UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

ALTHIERES JOSÉ FURTADO

PIGEON PEA INTERCROPPING WITH PASTURES AS MITIGATION STRATEGY FOR EMISSIONS OF GREENHOUSE GASES (GHG)

Pirassununga, SP
December 2022

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PIGEON PEA INTERCROPPING WITH PASTURES AS MITIGATION STRATEGY FOR EMISSIONS OF GREENHOUSE GASES (GHG)

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Advisor: Ph.D. Paulo Henrique Mazza Rodrigues

Co-advisor: Ph.D. Patrícia Perondi Anchão Oliveira

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São Paulo, 17th February 2022

CERTIFIED

We certify that the Research "PIGEON PEA INTERCROPPING WITH PASTURES AS EMISSIONS OF GREENHOUSE GASES (GHG) MITIGATION STRATEGY", protocol number CEUAx 6228200521 (ID 001914), under the responsibility Paulo Henrique Mazza Rodrigues, agree with Ethical Principles in Animal Research adopted by Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (University of São Paulo), and was approved in the meeting of day September 17, 2021.

Certificamos que o protocolo do Projeto de Pesquisa intitulado "CONSÓRCIO DE FEIJÃO-GUANDU COM PASTAGENS COMO ESTRATÉGIA DE MITIGAÇÃO DE EMISSÕES DE GASES DE EFEITO ESTUFA (GEE).", protocolado sob o CEUAx nº 6228200521, sob a responsabilidade de Paulo Henrique Mazza Rodrigues, está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, e foi aprovado na reunião de 17 de setembro de 2021.

Prof. Dr. Marcelo Bahia Labruna
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Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Embrapa
Pecuária Sudeste

CERTIFICADO

CEUA PRT Nº 03/2020

Certificamos que a proposta de pesquisa intitulada "Práticas estratégicas para a mitigação da emissão de gases e efeito estufa em sistemas pastoris da Região Sudeste brasileira: Bovinos machos fistulados para o experimento com leguminosa (guandu)", registrado com o número 20.19.00.047.00.00, sob responsabilidade da pesquisadora Dra. Patrícia Perondi Anchão Oliveira, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009 e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovada pela Comissão de Ética no Uso de Animais da Embrapa Pecuária Sudeste.

We certify that the research proposal "Strategic practices for mitigating greenhouse gas emissions in grassland systems of the Brazilian Southeast: Male fistulated cattle for the experiment with legume (pigeon pea)*" has been registered under the responsibility of Dr.* Patrícia Perondi Anchão Oliveira *(number* 20.19.00.047.00.00*) which involves production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), is in accordance with the Brazilian Federal Law on Animal Experimentation (Law 11.794, enacted on 8th October 2008), Decree 6.899 (enacted on 15th July 2009) as well as with the corresponding rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was approved by the Committee of Animal Use on Experimentation of Embrapa Southeast Livestock.*

São Carlos, 14 de Dezembro de 2020.

Dr Alexandre Rossetto Garcia
Presidente da Comissão de Ética no Uso de Animais
Embrapa Pecuária Sudeste

Finalidade	Pesquisa Científica
Vigência da Autorização	21/09/2019 a 31/12/2023
Espécie / Linhagem / Raça	Bovino / Nelore / *Bos Indicus*
Número de Animais	12
Peso / Idade	18-24 meses
Sexo	Macho
Origem	Embrapa Pecuária Sudeste

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Embrapa

Pecuária Sudeste

CERTIFICADO

CEUA PRT Nº 04/2019

Certificamos que o projeto de Pesquisa intitulado: "Práticas estratégicas para a mitigação da emissão de gases de efeito estufa em sistemas pastoris da Região Sudeste brasileira", registrado com o número 20.19.00.047.00.00, sob responsabilidade do pesquisador científico Dra. Patrícia Perondi Anchão Oliveira, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009 e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela Comissão de Ética no Uso de Animais da Embrapa Pecuária Sudeste.

We hereby declare that the research project titled "Strategic practices for mitigating greenhouse gas emissions in grassland systems of the Brazilian Southeast" has been registered under the responsibility of Dra. Patrícia Perondi Anchão Oliveira (number 20.19.00.047.00.00) involving production, management or utilization of animals from phylum Chordata, subphylum Vertebrata (except humans). The described experimental protocol is in accordance to the Brazilian Federal Law on Animal Experimentation (#11.794, enacted on 8th October 2008), to the Decree 6.899 (enacted on 15th July 2009) and the corresponding rules of National Council for Animal Experimentation Control (CONCEA), and it was approved by the Committee of Animal Experimentation of Embrapa Southeast Livestock.

São Carlos, 16 de Dezembro de 2019.

Dr Alexandre Rossetto Garcia
Presidente da Comissão de Ética no Uso de Animais
Embrapa Pecuária Sudeste

Finalidade	Pesquisa Científica
Vigência da Autorização	21/09/2019 a 31/12/2023
Espécie / Linhagem / Raça	Nelore (Bos Indicus)
Número de Animais	124
Peso / Idade	350 kg / 18-24 meses
Sexo	machos
Origem	Embrapa Pecuária Sudeste

DEDICATION

To God, for my life.

To my beloved parents, Bernadete and José Geraldo, for my education and values.

To my teachers, who guided me in my studies and academic achievements.

To my brother José Júnior, who has always been by my side.

In memory of my grandmother Maria Rosa do Lago, who always showed me the path I should follow.

To my friends and family, who always supported and encouraged me.

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To my dear parents Maria Bernadete de Lourdes Furtado and José Geraldo Furtado, who educated me and taught me my values. Thank you for the support to continue chasing my dreams.

To my dear brother, José Geraldo Furtado Júnior, who was always by my side in the most difficult moments.

To my advisor Prof. Paulo Henrique Mazza Rodrigues and co-advisor Dra. Patricia Perondi Anchão Oliveira, who guided me and gave me all the support for this research. Thank you for your patience and teachings.

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EPIGRAPH

“Failure is simply the opportunity to begin again, this time more intelligently.”

Henry Ford

RESUMO

FURTADO, A. J. **Consórcio de feijão guandu com pastagens como estratégia de mitigação de gases de efeito estufa (GEE)**. 2022. 75 p. Dissertação (Mestrado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2022.

O Brasil apresenta um dos maiores rebanhos do mundo, com cerca de 224 milhões de cabeças e o metano (CH_4) entérico emitido pelos ruminantes é um dos principais gases de efeito estufa (GEE). Neste aspecto, formas de se mitigar as emissões de CH_4 vêm sendo pesquisadas e implementadas. A recuperação das pastagens, intensificação e consórcio entre pastagens e leguminosas são estratégias de manejo e produção animal que apresentam potencial de mitigação das emissões de GEE. O objetivo geral deste estudo foi avaliar os efeitos da integração feijão guandu com pastagens tropicais para alimentação de bovinos *Nelore* e comparar variáveis de produção animal e emissão de CH_4 entérico com outros sistemas, baseados em pastagens durante a estação das secas e das chuvas. A pesquisa foi desenvolvida na Embrapa Pecuária Sudeste, em São Carlos, SP, em duas estações distintas no ano de 2021: águas (janeiro) e secas (julho). Trinta e seis novilhos da raça *Nellore* (221 ± 10 kg de peso vivo entre 15 e 16 meses de idade) foram distribuídos aleatoriamente em três tratamentos com três repetições (piquetes de 1,5 hectares): 1) pastagem degradada de *Urochloa decumbens* cv. Basilisk (DEG); 2) pastagem com mistura de *U. decumbens* cv. Basilisk e *U. brizantha* cv. Marandu com fertilização nitrogenada (200 kg N-ureia ha^{-1} ano^{-1}) (REC); e 3) pastagem com mistura de gramíneas tropicais (*U. decumbens* cv. Basilisk e *U. brizantha* cv. Marandu) e a leguminosa feijão guandu (*Cajanus cajan* cv. BRS Mandarim) (MIX). A emissão de CH_4 foi estimada pela técnica do gás traçador hexafluoreto de enxofre (SF_6) e o consumo de matéria seca (CMS) determinado utilizando marcadores internos (FDNi - fração insolúvel da fibra em detergente neutro) e externos (TiO_2 - dióxido de titânio). As forragens foram coletadas por simulação de pastejo, enquanto as fezes foram coletadas diariamente por defecação espontânea. A qualidade nutricional das forragens foi determinada, o desempenho animal foi acompanhado mensalmente e a taxa de lotação ajustada pela técnica “put and take”. O modelo estatístico considerou os tratamentos e as estações do ano como efeitos fixos e a interação tratamento×estação foi testada. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Fisher a 5% no software estatístico SAS. Os resultados deste trabalho indicaram que a integração feijão guandu com gramíneas tropicais é uma estratégia interessante para produção sustentável de bovinos em pastagem, pois no tratamento MIX a forragem apresentou melhor composição nutricional em relação aos demais tratamentos, os animais consumiram menos suplemento mineral e mesmo assim apresentaram melhor desempenho, além de redução nas emissões de CH_4 entérico que chegou a 70% quando expressa por ganho de peso diário e comparada ao tratamento DEG.

Palavras-chave: bovinos, brachiaria, *Cajanus cajan*, metano, *Urochloa*

ABSTRACT

FURTADO, A. J. **Pigeon pea intercropping with pastures as mitigation strategy for emissions of greenhouse gases (GHG)**. 2022. 75 p. Dissertation (Master's Degree) - College of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, 2022.

Brazil has one of the largest cattle herds in the world, with approximately 224 million heads and the enteric methane (CH_4) emitted by ruminants is one of the main greenhouse gases (GHG). Therefore, strategies to mitigate CH_4 emissions have been studied and implemented. The recovery and intensification of pastures and intercropping tropical pastures with legumes are some of the practices that have potential to mitigate GHG emissions. The objective of this study was to evaluate the effects of intercropping pigeon pea (*Cajanus cajan*) with tropical pastures for feeding *Nellore* cattle and to compare performance variables and enteric CH_4 emissions with other pasture-based systems during the dry and rainy seasons of 2021. The study was carried out at Embrapa Pecuária Sudeste, in São Carlos, SP, in two distinct seasons of 2021: rainy (January) and dry (July). Thirty-six *Nellore* steers (221 ± 10 kg of body weight and 15-16 months) were randomly distributed in three treatments with three replicates (paddocks 1.5 hectares each): 1) a degraded pasture of *Urochloa decumbens* cv. Basilisk (DEG); 2) a recovered and fertilized (200 kg N ha^{-1} $year^{-1}$) pasture stablished with *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu (REC); and 3) a intercropped of tropical grasses (*U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu) and the legume pigeon pea (*Cajanus cajan* L. Millsp. cv. BRS Mandarim) (MIX). CH_4 emissions were estimated using the sulfur hexafluoride (SF_6) tracer gas technique and dry matter intake (DMI) determined using internal (iNDF – indigestible neutral detergent fiber) and external (TiO_2 – titanium dioxide) markers. Forages were collected by hand plucking using the methodology of grazing simulation with observations of ingestive behavior, and feces were collected after voluntary defecation. The nutritional quality of the forages was determined, animal performance was monthly monitored, and the stocking rate adjusted by the "put and take" technique. The statistical model considered treatments and seasons as fixed effects, and the interaction treatment×season was tested. Data were subjected to analysis of variance and mean compared by Fisher test at 5% significance level in SAS software. The results indicate that intercropping pigeon pea with tropical grasses is an interesting strategy for sustainable livestock production based on pastures. In the MIX treatment the forage presented better nutritional composition, the animals consumed less mineral supplement while presenting better animal performance. In addition, there was a reduction in CH_4 emissions up to 70% when expressed per average daily weight gain in comparison to DEG treatment.

Keywords: brachiaria, *Cajanus cajan*, cattle, methane, *Urochloa*

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ABREVIATIONS

% = Percentage
® = Trademark
°C = Degrees Celsius
µm = Micron
ABW = Average body weight
ADF = Acid detergent fiber
ADG = Average daily weight gain
ADIN = Acid-detergent insoluble nitrogen
AICC = corrected Akaike information criterion
Al = Aluminum
Ash = Mineral matter
AU = Animal unity
BW = Body weight
cal = Calorie
CEUA = Committee for the Use and Care of Institutional Animals
CH_4 = Methane
cm = Centimeter
CO_2 = Carbon dioxide
CP = Crude protein
CT = Condensed tannins
d = Day
DM = Dry matter
DMD = Dry matter digestibility
DMI = Dry matter intake
EMBRAPA = Brazilian Agricultural Research Corporation
EE = Ether extract
FAPESP = São Paulo Research Foundation
FCR = Feed conversion ratio
FE = Feed efficiency
Fe = Iron
g = Gram
GE = gross energy
GEI = Gross energy intake
GHG = Greenhouse gases
H = Hydrogen
ha = Hectare
HT = Hydrolysable tannins
IBGE = Brazilian Institute of Geography and Statistics
iNDF = Indigestible neutral detergent fiber
IZ/APTA = Institute of Animal Science of the Paulista Agency for Agribusiness Technology
K = Potassium
kg = Kilogram
LI = Light interception

Lig = Lignin
Mcal = Megacalorie
mg = Milligrams
mm = Millimeter
N = Nitrogen
N_2O = Nitrous oxide
NaCl = Sodium chloride
NDF = Neutral detergent fiber
NDIN = Neutral-detergent insoluble nitrogen
NPN = Nonprotein nitrogen
OM = Organic matter
P = Phosphorus
PSI = Pounds per square inch
PVC = Polyvinyl chloride
SEEG = Removal Estimating System
SEM = Standard error of the means
SF_6 = Sulphur hexafluoride
SP = São Paulo
spp = Specie
SR = Stocking rate
syn = Synonym
TiO_2 = Titanium dioxide
U. = *Urochloa*
UC Davis = University of California, Davis
USA = United States of America
USP = University of São Paulo
Ym = Percentage of gross energy ingested converted to methane

SUMMARY

1. INTRODUCTION

The livestock activity is facing many challenges such as the need to increase food production to meet the growing world population (FAO, 2017; GILLER et al., 2021) while adapting to environmental and economic changes by improving animal performance in more sustainable production systems (SAKITA et al., 2022). Among the issues surrounding the growth of this sector is the increased use and degradation of natural resources, directly contributing to worsen the global climate change scenario due the emission of greenhouse gases (GHG), depleting water resources, causing soil erosion and impairing natural habitats (IPCC, 2022).

Evidence of human-induced climate change and the important contribution of the livestock sector to GHG emissions highlights the need to better understanding the sources of emissions and potential strategies available for their mitigation (IPCC, 2022). Among the GHGs, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are the most important in the context of the agricultural activity. Although the concentrations of CH_4 and N_2O in the atmosphere are lower than that of CO_2, they have a warming potential of 27.2 and 273 times greater compared to CO_2 (IPCC, 2022). It is also important to note that CH_4 has 8 to 12 years lifetime in the atmosphere and an annual increase rate of 1.0% (HÜTSCH, 2001). Additionally, CH_4 can be classified as a “short lived climate pollutant” having a relatively short lifetime in the atmosphere compared to CO_2, which can remain for periods up to 10,000 years until returning in the global carbon cycle (ARTAXO, 2014).

In this context, special attention should be given to Brazilian livestock, which has been the target of numerous criticisms related to climate change, mainly because it depends on extensive pasture areas, often in some stages of degradation, in a production system that emits a high amount of GHGs per unit of produced product (IPCC, 2007; MACHADO et al., 2011). Despite this, Brazilian livestock plays a fundamental role in the economy, where cattle production represents 83.9% of total animal production (89% beef cattle and 11% milk production; DE MARCHI et al., 2016). Brazil has one of the largest herds in the world (FAO, 2017), with approximately 224.60 million heads (IBGE, 2022), and posing as the world's largest exporter of beef, with 2.48 million tons in 2021

(ABIEC, 2022). In addition, Brazil is the second largest producer of meat, with 9.7 million tons per year, representing 13.7% of world production (ABIEC, 2022) and in the dairy sector is the fourth largest producer of milk, with 35.124 billion liters per year (FAOSTAT, 2017).

The GHGs emissions related to livestock activity in Brazil are of concern. Enteric CH_4 emissions correspond to 60.1% of total anthropogenic emissions of this gas, while fermentation and decomposition of waste correspond to 5.5% (BERCHIELLI et al., 2012). Also, according to Emission and Removal Estimating System (SEEG) in 2020, the Brazilian bovine herd was responsible for approximately 5.5% of the total CH_4 produced worldwide.

Aiming at harmony between environment, society, and economy, cattle production previously performed extensively can now be conducted with better planning, making use of management strategies, pasture maintenance, and good agricultural practices that allow livestock activity to be more efficient. This can generate better quality products, with better production efficiency and reduced damage to the environment (ABRÃO et al., 2016).

Among management strategies and agricultural practices, the use of legumes in pastoral systems has great potential to contribute to a more sustainable livestock production and to the recovery of degraded pastures. As an example, there is the use of the consortium between pigeon pea (*Cajanus cajan*) and tropical grasses, which contributes to greater forage production and availability, and pasture quality for feeding the animals, thus reducing the need of nitrogen fertilizers and protein/mineral supplements, especially during the dry season of the year (OLIVEIRA et al., 2017; CASTRO-MONTOYA and DICKHOEFER, 2020). In drier seasons, pigeon pea still has a high capacity to retain leaves after flowering, reaching a production of 12 tons per hectare per year, with its leaves and thinner branches serving as a protein supplement with values of crude protein varying between 16 and 20 % (PALUDO et al., 2012).

However, studies are still needed to evaluate pasture production systems with intercropping tropical pastures and pigeon pea in relation to animal performance, dry matter intake, and enteric CH_4 emissions from the Brazilian herd, thus allowing a better understanding of the sustainability and environmental consequences of this production system.

2. HYPOTHESIS AND OBJECTIVES

The hypothesis was that the use of pigeon pea in consortium with tropical grasses is an interesting strategy for feeding *Nellore* cattle, especially in the dry season of the year, contributing to the reduction of GHGs emissions, allowing land use intensification and increasing animal productivity.

In this sense, the general objective was to evaluate the effects of introducing pigeon pea in tropical grass pastures as an intercropped system to feed *Nellore* cattle, and thus compare animal production parameters and enteric CH_4 emission with other commonly used systems based on pasture, during the dry (May-October) and rainy (November-April) seasons of the year.

The specific objectives comprised:

- Determine DMI using internal (indigestible neutral detergent fiber; iNDF) and external (titanium dioxide; TiO_2) markers;

- Compare stocking rate and animal performance among the three production systems;

- Quantify the production and emission of enteric CH_4 through the sulfur hexafluoride (SF_6) tracer gas technique and compare the CH_4 intensity among the systems.

It is important to noteworthy that this study is part of the thematic project "Strategic practices to mitigate greenhouse gas emissions in southeastern Brazilian pasture systems" (São Paulo Research Foundation, FAPESP #2017/20084-5), a partnership of University of São Paulo (USP), Brazilian Agricultural Research Corporation (EMBRAPA), Institute of Animal Science of the Paulista Agency for Agribusiness Technology (IZ/APTA), University of California - Davis (UC Davis) and Brazilian Institute of Geography and Statistics (IBGE).

3. LITERATURE REVIEW

3.1. Livestock production based on pastures and consortium with legumes

An increasing global population and improved standards of living provide a market for high-quality ruminant protein in meat and milk (McADAM et al., 2022). Around 1.5 billion cattle are present worldwide (FAOSTAT, 2020). In 2019, there were 70 million tonnes of beef consumed and by 2023 that number is expected to rise to 74 million tonnes. Beef is a high-quality source of protein that provides highly desirable eating experiences (GREENWOOD, 2021). The USA (17%), Europe (15%), Brazil (13%), China (9%), Argentina (4%), India (4%), and Australia (4%) are the top beef-producing nations or regions. In 2018/2019, Brazil accounted for 20% of global beef exports, followed by Australia (16%), India (15%), USA (13%), New Zealand (6%), Argentina (6%), and Canada (5%), with the rest of the world supplying the remaining 18% of beef for export (GREENWOOD, 2021).

Brazil has had the highest rate growth in beef production of any of these beef production countries since 2008 (FAOSTAT, 2020). According to IBGE (2022), in 2021 Brazil had 224.6 million head of cattle, the majority of which were *Bos taurus indicus*. Despite being stable in recent years (2017 to 2020), cattle numbers have increased by 35% since 1998. The Midwest is the region with the greatest cattle increase in number of heads in Brazil, followed by the North, Southeast, then Northeast and South regions (ABIEC, 2019). Approximately 162 million hectares, or 19% of Brazil's 852 million hectares, are grazing pastures (ABIEC, 2019). Of these pastures, 137 million ha (84%) is classified as in good condition, 9.7 (6%) million ha is pasture requiring recovery, 4.2 million ha (2.6%) is pasture in an advance stage of biological or agricultural deterioration, and 11.8 million ha (7.3%) includes grain or other crops integrated with livestock (GREENWOOD, 2021).

According to ABIEC (2019), Brazilian pasture area decreased from 192 to 162 million ha between 1990 and 2018, increasing the area for agricultural production, reforestation, and urban development. However, deforestation and beef production have been rising in the Brazilian Amazon, which has adverse implications for GHG production and hence climate change (VALE et al., 2019). Currently, in all but the most southerly region of Brazil, the predominant plant forage grass species are of the genus *Urochloa* (syn. *Brachiaria* spp.), which are estimated to occupy around 90 million ha, and the Marandu cultivar of *U. brizantha* is grown in almost half of this area (JANK et al., 2014). In addition, beef cattle producers seldom use fertilizers for these pastures in tropical regions of Brazil (BODDEY et al., 2020).

Pasture-based systems benefit from the fact that ruminants do not require concentrates, such as grain, as they can obtain energy from the cellulose of forages and other feeds that cannot be fully digested by swine or poultry (VAN SOEST, 2018), nor compete with humans, contributing to the food security of the growing world population. However, livestock production in pasture-based systems in the tropics is a complex and interactive system, where the forage is a basal nutritional resource of high complexity because its ability to supply substrates for animal production fluctuates both qualitatively and quantitatively throughout the year (DETMANN et al., 2014; ALMEIDA et al., 2022).

The most discrepant and visible differences in pasture composition occur between rainy (medium to high-quality forage) and dry seasons (low-quality forage). In plant tissues, lignification decreases while crude protein (CP) levels increase during the rainy season. On the other hand, lignification increases and the content of CP decreases during the dry season (ALMEIDA et al., 2022). It is important to keep this in mind because past studies demonstrated that the rumen needs a minimum of 70-80 g/kg of dietary CP for adequate microbial growth and requires around 100 g/kg of dietary CP to promote maximum microbial growth, directly impacting neutral detergent fiber (NDF) digestibility, intake, and performance (DETMANN et al., 2014). No matter what season of the year, the forage that is being used for grazing does not provide a balanced diet since its nutritional characteristics do not meet the adequate requirements for a high animal performance (ALMEIDA et al., 2022). As a result, supplementing grazing cattle is a successful strategy for addressing pasture nutritional deficiencies and might be considered a practical strategy to mitigate the seasonality of feeding and enhancing animal performance (BOVAL et al., 2015).

The live weight gain of cattle raised on tropical pastures is low when compared to temperate pastures (WINTER et al., 1991). The tropical pastures production, in terms of mass, can be higher than that from temperate grasses. But, because of low quality, the results are lower stocking rates and lower levels of utilization as set by the phenology of the plant, resulting in low production per hectare (POPPI et al., 2018). To meet market demands for younger animals, with adequate fat cover and carcass composition, it is necessary to increase the annual body weight gain mainly in the dry season, and this may be achieved by introducing legumes into the pasture (POPPI et al., 2018).

In contrast to tropical grasses, perennial legumes fix their own nitrogen (N), present high nutritional quality and can be productive for multiple years after establishment without additional cultivation or planting (McADAM et al., 2022). With a suitable management, beef production can be equal or greater from mixed than from grass-alone pastures fertilized with 120 or 150 kg N ha^{-1} $year^{-1}$, and a reduction of enteric CH_4 emissions may occur due the presence of condensed tannins in forage legumes (BODDEY et al., 2020). Nevertheless, legumes can have a residual effect of increasing soil N due to the large litter biomass deposited (CADISH et al., 1994). Additional benefits are increased ecosystem biodiversity, improved soil fertility and increased soil organic matter (OM), which may also contribute to further GHG mitigation by CO_2 sequestration for as long as two decades (BODDEY et al., 2020). Because of that, the use of forage legumes in mixed pastures for tropical regions is emerging as a feasible strategy to keep livestock production at acceptable levels with reduced GHG emissions rates.

Tropical forage legumes were first used on a wide scale starting well before the 1950s in the state of Queensland, Australia (SHAW, 1961). The *Stylosanthes humilis* was introduced into thousands ha of pasture and made an important contribution to extensive production in northern Australia (GARDENER et al., 1993 b). However, In Brazil, it was mistakenly believed for a very long time that the lack of persistence of legumes in mixed pastures was caused by physiologic incompatibility between the tropical grass (C_4) and the legume (C_3). Brazilian scientists, technologists, and farmers began to believe that mixed pastures couldn't be used because of this incompatibility theory (BODDEY et al., 2020). In the past, the use of grass and legume in mixed pastures reached harmony in the early years, but over the time, the proportion of the legume in the sward decreased until it disappeared. However, this was linked to adoption of crown-forming legumes, which have shown low persistence mainly when the management of this pasture is interrupted (ALVES et al., 2016; MENEZES et al., 2015).

According to recent studies, the physiological differences between grasses and legumes do not pose a barrier for their compatibility (ANDRADE et al., 2012; GOMES et al., 2018; TAMELE et al., 2018). Compatibility between species can be defined as the ability of two species to form a harmonic and stable mixed sward (ANDRADE et al., 2012). Under tropical conditions, the legume content is thought to be between 20% and 45% of the total forage mass (THOMAS, 1992, 1995). In addition, legume species of the genera *Stylosanthes*, *Centrosema*, *Arachis*, *Desmodium* and others are tolerant to acidic soil conditions, but they often compete with the forage grass when phosphorus (P) and potassium (K) fertilization are increased (CADISCH et al., 1993).

Menezes et al. (2015) and Alves et al. (2016) studied *U. brizantha* cv. Xaraés mixed with *Stylosanthes guinanenis* cv. Mineirão with different botanical composition of the pasture (24%, 34%, 45% and 52% of legume in the forage mass) under rotational stocking. Two years after the legume disappearance, they found positive linear responses in grass accumulation and stocking rates due to N and OM fixation of legumes in the pasture. When the proportion of legume in the previous pasture grew from 24% to 52%, the rate of grass accumulation increased by 35% and the stocking rate increased by 16% (MENEZES et al., 2015; ALVES et al., 2016).

The early studies of Grof (1985) and Lascano and Thomas (1988) in Colombia showed that the legume *Arachis pintoi* (forage peanut) was compatible with at least four *Urochloa* species (*U. brizantha*, *U. humidicola*, *U. dictyoneura* and *U. ruziziensis*) and concluded that this legume is a very attractive choice for increasing cattle production without reliance on N fertilizer or expensive feed supplements.

A long-term experiment (9 years) was conducted in Brazil with forage peanut and Marandu grass consisting in two treatments: *U. brizantha* cv. Marandu pasture fertilized with N and a mixture of *U. brizantha* cv. Marandu and forage peanut (*A. pintoi* cv.

Belomonte) pasture. The authors found that the average daily live weight gain (ADG) per ha was 28.7% greater in the mixed pasture than in the N-fertilized grass-alone pasture (PEREIRA et al., 2018). *U. brizantha* cv. Marandu and forage peanut were also studied to evaluate the height management (10, 20, 30 or 40 cm) after the establishment of a mixed pasture and the 20-25 cm canopy heigh showed a desirable botanical composition from 20% to 45% of legume in the forage mass and thus was considered the ideal defoliation intensity to mixed canopies (TAMELE et al., 2018).

Under rotational stocking, Marandu grass and forage peanut canopies were evaluated for different management targets based on light interception (LI) and, like recommendations for grasses, interruption of the rest period is currently recommended when the mixed canopy attains 95% LI (PEREIRA et al., 2017; GOMES et al., 2018). In addition, higher legume removal rate was found in 100% LI resulting in lower post-grazing legume mass and the recommendation is that the height at initial stocking of this mixture pasture should be from 24 to 30 cm, with a stubble height of 15 cm (GOMES et al., 2018).

3.2. Potential use of pigeon pea (*Cajanus cajan*) in pasture-based systems

Supplement low-quality feeds with leguminous forage in ruminant diets can be considered to offset the limitations associated with low feed quality in systems where livestock are increasingly becoming dependent on low-quality roughages. In this context, the use of leguminous forage as an alternative source of protein has become an urgent research topic globally, and one of the potential species being evaluated is pigeon pea (*Cajanus cajans*) (TULU et al., 2021).

Pigeon pea is a drought tolerant legume grown mainly in the semi-arid tropics through since is well adapted to several environments (TROEDSON et al., 1990). It is a diploid plant (2n = 22) belonging to the *Cajaninae* sub-tribe of the tribe *Phaseoleae*, which also contains soybean (*Glycine max*), field bean (*Phaseolus vulgaris*) and mungbean (*Vigna radiata*) (YOUNG et al., 2003). Of the 32 species that fall under the *Cajaninae* sub-tribe, pigeon pea is the only one that has been known to be grown as a food crop. According to Hillocks et al. (2000), pigeon pea accounts for around 5% of global production of legumes, with over 70% of that production occurring in India. Eastern Africa and the Americas both produce a significant amount of pigeon peas (ODENY et al., 2007). About 3.6 million tonnes of pigeon peas seeds are produced annually, with a market worth at around US$1,600 million (FAOSTAT, 2007).

Pigeon pea's origin is still a matter of debate; however, it is most likely that immigrants from India who went to Africa in the 19th century to work as railway laborers and storekeepers introduced the legume into East Africa (HILLOCKS et al., 2000). Following that, it moved up the Nile Valley into West Africa and ultimately the Americas, increasingly becoming an important subsistence crop for smallholder's farmers (JOHANSEN et al., 1993; ODENY et al., 2007). The legume is grown exclusively in rainfed environments at various latitudes, altitudes, and temperatures (SILIM et al., 2006). Although it is mostly grown in parts of Africa that receive between 500 and 1000 mm of rain per year (NIEUWOLT, 1977), it is reported to have a wide adaptability to different climates and soil (TROEDSON et al., 1990).

Deep roots and osmotic adjustment in the leaves of pigeon pea are attributes that allowed withstand severe drought better than many legumes (FLOWER and LUDLOW, 1987; SUBBARAO et al., 2000). Its stomata regulation and osmotic adjustment, which is less energy demanding, allow root growth to proceed under drought conditions (NUNES et al., 2008), maintaining its leaf water content avoiding tissue dehydration, and reducing the effects of drought on dry matter yield.

The legume also maintains photosynthetic function under stress better to other drought-tolerant legumes such as cowpea (*Vigna unguiculata*) (LOPEZ et al., 1987), and its unique polycarpic flowering habit further enables the legume to shed reproductive structures in response to stress (MLIGO and CRAUFURD, 2005). According to Peoples et al. (1995), pigeon pea has the capacity to fix up to 235 kg of N per hectare and produces more N per unit area from plant biomass than many other legumes, being its N-fixing ability a desirable attribute for environmentally sustainable agricultural production.

Pigeon pea rarely needs inoculation since it can nodulate on *Rhizobium*, which is naturally present in most soils, while other legumes need inoculation to maximize their capacity for fixing N (FARIS, 1990). Even if pigeon pea is inoculated, the effectivity of vesicular-arbuscular mycorrhizae fungi has been found to the highest when compared to cowpea and *Arachis hypogea* (AHIABOR and HIRATA, 1994).

Unlike other legumes where growth has been reported to be limited by P, pigeon pea is cited as one of the few species that can utilize iron-bound P efficiently (AE et al., 1990; SUBBARAO et al., 1997). When cultivated on low P soils with aluminum (Al), pigeon pea is also more effective at absorbing P when compared to other legumes (AE et al., 1990). However, under drought conditions, salinity is a significant issue, and pigeon pea is relatively sensitive to salinity (TROEDSON et al., 1990).

Additionally, pigeon pea is also widely used as fodder and feed for livestock (RAO et al., 2002), being its foliage an excellent fodder with high nutritional value (ONIM et al., 1985). Animals are also fed with it seeds (WALLIS et al., 1986), and its fodder has been demonstrated to increase the intake of low-quality forages resulting in high animal live weight due to its relatively higher N content (KARACHI and ZENGO, 1998; SHENKUTE et al., 2013).

The higher number of branches and stem diameter in the regrowth of pigeon pea qualifies the legume as a potential climate-smart crop with the ability to maintain stable growth under drought conditions (TENAKWA et al., 2022), improve soil fertility and prevent soil erosion (SHUMUYE et al., 2016). The fodder is highly digestible and may be used in ruminant diets as a protein supplement even at high levels of inclusion without any detrimental effect (CORRIHER et al., 2007), despite having antinutritional factors such as proteinase inhibitors and tannins (CORRIHER et al., 2010).

Promising results with pigeon pea (*Cajanus cajan* cv. BRS Mandarim) in pasture-based production systems are being found at Embrapa Pecuária Sudeste (São Carlos, Brazil). This has been observed specially because of recovery of degraded pastures, forming a consortium with tropical grasses (*Urochloa* spp. and *Panicum* spp.), presenting around 3 years of persistence in the cultivated area, and allowing higher stocking rates during the dry seasons (OLIVEIRA et al., 2022). Pigeon pea has low palatability in its vegetative phase, which coincides with the rainy season. Therefore, the animals preferentially consume only the tropical grasses, favoring the growth and N biological fixation of the legume. Later, in the dry season, when Pigeon pea blooms and reaches it reproductive phase, the acceptability improves, and the animals start to consume it as an important source of protein (OLIVEIRA et al., 2017, 2022). At the end of the dry season, the pigeon pea stubbles are deposited in the pasture surface and act like green manure, providing more than 200 kg/ha of N (OLIVEIRA et al., 2017).

In addition to controlling infesting weeds, the consortium pigeon pea and tropical grasses was found to improve individual live weight gain, stocking rate and weight gain per area, while reducing the time needed until the slaughter of *Nellore* steers (OLIVEIRA et al., 2017, 2022). These results, together with the fact the pigeon pea may present tannins in its composition (CORRIHER et al., 2010), highlights the possibility of mitigating enteric CH_4 emissions in pasture-based systems where this legume was introduced.

3.3. Estimating feed intake with internal (iNDF) and external (TiO_2) markers

The estimation of feed intake is crucial for livestock production systems because they let managers decide on the quantity and timing to provide supplemental nutrients, which frequently represent the biggest out-of-pocket expense for ruminant production based on pastures (SMITH et al., 2021). Additionally, an estimation of intake in relation to forage availability are needed for the sustainable management of pastures in order to determine the proper stocking rates and evaluate the effects of grazing on the ecosystem. The physiological state of the ruminant changes over time, causing variations in feed intake and their nutritional requirements, affecting the demand for supplemental nutrients, and making these management decisions even more crucial (SMITH et al., 2021).

The topic of feed intake has received a lot of attention (COOK, 1964; CORDOVA et al., 1978; COTTLE, 2013; LIPPKE, 2002; MAYES and DOVE, 2000). However, there isn’t yet general agreement on how to best measure or account for this process. In ruminants, the selection of dietary components and sorting of completely mixed feeds is well-documented (DUNCAN and YOUNG, 2002; MILLER-CUSHON and DeVRIES, 2017). This reflects in a propensity to choose particular plants or plant parts while grazing, individual preferences, social pressure to eat or stay away from particular plants (or parts), acquired habits, and other elements could all play a role in this process (SMITH et al., 2021).

There have been numerous attempts to create mathematical models that can estimate feed intake in ruminants (NRC, 1987; FISHER, 1996; COLEMAN et al., 2014, NASEM, 2016), and these models rely on direct measurements of intake, as well as experiments that use inert markers to forecast nutrient fluxes and intake. At least until new techniques become available, the use of internal and external markers is the best method researchers now have at their disposal for estimating feed intake (SMITH et al., 2021). In addition to being used to estimate feed intake, markers are also used to measure total feces production, nutrient digestibility, and the flux of digesta (SALMAN et al., 2010).

According to Smith et al. (2021), a marker must satisfy the following requirements: *i)* it must not be absorbed; *ii)* it must not interfere with or be impacted by any digestive processes; *iii)* it must be similar to or directly related to a dietary component; and *iv)* sample analysis must have sufficient specificity and sensitivity (FAHEY and JUNG, 1983). In addition, researchers must utilize suitable experimental design, experimental units, statistical analysis, and critical evaluation of results beyond the initial step of choosing a marker to make sure the results are physiologically feasible (TITGEMEYER, 1997).

Internal markers are naturally occurring components of a feed resource that meet the basic criteria for markers, and most of these components are associated to the cell wall and its constituents. Components of the cell wall fibrous portion that are indigestible have been studied for their potential use on estimating feed intake and digestibility, particularly in ruminant animals (SMITH et al., 2021).

In 1969, the notion of indigestible cellulose as an internal marker was conceived by Wilkins (1969); however, most assays of forage account for cellulose indirectly by classifying fiber into relative fractions (SMITH et al., 2021). The indigestible neutral detergent fiber (iNDF) was promoted by Lippke et al. (1986) as an adequate substitute for indigestible cellulose, and iNDF was presented as the residue remaining after *in vitro* or *in situ* digestion for 6 to 8 days followed by neutral detergent extraction (SMITH et al., 2021).

To estimate feed intake on pasture using internal markers is necessary to know the degree of recovery of these indicators in digestibility assays (KOZLOSKI et al., 2009). The degree of internal markers recovery, the precision and accuracy of digestibility analysis and feed intake estimation have been variable (BERCHIELLI et al., 2005), mainly because these fractions are not uniform and constant chemical entities in feeds and do not have a standard method for their determination in the laboratory (LIPPKE, 2002). However, these problems do not restrain the use of internal markers if a recovery assay is performed in each experiment (CARVALHO et al., 2007).

Administering inert or indigestible markers to animals as a means of estimating digesta fluxes has been a crucial tool in nutrition studies for a very long time (OWENS and HANSON, 1992; SMITH et al., 2021). Bergeim (1926) first described the potential of external markers usage and utility. The most often used external markers, according to a search in the Journal of Animal Science from 1910 to November 2020, were chromic oxide, n-alkanes, and titanium dioxide (TiO_2) (SMITH et al., 2021).

Miller et al. (1976) first noted the use of TiO_2 as a potential indicator of soil intake, and TiO_2 had not been widely used in ruminant studies until 1988, when Hafez et al. (1988) described the use as an external marker in dairy cows. Although dosages can differ, it has been shown that 10 g/d in cattle and 5 g/d in sheep are the most frequently reported doses (MEYERS et al., 2006; TITGEMEYER et al., 2001; SCHOLLJEGERDES and KRONBERG, 2008, 2010; SCHOLLJEGERDES et al., 2014). Like other external markers, sampling timepoints have varying fecal concentrations during a 24-h period. Maximum fecal recovery rates reportedly reached equilibrium after at least 5 days of TiO_2 administration (GLINDEMANN et al., 2009).

In dairy cattle, Hafez et al. (1988) found average fecal recovery rates of 98% (95 to 101%), and these recovery results were similar to the recovery rates reported by Titgemeyer et al. (2001) in steers fed corn-based diets. Additionally, Titgemeyer et al.

(2001) evaluated the fecal recovery of chromic oxide and discovered that TiO_2 consistently had a higher fecal recovery following 21 days of dosing, with 98.3 and 116.2% recovery for each compound. Glindemann et al. (2009) evaluated TiO_2 as an inert marker for estimating feces excretion in grazing sheep and concluded that the variation in feces concentration was smaller and accuracy prediction of feces excretion was higher with twice than once daily TiO_2 administration and grab sampling. Finally, TiO_2 estimates of fecal recovery rates and digestibility fall well within the scope of what has been reported for chromic oxide (SMITH et al., 2021) and in terms of external indicators, TiO_2 is a good choice for studies on ruminant nutrition.

3.4. Enteric CH_4, rumen microbiota and tannins

Methane (CH_4) is one of the major GHG responsible for at least 14% of total GHG emissions, with a global warming potential of 21-25 times greater than that of CO_2 (IPCC, 2022). The CH_4 emission from agriculture is estimated to be 50-60% of the global emission, being ruminants one of the majors responsible of these emissions (WANAPAT et al., 2015; KUMARI et al., 2016).

In Latin America, CH_4 emissions by cattle are highly variable and ranged from 48.5 to 656 g/day per head, with an average of 337, 202 and 146 g/head/day for dairy, mature and growing cattle, respectively (BENAOUDA et al., 2020). This variation can be attributed to wide ranges in DMI (2.04 to 20.6 kg/ day), forage digestibility and level of forage inclusion in the diet. It is important to consider that enteric CH_4 emissions also represent a loss of energy for ruminants and thus reduces feed efficiency (BENAOUDA et al., 2020). The gross energy lost in the form of CH_4 in grazing animals is around 6 to 18%. For animals in feedlot these losses are between 5 and 6%, and for high-producing dairy cows, the losses are around 2 to 12% (JONHSON and JONHSON, 1995; STELLA et al., 2020).

The rate of enteric CH_4 emissions and feed efficiency are key factors for decreasing the ecological impacts of the meat industry and are directly linked to the symbiotic processes between the host and its associated microbial communities, also known as the microbiota (ANDRADE et al., 2022). Even though studies targeting these phenotypes have been published over the last few years, only recently the microbiota started to be considered as an important subject to increase the production efficiency and reduce the cost and environmental impacts of livestock production (PERES ASSUMPÇÃO, 2021). The rumen is inhabited by a diversity of microorganisms, including bacteria, Archaea, fungi and protozoa. The interaction between these microbes is fundamental to promote the breakdown and fermentation of different feed components ingested by the ruminant host (CUNHA et al., 2017).

To reduce the environmental impact of CH_4 and improve the energy efficiency of ruminants, allowing to increase productivity and decrease GHG intensity, various strategies can be adopted. Genetic selection, better pasture managements, increase the supply of energy and non-structural carbohydrates in their diets, use of ionophores, essential oils, calcium and ammonium nitrate, exogenous enzymes, CH_4 inhibitors and tannins are examples of these strategies (VLAMING, 2008; TEDESCHI et al., 2022).

Tannins are polyphenolic polymers with molecular weights between 500 and 20,000 Daltons, generally water soluble except for some high molecular weight structures (PATRA and SAXENA, 2011) and are widely distributed in trees, shrubs, legumes, cereals, and grains (PERES ASSUMPÇÃO, 2021). According to their solubility, tannins are divided into hydrolysable (HT) and condensed (CT) tannins. High CT content are known as antinutritional factor in ruminant diets, reducing intake and growth performance and negatively altering carcass characteristics (REED, 1995). One of the main characteristics of CT is to bind nutrients (mainly proteins) and form soluble or insoluble tannin-protein complexes, hence reducing protein digestion (PIÑERO-VÁZQUEZ et al., 2015). However, ruminant animals present greater tolerance to tannins since the action of ruminal microorganisms can degrade several anti-nutritional factors into simpler and non-toxic compounds (SELINGER et al., 1996).

Several studies have demonstrated that CH_4 mitigation by CT both *in vitro* and *in vivo* is related to a decrease of methanogenic Archaea communities (TAVENDALE et al., 2005; BHATTA et al., 2009; TAN et al., 2011; CIESLAK et al., 2012; TSEU et al., 2020; PERNA JUNIOR et al., 2022). Tan et al. (2011) found that a CT inclusion of 20 to

60 g kg^{-1} DM resulted in a linear reduction in total methanogens with a corresponding decrease in CH_4 production. A literature review conducted by Piñero-Vázquez et al. (2015) on the potential of CT in reducing CH_4 emissions showed a reduction in methanogenic archaea and protozoa population by as much as 33% and 79%, respectively. They also found that CT bind proteins and polysaccharides, forming complexes that reduce the digestibility of dry matter (DM) and OM, as well as the production of H_2 used by archaea to form CH_4 (PIÑERO-VÁZQUEZ et al., 2015).

According to Mueller-Harvey (2006), due to the highly heterogeneous phenolic chemical structures in legumes, CT from different plant species may cause different responses to nutrients availability and their use by ruminants, even when consumed at the same concentration (DENTINHO and BESSA, 2016). In a study characterizing and evaluating the biological activity of CT from tropical forage legumes, Pereira et al. (2018) concluded that offering leaves of pigeon pea in ruminant diets should be beneficial due its CT concentration (33 g kg^{-1} DM).

4. MATERIAL AND METHODS

4.1 Location, treatments, and experimental design

The study was approved and followed the guidelines of the Committee for the Use and Care of Institutional Animals (CEUA) of Embrapa (nº 05/2016) and College of Veterinary Medicine and Animal Science of University of São Paulo (nº 6228200521), being conducted at Embrapa Pecuária Sudeste, São Carlos, SP, Brazil.

The treatments consisted of three pasture-based systems: 1) Degraded pasture (DEG) of *Urochloa decumbens* Stapf cv. Basilisk; 2) Recovered pasture (REC) stablished with a mixture of *U. decumbens* cv. Basilisk and *U. brizantha* (Hochst ex A. Rich) Stapf cv. Marandu managed with a moderate stocking rate; and 3) Intercropped pasture (MIX), a mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim also using a moderate stocking rate. Each treatment was distributed in three grazing units (1.5 ha, Figure 1) in a completely randomized design, totaling 9 grazing units (13.5 ha in total).

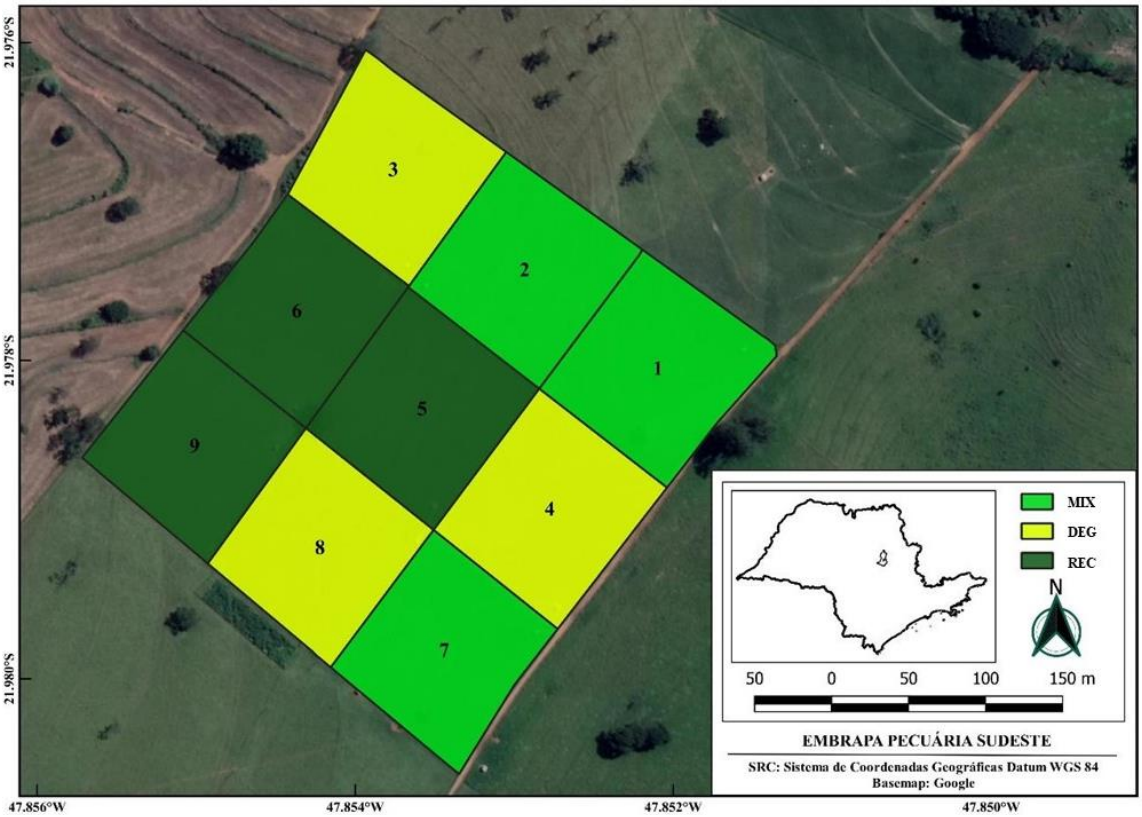


Figure 1 - Aerial view of the experimental area. 21°59’ S 47°51’ W 892m asl. Source: Adapted from Google Earth and SRC.

A total of 36 *Nellore* steers from the herd of Embrapa Pecuária Sudeste (221 ± 7 kg of body weight and 15-16 months of age) were used as experimental animals randomly distributed in the grazing units [12 animals per treatment – nine non-fistulated (testers animals) and three fistulated]. In each treatment, nine animals were monitored for performance evaluation, six were used for CH_4 measurements using the SF_6 tracer gas technique, and three monitored for dry matter intake (DMI) measurements. A variable number of “non-experimental” animals were used to adjust the stocking rate by the “put and take” technique as described by Mott and Lucas (1952) considering the visual assessment of the forage mass availability in each grazing unit (COSTA and QUEIROZ, 2013).

During the experimental period that lasted from July 2020 to July 2021, samples for assessing forage quality, DMI and CH_4 emissions were collected in two seasons: rainy (January) and dry (July). For performance evaluations the animals were monthly monitored. All pastures were established in 1996 with *U. decumbens* cv. Basilisk and the MIX treatment was established in 2011 by overseeding pigeon pea and replanting every three years due to the decline in plant population over the years. Initially the pigeon pea stand is around 75,000 plants per hectare, however this number may decrease over the years due to animal trampling and grazing, adverse weather conditions, plant senescence, leaf-cutting ant attack, and others (OLIVEIRA et al., 2017).

During the dry season, the average temperature was 20.4ºC and the average cumulative rainfall was 156 mm, while during the rainy season the average temperature was 22.9ºC and the average cumulative rainfall was 868 mm according to the climatic data obtained from an automatic weather station located near the experimental site.

All pastures were managed under continuous grazing, and nitrogen fertilization (200 kg of N-urea per ha, divided in five applications during the rainy season) was applied only for the REC system. REC and MIX pastures were corrected and fertilized with superphosphate and potassium and managed to maintain a specific intermediate height for each grass species, as recommended by Costa and Queiroz (2013). DEG systems were not corrected, fertilized nor managed to maintain a specific height, and when it was not possible to include regulatory animals to adjust stocking rate, only experimental animals were kept in the pastures. Mineral supplement was provided *ad libitum* throughout the year (Table 1). During the dry season MIX animals received the mineral formulation (50% NaCl and 50% Minerthal® mineral mix), while REC and DEG animals received an adaptation formulation for 14 days and then an energy-protein supplementation composed by corn meal, NaCl, mineral mix and urea. During the rainy season all animals received only the mineral formulation.

Table 1 - Formulation and composition of the mineral supplements.

Ingredients (%)	Supplementation: Rainy Season	Adaptation[3]	Dry Season	
	Mineral	Energetic-Protein	Energetic-Protein	Mineral
Ground corn	-	55	48	-
NaCl	50	20	15	50
Mineral mixture[1]	50	15	15	50
Urea[2]	-	10	22	-
Estimated Chemical Composition				
CP	-	49.33	97.15	-
NPN	-	28	61.6	-
NDF	-	5.01	7.30	-
ADF	-	1.53	1.67	-
Lig	-	1.26	1.25	-
EE	-	1.66	1.29	-
Ash	90.68	56.6	22.60	88.22

[1]Minerthal® quantity per kg of product: 200 g of calcium, 160 g of phosphorus, 60 g of sulfur, 185 g of sodium, 200 mg of cobalt, 2.5 g of copper, 1.6 g of fluorine, 125 mg of iodine, 2.25 g of manganese, 50 mg of selenium, 7.5 g of zinc; [2]Heringer ®; [3]Adaptation supplement provided for 14 days for adaptation of the rumen microbiota; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; Lig: lignin; EE: ether extract; Ash: mineral matter.

Monthly the number of animals in each pasture and their weight were monitored to allow estimate stocking rate [expressed as number of animals or AU (450kg of body weight) per ha], animal performance (kg of average body weight gain per day, ADG) and productivity parameters (kg of body weight per ha).

4.2. Forage sampling and chemical analysis

For collecting samples of forages, the methodology of grazing simulation with observations of ingestive behavior described by Sollenberger et al. (1995) was used. The forages (pastures and pigeon pea) were collected by hand plucking (±150 g of fresh matter) in three consecutive days, observing the animals for approximately 24 minutes, and using scissors to cut the portion of forages in which the animals were consuming. Samples were stored in paper bags (18 cm × 44 cm), weighed, and then dried in a forced ventilation oven at 65 °C for 72 h (pigeon pea samples were dried at 40 °C until the sample weight became constant to not compromised tannin analysis), milled to 1 mm in a Willey-type mill and subjected to chemical analysis at the Animal Nutrition Laboratory of Embrapa Pecuária Sudeste.

Forages chemical analysis were based on the content of DM (DM at 105 ºC; Method 934.01, AOAC, 1990). Concentrations of mineral matter (MM; Method 923.03 – hereafter called Ash), crude protein (CP; Method 920.87) and ether extract (EE; Method 920.85) were determined according to AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Lig), as well as neutral-detergent insoluble nitrogen (NDIN) and acid-detergent insoluble nitrogen (ADIN) were analyzed according to Goering and Van Soest (1970). Gross energy (GE) was determined using a bomb calorimeter (IKA WERKE, model C 500). Condensed tannins (CT) was evaluated using the methodology proposed by Makkar (2003). The isotope ratio of C ($^{13}C/^{12}C$) of forages samples were determined using a continuous-flow isotope ratio mass spectrometer (Delta Plux, ThermoFisher Scientific, Bremen, Germany) coupled to an elemental analyzer (CHN-1110, Carlo Erba, Rodano, Italy) at the Laboratory of Isotope Ecology of the Center for Nuclear Energy in Agriculture (LEI-CENA/USP), and calculated as:

$$\delta\ (‰) = [(R\text{sample} / R\text{stantard}) - 1] \times 1000$$

where: R is the ratio of $^{13}C/^{12}C$ and Pee Dee Belemnite is the internationally recognized standard.

4.3. Dry matter intake and dry matter digestibility

The total DMI (kg DM/day) was estimated by the sum of forage and mineral supplement consumed by the animals:

$$\mathrm{DMI} = \mathrm{DMI_s} + \mathrm{DMI_f}$$

where: DMI = total dry matter intake (kg DM/ day); DMI_f = forage dry matter intake (kg DM/ day); DMI_s = mineral supplement intake (kg).

The mineral supplement intake was estimated by the difference between the amount provided and the amount of supplement leftovers in the trough after five days. For this measurement a digital scale (1-10000g) was used, and the calculation followed the equation:

$$\mathrm{DMI_s} = \frac{\left[\frac{(\mathrm{DMI_{sSupplied}} - \mathrm{DMI_{sLeftovers}})}{5_{(\mathrm{days})}}\right]}{\mathrm{Total\ Weight}}$$

where: DMI_s = mineral supplement intake (kg); $DMI_{sSupplied}$ = total supplement provided (kg); $DMI_{sLeftovers}$ = mineral supplement leftovers after 5 days (days); Total Weight = total weight of animals with access to that trough (kg).

To determine the forage DMI (DMIf) indirect methods with external (titanium dioxide, TiO2) and internal (indigestible neutral detergent fibre, iNDF) markers were used. TiO2 in small paper capsules was instilled with the aid of an oral applicator. The external marker was administered for 10 days in the amount of 15 g per animal per day. In the last 5 days of TiO2 administration, feces samples were collected after spontaneous defecation in the paddocks (Figure 2). The feces samples were frozen in properly identified plastic bags, then thawed, homogenized, and dried (65º C for five days).

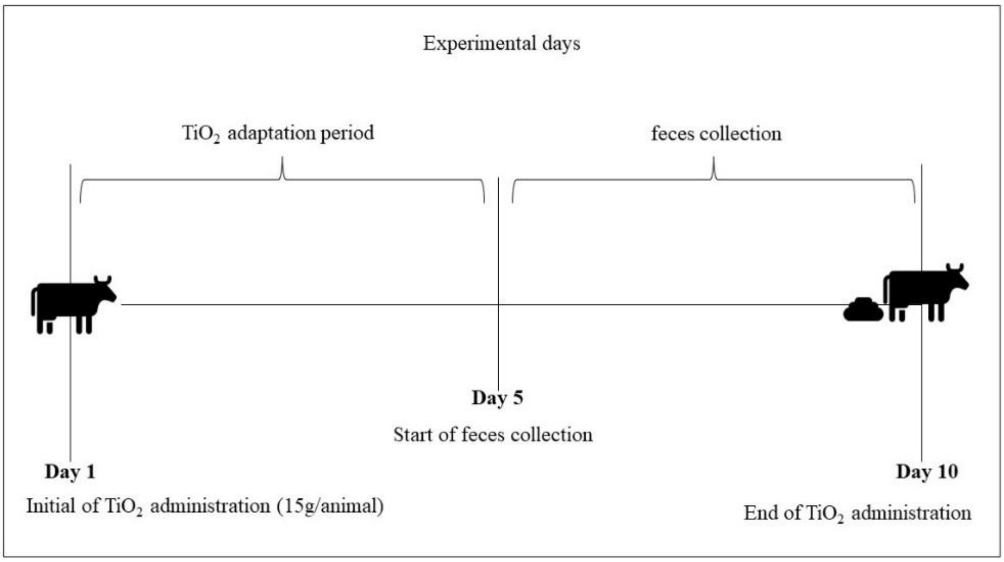


Figure 2 - Schematic representation of TiO_2 administration during the experimental period. Adapted from Pasquini Neto (2022).

After drying, the samples were ground in a Willey-type knife mill with 2 mm sieves. Subsequently, analysis of TiO_2 and iNDF were performed using the technique described by Mertens (1993) and Titgemeyer et al. (2001), and DMI_f was calculated according to the equation:

$$\mathrm{DMI_f} = \frac{\left[\mathrm{iNDF_{(feces)}} \times \text{ fecal output}\right]}{\mathrm{iNDF_{(forages)}}}$$

where: DMI_f = forage dry matter intake (kg DM/day); fecal output = TiO_2 supplied/TiO_2 recovered in feces (kg/day); $iNDF_{(feces)}$ = feces content of indigestible neutral detergent fiber (%); $iNDF_{(forages)}$ = forages content of indigestible neutral detergent fiber (%).

The dry matter digestibility (DMD) was calculated through an indirect method, using the following equation adapted from Givens et al. (2000):

$$\mathrm{DMD} = 100 - \left(100 \times \left(\frac{\text{fecal output}}{\mathrm{DMI_{Total}}}\right)\right)$$

where: DMD = dry matter digestibility (%); DMI_{total} = total dry matter intake (kg); and fecal output = TiO_2 supplied/TiO_2 recovered in feces (kg/day).

Feces samples were also analyzed for their C isotopic composition as previously described and the principle of isotopic differences between C_3 and C_4 plants was used to estimate the intake proportion of each forage (tropical grasses – C_4; and pigeon pea – C_3) following the equation described by Norman et al. (2009) and Ovani et al. (2022):

$$C_4\ (\%) = 100 - \left(100 \times \left(\frac{(\mathrm{A-B-D})}{(\mathrm{C-B})}\right)\right)$$

where: A = $\delta^{13}C$ value in feces; B = $\delta^{13}C$ value of the C_4 plant; C = $\delta^{13}C$ value of the C_3 plant; D = fraction factor for $\delta^{13}C$ in feces.

4.4. Animal performance

To determine performance variables, the animals were weighed on a hydraulic trunk with built-in scale (Parede Móvel Hidráulico/idBeck 3.0 - BechHouser®, 2009) after 16 h of fasting and this was repeated every 28 days. The individual performance was evaluated by animals' average daily gain (ADG) obtained by dividing the body weight (BW) difference between two successive weighing by the interval of days between measurements, according to the equation:

$$\mathrm{ADG} = \frac{\mathrm{fBW} - \mathrm{iBW}}{\mathrm{IW}}$$

where: ADG = average daily gain (kg); fBW = final BW, most recent animal weight (kg); iBW =initial BW, animal weight from previous weighing (kg); IW = interval between weighing (days).

The first and last weighing expressed as kg/animal were considered as the initial average body weight (ABWinitial) and final average body weight (ABWfinal), respectively, and were used to determine the average live weight gain per area (kg/ha). Through the weighing performed every 28 days, it was possible to adjust the stocking rate (SR) according to the forage availability using the "put and take" technique (MOTT and LUCAS, 1952). The SR was expressed in animal unit (AU), assuming that one AU is equivalent to 450 kg for animals of the Zebu breed according to the equation:

$$\mathrm{SR} = \frac{\left(\frac{\mathrm{BWtotal}}{\mathrm{AU}}\right)}{\mathrm{Area}}$$

where: SR= stocking rate (AU ha^{-1}); BWtotal = total body weight of tracers and regulators animals present in the experimental area (kg); AU = Animal unit (450 kg); Area = experimental unit area (ha^{-1}).

The feed conversion ratio (FCR), and feed efficiency (FE) were calculated using the following formulations:

$$\mathrm{FCR} = \frac{\mathrm{DMI}}{\mathrm{ADG}}$$

$$\mathrm{FE} = \frac{\mathrm{ADG}}{\mathrm{DMI}}$$

where: FCR = feed conversion ratio (kg DMI/kg ADG); DMI = dry matter intake (kg DM/day); ADG = average daily weight gain (kg); FE = feed efficiency (kg ADG/kg DMI).

4.5. Enteric CH_4 emission

The SF_6 tracer gas technique (JOHNSON et al., 1994 a,b; PRIMAVESI et al., 2004) was used for measuring enteric CH_4 emissions from rumination, eructation and breathing. As described by Berndt et al. (2013), the technique comprises the administration of a permeation tube (capsule) through the animals’ oral cavity being then housed in the rumen or reticulum. The capsule releases a known amount of SF_6, which is then eructed together with CH_4 (BERNDT et al., 2013). Fourteen days before gas sampling the animals were fitted with gas collection halters to allow acclimatization in an adaptation period (Figure 3). Seventy-two hours prior the sampling period a small brass permeation tube was placed in the rumen allowing the tracer gas to equilibrate in the ruminal environment. Each animal was sampled daily (24h) for five consecutive days. The gas samples were obtained continuously through a capillary tube connected to a collecting container placed on the neck of the animal, as shown in Figure 3. A halter with a 0.127 mm stainless steel capillary tube and a 15 µm in-line filter was placed on the animal’s head and connected to an evacuated sampling vessel. Before the experiment, collection canisters made of polyvinyl chloride (PVC) were attached to a vacuum pump in the laboratory to create a negative pressure (around -13.15 psi). As the vacuum in the sampling vessel slowly dissipated, the negative pressure continuously drew the air sample around the animal’s mouth and nose.

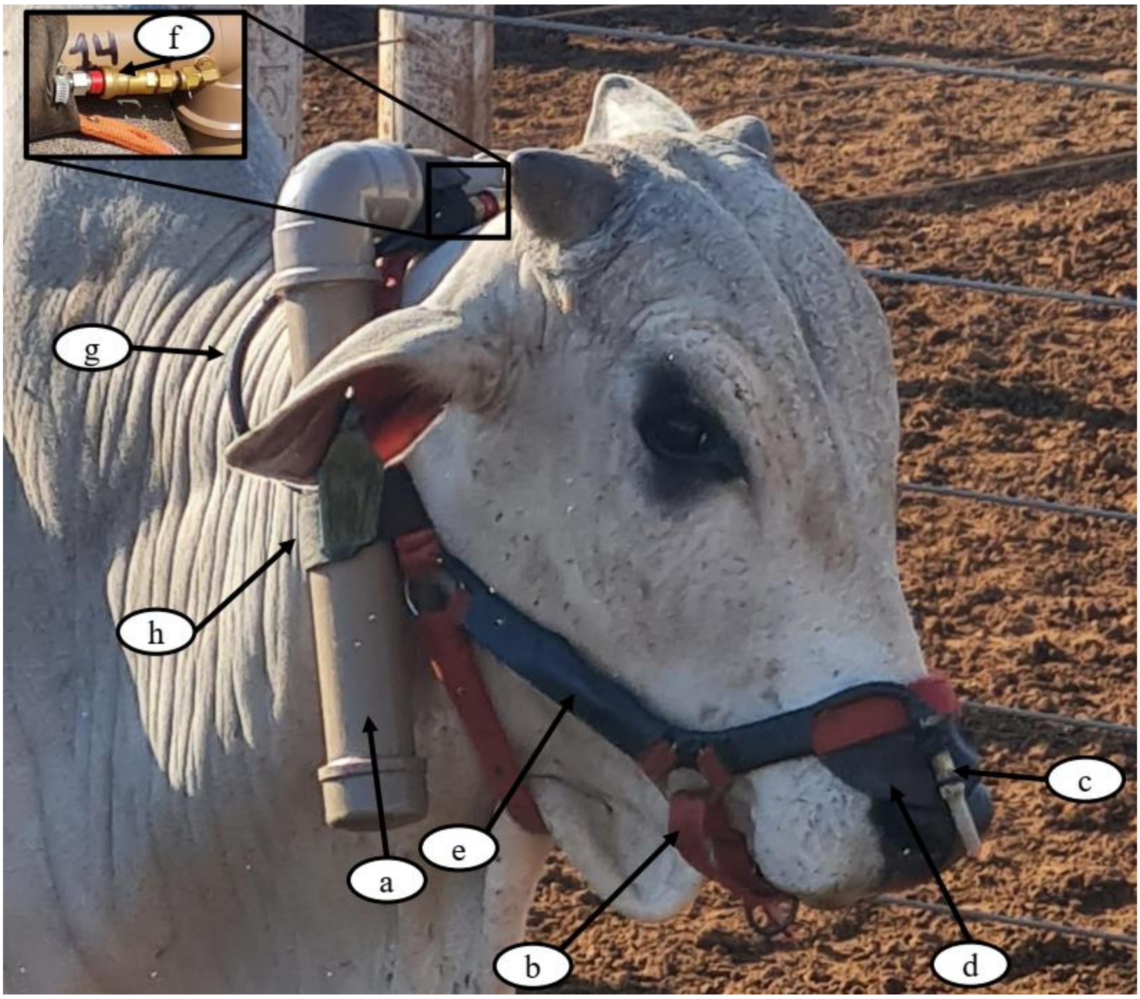


Figure 3 - Animal with halter and yokes: (a) PVC canister; (b) halter; (c) filter; (d) muzzle protector; (e) stainless steel capillary tubing; (f) quick-connect coupling; (g) tubing; (h) Velcro strip. Source: Adapted from Deramus et al. (2003).

Additional PVC canisters were placed near the experimental pastures to monitor the ambient daily concentration of CH_4 and SF_6 during each sampling period. Sampling was performed daily at 07:00 h when the animals were removed from the paddocks and transferred to the working facilities of Embrapa Pecuária Sudeste. After the gas sampling, pure nitrogen was added to the canisters and then CH_4 and SF_6 were measured using gas chromatographs (Agilent HP-6890, Delaware, USA; and Shimadzu GC-2014, Columbia, MD, USA).

The CH_4 flux was calculated according to Westberg et al. (1998), using the following equation:

$$QCH_4 = QSF_6 \times \left(\frac{[(CH_4)_Y - (CH_4)_b]}{[(SF_6)_Y - (SF_6)_b]}\right)$$

where: QCH_4= CH_4 emission rate per animal; QSF_6 = known SF_6 emission rate from the capsule in the rumen; $(CH_4)_Y$ = CH_4 concentrations in the collection device; $(CH_4)_b$ = basal concentration of CH_4; $(SF_6)_Y$ = SF_6 concentration in the collection device and $(SF_6)_b$= basal SF_6 concentration in the ambient.

The gross energy intake (GEI) was calculated by multiplying DMI (kg) and diet GE (MJ/kg), and the CH_4 conversion rate (Ym, the percentage of GEI converted to CH_4) was calculated using the following equation, considering the heat value of CH_4 as 55.6 MJ/kg:

$$\text{Ym}\ (\%) = \frac{(CH_4 \times 55.6)}{GEI} \times 100$$

4.6. Statistical analysis

For the statistical analysis the grazing units (paddocks) were considered the experimental units for data obtained by area, and individual steers were considered the experimental units for data obtained per animal. Data were analyzed using the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Before the analysis, outliers were identified, and the normality of residuals tested (Shapiro-Wilk). When the normality assumption was not accepted, the logarithmic transformation was applied, then the data were analyzed using the mixed procedure (PROC MIXED) testing different covariance structures and chosen the best fitting model based on the lowest value of the corrected Akaike information criterion (AICC) (WANG AND GOONEWARDENE, 2004). The statistical model included the three pasture-based grazing systems and seasons (dry and rainy) as fixed effects, and the interaction between treatment and season was tested. Fixed effects were considered significant at the 5 %, and in the face of treatments×seasons interaction the effects of one factor within the other were evaluated using the SLICE command of PROC MIXED. Finally, all means were estimated according to the least squares test (LSMEANS) and the multiple comparisons were performed using the GLIMMIX procedure applying the Fisher's test through the PDIFF LINES option.

5. RESULTS

The chemical composition, content of condensed tannins and *in vitro* dry matter digestibility of pigeon pea sampled during the dry and rainy seasons through hand plucking following the methodology of grazing simulation are presented in Table 2.

Table 2 – Chemical composition and condensed tannins content of *Cajanus cajan* spp. during the rainy and dry seasons of the experimental period.

Seasons	CP (%)	NDF (%)	ADF (%)	Lig (%)	EE (%)	Ash (%)	NDIN (%)	ADIN (%)	GE (cal/g)	CT[1]
Rainy	17.8	42.4	26.8	12.3	5.7	5.2	0.93	0.25	4431.3	23.7
Dry	24.3	43.9	28	12.4	5.6	5.8	0.95	0.25	4509.8	87.9
Average	21	43.2	27.4	12.4	5.7	5.5	0.94	0.25	4470.5	55.8
SEM	1.8	0.8	0.4	0.4	0.1	0.1	<0.1	<0.1	94.27	15.11

Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (Lig), ether extract (EE), mineral matter (Ash), neutral detergent insoluble nitrogen (NDIN), acid-detergent insoluble nitrogen (ADIN), gross energy (GE) expressed as calorie per gram (cal/g) and condensed tannins (CT[1]), expressed as eq-g leucocyanidin/kg DM. SEM: Standard error of the mean.

The chemical composition, condensed tannins content and dry matter digestibility of the forages in the different pasture-based systems, considering the proportion of *Urochloa* spp. (C_4) and pigeon pea (C_3) intake for MIX treatment estimated by stable isotopes are presented in Table 3. The isotopic results [$\delta^{13}C$ value in feces = -13.8 ± 0.23‰ in the rainy season, and -18.7 ± 1.33‰ in the dry season; $\delta^{13}C$ value of the C_4 plant = -13.7 ± 0.17‰; $\delta^{13}C$ value of the C_3 plant = -26.1 ± 0.08‰] indicated that there was no intake of the legume during the rainy season; however, during the dry season the intake of pigeon pea reached around 65% (64.8 ± 0.40) of the diet in the MIX treatment.

Treatment affected all evaluated variables, which except for CP, ADF and ADIN were also affected by the different seasons ($P < 0.05$). When compared to DEG and REG, the MIX treatment present higher values of CP, Lig, EE, NDIN, ADIN, GE and CT (P <

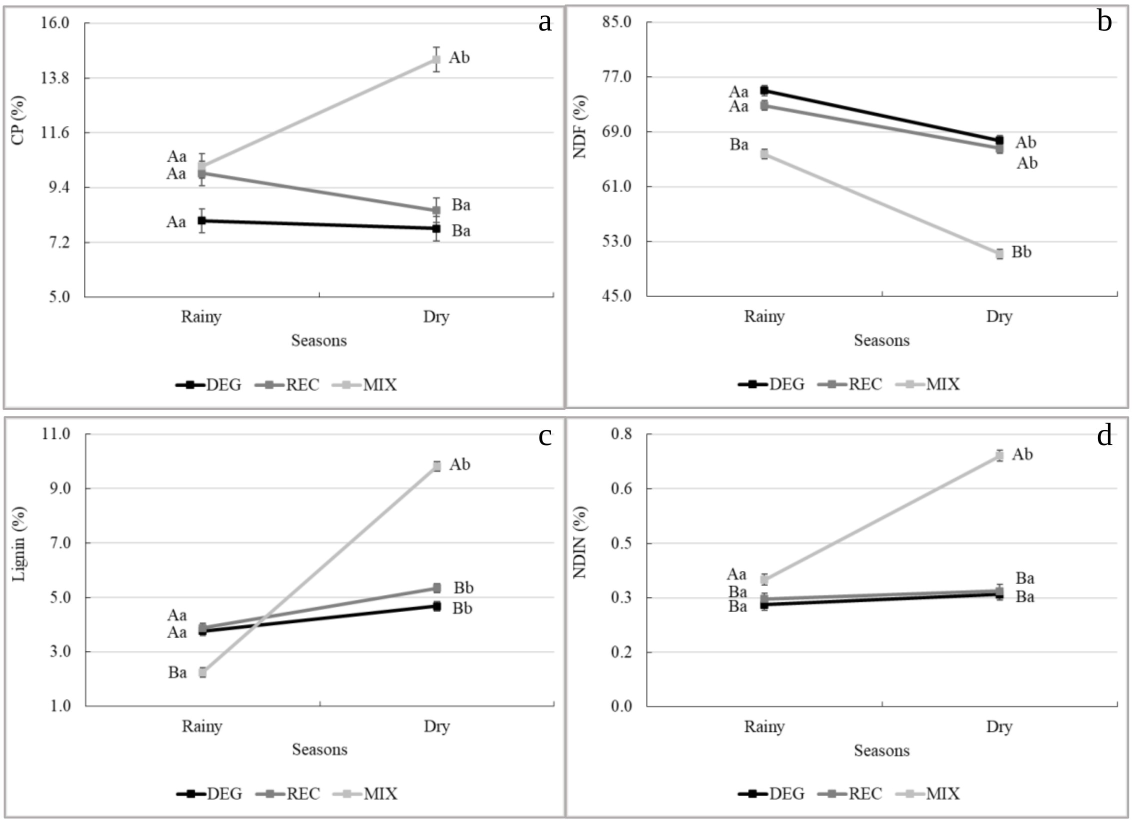
0.05). On the other hand, lower values of NDF, ADF and Ash were found for MIX when compared to DEG and REG ($P < 0.05$). During the dry season were found higher values of Lig, EE, NDIN, GE and CT, and lower values of DMD when compared to rainy season ($P < 0.05$).

Table 3 – Chemical composition, condensed tannins content and dry matter digestibility of the forages collected by hand-plucking in the different pasture-based systems.

Effects		Nutritive Composition										
Treat.	Seasons	CP (%)	NDF (%)	ADF (%)	Lig (%)	EE (%)	Ash (%)	NDIN (%)	ADIN (%)	GE (cal/g)	CT[1]	DMD (%)
DEG		7.9[b]	71.4[a]	40.1[a]	4.22[b]	2.25[b]	8.60[a]	0.32[b]	0.05[b]	3611.6[b]	0.32[b]	51.2
REC		9.2[b]	69.7[a]	40.5[a]	4.61[b]	1.85[b]	8.32[a]	0.33[b]	0.05[b]	3649.1[b]	0.25[b]	55.3
MIX		12.4[a]	58.4[b]	33.7[b]	6.03[a]	3.04[a]	7.89[b]	0.56[a]	0.20[a]	3969.0[a]	29.35[a]	59.8
	Rainy	9.2	71.2	38.7	3.30	2.11	8.87	0.33	0.09	3660.3	0.79	67.8
	Dry	10.3	61.9	37.6	6.61	2.64	7.68	0.47	0.11	3826.3	19.20	43.1
Average		9.8	66.5	38.2	4.96	2.38	8.27	0.4	0.1	3743.3	9.97	55.4
SEM		0.5	0.7	0.5	0.17	0.13	0.11	<0.01	<0.01	33.2	1.27	3.2
Statistical Probabilities (P value)												
Treatm.		**0.016**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	0.061
Seasons		0.087	**<.001**	0.227	**<.001**	**0.013**	**<.001**	**<.001**	0.201	**0.004**	**0.011**	**<.001**
Treat×Season		**0.003**	**0.011**	0.068	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.004**	**<.001**	**0.006**

[a, b, c] Different lowercase letters in the same column represent treatments that differ from each other ($P \leq 0.05$) by Fisher's test. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) lignin (Lig), ether extract (EE), mineral matter (Ash), neutral-detergent insoluble nitrogen (NDIN) and acid-detergent insoluble nitrogen (ADIN), gross energy expressed as calorie per gram (GE) condensed tannins (CT[1]), expressed as eq-g leucocyanidin/ kg DM and dry matter digestibility (DMD). DEG: degraded pasture of *Urochloa decumbens* cv. Basilisk; REC: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu, fertilized with 200 kg of N-urea ha^{-1} year; MIX: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim. SEM: Standard error of the mean.

The decomposition of the treatment×season interaction is present in Figure 4. Similar results of CP, GE, CT and DMD were found among treatments during rainy season (Figure 4a, 4h, 4i and 4j) (P > 0.05). However, during the dry season, higher values of these variables were found for MIX when compared to REG and DEG treatments (Figure 4a, 4h, 4i and 4j) (P < 0.05). During both seasons, MIX presented higher values of ADIN (Figure 4e) and lower values of NDF (Figure 4b) when compared to REG and DEG treatments (P < 0.05). The Lig content in the MIX treatment was lower in the rainy and higher in the dry season when compared to REG and DEG (P < 0.05) (Figure 4c). During the rainy season, MIX presented lower values of EE; however, higher value than DEG and REG treatments was found in the dry season (Figure 4f). The highest content of Ash was found in the MIX treatment during rainy season; however, the lowest value was found for that treatment during the dry season of the experimental period (P<0.05) (Figure 4g).



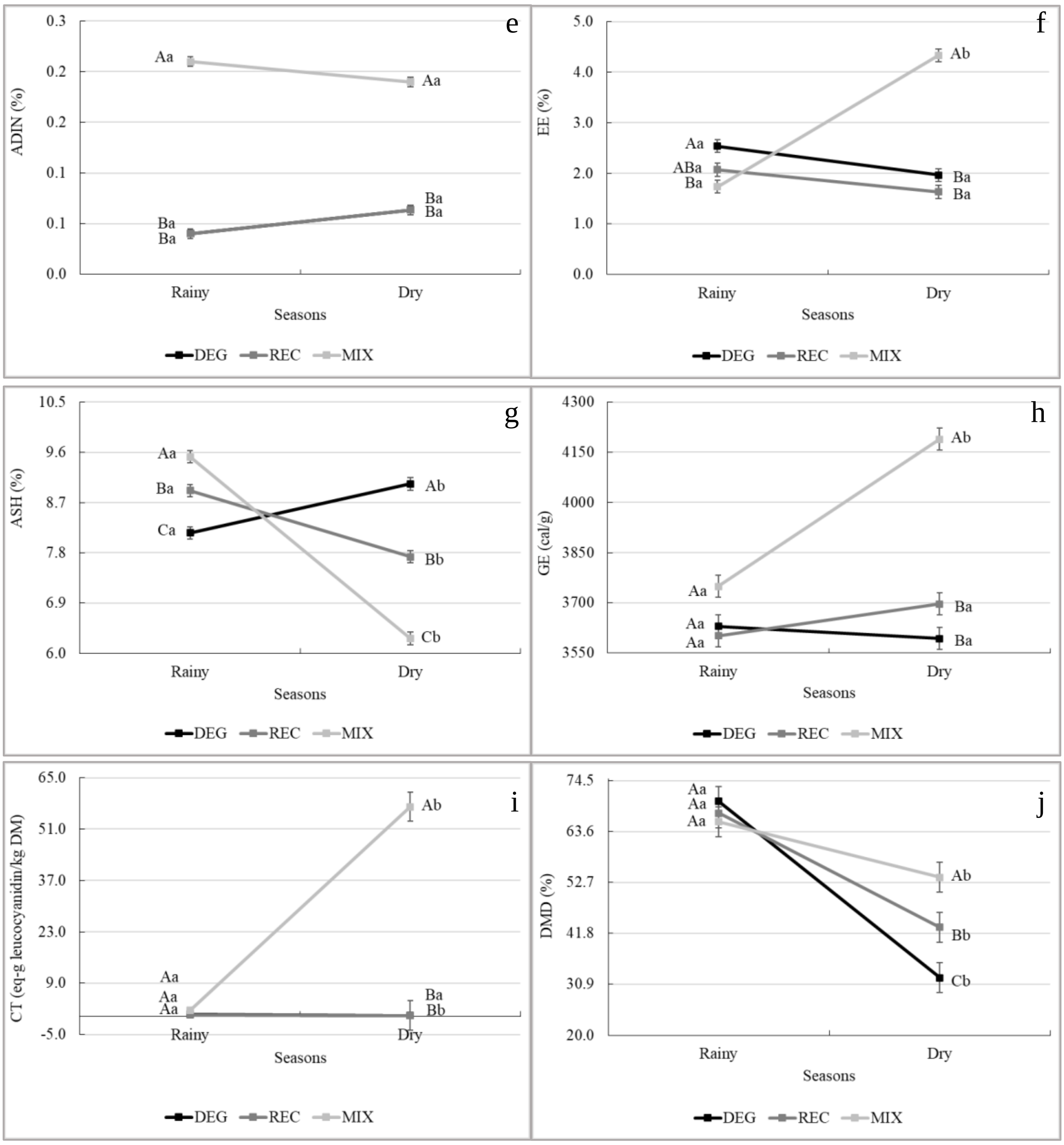


Figure 4 – Decomposition of the treatment×season interaction for the chemical composition, condensed tannins content and dry matter digestibility of the forages collected by hand-plucking in the different pasture-based systems. Different capital letters indicate statistical differences among treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test ($P \leq 0.05$). Vertical bars are standard error of the mean.

The average values of forage and mineral supplement DMI during the experimental period are presented in Table 4. When the DMI was expressed as %ABW, no effect of treatment (P > 0.05) was found for forage, total, and supplement DMI. Nevertheless, when expressed as kg DM/ day, the DMI was lower in the MIX treatment (P < 0.05) as compared to the other treatments. In addition, when expressed as kg DM/day, higher values of forage and total DMI were found in the rainy season (P < 0.05), while when expressed as %ABW, higher supplement DMI was found in the dry season (P < 0.05).

Table 4 – Average values of forage, mineral supplement and total DMI in the different pasture-based systems during the experimental period.

Effects		Variables						
Treat.	Seasons	Forage DMI		Supplement DMI		Total DMI		$DMI/BW^{0.75}$
		kg/day	%ABW	kg/day	%ABW	kg/day	%ABW	kg/kg
DEG		7.12	2.33	0.07[a]	0.023	7.20	2.42	0.103
REC		7.56	2.20	0.07[a]	0.025	7.63	2.27	0.102
MIX		8.24	2.29	0.04[b]	0.015	8.28	2.32	0.102
	Rainy	9.62	2.59	0.05	0.011	9.67	2.64	0.120
	Dry	5.66	1.96	0.07	0.027	5.73	2.04	0.084
Average		7.64	2.27	0.06	0.021	7.70	2.29	0.102
SEM		1.65	0.24	0.005	0.002	0.83	0.23	0.010
Statistical Probabilities (P value)								
Treat.		0.7689	0.9503	**0.0068**	0.2158	0.7856	0.9492	0.9922
Seasons		**0.0118**	0.0903	0.0552	**0.0420**	**0.0132**	0.0965	0.0544
Treat. × Season		0.3145	0.2009	0.1384	0.9399	0.3245	0.2088	0.5642

[a, b, c] Different lowercase letters in the same column represent treatments that differ from each other (P ≤ 0.05) by Fisher's test. DMI: dry matter intake; ABW: average body weight; $BW^{0.75}$: metabolic body weight. DEG: degraded pasture of *Urochloa decumbens* cv. Basilisk; REC: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu, fertilized with 200 kg of N-urea ha^{-4} year; MIX: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim. SEM: Standard error of the mean.

The average values of ADG, body weight (ABW) and stocking rate in the three different pasture-based treatments in the rainy or dry seasons during the experimental period are presented in Table 5, as well as the statistical probabilities. Higher values of ADG and ABW were observed in the MIX treatment when compared to DEG and REC (P < 0.05), while higher stocking rate was observed in the REC treatment (P < 0.05). The rainy season presented higher values of ADG and ABW when compared to the dry season (P < 0.05), but no differences between seasons were observed for stocking rate expressed as AU/ha

$(P > 0.05)$. When expressed as number of animals per hectare, higher stocking rate was observed in the dry season of the year $(P < 0.05)$.

Table 5 – Average values of initial and final body weight, average daily gain, average live weight, stocking rate, feed conversion ratio and feed efficiency in the different pasture-based systems and seasons of the experimental period.

Effects		Variables							
Treat.	Seasons	iBW	fBW	ADG	ABW	Stocking rate		FCR	FE
		kg	kg	kg/ani/day	kg/ani	ani/ha	AU/ha	kg/kg	kg/kg
DEG		220.7	344.1[b]	0.302[c]	313[b]	2.5[c]	1.5[c]	37.9	0.038
REC		222.8	368.2[b]	0.387[b]	323[b]	4.5[a]	3.0[a]	50.5	0.043
MIX		230.5	401.9[a]	0.478[a]	366[a]	4.0[b]	2.6[b]	28.5	0.062
	Rainy	*	*	0.667	379	3.5	2.4	17.2	0.073
	Dry	*	*	0.112	289	3.8	2.3	60.2	0.022
Average		224.4	371.4	0.369	334	3.7	2.38	38.8	0.048
SEM		11.9	10.5	0.013	6.6	0.07	0.080	8.1	0.009
Statistical Probabilities (P value)									
Treat.		0.8339	**0.0073**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	0.4768	0.4135
Seasons		*	*	**<.0001**	**<.0001**	**0.0031**	0.4376	**0.0175**	**0.0063**
Treat. × Season		*	*	**0.0055**	**0.0415**	**<.0001**	**<.0001**	0.4708	0.5642

[a, b, c] Different lowercase letters in the same column represent treatments that differ from each other (P ≤ 0.05) by Fisher's test. * Data not presented by season. ADG: Average daily weight gain; ABW: Average live weight; AU: Animal unit (450 kg of body weight), Feed-Conversion Ratio (FCR), Feed Efficiency (FE), DEG: degraded pasture of *Urochloa decumbens* cv. Basilisk; REC: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu, fertilized with 200 kg of N-urea ha^{-4} year; MIX: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim. SEM: Standard error of the mean.

Interaction between season and treatments were found for ADG, ABW and stocking rate parameters (P < 0.05) (Figure 5 a-d). All treatments presented greater ADG and ABW values in the rainy season (P < 0.05). However, for the MIX treatment, higher value of stocking rate was observed in the dry season of the year (P < 0.05), which could be related to a greater forage biomass usually obtained when including *Cajanus cajan* in pasture systems. In fact, MIX treatment showed higher values of ADG and ABW when compared to DEG and REC in both seasons (P < 0.05). However, the highest stocking rate was observed in the REC treatments during the rainy season (P < 0.05).

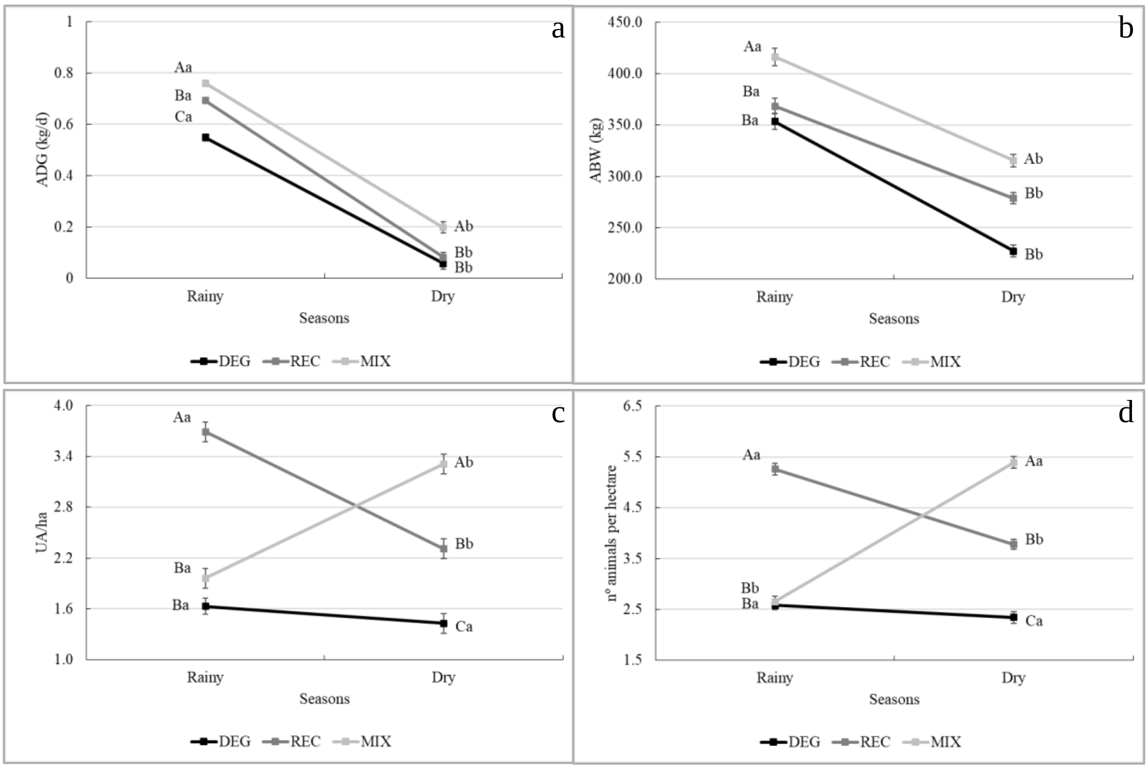


Figure 5 – Decomposition of the treatment×season interaction for ADG, ABW and stocking rate in the different pasture-based systems during the experimental period. Different capital letters indicate statistical differences among treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test (P ≤ 0.05). Vertical bars are standard error of the mean.

The average values of enteric CH_4 emissions using the SF_6 tracer gas technique in the different treatments and seasons, as well as the statistical probabilities are presented in Table 6. Except when CH_4 was expressed as g per kg of live weight ($P > 0.05$), the emissions in the rainy seasons were greater when compared to the dry season of the year ($P < 0.05$). The highest CH_4 emissions were found in the DEG treatment when expressed per ADG or per AU per hectare ($P < 0.05$), while the lowest CH_4 intensity was observed for MIX treatment when expressed per ADG or hectare ($P < 0.05$). No effect of treatment nor season was found for CH_4 per DMI or Ym ($P > 0.05$). When expressed as CH_4/GEI, higher emission was found in the rainy season of the experimental period ($P < 0.05$).

Table 6 – Average values of CH_4 emissions and Ym in the different pasture-based systems and seasons of the experimental period.

Effects		Variables							
Treat.	Seasons	CH_4/Ani g/Ani.day	CH_4/ADG g/kg/Ani.day	CH_4/ABW g/kg.BW	CH_4/ha[†] kg/ha.season	CH_4/AU[†] kg/AU.season	CH_4/DMI g/kg	CH_4/GEI MJ	Ym %
DEG		222.4	2022.7[a]	0.694	84.1	73.23	31.4	108.7	12.5
REC		218.7	1053.6[b]	0.701	141.0	66.30	30.5	115.4	11.1
MIX		204.2	614.1[c]	0.616	121.1	63.87	26.7	137.0	9.3
	Rainy	236.6	351.8	0.697	122.5	66.83	25.4	147.7	9.6
	Dry	193.6	2108.4	0.644	108.3	68.77	33.6	93.0	12.3
Average		215.1	1254.5	0.67	115.38	67.80	29.5	120.4	10.9
SEM		8.3	97.2	0.05	10.81	3.34	3.3	14.6	1.2
Statistical Probabilities (P value)									
Treat.		0.3020	**<.0001**	0.1813	0.0606	0.3623	0.6901	0.5222	0.3334
Seasons		**0.0002**	**<.0001**	0.1990	0.4169	0.7192	0.1037	**0.0212**	0.1343
Treat. × Season		**0.0155**	**<.0001**	**0.0286**	0.4854	0.1248	**0.0270**	0.2683	**0.0274**

[†] The CH_4/ABW values of testers animals in each treatment were used to calculate the emissions variables per hectare and animal unit. [a, b, c] Different lowercase letters in the same column represent treatments that differ from each other ($P \leq 0.05$) by Fisher's test. CH_4/Ani: methane emissions by animal; CH_4/ADG: methane emissions by average daily weight Gain; CH_4/ABW: methane emissions by average body weight; CH_4/ha: methane emissions by hectare; CH_4/AU: methane emissions by animal unit (450 kg of body weight), gross energy intake (GEI) and percentage of gross energy in feed converted to methane (Ym). DEG: degraded pasture of *Urochloa decumbens* cv. Basilisk; REC: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu, fertilized with 200 kg of N-urea ha^{-1} year; MIX: mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim. ADE: Standard error of the mean.

Interaction between season and treatments were found for the enteric CH_4 emission parameters and Ym ($P < 0.05$) (Figure 6 a-f). No differences among treatments were found in the rainy season of the experimental period. During the dry season lower values of enteric CH_4 emissions and Ym were found for MIX when compared to both DEG and REC treatments ($P < 0.05$) (Figure 6 a-f). Also, during the dry season, lower emissions of CH_4 per ADG were found for REC when compared to DEG treatment ($P > 0.05$) (Figure 6b).

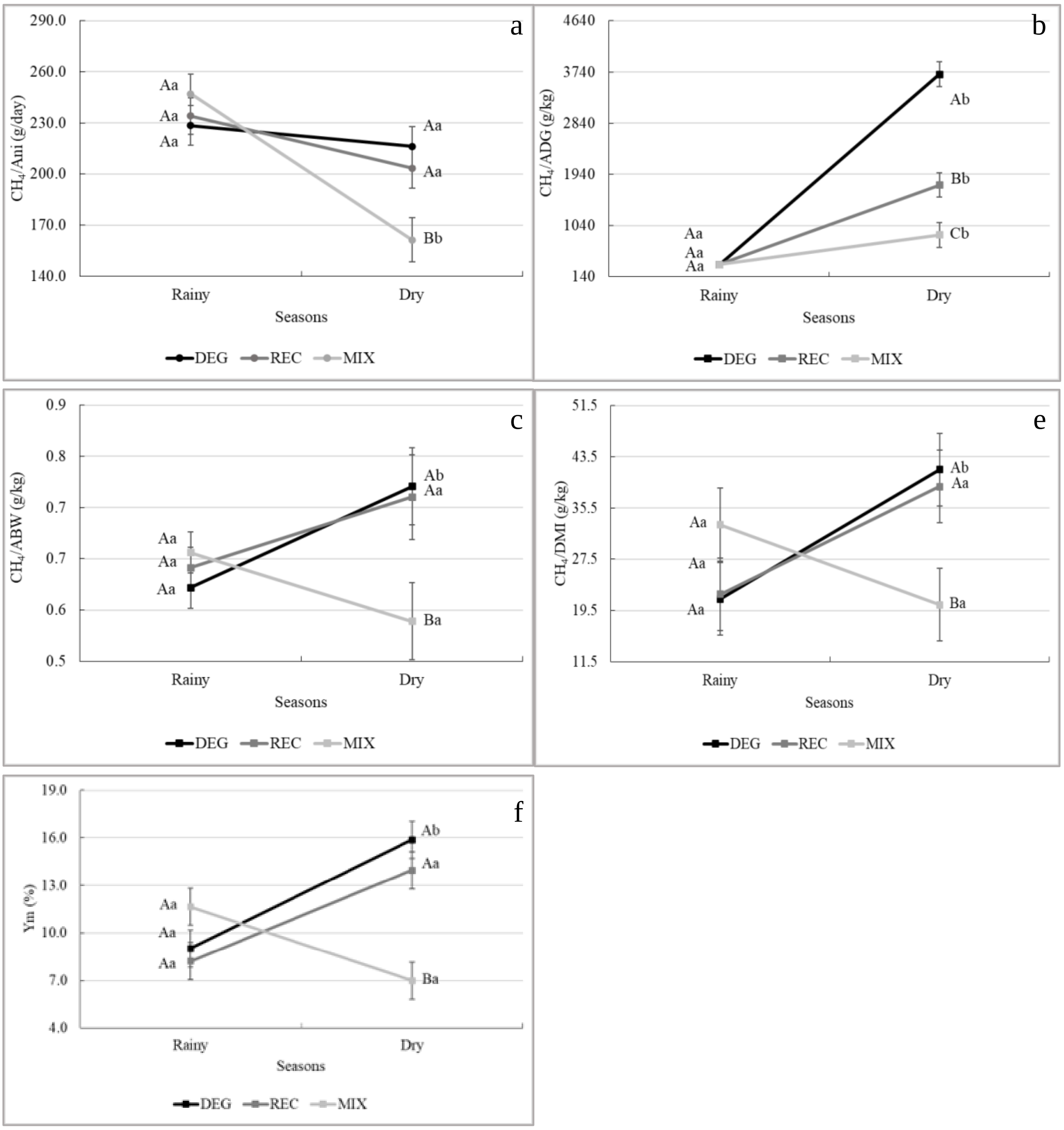


Figure 6 – Decomposition of the treatment×season interaction for enteric CH_4 emissions and Ym in the different pasture-based systems during the experimental period. Different capital letters indicate statistical differences among treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test ($P \leq 0.05$). Vertical bars are standard error of the mean.

6. DISCUSSION

The hand plucking technique was used for sampling the forage in the different treatments, and the isotopic analysis allowed estimating the proportion of *Urochloa* spp. and pigeon pea intake in the MIX treatment. A recent review article by Castro-Montoya and Dickhoefer (2020) pointed that there are 18 *in vivo* trials with pigeon pea fed to ruminants, and to the best of our knowledge, this is the first study reporting the nutritional quality of a diet composed by *Urochloa* spp. and pigeon pea in an intercropped pasture-legume system for feeding *Nellore* cattle in the Southeast of Brazil.

An efficient digestion by ruminal microorganisms requires at least 7% of CP (MEDEIROS et al., 2015) and during both seasons the CP values of all treatments were above the minimum and consistent with the values reported by dos Santos et al. (2022) evaluating *Urochloa* spp. fertilized, unfertilized and intercropped with the legume *Desmodium ovalifolium*. The mean CP value of the MIX treatment was higher than that reported by dos Santos et al. (2022) for the *Urochloa* spp. and *Desmodium ovalifolium* intercropped system. During the dry season, the CP value of MIX was slightly lower than those reported by Brown et al. (1988), Masama et al. (1997), Rodrígues et al. (2010), Oliveira et al. (2017), Miano et al. (2020), Castro-Montoya and Dickhoefer (2020) and Hampel et al. (2021) (17 to 24% CP), but this is due the fact that these authors report values exclusively for the legume pigeon pea, while the values presented in this study consider the proportion of pigeon pea and *Urochloa* spp. consumed by the animals. The NDIN and ADIN values were higher in MIX, which can be attributed to the higher CP content of this treatment, and this value was lower than those reported by Valadares Filho et al. (2018) for *Cajanus cajan* green forage.

In forage diets, the NDF content is one of the determinants of forage intake (BAUMONT et al., 2004). During the rainy season, DEG and REG treatments showed higher NDF values than those reported by Costa et al. (2014), Abdalla Filho et al. (2019) and dos Santos et al. (2022) evaluating *Urochloa* spp. The mean NDF value of the MIX treatment was lower those reported by authors evaluating pastures intercropped with *Desmodium ovalifolium* (DOS SANTOS et al., 2022) and pigeon pea (HAMPEL et al., 2021). Also, the lowest NDF value of the MIX treatment during the dry season is in line with those reported by Alves et al. (2014) and Pereira et al. (2018) evaluating the nutritional quality of *Cajanus cajan*. During the rainy season, the forage from the DEG and REC treatments presented higher ADF content than those found by Abdalla Filho et al. (2019) and Costa et al. (2014), while ADF value of MIX was lower than those reported by Alves et al. (2014) and Pereira et al. (2018), and higher than the ADF value reported for a consortium of *Panicum maximum* and pigeon pea by Hampel et al. (2021). In addition, Lig values of the MIX treatment were lower than those found by Hampel et al. (2021).

The mean EE values of DEG and REC treatments are similar to those reported by Sá et al. (2010) for C_4 pastures composed mainly of *Urochloa* spp. The EE values of the MIX treatment are similar to those found by Vitti et al. (2005) and Castro-Montoya and Dickhoefer (2020) evaluating the nutritional quality of pigeon pea. The Ash value of the MIX treatment is lower than that reported by Hampel et al. (2021) for *Panicum maximum* intercropped with pigeon pea. During the dry season, MIX presented Ash value similar to those reported by Vitti et al. (2005) and higher than Miano et al. (2020) evaluating the nutritional quality of pigeon pea. It is important to mention that it is not uncommon for Ash values to be overestimated due possible soil contamination at the time of sampling (NES, 1975). In both seasons, the GE content of DEG and REC treatments were similar to those found by Mora et al. (2016), while GE of the MIX treatment was similar to those for pigeon pea green forage in the Brazilian Tables of Feed Composition for Cattle (VALADARES FILHO et al., 2018).

In both seasons, the TC content of DEG and REC treatments were higher than other tropical grasses reported by Bueno et al. (2015), while MIX treatment presented values higher than those found by Pereira et al. (2018) for pigeon pea, and lower than those found by Hampel et al. (2021) for *Panicum maximum* intercropped with pigeon pea. Some studies have shown that feed consumption by ruminants can be reduced when the concentration of TC exceeds 50 g CT/kg DM, due to the reduction in acceptability and conditioned aversion (FRUTOS et al., 2004; MUELLER-HARVEY, 2006). As the level of CT observed here for all treatments were below this value, no negative effect was seen on the consumption of the diet, as other authors have shown when using diets with similar CT content, irrespective of the plant used (BARRY and DUNCAN, 1984; WAGHORN et al., 1994; AERTS et al., 1999; ABDALLA FILHO et al., 2017;). In addition, according to Perna Junior et al. (2022), values around 20 to 45 g CT/kg DM are sufficient to interfere in the digestive process of ruminants.

The DMD of the MIX treatment during the dry season was higher than that reported for pigeon pea green forage (VALADARES FILHO et al., 2018), while the DMD value of REC was similar to those found by Dias et al. (2016) and Euclides et al. (2021). During the dry season the DMD of MIX was higher than both DEG and REC treatments; a similar result to that found by Epifanio et al. (2019) evaluating *Urochloa* spp. intercropped with *Stylosanthes* spp., which reported an increase in digestibility when compared to pastures composed only with grasses. A possible explanation for the higher DMD value of MIX is some of the associative effects between forages on feed digestion (NIDERKORN and BAUMONT, 2009). Increased digestion when a low-quality forage is supplemented by a legume with high nitrogen content can be attributed to the stimulation of the microbial activity and modification of digestive processes in the rumen, including proteolysis and CH_4 production when secondary metabolites such as tannins, saponins or polyphenol oxidase are present in low quantities (NIDERKON and BAUMONT, 2009).

During the dry season, forage and total DMI were lower than that found in the rainy season. These results can be justified by the structure of the vegetation, lower acceptability, presence of antinutritional compounds, lower passage rate of food through the gastrointestinal tract and lower forage availability in the dry season of the year, in addition to factors inherent to the animals like breed, sex and age (WHITEMAN, 1980;

CROWDER and CHHEDA, 1982). The DMI of REC was lower than that found by Meo-Filho et al. (2022). For all treatments, the DMI during the rainy season were approximately 1 kg lower than that described by Barioni et al. (2007) in DMI tables for *Nellore* steers under grazing conditions. In the dry season, DMI for all treatments were similar to those reported by (BARIONI et al., 2007). In a meta-analytical approach evaluating zebu animals grazing *Urochloa* spp. with mineral and energy/protein supplementation (ALMEIDA et al., 2022), DMI results were lower than those found in this study, with the average performance of animals consuming only mineral supplementation similar to those observed in the DEG treatment. In addition, energy/protein supplement consumption was around 1 kg per animal (ALMEIDA et al., 2022), a value above that found in the DEG and REC treatments. The weight gain of the animals receiving energy/protein supplement, around 580g per day, was greater than that observed in the treatments of this study.

The daily DMI is a very important factor ensuring the release of nutrients for maintenance and production. Tulu et al. (2021) found considerable variations in DMI among pigeon pea genotypes. Usually during the dry season, tropical grasses present low nutritional quality and forage availability, and these could explain the lower forage and total DMI found in this study during in this season. Also in the dry season, animals preferentially consume more supplements to enhance the use of diet’ substrates and optimize animal performance and feed efficiency by ameliorating the pastures nutritional composition (BOVAL and DIXON 2012; BOVAL et al. 2014) and higher intake of supplement was found during the dry season when expressed as %ABW. However, when comparing the different treatments, lower supplement DMI was found for the pasture with pigeon pea. This could be attributed to some of the pigeon pea characteristics since it is a legume that reaches it reproductive phase and improved acceptability of its pods and oldest leaves during the dry season of the year, being consumed as an important source of protein (OLIVEIRA et al. 2017; 2022), thus reducing the need of mineral supplements (OLIVEIRA et al., 2023). In times of scarcity and high prices for protein mineral supplements the introduction of this legume in pasture systems is even more relevant.

The similar iBW evidenced the animals' weight uniformity among the treatments, while higher fBW and ADG in the MIX treatment when compared to DEG and REC indicate greater performance in pasture intercropped with pigeon pea. In both seasons, the animals from MIX showed higher ADG, and higher performance of cattle on pastures intercropped with legumes was also found by Machado and Sales (2020) when comparing to pastures exclusively with *Urochloa* spp. Both forage DMI and ADG were in line with those described by Oliveira et al. (2017) for a consortium system using pigeon pea. The ADG values of MIX were higher than those found by dos Santos et al. (2022) using higher stocking rates.

It is important to consider that pigeon pea has the ability to fix N and add organic matter to the soil, factors that can contribute to a greater forage nutritional quality and availability to the animals. This legume also contributes to the recovery of degraded pastures (OLIVEIRA et al., 2017) that represent approximately 70% of pasture areas in Brazil (DIAS-FILHO, 2011; BORGHI et al., 2018). In the DEG treatment, which represents a pasture with some level of degradation, the stocking rate expressed both as number of animals per hectare and AU per hectare were lower than the other treatments; a fact that could be related to the low persistence and biomass production of the tropical grass in a soil without proper nutritional management as found by dos Santos et al. (2022). The REC treatment that received nitrogen fertilization showed higher stocking rate during the rainy season, with values similar to those found by Meo-Filho et al. (2022) evaluating an fertilized intensive pasture under rotational grazing with liming application. However, during the dry season, REC treatment had a lower stocking rate than MIX. During the dry season, the seasonality of production and nutritional quality of tropical grasses occurs (WHITEMAN, 1980; CROWDER and CHHEDA, 1982), reducing the pasture support capacity, while it is in this period that pigeon pea begins to be consumed as an important source of forage for the animals, enabling a higher stocking rate (OLIVEIRA et al., 2017). Considering seasons, higher feed conversion ratio was found during the dry season, and this is justified by the poorer nutritional quality of the forages. In the same line, the greater feed efficiency found in the rainy season is justified by the better nutritional quality of the forage to which the animals had access during this season (DIAS et al., 2020).

Decreasing the emissions of enteric CH_4 from ruminant production is strategic to limit the global temperature increase to 1.5ºC by 2050 (BEAUCHEMIN et al., 2022). During the dry season, when pigeon pea consumption was observed, CH_4 emissions expressed per animal, per ADG, per ABW and per DMI were lower in the MIX treatment, which can be attributed to some of its nutritional quality and CT content. The effect of tannins on the reduction in enteric CH_4 production is usually related to its direct action by inhibiting the activity of methanogenic microorganisms and/or reducing the digestibility of rumen fiber fractions (PATRA and SAXENA, 2011). Also, it is important that the benefits of the reduction in the emission of CH_4 do not hide the possible harmful effects of tannins on nutrient digestibility and production parameters (HRISTOV et al., 2013). Further *in vitro* studies using tannin-binding agents (*e.g.* polyethylene glycol) evaluating the effects of pigeon pea on diet degradability, ruminal fermentation parameters, ruminal microorganisms and potential of CH_4 mitigation may contribute to elucidate the results found in this study. Berhanu et al. (2019) evaluated the *in vitro* potential for mitigating CH_4 emissions from several legumes, including pigeon pea, and found lower production of total gases as well CH_4.

When expressed per ADG, the highest CH_4 emission was found for DEG during the dry season of the year, which can be explained by the reduced performance results of this treatment. During the dry season, MIX treatment showed higher performance results, which contributed to the lower emission intensity found in the system with the inclusion of pigeon pea. When expressed as a percentage of the gross energy intake (Ym), similar values among treatments were found during the rainy season. However, in the dry season, lowest Ym was found in the treatment with pigeon pea, once again indicating the potential that this intercropped system has in contributing to the sustainability of livestock production based on pastures.

Finally, the results of this study highlight the fact that the inclusion of pigeon pea in pasture-based systems can represent advantage not only for cattle farmers raising animals with greater performance, but also for Brazil as a country, which made a commitment to reduce CH_4 emissions by 30% by 2030 during the 26^{th} UN Climate Change Conference of the Parties (COP26), in Glasgow, Scotland.

7. CONCLUSION

The hypothesis that pigeon pea intercropped with tropical grasses is an interesting strategy for feeding *Nellore* cattle was confirmed since the MIX treatment was able to meet the nutritional requirements of the animals. In this treatment, animals presented lower intake of mineral supplement, higher performance, and reduced emissions of enteric CH_4 when compared to other pasture-based systems commonly used in Brazil.

8. REFERENCES

ABDALLA FILHO, A. L. *et al.* CO_2 fertilization does not affect biomass production and nutritive value of a C_4 tropical grass in short timeframe. **Grass and Forage Science**, v. 74, p. 670-677, 2019.

ABDALLA FILHO, A. L. *et al.* Performance, metabolic variables and enteric methane production of Santa Ines hair lambs fed *Orbignya phalerata* and *Combretum leprosum*. **Journal of Animal Physiology and Animal Nutrition**, v. 101, p. 457-465, 2017.

ABIEC. Beef Report. **Brazilian Livestock Profile,** ABIEC – Brazilian Beef Exporters Association, Sao Paulo, Brazil, 2019.

ABIEC. Beef Report. **Brazilian Livestock Profile,** ABIEC – Brazilian Beef Exporters Association, Sao Paulo, Brazil, 2022.

ABRÃO, F. O.; FERNANDES, B. C.; PESSOA, M. S. Produção sustentável na bovinocultura: princípios e possibilidades. **Revista Brasileira de Agropecuária Sustentável**, v. 6, n. 4, p. 61-73, 2016.

AE, N. A. *et al*. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. **Science**, v. 248, p.477-480, 1990.

AERTS, R. J.; BARRY, T. N.; MCNABB, W. C. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. **Agriculture, Ecosystems & Environment**, v. 75, p. 1-12, 1999.

AHIABOR, B. D.; HIRATA, H. Characteristic responses of three tropical legumes to the inoculation of two species of VAM fungi in Andosol soils with different fertilities. **Mycorrhiza**, v. 5, p. 63–70, 1994.

ALMEIDA, D. *et al.* Performance of *Bos indicus* beef cattle supplemented with mineral or with concentrates in tropical *Urochloa decumbens* pastures: A meta-regression approach. **Animal Feed Science and Technology,** v. 283, n. 115178, 2022.

ALVES, F. J. S. *et al.* Produção de biomassa e valor nutricional do *Cajanus cajan* cv. Mandarin sob manejo orgânico e convencional. **XXIV Congresso Brasileiro De Zootecnia**, p. 1-3, 2014.

ALVES, F. J. S. *et al.* Residual effects of stylo on the morphogenetic and structural characteristics of palisade grass pasture. **Grassland Science**, v. 62, p. 151-159, 2016.

ANDRADE, B. G. N. *et al.* Stool and ruminal microbiome components associated with methane emission and feed efficiency in Nelore beef cattle. **Frontiers in Genetics**, v. 13, n. 812828, p. 1-12, 2022.

ANDRADE, C. M. S. *et al.* Productivity, utilization efficiency and sward targets for mixed pastures of Marandu grass, forage peanut and tropical kudzu. **Revista Brasileira De Zootecnia**, v. 41, p. 512-520, 2012.

AOAC. **Official methods of analysis.** 15th ed. Association of Official Analytical Chemists. Washington, DC, USA, 1990.

ARTAXO, P. Dossiê do clima – Mudanças climáticas e o Brasil. **Revista USP**, São Paulo, n. 103, p. 8-12, 2014

ARAÚJO, T. L. R. *et al*. Effects of replacing cottonseed meal with corn dried distillers' grain on ruminal parameters, performance, and enteric methane emissions in young *Nellore* bulls reared in tropical pastures. **Animals**, v. 11, n. 2959, 2021.

BARIONI, L. G. *et al.* **Tabelas de estimativa de ingestão de matéria seca de bovinos de corte em crescimento em pastejo**. Planaltina, DF: p. 8, 2007.

BARRY, T. N.; DUNCAN, S. J. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. Voluntary intake. **British Journal of Nutrition,** v. 51, p. 485-491, 1984.

BAUMONT, R. *et al.* A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. **Animal Feed Science and Technology**, v. 112, p. 5-28, 2004.

BEAUCHEMIN, K. A. *et al.* Invited review: current enteric methane mitigation options. **Journal Dairy Science**, v. 105, p. 9297-9326, 2022.

BENAOUDA, M. *et al.* Development of mathematical models to predict enteric methane emission by cattle in Latin America. **Livestock Science,** v. 241, n. 1041772, 2020.

BERCHIELLI, T. T. *et al.* Produção de metano entérico em pastagens tropicais. **Revista Brasileira de Saúde e Produção Animal**, v. 13, n. 4, p. 954-968, 2012.

BERCHIELLI, T. T.; OLIVEIRA, S. G.; CARRILHO, E. N. V. *et al.* Comparação de marcadores para estimativas de produção fecal e fluxo de digesta em bovinos. **Revista Brasileira de Zootecnia**, v. 34, n. 3, p. 987-996, 2005.

BERGEIM, O. Intestinal chemistry: IV. A method for the study of food utilization or digestibility. **Journal of Biological Chemistry**, v. 70, p. 29-33, 1926.

BERHANU, Y. *et al.* Methane emissions from ruminant livestock in Ethiopia: promising forage species to reduce CH_4 emissions. **Agriculture,** v. 9, n. 130, 2019.

BERNDT, A.; SOLÓRZANO, L. A. R.; SAKAMOTO, L. S. Pecuária de corte frente à emissão de gases de efeito estufa e estratégias diretas e indiretas para mitigar a emissão de metano. **VI Simpósio de Nutrição de Ruminantes**, Botucatu, p. 3-19. 2013.

BHATTA, R. *et al.* Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. **Journal Dairy Science**, v. 92, p. 5512-5522, 2009.

BLAXTER, K. L.; CLAPPERTON, J. L. Prediction of the amount of methane produced by ruminants. **British Journal of Nutrition,** v. 19, p. 511-522, 1965.

BODDEY, R. M. *et al.* Forage legumes in grass pastures in tropical Brazil and likely impacts on greenhouse gas emissions: A review. **Grass and Forage Science**, v. 75, p. 357-371, 2020.

BORGHI, E. *et al.* **Recuperação de pastagens degradadas. In M. M. Nobre & I. R. Oliveira, Agricultura de Baixo Carbono Tecnologias e Estratégias de Implantação**. (p. 105-138). Embrapa Milho e Sorgo, 2018.

BOVAL, M.; DIXON, R.M. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. **Animal**, v. 6, p. 748-762, 2012.

BOVAL, M.; EDOUARD, N.; SAUVANT, D. A meta-analysis of nutrient intake, feed efficiency and performance in cattle grazing on tropical grasslands. **Animal**, v. 9, p. 973-982, 2015.

BOVAL, M.; EDOUARD, N.; SAUVANT, D. Mixed grazing systems of goats with cattle in tropical conditions: an alternative to improving animal production in the pasture. **Animal**, v. 8, p. 1282-1289, 2014.

BROWN, D. *et al.* Intake, selection, apparent digestibility and chemical composition of *Pennisetum purpureum* and *Cajanus cajan* foliage as utilized by lactating goats. **Small Ruminant Research**, v. 1, p. 59-65, 1988.

BUENO, I. C. S. *et al. In vitro* methane production and tolerance to condensed tannins in five ruminant species. **Animal Feed Science and Technology**, v. 205, p. 1-9, 2015.

CADISCH, G. *et al.* Effects of phosphorus and potassium on N_2 fixation (^{15}N-dilution) of field-grown *Centrosema acutifolium* and *C. macrocarpum*. **Field Crops Research**, v. 31, p. 329-340, 1993.

CADISCH, G.; SCHUNKE, R. M.; GILLER, K. E. Nitrogen cycling in a pure grass pasture and a grass-legume mixture on a red latosol in Brazil. **Tropical Grasslands**, v. 28, p. 43-52, 1994.

CARVALHO, P. C. F. *et al*. Avanços metodológicos na determinação do consumo de ruminantes em pastejo. **Revista Brasileira de Zootecnia**, v. 36, p. 151-170, 2007.

CASTRO-MONTOYA, J.M.; DICKHOEFER, U. The nutritional value of tropical legume forages fed to ruminants as affected by their growth habit and fed form: A systematic review. **Animal Feed Science and Technology**, v. 269, n. 114641, 2020.

CIESLAK, A. *et al.* Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. **Animal Feed Science and Technology**, v. 176, p. 102-106, 2012.

COLEMAN, S. W. *et al*. Difficulties associated with predicting forage intake by grazing beef cows. **Journal of Animal Science**, v. 92, p. 2775-2784, 2014.

COOK, C. W. Symposium on nutrition of forage and pastures: Collecting forage samples representative of ingested material of grazing animals for nutritional studies. **Journal of Animal Science**, v. 23, p. 265-270, 1964.

CORDOVA, F. J.; WALLACE, J. D.; PIEPER, R. D. Forage intake by grazing livestock: A review. **Journal of Range Management**, v. 31, p. 430-438, 1978.

CORRIHER, V. A. *et al.* Performance of beef heifers and digestibility of steers fed whole cotton seed, corn gluten feed and pigeon peas. **Journal of Animal Science**, Abstract, v. 85, n. 617, 2007.

CORRIHER, V. A. *et al.* Pigeon peas as a supplement for lactating dairy cows fed corn silage-based diets. **Journal of Animal Science**. v. 93, p. 5309-5317, 2010.

COSTA, J. A. A.; QUEIROZ, H.P. **Régua de manejo de pastagens.** Campo Grande, MS: Embrapa Gado de Corte, 2013. (Comunicado técnico, 125).

COSTA, N. L. et al. Produtividade e composição química da *Brachiaria humidicola* sob diferimento nos cerrados de Rondônia. **PUBVET**, v. 8, n. 7, 2014.

COTTLE, D. J. The trials and tribulations of estimating the pasture intake of grazing animals. **Animal Production Science**. v. 53, p. 1209-1220, 2013.

CROWDER, L. V.; CHHEDA, H. R. **Tropical Grassland Husbandry**. London and New York: Longmans. 1982, p. 562. Experimental Agriculture.

CUNHA, C. S. *et al.* Assessing the impact of rumen microbial communities on methane emissions and production traits in Holstein cows in a tropical climate. **Systematic and applied microbiology**, v. 40(8), p. 492-499, 2017.

DE MARCHI, J. J. A. A. *et al*. Effect of seasons on enteric methane emissions from cattle grazing *Urochloa brizantha*. **Journal of Agricultural Science**, v. 8, n. 4, p. 106, 2016.

DENTINHO, M. T. P.; BESSA, R. J. B. Effect of tannin source and pH on stability of tannin-protein and fiber complexes. **Revista de Ciências Agrárias**, v. 39, p. 114-121, 2016.

DERAMUS, H. A. *et al.* Methane emissions of beef cattle on forages: Efficiency of grazing management systems. **Journal of Environmental Quality**, v. 32, p. 269-277, 2003.

DETMANN, E. *et al.* An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science**, v. 162, p. 141-153, 2014.

DIAS, A. M. *et al*. **Perspectivas e desafios para a produção de leite e carne no cerrado**. 1. ed. Rio de Janeiro, e-Publicar. p. 40-66, v. 1, 2020.

DIAS, A. M.; GOMES, E.N.O.; ÍTAVO, L.C.V. Herbage allowance effects on the characteristics of *Brachiaria brizantha* cv. Marandu pastures and the production and economic viability of *Nellore* heifers. **Semina: Ciências Agrárias**. v. 37, p. 2301-2312, 2016.

DIAS-FILHO, M. B. Degradação de pastagens: processos, causas estratégias de recuperação / Moacyr Bernardino Dias-Filho. 4. Ed., Belém, PA, 2011.

DOS SANTOS, C. A. *et al.* Productivity of beef cattle grazing *Brachiaria brizantha* cv. Marandu with and without nitrogen fertilizer application or mixed pastures with the legume *Desmodium ovalifolium*. **Grass and Forage Science**, p. 1-14, 2022.

DUNCAN, A. J.; YOUNG, S. A. Can goats learn about foods through conditioned food aversions and preferences when multiple food options are simultaneously available? **Journal of Animal Science**, v. 80, p. 2091-2098, 2002.

EPIFANIO, P. S. *et al.* Productive and nutritional characteristics of *Brachiaria brizantha* cultivars intercropped with *Stylosanthes* cv. Campo Grande in different forage systems. **Crop & Pasture Science**, v. 70, p. 718-729, 2019.

EUCLIDES, V. P. B. *et al.* **Valor nutritivo, estrutura do dossel e desempenho animal de alguma cultivares de *Panicum maximum* e *Brachiaria* spp. submetidas à diferentes estratégias de manejo.** Campo Grande, MS: Embrapa Gado de Corte, p. 102, 2021. (Documentos, 292).

FAGUNDES, J. L. *et al.* Acúmulo de forragem em pastos de *Brachiaria decumbens* adubados com nitrogênio. Brasília, DF: Embrapa. **Pesq. agropec. bras**., Brasília, v.40, n.4, p.397-403, abr. 2005.

FAHEY, G. C. J. R.; JUNG, H. G. Lignin as a marker in digestion studies: a review. **Journal of Animal Science**, v. 57, n. 1, p. 220-225, 1983.

FAO. **The future of food and agriculture – Trends and challenges**. Rome, Italy, 2017.

FAOSTAT. Statistical database: **Agricultural Data, Food and Agricultural Organisation of the United Nations**, Rome, Italy, 2007.

FAOSTAT. Statistical database: **Agricultural Data, Food and Agricultural Organisation of the United Nations**, Rome, Italy, 2017.

FAOSTAT. Statistical database: **Agricultural Data, Food and Agricultural Organisation of the United Nations**, Rome, Italy, 2020.

FARIS, D.G., SINGH, U. **Pigeon pea: Nutrition and products**. In: Nene, Y.L., Hall, S.D., Sheila, V.K. (Eds.). *The Pigeon pea*. Wallingford, UK.CAB International, p. 401-434, 1990.

FISHER, D. W. Modeling ruminant feed intake with protein, chemostatic, and distention feedbacks. **Journal of Animal Science**. v. 74, p. 3076-3081, 1996.

FLOWER, D. J.; LUDLOW, M. M. Variation among accessions of pigeon pea (*Cajanus cajan*) in osmotic adjustment and dehydration tolerance of leaves. **Field Crops Research**, v. 17, p. 229-243, 1987.

FRUTOS, P. *et al.* Review. Tannins and ruminant nutrition. **Spanish Journal of Agricultural Research**, v. 2, p. 191-202, 2004.

GARDENER, C. J.; MCCASKILL, M. R.; MCIVOR, J. G. Herbage and animal production from native pastures and pastures oversown with *Stylosanthes hamata*. 1. Fertiliser and stocking rate effects. **Animal Production Science**, v. 33, p. 561-570, 1993 a.

GARDENER, C. J.; MCIVOR, J. G.; JANSEN, A. Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. **Journal of Applied Ecology**, v. 30, p. 63-74, 1993 b.

GILLER, K. E. *et al*. The future of farming: Who will produce our food? **Food Science**, v. 13, p. 1073-1099, 2021.

GIVENS, D. I. *et al.* **Forage Evaluation in Ruminant Nutrition.** Cabi, New York, 2000.

GLINDEMANN, T. *et al.* Evaluation of titanium dioxide as an inert marker for estimating faecal excretion in grazing sheep. **Animal Feed Science and Technology**. v. 152, p. 186-197, 2009.

GOERING, H. K. & VAN SOEST, P. J. **Forage fiber analysis** (apparatus, reagents, procedures and some applications). Agricultural Research Service, U.S. Department of Agriculture Handbook, n. 39, 1970.

GOMES, F. K. *et al.* Effects of grazing management in brachiaria grass-forage peanut pastures on canopy structure and forage intake. **Journal of Animal Science**, v. 96, p. 3837-3849, 2018.

GREENWOOD, P. L. Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. **Animal**, v. 15, n. 100295, 2021.

GROF, B. Forage attributes of the perennial groundnut *Arachis pintoi* in a tropical savanna environment in Colombia. **Proceedings of the XV International Grassland Congress**, p. 168-170, 1985.

HAFEZ, S.; JUNGE, W.; KALM, E. Estimate of digestibility in dairy cows using an indicator method in comparison to the Hohenheimer feed value test. **Archiv. Für Tierernährung**, v. 38, p. 929-945, 1988.

HAMPEL, V. S. *et al*. Tropical grass and legume pastures may alter lamb meat physical and chemical characteristics. **Tropical Animal Health and Production**, v. 53, p. 427, 2021.

HILLOCKS, R. J. *et al.* Diseases and pests of pigeon pea in eastern Africa. **International Journal of Pest Management**, v. 46, p. 7-18, 2000.

HRISTOV, A. N. *et al.* **Mitigation of greenhouse gas emissions in livestock production; a review of technical options for non-co2 emissions**. 2013. FAO Animal Production and Health Paper. n. 177. Food and Agriculture Organization, Rome, Italy. p. 206. Retrieved on 28 November 2022 from: http://www.fao.org/docrep/018/i3288e/i3288e.pdf.

HÜTSCH, B. W. Methane oxidation in non-flooded soils as affected by crop production. **European Journal of Agronomy**, v. 14, p. 237-260, 2001.

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Censo Agropecuário 2017:** Resultados Definitivos. IBGE: Rio de Janeiro, Brasil, 2017. Retrieved on 28 November 2022 from: https://biblioteca.ibge.gov.br/visualizacao/periodicos/3096/agro_2017_resultados_definitivos.

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTTÍSTICA. **Censo Agropecuário 2021:** Resultados Definitivos. IBGE: Rio de Janeiro, Brasil, 2022. Retrieved on 28 November 2022 from: https://www.ibge.gov.br/explica/producao-agropecuaria/.

IPCC. Summary for Policymakers. **Climate Change 2007**. The Physical Science Bass. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.

IPCC. Summary for Policymakers. **Climate Change 2022**. Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2022.

JANK, L. *et al.* The value of improved pastures to Brazilian beef production. **Crop and Pasture Science**, v. 65, p. 1132-1137, 2014.

JOHANSEN, C.; SILIM, S. N.; LAXMAN, S. Towards a database for pigeon pea in Africa. **International Pigeon pea Newsletter**, v. 18, p. 2-5, 1993.

JOHNSON, K. A.; JOHNSON, D. E. Methane emissions from cattle**. Journal of Animal Science**, v.73, p.2483-2492, 1995.

JOHNSON, K. *et al.* **Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique**. p. 335-338. J. F. Aguilera, ed. Energy metabolism of farm animals (EAAP Publication n. 76). 1994 b. Servicio de Publicaciones, Consejo Superior de Investigaciones Cientificas, Granada, Spain.

JOHNSON, K. *et al.* Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. **Environmental Science & Technology**. v. 28, n. 359362, 1994.

KARACHI, M.; ZENGO, M. Legume forages from pigeon pea, Leucaena and sesbania as supplements to natural pastures for goat production in western Tanzania. **Agroforestry Systems**, v.39, p. 13-21, 1998.

KOZLOSKI, G. V. *et al.* Faecal nitrogen excretion as an approach to estimate forage intake of wethers. **Journal of Animal Physiology and Animal Nutrition,** v. 98, p. 659-666, 2009.

KUMARI, S. *et al*. Projection of methane emissions from livestock through enteric fermentation: A case study from India. **Environmental Development**. v. 20, 2016.

LARRAÍN, R. E. *et al.* Finishing steers with diets based on corn, high tannin sorghum, or a mix of both: feedlot performance, carcass characteristics, and beef sensory attributes. **Journal of Animal Science**, v. 87, p. 2089-2095, 2009.

LASCANO, C. E.; THOMAS, D. Forage quality and animal selection of *Arachis pintoi* in association with tropical grasses in the Eastern Plains of Colombia. **Grass and Forage Science**, v. 43, p. 433-439, 1988.

LIPPKE, H. Estimation of forage intake by ruminants on pasture. **Crop Science**, v. 42, n. 2, p. 869-72, 2002.

LIPPKE, H. Regulation of voluntary intake of ryegrass and sorghum forages in cattle by indigestible neutral detergent fiber. **Journal of Animal Science**, v. 63, p. 1459-1468, 1986.

LOPEZ, F. B.; SETTER, T. L.; MCDAVID, C. R. Carbon dioxide and light response of photosynthesis in cowpea and pigeon pea during water deficit and recovery. **Plant Physiology**, v. 85, p. 990-995, 1987.

MACADAM, J. W. Increased nitrogen retention and reduced methane emissions of beef cattle grazing legume vs. grass irrigated pastures in the mountain west USA. **Agronomy**, v. 12, n. 304, 2022.

MACHADO, M. L. C.; SALES, M. F. L. Performance of intact and castrated nelore steers grazing *Brachiaria humidicola* (Rendle) Schweick (Poaceae) alone and intercropped with forage peanut. **Brazilian Animal Science**, v. 21, n. 51379, 2020.

MACHADO, P. A. S. *et al.* Nutritional and productive parameters of beef cattle on pasture fed different amounts of supplement. **Revista Brasileira de Zootecnia**, v. 40, p. 1303-1312, 2011.

MAKKAR, H. P. S. Quantificatin of tannins in tree and shrub foliage: a laboratory manual. Springer, Netherlands, 2003.

MASAMA, E. *et al*. Effects of supplementation with foliage from the tree legumes *Acacia angustissima*, *Cajanus cajan*, *Calliandra calothyrsus* and *Leucaena leucocephala* on feed intake, digestibility and nitrogen metabolism of sheep given maize stover ad libitum. **Animal Feed Science and Technology**, v. 69, p. 233-240, 1997.

MAYES, R. W.; DOVE, H. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. **Nutrition Research Reviews**, v. 13, p. 107-138, 2000.

MEDEIROS, S. R. *et al.* **Nutrição de bovinos de corte:** fundamentos e aplicações. Brasília, DF: Embrapa, p. 176, 2015.

MENEZES, R. C. *et al.* Xaraés palisadegrass remains productive after the disappearance of stylo in tropical legume-grass pasture. **Scientia Agricola**, v. 72, p. 484-488, 2015.

MEO-FILHO, P. *et al.* Can intensified pasture systems reduce enteric methane emissions from beef cattle in the Atlantic Forest biome? **Agronomy**, v. 12, n. 2738, 2022.

MERTENS, D. R. Kinetics of cell wall digestion and passage in ruminants. Pages 535–570 in **Forage Cell Wall Structure and Digestibility**. ed. American Society of Agronomy, Madison, WI. 1993.

MEYERS, W. D. *et al.* Excretion patterns of titanium dioxide and chromic oxide in duodenal digesta and feces of ewes. **Small Ruminant Research**, v. 63, p. 135-141, 2006.

MIANO, A. C. *et al.* Evaluating new lines of pigeon pea (*Cajanus cajan* L.) as a human food source. **Journal of Food Processing and Preservation**, v. 44, n. 14517, p. 1-9, 2020.

MILLER, J. K.; MADSEN, F. C.; HANSARD, S. L. Absorption, excretion, and tissue deposition of titanium in sheep. **Journal of Dairy Science**, v. 59, p. 2008-2010, 1976.

MILLER-CUSHON, E. K.; DEVRIES, T. J. Feed sorting in dairy cattle: Causes, consequences, and management. **Journal of Dairy Science**, n. 41724183, 2017.

MLIGO, J. K.; CRAUFURD, P. Q. Adaptation and yield of pigeon pea in different environments in Tanzania. **Field Crops Research**, v. 94, p. 43-53, 2005.

MORA, B. V.; GALLEGOS, E. C.; BARRAGÁN, H. B. Rendimiento y degradabilidad ruminal de matéria seca y energía de diez pastos tropicales cosechados a cuatro edades. **Revista Mexicana de Ciencias Pecuarias**, v. 7, p. 141-158, 2016.

MOTT, G. O.; LUCAS H. L. The design, conduct, and interpretation of grazing trials on cultivated and improved pastures. 1952. In: Wagner R. E., Myers W. M., Gaines S. H. and Lucas H. L. (eds) **Proceedings 6th International Grassland Congress**, Pennsylvania State College, Pa., 17-23 August 1952, p. 1380–1385. Hershey, Pennsylvania, USA: Pennsylvania State College.

MOTT, G. O.; MOORE, J.E. **Evaluating forage production.** In: Forages - The science of grassland agriculture, 4th ed. Iowa State University Press, Ames, IA, USA. p. 422-429, 1985.

MUELLER-HARVEY, I. Unravelling the conundrum of tannins in animal nutrition and health. **Journal of the Science of Food and Agriculture**, v. 86, p. 2010-2037, 2006.

NASEM. **Nutrient Requirements of Beef Cattle**. 8th rev. ed. Natl. Acad. Press, Washington, DC, 2016.

NES, P. A correction for soil contamination of herbage samples by dry ashing. **New Zealand Journal of Agricultural Research**, v. 18, n. 1, p. 69-71, 1975.

NIDERKORN, V.; BAUMONT, R. Associative effects between forages on feed intake and digestion in ruminants. **Animal**, v. 3, n. 7, p. 951-960, 2009.

NIEUWOLT, S., **Tropical Climatology: An Introduction to the Climates of the Low Altitudes**. John Wiley, Chichester. 1977.

NORMAN, H. C. *et al.* Stable carbon isotopes accurately predict diet selection by sheep fed mixtures of C_3 annual pastures and saltbush or C_4 perennial grasses. **Livestock Science,** v. 121, p. 162-172, 2009.

NRC. Predicting Feed Intake of Food-Producing Animals. Natl. Acad. Press, Washington, 1987.

NUNES, C. *et al.* Physiological responses of the legume model *Medicago truncatula* cv. Jemalong to water deficit. **Environmental and Experimental Botany**, v. 63, p. 289-296, 2008.

ODENY, D. A. The potential of pigeon pea (*Cajanus cajan* (L.) Millsp.) in Africa. **Natural Resources Forum**, v. 31, p. 297-305. 2007.

OLIVEIRA, P. P. A. *et al.* **Avaliação de diferentes materiais genéticos de feijão-guandu em consórcio com capim-marandu na recuperação de pastagens degradadas**. p. 23. 2022. Boletim de pesquisa e desenvolvimento 52, Embrapa Pecuária Sudeste, São Carlos, SP.

OLIVEIRA, P. P. A. *et al*. Forage intake of *Nellore* steers grazing a *Cajanus Cajan* legume-grass mixed pasture. IGC International Grassland Congress. **Abstract**. 2023.

OLIVEIRA, P. P. A.; MATTA, F. de P.; GODOY, R. **Consorciação com guandu na recuperação de pastagens degradadas, uma tecnologia de duplo propósito: adubação verde e pastejo consorciado diferido**. Boletim técnico. São Carlos, SP: Embrapa Pecuária Sudeste, p. 6, 2017.

ONIM, J. F. M.; SEMENYE, P. P.; FITZHUGH, H. A. Research on feed resources for small ruminants on smallholder farms in Western Kenya. In: Kategile, J.A., Said, A.N., Dzowela, B.H. (Eds.), **Animal Feed Resources for Small-scale Livestock Producers**. Proceedings of the second PANESA workshop, held in Nairobi, Kenya, p. 11-15, 1985.

OVANI, V. S. *et al.* Use of internal markers to estimate feed intake and selection of forage in sheep fed grass and legume hay. **Animal Feed Science and Technology** v. 283, n. 115177, 2022.

PALUDO, A. *et al.* Feijão guandu em três alturas de corte na alimentação de ruminantes. **Nutritime Revista Eletrônica**, v. 9, n. 5, p. 1981-1994, 2012.

PASQUINI NETO, R. **Efeitos da intensificação e integração como estratégias para o manejo sustentável das pastagens nos sistemas de produção de bovinos de corte da raça Nelore: produtividade da forragem, desempenho animal e consumo alimentar**. 2022. Dissertação de mestrado, Departamento de Nutrição e Produção Animal. Universidade de São Paulo.

PATRA, A. K.; SAXENA, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. **Journal of the Science of Food and Agriculture**, v. 91, p. 24-37, 2011.

PEOPLES, M. B.; HERRIDGE, D. F.; LADHA, J. K., Biological nitrogen fixation: An efficient source of Nitrogen for sustainable agricultural production? **Plant and Soil**, v. 174, p. 3-28, 1995.

PEREIRA, A. V.; LÉDO, F. J. S.; MACHADO, J. C. BRS Kurumi and BRS Capiaçu-New elephant grass cultivars for grazing and cut-and-carry system. **Crop Breeding and Applied Biotechnology**, v. 17, n. 1, p. 59-62, 2017.

PEREIRA, L. E. T.; NISHIDA, N. T.; CARVALHO, L. T. **Recomendações para correção adubação de pastagens tropicais.** FZEA/USP, Pirassununga, SP, 56 p., 2018.

PERES ASSUMPÇÃO, A. H. **O uso individual e associado de monensina, taninos e nitrato de cálcio na alimentação de bovinos promove a redução do gás metano.** 97 f. Dissertação (Mestrado em Ciências) - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, São Paulo, 2021.

PERNA JUNIOR, F. *et al***.** Use of tannin extract as a strategy to reduce methane in Nellore and Holstein cattle and its effect on intake, digestibility, microbial efficiency and ruminal fermentation. **Journal of Animal Physiology and Animal Nutrition**. P.1–14, 2022.

PIÑERO-VÁZQUEZ, A. T. *et al*. Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. **Archives of Veterinary**, v. 47, p. 263-272, 2015.

POPPI, D. P. *et al.* Challenges of beef cattle production from tropical pastures. **Revista Brasileira de Zootecnia**, v. 47, n. 20160419, 2018.

PRIMAVESI, O. *et al.* **SF_6-tracer technique for bovine ruminal methane field measurements: adaptations to Brazilian conditions**. Document 39. Embrapa Pecuária Sudeste, São Carlos, 2004.

RAO, S. C.; COLEMAN, S. W.; MAYEUX, H. S. Forage production and nutritive value of selected pigeon pea ecotypes in the southern great plains. **Crop Science**, v. 42, p. 1259-1263, 2002.

REED, J. D. Nutritional toxicology of tannins and related polyphenols in forage legumes. **Journal of Animal Science**. v. 73, p. 1516-1528, 1995.

RODRÍGUES, A. A. *et al*. Chemical composition, in vitro degradability, and intake and digestibility by goats of pigeon pea (*Cajanus Cajan* cv. Guerrero) and guinea-grass hay mixtures, **Journal of Agricultural Science**, v. 94, p. 269-273, 2010.

SÁ, J. F. Fracionamento de carboidratos e proteínas de gramíneas tropicais cortadas em três idades. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia,** v. 62, n. 3, p. 667-676, 2010.

SAKITA, G. Z. *et al.* Treating tropical grass with fibrolytic enzymes from the fungus *Trichoderma reesei*: Effects on animal performance, digestibility and enteric methane emissions of growing lambs. **Animal Feed Science and Technology**, v. 286, n. 115253, 2022.

SALMAN, A. K. D. *et al.* **Metodologias para avaliação de alimentos para ruminantes domésticos**. 2010. p.21, (Documentos-136/ Embrapa Rondônia).

SCHOLLJEGERDES, E. J.; KRONBERG, S. L. Effects of supplemental ground flaxseed fed to beef cattle grazing summer native range on the northern Great Plains. **Journal of Animal Science**. v. 88, p. 2108-2121, 2010.

SCHOLLJEGERDES, E. J.; KRONBERG, S. L. Influence of level of supplemental whole flaxseed on forage intake and site and extent of digestion in beef heifers consuming native grass hay. **Journal of Animal Science**. v. 86, p. 2310-2320, 2008.

SCHOLLJEGERDES, E. J.; LEKATZ, L. A.; VONNAHME, K. A. Effects of short-term oilseed supplementation on plasma fatty acids composition, progesterone and prostaglandin F metabolite in lactating beef cows. **Animal**, v. 8, p. 777-785, 2014.

SELINGER, L. B.; FORSBERG, C. W.; CHENG, K. J. The Rumen: A Unique Source of Enzymes for Enhancing. **Anaerobe**, v. 2, p. 263-284, 1996.

SHAW, N. H. Increased beef production from Townsville lucerne (*Stylosanthes sundaica* Taub.) in the spear grass pastures of central coastal Queensland. **Australian Journal of Experimental Agriculture and Animal Husbandry**, v. 1, p. 73-80, 1961.

SHENKUTE, B. *et al*. Performance of Arsi-Bale kids supplemented with graded levels of pigeon pea in dry season in Mid Rift valley of Ethiopia. **African Journal of Agricultural Research**, v. 8, p. 2366-2370, 2013.

SHUMUYE, B.; GUESH G.; MINISTER, B. Effect of graded level of pigeon pea (*Cajanus cajan*) leaves on growth performance of sheep. **International Journal of Agricultural Science Research**, v. 5, p. 035-039, 2016.

SILIM, N. M. *et al.* Integrated management of postharvest pests of pigeon pea: Status and potential. 2006. In: **Status and potential of pigeon pea in Eastern and Southern Africa: Proceedings of a regional workshop**, 12-15 Sept. 2000, Nairobi, Kenya (Silim SN, Mergeai G, Kimani PM eds.). B-5030 Gembloux, Belgium: Gembloux Agricultural University; and Patancheru 502 324, Andhra Pradesh, India. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

SMITH, W. B. *et al.* **Board-invited review: Understanding intake on pastures: How, why, and a way forward.** 2021. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved.

SOLLENBERGER, L. E. *et al.* Evaluating forage production and quality. **The Science Grassland Agriculture**, p. 97-110, 1995.

STELLA, L. A.; ZUBIETA, A. S.; GOMES, B. C. K. Estratégia do uso de cereais na redução da produção de metano entérico em ruminantes. **Nutritime Revista Eletrônica**, on-line, Viçosa, v. 17, n. 2, p. 8669-8673, 2020.

SUBBARAO, G. V.; CHAUHAN, Y. S.; JOHANSEN, C. Patterns of osmotic adjustment in pigeon pea its importance as a mechanism of drought resistance. **European Journal of Agronomy**, v. 12, p. 239-249, 2000.

SUBBARAO, G.V.; AE, N.; OTANI, T. Genetic variation in acquisition, and utilization of phosphorus from iron-bound phosphorus in pigeon pea. **Soil Science and Plant Nutrition**, v. 43, p. 511-519, 1997.

TAMELE, O. H. *et al.* Optimal defoliation management of brachiaria grass–forage peanut for balanced pasture establishment. **Grass and Forage Science**, v. 73, p. 522-531, 2018.

TAN, H. Y. *et al.* Effects of condensed tannins from Leucaena on methane production, rumen fermentation and populations of methanogens and protozoa in vitro. **Animal Feed Science and Technology**. v. 169, p. 185-193, 2011.

TAVENDALE, M. H. *et al.* Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa and effects of extractable condensed tannin fractions on methanogenesis. **Animal Feed Science and Technology**, v. 123, n. 124, p. 403-419, 2005.

TEDESCHI, L. O. *et al.* Quantification of methane emitted by ruminants: a reviewof methods. **Journal of Animal Science**, v. 100, n. 7, 2022.

TENAKWA, E. A. *et al.* Pigeon pea (Cajanus cajan) fodder cutting management in the Guinea Savanna Agro-Ecological Zone of Ghana. **Agroforestry Systems**, v. 96, p. 1-10, 2022.

THOMAS, R. J. Role of legumes in providing N for sustainable tropical pasture systems. **Plant and Soil**, v. 174, 1995.

THOMAS, R. J. The role of the legume in the nitrogen cycle of productive and sustainable pastures. **Grass and Forage Science**, v. 47, p. 133-142, 1992.

TITGEMEYER, E. C. Design and interpretation of nutrient digestion studies. **Journal of Animal Science**, v. 75, p. 2235-2247, 1997.

TITGEMEYER, E.C. *et al.* Evaluation of titanium dioxide as a digestibility marker in cattle. **Journal of Animal Science**, v. 79, p. 1059-1063, 2001.

TSEU, R. J. et al. Effect of tannins and monensin on feeding behaviour, feed intake, digestive parameters and microbial efficiency of nellore cows. **Italian Journal of Animal Science**, v. 19, p. 262-273, 2020.

TROEDSON, R. J.; WALLIS, E. S.; SINGH, L. Pigeon pea: Adaptation. In: Nene, Y., Hall, S.D., Sheila, V.K. (Eds.), **The Pigeon Pea**, CABI, Wallingford, p. 159-177, 1990.

TULU, A. *et al.* Comparative evaluations of selected pigeon pea (*Cajanus cajan*) genotypes for biomass yield, nutrient composition, and dry matter intake under diverse locations of tropical Africa. **Advances in Agriculture**, v. 2021, n. 5516662, p. 5, 2021.

VALADARES, FILHO, S. C. *et al.* CQBAL 4.0 **Brazilian Feed Composition Tables for Ruminants**. 2018. Retrieved on 28 November 2022 from: www.cqbal.com.br.

VALE, P. *et al.* The expansion of intensive beef farming to the Brazilian Amazon. **Global** HÜTSCH **Environmental Change**, v. 57, n. 101922, 2019.

VAN SOEST, P. J. V. **Nutritional Ecology of the Ruminant**. Cornell University Press, 2018.

VITTI, D. M. S. S. *et al.* Do all tannins have similar nutritional effects? A comparison of three Brazilian fodder legumes. **Animal Feed Science and Technology**, v. 119, p. 345-361, 2005.

VLAMING, J. B. **Quantifying variation in estimated methane emission from ruminants using the SF_6 tracer technique.** 2008, 182 p. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy In Animal Science At Massey University, Palmerston North, New Zealand, 2008.

WAGHORN, G. C. *et al.* Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. Nitrogenous aspects. **The Journal of Agricultural Science**, v. 123, p. 109-119, 1994.

WALLIS, E. S. *et al.* Varietal improvement of pigeon pea for small-holder livestock production systems. **Proceedings of workshop on Crop-Livestock Systems Research**, Khon Kaen, Thailand, 1986.

WANAPAT, M. *et al.* Dietary sources and their effects on animal production and environmental sustainability. **Animal Nutrition**, v.1, p. 96-103, 2015.

WANG, L. A.; GOONEWARDENE, Z., 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. **Canadian Journal of Animal Science**, v. 84, p. 1-11, 2004.

OWENS, F. N.; HANSON, C. F. External and internal markers for appraising site and extent of digestion in ruminants. **Journal of Dairy Science**, v. 75, p. 2605-2617, 1992.

WESTBERG, H. *et al.* **SF_6 tracer technique: methane measurement from ruminants**. Washington State University, Pullman, Washington, 1998.

WHITEMAN, P. C. **Tropical Pasture Science**, Oxford University Press, 1980.

WILKERSON V. A.; Casper D. P. The prediction of methane production of Holstein cows by several equations. **Journal of Dairy Science**, v. 78, p. 2402-2414, 1995.

WILKINS, B. J. The potential digestibility of cellulose in forage and faeces. **Journal of Agricultural Science,** v. 73, p. 57-64,1969.

WINTER, W. H.; WINKS, L.; SEEBECK, R. M. Sustaining productive pastures in the tropics 10. Forage and feeding systems for cattle. **Tropical Grasslands**, v. 25, p. 145-152, 1991.

YOUNG, N. D.; MUDGE, J.; ELLIS, T. N. Legume genomes: More than peas in a pod. **Current Opinion in Plant Biology**, v. 6, p. 199-204, 2003.