$ in legume monoculture with 56-d harvesting interval with values ranging from 123 to 280 kg N ha^{-1} yr^{-1} in pure stands (DUBEUX et al., 2017b). Also, Jaramillo et al. (2021) reported that rhizoma peanut mixed with bahiagrass planted by strips had 16 kg N ha^{-1} season^{-1} that was considered low. They explained that these results likely occurred because of the planting arrangements that resulted in a cattle selection reflected in lower herbage accumulation and BNF contribution of the legume. A pure legume stand has more potential to fix N_2 than a grass mixture because of competition for moisture, nutrients, and light (EVERS, 2011). However, the inclusion of legumes on non-fixing species pastures present benefits, for instance the reduction of cost with N fertilizers and mitigate the negative environmental impacts caused by N losses (SANTOS et al., 2018).

8. Cattle supplementation

Forages consumed by Florida beef cattle vary in quality, maturity, seasons, and management. When forage quality is low, just forage cannot provide the nutrient requirements to promote animal performance, and the protein and energy supplementation is needed (MOORE; KUNKLE, 1998). The reasons for using supplementation to animals consuming forages are correcting nutrient deficiencies, conserving forage, improving forage utilization, improving animal performance, increasing economic return, and managing animal behavior (KUNKLE et al., 1999). The way to use supplement should be clever, since that must be with a minimal input and improve the use of forage (MCCOLLUM III, 1997). In nutrient-deficient scenario, the protein supplementation is the most important priority (SILVIA-MARQUES et al., 2019) and typically required to obtain goals in animal performance for growing cattle (ADAMS et al., 2022) and protein supply do increase animal performance (DELCURTO et al., 1989), and in general way, the beef cattle requirement range from 70 to 100 g d^{-1} (MOORE et al., 1991).

Supplement can affect intake and animal performance of animal fed by low quality forages with low CP (MOORE; KUNKLE, 1995). There is a factor that is very related with the response of protein supplementation, that is the digestible organic matter and crude protein ratio (DOM:CP) or total digestible nutrient and crude protein ratio (TDN:CP) (MOORE; KUNKLE, 1995; MOORE; KUNKLE, 1998). The DOM:CP is the relationship between energy and protein of the diet, which can be balanced or unbalanced, and the threshold is between 6 to 8 (MCCOLLUM III, 1997). In cases where the forage has low CP, below 70 g kg^{-1} the protein, protein supplementation has effect on animal performance, and this relation can be greater than 8.5. In this case, the intake of forage and animal performance will increase. If this relationship is less than 8, the effect is often not observed (MOORE et al., 1991). Several studies in supplementation strategies with low-quality presented the DOM:CP in the diet (HOLDERBAUM et al., 1991, LIMA et al., 1999, NEWMAN et al., 2002; VENDRAMINI et al., 2010; WALLAU et al., 2020).

The effect of supplementation can vary and can be related to forage quality and characterization of the supplement (ADAMS et al., 2022). The effect of supplement can be additive, when the forage intake did not change with the increase of supplementation, and substitutive when the forage decreases with the inclusion of supplementation (MCCOLLUM III, 1997). However, according to Moore and Kunkle (1995), there is a combined effect, that is the combination of the two effects observed in the supplement uses, which is noticed by the voluntary forage intake depression, but with the improvement of the total intake. It is possible to reach desirable results using supplement strategies (BEATY et al., 1994) maximized forage intake and animal performance. Vendramini and Arthington (2010) found great increase on average daily gain of beef heifers on stockpiled limpograss pastures supplemented with concentrate. To meet the animal requirements and to overcome the nutrient requirements is not easy because of forage conditions and environmental issues, but the most flexible factor to count in this scenario is the supplement inputs (HERSOM, 2011). The use of low-quality forages has some limitation, and the use of strategies to change this reality is accessible and must be understandable since the only use of supplementation do not solve the problem and can provide results that is not expected, in case of excess or scarcity.

9. REFERENCES

Abreu, D., Dubeux, J. C. B., Queiroz, L. D., Jaramillo, D., Da Silva Santos, E. R., van Cleef, F., Vela-Garcia, C., DiLorenzo, N., & Ruiz-Moreno, M. (2022). Supplementation of Molasses-Based Liquid Feed for Cattle Fed on Limpograss Hay. *Animals*, *12*(17), 2227. https://doi.org/10.3390/ani12172227

Adams, J. M., Robe, J., Grigsby, Z., Rathert-Williams, A., Major, M., Lalman, D. L., Foote, A. P., Tedeschi, L. O., & Beck, P. A. (2022). Effects of supplementation rate of an extruded dried distillers' grains cube fed to growing heifers on voluntary intake and digestibility of bermudagrass hay. *Journal of Animal Science*, *100*(5), skac097. https://doi.org/10.1093/jas/skac097

Adjesiwor, A. T., Islam, M. A., Zheljazkov, V. D., Ritten, J. P., & Garcia y Garcia, A. (2017). Grass-Legume Seed Mass Ratios and Nitrogen Rates Affect Forage Accumulation, Nutritive Value, and Profitability. *Crop Science*, *57*(5), 2852–2864. https://doi.org/10.2135/cropsci2016.09.0776

Aguiar, A. D., Vendramini, J. M. B., Arthington, J. D., Sollenberger, L. E., Caputti, G., Sanchez, J. M. D., Cunha, O. F. R., & da Silva, W. L. (2015). Limited creep-feeding supplementation effects on performance of beef cows and calves grazing limpograss pastures. *Livestock Science*, *180*, 129–133. https://doi.org/10.1016/j.livsci.2015.08.008

Agyin-Birikorang, S., Newman, Y. C., Obour, A. K., & Kasozi, G. N. (2012). Agro-ecological nitrogen management in soils vulnerable to nitrate leaching: A case study in the Lower Suwannee Watershed. *Nutrient Cycling in Agroecosystems*, *92*(1), 91–105. https://doi.org/10.1007/s10705-011-9474-9

Anthony, W. B., & Harris, R. R. (2015). Effects of Legumes on Performance of Grazing Animals. In C. S. Hoveland, W. E. Knight, & G. C. Marten (Eds.), *ASA Special Publications* (pp. 73–84). American Society of Agronomy, Crop Science Society of America. https://doi.org/10.2134/asaspecpub28.c4

Asem-Hiablie, S., Rotz, C. A., Stout, R., & Place, S. (2018). Management characteristics of beef cattle production in the eastern United States. *The Professional Animal Scientist*, *34*(4), 311–325. https://doi.org/10.15232/pas.2018-01728

Ball, D. M., Hoveland, C. S., & Lacefield, G. D. (2015). Southern Forages: Modern Concepts fo r Forage Crop Management.

Barneze, A. S., Whitaker, J., McNamara, N. P., & Ostle, N. J. (2020). Legumes increase grassland productivity with no effect on nitrous oxide emissions. *Plant and Soil, 446*(1–2), 163–177. https://doi.org/10.1007/s11104-019-04338-w

Beaty, J. L., Cochran, R. C., Lintzenich, B. A., Vanzant, E. S., Morrill, J. L., Brandt, R. T., & Johnson, D. E. (1994). Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. *Journal of Animal Science*, *72*(9), 2475–2486. https://doi.org/10.2527/1994.7292475x

Blount, A. R., Wallau, M., Rios, E., Vendramini, J. M. B., Dubeux, J. C. B., Babar, A., Mulvaney, M., & Quesenberry, K. H. (2020). *2020 Cool-Season Forage Variety Recommendations for Florida*. 6.

Boller, B., Posselt, U. K., & Veronesi, F. (Eds.). (2010). *Fodder Crops and Amenity Grasses*. Springer New York. https://doi.org/10.1007/978-1-4419-0760-8

Bordeleau, L. M., & Prévost, D. (1994). Nodulation and nitrogen fixation in extreme environments. *Plant and soil*, *161*(1), 115-125.

Brink, G. E., Pederson, G. A., Sistani, K. R., & Fairbrother, T. E. (2001). Uptake of Selected Nutrients by Temperate Grasses and Legumes. *Agronomy Journal, 93*(4), 887–890. https://doi.org/10.2134/agronj2001.934887x

Bruijn, F. J. de, & Hungria, M. (2022). Biological nitrogen fixation. *Good Microbes in Medicine, Food Production, Biotechnology, Bioremediation, and Agriculture*, 466-475.

Butler, T. J., Biermacher, J. T., Kering, M. K., & Interrante, S. M. (2012). Production and Economics of Grazing Steers on Rye-Annual Ryegrass with Legumes or Fertilized with Nitrogen. *Crop Science*, *52*(4), 1931–1939. https://doi.org/10.2135/cropsci2011.11.0611

Byrnes, B. H. (1990). Environmental effects of N fertilizer use ? An overview. *Fertilizer Research*, *26*(1–3), 209–215. https://doi.org/10.1007/BF01048758

Casler, M. D., Kallenbach, R. L., & Brink, G. E. (2020). Cool-Season Grasses for Humid Areas. *Forages: The Science of Grassland Agriculture, 2*, 297-311.

Carlsson, G., & Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. *Plant and soil*, *253*(2), 353-372.

Castillo, M. S., Sollenberger, L. E., Mullenix, M. K., Blount, A. R., Williams, M. J., & Mackowiak, C. L. (2015). Grazing Management Affects Establishment Performance of Rhizoma Peanut Strip Planted into Bahiagrass Pasture. *Crop Science*, *55*(5), 2384–2389. https://doi.org/10.2135/cropsci2015.02.0090

Cooper JE, Scherer HW (2012) Nitrogen fixation. In: Marschner H (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, Cambridge, Massachusetts, pp 389–408

Cox, S., Peel, M. D., Creech, J. E., Waldron, B. L., Eun, J.-S., Zobell, D. R., Miller, R. L., & Snyder, D. L. (2017). Forage Production of Grass-Legume Binary Mixtures on Intermountain Western USA Irrigated Pastures. *Crop Science*, *57*(3), 1742–1753. https://doi.org/10.2135/cropsci2016.04.0235

Crème, A., Rumpel, C., Gastal, F., de la Luz Mora Gil, M., & Chabbi, A. (2016). Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. *Plant and Soil*, *402*(1–2), 117–128. https://doi.org/10.1007/s11104-015-2740-x

Cuomo, G. J., Johnson, D. G., Forcella, F., Rudstrom, M. V., Lemme, G. D., & Martin, N. P. (1999). Pasture Renovation and Grazing Management Impacts on Cool-Season Grass Pastures. *Journal of Production Agriculture*, *12*(4), 564–569. https://doi.org/10.2134/jpa1999.0564

DelCurto, T. (1989). *Protein and energy supplementation of beef cattle grazing dormant, tallgrass prairie forage* (Order No. 9016516). Available from ProQuest Dissertations & Theses Global. (303793682). Retrieved from https://login.lp.hscl.ufl.edu/login?url=https://www.proquest.com/dissertations-theses/protein-energy-supplementation-beef-cattle/docview/303793682/se-2

Drouillard, J. S. (2018). Current situation and future trends for beef production in the United States of America—A review. *Asian-Australasian Journal of Animal Sciences*, *31*(7), 1007–1016. https://doi.org/10.5713/ajas.18.0428

Dubeux, J. C. B., Blount, A. R. S., Mackowiak, C., Santos, E. R. S., Pereira Neto, J. D., Riveros, U., Garcia, L., Jaramillo, D. M., & Ruiz-Moreno, M. (2017a). Biological N $_2$ Fixation, Belowground Responses, and Forage Potential of Rhizoma Peanut Cultivars. *Crop Science*, *57*(2), 1027–1038. https://doi.org/10.2135/cropsci2016.09.0810

Dubeux, J. C. B., Blount, A. R. S., Mackowiak, C., Santos, E. R. S., Pereira Neto, J. D., Riveros, U., Garcia, L., Jaramillo, D. M., & Ruiz-Moreno, M. (2017b). Biological N $_2$ Fixation, Belowground Responses, and Forage Potential of Rhizoma Peanut Cultivars. *Crop Science*, *57*(2), 1027–1038. https://doi.org/10.2135/cropsci2016.09.0810

Dubeux, J. C. B., DiLorenzo, N., Blount, A., Mackowiak, C., Santos, E. R. S., Silva, H. M. S., Ruiz-Moreno, M., & Schulmeister, T. (2016). Animal Performance and Pasture Characteristics on Cool-Season Annual Grass Mixtures in North Florida. *Crop Science*, *56*(5), 2841–2852. https://doi.org/10.2135/cropsci2016.03.0141

Dubeux, J. C. B., & Sollenberger, L. E. (2020). Nutrient cycling in grazed pastures. In *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 59–75). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00004-9

Dubeux, J. C. B., Sollenberger, L. E., Vendramini, J., Wallau, M. O., Blount, A. R. S., Garcia-Jimenez, L., Santos, E., & Jaramillo, D. (2018). Strip-planting Rhizoma Peanut into Grazing Systems. *EDIS*, *2018*(4). https://doi.org/10.32473/edis-ag421-2018

Evers, G. W. (1985). Forage and Nitrogen Contributions of Arrowleaf and Subterranean Clovers Overseeded on Bermudagrass and Bahiagrass ^{1}. *Agronomy Journal*, *77*(6), 960–963. https://doi.org/10.2134/agronj1985.00021962007700060030x

Evers, G. W., Smith, G. R., & Hoveland, C. S. (1997). Ecology and Production of Annual Ryegrass. In F. M. Rouquette & L. R. Nelson (Eds.), *CSSA Special Publications* (pp. 29–43). Crop Science Society of America. https://doi.org/10.2135/cssaspecpub24.c3

Evers, G. W., & Newman, Y. C. (2008). Arrowleaf, Crimson, Rose, and Subterranean Clover Growth with and without defoliation in the Southeastern United States. *Agronomy Journal*, *100*(1), 221–230. https://doi.org/10.2134/agronj2007.0067

Evers, G. (2011a). The Interaction of Annual Ryegrass and Nitrogen on Arrowleaf Clover in the Southeastern United States. *Crop Science*, *51*(3), 1353–1360. https://doi.org/10.2135/cropsci2010.02.0049

Evers, G. W. (2011b). Forage Legumes: Forage Quality, Fixed Nitrogen, or Both. *Crop Science*, *51*(2), 403–409. https://doi.org/10.2135/cropsci2010.06.0380

Ferguson, B. J., Indrasumunar, A., Hayashi, S., Lin, M.-H., Lin, Y.-H., Reid, D. E., & Gresshoff, P. M. (2010). Molecular Analysis of Legume Nodule Development and Autoregulation. *Journal of Integrative Plant Biology*, *52*(1), 61–76. https://doi.org/10.1111/j.1744-7909.2010.00899.x

Ferguson, B. J., Mens, C., Hastwell, A. H., Zhang, M., Su, H., Jones, C. H., Chu, X., & Gresshoff, P. M. (2019). Legume nodulation: The host controls the party. *Plant, Cell & Environment*, *42*(1), 41–51. https://doi.org/10.1111/pce.13348

Galyean, M. L., Ponce, C., & Schutz, J. (2011). The future of beef production in North America. *Animal Frontiers*, *1*(2), 29–36. https://doi.org/10.2527/af.2011-0013

Carlsson, G., & Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. *Plant and soil*, *253*(2), 353-372.

Garay, A. H., Sollenberger, L. E., Staples, C. R., & Pedreira, C. G. S. (2004). 'Florigraze' and 'Arbrook' Rhizoma Peanut as Pasture for Growing Holstein Heifers. *Crop Science, 44*(4), 1355–1360. https://doi.org/10.2135/cropsci2004.1355

Gates, R. N., Mislevy, P., & Martin, F. G. (2001). Herbage Accumulation of Three Bahiagrass Populations during the Cool Season. *Agronomy Journal*, *93*(1), 112–117. https://doi.org/10.2134/agronj2001.931112x

Garcia, L., Dubeux Jr, J. C., Sollenberger, L. E., Vendramini, J. M., DiLorenzo, N., Santos, E. R., ... & Ruiz-Moreno, M. (2021). Nutrient excretion from cattle grazing nitrogen-fertilized grass or grass–legume pastures. *Agronomy Journal*, *113*(4), 3110-3123.

George, J. R., Blanchet, K. M., & Gettle, R. M. (2015). Growing Legumes in Mixtures with Warm-Season Grasses. In K. J. Moore & B. E. Anderson (Eds.), *CSSA Special Publications* (pp. 67–82). Crop Science Society of America and American Society of Agronomy. https://doi.org/10.2135/cssaspecpub30.c5

Graham, P. H., & Vance, C. P. (2000). Nitrogen fixation in perspective: an overview of research and extension needs. *Field crops research, 65*(2-3), 93-106

Hancock, D. W., Hicks, R., Morgan, S. P., & Franks, R. W. (2008). Georgia forages: legume species. available in Microsoft Word - B1347-Georgia Forages Legume Species.doc accessed (12/30/2022)

Havilah. (n.d.). Forages and Pastures: Annual Forage and Pasture Crops—Species and Varieties. In *Reference Module in Food Science*. https://doi.org/10.1016/B978-0-08-100596-5.21855-6

Hersom, M. (2007). Basic Nutrient Requirements of Beef Cows: AN190/AN190, 10/2007. *EDIS, 2007*(20). https://doi.org/10.32473/edis-an190-2007

Holderbaum, J. F., Sollenberger, L. E., Moore, J. E., Kunkle, W. E., Bates, D. B., & Hammond, A. C. (1991). Protein Supplementation of Steers Grazing Limpograss Pasture. *Journal of Production Agriculture*, *4*(3), 437–441. https://doi.org/10.2134/jpa1991.0437

Hoveland, C. S. (1960). Ball clover. accessed 0355LEAF.pdf (auburn.edu)

Hughes, A. L., Hersom, M. J., Vendramini, J. M. B., Thrift, T. A., & Yelich, J. V. (2010). Comparison of Forage Sampling Method to Determine Nutritive Value of Bahiagrass Pastures. *The Professional Animal Scientist*, *26*(5), 504–510. https://doi.org/10.15232/S1080-7446(15)30638-0

Jaramillo, D. M., Dubeux, J. C. B., Mackowiak, C., Sollenberger, L. E., DiLorenzo, N., Rowland, D. L., Blount, A. R. S., Santos, E. R. S., Garcia, L., & Ruiz-Moreno, M. (2018). Annual and Perennial Peanut Mixed with 'Pensacola' Bahiagrass in North Florida. *Crop Science*, *58*(2), 982–992. https://doi.org/10.2135/cropsci2017.09.0542

Jaramillo, D. M., Dubeux, J. C., Sollenberger, L. E., Vendramini, J. M., Mackowiak, C., DiLorenzo, N., ... & Ruiz-Moreno, M. (2021). Water footprint, herbage, and livestock responses for nitrogen-fertilized grass and grass–legume grazing systems. *Crop Science*, *61*(5), 3844-3858.

Juwarkar A.S., Asha Juwarkar, P. Khanna, VI.7 - Use of selected waste materials and biofertilizers for industrial solid waste reclamation,Editor(s): Irena Twardowska,Waste Management Series,Elsevier,Volume 4,2004,Pages 911-948

Knight, W. E. (1985). Crimson clover. *Clover science and technology*, *25*, 491-502.

Kohmann, M. M., Sollenberger, L. E., Dubeux, J. C. B., Silveira, M. L., Moreno, L. S. B., Silva, L. S., & Aryal, P. (2018). Nitrogen Fertilization and Proportion of Legume Affect Litter Decomposition and Nutrient Return in Grass Pastures. *Crop Science*, *58*(5), 2138–2148. https://doi.org/10.2135/cropsci2018.01.0028

Ledgard, S. F., & Steele, K. W. (1992). Biological nitrogen fixation in mixed legume/grass pastures. *Plant and soil*, *141*(1), 137-153.

Lemus, R., White, J. A., & Morrison, J. I. (2021). Impact of cool-season legume interseedings or timed nitrogen applications on yield and nutritive value of annual ryegrass. *Crop, Forage & Turfgrass Management*, *7*(1). https://doi.org/10.1002/cft2.20090

Lima, G. F. C, Sollenberger, L. E., Kunkle, W. E., Moore, J. E., & Hammond, A. C. (1999). Nitrogen Fertilization and Supplementation Effects on Performance of Beef Heifers Grazing Limpograss. *Crop Science*, *39*(6), 1853–1858. https://doi.org/10.2135/cropsci1999.3961853x

Lloveras, J., & Iglesias, I. (2001). Morphological development and forage quality changes in crimson clover (Trifolium incarnatum L.). *Grass and Forage Science*, *56*(4), 395-404.

Lüscher, A., Mueller-Harvey, I., Soussana, J. F., Rees, R. M., & Peyraud, J. L. (2014). Potential of legume-based grassland–livestock systems in Europe: a review. *Grass and forage science*, *69*(2), 206-228.

McCollum III,. (1997). *Supplementation Strategies for Beef Cattle*. https://hdl.handle.net/1969.1/87802

Moore, J. E., Kunkle, W. E., & Brown, W. F. (1991). Forage quality and the need for protein and energy supplements. *Florida Beef Cattle Short Course*, *40*.

Moore, J. E., & Kunkle, W. E. (1995). Improving forage supplementation programs for beef cattle. In Proceedings of the 6th Annual Florida Ruminant Nutrition Symposium (pp. 65–74). Gainesville: University of Florida.

Moore, J. E., & Kunkle, W. E. (1998). *Balancing Protein and Energy in Forages*. 8.

Morris, D. R., Weaver, R. W., Smith, G. R., & Rouquette, F. M. (1990). Nitrogen transfer from arrowleaf clover to ryegrass in field plantings. *Plant and Soil*, *128*(2), 293–297. https://doi.org/10.1007/BF00011122

Muir, J. P., Pitman, W. D., & Foster, J. L. (2011). Sustainable, low-input, warm-season, grass–legume grassland mixtures: Mission (nearly) impossible?. Grass and Forage Science, 66(3), 301-315.

Muir, J. P., Pitman, W. D., Dubeux, J. C., & Foster, J. L. (2014). The future of warm-season, tropical and subtropical forage legumes in sustainable pastures and rangelands. *African Journal of Range & Forage Science*, *31*(3), 187–198. https://doi.org/10.2989/10220119.2014.884165

Mullenix, M. K., Bungenstab, E. J., Lin, J. C., Gamble, B. E., & Muntifering, R. B. (2012). CASE STUDY: Productivity, quality characteristics, and beef cattle performance from cool-season annual forage mixtures. *The Professional Animal Scientist*, *28*(3), 379–386. https://doi.org/10.15232/S1080-7446(15)30371-5

Mullenix, M. K., & Rouquette, F. M. (2018). Review: Cool-season annual grasses or grass–clover management options for extending the fall–winter–early spring grazing season for beef cattle. *The Professional Animal Scientist*, *34*(3), 231–239. https://doi.org/10.15232/pas.2017-01714

Mullenix, M. K., Sollenberger, L. E., Blount, A. R., Vendramini, J. M. B., Silveira, M. L., & Castillo, M. S. (2014). Growth Habit of Rhizoma Peanut Affects Establishment and Spread when Strip Planted in Bahiagrass Pastures. *Crop Science*, *54*(6), 2886–2892. https://doi.org/10.2135/cropsci2014.03.0254

Mullenix, M. K., Sollenberger, L. E., Wallau, M. O., Blount, A. R., Vendramini, J. M. B., & Silveira, M. L. (2016). Herbage Accumulation, Nutritive Value, and Persistence Responses of Rhizoma Peanut Cultivars and Germplasm to Grazing Management. *Crop Science*, *56*(2), 907–915. https://doi.org/10.2135/cropsci2015.08.0507

Newman, Y. C., Mackowiak, C. L., Blount, A. R., & Ferrell, J. (2009). Perennial Peanut: A Quick Reference: SS-AGR-319/AG329, 3/2009. *EDIS*, *2009*(2). https://doi.org/10.32473/edis-ag329-2009

Newman, Y. C., Sollenberger, L. E., Kunkle, W. E., & Chambliss, C. G. (2002). Canopy Height and Nitrogen Supplementation Effects on Performance of Heifers Grazing Limpograss. *Agronomy Journal*, *94*(6), 1375–1380. https://doi.org/10.2134/agronj2002.1375

Newman, Y., Vendramini, J., & Blount, A. (2010). Bahiagrass (Paspalum notatum): Overview and Management: SS-AGR-332/AG342, 5/2010. *EDIS*, *2010*(4). https://doi.org/10.32473/edis-ag342-2010

Owens, C. P., & Tezcan, F. A. (2018). Conformationally gated electron transfer in nitrogenase. Isolation, purification, and characterization of nitrogenase from Gluconacetobacter diazotrophicus. In *Methods in enzymology* (Vol. 599, pp. 355-386). Academic Press.

Pederson, G. A., & Quesenberry, K. H. (1998). Clovers and other forage legumes. *Plant and nematode interactions*, *36*, 399-425.

Quesenberry, K.H., L.E. Sollenberger, and Y.C. Newman. 2004. Limpograss. In: L.E. Moser, et al., editors, Warm-season (C4) grass monograph. ASA, CSSA, SSSA, Madison, WI. p. 809–832.

Quesenberry, K. H., Sollenberger, L. E., Vendramini, J. M. B., Wallau, M. O., Blount, A. R., & Acuña, C. A. (2018). Registration of 'Kenhy' and 'Gibtuck' Limpograss Hybrids. *Journal of Plant Registrations*, *12*(1), 19–24. https://doi.org/10.3198/jpr2017.03.0012crc

Rodriguez, D. G. P., Bullock, D. S., & Boerngen, M. A. (2019). The origins, implications, and consequences of yield-based nitrogen fertilizer management. *Agronomy Journal, 111*(2), 725-735.

Rouquette Jr, F. M. (2017). Invited Review: Management strategies for intensive, sustainable cow-calf production systems in the southeastern United States: Bermudagrass pastures overseeded with cool-season annual grasses and legumes. *The Professional Animal Scientist*, *33*(3), 297-309.

Rouquette, M., Corriher-Olson, V., & Smith, G. R. (2020). 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* (pp. 123–187). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00007-4

Santos, E. R. S., Dubeux, J. C. B., Mackowiak, C. L., Blount, A. R. S., Sollenberger, L. E., Jaramillo, D. M., Garcia, L., Abreu, D. S., Souza, R. T., & Ruiz-Moreno, M. (2021). Herbage responses and nitrogen agronomic efficiency of bermudagrass–legume mixtures. *Crop Science*, *61*(5), 3815–3829. https://doi.org/10.1002/csc2.20552

Santos, E. R. S., Dubeux, J. C. B., Sollenberger, L. E., Blount, A. R. S., Mackowiak, C., DiLorenzo, N., Jaramillo, D. M., Garcia, L., Pereira, T. P., & Ruiz-Moreno, M. (2018). Herbage Responses and Biological N_2 Fixation of Bahiagrass and Rhizoma Peanut Monocultures Compared with their Binary Mixtures. *Crop Science*, *58*(5), 2149–2163. https://doi.org/10.2135/cropsci2018.02.0128

Schils, R. L. M., Boxem, T. J., Jagtenberg, C. J., & Verboon, M. C. (2000). The performance of a white clover based dairy system in comparison with a grass/fertiliser-N system. II. Animal production, economics and environment. *NJAS-Wageningen Journal of Life Sciences, 48*(3), 305-318.

Seo, S., Lee, J. K., Shin, D. E., & Chung, E. S. (1997). Effects of grass/legume pasture on forage production, forage nutritive values and live weight gain of the grazing heifer. *Asian-Australasian Journal of Animal Sciences*, *10*(3), 289-292.

Shepard, E. M., Sollenberger, L. E., Kohmann, M. M., da Silva, L. S., Harling, J. F., Dubeux, J. C. B., & Vendramini, J. M. B. (2022). Establishing rhizoma peanut–bahiagrass mixtures. *Agrosystems, Geosciences & Environment*, *5*(3). https://doi.org/10.1002/agg2.20285

Silva-Marques, R. P., Zervoudakis, J. T., Nakazato, L., Hatamoto-Zervoudakis, L. K., da Silva Cabral, L., do Nascimento Matos, N. B., da Silva, M. I. L., & Feliciano, A. L. (2019). Ruminal Microbial Populations and Fermentation Characteristics in Beef Cattle Grazing Tropical Forage in Dry Season and Supplemented with Different Protein Levels. *Current Microbiology*, *76*(3), 270–278. https://doi.org/10.1007/s00284-019-01631-w

Silveira, M. L., Obour, A. K., Arthington, J., & Sollenberger, L. E. (2011). The cow-calf industry and water quality in South Florida, USA: A review. *Nutrient Cycling in Agroecosystems*, *89*(3), 439–452. https://doi.org/10.1007/s10705-010-9407-z

Sleugh, B., Moore, K. J., George, J. R., & Brummer, E. C. (2000). Binary Legume–Grass Mixtures Improve Forage Yield, Quality, and Seasonal Distribution. *Agronomy Journal*, *92*(1), 24–29. https://doi.org/10.2134/agronj2000.92124x

Smith, A. P., Christie, K. M., Rawnsley, R. P., & Eckard, R. J. (2018). Fertiliser strategies for improving nitrogen use efficiency in grazed dairy pastures. *Agricultural Systems*, *165*, 274–282. https://doi.org/10.1016/j.agsy.2018.06.017

Sollenberger, L. E., Ocumpaugh, W. R., Euclides, V. P. B., Moore, J. E., Quesenberry, K. H., & Jones, C. S. (1988). Animal Performance on Continuously Stocked ‘Pensacola’ Bahiagrass and ‘Floralta’ Limpograss Pastures. *Journal of Production Agriculture*, *1*(3), 216–220. https://doi.org/10.2134/jpa1988.0216

Sollenberger, L. E., Vendramini, J. M., Pedreira, C. G., & Rios, E. F. (2020). Warm-Season Grasses for Humid Areas. *Forages: The Science of Grassland Agriculture*, *2*, 331-345.

Springer, T. L., Gillen, R. L., & McNew, R. W. (2007). Combining Ability of Binary Mixtures of Introduced, Cool- and Warm-Season Grasses and Legumes. *Crop Science*, *47*(6), 2540–2546. https://doi.org/10.2135/cropsci2006.12.0773

Stewart, R. L., Sollenberger, L. E., Dubeux, J. C. B., Vendramini, J. M. B., Interrante, S. M., & Newman, Y. C. (2007). Herbage and Animal Responses to Management Intensity of Continuously Stocked Bahiagrass Pastures. *Agronomy Journal*, *99*(1), 107–112. https://doi.org/10.2134/agronj2006.0167

USDA Economic Research Service. Livestock and meat dome- stic data: Livestock and poultry slaughter. United States Depart- ment of Agriculture; c2018 [cited 2022 Sep 9]. Available from: http://www.ers.usda.gov

Vance, C. P. (1998). Legume symbiotic nitrogen fixation: agronomic aspects. In *The rhizobiaceae* (pp. 509-530). Springer, Dordrecht.

Van Keuren, R. W., & Hoveland, C. S. (2015). Clover Management and Utilization. In N. L. Taylor (Ed.), *Agronomy Monographs* (pp. 325–354). American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. https://doi.org/10.2134/agronmonogr25.c12

Vendramini, J. M. B., & Arthington, J. D. (2010). Supplementation Strategies Effects on Performance of Beef Heifers Grazing Stockpiled Pastures. *Agronomy Journal*, *102*(1), 112–117. https://doi.org/10.2134/agronj2009.0207

Vendramini, J. M. B., Sollenberger, L. E., de Oliveira, F. C. L., Herling, V. R., Gomes, V. C., Sanchez, J. M. D., & Yarborough, J. K. (2019). Herbage Characteristics of Continuously

Stocked Limpograss Cultivars under Stockpiling Management. *Crop Science*, *59*(6), 2886–2892. https://doi.org/10.2135/cropsci2019.05.0299

Vendramini, J., & Moriel, P. (2020). Management of forages and pastures in Lower-South: I-10 Corridor. In *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 101–122). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00006-2

Wallau, M. O., Sollenberger, L. E., Vendramini, J. M. B., Mullenix, M. K., Quesenberry, K. H., Gomide, C. A. M., Costa e Silva, V., & DiLorenzo, N. (2015). Herbage Accumulation and Nutritive Value of Limpograss Breeding Lines Under Stockpiling Management. *Crop Science*, *55*(5), 2377–2383. https://doi.org/10.2135/cropsci2014.11.0797

Wallau, M. O., Vendramini, J. M. B., Sollenberger, L. E., Santen, E., Aguiar, A. D., & Cunha, O. F. R. (2020). In situ dry matter and crude protein disappearance dynamics in stockpiled limpograss. *Crop Science*, *60*(4), 2159–2166. https://doi.org/10.1002/csc2.20144

Wallau, M., Vendramini, J., Dubeux, J., & Blount, A. (2019). Bahiagrass (Paspalum notatum Flueggé): Overview and Pasture Management. *EDIS*, *2019*(4), 10. https://doi.org/10.32473/edis-ag342-2019

Zemenchik, R. A., Albrecht, K. A., & Shaver, R. D. (2002). Improved Nutritive Value of Kura Clover– and Birdsfoot Trefoil–Grass Mixtures Compared with Grass Monocultures. *Agronomy Journal*, *94*(5), 1131–1138. https://doi.org/10.2134/agronj2002.1131

CHAPTER 2

HERBAGE RESPONSES AND ANIMAL PERFORMANCE OF NITROGEN-FERTILIZED GRASS AND GRASS-LEGUME GRAZING SYSTEMS

ABSTRACT

The inclusion of forage legumes as sustainable option in grazing systems may reduce N fertilizer inputs and increase animal performance. This study evaluated herbage responses and animal performance of steers in contrasting grazing systems in North Florida during the cool and warm seasons of 2020 and 2021. Treatments were three grazing systems: 1. Grass+N that consisted of N-fertilized bahiagrass (*Paspalum notatum Flugge*) in the warm season (112 kg N ha^{-1}) and overseeded with a N-fertilized mixture of annual ryegrass (*Lolium multiflorum* Lam.) and oat (*Avena sativa* L.) in the cool season 112 kg N ha^{-1}; total annual input of N fertilizer = 224 kg N ha^{-1}); 2. Grass+Clover was unfertilized bahiagrass in the warm season, overseeded by a mixture of annual ryegrass+oat+clover (*Trifolium* spp.) fertilized with 34 kg N ha^{-1} in the cool season; 3. Grass+Clover+RP consisted of the mixture of bahiagrass with 'Ecoturf' rhizoma peanut (*Arachis glabrata* Benth.) during the warm season, overseeded with annual ryegrass+oat+clover mixture plus 34 kg N ha^{-1} in the cool season. Treatments were replicated three times in a randomized complete block design. The herbage mass of cool-season and warm-season did not differ among treatments. Herbage accumulation in cool season presented a treatment x evaluation interaction. In the warm season, the herbage accumulation did not differ among treatments and presented an evaluation effect. The N fertilizer did not affect the crude protein concentration (CP) and in vitro digestible organic matter (IVDOM) in both seasons. The clover N derived from atmosphere (%Ndfa) was 69% and biological nitrogen fixation (BNF) was 33 kg N ha^{-1} season^{-1}. Rhizoma peanut %Ndfa was 64% and the BNF 63 kg N ha^{-1} season^{-1}. The average daily gain (ADG), gain per area (GPA), stocking rate and herbage allowance in the cool season did not differ across treatments and averaged 0.87 kg d^{-1}, 308 kg ha^{-1}, 2.69 AU ha^{-1}, and 1.06 kg DM kg^{-1} BW, respectively. In the warm season, rhizoma peanut improved the ADG (0.34 kg d^{-1} vs. 0.12 kg d^{-1} for the N-fertilized bahiagrass). During the summer, GPA of rhizoma peanut+bahiagrass pastures was superior to N-fertilized bahiagrass pasture (257 kg ha^{-1} vs. 101 kg ha^{-1}). The stocking rate and herbage allowance were similar for all the treatments and averaged 3.2 AU ha^{-1} and 0.99 kg DM kg^{-1} BW, respectively. Annual cattle performance, ADG, and GPA was not different among treatments. The ADG with the inclusion of GR+CL+RP was 0.6 kg d^{-1} and the GR+N was 0.5 kg d^{-1}. The GPA in the GR+CL+RP was 550 kg ha^{-1} and the GR+N was 447 kg ha^{-1}. Integration of legumes into grazing systems in the warm and cool seasons contributes to the development of a sustainable grazing system, reducing 85% of the N fertilizer application.

Keyword: *Beef; forage; grazing; performance; sustainability; legume.*

1. INTRODUCTION

Nitrogen fertilizer is crucial for forage yield in grazing systems, especially in grass monocultures. The increasing costs of commercial N fertilizers and environmental issues associated with improper fertilization management are raising the awareness to improve N use efficiency to mitigate N losses from grassland ecosystems (SILVEIRA et al., 2015). Excess of N fertilizer application might lead to environmental pollution (DIMKPA et al., 2020) by volatilization of ammonia (NH_3) and nitrous oxide (N_2O) emissions (WOODLEY et al., 2020). Therefore, improving N management is a great way to elevate profitability and reduce N losses (SMITH et al., 2018). Moreover, the influence of unexpected market oscillations in the commercial N affects farmers' profitability (SANTOS et al., 2018).

The incorporation of legumes as a component in mixed pastures have been used as an alternative to reduce the N inputs (JARAMILLO et al., 2021), because of BNF (SOUMARE et al., 2020). The BNF is the symbiotic interaction between nitrogen-fixing "Rhizobia" and legume plants, and due to this symbiosis, the BNF is a feasible alternative to commercial N fertilizers (BRUIJN et al., 2015). Hence, the association of N-fixing legumes and grasses is desirable and might help to enhance the productivity and profitability of grazing systems (DUBEUX; SOLLENBERGER et al., 2020). Integrating legumes into grass systems provide some benefits to livestock, including improved diet digestibility and forage intake (MUIR et al., 2011), greater forage CP concentration (SALOMON, 2022) and animal performance (CUOMO et al., 2005), provision of N to grass-based grazing system (Mullenix et al., 2016), as well as contribute to other ecosystem services (DUBEUX et al., 2018). For that reason, the inclusion of legumes into a grass monoculture might enhance profitability of grass-legume mixtures compared with grass monocultures (BALL et al., 2015).

The livestock operation in the U.S. southeastern region is based on warm-season grasses such as bermudagrass and bahiagrass, along with cool-season annual legumes and grasses including arrowleaf clover and annual ryegrass to provide forage during autumn and winter (SANDERSON et al., 2012). In Florida particularly, the most used cultivars of bahiagrass are 'Argentine' and 'Pensacola' (VENDRAMINI; MORIEL, 2020), the popularity of bahiagrass could be related to its adaption to low soil fertility and basic input management needs (NEWMAN et al., 2010), as well as its grazing tolerance (ROUQUETTE et al., 2020). The inclusion of rhizoma peanut, which is well adapted to Florida conditions and is used as a forage in grazing systems might be a sound strategy to diversify livestock systems (VENDRAMINI; MORIEL 2020). This forage legume is characterized by its rhizome propagation and time of establishment (ARYAL et al., 2021), nutritive value, productive, persistence, and adaptation to a wide range of grazing managements (ORTEGA-S et al., 1992).

During the cool-season, when the warm-season grasses are dormant (ROUQUETTE, 2020), cool-season annual forages can be established during the fall by broadcasting on warm-season pastures as bahiagrass (DILLARD et al., 2018). Cool-season annual forages can provide forages with great nutritive values for later winter to early spring (HAN et al., 2018) when herbage from perennial forages is scarce, thus optimizing the use of stored feed during the winter period (DILLARD et al., 2018). The most common cool-season legume present in southern pastures are clovers, which are used as a component to integrate an annual cool-season grass mixture as annual ryegrass (VENDRAMINI; MORIEL, 2020). The annual ryegrass is a key component for forage-based livestock production in the southern U.S. (LEMUS et al., 2021). This markable forage crop is very versatile, presenting an elevated nutritive value, and tolerates intensive grazing (ROUQUETTE, 2020). Beyond that, annual ryegrass has a great development among dormant warm-season perennial grasses and an excellent performance when mixed with clovers (ROUQUETTES et al., 1997), which provide an earlier grazing than clover alone and decreasing the risk of bloating (EVERS et al., 1997).

Since the increasing costs of N fertilizer are concerning producers, the strategy of reduction of N inputs and integration of legumes in grazing systems is being considered (LEMUS et al., 2020). The goal of most producers is to reach high levels of forage production, high forage quality, and a great animal performance in grazing systems, sustainability, and profitability. The hypothesis of this study was that the inclusion of legumes in grazing systems may reduce N fertilizer input in grass-legume pastures, not affecting herbage and cattle growth performance. The objective of this study was to assess the herbage responses, nutritive value, and animal performance in three contrasting grazing systems, during the warm and cool seasons.

2. MATERIAL AND METHODS

2.1 Experimental site, animal management and treatments

This study approved by the University of Florida, Institutional Animal Care & Use Committee (IACUC) with the ID: IACUC201709924. This experiment was performed at the University of Florida, North Florida Research and Education Center located in Marianna, FL (30˚52'N, 85˚11' W, 35 m asl), during the cool and warm seasons of 2020 and 2021. The cool season assessment occurred from January to mid-May, and the warm season from mid-May to October. Soils were sampled to a depth of 0-15 cm at the experimental site. Soils were classified as Orangeburg loamy sand (fine-loamy kaolinitic, thermic Typic Kandiudults), with a pH of 5.7. Average Mehlich-I extractable soil P, K, Mg, Ca concentration at the beginning of the experiment were 26, 99, 43, and 224 mg kg^{-1}, respectively. Soil organic matter was 15.4 g kg^{-1}, and estimated cation-exchange capacity was 3.8 meq 100 g^{-1}.

The experiment was carried out in a randomized complete block design with three treatments and three replicates. Treatments were three grazing systems including warm-season perennial forages and cool-season annual and both seasons are described in Figure 2.1. The pastures were considered experimental unit, and each pasture measured 0.85 ha. The treatments consisted in Grass+N (GR+N), Grass+Clover (GR+CL), and Grass+Clover+Rhizoma peanut (GR+CL+RP). GR+N consisted of N-fertilized 'Argentine' bahiagrass during the summer (112 kg N ha^{-1}, split equally in two applications using urea as N source) overseeded with a N-fertilized (112 kg N ha yr^{-1}, applying 34 kg N ha^{-1} 3-wk. after planting using urea and 78 kg N ha^{-1} in February using a slow-release N commercially marketed as 'ESN') mixture of 'Prine' annual ryegrass and 'RAM' oat. Total annual N fertilization for GR+N was 224 kg N ha^{-1}. The treatment GR+CL consisted of bahiagrass receiving no N during the summer that was overseeded in the fall with ryegrass-oat-clover mixture, consisting of 'Dixie' Crimson, 'Southern Belle' red, and ball clovers seeded at rates of 16.8, 6.7, and 3.4 kg ha^{-1}, respectively. This treatment received an N-fertilizer application (34 kg N ha^{-1}) only in the Fall, 3 weeks after planting. Finally, the treatment GR+CL+RP consisted of the incorporation of 'Ecoturf' rhizoma peanut in the mixture with 'Argentine' bahiagrass in the summer. The rhizoma peanut present was strip-planted and established simultaneously with bahiagrass on 12 June 2014 (Jaramillo et al., 2021b). The The GR+CL+RP did not receive N-fertilizer during the summer, but in the fall the pastures were overseeded to a similar ryegrass, oat, and clover mixture and the winter N-fertilizer application was the same as for GR+CL.

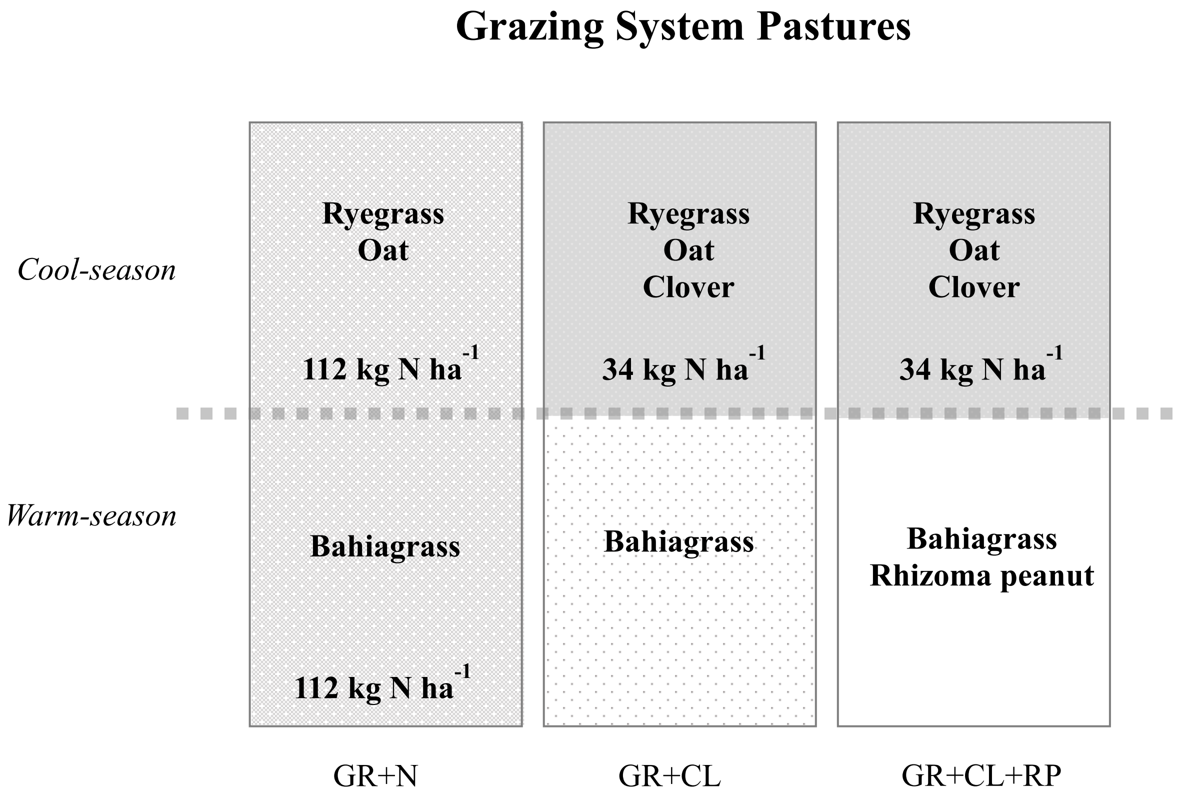


Figure 2.1. GR+N, N-fertilized bahiagrass during the warm season overseed with ryegrass+oat during the cool; GR+CL, bahiagrass during the warm season overseeded with ryegrass+oat plus a mixture of clovers during the cool season; GR+CL+RP, bahiagrass plus a mixture of rhizoma peanut during the warm season overseeded with ryegrass-oat-clover mixture during the cool season. In the summer, the fertilization was split in two equal portions (56 kg N ha^{-1}). In the winter, were applied 34 kg N ha^{-1} in the first moment and then 78 kg N ha^{-1} were applied.

The cool-season forages were seeded in early November of each year. All the pastures were fertilized with 34 kg N, 45 kg P, 56 kg K, and 13.5 kg S ha^{-1} after 4 wk of seeding in both years. The grazing system of this study has been operating for, approximately 7 years. Treatment design, seeding rate, N fertilization application and pasture characterization of previous years were also described by Garcia et al. (2021) and Jaramillo et al. (2021). The planting and the fertilization dates are presented in the Table 2.1.

Table 2.1 Nitrogen fertilization, herbicide management and planting period for the grazing systems in cool and warm-season.

	2020	2021		
Cool-season	Months		kg N ha^{-1}	Systems
Overseeding	November	October		All
1st Fertilization	December	December	34	All
2nd Fertilization	February	February	78	Grass+N
Warm-season				
1st Fertilization	May	June	56	Grass+N
2nd Fertilization	July	August	56	Grass+N

All = Grass+N, N-fertilized bahiagrass during the warm season overseed with ryegrass and oat during the cool; Grass+Clovers, bahiagrass during the warm season overseeded with ryegrass+oat plus a mixture of clovers during the cool season; Grass+Clovers, rhizoma peanut and bahiagrass during the warm season overseeded with ryegrass-oat-clover mixture during the cool season. In the summer, the fertilization was split in two equal portions (56 kg N ha^{-1}). In the winter, were applied 34 kg N ha^{-1} in the first moment and then 78 kg N ha^{-1} were applied. Periods was Early-February, Late-May, Early-June, Early-July, Early-August, Late-October, Early-November, Early-December.

Water, shade, and a mineral supplement mixture (Ca = min. 150 and max. 190 g kg−1, P = min. 30 g kg−1, NaCl = min. 150 and max. 180 g kg−1, Mg = min. 100 g kg−1, Zn = min. 2,800 mg kg−1, Cu = min. 1,200 mg kg−1, I =min 68 mg kg−1, Se = 30 mg kg−1, Vitamin A = 308,370 units per kg, Vitamin D3 = 99,119 units per kg; Special Mag, W.B. Fleming Company) were available for cattle in each pasture. In April of 2020 was applied 3.5 L per ha of pendimethalin on RP strips in all GR+CL+RP treatments pastures. In June of 2020, was applied 3.5 L per ha of pendimethalin plus 0.27 L per ha of imazapic on RP strips in all GR+CL+RP treatments pastures. In July of 2020, was applied 9.3 L of aminopyralid in all GR+CL and GR+N treatments pastures. In March and May of 2021, was applied 9.3 L per ha of pendimethalin in all pastures. In August of 2021, was applied 1.48 L of rinskor active with ¼ of non-ionic surfactant (NIS) per 378 L in all GR+CL and GR+N treatments pastures.

Pastures were continuously stocked during the grazing trial using an adjustable stocking rate that was based on HA. Each pasture had two testers crossbred (Angus × Brahman) yearling steers that remained on the pastures during the experimental period. The initial body weight (BW) of tester steers was 293 ± 23 kg and 291 ± 21 kg for 2020 and 2021 respectively. The same animals remained on their corresponding pasture during the months of grazing each year in both seasons.

2.2 Herbage responses: Cool-season and warm-season

2.2.1 Herbage mass, herbage allowance, and herbage accumulation rate

Herbage mass (HM) was determined using the double-sampling method (HAYDOCK; SHAW 1975; WILM et al., 1944). Every 14 days a rising disk meter was used to obtain the heights (in cm) of 30 aleatory points in each pasture, except for the treatment GR+CL+RP in the warm season, that were obtained 60 disk heights, which 30 points in each strip type (bahiagrass or rhizoma peanut). The disk heights (cm) were the indirect measurements, which were calibrated with harvested samples every 28 d. Forage samples were clipped at a 5-cm stubble height using a 0.25 m^2 metallic ring and dried at 55 °C for 72 h and recorded the dry weight. Regression equations were developed for each pasture using 18 paired samples (rising disk meter and its respective harvested sample). Each prediction equation had 18 points for grass only and 18 for legume. After developing the equations, the average disk heights of each pasture were used as the independent variable to estimate herbage mass.

The herbage allowance (HA), kg DM kg^{-1} BW, was estimated every 14 days using the method described by (SOLLENBERGER et al., 2005). Put-and-take animals were used to adjust the stocking rate during the entire study period with the goal of maintaining a similar herbage allowance among treatments in each block. The target herbage allowance was 1.0 kg DM kg^{-1} BW for the cool season and 1.5 kg DM kg^{-1} BW for the warm season.

The herbage accumulation rate (HAR) was determined using four exclusion cages per experimental unit placed at the initial sampling date. In the warm season, eight cages per pasture were placed at random sites in the GR+CL+RP pasture, four in each strip type (bahiagrass or rhizoma peanut). After 14 days, the cages were moved to a new location in the pasture, and the previous and new canopy heights were taken by falling disk meter (VENDRAMINI et al., 2012). The same equation used to obtain the HM was used to calculate the pre- and post-HM inside the cage.

The HAR (kg ha^{-1} d^{-1}) was calculated as the change in HM for 14 days that the cage was in a single location in the pasture. The total HAR in the pastures with legume was calculated for each component in the sward by multiplying the HAR by the proportion of grass or legume (only in the legume-containing treatments) in each pasture obtained from the botanical composition (BC, % of dry weight). In the warm season, the HAR of the RP was estimated by multiplying the proportion of RP in the BC by the HAR of RP from the evaluation period considering the RP strip area.

The botanical composition (BC) was calculated by the dry weight rank method described by (MANNETJE; HAYDOCK, 1963). A metallic ring (0.25-m^2) was randomly placed on the pasture followed by a visual estimation (% of dry-weight, DW) where all species presents were classified and recorded as either grass (ryegrass, oat), legume (clovers), or weeds for evaluation in the cool season. In the warm season the components were grass (bahiagrass and other grass) or legume (rhizoma peanut). This was estimated by ranks whereby the most abundant species took the first place, followed by the second and third respectively on dry weight basis. This procedure was repeated 60 times in each pasture. The BC in the cool season of the first year was estimated in March, and the second year in April while for the warm season it was done in August and September for the first and second year respectively.

2.2.2 Nutritive value

To determine forage nutritive value, hand-plucked samples were taken to analyze herbage crude protein (CP) and in vitro digestible organic matter (IVDOM), in both seasons. Grass and legume samples were collected every 14 days at each pasture, collected in different points to represent the entire pasture and simulating grazing behavior. All the samples were dried at 55 °C for 72 h and ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley laboratory Mill, Thomas Scientific, Swedesboro, NJ). To determine the IVDOM of the herbage material, the two-stage technique described by (MOORE; MOTT, 1974) was used. Subsamples were taken and ball-milled in a Retsch Mixer Mill MM400 (Retsch, Haan, Germany) at 25 Hz for 9 min to reduce the particle size under 100 μm. Samples of approximately 5 mg were analyzed for total C, N, $\delta^{13}C$, and $\delta^{15}N$ through the Dumas dry combustion method using a CHNS analyzer vario MICRO Cube (Elementar, Frankfurt, Germany) coupled to an isotope ratio mass spectrometer (IRMS) using an IsoPrime100 (Elementar, Frankfurt, Germany). The $^{13}C/^{12}C$ ratios are presented in the conventional delta (δ) notation, in per mil (‰) relative to the Pee Dee Belemnite (PDB). The IRMS provide the $\delta^{13}C$, $\delta^{15}N$ and the concentration of both elements in the sample. Once the concentration of N was obtained, crude protein (CP) of all samples could be estimated multiplying total N concentration by 6.25 factor.

2.2.3 Biological N_2 fixation

The BNF was determined for clovers and RP using the natural abundance technique (FREITAS et al., 2010). Reference plants (n=5) were collected every 28 days and were classified to the species level, dried at 55 °C for 72 h and ground to pass a 2-mm screen and ball-milled. The proportion of plant N derived from the atmosphere (%Ndfa) was estimated using Equation 1 described by (Shearer and Kohl, 1986):

$$\frac{\%\mathrm{Ndfa} = \delta^{15}\mathrm{N}_{\mathrm{reference\ plant}} - \delta^{15}\mathrm{N}_{\mathrm{N2\ \ fixing\ legume}}}{\delta 15\mathrm{N\ reference\ plant} - \mathrm{B}} \quad 100 \tag{1}$$

Where the $\delta^{15}N_{\text{reference}}$ is the $\delta^{15}N$ value for the non-N_2 – fixing reference plant, $\delta^{15}N_{N2\text{ – fixing legume}}$ is the $\delta^{15}N$ value for the N_2 – fixing (clover and RP) and *B* is the $\delta^{15}N$ value for N_2 – fixing plant grown in absence of inorganic N. In the cool season, the *B* value used was -1.96 ‰ and was the lowest value of clover obtained in this study. In the warm season, the *B* value used was – 1.41‰, as reported by (Okito et al., 2004) for *Arachis hypogea L*. The shoot N accumulation was estimated by multiplying herbage accumulation by legume N concentration. Herbage BNF was estimated by multiplying shoot N accumulation for the %Ndfa. The seasonal BNF was then estimated by multiplying the herbage BNF by the number of days within both season for each year.

2.2 Animal performance

2.3.1 Average daily gain, gain per area, and stocking rate

The determination of animal performance in cool and warm seasons followed the same methodologies. To obtain the BW, all the tester steers were weighed at the initiation of the experiment and every 28 days thereafter. Weights were taken at 0800 h following a 16-h fasting period. Average daily gain (ADG) was calculated for each 28-d period by dividing the average weight gain of the two animals, in each pasture, during that specific period by the number of days (kg head^{-1} d^{-1}). The ADG over the entire year, both seasons, was determined as a weighted average based on ADG per given season and year and the length of the season per given year. Grazing days were calculated by multiplying the total number of animal units (AU, 350 kg BW) in each pasture (both tester and put-and-take) by the number of days within each period, and subsequently summing all the animal days at the end of the season. Gain per area (GPA) (kg ha^{-1}) was calculated by multiplying ADG by the number of grazing days per hectare within each period. Stocking rate was calculated by dividing the grazing days by the total number of days within each season.

2.4 Statistical analyses

All responses variables were analyzed using procedures PROC GLIMMIX as implemented by SAS (SAS/STAT 15.1, SAS Institute). Pastures were considered experimental units for all output variables. For herbage responses the variables herbage mass, herbage accumulation, nutritive value, %Ndfa, and BNF were considered repeated measures. For responses including ADG, GPA, and stocking rate the model included treatment, evaluation period, and their interaction as fixed effects. Block, year, and block x treatment were considered random effects. Differences were considered significant at P ≤ .05.

3. RESULTS

3.1 Herbage responses

3.1.1 Cool season

The total herbage mass for the cool-season treatments did not differ significantly (P>0.05) and present an average of 1236 kg DM ha^{-1}. The herbage accumulation rate showed a treatment x evaluation interaction W(P=.03). Interaction occurred because GR+N peaked earlier compared with the other treatments.

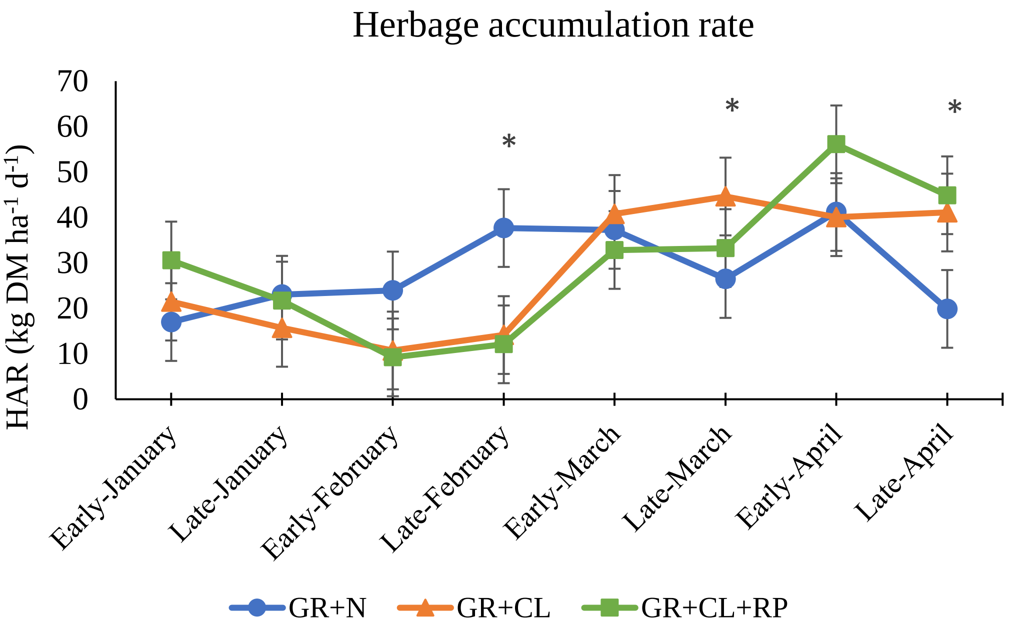


Figure 2.2. Treatment x evaluation date interactions (P=0.03) for total herbage accumulation rate (HAR) during cool season. Error bars denote standard errors. *Significant at the 0.05 probability level according to Least Significant Difference.

Grass differed among treatments in the cool season (P=0.0001) in the botanical composition Table 2.1. As was expected, the grass in the treatment GR+N presented the highest proportion compared to legume-mixture that averaged 62%. The legumes differed among treatments (P=.0001) and presented the same proportion in treatments GR+CL and GR+CL+RP, because the GR+N is monoculture.

Table 2.2 Botanical composition (BC) during cool-season in three grazing systems from 2020–2021.

	Treatment^2			
	GR+CL	**GR+CL+RP**	SEM	P Value
Grass %	64b	60b	22.9	.0001
Legume %	28 a	31 a	23.3	.0001

[a]Grass+N, N-fertilized bahiagrass during the warm season overseeded with annual ryegrass and oat during the cool season; Grass+Clover, bahiagrass during the warm season overseeded with ryegrass–oat plus a mixture of clovers during the cool season; Grass+Clover+RP, rhizoma peanut and bahiagrass during the warm season overseeded with ryegrass–oat–clover mixture during the cool season.
Means are averages of values across years.
Means not sharing a common letter are significantly different at P ≥ .05 according to Least Significant Difference.

3.1.1 Warm season

In the warm season, the total herbage mass did not differ among treatments (P=0.47) and averaged 1682 kg DM ha^{-1}. However, there was an evaluation effect (P<0.0001) on herbage mass and the results are presented in the Figure 3.3a. The evaluations are defined as 1 middle of May, 2-3 June, 4-5 July, 6-7 August, 8-9 September, and 10-11 October. The herbage mass in the first two evaluations was around 1000 kg DM ha^{-1}, which increased the yield with time and peaked at 2500 kg DM ha^{-1} in August and September and decreased in October (1600 kg DM ha^{-1}). The herbage accumulation rate (HAR) did not present a treatment effect (P=0.17) and the means were 34, 29, and 37 kg ha^{-1} d^{-1} for GR+N, GR+CL, and GR+CL+RP, respectively. However, there was an evaluation date effect (P<0.0001) on HAR. In late June, it was observed the least herbage accumulation rate, following an increase until reaching the peak in late July (54 kg ha^{-1} d^{-1}), decreasing thereafter with the advance of the season. The herbage accumulation rate is presented in the Figure 3b. The of grass proportion differed among treatments (P=0.0001). The botanical composition for bahiagrass in the GR+N and GR+CL average 76%. In the GR+CL+RP the proportion of bahiagrass and rhizome peanut was 48% and 38%, respectively.

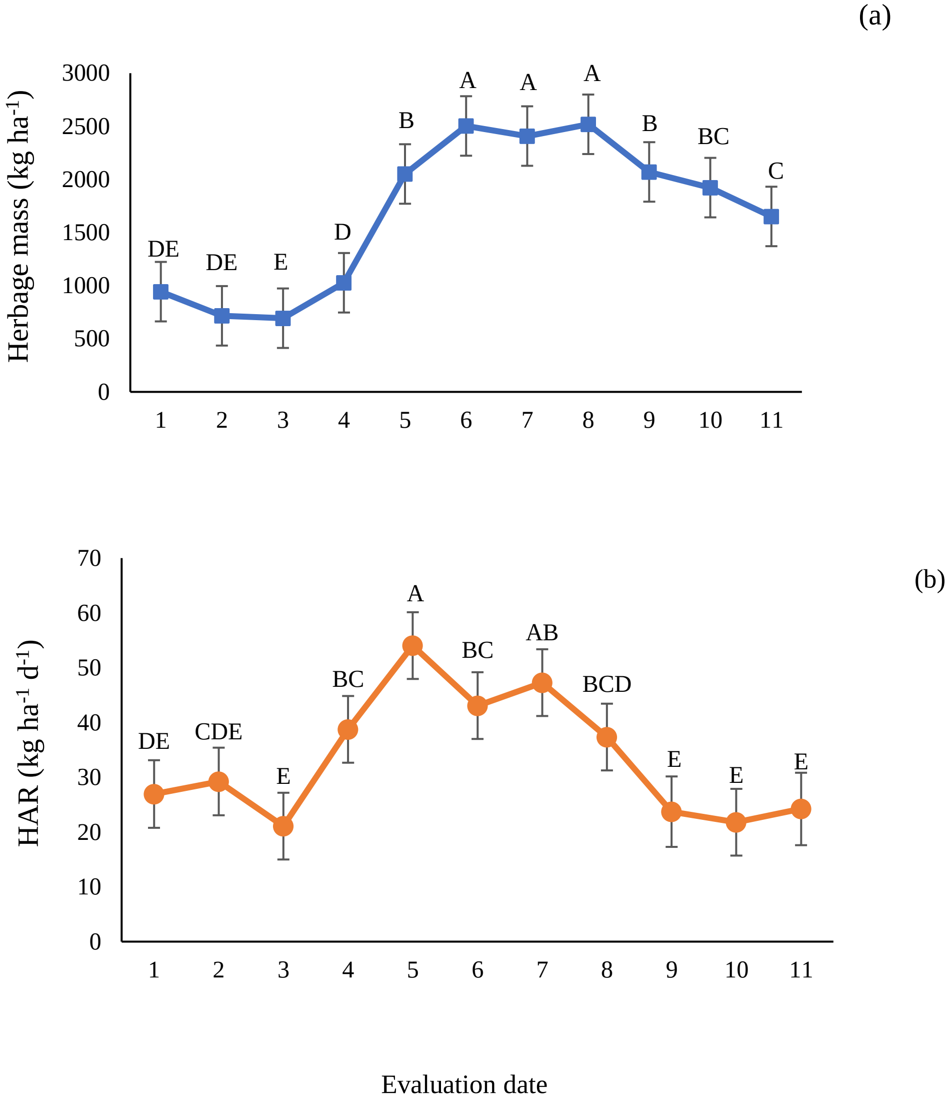


Figure 3.3. Warm season evaluation date effect (P<.0001) on herbage mass (a) and evaluation effect (P<.0001) herbage accumulation rate (HAR) (b). Evaluation numbers indicate evaluation date, and are as follows: 1, early May; 2, late May; 3, Early June; 4, late June; 5, early July; 6, late July; 7, early August; 8, late August; 9, early September; 10, late September; 11, early October; 12, late October. DM, dry matter. Error bars denote standard errors. *Significant at the .05 probability level according to Least Significant Difference.

3.2 Nutritive value

3.2.1 Cool season

During the cool season, the CP concentration in the grass component (Figure 3.4a) did not differ among treatments (P =0.62), however differed across evaluation dates (P=0.001). The values of CP fluctuated throughout the season, and the greatest CP concentration was 263 g kg^{-1} in late February, and the least concentration was observed in late April, with the end of the season. The IVDOM of cool-season grasses presented differences (P=0.01) among treatments, and the treatment GR+N had the greatest value (720 g kg^{-1}), with GR+CL and GR+CL+RP not differing significantly (691 and 675 g kg^{-1}, respectively). There was an evaluation date difference (P<0.0001), and the values varied during the season (Figure 3.4b). The greatest value was 737 g kg^{-1} in late January and maintained around 700 g kg^{-1} during four evaluations when dropped and reached 596 g kg^{-1} in early May. The clover CP concentration was different across evaluation dates (P=0.0001, Figure 5a). The CP concentration was constant during almost the entire cool season, with average from January to early April around 264 g kg^{-1}, and then dropped to 198 g kg^{-1} in late April and early May, with the end of the cool season.

The IVDOM of clover differed across evaluation dates during cool season (P=0.01). The means were not different until March. In early March, the IVDOM reached the greatest value, 742 g kg^{-1}, and in April decreased, to reach 624 g kg^{-1} in early May, the last evaluation (Figure 3.5b).

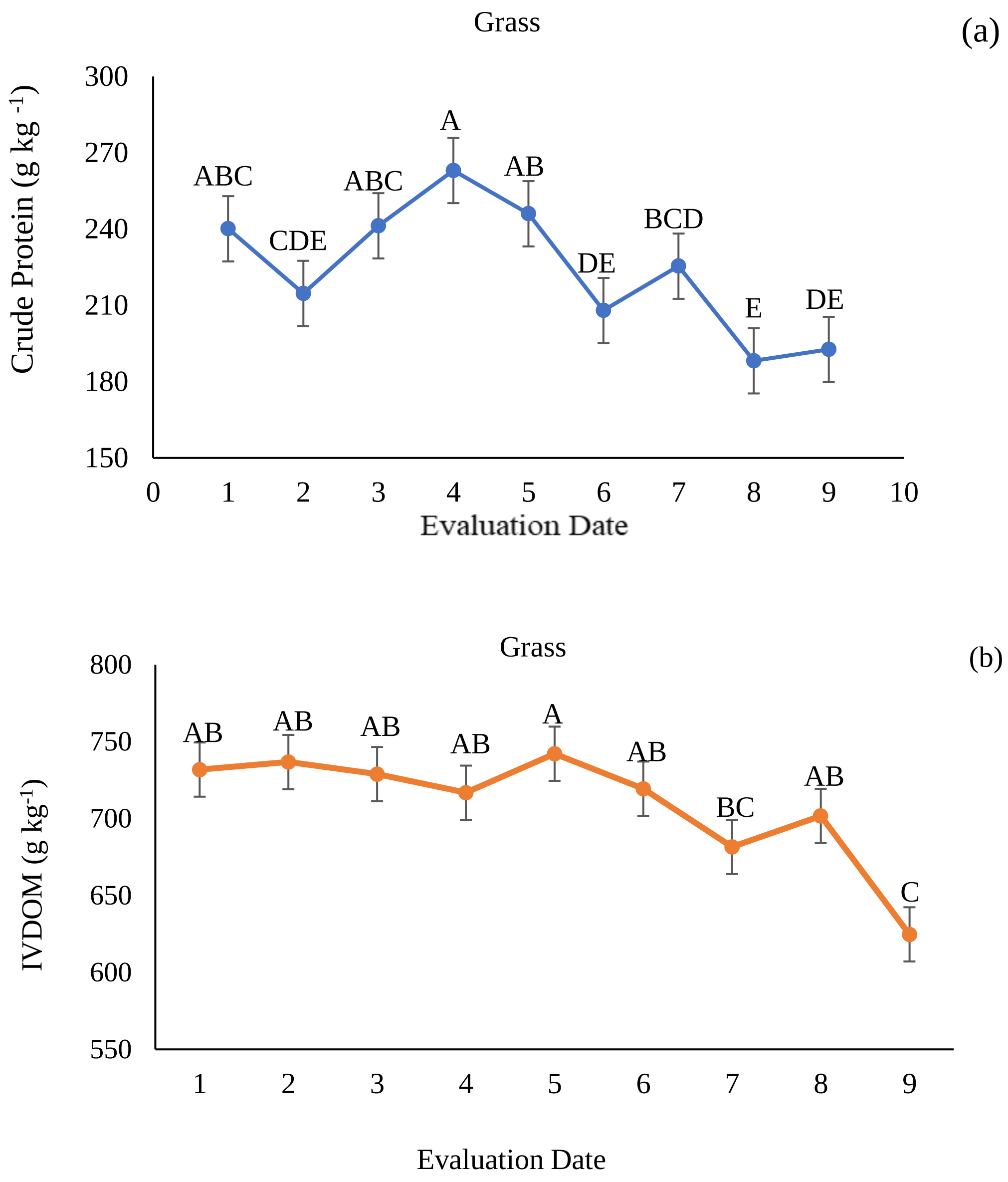


Figure 2.4. Cool season evaluation date effects (P=.001) on grass crude protein concentrations (a) and evaluation effect on legume (<.001) in vitro digestible organic matter (IVDOM) concentrations (b). Evaluation numbers indicate evaluation date, and are as follows: 1, early January; 2, late January; 3, early February; 4, late February; 5, early March; 6, late March; 7, early April; 8, late April; 9, early May. Error bars denote standard error. Means not sharing a common letter are significantly different at the .05 probability level according to Least Significant Difference.

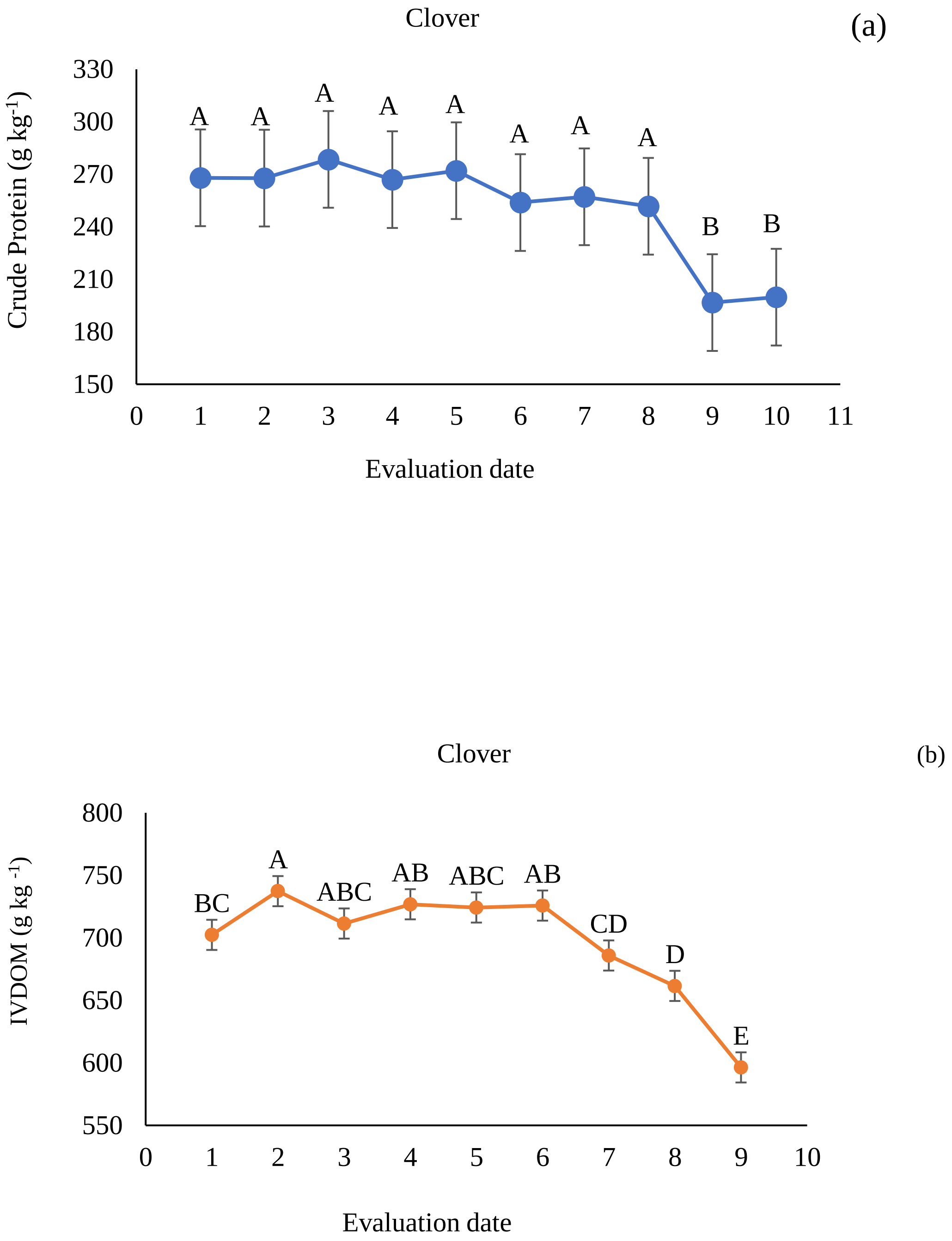
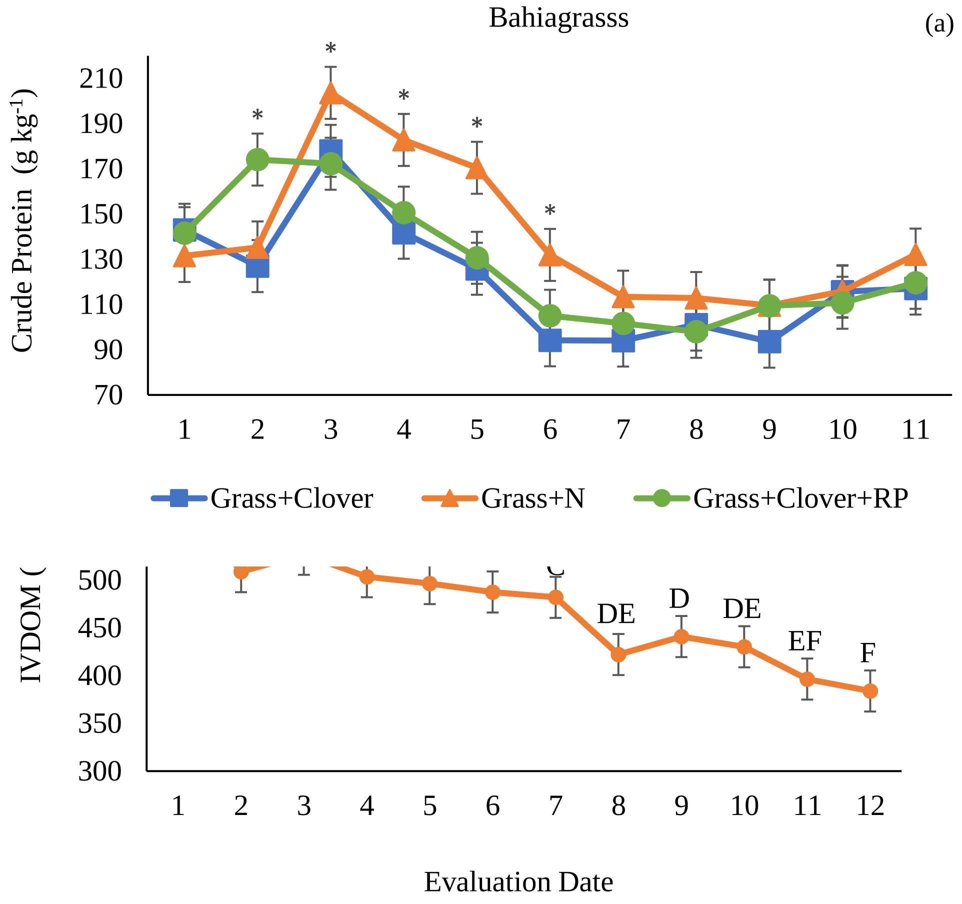


Figure 2.5. Cool season evaluation date effects (P=.001) on clover crude protein concentrations (a) and evaluation effect on legume (<.001) in vitro digestible organic matter (IVDOM) concentrations (b). Evaluation numbers indicate evaluation date, and are as follows: 1, early January; 2, late January; 3, early February; 4, late February; 5, early March; 6, late March; 7, early April; 8, late April; 9, early May. Error bars denote standard error. Means not sharing a common letter are significantly different at the .05 probability level according to Least Significant Difference.

3.2.2 Warm season

In the warm season, it was observed a treatment x evaluation date interaction (P=0.01) for grass CP (Figure 3.6a). The differences were observed from early June to late August. In the second evaluation, the treatment GR+CL+RP presented the greatest CP value (203 g kg^{-1}). From early June to early August, the GR+N treatment had the greatest CP concentration, where for this treatment the greatest value was 203 g kg^{-1} (evaluation 3), being the highest CP concentration until early August. In early June, there was the CP peak for GR+CL and GR+N. As expected, with the advance of the season, from early July until the end of the season, the CP was depressed. Following the same pattern of the CP concentration, the IVDOM of the grass component in the warm season presented an evaluation effect (P<0.0001). The greatest value obtained was 591 g kg^{-1} of IVDOM in late May, and then dropped to 508 g kg^{-1} early June. It was observed a significant reduction on IVDOM throughout the warm season, and the least value obtained was 384 g kg^{-1} in late October (Figure 3.6b). The CP concentration of rhizoma peanut presented an evaluation effect (P=0.02). The CP concentration oscillated during the season; the peak was observed late June when reached 204 g kg^{-1} (Figure 7a). The CP concentration was maintained up to 170 g kg^{-1} and below 200 g kg^{-1} during most of the season. As observed in the CP concentration, the IVDOM of rhizoma peanut also presented an evaluation effect (P<.0001), and the greatest value was obtained in late May, that was 800 g kg^{-1} and dropped following by evaluations. For most of the season, the IVDOM oscillated from 600 to 700 g kg^{-1} (Figure 3.7b).

Figure 2.6. Warm season treatment x evaluation date interactions (P=.01) on crude protein concentrations (a) and evaluation effect on grass (<.001) in vitro digestible organic matter (IVDOM) concentrations (b). Evaluation numbers indicate evaluation date, and are as follows: 1, early May; 2, late May; 3, Early June; 4, late June; 5, early July; 6, late July; 7, early August; 8, late August; 9, early September; 10, late September; 11, early October; 12, late October. DM, dry matter. Error bars denote standard errors. *Significant at the .05 probability level according to Least Significant Difference.



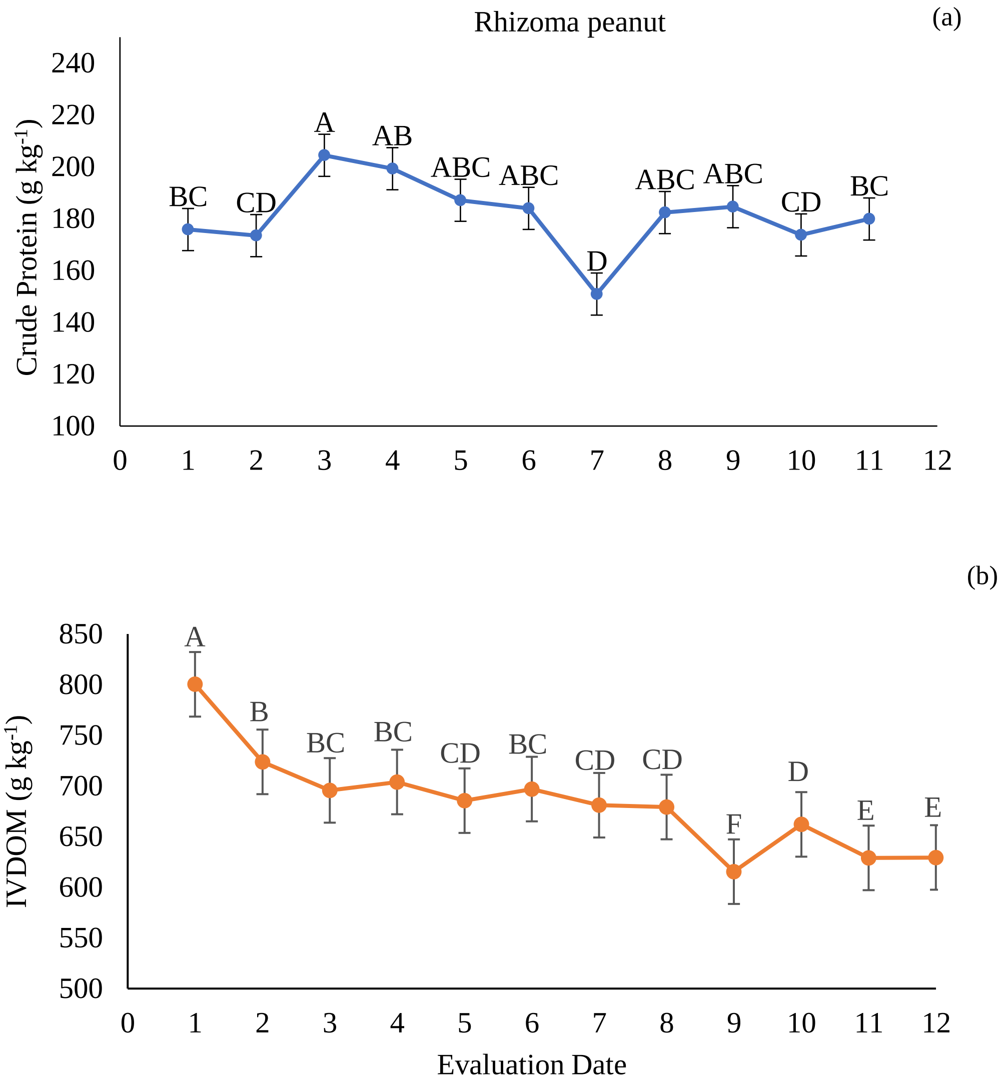


Figure 2.7. Warm season treatment x evaluation date interactions (P=.01) on crude protein concentrations (a) and evaluation effect on legume (<.001) in vitro digestible organic matter (IVDOM) concentrations (b). Evaluation numbers indicate evaluation date, and are as follows: 1, early May; 2, late May; 3, Early June; 4, late June; 5, early July; 6, late July; 7, early August; 8, late August; 9, early September; 10, late September; 11, early October; 12, late October. DM, dry matter. Error bars denote standard errors. Significant at the .05 probability level according to Least Significant Difference.

3.3 Nitrogen derived from atmosphere and biological N_2 fixation

3.3.1 Cool season

Nitrogen derived from atmosphere (Ndfa) percentage differed on evaluation date in the cool season (P=0.03). The %Ndfa increased with the progress of the season and in early January was observed the least value (45.7%), following to the highest value that was 86.3% in early April (Figure 8a). The biological N_2 fixation (BNF) presented an evaluation effect (P=0.02) and oscillated during the cool season. The difference in evaluation occurred specially from February to March, where in the evaluation of March the BNF was 10.75 kg N ha^{-1}, and in February was 2.85 kg N ha^{-1} (Figure 8b).

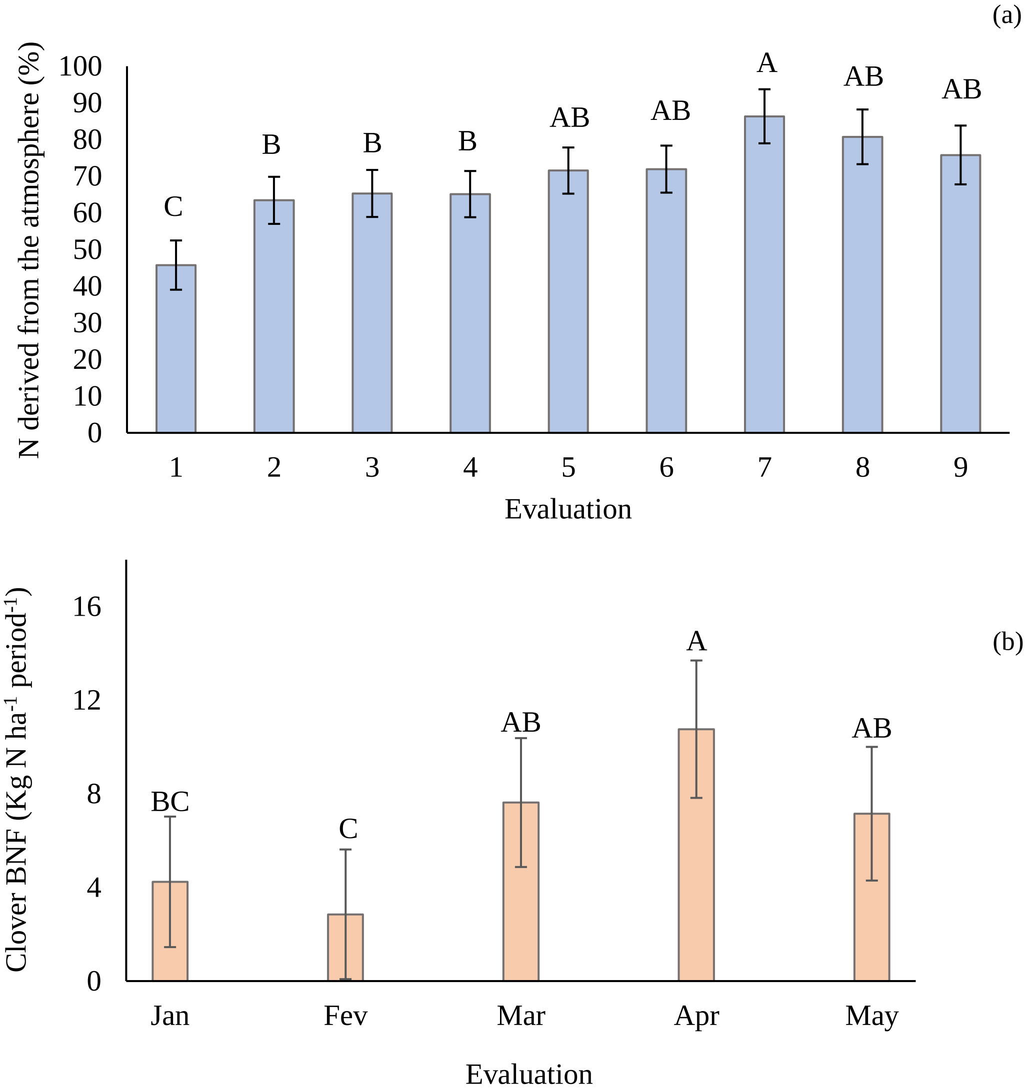


Figure 2.8. Evaluation effect on clover percentage of N_2 derived from atmosphere (Ndfa) (P=.03; a) and evaluation effect on clover biological N_2 fixation (BNF) (P=.02; b). a) evaluation numbers indicate evaluation date, and are as follows: 1, early January; 2, late January; 3, early February; 4, late February; 5, early March; 6, late March; 7, early April; 8, late April; 9, early May (a). b) January; February; March; April; May. Error bars denote standard error. Means not sharing a common letter are significantly different at the .05 probability level according to Least Significant Difference.

3.3.2 Warm season

The %Ndfa differed on evaluation date in warm season ($P<0.0001$; Figure 9). The %Ndfa changed during the warm season and increased with time. The least Ndfa was observed in early June, which was 53%. The highest value obtained was in October, the end of the season, when the Ndfa was 79%. In the warm season, the BNF did not differ among evaluation dates ($P=0.15$) and the fixation was 63 kg N ha^{-1} $season^{-1}$.

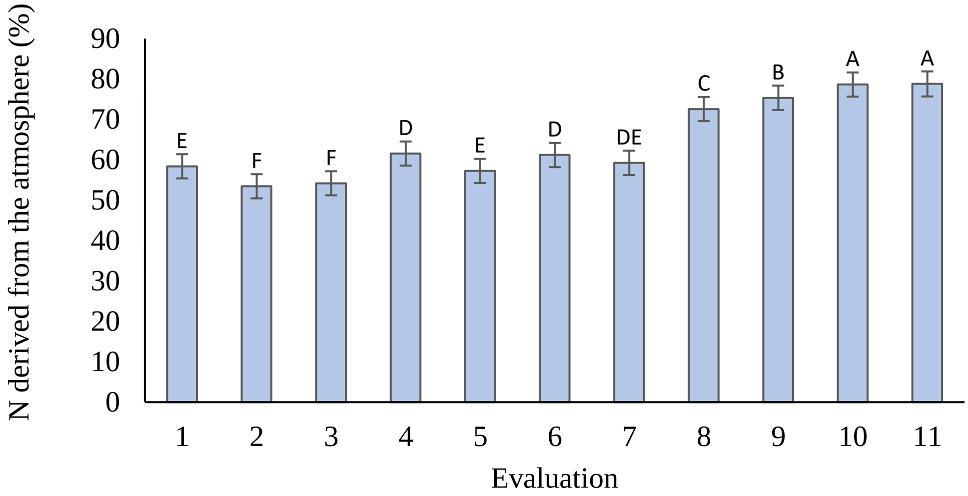


Figure 2.9. Evaluation effect percentage of N derived from atmosphere in rhizome peanut ($P<.0001$). Error bars denote standard error. Means not sharing a common letter are significantly different at the .05 probability level according to Least Significant Difference.

3.4 Animal performance

3.4.1 Cool-season

After two consecutive cool grazing seasons (2020 to 2021) the average daily gain (ADG), gain per area (GPA), stocking rate and herbage allowance did not differ ($P>0.05$) among treatments (Table 2.3). The ADG, GPA, stocking rate, and herbage allowance averaged 0.87 kg d^{-1}, 308 kg ha^{-1}, 2.69 AU ha^{-1}, and 1.06 kg DM kg^{-1} BW, respectively.

3.4.2 Warm-season

However, after two warm grazing seasons, the ADG differed among treatments ($P=0.02$). The treatment GR+CL+RP presented the greatest ADG, that was 0.34 kg hd^{-1} d^{-1} superior to GR+N and 0.16 kg hd^{-1} d^{-1} superior to GR+CL. The GPA differed among treatments ($P=0.008$). The treatment GR+CL+RP showed the greatest gain, which was 0.19 kg d^{-1} greater than GR+N and

GR+CL. The stocking rate did not present differences among treatments (P=0.14), with an average of 3.2 AU ha^{-1}. The herbage allowance of warm season did not differ among treatments (P=0.75) and averaged 0.99 kg DM kg^{-1}.

3.4.3 Annual grazing

The annual grazing season animal performance, that is the combination of cool and warm seasons, did not present any difference among treatments in ADG (P=0.15). The average of all treatments was 0.54 kg hd^{-1} d^{-1}. The GPA differed among treatments (P=0.02), with the treatment GR+CL+RP presenting the greatest GPA, and the GR+N and GR+CL had an average of 0.43 kg ha^{-1}. The stocking rate presented differences among treatments (P=0.04). The treatment GR+N presented the greatest value and the other treatment averaged 2.2 AU ha^{-1}.

Table 2.3. Average daily gain (ADG), gain per area (GPA), stocking rate (AU ha−1), and herbage allowance (kg DM kg−1 BW) in Grass+Clover, Grass+N, and Grass+Clover+RP pastures during cool and warm seasons from 2020–2021, and the year average.

	Treatment[a]				
	GR+CL	**GR+N**	**GR+CL+RP**	SEM	P Value
Cool Season					
ADG, kg hd^{-1} d^{-1}	0.89	0.87	0.86	0.12	0.95
GPA, kg ha^{-1}	286	346	293	44.36	0.15
Stocking rate, AU[b] ha^{-1}	2.35	3.34	2.40	0.34	0.08
HA, kg DM kg^{-1} BW	1.07	1.03	1.08	0.02	0.20
Warm Season					
ADG, kg hd^{-1} d^{-1}	0.18b	0.12b	0.34a	0.02	0.02
GPA, kg ha^{-1}	127b	101b	257a	24.5	.008
Stocking rate, AU[b] ha^{-1}	3.05	3.35	3.21	0.10	0.14
HA, kg DM kg^{-1} BW	1.00	0.98	0.99	0.04	0.75
Annual					
ADG, kg hd^{-1} d^{-1}	0.53	0.50	0.60	0.07	0.15
GPA, kg ha^{-1}	413	447	550	33.4	0.02
Stocking rate, AU[b] ha^{-1}	2.19b	2.82a	2.26b	0.16	0.04

Means not sharing a common letter are significantly different at $P \geq .05$ according to Least Significant Difference. [a]Grass+N, N-fertilized bahiagrass during the warm season overseeded with annual ryegrass and oat during the cool season; Grass+Clover, bahiagrass during the warm season overseeded with ryegrass–oat plus a mixture of clovers during the cool season; Grass+Clover+RP, rhizoma peanut and bahiagrass during the warm season overseeded with ryegrass–oat–clover mixture during the cool season; HA, Herbage allowance; SEM, standard error of the mean; hd, head; DM, dry matter; BW, bodyweight. [b]AU (animal unit); 1 AU = 350 kg BW.

4. DISCUSSION

4.1 Herbage responses

4.1.1 Cool-season

The incorporation of clovers into annual ryegrass and oats mixed pastures in this study presented the same ($P>0.05$) biomass production of N fertilized pastures. Nitrogen fertilizer enhances annual ryegrass productivity in pastures (EVERS et al., 2010), but pastures composed by clover and grass could provide the same productivity, and this mixture may improve the animal performance.

The HAR of the treatments GR+CL and GR+CL+RP increased from early March until the end of the cool season. This result can be explaining because the ball clover seasonal production is from March to May and red clover from April to the end of the season, while crimson clover has an earlier production and covered the begin of the season, that was January (BALL et al., 2015). In late April, the GR+N presented the lowest HAR that was 20 kg DM ha^{-1} d^{-1} while the GR+CL and GR+CL+RP presented 43 kg DM ha^{-1} d^{-1}. The presence of ball and red clover provided forage during this period that the grass was decreasing the production, since the annual ryegrass decreases the production from March to April (BALL et al., 2015; DUBEUX et al., 2016). This result is in accordance with Jaramillo et al. (2021) that assessed cattle performance on rye and oats pastures with the same combination of clovers and N fertilizer amount used in this study. The grass-clover mixture in their trial reached the peak in late April, which also was observed in this present study. They found a superior HAR, that was 70 kg DM ha^{-1} d^{-1} vs 40 kg DM ha^{-1} d^{-1} found in this study. However, the HAR of ryegrass-oat mixture was close to 31.5 kg DM ha^{-1} d^{-1} of two seasons grazing period reported by Dubeux et al. (2016) obtained in a grazing study of small grain-annual ryegrass pastures. The HAR The proportion of clovers observed in this study (averaged 30%) is in the range 20-45 % proposed by Thomas (1992) which legumes could provide the benefits in relation to N requirement for a productive and sustainable pasture in temperate or tropical situation. According with Ledgard et al. (2000) the defoliation management and N application are determinant in the proportion of legume in mixture pastures, because the proportion of legumes can affect the total N_2 fixation. Thus, in this present study, the animal defoliation affected proportion of clovers in the pasture, which could be altered the proportion of legume, reflecting the BNF.

4.1.2 Warm-season

In the warm season, N fertilizer did not affect HM and HAR and the inclusion of rhizoma peanut into bahiagrass pastures presented the same result of non-fertilized and fertilized bahiagrass pastures. This result indicated the possibility to reduce N inputs by replacing 112 kg N ha^{-1} by incorporating rhizoma peanut. The HM and HAR were different throughout the season and increased with until the peak occurred between July and August, that is the peak of the warm season. This data corroborates with data from Jaramillo et al. (2021) who observed greater HAR of all treatments between late July and early September. The same biomass among the treatments is positive since the inclusion of rhizoma peanut can provide forage during the most part of the season, which may reflect on animal performance. The proportion of rhizoma peanut in this study was 38% and is inside the range proposed by Thomas et al. (1992) which could consider this proportion of RP adequate to provide N benefits to mixture pasture. The proportion of legume in the pasture it is an important factor to obtain the N_2 atmospheric fixation by legumes.

4.2 Nutritive value

4.2.1 **Cool season**

The nutritive value of grass and legume across all treatments were not different. The same result was observed in the clover across all treatments. This is an important result, indicating that the practice of incorporating clover with annual cool season grasses can partially replace the N fertilizer since the nutritive value of the pastures were the same across treatments. Our CP and IVDOM of ryegrass-oat values were close to the ones reported by Dubeux et al. (2016) and Jaramillo et al. (2021). However, as expected, it was observed an evaluation effect on forage nutritive value throughout the grazing season. CP concentration of annual ryegrass with N or legume decreases with the advance of maturity (LEMUS et al., 2021).

In a cool-season annual forage mixtures grazing trial, Mullenix et al. (2012) observed the reduction of CP concentration and IVDDM during the cool season. Butler et al. (2012) also reported the reduction of CP and digestibility of ryegrass-oat mixtures. They observed that ryegrass-oat-legume in January had 183 g kg^{-1} and peaked in March (293 g kg^{-1}), decreasing to 135 g kg^{-1} in April. The ryegrass-oat-N peaked by March (341 g kg^{-1}) decreasing to 138 g kg^{-1} in April. Clover CP concentration were maintained during the whole period and decreased because of maturity. The reduction in IVDOM is also related with advanced maturity and was expected because of growth dynamic.

4.2.2 Warm season

The CP concentration of fertilized bahiagrass treatment maybe was the greatest during most of the season. The increase was observed right after the beginning of the season (late-June) and reached 203 g kg^{-1} and was decreasing with the progress of the warm-season reaching 120 g kg^{-1} in October. Perhaps this is related to the presence of remaining cool-season forages in the pasture that matches with these evaluations. The difference among the CP of the treatments during the evaluation periods was late June to early August. However, the N fertilizers did not change the IVDOM of bahiagrass and did differ among treatments. It was observed in the first evaluation a high value (590 g kg^{-1}), with dropped drastically in the second evaluation to 508 g kg^{-1}. Probably, this can also be related to the presence of cool-season forages in the samples, increasing the digestibility in this evaluation, in early June, when it is more difficult to detect the presence of the cool-seasons forages. Our values of CP and IVDOM for bahiagrass was in accordance with Jaramillo et al. (2021), which also found that bahiagrass decreased nutritive value with time and the values ranged from 160 to 80 g kg^{-1} and 500 to 400 g kg^{-1}, from May to October, respectively, for CP and IVDOM. As expected, the CP and IVDOM of rhizoma peanut presented variation with the advance of growing period. The IVDOM declined from 800 g kg^{-1} in May to 630 g kg^{-1} in October.

4.3 Nitrogen derived from atmosphere and biological nitrogen fixation

4.3.1 Cool season

Determining the percentage of Ndfa allows the estimation of how much N_2 atmospheric the legume is fixing, especially in mixture with grasses, which 80% depending on location, yield, and management (CARLSSON; HUSS-DANELL et al., 2003). In the cool season, the % Ndfa of this study agrees with what was reported by Blink (1990) that found a %Ndfa of crimson clover around 77%. Krinstesen et al. (2022) reported a %Ndfa in a range of 65 to 80% of white and red clover fertilized with 100 kg N ha^{-1}. This range is in concordance with the clover %Ndfa found in this trial, which reached 86% in a treatment with 34 kg N ha^{-1}, since that N fertilization can affect N_2-fixation activity (Kristensen et al., 2022b). The BNF of clovers in monoculture, in Southeast U.S, was reported by BLINK (1990). They reported that clover can fix around 155 kg N ha^{-1}, that was superior to the result obtained in this present study, that presented an average of 63 kg N ha^{-1} for the season. A similar result was observed by Jaramillo et al. (2021), which related the low fixation to the grazing effects on clover biomass, since it is related to the fixation as well as the proportion of legume.

4.3.2 Warm season

In the warm season, the %Ndfa of rhizoma peanut was below the one reported by Dubeux et al. (2016) until September that reached up to 67%, which was close to the lowest value found by the authors. The BNF of rhizoma peanut average of the two years obtained in this trial was 53 kg N ha^{-1} season^{-1}, different from the one reported by Dubeux et al. (2016) that indicated that rhizoma peanut N_2 fixation can range from 123 to 280 kg N ha^{-1} yr^{-1} in pure stands. In the current study, however, RP was not in monoculture, but rather in a mixed stand with bahiagrass. Our BNF of RP is greater than the one reported by Jaramillo et al. (2021), who attributed the low BNF (16 kg N kg N ha^{-1} season^{-1}) to the preference of cattle for rhizoma peanut, which negatively affected the herbage accumulation rate, decreasing the contribution via BNF. The amount of N fixed in grass-legumes mixtures is directly related to the legume proportion (DUBEUX; SOLLENBERGER, 2020).

4.4 Animal performance

4.4.1 Cool-season

During the entire cool season, the ADG was the same across treatments. The result of ADG in ryegrass-oat is lesser than the one reported by Mullenix et al. (2012) who observed during the cool season ADG of 1.39 kg hd^{-1} d^{-1}. Also, the GPA observed in this present study was superior to the ones reported by Jaramillo et al. (2021) and the GPA obtained by rye-oat+N system was 285 kg ha^{-1} compared with 346 kg ha^{-1} provided by annual ryegrass-oat+N system. Based on this, annual ryegrass in a mixture with oat and clover had greater result of animal performance in the cool-season in relation to the previous study of Jaramillo et al. (2021) with rye-oat-clover. The use of clovers in the system contributed to the reduction of N fertilizer during the cool season. The grasses plus N system counted with a fertilization of 112 kg N ha^{-1} during the whole season, compared to the grass-legume rate of 34 kg N ha^{-1}.

4.4.2 Warm-season

During the warm season grazing period, the use of N fertilizer did not affect ADG. The incorporation of rhizoma peanut improved the ADG in 0.22 kg hd^{-1} d^{-1} and GPA in 156 kg ha^{-1}, which is important for producers since the inclusion of legumes are, sometimes, limited by investments and time of establishment and they are concerned about profitability. The application of rhizoma peanut into bahiagrass showed a great result compared to the N fertilized monoculture, which can be considered a feasible alternative to improve animal performance, reducing the need of N fertilizers. Although our treatments were equal and the rhizoma peanut and bahiagrass mixture was the best system, our results were inferior to the ones reported by Jaramillo et al. (2021). The herbage mass and accumulation of this present trial were inferior to the previous study, and this could be related with the dry period obtained in 2020 and 2021 compared with the same period in 2016-2019.

4.4.3 Annual grazing

In the annual grazing season, the inclusion of legumes in the system confirmed the reduction of N inputs and the feasibility of legume incorporation into grass pastures, once the ADG and GPA were the same across treatments. In grass monoculture systems, the N fertilization could be 224 kg N ha^{-1}, considering the management proposed by this study. The incorporation of legumes into grasses pastures promoted an equal gain in cool season compared with grass N fertilized treatment. In warm season, the incorporation of legumes into grass pastures promoted a superior animal performance compared to the grass only plus N fertilizer. This reflected in the annual N input because it was possible to reduce N fertilizer application by 190 kg N ha^{-1}, considering that the grass + N was 224 kg N ha^{-1} yr^{-1} and for the grass-clover systems the rate was only 34 kg N ha^{-1}. Additionally, looking at the warm season, the results was even better, because the incorporation of rhizoma peanut into bahiagrass pastures improved the ADG and GPA in relation to the N-fertilized treatment. In summary, for the whole year, the most important result is that the system can be profitable by incorporating legumes during the whole year. It is possible obtain the same gain using less nitrogen.

5. CONCLUSION

In the cool season, the use of annual ryegrass-oat associated with clovers and fertilized by 34 kg N ha^{-1} had the same performance of grass only N-fertilized treatment (112 kg N ha^{-1})

regarding the animal responses. Although annual ryegrass is well cultivated in the southeast United States, ryegrass presented a great herbage response in oat and clover mixtures. Based on results of this trial, ryegrass had a superior result in animal performance compared to a previous study in the same system with rye. The participation of rhizoma peanut during the summer improved the animal performance compared with fertilized and non-fertilized bahiagrass pastures, and the continuity of RP in the system is showing the success of RP establishment in this system, in Florida.

The incorporation of legumes into grass pastures beyond increased animal performance, warranted a satisfactory herbage production throughout seasons, with potential to increase transference of N_2 atmospheric into the system, thus reducing dependence of off-farms inputs avoiding N losses caused by high levels of fertilizer. Integration of legumes into grass system pastures, in warm and cool seasons, may contributes to the development of a sustainable grazing system, reducing by 85% the N fertilizer application. Annual cattle performance demonstrated a superior animal performance grass-legume systems in relation to N fertilizer grass pasture treatment, demonstrating the viability of this process of sustainable intensification of grazing systems.

6. REFERENCES

Aryal, P., Sollenberger, L. E., Kohmann, M. M., da Silva, L. S., Cooley, K. D., & Dubeux Jr, J. C. (2021). Plant growth habit and nitrogen fertilizer effects on rhizoma peanut biomass partitioning during establishment. *Grass and Forage Science*, *76*(4), 485-493.

Cuomo, G. J., Rudstrom, M. V., Johnson, D. G., Anderson, J. E., Singh, A., Peterson, P. R., & Sheaffer, C. C. (2005). Nitrogen fertilization impacts on stand and forage mass of cool-season grass-legume pastures. *Forage & Grazinglands*, *3*(1), 1-10.

Dillard, L. S., Hancock, D. W., Harmon, D. D., Kimberly Mullenix, M., Beck, P. A., & Soder, K. J. (2018). Animal performance and environmental efficiency of cool- and warm-season annual grazing systems. *Journal of Animal Science*, *96*(8), 3491–3502. https://doi.org/10.1093/jas/sky025

Dimkpa, C. O., Fugice, J., Singh, U., & Lewis, T. D. (2020). Development of fertilizers for enhanced nitrogen use efficiency – Trends and perspectives. *Science of The Total Environment*, *731*, 139113. https://doi.org/10.1016/j.scitotenv.2020.139113

Dubeux, J. C. B., Sollenberger, L. E., Vendramini, J., Wallau, M. O., Blount, A. R. S., Garcia-Jimenez, L., Santos, E., & Jaramillo, D. (2018). Strip-planting Rhizoma Peanut into Grazing Systems. *EDIS*, *2018*(4). https://doi.org/10.32473/edis-ag421-2018

Enriquez-Hidalgo, D., Gilliland, T. J., & Hennessy, D. (2016). Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. *Grass and Forage Science*, *71*(4), 559–574. https://doi.org/10.1111/gfs.12201

Evers, G. W., Smith, G. R., & Hoveland, C. S. (1997). Ecology and Production of Annual Ryegrass. In F. M. Rouquette & L. R. Nelson (Eds.), *CSSA Special Publications* (pp. 29–43). Crop Science Society of America. https://doi.org/10.2135/cssaspecpub24.c3

Freitas, A. D. S., Sampaio, E. V. S. B., Santos, C. E. R. S., & Fernandes, A. R. (2010). Biological nitrogen fixation in tree legumes of the Brazilian semi-arid caatinga. Journal of Arid Environments, 74(3), 344–349. https://doi.org/10.1016/j.jaridenv.2009.09.018

Garcia, L., Dubeux Jr, J. C., Sollenberger, L. E., Vendramini, J. M., DiLorenzo, N., Santos, E. R., ... & Ruiz-Moreno, M. (2021). Nutrient excretion from cattle grazing nitrogen-fertilized grass or grass–legume pastures. *Agronomy Journal*, *113*(4), 3110-3123.

Han, K. J., Smith, D. J., & Pitman, W. D. (2018). Potential of Cool-Season Species as Cover Crops and Forage in the Southeastern United States. *Crop, Forage & Turfgrass Management*, *4*(1), 1–7. https://doi.org/10.2134/cftm2017.05.0038

Haydock, K., & Shaw, N. (1975). The comparative yield method for estimating dry matter yield of pasture. Australian Journal of Experimental Agriculture, 15, 663–670

Jaramillo, D. M., Dubeux, J. C. B., Sollenberger, L., Mackowiak, C., Vendramini, J. M. B., DiLorenzo, N., Queiroz, L. M. D., Santos, E. R. S., Garcia, L., Ruiz-Moreno, M., & Santen, E. (2021). Litter mass, deposition rate, and decomposition in nitrogen-fertilized or grass–legume grazing systems. *Crop Science*, *61*(3), 2176–2189. https://doi.org/10.1002/csc2.20475

Jaramillo, D. M., Dubeux, J. C., Sollenberger, L. E., Vendramini, J. M., Mackowiak, C., DiLorenzo, N., ... & Ruiz-Moreno, M. (2021b). Water footprint, herbage, and livestock responses for nitrogen-fertilized grass and grass–legume grazing systems. *Crop Science*, *61*(5), 3844-3858.

Kristensen, R. K., Fontaine, D., Rasmussen, J., & Eriksen, J. (2022). Contrasting effects of slurry and mineral fertilizer on N2-fixation in grass-clover mixtures. *European Journal of Agronomy*, *133*, 126431. https://doi.org/10.1016/j.eja.2021.126431

Mannetje, L. T., & Haydock, K. P. (1963). The dry‐weight‐rank method for the botanical analysis of pasture. *Grass and Forage Science*, *18*(4), 268-275.

Muir, J. P., Pitman, W. D., & Foster, J. L. (2011). Sustainable, low‐input, warm‐season, grass–legume grassland mixtures: Mission (nearly) impossible?. *Grass and Forage Science*, *66*(3), 301-315.

Ledgard, S., Schils, R., Eriksen, J., & Luo, J. (2009). Environmental impacts of grazed clover/grass pastures. *Irish journal of Agricultural and food research*, 209-226.

Lemus, R., White, J. A., & Morrison, J. I. (2021). Impact of cool-season legume interseedings or timed nitrogen applications on yield and nutritive value of annual ryegrass. *Crop, Forage & Turfgrass Management*, *7*(1). https://doi.org/10.1002/cft2.20090

Moore, J. E., & Mott, G. O. (1974). Recovery of residual organic matter from in vitro digestion of forages. *Journal of Dairy Science*, *57*(10), 1258-1259https://doi.org/10.3168/jds.S0022-0302(74)85048-4

Mullenix, M. K., Bungenstab, E. J., Lin, J. C., Gamble, B. E., & Muntifering, R. B. (2012). CASE STUDY: Productivity, quality characteristics, and beef cattle performance from cool-season annual forage mixtures. The Professional Animal Scientist, 28(3), 379–386. https://doi.org/10.15232/S1080-7446(15)30371-5

Mullenix, M. K., Sollenberger, L. E., Wallau, M. O., Blount, A. R., Vendramini, J. M. B., & Silveira, M. L. (2016). Herbage Accumulation, Nutritive Value, and Persistence Responses of Rhizoma Peanut Cultivars and Germplasm to Grazing Management. *Crop Science*, *56*(2), 907–915. https://doi.org/10.2135/cropsci2015.08.0507

Newman, Y., Vendramini, J., & Blount, A. (2010). Bahiagrass (Paspalum notatum): Overview and Management: SS-AGR-332/AG342, 5/2010. *EDIS*, *2010*(4). https://doi.org/10.32473/edis-ag342-2010

Okito, A., Alves, B. R. J., Urquiaga, S., & Boddey, R. M. (2004). Isotopic fractionation during n2 fixation by four tropical legumes. Soil Biology and Biochemistry, 36(7), 1179–1190. https://doi.org/10.1016/j. soilbio.2004.03.004

Ortega-S, J. A., Sollenberger, L. E., Quesenberry, K. H., Jones, C. S., & Cornell, J. A. (1992). Productivity and persistence of rhizoma peanut pastures under different grazing managements. Agronomy Journal, 84, 799–804. https://doi.org/10.2134/agronj1992.000219620

Rouquette Jr, F. M. (2017). Invited Review: Management strategies for intensive, sustainable cow-calf production systems in the southeastern United States: Bermudagrass pastures overseeded with cool-season annual grasses and legumes. *The Professional Animal Scientist*, *33*(3), 297-309.

Rouquette, M., Corriher-Olson, V., & Smith, G. R. (2020). 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* (pp. 123–187). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00007-4

Rouquettes, F. M., Bransby, D. I., & Riewe, M. E. (2015). Grazing Management and Use of Ryegrass. In F. M. Rouquette & L. R. Nelson (Eds.), *CSSA Special Publications* (pp. 79–99). Crop Science Society of America. https://doi.org/10.2135/cssaspecpub24.c6

Sanderson, M. A., Jolley, L. W., & Dobrowolski, J. P. (2012). Pastureland and hayland in the USA: Land resources, conservation practices, and ecosystem services. Conservation outcomes from pastureland and hayland practices: Assessment, recommendations, and knowledge gaps. Allen Press, Lawrence, KS, 25-40.

Santos, E. R. S., Dubeux, J. C. B., Sollenberger, L. E., Blount, A. R. S., Mackowiak, C., DiLorenzo, N., Jaramillo, D. M., Garcia, L., Pereira, T. P., & Ruiz-Moreno, M. (2018). Herbage Responses and Biological N_2 Fixation of Bahiagrass and Rhizoma Peanut Monocultures Compared with their Binary Mixtures. *Crop Science*, *58*(5), 2149–2163. https://doi.org/10.2135/cropsci2018.02.0128

Shearer, G., & Kohl, D. H. (1986). N2-fixation in field settings: Estimations based on natural 15n abundance. Functional Plant Biology, 13(6), 699–756.

Silveira, M. L., Vendramini, J. M. B., Sellers, B., Monteiro, F. A., Artur, A. G., & Dupas, E. (2015). Bahiagrass response and N loss from selected N fertilizer sources. *Grass and Forage Science*, *70*(1), 154–160. https://doi.org/10.1111/gfs.12078

Sollenberger, L. E., Moore, J. E., Allen, V. G., & Pedreira, C. G. S. (2005). Reporting forage allowance in grazing experiments. Crop Science, 45(3), 896–900. https://doi.org/10.2135/cropsci2004.0216

Smith, A. P., Christie, K. M., Rawnsley, R. P., & Eckard, R. J. (2018). Fertiliser strategies for improving nitrogen use efficiency in grazed dairy pastures. *Agricultural Systems*, *165*, 274–282. https://doi.org/10.1016/j.agsy.2018.06.017

Solomon, J. K. (2022). Legumes for animal nutrition and dietary energy. In Advances in Legumes for Sustainable Intensification (pp. 227-244). Academic Press.

Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. (2020). Exploiting Biological Nitrogen Fixation: A Route Towards a Sustainable Agriculture. *Plants*, *9*(8), 1011. https://doi.org/10.3390/plants9081011

Thomas, R. J. (1992). The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage Science*, *47*(2), 133–142. https://doi.org/10.1111/j.1365-2494.1992.tb02256.x

Vendramini, J. M. B., Sollenberger, L. E., Lamb, G. C., Foster, J. L., Liu, K., & Maddox, M. K. (2012). Forage accumulation, nutritive value, and persistence of ‘Mulato II’ brachiariagrass in northern Florida. Crop Science, 52(2), 914–922. https://doi.org/10.2135/cropsci2011.06.0338

Vendramini, J., & Moriel, P. (2020). Management of forages and pastures in Lower-South: I-10 Corridor. In *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 101–122). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00006-2

Wilm, H. G., Costello, D. F., & Klipple, G. E. (1944). Estimating forage yield by the double-sampling method. Agronomy Journal, 36(3), 194203. https://doi.org/10.2134/agronj1944.00021962003600030003x

Woodley, A. L., Drury, C. F., Yang, X. Y., Phillips, L. A., Reynolds, D. W., Calder, W., & Oloya, T. O. (2020). Ammonia volatilization, nitrous oxide emissions, and corn yields as influenced by nitrogen placement and enhanced efficiency fertilizers. *Soil Science Society of America Journal*, *84*(4), 1327–1341. https://doi.org/10.1002/saj2.20079

CHAPTER 3

LIMPOGRASS SILAGE ASSOCIATED WITH SUPPLEMENT AS AN ALTERNATIVE FEED TO GROWING HEIFERS IN NORTH FLORIDA

ABSTRACT

Limpograss (*Hemarthria altissima*) is a warm-season perennial grass that has the potential to feed beef cattle during the scarcity periods with a considerable digestibility in late maturity, but because of the low crude protein concentration, protein supplementation is needed. The aim of this trial was to evaluate intake, nutrient digestibility, and animal performance of beef heifers fed limpograss silage, cultivar 'Gibtuck', plus different levels of a range cube (Cube; 32% CP and 68% TDN) supplementation. Twenty-four crossbred Angus x Brahman heifers (330 ± 16 kg live weight) were blocked by initial weight and then housed in a single pen and submitted to four different treatment; 1) control, no supplementation and *ad libitum* access to silage of limpograss; 2) 1.4 kg of Cube supplementation and *ad libitum* access to limpograss silage; 3) 2.8 kg of Cube supplementation and *ad libitum* access to limpograss silage: 4) 4.2 kg of cube supplementation and *ad libitum* access to limpograss silage. Silage dry matter intake, organic matter intake, and crude protein intake increased linearly ($P=0.05$) with the increasing levels of supplement, while the neutral detergent fiber intake decreased linearly ($P=0.05$). Apparent total tract digestibility of DM increased linearly with supplementation levels ($P<0.05$), with a quadratic increase for the OM digestibility ($P<0.05$), and a quadratic effect for the CP digestibility ($P<0.01$). The NDF digestibility presented a quadratic decrease ($P<0.01$). The average daily gain (ADG) present a quadratic effect ($P=0.01$) with the inclusion of supplementation (0.04, 0.42, 0.6, and 0.64 kg d^{-1} to 0, 1.4, 2.8, and 4.2 kg of supplement, respectively). The supplement improved diet digestibility and total DM intake, but reduced silage intake indicating a combined effect of the supplementation (substitutive and additive effect). Thus, it is concluded that the use of limpograss silage associated with supplementation can improve diet digestibility and increase animal performance of growing heifers and can be used in livestock production systems in North Florida.

Keywords: *Animal performance; forage intake; nutritive value; supplementation; protein; weight gain*

1. INTRODUCTION

Seasonality in forage production is a challenge observed in livestock systems based on pastures (Wallau et al., 2015) and livestock systems in the southeastern United States are mainly based on perennial warm-season grasses that are widely planted over tropical and subtropical climates regions (VENDRAMINI; MORIEL, 2020). The production and nutritive value of those forages are limited during the winter, and supplementation is often needed to maintain adequate nutritional animal status (VENDRAMINI; ARTHINGTON, 2010). Because warm-season forages become dormant in the pastures in late fall and winter reflecting the effects of short days, cooler temperatures, and frosts (Blount et al., 2020), some alternatives are required to overcome this scenario. Most warm-season grasses have the potential to increase the production during the growth period, which is common in C4 plants due to the photosynthetic system associated with the high efficiency in water and N-use. Thus, the practice of conserving forage during periods of greater production to use in the winter is a viable alternative (VENDRAMINI et al., 2010).

Limpograss [*Hemarthria altissima* (Poir.) Stapf & Hubb.] is a warm-season perennial grass native from southern Africa and cultivated in tropical and subtropical regions (VENDRAMINI; MORIEL, 2020). This grass was introduced in Florida in 1964 and cultivars were produced and selected for superior yield, persistence under grazing defoliation, improved nutritive value, and utility as stockpiled forage (QUESENBERRY et al., 2018). Limpograss usually has low crude protein concentration (CP), especially when stockpiled, with CP ranging from 34 to 125 g kg^{-1} (SOLLENBERGER et al., 1988; VENDRAMINI et al., 2010; AGUIAR et al., 2015; WALLAU et al., 2015; QUESENBERRY et al., 2018). Limpograss decline slowly its digestibility with maturity when compared to other warm-season perennial grasses (Santos et al., 2022), with IVDOM ranging from 520 to 594 g kg^{-1} (WALLAU et al., 2015; SANTOS et al., 2022; QUESENBERRY et al., 2018).

Limpograss has been used in grazing studies as stockpiling (Sollenberger et al.,1988; Newman; Sollenberger, 2005; Vendramini et al., 2019) in subtropical regions as an option during the winter (ABREU et al., 2022). Because the potential of limpograss to initiate growth after a frost and reasonable digestibility during late stages of maturity, stockpiling is considered a great alternative (VENDRAMINI; MORIEL 2020). Although stockpiling limpograss during the fall seems to be a viable option, surplus of forage production during the summer months can be stored as silage. The use of this material as silage could be useful to overcome the fodder shortage, since silage is a feasible alternative to overcome the weather-related limitations to conserve warm-season grasses in Florida (VENDRAMINI et al., 2010). Therefore, it might be a viable option to use limpograss silage as a new alternative to bridge the forage shortage window between seasons.

Although limpograss present a potential to be an alternative to livestock operations, protein is often the most limiting nutrient in animal performance. When forage is available during scarce scenarios and CP is a limiting factor, supplementation must be required to elevate diet CP and maintain or improve animal performance (AGUIAR et al.,2015; ABREU et al., 2022; SANTOS et al., 2022). Therefore, experiments with warm-season perennial grass conserved as an alternative to feed beef cattle plus protein supplementation may contribute to elaborate strategies that probably can mitigate the effects of the fall gap in the North Florida livestock production.

The hypothesis of this study was that feeding limpograss silage associated with protein supplementation could increase heifer growth performance, and that limpograss silage can be established as a useful alternative during the forage shortage between warm and cool seasons in southeast U.S. Therefore, the objective of this study was to evaluate the effect of supplementation on intake, digestibility, and animal performance of developing heifers fed on a limpograss silage-based diet.

2 MATERIAL AND METHODS

2.1 Experimental design, animals, and treatments

This study was approved by the University of Florida, Institutional Animal Care & Use Committee (IACUC) with the ID: IACUC202100000035. The experiment was performed at North Florida Research and Education Center located in Marianna, FL (30˚52’N, 85˚11’ W, 35 m asl), in the Feed Efficiency Facility (FEF) from April to July and lasted for 63 days. Twenty-four crossbred Angus x Brahman heifers with initial body weight (BW) of 330 ± 16 kg were used in this experiment, and before the beginning of the trial, all of them were weighted to obtain the initial weight. The animals were arranged in a randomized complete block design; the initial weight was used as a blocking criterion. All heifers were housed together in a pen (21.94 m x 14.94 m) with watering system. They were submitted to four different treatment: 1) control, with no supplement and *ad libitum* access to *Hermathria altissima* cv. Gibtuck silage (limpograss silage); 2) 1.4 kg of cube supplement (32%CP and 68% TDN) in dry matter (DM) basis *and ad libitum* access to limpograss silage; 3) 2.8 kg of cube supplement (DM basis) and *ad libitum* access to limpograss silage; 4) 4.2 kg of cube supplement (DM basis) and *ad libitum* access to limpograss silage.

The limpograss cv. Gibtuck used to make the silage was planted in early June of 2022 at NFREC Marianna, and fertilized with 67 kg N, 45 kg P, 90 kg K, and 16.8 kg S ha^{-1}. After 12 weeks, in early-September, the limpograss was harvested and chopped using a Claas JAGUAR 860 forage harvester (Claas, Harsewinkel, Germany), and then ensiled in bag (3.65 meters diameter) using silage bagger Versa model ID1017 (Versa Internal Density®, Astoria, OR), which was possible to produce 150 tons in 9 acres. Limpograss samples were collected before the ensiling process for chemical analysis. The period between the ensiling and silo opening was 8 months. In late-April of 2022, the silo bag was open, and the silage was offered daily to the heifers, once a day in the morning for 63 d. The silage was placed in the GrowSafe® feed bunk located at the pen in the FEF. Particle size distribution of limpograss silage was determined using the Penn State Particle Separator (PSPS) technique. The PSPS is a set of three sieves with fractions > 19.0, > 8.0, < 8.0 mm, and a bottom pan. Three samples of silage were collected from each feed bunk, at different points right after the offer time. After the two rounds of shaking, the material of each sieve and bottom pan were weighted and dried at 55°C for 72 h to determine the DM concentration on each separator sieve (WHITNEY et al., 2011).

A commercial range cube supplement with 32% CP was used in this trial as a protein supplementation, which was obtained from a commercial plant in South Florida. The supplement was offered using the Super Smart Feed (SSF; C-lock inc. Rapid City, SD, US), an automatic feeding system, which was placed at the pen side with two trays available and set to offer the supplement every day after 12:00 am, since the system was restarted. Samples of cube and silage were sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) to preliminary nutrient composition. The diet nutrient composition is presented in Table 3.1.

Table 3.1. Chemical composition of limpograss silage and cube

Item	Limpograss Silage	Cube
DM g kg^{-1} DM	416	900
OM g kg^{-1} DM	930	910
CP g kg^{-1} DM	73	320
NDF g kg^{-1} DM	674	301
TDN g kg^{-1} DM	-	680
$\delta\,^{13}C$, ‰	-15.04	-28.21
$\delta\,^{15}N$, ‰	2.52	3.26
Calcium %	-	.51
Phosphorus %	-	1.03
Magnesium %	-	.43
Potassium %	-	1.16
Sodium %	-	.120
Sulfur %	-	.39
Lactic Acid, % DM[1]	4.35	-
Acetic Acid, % DM[1]	0.53	-
Lactic/Acetic Ratio[1]	8.15	-
Propionic Acid, % DM[1]	0.00	-
Butyric Acid, % DM[1]	0.00	-
IsoButyric, % DM[1]	0.00	-
Total Acids, % DM[1]	4.89	-
pH[1]	3.80	-
NH_3, CP equivalent %, DM[1]	0.26	-
NH_3-N, % of total N[1]	3.61	-
VFA score[1]	8.32	-
PSPS Particle size, %[2]		
>19.0 mm	7.3	-
>8.0 mm	46.3	-
<8.0 mm	37.2	-
<4.0 mm	9.1	-

[1] Dairy One Forage Laboratory. DM, Dry matter; OM, Organic matter; CP, Crude protein; NDF, Neutral detergent fiber.

[2] Penn State Separator.

2.2 Intake and average daily gain

All heifers had an electronic radio frequency identification ear tag, which identifies each animal in both systems used in this trial. The GrowSafe® intake system measured silage intake and the SSF system measured supplement intake. The pen was equipped with three pairs of GrowSafe feed bunk (GrowSafe® System Ltd., Airdrie, AB, Canada) to record intake by weight change, measured to the nearest gram. In this system, each animal with an electronic identification (EID ear tag) had the feed behavior monitored and the intake was recorded during all the experiment. The silage was offered *ad libitum*, which means that orts were allowed, and every day before offer a new silage, the orts were discarded. For the protein supplement, the SSF system offered the amount of supplement pre-determined for each treatment computing the intake of each animal per day. The machine was filled up with supplement, and then the system released small portions of the amount of supplement to each animal accessing the tray gate; this process avoids other animals that were not in the tray to consume the supplement that was not for them. Throughout the experiment period, the animal live weight was assessed every 14 days. In this trial, fasting was not applied before weighing the animals. Rather, they were weighed over two consecutive days to obtain an average live weight (WARREN et al., 2008). To calculate the ADG, it was considered the difference between the final and initial body weight divided by the number of days of the animal performance trial. In the whole trial, all the weighing were recorded at the same time, which occurred between 8:00 am and 10:00 am, before the silage offer time, to reduce the disturbance over animal feeding behavior. Gain to feed ratio (G:F) was calculated by dividing the ADG by the total dry matter intake.

2.3 Apparent total tract digestibility

At the beginning of the trial, there were five days of adaptation to facilities, SSF, and all the animals had the same amount of supplement available in the machine (1.4 kg DM d^{-1}) and silage *ad libitum* in the bunk (Figure 3.1). After the adaptation period all the heifers were submitted to the diets. After 15 days, samples of feed (silage and concentrate) and feces were collected for five consecutive days to determine apparent total tract digestibility of DM, OM, CP, and NDF. Feed samples were collected once daily in three different points from the feed bunk and from the super smart feed. The fecal samples were collected twice a day, at 8:00 am and 4:00 pm, directly from the rectal ampoule.

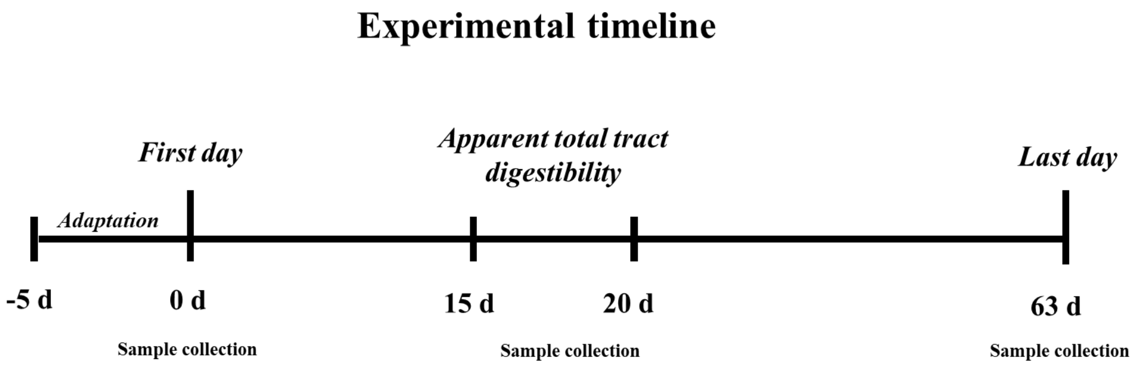


Figure 3.1. Growing heifer's animal performance timeline. Adaptation period consisted of 5 days during which the animals were familiarized to the machinery and feed. All the animals accessed 1.4 kg DM d^{-1} of supplement and silage *ad libitum*. First day consisted of the day zero of the experiment. Day 15th consisted of the first day of apparent total tract digestibility assay. Last day consisted of the end of the experiment. Sample collection consisted of fecal, urine, blood, ruminal fluid, and feed sample collection twice a day, except for feed, ruminal fluid, and blood.

All the samples collected were stored in labeled plastic bags and stored frozen at -20°C after sampling. The samples were thawed and dried at 55°C until constant weight, ground in a Wiley mill Model 4 (Thomas-Wiley laboratory Mill, Thomas Scientific, Swedesboro, NJ). to pass through a 2-mm screen and pooled within heifer for further determination of nutrient content, digestibility, and marker concentration. Indigestible NDF (iNDF) was used as an internal marker to obtain the apparent total tract digestibility of DM, OM, CP, and NDF were calculated as follows:

$$100 - 100 \times [(\textit{marker concentration in feed/marker concentration in feces}) \times (\textit{nutrient concentration in feces/nutrient concentration in feed})].$$

2.4 Laboratory analyses

To obtain DM and OM, approximately 1 g of feed and feces were weighed in duplicate, dried in an oven at 100°C for 24 h, and subsequently submitted at 550°C for 6 h in an ashing oven. To obtain the NDF concentration, approximately 0.5 g of feed and feces were weighed in duplicate inside of F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed by using heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991) in an Ankom 200 Fiber Analyzer (Ankom

Technology Corp.) To obtain the concentrations of iNDF in feed and feces samples were weighed (0.5 g) into Ankom F57 filter bags and then incubated (*in situ)* within the rumen of a cannulated steer for 288 h (12 d). After that, samples were washed, dried, and performed NDF analysis as described by Cole et al. (2011) and Krizsan and Huhtanen (2013).

Blood samples were collected during the first day, digestibility period, and the last day of the experiment. The animals were bled from the jugular vein when they were in the squeeze chute. Samples were collected into 10-mL sodium heparinized vacutainers (BD), labeled, and placed on ice. Blood was centrifuged at 2360 g for 15 min using a Beckman Coulter Allegra X-22R centrifuge (Beckman Coulter, Pasadena, CA). Red blood cells (RBC) and plasma were separated, and the plasma were stored at -20°C and then freeze dried for further determination of N, $\delta^{15}N$, C, and $\delta^{13}C$. The urine samples were also collected after manually stimulation to induce urination. The samples were collected into a plastic cup and then transferred to a labeled conical tube placed on ice.

To obtain the washed microbial pellet, ruminal fluids were collected in the first day of the digestibility period. The ruminal fluid was obtained through oral stomach tubing from each animal using an electric vacuum pump (RAMOS-MORALES et al., 2014; MUIZELAAR et al., 2020). The device used was an oral-stomach probe RUMINATOR (Profs Products, Wittibreut, Germany). The pump consisted of a pumping unit and a fluid glass container (GEISHAUSER et al., 2019). To avoid saliva contamination, the first suction fluid was discarded, and then the second fluid was collected and passed through two layers of cheesecloth fabric (grade 10, threads 20x12) and saved in a plastic bottle labeled and directly taken to the lab.

To obtain the microbial pellet to assess the microbial N, the samples were submitted to a differential centrifugation procedure using a Beckman Coulter Allegra X-22R centrifuge (Beckman Coulter, Pasadena, CA). An aliquot of 200 ml of the rumen fluid was dispensed into a Beckman 250-ml centrifugation tube and performing a “slow step” and then centrifuged at about 1.000 x g for 10 min at 4°C. After the slow process, the supernatants were placed into a Beckman 50-ml centrifuge tubes and the initial pellets were discarded. To promote the formation of bacterial pellet at the bottom of the tube, it was performed the “fast step” centrifugation process, and the supernatant was discarded; the pellets were centrifuged at about 20.000 x g for 20 min at 4°C and washed with 0.9% w/v NaCl. This washing step was repeated two more times. After centrifugations, the pellets were washed with distilled water and then freeze dried to assess the N, $\delta^{15}N$, C, and $\delta^{13}C$.

2.5 Isotopes analyses

Stable isotopic analyses were performed at the Forage Laboratory from the University of Florida, North Florida Research and Education Center. Subsamples of RBC, plasma, urine, fecal and ruminal microorganisms were collected and ball-milled in a Retsch Mixer Mill MM400 (Retsch, Haan, Germany) at 25 Hz for 9 min to reduce the particle size under 100 µm. Samples of approximately 5 mg were analyzed for total C, N, $\delta^{13}C$, and $\delta^{15}N$ through the Dumas dry combustion method using a CHNS analyzer vario MICRO Cube (Elementar, Frankfurt, Germany) coupled to an isotope ratio mass spectrometer (IRMS) using an IsoPrime100 (Elementar, Frankfurt, Germany). The $^{13}C/^{12}C$ ratios are presented in the conventional delta (δ) notation, in per mil (‰) relative to the Pee Dee Belemnite (PDB). The IRMS provide the $\delta^{13}C$, $\delta^{15}N$ and the concentration of both elements in the sample. Once the concentration of N was obtained, crude protein (CP) of all samples could be estimated multiplying total N concentration by 6.25 factor.

2.6 Statistical analysis

All the responses variables were analyzed as a randomized complete block design. Animals in the treatments were considered random effects. Treatments were considered fixed effects. The nutrient intake, apparent total tract digestibility, and average daily gain variables were tested for linear or quadratic effects using SAS PROC REG (SAS/STAT 15.1, SAS Institute). The ^{13}C and ^{15}N values of fecal, urine, ruminal microorganisms, red blood cells, and plasma were analyzed by fitting mixed models using SAS PROC MIXED. The variables of ^{13}C and ^{15}N from fecal, urine, ruminal microorganisms, red blood cells, and plasma variables reported are LSMEANS. The discrimination and means comparisons among limpograss before ensiling, limpograss silage, and sample types from the heifers fed on limpograss silage only was compared using a T-TEST. The differences were considered significant at $P \leq .05$.

3 RESULTS

3.1 Intake

The inclusion of the range cube protein supplement affected the limpograss silage ($P=0.05$) (Table 3.2). It was observed a combined effect with the inclusion of the supplement (Figure 3.1). As expected, the supplement DMI reached the threshold that was set ($P<0.01$). Both total DMI

(P=0.01) and total DMI as % BW (P=0.01) presented a quadratic effect with the increase of supplement levels.

The inclusion of supplement linearly decreased silage intake (P=.05). Silage OM intake was affected by the inclusion of supplement, decreasing linearly (*P*=.05), and the OM intake of the supplement increased quadratically (*P*<.01). Silage CP intake reduced linearly with increasing supplementation levels (*P*=.05). As expected, the supplement CP intake increased quadratically (*P*<.01) with increasing levels of the supplement. Silage NDF intake was negatively affected by the inclusion of supplement (*P*=.05). However, the supplement NDF intake increased quadratically (*P*<.01) because the amount of supplement was increased.

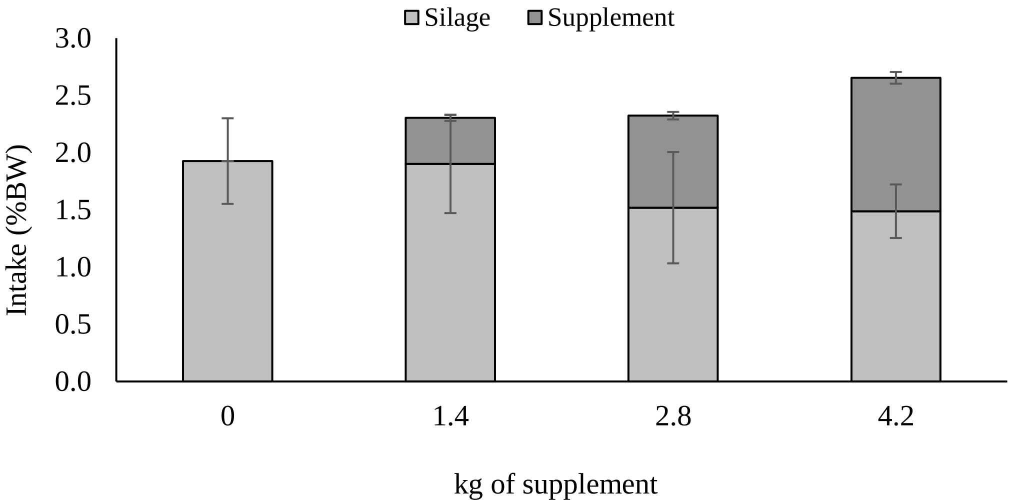


Figure 3.2. Dry matter intake (% BW) of growing heifers crossbred Angus fed on limpograss silage and protein supplement in kg d^{-1}. ^{1}0 = Ad libitum access to limpograss silage. 1.4 = Ad libitum access to limpograss silage and supplementation of 1.4 kg DM d^{-1} of range cube (32% CP, DM basis). 2.8 = Ad libitum access to limpograss silage and supplementation of 2.8 kg DM d^{-1} range cube (32% CP, DM basis). 4.2 = Ad libitum access to limpograss silage and supplementation of 4.2 kg DM d^{-1} of range cube (32% CP, DM basis). Limpograss silage had a linear effect (*P*=.02), SE =0.03; protein supplement had a quadratic effect (*P*<.01), SE =.02.

3.2 Animal performance

The inclusion of protein supplementation in limpograss silage-based diet improved the average daily gain of growing heifers following a quadratic effect (*P*= .01; Table 3.3). The gain observed by the first level of inclusion was 10 times greater than the gain in the control treatment, presenting a quadratic effect thereafter until the last level. Gain to feed ratio did not differ among the treatments (*P*=.07).

Table 3.2. Effects of different levels of cubes (32% crude protein) on the intake and average daily gain and gain to feed ratio of diets with limpograss silage ad libitum.

	kg of cubes*						
	0	1.4	2.8	4.2	SEM	*L*	*Q*
Intake, kg d^{-1}							
DM							
Silage	6.4	6.5	5.2	5.2	0.01	0.05	0.16
Supplement	-	1.4	2.8	4.0	0.01	<0.01	<0.01
Total	6.4	7.9	8.0	9.2	0.12	0.02	0.01
Total, %BW	1.9	2.3	2.3	2.6	0.03	0.06	0.02
OM							
Silage	6.0	6.0	4.9	4.8	0.11	0.05	0.16
Supplement	-	1.4	2.7	4.0	0.01	<0.01	<0.01
Silage, %BW	1.8	1.8	1.4	1.4	0.03	0.02	0.08
Supplement, %BW	-	0.36	0.7	1.1	0.02	<0.01	<0.01
Total, %BW	1.8	2.13	2.14	2.4	0.03	0.07	0.03
CP							
Silage	0.5	0.5	0.4	0.4	0.08	0.05	0.15
Supplement	-	0.48	0.97	1.42	0.04	<0.01	<0.01
Silage, %BW	0.1	0.1	0.1	0.1	0.02	0.02	0.08
Supplement, %BW	-	0.13	0.26	0.37	0.01	<0.01	<0.01
Total, %BW	0.14	0.27	0.37	0.48	0.02	<0.01	<0.01
NDF							
Silage	4.3	4.4	3.5	3.5	0.08	0.05	0.16
Supplement	-	0.9	1.9	2.7	0.01	<0.01	<0.01
Silage, %BW	1.3	1.3	1.0	1.0	0.02	0.02	0.08
Supplement, %BW	-	0.3	0.5	0.8	0.02	<0.01	<0.01
Total, %BW	1.3	1.5	1.6	1.8	0.02	0.05	0.02
ADG kg d^{-1}	0.04	0.42	0.60	0.64	0.03	0.06	0.01
G: F	0.01	0.05	0.08	0.07	0.005	0.07	0.10

*0 = Ad libitum access to limpograss silage. 1.4 = Ad libitum access to limpograss silage and supplementation of 1.4 kg DM d^{-1} of cube (32% CP, DM basis). 2.8 = Ad libitum access to limpograss silage and supplementation of 2.8 kg DM d^{-1} cube (32% CP, DM basis). 4.2 = Ad libitum access to limpograss silage and supplementation of 4.2 kg DM d^{-1} of cube (32% CP, DM basis). DM, Dry matter; OM, Organic matter; CP, Crude protein; NDF, Neutral detergent fiber; ADG, average daily gain; G: F, gain to feed.

3.3 Apparent total tract digestibility

The digestibility of DM linearly increased (*P*=.01), while OM and CP presented a quadratic effect (*P*=0.1) and (*P*<.01) respectively, with the inclusion of supplement (Table 3.3). The inclusion of 1.4 kg of supplement increased the DM digestibility in 20 g kg^{-1}, and 48 g kg^{-1} when the inclusion was 2.8 kg. The difference between the DM digestibility of the treatment with only silage and the treatment with silage and 4.2 kg of supplement was 64 g kg^{-1}. The CP digestibility had a great increase with inclusion of supplement, and the difference between only silage and 4.2 kg of supplement was 395 g kg^{-1}. The NDF digestibility decreased with the inclusion of the supplement and had a quadratic effect (*P*<.01).

Table 3.3. Effects of different levels of cubes (32% crude protein) on the apparent total tract digestibility of heifers fed on limpograss silage-based diet and DOM:CP ratio of the diet

	kg of Cubes*						
	0	1.4	2.8	4.2	SEM	*L*	*Q*
Digestibility							
DM g kg^{-1}	543	563	591	607	0.21	.01	.09
OM g kg^{-1}	574	593	620	638	0.23	.02	.01
CP g kg^{-1}	269	488	616	665	0.78	<.01	<.01
NDF g kg^{-1}	620	614	608	604	0.01	<.01	<.01
DOM: CP	7.8	5.0	3.8	3.4			

*0 = Ad libitum access to limpograss silage. 1.4 = Ad libitum access to limpograss silage and supplementation of 1.4 kg DM d^{-1} of cube (32% CP, DM basis). 2.8 = Ad libitum access to limpograss silage and supplementation of 2.8 kg DM d^{-1} cube (32% CP, DM basis). 4.2 = Ad libitum access to limpograss silage and supplementation of 4.2 kg DM d^{-1} of cube (32% CP, DM basis). **Standard error of treatment means, n = 6 animals per treatment. L= linear effect; Q = quadratic effect. DM, Dry matter; OM, Organic matter; CP, Crude protein; NDF, Neutral detergent fiber; DOM: CP, ration between organic matter digestibility and CP. Standard error of treatment means, n = 6 animals per treatment. L= linear effect; Q = quadratic effect.

The NDF digestibility decreased quadratically with the reduction of DOM:CP ratio (Figure 3.2). The reduction of NDF digestibility decreased in 20 g kg $^{-1}$ with the inclusion of 4.2 kg of supplement in the diet, reaching a DOM:CP ratio of 3.4.

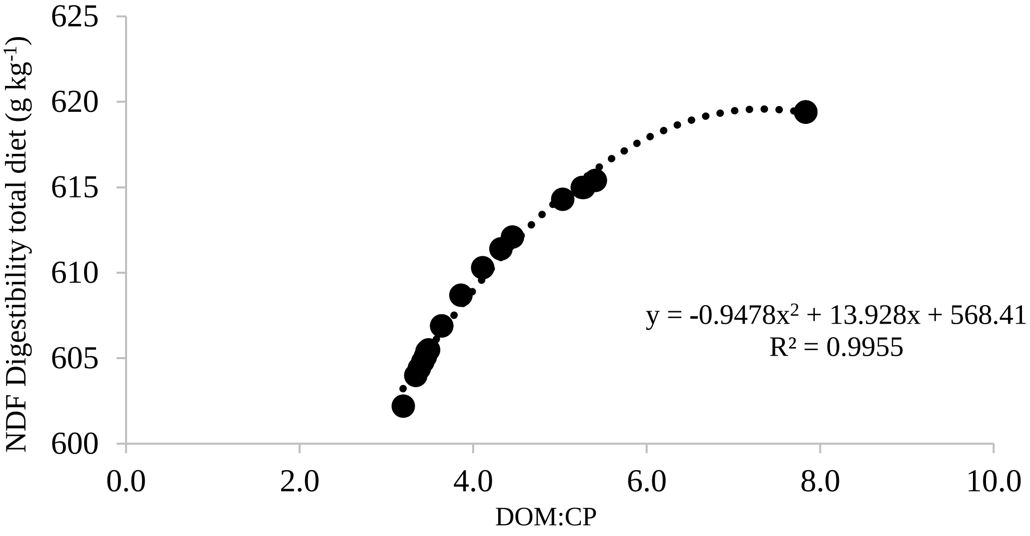


Figure 3.3. Relationship between DOM and CP affects and the neutral detergent fiber digestibility.

3.4 Isotopes responses

The values of δ ^{13}C and δ ^{15}N discrimination (Δ) of limpograss in the animal performance trial are presented in the Figure 3.4. The ^{13}C discrimination analysis indicated that limpograss silage is more depleted (*P*=.02) than fresh limpograss before the ensiling process with a Δ 2.27 ‰. When comparing silage and animal samples, silage δ ^{13}C was different from red blood cells (RBC; *P*<.01) and fecal samples (*P*=.01). The δ^{13}C for plasma, urine, and microbial pellets did not differ from limpograss silage (P<.01). The RBC discrimination was Δ 1.81 ‰ and the fecal samples had the greatest, Δ 3.17 ‰.

In the ^{15}N analysis, the δ ^{15}N of the fresh limpograss was similar to the limpograss silage (*P*=.2). All sample types presented a discrimination and was different from the silage (*P*<.01) regarding δ ^{15}N. Urine δ ^{15}N was more depleted than the rest of the sample types, and the value was closer to the diet (Δ -0.95‰). The discrimination of samples of RBC, plasma, fecal, and ruminal microorganisms to limpograss silage were Δ 2.17‰, 4.05‰, 3.56‰, and 2.01‰, respectively.

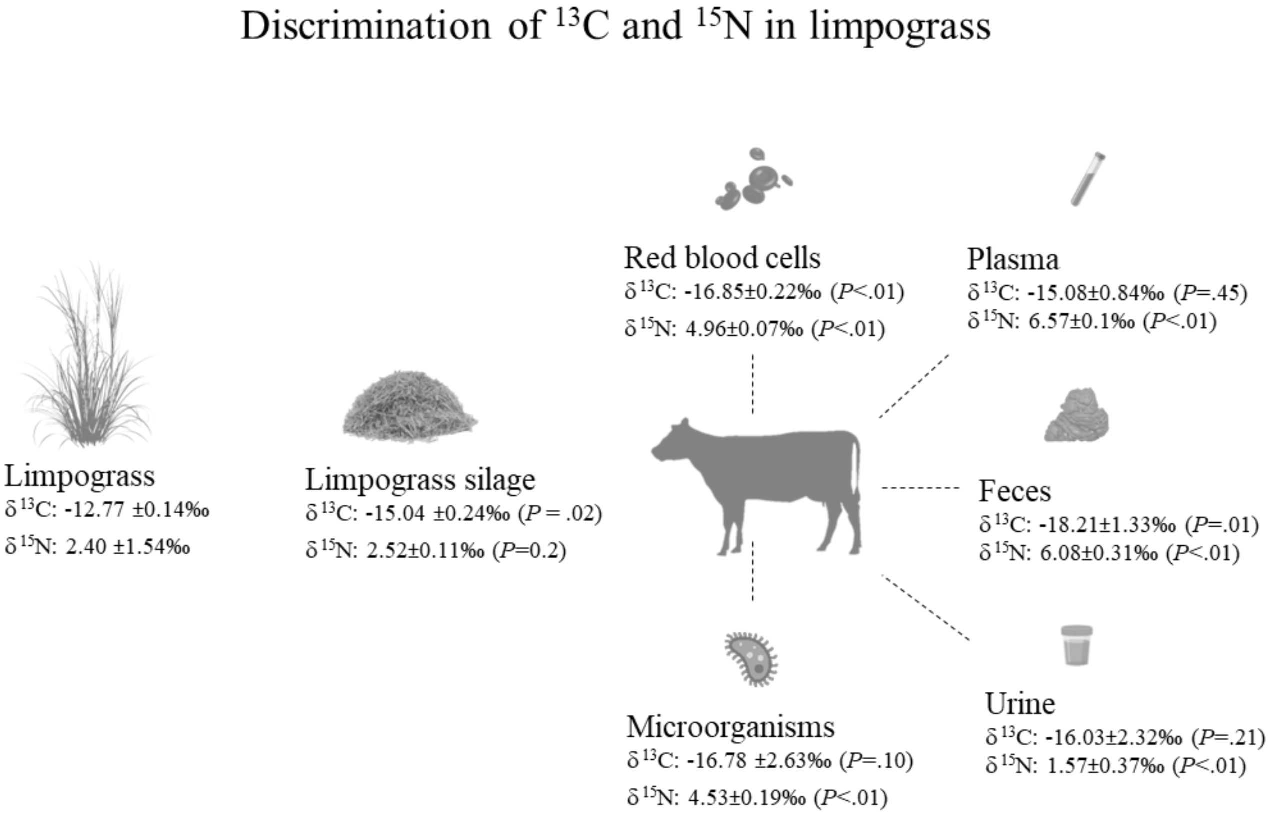


Figure 3.4. Discrimination of ^{13}C and ^{15}N in limpograss, limpograss silage, and RBC, plasma, feces, urine, microorganisms of the only silage treatment. The means of $\delta\ ^{13}$C and $\delta\ ^{15}$N from limpograss before and after ensiling were considered different at ($P<.05$). The means of $\delta\ ^{13}$C and $\delta\ ^{15}$N among limpograss silage and RBC, plasma, feces, urine, microorganisms were considered different at ($P<.05$).

The results of δ^{13}C and δ^{15}N of red blood cells (RBC), plasma, urine, ruminal microorganisms (RM), and fecal samples are presented in Table 3.4. The inclusion of supplement affected the δ^{13}C for RBC (*P*=.03), plasma (*P*=.09), urine (*P*=.05), RM (*P*=.01), and fecal (*P*=.02) samples. All sample types became more depleted in $\delta\ ^{13}$C, becoming more negative, with the inclusion of supplement. The inclusion of supplement into limpograss silage-based diet did not affect the $\delta\ ^{15}$N values for RBC (*P*=.66), plasma (*P*=.51), urine (*P*=.82), RM (*P*=.10), and fecal (*P*=.58) samples. All sample types did not have their $\delta\ ^{15}$N affected by supplementation level.

Table 3.4. Effects of different levels of range cube supplementation (32% crude protein) on RBC, plasma, ruminal microorganisms, urine, and fecal samples of heifers fed on diets with limpograss silage ad libitum.

Sample type	kg of cubes[*]				*P* value
	0	1.4	2.8	4.2	
			$\delta^{13}C$ ‰		
RBC	-16.85±0.2a[2]	-17.11±0.2a	-17.25±0.2ab	-17.78±0.2b	.03
Plasma	-14.93±0.68a	-16.41±0.68ab	-17.79±0.68b	-18.31±0.73b	.009
Urine	-16.03 ±0.82a	-16.26 ±0.75a	-17.78±0.75ab	-18.95±0.75b	.05
Fecal	-18.21±0.65a	-19.79±0.56ab	-20.90±0.56bc	-21.02±0.71c	.01
Microorganisms	-16.77± 0.95a	-18.57± 0.95ab	-19.78±0.95b	-21.24±0.95b	.02
			$\delta^{15}N$ ‰		
RBC	5.02±0.10	5.02±0.10	4.87±0.10	4.9±0.1	.66
Plasma	7.11±0.1	6.92±0.1	7.04±0.1	7.12±0.15	.51
Urine	1.92±0.55	1.32±0.45	1.65±0.45	1.35±0.45	.82
Fecal	5.65±0.20	6.0±0.21	6.24±0.20	6.36±0.20	.10
Microorganisms	4.29±0.23	4.75±0.23	4.61±0.23	4.47±0.23	.58

[*] 0 = Ad libitum access to limpograss silage. 1.4 = Ad libitum access to limpograss silage and supplementation with 1.4 kg DM d^{-1} range cube with 32% CP. 2.8 = Ad libitum access to limpograss silage and supplementation with 2.8 kg DM d^{-1} range cube with 32% CP. 4.2 = Ad libitum access to limpograss silage and supplementation with 4.2 kg DM d^{-1} range cube with 32% CP. Values of RBC, and plasma are referent to sampling after 63 days of animal performance. Values of urine, fecal, and microorganisms are referent to sampling after 15 days of diet adaptation.

[2] Means followed by the same letter within rows are not different (*P*>0.05).

4 DISCUSSION

4.1 Nutrient intake

Protein supplementation can often improve the intake and performance of animal fed low-quality forages (MOORE; KUNKLE, 1995). In this current study, the limpograss silage voluntary DMI decreased with the inclusion of protein supplementation. Our study has hypothesized that protein supplementation incorporated into limpograss silage-based diet can improve the response of growing heifers, meanwhile providing evidence that limpograss silage can be a feasible alternative to bridge the forage fall gap in beef cattle operations in North Florida. Limpograss during late maturity has low CP; therefore, protein supplementation is recommended to meet the nutrient requirements (VENDRAMINI; ARTHINGTON, 2010; AGUIAR et al., 2015; WALLAU et al., 2020). Usually, supplementation improves forage intake and additionally provides nutrients, which can overcome the forage nutrient deficit, and may increase the voluntary intake, digestibility, and animal performance (MOORE, 1980; MOORE et al., 1999; PATERSON et al., 1994). According to SOUSA et al. (2022), protein supplementation has the potential to increases the dietary CP up to the minimum requirement to improve the microbial growth and fiber degradation (80 to 100 g kg^{-1}) which may increase the forage intake. Generally, the addition of protein (nitrogen) affects forage intake, especially in low-quality forages that have less than 6 and 8% of CP (NRC, 2016). So, it was expected an improvement on forage voluntary intake with the protein supplementation; however, total intake increased, but silage intake decreased after the initial level of supplementation.

One possible reason that can explain the reduction of silage DMI and increasing of total intake in this present study is that the inclusion of supplement improved diet quality, by the inclusion of rapid and available digestible protein in the rumen, promoting fermentation and increasing passage rate. Voluntary intake is limited by physical conditions in the tract and specially by the digesta accumulated in the rumen (ALLISON, 1985). According to Mertens and Grant (2020), the physical limitation occurs when the product of the intake (forage) is equal to the animal fill-processing capacity, and intake and passage rate are connected. The passage rate reduced the fill effect, and the heifers eating supplement had more conditions to increase the total intake and reduce the forage intake. The inclusion of supplement, that had a different diet composition compared with the limpograss silage, might have affected the silage DMI. This can be related with the filling effect, once the "space" occupied by silage was replaced by concentrate. All the heifers had the accesses to concentrate right after 12 am, eating the supplement before the silage offer. They reached the filling effect by the inclusion of supplement and reduced the voluntary intake of forage. In other words, it was observed a substitutive effect, because the animals were consuming less silage while eating the supplement offered, which was consumed entirely. Substitution, however, was not observed in a 1 by 1 change, or unit by unit, because the amount of supplement was not the same of the silage that was not consumed.

The substitutive effect can be negative when the amount of the voluntary forage intake with supplement is greater than the amount of intake without supplement, or positive when the voluntary forage intake with supplement is not greater than without supplement (MOORE et al., 1999). This effect can be observed in the total DMI that was increased with the inclusion of supplement. In this case, it can be considered that protein supplementation had a combined effect, that is the association between additive and substitutive effects (MOORE, 1980; MOORE et al., 1999). The combined effect was observed because the reduction of forage DMI did not affect the total DMI and OM intake, which were increased. This result is related to the hypothesis because a theorical drastic reduction of forage DMI is not desirable, as a robust substitutive effect, which is 1 unit of concentrate replacing 1 unit of forage, without increase the total intake. Thus, the amount of supplement that is offered need to be evaluated nutritionally and economically, since generally the amount of supplement is restricted, while the amount of forage is ad libitum (MOORE, 1980). The reduction of silage in this current study, however, was not negative, and the combined effect increased the total intake once the amount of supplement included was not superior or equal to the reduction obtained in silage. This result agrees with the assumption of the use of supplement for low-quality forages since the total intake was increased.

Some studies reported an enhance of forage intake of low-quality forages by protein supplementation (Bohnert et al., 2011; Cappellozza et al., 2021; Shreck et al., 2021; Wickersham et al., 2008), with forages presenting a range of 29 to 46 g CP kg^{-1}, respectively. Sousa et al. (2022) did not observe any effect of supplement in the DMI of forages (average of 58 g kg^{-1} of CP). Also, a recent study published by Abreu et al. (2022) reported that molasses-based liquid supplementation did not affect the limpograss hay (40 g kg^{-1} of CP) intake as well. Although our limpograss silage presented 73 g kg^{-1} of CP, that is considered the minimum value to the ruminal microorganism condition (Moore, 1991), our results were different, and it was observed a linear reduction of forage DMI with the inclusion of supplement. As was observed in this current trial, Adams et al. (2022) reported a reduction of voluntary intake of bermudagrass hay (105 g kg of CP) offered *ad libitum* supply by loose and extruded dried distillers' grains cubes, where the DOM: CP < 7 resulted in the reduction of the voluntary forage intake.

Low-quality forages fed alone requires supplementation, and some factors can affect the responses of supplementation in animal performance, such as intake and forage quality and composition (MOORE; KUNKLE, 1998). Forage DMI has a negative relation to the DOM: CP ratio, which the threshold value is greater than 6 to 8 (MCCOLLUM III, 1997). In most of the cases, low-quality forages has the DOM: CP up to 7, and it means that the forage has a deficit of protein relative to available energy, which may promotes the reduction of forage intake (MOORE; KUNKLE, 1998). Most of the research in the literature considered the DOM:CP threshold > 7, reported by Moore and Kunkle (1995), and it is possible to find some studies that reports the DOM:CP in limpograss trials (HOLDERBAUM et al., 1991, LIMA et al., 1999, NEWMAN et al., 2002; VENDRAMINI et al., 2010, and WALLAU et al., 2020). The effect of supplement on forage intake is not fixed, can vary in response to forage quality and supplement composition (ADAMS et al., 2022). Moore et al. (1999) in a data base study constructed to describe and estimate supplementation effects in beef cattle forage intake reported that in most of trials, the supplement decreased the forage intake of improved warm season, and when forage was the only feed and intake was greater than 1.75 % of BW, the supplement reduced forage intake. Therefore, probably this is related with the reduction of forage DMI in this current trial, since it was observed that the treatment without supplement, forage intake was 1.93% BW. Also, the DOM: CP obtained by this current study was greater than 7, and it is at the threshold suggested by Moore and Kunkle (1995) whereas the supplementation may improve the intake and animal performance.

The amount of supplement offered can be related with the forage reduction, and according to Moore and Kunkle (1998), when the DOM: CP is > 7 and a small amount of supplement (e.g., 0.5% BW) may increase the intake, and the nutrients provided by the association between forage and concentrate will improve the animal responses. They also reported that, when there is no deficit of protein, and large amounts of supplements are consumed (more than 0.8 % of BW), the voluntary intake and animal performance will be less than expected. Perhaps, the DOM: CP ratio is related with the forage voluntary intake depression. This effect was observed in this current trial, at the first level of supplementation, which was offered 0.4 % of BW of supplement and the intake of forage was not changed. However, the forage intake was reduced when the supplementation level was 0.8% BW. Although the DOM: CP was between 7 and 8, the result is demonstrating a combination of factors (physical and nutritional), which the reduction of voluntary forage intake and the increase of total intake was observed, and the animal performance was positive. It is important to consider that the current trial used a self-feeding system, which was possible to measure the individual diet ingredient intake (by each animal in the pen) and then, to assess the total diet intake and digestibility once the interaction of forage and cube in the rumen must be considered. The approach to feed and measure the animal intake, and the use of self-feeding apparatus was positive in this trial. The use of the two machines (GrowSafe® and Super smart feed) at the same time, provided some advantages, such as less impact on animal behavior, less time consuming in feeding process, and data recording.

4.2 Animal performance

Animal fed low-quality forages may respond to supplementation strategies, increasing intake and animal performance (MOORE et al., 1999). Heifers fed limpograss silage only maintained body weight, even considering limpograss being a low-quality warm season perennial grass. The silage alone (control) presented a gain of 0.04 kg d^{-1} only. Perhaps the maintenance of the weight can be considered a strategy for dry cows, since limpograss silage has a feeding potential for mature beef cows during periods of scarcity. However, the supplementation is deemed necessary in limpograss diets for animal maintenance or performance (SANTOS et al. 2022). Additionally, as expected, the inclusion of levels of range cube protein supplement improved the average daily gain of the heifers fed on limpograss silage-based diet. This predicted result is based on and validate by the premise that low-quality forages with DOM: CP greater than 7 respond to supplementation and increase animal performance (Moore; Kunkle, 1995) in the case of the current study, animal weight gain.

As discussed before, although the forage intake had decreased, the total intake increased, which can be related with the supplementation strategy, inclusion of concentrate, that increased the average daily gain. The additional gain can be related with the availability of readily available protein in the rumen provided by the supplement, which improved the diet digestibility, resulting in high levels of nutrient assimilated by the animals. The gain between the level 1.4 and the 2.8 was considerable, however the level 4.2 may not be economical because of the quadratic response. It is noticeable that the inclusion of high levels of protein supplementation is not profitable. According to Hersom et al. (2011), every additional unit of input generate an expected small amount of output, so the second level of supplement has lesser effect on the performance as much as the first level had, and the next will not had as the previous and so on. The author suggest that is necessary to find the point at which cattle performance and cost expenses are optimized.

In Florida, many trials were conducted to assess the animal performance of animals with limpograss diet with or without supplementation. Sollenberger et al. (1988) compared the ADG of limpograss and bahiagrass (*Paspalum notatum* Flugge) pastures grazed by yearling steers under continuous stocking. They did not find any difference between grasses in two years of research. They reported a gain of 0.33 kg d^{-1} on limpograss and 0.38 kg d^{-1} on bahiagrass. Vendramini and Arthington (2010) evaluating the performance of beef yearling heifers (338 kg LW) grazing stockpiled limpograss pastures supplemented with cottonseed meal (480 g CP kg^{-1}) observed an increase of ADG with the supplement strategy. They found an ADG of 0.14 kg d^{-1} in animals just grazing limpograss and 0.44 and 0.64 kg d^{-1} receiving 1.1 and 2.2 kg $head^{-1}$ d^{-1} of cottonseed meal, respectively. Newman et al. (2002) evaluated ADG of beef heifers and sward characteristics on continuously stocked limpograss pastures grazed to different heights and receiving 0 or 0.8 kg d^{-1} of 44% CP corn-urea meal as a supplementation. They reported an increase on ADG by supplementation, approximately 0.2 kg d^{-1} was the difference, where the gains of supplemented heifers were not affected by canopy heights. Lima et al. (1999) assessed the ADG of heifers (350 kg LW) on fertilized limpograss pastures with two types of supplementations. The pasture fertilized with 50 kg N ha^{-1} with no supplementation had the least gain, 0.06 kg d^{-1}, close to the results found for the control treatment in the current trial. With the inclusion of corn-urea (0.41 kg d^{-1}) and corn-urea plus rumen-undegraded protein (0.56 kg d^{-1}), results are in concordance with the values obtained by this present study. The increase of fertilization to 150 kg ha^{-1} affected the ADG, whereas the no-supplemented treatment increased to 0.36 kg d^{-1}, while corn-urea (0.39 kg d^{-1}) and corn-urea plus rumen-undegraded protein (0.47 kg d^{-1}) showing no difference to the supplemented treatment. In their study, with the highest N application, the DOM:CP decreased in relation to the lower N application, which means that the response to supplementation would be what was expected.

The change in DOM:CP can alter the response to supplementation, and this effect is probably related with the low gain in the highest level of supplementation in this current trial, since the DOM:CP was affected by the increase in supplementation. Brown and Adjei (2001) assessed the animal performance of steers grazing limpograss supplemented by molasses-urea and hydrolyzed poultry feather meal. They found ADG of 0.30 kg d^{-1} for only pasture and 0.40 kg d^{-1} for protein supplementation. Holderbaum et al. (1991) determined the animal performance of steers on pastures of limpograss supplemented with corn-urea meal (210 g kg^{-1} CP – low: and 500 g kg^{-1} CP - high). No supplementation had the lowest gain, 0.03 kg d^{-1} and with the inclusion of supplementation the gains increased to 0.53 and 0.59 kg d^{-1} for low and high, respectively. They reported that the inclusion of supplementation increased significantly the ADG, but the level of CP did differ, which means that the highest level is not feasible. The authors suggested that effectiveness of urea as a supplemental source of N is dependent on the diet balance of energy and protein, the DOM: CP.

Thus, the strategy for using limpograss as an alternative to overcome the difficult periods of forage scarcity in beef cattle is attractive. Limpograss is resilient to cool season and has the potential to grow, great production, and stable TDN concentration during late maturity that results in animal gain (VENDRAMINI, 2008). To improve animal gain in limpograss pastures, Sollenberger et al. (1988) suggested high level of CP supplementation. The strategic supplementation of the appropriate supplement feed in specific moment often has positive outcome. Del Curto et al. (1989) evaluated the effect of protein supplementation on intake and ADG of beef cows (454 kg BW) fed by dormant range forage and concluded that was possible to reduce the weight losses in the winter when increasing the CP supplement. The protein supplementation to stimulate performance, milk production, and reproductive performance is mandatory to sustainable and viable beef cattle herds (HERSOM et al., 2011). The feed to gain calculated for the diets did not differ among the treatments and did not present any statistical difference.

4.3 Apparent total tract digestibility

Limpograss has low CP protein concentration, especially in late maturity, and because stems and leaves are different in CP concentration, the leaf:stem ratio can change it, decreasing limpograss CP significantly (VENDRAMINI et al., 2008; VENDRAMINI; MORIEL, 2020). The CP concentration in this trial was in the range reported by Vendramini and Moriel (2020), around 73 g kg^{-1}; however, the value obtained was greater than 39 g kg^{-1} of CP reported by Abreu et al. (2022) for limpograss hay and 40 g kg^{-1} of CP reported by Wallau et al. (2020) for limpograss pastures stockpiling, but lesser than reported by Vendramini and Arthington (2010) that was 120 g kg^{-1} of CP. The limpograss silage apparent total tract digestibility of OM obtained in this study was 574 g kg^{-1} of OM, a reasonable value considering that was a warm-season perennial grass in an advanced stage of maturity. This result is in concordance with Vendramini and Moriel (2020) statement, which limpograss usually has great digestibility after long regrowth intervals and the result found in this current research was greater than the IVDOM of 523 g kg^{-1} OM found by Wallau et al. (2020), which evaluated the cultivar ‘Gibtuck’ in a stockpiling pasture with 12 weeks of age. Vendramini and Arthington (2010) found the IVDOM of limpograss cultivar Floralta 500 g kg^{-1} of OM. The limpograss used to make the silage of this study was harvested with 84 days.

In fact, the range cube protein supplement used in this current trial improved the digestibility of nutrients in limpograss silage-base in total diet, except for NDF. The interaction between forage and supplement affecting the digestibility was also reported by some researchers. Beaty et al. (1994), assessing the effect of protein concentration and frequency of supplementation of low-quality forages, found an increase from 487 to 545 g kg^{-1} for total DM digestibility in response to protein supply that increased from 12 to 39% CP. Adams et al. (2022) reported an increase in DM digestibility of bermudagrass hay offered *ad libitum* supply by loose and extruded dried distillers' grains cubes in heifers, where bermudagrass only was 400 g kg^{-1} and the total diet digestibility with the high level of inclusion of protein supplementation was 550 g kg^{-1}. Abreu et al. (2022) reported an increase in DM and OM digestibility of diet based on limpograss hay supplemented by molasses (32% CP). The authors reported an increase from 285 g kg^{-1} to 334 g kg^{-1} in DM digestibility and 346 g kg^{-1} to 389 g kg^{-1} of OM after supplementation of 0.9 kg d^{-1} of molasses (32% CP).

In this current study, some factors must be considered to explain the effects reported. It is important to consider that the integration of different feed into the rumen system promotes changes in the rumen environment, which modifies the digestibility of feeds as was described by DOYLE et al. (2005). Low-quality forages usually have low concentrations of CP, which can reduce efficiency of fiber-digesting microorganisms. Perhaps, this effect can be explained by the extra protein availability in the rumen and the supplementation strategy, which favored the microorganism growth, resulting in the increase of digestibility (MERTENS; GRANT, 2020). The increase in DM, OM, and CP digestibility resulted from the inclusion of supplement, which is easily digested. However, the fiber digestion by ruminal microorganisms is a relative slow process. The material retained in the rumen reduces the intake because of the filling effect (NRC, 2016), which was observed after the inclusion of supplement that permitted the increase in total intake, likely due to the elevation of passage rate. The elevation of passage rate, that is the reduction of retention time, reduce fiber degradation, explaining the reduction observed for NDF digestibility (Table 3.3). Perhaps, a possible explanation not evaluated in the current study is a depression of the pH caused by the interaction of concentrate and forage in the rumen. The elevation of concentrate levels in ruminant diets can drop the rumen pH below 6.2 because of the use of rapidly fermentable carbohydrates by microorganisms (MOULD et al., 1983). Due to the rapid degradability, microorganism may utilize more the concentrate instead of digesting fiber, promoting the fermentation of rapidly fermentable substrates first and then decreasing the fiber digestibility (MERTENS; GRANT, 2020). Also, there is a strong relationship (R^2 0.99) between the NDF digestibility reduction and the DOM: CP. The elevation of supplement in the limpograss silage diet shifted the ratio towards 3.4 energy and protein balance because the CP of the diet increased. Therefore, the elevation of supplementation levels improved the digestion, promoted the passage rate, and then reduced the fiber digestibility. Greater nutrient digestibility improves nutrient assimilation to meet the nutritional requirements, which will permit the animal response to reach the productive potential.

4.4 Isotopes responses

The inclusion of supplement affected the $\delta^{13}C$ value, leading to depleted $\delta^{13}C$. Generally, the C3 plants present natural abundance of ^{13}C around -28 to -31‰ and C4 plants a range of -12 to -14‰, which can be used to identify functional groups in different proportion and samples (JARAMILLO et al., 2022; PEREIRA NETO et al., 2019). Limpograss is a C4 plant and presented a $\delta^{13}C$ value of -12‰ in this current study. The limpograss silage presented a smaller value than the limpograss before ensiling. The difference between fresh limpograss and the limpograss ^{13}C signal probably is a result of a fractionation in the ensiling fermentation, which could be, theoretically, related with the anaerobic action of microorganisms in the silo. Natural abundance variation of stable isotopes is entrenched in fractionation that occurs in chemical, biological, and physical processes resulting in specific isotopic signature in biological materials that permit to identify the sources (GANNES et al., 1998). However, this is just a hypothesis because an appropriate trial must be conducted to assess the ^{13}C fractionation in ensiling fermentation. Plasma had the same value of ^{13}C of the silage and did not preset any discrimination. The fecal presented the greatest discrimination in the digestion process of the limpograss silage, what was expected because it is where the discrimination occurs more, maybe because of the fractionation (PEREIRA NETO et al., 2019). In this trial, the inclusion of supplement in the limpograss silage diet resulted in samples more depleted in $\delta^{13}C$ for all sample types. This result was expected since the inclusion of a C3 source, into a C4 source can generate a mixture and then a different $\delta^{13}C$ (Fry, 2006), and the signal will depend on the proportion of the sources. The microorganisms presented the most realistic value to match the diet values, because the microorganisms are digesting the diet and can reflect the signal with less discrimination.

Generally, the $\Delta^{15}N$, difference between diet and animal samples, are more enriched and can be in the range of 3 to 5‰, caused by fractionation during deamination and transamination

(GANNES et al., 1998). All the sample types did not change δ^{15}N with the inclusion of supplement and maybe it is because the ^{15}N sources had similar values. Concentration of N in the sample can also be another source of variation, affecting the results. It is possible to observe that the δ ^{15}N became more enriched from the diet to animal samples (STEELE; DANIEL, 1978). Among all sample types for δ^{15}N, urine had the most discrimination, likely related to greater excretion of ^{14}N used in the metabolism (GUARNIDO-LOPEZ et al., 2021).

5. CONCLUSION

The cube supplementation provide a combined effect, reducing voluntary silage intake but increasing total dry matter intake. The apparent total tract digestibility of nutrients increased with the inclusion of protein supplementation, except for the NDF digestibility. The inclusion of protein supplementation improved animal performance, and based on our results, the most feasible level of range cube protein supplementation to supplement growing heifers fed on limpograss silage is 2.8 kg d-1, resulting in a gain of 0.6 kg d^{-1}. The results of δ ^{13}C of limpograss discrimination before and after ensiling process brought some questions about what happen into fractionation of ^{13}C in fermentations processes, especially between limpograss before and after ensiling, and further experiments could provide more detailed information. Studies assessing limpograss as silage to feed beef cattle are scarce, therefore, the results presented by this trial are novel and indicate that limpograss silage can be an alternative to feed growing heifers associated with protein supplementation. This feeding strategy can be extrapolated to feed mature cows in cow-calf operations in North Florida during the fall gap.

6. REFERENCES

Abreu, D., Dubeux, J. C. B., Queiroz, L. D., Jaramillo, D., Da Silva Santos, E. R., van Cleef, F., Vela-Garcia, C., DiLorenzo, N., & Ruiz-Moreno, M. (2022). Supplementation of Molasses-Based Liquid Feed for Cattle Fed on Limpograss Hay. *Animals*, *12*(17), 2227. https://doi.org/10.3390/ani12172227

Adams, J. M., Robe, J., Grigsby, Z., Rathert-Williams, A., Major, M., Lalman, D. L., Foote, A. P., Tedeschi, L. O., & Beck, P. A. (2022). Effects of supplementation rate of an extruded dried distillers' grains cube fed to growing heifers on voluntary intake and digestibility of bermudagrass hay. *Journal of Animal Science*, *100*(5), skac097. https://doi.org/10.1093/jas/skac097

Aguiar, A. D., Vendramini, J. M. B., Arthington, J. D., Sollenberger, L. E., DiLorenzo, N., & Hersom, M. J. (2015). Performance of beef cows and calves fed different sources of rumen-degradable protein when grazing stockpiled limpograss pastures. *Journal of Animal Science*, *93*(4), 1923–1932. https://doi.org/10.2527/jas.2014-8599

Allison, C. D. (1985). Factors affecting forage intake by range ruminants: a review. *Rangeland Ecology & Management/Journal of Range Management Archives*, *38*(4), 305-311

Beaty, J. L., Cochran, R. C., Lintzenich, B. A., Vanzant, E. S., Morrill, J. L., Brandt, R. T., & Johnson, D. E. (1994). Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. *Journal of Animal Science*, *72*(9), 2475–2486. https://doi.org/10.2527/1994.7292475x

Blount, A. R., Wallau, M., Rios, E., Vendramini, J. M. B., Dubeux, J. C. B., Babar, A., Mulvaney, M., & Quesenberry, K. H. (2020). *2020 Cool-Season Forage Variety Recommendations for Florida*. 6.

Bohnert, D. W., DelCurto, T., Clark, A. A., Merrill, M. L., Falck, S. J., & Harmon, D. L. (2011). Protein supplementation of ruminants consuming low-quality cool- or warm-season forage: Differences in intake and digestibility. *Journal of Animal Science*, *89*(11), 3707–3717. https://doi.org/10.2527/jas.2011-3915

Brown, W. F., & Adjei, M. B. (2001). Urea and(or) feather meal supplementation for yearling steers grazing limpograss (Hemarthria altissima var. 'Floralta') pasture. *Journal of Animal Science*, *79*(12), 3170. https://doi.org/10.2527/2001.79123170x

Cappellozza, B. I., Bohnert, D. W., Reis, M. M., Swanson, K. C., Falck, S. J., & Cooke, R. F. (2021). Influence of amount and frequency of protein supplementation to steers consuming low-quality, cool-season forage: Intake, nutrient digestibility, and ruminal fermentation. *Journal of Animal Science*, *99*(6), skab112. https://doi.org/10.1093/jas/skab112

Cole, N. A., K. McCuistion, L. W. Greene, and F. T. McCollum. 2011. Effects of concentration and source of wet distillers grains on digestibility of steam-flaked corn-based diets fed to finishing steers. Prof. Anim. Sci. 27:302–311.

Doyle, P. T., Francis, S. A., & Stockdale, C. R. (2005). Associative effects between feeds when concentrate supplements are fed to grazing dairy cows: A review of likely impacts on metabolisable energy supply. *Australian Journal of Agricultural Research*, *56*(12), 1315. https://doi.org/10.1071/AR05087

Fry, B. (2006). *Stable isotope ecology* (Vol. 521, p. 318). New York: Springer.

Gannes, L. Z., del Rio, C. M., & Koch, P. (1998). Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *119*(3), 725–737. https://doi.org/10.1016/S1095-6433(98)01016-2

Geishauser, T. (2019). An Instrument for Collection and Transfer of Ruminal Fluid and for Administration of Water Soluble Drugs in Adult Cattle. *The Bovine Practitioner*, *1993*(27), 27-42. https://doi.org/10.21423/bovine-vol1993no27p27-42

Guarnido-Lopez, P., Ortigues-Marty, I., Taussat, S., Fossaert, C., Renand, G., & Cantalapiedra-Hijar, G. (2021). Plasma proteins δ15N vs plasma urea as candidate biomarkers of between-animal variations of feed efficiency in beef cattle: Phenotypic and genetic evaluation. *Animal*, *15*(8), 100318. https://doi.org/10.1016/j.animal.2021.100318

Hersom, M., Alva, E., Thrift, T., & Yelich, J. (n.d.). *Strategic Supplementation of Beef Cows to Correct for Nutritional Imbalances*. 14.

Holderbaum, J. F., Sollenberger, L. E., Moore, J. E., Kunkle, W. E., Bates, D. B., & Hammond, A. C. (1991). Protein Supplementation of Steers Grazing Limpograss Pasture. *Journal of Production Agriculture*, *4*(3), 437–441. https://doi.org/10.2134/jpa1991.0437

Jaramillo, D. M., Dubeux, J. C. B., Ruiz-Moreno, M., DiLorenzo, N., Vendramini, J. M. B., Sollenberger, L., Mackowiak, C., Queiroz, L. M. D., Abreu, D. S., Garcia, L., Santos, E. R. S., & Kieke, B. A. (2022). Stable isotopes of C and N differ in their ability to reconstruct diets of cattle fed C3–C4 forage diets. *Scientific Reports*, *12*(1), 17138. https://doi.org/10.1038/s41598-022-21051-4

Krizsan, S.; Huhtanen, P. Effect of Diet Composition and Incubation Time on Feed Indigestible Neutral Detergent Fiber Concentration in Dairy Cows. J. Dairy Sci. 2013, 96, 1715–1726.

Lima, G. F. C, Sollenberger, L. E., Kunkle, W. E., Moore, J. E., & Hammond, A. C. (1999). Nitrogen Fertilization and Supplementation Effects on Performance of Beef Heifers Grazing Limpograss. *Crop Science*, *39*(6), 1853–1858. https://doi.org/10.2135/cropsci1999.3961853x

McCollum III,. (1997). *Supplementation Strategies for Beef Cattle*. https://hdl.handle.net/1969.1/87802

Mertens, D. R., & Grant, R. J. (2020). Digestibility and Intake. In K. J. Moore, M. Collins, C. J. Nelson, & D. D. Redfearn (Eds.), *Forages* (1st ed., pp. 609–631). Wiley. https://doi.org/10.1002/9781119436669.ch34

Moore, J.E. (1980). Forage Crops. In Crop Quality, Storage, and Utilization, C.S. Hoveland (Ed.). https://doi.org/10.2135/1980.cropquality.c3

Moore, J. E., Kunkle, W. E., & Brown, W. F. (1991). Forage quality and the need for protein and energy supplements. *Florida Beef Cattle Short Course, 40.*

Moore, J. E., Brant, M. H., Kunkle, W. E., & Hopkins, D. I. (1999). Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. *Journal of Animal Science*, *77*(suppl_2), 122. https://doi.org/10.2527/1999.77suppl_2122x

Moore, J. E., & Kunkle, W. E. (1995). Improving forage supplementation programs for beef cattle. In Proceedings of the 6th Annual Florida Ruminant Nutrition Symposium (pp. 65–74). Gainesville: University of Florida.

Moore, J. E., & Kunkle, W. E. (1998). *Balancing Protein and Energy in Forages*. 8.

Mould, F. L., Ørskov, E. R., & Mann, S. O. (1983). Associative effects of mixed feeds. I. effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. *Animal Feed Science and Technology*, *10*(1), 15–30. https://doi.org/10.1016/0377-8401(83)90003-2

Muizelaar W, Bani P, Kuhla B, Larsen M, Tapio I, Yáñez-Ruiz D, van Gastelen S. Rumen fluid sampling via oral stomach tubing method. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, Dewhurst R, Ferguson H, Terré M, Kuhla B, (editors). Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2020-. DOI: 10.5680/mcpb008

Newman,Y. C., & Sollenberger, L. E. (2005). Grazing Management and Nitrogen Fertilization Effects on Vaseygrass Persistence in Limpograss Pastures. *Crop Science*, *45*(5), 2038–2043. https://doi.org/10.2135/cropsci2004.0736

Newman, Y. C., Sollenberger, L. E., Kunkle, W. E., & Chambliss, C. G. (2002). Canopy Height and Nitrogen Supplementation Effects on Performance of Heifers Grazing Limpograss. *Agronomy Journal*, *94*(6), 1375–1380. https://doi.org/10.2134/agronj2002.1375

National Academies of Sciences, Engineering, and Medicine. (2016). Nutrient requirements of beef cattle.

Paterson, J. A., Belyea, R. L., Bowman, J. P., Kerley, M. S., & Williams, J. E. (1994). The impact of forage quality and supplementation regimen on ruminant animal intake and performance. *Forage quality, evaluation, and utilization*, 59-114.

Pereira Neto, J. D., Batista Dubeux, J. C., Ramos de Carvalho, F. F., Ferreira dos Santos, M. V., Ruiz-Moreno, M., & da Silva Santos, E. R. (2019). Tracing back sheep diet composition feeding grass-legume mixtures using fecal δ13C. *Small Ruminant Research*, *175*, 7–14. https://doi.org/10.1016/j.smallrumres.2019.02.006

Quesenberry, K. H., Sollenberger, L. E., Vendramini, J. M. B., Wallau, M. O., Blount, A. R., & Acuña, C. A. (2018). Registration of 'Kenhy' and 'Gibtuck' Limpograss Hybrids. *Journal of Plant Registrations*, *12*(1), 19–24. https://doi.org/10.3198/jpr2017.03.0012crc

Ramos-Morales, E., Arco-Pérez, A., Martín-García, A. I., Yáñez-Ruiz, D. R., Frutos, P., & Hervás, G. (2014). Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats. *Animal Feed Science and Technology*, *198*, 57-66.

Silva Santos, E. R., Dubeux, J. C., Jaramillo, D. M., Garcia, L., Vendramini, J., DiLorenzo, N., ... & Ruiz-Moreno, M. (2021). Herbage accumulation and nutritive value of stockpiled limpograss and 'Tifton 85'bermudagrass.

Shreck, A. L., Zeltwanger, J. M., Bailey, E. A., Jennings, J. S., Meyer, B. E., & Cole, N. A. (2021). Effects of protein supplementation to steers consuming low-quality forages on greenhouse gas emissions. *Journal of Animal Science*, *99*(7), skab147. https://doi.org/10.1093/jas/skab147

Sollenberger, L. E., Ocumpaugh, W. R., Euclides, V. P. B., Moore, J. E., Quesenberry, K. H., & Jones, C. S. (1988). Animal Performance on Continuously Stocked 'Pensacola' Bahiagrass and 'Floralta' Limpograss Pastures. *Journal of Production Agriculture*, *1*(3), 216–220. https://doi.org/10.2134/jpa1988.0216

Sousa, L. C. O., Palma, M. N. N., Franco, M. O., & Detmann, E. (2022). Does frequency of protein supplementation affect performance of cattle under grazing in tropical pastures? *Animal Feed Science and Technology*, *289*, 115316. https://doi.org/10.1016/j.anifeedsci.2022.115316

Steele, K. W., & Daniel, R. M. (1978). Fractionation of nitrogen isotopes by animals: A further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. *The Journal of Agricultural Science*, *90*(1), 7–9. https://doi.org/10.1017/S002185960004853X

Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991, 74, 3583–3597

Vendramini, J. M. B. (2008). Use of Limpograss in Grazing Systems in Florida. *Forage and Grazinglands*. https://doi.org/10.1094/FG-2008-1212-01-RV

Vendramini, J. M. B., Desogan, A. A., Silveira, M. L. A., Sollenberger, L. E., Queiroz, O. C. M., & Anderson, W. F. (2010). Nutritive Value and Fermentation Parameters of Warm-Season Grass Silage1. *The Professional Animal Scientist*, *26*(2), 193–200. https://doi.org/10.15232/S1080-7446(15)30580-5

Vendramini, J. M. B., & Arthington, J. D. (2010). Supplementation Strategies Effects on Performance of Beef Heifers Grazing Stockpiled Pastures. *Agronomy Journal*, *102*(1), 112–117. https://doi.org/10.2134/agronj2009.0207

Vendramini, J. M. B., Sollenberger, L. E., de Oliveira, F. C. L., Herling, V. R., Gomes, V. C., Sanchez, J. M. D., & Yarborough, J. K. (2019). Herbage Characteristics of Continuously Stocked Limpograss Cultivars under Stockpiling Management. *Crop Science*, *59*(6), 2886–2892. https://doi.org/10.2135/cropsci2019.05.0299

Vendramini, J., & Moriel, P. (2020). Management of forages and pastures in Lower-South: I-10 Corridor. In *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 101–122). Elsevier. https://doi.org/10.1016/B978-0-12-814474-9.00006-2

Wallau, M. O., Sollenberger, L. E., Vendramini, J. M. B., Mullenix, M. K., Quesenberry, K. H., Gomide, C. A. M., Costa e Silva, V., & DiLorenzo, N. (2015). Herbage Accumulation and Nutritive Value of Limpograss Breeding Lines Under Stockpiling Management. *Crop Science*, *55*(5), 2377–2383. https://doi.org/10.2135/cropsci2014.11.0797

Wallau, M. O., Vendramini, J. M. B., Sollenberger, L. E., Santen, E., Aguiar, A. D., & Cunha, O. F. R. (2020). In situ dry matter and crude protein disappearance dynamics in stockpiled limpograss. *Crop Science*, *60*(4), 2159–2166. https://doi.org/10.1002/csc2.20144

Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., & Wood, J. D. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science*, *78*(3), 256–269. https://doi.org/10.1016/j.meatsci.2007.06.008

Wickersham, T. A., Titgemeyer, E. C., Cochran, R. C., Wickersham, E. E., & Moore, E. S. (2008). Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage1. *Journal of Animal Science*, *86*(11), 3089–3099. https://doi.org/10.2527/jas.2007-0326

Whitney, T. R., Lee, A. E., Williamson, M. G., Swening, C. D., & Noland, R. L. (2011). Use of the Penn State particle separator to determine if molasses can reduce sorting of ground juniper when juniper is used as a feed intake limiter for lambs. Animal feed science and technology, 168(1-2), 21-29. 10.1016/j.anifeedsci.2011.03.010