UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

ANALISA VASQUES BERTOLONI

Performance evaluation, carcass, and meat quality of Nellore heifers in different grazing systems as a methane emission mitigation strategy

Pirassununga

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Corrected Version

Thesis presented to the College of Animal Science and Food Engineering of the University of São Paulo, as a part of the requirements for obtaining the Doctorate degree in Sciences from the postgraduate program in Animal Science.

Area of concentration: Animal Productivity and Quality

Advisor: Prof. Paulo Henrique Mazza Rodrigues, Ph.D.

Co-advisor: Prof. Angélica Simone Cravo Pereira, Ph.D.

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UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação do desempenho, carcaça e qualidade da carne de novilhas Nelore em diferentes sistemas de pastejo como estratégia de mitigação das emissões de metano", protocolada sob o CEUA nº 3455101019 (ID 001375), sob a responsabilidade de **Paulo Henrique Mazza Rodrigues** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está 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, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 18/12/2019.

We certify that the proposal "Performance evaluation, carcass and meat quality of Nelore heifers in different grazing systems as a methane emission mitigation strategy", utilizing 48 Bovines (48 females), protocol number CEUA 3455101019 (ID 001375), under the responsibility of **Paulo Henrique Mazza Rodrigues** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of july 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 12/18/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 06/2019 a 06/2021 Área: Zootecnia

Origem:	Prefeitura do Campus USP Fernando Costa						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	19 a 21 meses	N:	48
Linhagem:	Nelore			Peso:	350 a 400 kg		

Local do experimento: Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Campus de Pirassununga - São Paulo.

Pirassununga, 05 de novembro de 2020

Profa. Dra. Cristiane Gonçalves Titto
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
Universidade de São Paulo - FZEA/USP

Profa. Dra. Daniele dos Santos Martins
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
Universidade de São Paulo - FZEA/USP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Performance evaluation, carcass and meat quality of Nelore heifers in different grazing systems as a methane emission mitigation strategy

Pesquisador: ANALISA VASQUES BERTOLONI

Área Temática:

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Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.990.041

Apresentação do Projeto:

O projeto de pesquisa "Performance evaluation, carcass and meat quality of Nelore heifers in different grazing systems as a methane emission mitigation strategy" envolve a utilização de seres humanos em testes sensoriais onde haverá avaliação sensorial (degustação) de carne de animais Nelore.

Objetivo da Pesquisa:

O objetivo da pesquisa é avaliar os efeitos das práticas de manejo de pastagens (pastejo rotacionado e diferido) sobre o desempenho, as características da carcaça, e a qualidade da carne de animais que receberão suplementação.

Avaliação dos Riscos e Benefícios:

A avaliação de riscos e benefícios está adequada.

Comentários e Considerações sobre a Pesquisa:

A pesquisadora justificou o não detalhamento do orçamento do projeto com uma "declaração", na qual relata que o projeto é parte de um projeto temático sendo parte do programa de inovação tecnológica da FAPESP com pesquisas sobre mudanças climáticas da globais. Que há uma verba de R$ 1.346.615,63 para serem utilizados em tais projetos.

ANALISA VASQUES BERTOLONI

Performance evaluation, carcass, and meat quality of Nellore heifers in different grazing systems as a methane emission mitigation strategy

Doctoral thesis presented to the Faculty of Animals Science and Food Engineering, University of São Paulo, as part of the requirements to obtain the degree of Doctor of Science of the Animal Science Graduate Program.

Concentration area: Animal Quality and Productivity

***Date of approval:* ____/____/_____**

Thesis Defense Committee Members

President:
Institution:

Prof. Dr.
Institution:

Prof. Dr.
Institution:

Prof. Dr.
Institution:

Prof. Dr.
Institution:

DEDICATÓRIA

Dedico este trabalho aos meus pais Sidney e Lurdes e as minhas
irmãs Michele e Mayara, que sempre apoiaram meus sonhos.
Ao meu namorado e aos amigos e amigas, que
me encorajaram a chegar até aqui.

AGRADECIMENTOS

A Deus, por ter me dado forças nas horas mais difíceis, permitindo que eu chegasse até aqui.

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“Você enfrentará muitas derrotas na vida, mas nunca se deixe ser derrotado.”

(Maya Angelou)

ABSTRACT

BERTOLONI, A.V. **Performance evaluation, carcass, and meat quality of Nellore heifers in different grazing systems as a methane emission mitigation strategy.** 2023. 80 p. Doctoral Thesis – Faculdade de Zootecnia e Engenharia de alimentos, Universidade de São Paulo, 2023.

Brazil occupies a prominence position in cattle production, being considered one of the most important producers and exporters of beef in the world. In order to meet the demand of a growing population, the livestock sector needs to efficiently increase its production while reducing their environmental impacts, which is the focus of numerous criticisms. One of the strategies that can be adopted to reduce the effect of low forage availability due to drought seasonality is the use of deferred stocking associated with nutritional supplementation, aiming to improve animal efficiency. The objective of this study was to evaluate performance, carcass characteristics and meat quality of Nellore heifers. Forty-eight Nellore heifers, with an initial weight of 348 ± 30 kg and 18-21 months old, were used in a randomized complete blocks design and the experimental period lasted 2 years, divided into 2 periods: year 1 and year 2. The treatments arrangement was a 2 × 2 ×4 factorial, as fallow: Factor 1) rotational stocking grazing system or deferred stocking grazing system; Factor 2) urea or ammonium nitrate supplementation; Factor 3) four seasons of the year. For post-slaughter data, only Factors 1 and 2 were considered in a 2 × 2 factorial arrangement. During the experimental period, forage and supplement intake, performance, and enteric methane (CH_4) emissions were measured. At the end of each experimental period, the animals were slaughtered for carcass characteristics and meat quality evaluations. Data were statistically analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) considering effects significant when $P \leq 0.05$. Interaction grazing system and season of the year was found ($P<0.05$) for average daily gain (ADG), with heifers kept in the deferred stocking grazing system presented higher ADG in the winter. During the autumn, heifers in the rotational stocking grazing system had higher ADG. Effect of nitrogen sources supplementation was found ($P<0.05$), and heifers supplemented with urea presented higher ADG. Grazing system and season interaction was also found for dry matter intake in relation to live weight (DMI_{TWL}) with heifers in the rotational stocking grazing system presenting higher values during the autumn ($P<0.05$). Effects of seasons were found for forage and total DMI ($P<0.05$) but not for supplement DMI ($P>0.05$). When expressed per ADG and live body weight (LBW), interaction between grazing system and season were found for CH_4 emissions. ($P<0.05$). The lowest values of daily CH_4 emission per animal was found in the winter ($P<0.05$), while the highest CH_4 emission per total DMI was found during the spring ($P<0.05$). No effect of N supplementation was found for CH_4 emissions ($P>0.05$). For the percentage of gross energy intake converted to enteric CH_4 emission (Y_m%), a triple interaction between grazing system, nitrogen source and season of the year was detected ($P<0.05$). In winter, the highest values of Ym were found for heifers that were in rotational stocking grazing system receiving urea as a nitrogen source. For spring season, the highest values of Y_m were found for heifers in the deferred stocking grazing system supplemented with ammonium nitrate and for heifers in the rotational stocking grazing system receiving urea. Deferred grazing system allowed higher hot carcass weight, cold carcass weight, dressing percentage, edible carcass portion, spare ribs and striploin ($P<0.05$), and higher subcutaneous fat thickness was found in the carcasses from heifers that received ammonium nitrate ($P<0.05$). Beef from heifers in the deferred stocking grazing system presented higher aroma, juiciness and flavor attributes evaluated in the sensory panel ($P<0.05$). Interaction grazing system and nitrogen source effects were found for tenderness and overall acceptance attributes ($P<0.05$). Overall, the use of ammonium nitrate as a nitrogen source showed similar results to the use of urea, while the deferred stocking grazing system proved to be an efficient intensification method as the performance of heifers was similar to the rotational stocking grazing system but presenting higher carcass dressing percentage and edible portions.

Key words: Deferred stocking, nitrate, supplementation, beef cattle.

RESUMO

BERTOLONI, A.V. **Avaliação do desempenho, carcaça e qualidade da carne de novilhas Nelore em diferentes sistemas de pastejo como estratégia de mitigação da emissão de metano.** 2023. 80 p. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de alimentos, Universidade de São Paulo, 2023.

O Brasil ocupa destaque na pecuária, sendo considerado um dos mais importantes produtores e exportadores de carne bovina do mundo. Para atender à demanda de uma população crescente, a pecuária precisa aumentar a produção de forma eficiente, reduzindo o impacto ambiental que é objeto de inúmeras críticas sobre a produção animal. Uma das estratégias que podem ser adotadas para reduzir o efeito da baixa disponibilidade de forragem devido à sazonalidade da seca é o uso de pastejo diferido associado à suplementação nutricional, que visa melhorar a eficiência animal. O objetivo deste projeto foi avaliar o desempenho, as características de carcaça e a qualidade da carne de novilhas Nelore terminadas em diferentes sistemas de pastagem. Foram utilizadas 48 novilhas da raça Nelore, com peso inicial de 348 ± 30 kg e 18 - 21 meses de idade. O delineamento experimental utilizado foi de blocos completos casualizados e a duração do experimento foi de 2 anos, divididos em 2 períodos: ano 1 e ano 2. Os tratamentos foram definidos por um arranjo fatorial 2 × 2 × 4, sendo: Fator 1) sistema de lotação rotacionada ou sistema de lotação diferida; Fator 2) suplementação convencional utilizando ureia ou suplementação alternativa com nitrato de amônio; Fator 3) quatro estações do ano. Para os dados de pós abate, foram considerados somente os fatores 1 e 2, compondo um arranjo fatorial 2 × 2. Durante o experimento foram mensurados o consumo de forragem, consumo de suplemento, ganho de peso corporal das novilhas e emissão de metano (CH_4) entérico. Ao final de cada ano experimental os animais foram abatidos para avaliação das características de carcaça e qualidade de carne. Os dados foram analisados por meio do SAS 9.4 (SAS Institute Inc., Cary, NC, EUA), sendo considerados efeitos significativos quando $P \leq 0{,}05$. Houve efeito de interação ($P<0{,}05$) para sistema de pastejo e estação do ano no ganho médio diário (GMD) dos animais, sendo possível observar que as novilhas mantidas no sistema de lotação diferida apresentaram maior GMD no inverno. No outono o efeito ocorreu de forma inversa, onde as novilhas do sistema de lotação rotacionada apresentaram maior GMD. As fontes de nitrogênio utilizadas interferiram no GMD das novilhas, de maneira que a suplementação com ureia resultou em maior GMD ($P<0{,}05$). No que diz respeito ao consumo das novilhas, o consumo de matéria seca (CMS) em relação ao peso vivo (CMSPV) apresentou efeito de interação entre sistema de pastejo e estação, mostrando que as novilhas do sistema de lotação rotacionada apresentam CMSPV superior no outono. O CMS de forragem e CMS total foram influenciados pelas estações ($P<0,05$), já o CMS de suplemento não foi afetado ($P>0,05$). Efeito da interação sistema de pastejo e estação do ano foram observadas para as variáveis de emissão de CH_4 por GMD e por peso vivo (PV) ($P<0,05$). As emissões diárias de CH_4 por animal apresentaram menores valores no inverno ($P<0,05$) e a primavera foi a estação que apresentou maior emissão de CH_4 por CMS total ($P<0.05$). As fontes de nitrogênio utilizadas não afetaram a produção de CH_4 ($P>0,05$). As emissões de CH_4 por características de carcaça não mostraram efeito significativo ($P>0,05$). Para a porcentagem de energia bruta ingerida convertida em emissão entérica de CH_4 (Y_m%), foi detectado efeito de interação tripla entre sistema de pastejo, fonte de nitrogênio e estação do ano ($P<0.05$). No inverno o maior valor de foi encontrado para as novilhas que estavam no sistema de pastejo em lotação rotacionada recebendo uréia como fonte de nitrogênio. Já na primavera, os maiores valores de Y_m foram encontrados para as novilhas do sistema de pastejo em lotação diferida suplementadas com nitrato de amônio e para as novilhas do sistema de pastejo em lotação rotacionada recebendo uréia. A pastagem diferida permitiu maior peso e rendimento de carcaça quente, e a espessura de gordura subcutânea foi maior nas carcaças das novilhas que receberam nitrato de amônio como fonte de nitrogênio. O sistema de lotação diferida proporcionou maior peso de carcaça fria, porção comestível da carcaça, ponta de agulha e contrafilé ($P<0,05$). Nenhuma variável de qualidade da carne foi afetada pelos sistemas de pastejo ou fontes de nitrogênio ($P>0,05$). Os atributos aroma, suculência e sabor da carne, avaliados no painel sensorial, receberam as maiores notas quando a carne era proveniente das novilhas do sistema de lotação diferida ($P<0,05$). Já os atributos maciez e aceitação global apresentaram efeito de interação entre sistema de pastejo e fonte de nitrogênio ($P<0,05$). O uso de nitrato como fonte de nitrogênio apresentou resultados similares ao uso da ureia. A pastagem diferida se mostrou um método de intensificação eficiente, pois o desempenho das novilhas foi semelhante ao sistema de pastejo rotacionado e proporcionou peso e porção comestível da carcaça superior.

Palavras-chave: lotação diferida, nitrato, suplementação, gado de corte.

FIGURES

TABLES

SUMARY

1. INTRODUCTION AND JUSTIFICATION

It is estimated that the world population will reach 9.6 billion in 2050 (CERRI et al., 2016) resulting in a 70% higher demand for animal products when compared to 2010. To meet this demand, total beef and milk production is expected to increase by 73% and 58%, respectively (FAO, 2011), since 34% of the animal protein consumed worldwide comes from beef, milk, and eggs (FAO, 2017). In this scenario, Brazil occupies the first position as beef exporter and is one of the world's largest beef producers, mainly from Nellore (*Bos indicus*) cattle (BATTISTELLI, 2012; SIQUEIRA et al., 2012a). Due to the favorable characteristics of adaptation, rusticity, and productivity in a predominant tropical climate, approximately 80% of the national cattle herd is formed by Nellore animals and their crossbreeds (ABIEC, 2014). A significant proportion of this herd consists of young heifers with one to three years of age (SEMMELMANN et al., 2001). In fact, the slaughter of female animals has grown in the country, representing 10.2% of the total slaughters until September of 2018 (IBGE, 2019).

The world beef market is increasingly focused on the quality, origin (traceability) and environmental issues related to animal products. Thus, alternatives to improve the productivity of the national cattle herd while reducing environmental impacts became mandatory. Therefore, the livestock sector must intensify its production efficiently with a land-saving effect, since the Brazilian beef production is based on tropical pastures (BEZABIH et al., 2014). However, due to physiological characteristics of tropical grasses and climatic conditions throughout the year in the country (180 days of dry and 180 days of rainy season), there is a production seasonality (SANTOS et al., 2009) where 80% of annual production is concentrated in the rainy season (ESTEVES et al., 1998). In addition, during the dry season, tropical pastures usually present values of crude protein (CP) lower than 7 %, the minimum required for rumen microbiota with potential for reducing voluntary intake and diet digestibility, animal performance and increased enteric methane (CH_4) emissions (ARCHIMÈDE et al., 2011).

Pasture deferral is a storage strategy, which has often been defined as the discontinuation of pasture use at the end of the growing season for a specific period to allow forage accumulation that can be employed during periods of scarcity (EUCLIDES, 2007). However, beef cattle allotted on deferred pasture may express lower performance or simply maintain their body weight as deferred forage usually has poor nutritional quality (SANTOS et al., 2004; GOMES JR. et al., 2002). In order to increase animal performance, supplementation strategies can be adopted to match the nutritional value of available forage and/or improve feed conversion for deferred pastures (EUCLIDES; MEDEIROS, 2005).

Another important issue related to livestock production is the emissions of greenhouse gases (GHG). In agricultural activity, the most important GHG are carbon dioxide (CO_2), CH_4, and nitrous oxide (N_2O). The CH_4 and N_2O concentrations in the atmosphere are lower than CO_2 (SNYDER, et al., 2008); however, CH_4 and N_2O have 25- and 296-times greater heating potential (IPCC, 2006). About 35 to 40% of all CH_4 produced by anthropic activities originate from ruminants (BEAUCHEMIN et al., 2008), with enteric fermentation contributing to 94% of these emissions (FAO, 2013).

As the demand for food production grows with the growing population, the emissions of GHG by the agricultural sector will also increase (O'MARA, 2011). However, if all producers adopted the mitigating methodologies applied by 10 to 25% of producers who have lower emission intensities on their properties, the emissions in the agricultural sector could be reduced between 18 and 30% (FAO, 2013). Through modifying ruminal fermentation, changing the roughage, type and amount of carbohydrate included in the diet, use of food additives or addition of lipids CH_4 production can be reduced (BERCHIELLI; MESSANA; CANESIN, 2012). To be considered efficient, the mitigation strategy must, in addition to promoting a persistent reduction in the emission of enteric CH_4, provide a lucrative increase in milk or beef production (GRAINGER et al., 2010).

Among techniques to reduce GHG emissions from grazing beef cattle, nutritional strategies, such as the ad of ionophores, glycerol, tannins, saponins, essential oils, lipids, vaccines, and antibiotics, as well as supplements to manipulate rumen fermentation, grazing systems strategies, and genetic improvement stands out, with the potential to result in more efficient production systems (MOHAMMED et al., 2004; BERNDT, 2010).

Nitrate (NO_3^-) is a nitrogen source that is a hydrogen sink in rumen and, when supplemented to ruminants' diet, has the potential to decrease rumen CH_4 emissions by reducing its formation (LENG; PRESTON, 2010). Currently, the effect of NO_3^- on the reduction of CH_4 production has already been evaluated in some studies with beef cattle, becoming increasingly important due to the potential for promoting better animal efficiency with superior final product quality while also reducing the environmental impact of the activity. This type of strategy is one of those that should be encouraged for improving the sustainability of livestock production.

2. HYPOTHESIS AND OBJECTIVES

The hypothesis of this study is that the adoption of deferred stocking grazing can increase animal performance, especially during the dry season of the year, by providing higher availability of forage mass. In addition, the use of ammonium nitrate as supplementation can reduce CH_4 production by ruminal fermentation and, consequently, reduce enteric CH_4 emission when compared to urea supplementation, resulting in increased animal performance and higher slaughter weight.

Furthermore, there may be an interaction between grazing systems and non-protein nitrogen sources. Despite the deferred grazing systems providing greater forage availability, the fiber ingested by the animals is of low quality, resulting in greater CH_4 production. Therefore, the deferred stocking grazing system and supplementation adopted during the dry season can result in beneficial environmental and performance effects, with higher live body weight (BW) gain, carcass traits and beef quality, while also reducing CH_4 per kg of produced beef.

Based on this, the main objective of this study was to evaluate the effects of pasture management practices (rotated or deferred grazing) on performance, carcass traits, and beef quality of animals receiving supplementation with urea or ammonium nitrate.

3. LITERATURE REVIEW

3.1. Overview of beef cattle production in Brazil

Brazil is considered an important global food supplier especially animal protein both as an importer and exporter. In 2021, Brazilian agribusiness recorded an 8.3% growth in GPD (Gross Domestic Product) compared to the previous year, reaching a share of 27.4% in total GDP (CEPEA, 2022). The livestock sector accounted for a turnover of R$913.14 billion, which represented a 14.9% growth from 2020 (ABIEC, 2022), playing a relevant role in the country's economy. With 196.4 million cattle heads on approximately 163.1 million hectares of pastures, Brazilian livestock recorded 39.1 million head slaughtered in 2021, of which 74.4% was destined for the domestic market (expressed as tonnes of carcass equivalent - TCE; ABIEC, 2022). Animals finished on pasture represent around 85% of total slaughter, emphasizing that the extensive grazing system is predominant in the production of beef cattle in Brazil (ABIEC, 2022).

Despite having the largest cattle herd in the world, Brazil still loses in the percentage of production to the United States, indicating that the cattle production system is founded on low-tech systems. These low-tech systems usually result in the degradation of pastures, low animal performance, increase in GHG emissions, and late animal slaughter, leading the livestock sector to an inefficient production system. According to the IBGE agricultural census in 2017, Brazil has large pasture areas, estimated at approximately 158.6 million hectares of natural and planted pastures. However, the average stocking rate for cattle varies below 1.0 animals per hectare, which represents a potential loss of more than US $ 10 billion in beef value, considering a stocking rate of 2.0 animals per ha, which could easily be obtained through improvements in pasture management, assuming that the national or international market could absorb such a large amount of beef at current prices (OLIVEIRA, 2015). Therefore, to meet the growing demand for food production, grazing systems need to become increasingly efficient, without causing damage to the environment and promoting ecological services (CARVALHO et al., 2010; SALTON et al., 2014).

As an example, Gimenes et al. (2011) evaluated management targets for Marandu palisade grass (*Urochloa brizantha* cv. Marandu) for more than 1 year. Treatments concisted of a combination of two rotational grazing frequencies (pre-grazing heights of 25 and 35 cm), fertilized with two levels of N (50 and 200 kg ha^{-1} per year). The authors found that pastures managed with a 25 cm pre-grazing height presented higher pasture support capacity resulting in higher stocking rates (3.13 *vs* 2.85 UA ha^{-1}), promoting greater daily weight gain (0.629 *vs* 0.511 kg day^{-1}) and weight gain per hectare (886 *vs* 674 kg ha^{-1}). Regarding fertilization, increases in leaves portion in the post-grazing forage mass, accumulation rates, stocking rate and weight gain per hectare were found for the 200 kg ha^{-1} of N.

In another study, Oliveira et al. (2018) evaluated animal growth and beef quality of beef cattle reared in different intensified and degraded grazing systems, in Brazilian Southeast under conditions of subtropical humid climate. The authors found that the intensification of the systems improved performance, muscular development, and growth of the animals. Carcass production also increased in intensified systems; however, the aspects of beef quality, colour and tenderness, did not differ among the evaluated systems.

Overall, in order to shorten the production cycle of cattle raised on tropical pastures, and make the livestock activity more competitive, efficient and sustainable, it is necessary to intensify grazing systems, ensuring the animals' nutritional requirements by improving forage availability and nutritional quality, and increasing stocking rates (SILVA et al., 2017).

3.2. Beef cattle supplementation under grazing systems

In pasture systems, forage is the main nutritional resource for ruminants. In the pasture production system, there are basically two types of animal management: the continuous grazing system and the rotational stocking grazing system. The available forage species, animal category and the producer's objectives will influence the management choice. In the continuous grazing system, a variable number of animals remain throughout the year in a determined area. In the rotational stocking grazing system, the animals are managed in different paddocks according to forage availability, stocking rate and pasture support capacity (MENEZES et al., 2017).

Due to the territorial extension in Brazil and favorable climate conditions for tropical grasses, a major part of beef cattle production is based on grazing tropical pastures; however, the increased age of slaughter in this type of system occurs due the seasonality of forage production and quality (EUCLIDES et al., 2005). Forage plants have seasonality of production, with excess production during the rainy season and scarcity during the drought (AMARAL et al., 2012). The rainy season (spring and summer) is characterized by more rainfall, fact that favors the production of forage as it allows for higher plant growth, increasing the availability of food for animals. On the other hand, in the dry season (autumn and winter) pasture production is scarce and of worse quality, due to the reduction in rainfall at this time of year (MENEZES et al., 2017). In addition, the nutritional quality of the forage is reduced in the dry season due to the increase of the plant's structural carbohydrates, which contributes to the reduction of the CP content. This fact can result in lower animal performance and higher emission of enteric CH_4, whether expressed per forage intake or animal product (ARCHIMÈDE et al., 2011).

Animal performance can be influenced because of the mass and nutritive value of forage when different pasture management strategies are used, as this can cause variations in the structure of the forage canopy (PEDREIRA et al., 2009). The deferral of pasture is a management strategy that aims at accumulating forage during the period of plant growth, where a certain area of the pasture is closed so that it can be used in times of scarcity. However, stockpiled pastures have low nutritional value despite being associated with a high forage mass (SOUSA et al., 2012). Therefore, to adjust the nutritional value of the available forage and/or improve the feed conversion of cattle, the adoption of supplementation is important since it could increase animal performance (EUCLIDES et al., 2007).

Supplementation aims to complement the nutritional value of forage so that the nutritional requirements of animals raised in pasture systems are met and the desired animal performance is achieved (EUCLIDES; MEDEIROS, 2005). During the rainy season, supplementation aims to enhance animal performance, and, with the adoption of proper pasture managements, it is possible to obtain animal performance greater than 1 kg per day as there is a greater forage accumulation during this season (BARBERO et al., 2015; THIAGO; SILVA, 2001). To achieve the expected results, supplementation needs to be planned correctly and for that some factors must be considered. These factors involve the requirements of animal category, time of the year, types of supplements (energy, protein or mixed) and the effects of compensatory growth on nutritional requirements and use of the diet. The main objective of correct pasture management aligned with animal supplementation is to provide that weight gain is always higher than that obtained in the previous period of growth (MENEZES et al., 2017).

Canesin et al. (2007) evaluated different supplementation frequencies (everyday, alternate days, or 5 days a week) for cattle kept on *Brachiaria brizantha* cv. Marandu pasture and found a linear increase in weight gain and a reduction in age at slaughter, regardless of the supplementation strategy. Fernandes et al. (2010) provided energetic protein supplementation (0.6% of live weight) to crossbred steers grazing *Brachiaria brizantha* cv. Marandu pasture during the rainy season. The authors found an 27% increase in the daily weight gain of supplemented animals (1.06 kg day^{-1}; $P<0.05$) compared to steers that were not supplemented (0.77 kg day^{-1}). In another study, the authors found a linearly increase in ADG ($P<0.10$) of cattle on deferred pastures receiving different supplement doses (0, 1, 2 and 3 kg animal^{-1} day^{-}

^{1}). The ADG of the animals ranged from 0.419 to 1.019 kg animal^{-1} day^{-1} and the authors concluded that animals that received a higher amount of supplementation presented higher performance and consequently, higher final weight (SANTOS et al., 2020).

In addition, the supplementation of grazing cattle can also be used as a strategy for providing ruminal modulators to the animal, also aiming to reduce CH_4 while improving animal performance (SIQUEIRA et al., 2012b). In order to provide non-protein nitrogen to the animals and at the same time mitigate CH_4 emissions, the use of NO_3^- is gaining attention as an alternative for replacing urea supplementation, since its use can provide nitrogen for ruminal bacteria and has effects in reducing enteric CH_4 production (LEE; BEAUCHEMIN, 2014).

3.3. Emission of Greenhouse Gases and Beef Production

Although agriculture is recognized for its importance in food production, discussions about the environmental impact caused by this activity have been raised and since the livestock sector has its extensive production systems residing in large areas of degraded pastures and generating a large amount of GHG per product unit, it has been subject to numerous criticisms (IPCC, 2007; MACHADO et al., 2011). Degraded pastures or those below their production potential results in low zootechnical indexes, causing greater amounts of GHGs emitted per kilogram of beef and/or milk produced (IPCC, 2007). Of the pastures cultivated in Brazil, it is estimated that 80% are established in degraded soils (BARCELLOS; VILELA, 2001; KLUTHCOUSKI; ADAIR, 2003).

According to the IPCC (2019), 23% of GHG emissions from anthropic activities are from agriculture, livestock, forestry, and other land uses. The main GHGs emitted by farming activities are CO_2, N_2O and CH_4 (IMAFLORA, 2014), with CH_4 and N_2O being the most impacting GHGs in the agricultural sector, in which enteric fermentation of ruminants is the largest contributor (57.5%), followed by agriculture (35%) and other activities (7.5%) (MCTI, 2016). Emissions of enteric CH_4 in Brazil correspond to 63.3% of the anthropogenic emissions of this gas (54.1% of beef cattle, 7.4% of dairy cattle and 1.9% of other species), while the decomposition of manure corresponds to 5.5% of these emissions (BRASIL, 2009). The Brazilian cattle herd is responsible for approximately 3.3% of the CH_4 produced worldwide by anthropic activities, representing 11.3% of the total enteric CH_4 emitted (BERCHIELLI et al., 2012).

The enteric CH_4 emission is a natural process intrinsic to ruminants and tends to follow the growth of the herd (BERNDT et al., 2013). In the rumen, the ingested food will be fermented by the bacteria generating short chain fatty acids (SCFA), mainly acetic, propionic, and butyric acids, which are then used by ruminants as an energy source. However, there is also the formation of undesirable compounds such as CO_2 and hydrogen (KOZLOSKI, 2009). The main hydrogen-producing metabolic pathways are those involved with acetate and butyrate production. Methanogenic bacteria use molecular hydrogen to obtain energy for their growth by reducing CO_2 to form CH_4, which is eructated or exhaled into the atmosphere (COTTLE et al., 2011). Therefore, to maintain adequate concentrations of hydrogen in the rumen, ruminal CH_4 production is a necessary metabolic pathway for ruminants, as the enzymatic processes involving nicotinamide adenosine diphosphate ($NADH + H^+ \leftrightarrow NAD^+$) can be inhibited even with traces of H_2 in the rumen, as these limit the sugar oxidation involved in the reduction of NAD^+ in NADH, when alternative routes for H_2 disposal are absent (MCALLISTER; NEWBOLD, 2008).

The emission of enteric CH_4 impacts the environment as an important GHG and is affected according to the level of intake, composition, and nutritional quality of the diet (WARNER et al., 2017). Furthermore, the production of enteric CH_4 represents energy losses for the animal, varying between 2 and 12% of the total gross energy intake (VAN SOEST et al., 1994). The nature and rate of fermentation of carbohydrates influence the production of CH_4 by ruminants, and forage-based diets favor greater production of acetate and increase the production of CH_4 per unit of fermentable organic matter (JOHNSON; JOHNSON, 1995).

Brazil has the highest growth rates in annual estimates of CH_4 emissions (2.12% per year) when we compare the performance of the 10 largest beef exporters in the world between 1988 and 2007 (MILLEN et al., 2011). However, as a result of the increase in animal productivity, from 1997 to 2014, the Brazilian beef cattle has shown a decrease in CH_4 intensity (kg CO_2-eq. / kg. eq. carcass), since over these years the animal population has increased around 32% and gross CH_4 emissions by 29%, while carcass production increased by around 142% (IBGE, 2018).

The CH_4 emissions are commonly expressed in terms of production per animal unit or production by gross energy intake. A strategy to reduce the negative impact of livestock production on global warming is to reduce CH_4 per unit of product, such as beef or milk (KNAPP et al., 2014). Data accuracy increases when GHG emissions are expressed per unit product of animal origin. The fact that most beef production in Brazil comes from pasture systems, contributes to the country having one of the highest emission intensities (kg CO_2-eq./kg beef). However, Brazil has a differential when compared to other countries, as tropical pastures soils have the capacity to act as a C sink and it is estimated that around 89% of the potential for mitigating GHG emissions is related to the soil C sequestration (OLIVEIRA et al., 2015).

Thus, the development of strategies that reduce energy losses from CH_4 production may increase weight gain or milk production, since there will be a better use of the energetic source in the diet, and at the same time may reduce the CH_4 emissions to the atmosphere (PRIMAVESI et al., 2004).

3.4. Use of nitrate for ruminants

To reduce energy losses and improve feed efficiency and animal productivity, the ruminant production system faces the challenge of developing diets and management systems that reduce CH_4 production (NARDONE et al., 2010). A strategy to reduce the production of enteric CH_4 is by providing alternative electron acceptors; these electrons will effectively consume the reducing equivalents generated during fermentation to resize the electron flow from CO_2 reduction to CH_4 (ANDERSON; RASMUSSEN, 1998). Nitrate (NO_3^-) is powerful inhibitor of methanogenesis in fermentative digestion systems. The NO_3^- conversion into ammonia by ruminal microorganisms is highly competitive with the production of CH_4, because it consumes eight H_2 electrons (LENG; PRESTON, 2010). Thus, if there is enough NO_3^- in the rumen, this route can become a remarkable hydrogen drain, so each mole of reduced NO_3^- would decrease the production of one mole of CH_4 (VAN ZIJDERVELDET et al., 2010).

Another advantage of using NO_3^- is that the ammonia generated in its conversion is used as a source of nitrogen (N) for bacterial growth (VAN ZIJDERVELDET et al., 2010), favoring the synthesis of microbial protein and, allowing the replacement of part of the dietary protein by non-protein nitrogen source (LEWIS, 1951; VAN ZIJDERVELDET et al., 2010; LI et al., 2013). The reactions of conversion of NO_3^- to ammonia consume less energy than the conversion of CO_2 to water and CH_4 (-598 kJ *vs* -131 kJ, respectively), providing retention of raw energy to the animal (LENG; PRESTON, 2010), showing that the reduction of NO_3^- is more energy efficient than methanogenesis (GUO et al., 2009). However, NO_3^- is reduced to nitrite (NO_2^-) by rumen microorganisms, and this component is toxic to animals if accumulated in large quantities in the rumen (LENG; PRESTON, 2010; BRUNING-FANN; KANEENE, 1993). Dietary NO_3^- levels, intake rates, ruminal reduction and rumen flow rates are all considered critical factors causing toxicity by NO_2^- (LEE; BEAUCHEMIN, 2014).

The accumulation of NO_2^- in the rumen can cause possible intoxication, as NO_3^- is quickly reduced to NO_2^- by the rumen microorganisms, but the rate of reduction of NO_2^- to ammonia is slower (IWAMOTO et al., 1999), which can result in the accumulation of this intermediate in the rumen, which will be absorbed into the bloodstream (BRUNING-FANN; KANEENE, 1993). Once in the blood, it will prevent cells from generating energy through the respiratory chain as it binds to hemoglobin, causing the oxidation of iron to form methemoglobin, which cannot transport oxygen to tissues (SANTOS, 2006). Borges (2018) evaluated calcium nitrate levels (0; 1.5; 3 and 4.5% on dry matter basis) in the diet of cannulated Nellore females and found no signs of intoxication. Similarly, Cassiano (2017) evaluated 0, 1, 2, and 3% levels of calcium nitrate (DM basis) in the diet of rumen-cannulated Nellore and Holsteins females, and clinical effects of nitrate intoxication were not found.

Troy et al. (2015) found a 22.6% reduction in CH_4 emissions with the inclusion of 21.5g of nitrate/kg of DM in the diet of crossbred steers (*Bos taurus*) fed roughage:concentrate (50:50). Using the same nitrate inclusion (21.5g of nitrate/kg DM) in a diet containing 550 forage (grass and whole crop barley silages): 450 concentrate for crossbred steers, Duthie et al. (2018) found a CH_4 reduction of 8% compared to the control treatment. Capelari, (2018) encountered a reduction of 8.6% in CH_4 production of crossbred Angus steers fed a mixed diet (50% high moisture corn; 30% silage; 15% of corn dry distiller's grains) and inclusion of 15g of nitrate /kg DM for 64 days. With the inclusion of 25 g of nitrate/kg of DM for crossbred steers fed a high forage diet, Alemu et al. (2019) achieved 17% CH_4 reduction. According to Lee and Beauchemin (2014), there are many studies with the addition of nitrates to the diet of ruminants, but few are with animals on pasture.

3.5. Beef Quality

Beef is considered a food of high nutritional quality, resulting from the continuous transformations that occur in the muscle after the animal is slaughtered (MONTE et al. 2012). Currently, consumers are increasingly interested in information about the product quality they will purchase, with concerns related to animal welfare and environmental impact of the livestock production system.

Tenderness, flavor, and juiciness are the main characteristics that define whether the consumer will consume the beef product. In addition to these characteristics, pH and water holding capacity are also evaluated when measuring the beef quality (LAWRIE, 2005). All these quality characteristics are affected by breed, age at slaughter, fed and the production system in which the animal is reared (SILVA SOBRINHO; SILVA, 2000). According to Zhang et al. (2010), gender may also affect beef quality since it influences muscle deposition and adipose tissue in the carcass. Generally, non-castrated males have a higher dressing percentage, as they produce more muscle tissue than fat, due to more efficient feed conversion when compared to females and castrated males (SEIDMAN et al., 1982; STEINHART, 1998). However, the beef of these animals tends to less tender with lower and fat deposition, impairing beef quality (MUELLER et al., 2019). In contrast, females and castrated males have better carcass quality due to higher intramuscular fat deposition (SEIDEMAN et al., 1982; DOS SANTOS et al., 2015).

As previously mentioned, most of the Brazilian herd is composed of *Bos indicus* cattle, and the beef quality of these animals has differences when compared to *Bos taurus* cattle, such as a lower rate of subcutaneous fat deposition and few or no deposition of intramuscular fat (CROUSE et al., 1989; PEREIRA et al., 2015; RODRIGUES et al., 2017). *Bos indicus* animals may have beef tenderness affected by the increased calpastatin activity, which reduces the rate of degradation of myofibrillar proteins during postmortem storage (WHIPPLE et al., 1990; KOOHMARAIE, 1992).

Another relevant factor is that most of the bovine protein produced in Brazil comes from animals kept in grazing systems. Beef from cattle raised on pasture has been valued by consumers, for presenting desirable nutritional characteristics such as higher levels of polyunsaturated fatty acids, a lower ratio of Omega 6: Omega 3 fatty acids, in addition to higher amounts of conjugated linoleic acid, when compared to that are produced in confinement (MEDEIROS, 2008).

To meet the consumers' demands, the search for improving beef quality in the market is increasingly encouraged. Brazil already has programs such as "O Pacto Sinal Verde para a Carne de Qualidade", "Programa de Novilho Precoce" and the "Carne Carbono Neutro" certification. These type of initiatives aims to recognize the producers that produce better quality beef products, consequently guiding towards more sustainable production systems in both environmental and economic point of view (EMBRAPA, 2023).

4. MATERIALS AND METHODS

4.1. Location and ethical issue

The experimental was conducted at College of Veterinary and Animal Science of the University of São Paulo (FMVZ/USP), in the Laboratory beef cattle, Pirassununga, São Paulo State, Brazil. The animals were handled and managed according by the Animals Use Ethic Committee (CEUA) of the College of Animal Science and Food Engineering – University of Sao Paulo (FZEA-USP), under the protocol number 3455101019.

4.2. Animals and experimental period

A total of the 48 Nellore heifers (24 animals per year), of approximately 348 kg (± 30 kg) in body weight (BW; at the beginning of the experiment) and 18-21 months old were used as experimental animals.

The experimental period lasted two years and, excluding post-slaughter data, all other variables were collected during four seasons the years (winter, spring, summer, and autumn). The first trial period started in June 2019 and ended in June 2020. The second trial period started in June 2020 and ended in June 2021. At the end of each period the animals were slaughtered with weights above 450 kg in BW.

4.3. Experimental design, pasture system and treatments

The experimental animals were randomly allotted to 8 modules, of which 4 modules were comprised of 6 paddocks with 0.3 ha each (rotational grazing paddocks) and other 4 modules (deferred grazing paddocks) with 1.8 ha each (Figure 1). Each treatment was allocated to an experimental unit in a randomized block design (blocks were formed as a function of terrain location). Treatments were consisted of two grazing systems and two nitrogen supplements, evaluated in all seasons of the year (2 × 2 × 4 factorial arrangement) in which: Factor 1) rotational stocking grazing system or deferred stocking grazing system; Factor 2) conventional supplementation using urea or alternative supplementation with ammonium nitrate; Factor 3) Four seasons of the year. Except for post-slaughter data, only factors 1 and 2 were considered, composing a 2 × 2 factorial arrangement.

The description of the treatments is described below:

1) Deferred stocking grazing system plus nutritional supplementation with urea (DG+U);
2) Rotational stocking grazing system plus nutritional supplementation whit urea (RG+U);
3) Deferred stocking grazing system plus nutritional supplementation with ammonium nitrate (DG+AN);
4) Rotational stocking grazing system plus nutritional supplementation with ammonium nitrate (RG+AN).

The experimental area has 14.4 ha divided into 8 experimental units and management corridors. The area was established in 1999 with *Brachiaria brizantha* cv. Marandu Syn. *Urochloa brizanta* cv. Marandu. Additionally, 6 ha (reserve pasture) were used to allocate extra animals, which were used during the seasons to adjust the stocking rate (Figure 1).

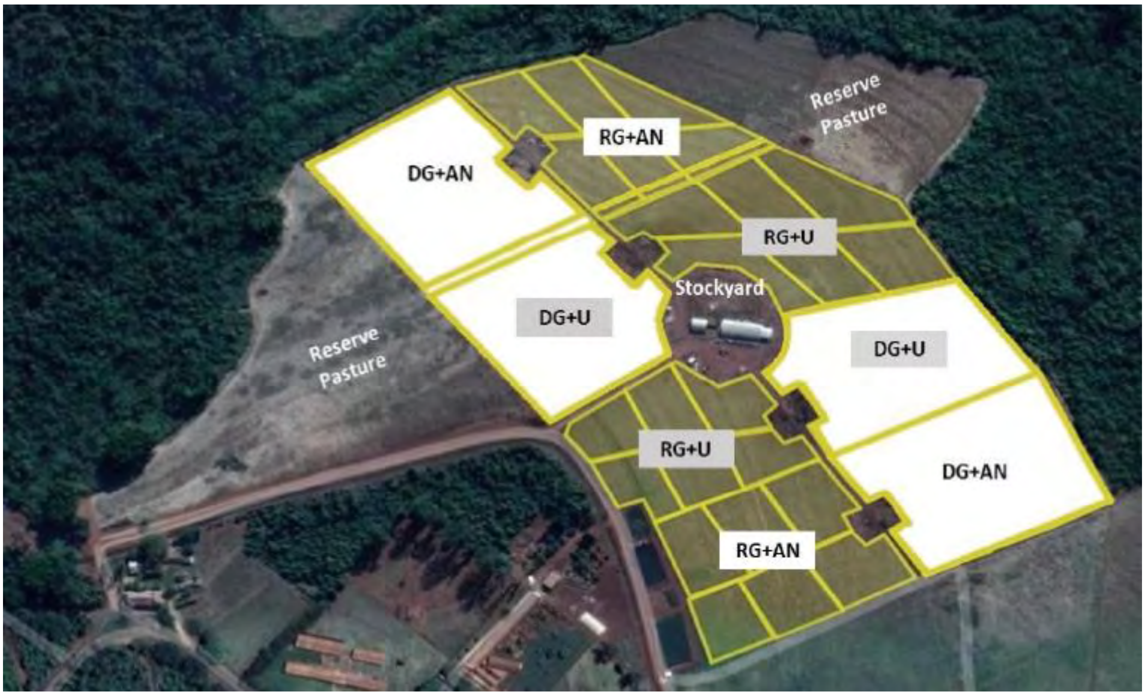
The experimental units received limestone and fertilizer recommendation for pastures based on soil analysis and calculated following Raij et al. (1997). The deferred stocking grazing systems (four experimental units) were deferred for 85 days at the end of the rainy season in the first year (March 23th, 2019) and were also deferred for 85 days at the end of the rainy season in the second year (March 26th, 2020).

After introducing the animals into the experimental units, the deferred stocking grazing systems were left to continuously grazing, while the other four experimental units were submitted to rotational stocking grazing, throughout the experimental period. The animals had free access to clean and fresh water in each experimental unit. In the rotational stocking grazing system, the dynamics at the paddocks was seven days of occupation and 35 days of resting per cycle.

Three heifers (testers) were used to evaluate performance in each experimental unit (six per treatment) and regulating animals were used to adjust the stocking rate using the put-and-take technique (MOTT; LUCAS, 1952).

Mineral supplements were formulated using Microsoft Excel and the composition of the ingredients was estimated according to the NRC (2016), for an expected consumption of 0.1% body weight per animal; however, the heifers had *ad libitum* access to the supplement. The composition of the supplements and forage is shown in Table 1 and Table 2.

Figure 1 - Aerial view of experimental area.



Laboratory of beef cattle. Source: personal archive.

Table 1 – Ingredient proportion and nutritional composition of the supplement provided during the adaptation period, rainy and dry seasons, using urea or nitrate as nitrogen source.

Ingredient		Adaptation		(Dry season)		Rainy (Season)	
		Urea	Nitrate	Urea	Nitrate	Urea	Nitrate
				(%)			
Ground corn		55	55	48	45	72	69
Urea		10	-	22	-	13	-
Salt		20	15	15	10	7	5
Mineral		15	15	15	15	8	8
Ammonium nitrate		-	15	-	30	-	18
			Nutritional composition				
CP	%	33.14	33.49	66.34	61.13	43.01	43.34
TDN	%	48.22	48.22	42.02	39.46	63.13	60.50
CF	%	1.27	1.27	1.10	1.04	1.66	1.59
EE	%	1.60	1.60	1.39	1.31	2.09	2.00
NDF	%	4.35	4.35	3.79	3.56	5.69	5.45
ADF	%	1.43	1.43	1.25	1.17	1.87	1.79
Ca	%	2.70	2.70	2.69	2.69	1.45	1.45
P	%	2.54	2.54	2.52	2.52	1.47	1.46
Na	%	7.81	5.86	5.86	3.91	2.74	1.96

Estimated Macro and micromineral composition for the urea and nitrate supplement adopted in adaptation period and dry season: 3.36 mg/kg of Selenium; 0.77 g/kg of magnesium; 3.29 g/kg of sulfur; 342.45 mg/kg of copper; 812.70 mg/kg of zinc; 291.00 mg/kg of molybdenum; 12.30 mg/kg of cobalt; 16.79 mg/kg of iodine; 402.90 mg/kg of Iron; 1.93 g/kg of potassium *adaptation period; 1.68 g/kg of potassium *dry season. **Estimated Macro and micromineral composition for the urea supplement adopted in rainy season:**1.79 mg/kg of Selenium; 1.01 g/kg of magnesium; 2.22 g/kg of sulfur; 182.64 mg/kg of copper; 433.44 mg/kg of zinc; 155.20 mg/kg of molybdenum; 6.56 mg/kg of cobalt; 8.96 mg/kg of iodine; 214.88 mg/kg of Iron; 2.52 g/kg of potassium. **Estimated Macro and micromineral composition for the ammonium nitrate supplement adopted in rainy season:** 1.79 mg/kg of Selenium; 0.97 g/kg of magnesium; 2.19 g/kg of sulfur; mg/kg of copper; 433.44 mg/kg of zinc; 155.20 mg/kg of molybdenum; 6.56 mg/kg of cobalt; 8.96 mg/kg of iodine; 214.88 mg/kg of Iron; 2.42 g/kg of potassium. *It was used Minerthal mineral.

Table 2 - Chemical composition of *Uruchloa brizantha* cv. Marandu during the two years of experimental period.

Fixed Effects^{1}			**Variables**^{2}									
Grazing	**N source**	**Season**	**CP (%)**	**NDF (%)**	**ADF (%)**	**LIG (%)**	**EE (%)**	**MM (%)**	**DIVMS (%)**	**NFC (%)**	**CE (%)**	**TDN (%)**
Deferred			10.97	65.45	33.06	2.51	3.32	10.10	76.05	10.50	18.16	68.11
Rotated			11.09	64.41	34.16	3.08	3.28	9.99	74.55	10.46	18.22	70.43
	Nitrate		10.98	65.01	33.33	2.70	3.33	10.21	75.90	10.50	18.15	69.82
	Urea		11.08	64.86	33.89	2.89	3.27	9.88	74.70	10.46	18.23	68.72
		Winter	9.54^{c}	66.56^{a}	36.39^{a}	3.99^{a}	3.43	9.83^{bc}	70.65^{c}	10.47^{b}	17.95^{b}	65.39^{b}
		Spring	10.69^{b}	66.81^{a}	34.21^{b}	3.54^{a}	3.32	9.60^{c}	66.59^{d}	9.43^{c}	18.17^{a}	67.55^{b}
		Summer	10.78^{b}	64.20^{b}	31.81^{c}	1.25^{c}	3.10	10.62^{a}	84.41^{a}	11.14^{a}	18.27^{a}	71.82^{a}
		Autumn	13.10^{a}	62.15^{c}	32.03^{c}	2.40^{b}	3.35	10.13^{b}	79.56^{b}	10.87^{a}	18.37^{a}	72.33^{a}
Average data												
Average			10.89	65.36	33.61	2.80	3.33	10.05	75.30	10.48	18.18	69.27
SEM			0.24	0.33	0.35	0.18	0.10	0.09	1.07	0.13	0.05	0.73
Statistic Probabilities												
Grazing			0.8588	0.1184	0.0615	0.0741	0.9197	0.4648	0.2439	0.8420	0.3997	**0.0097**
N source			0.8074	0.7430	0.3165	0.5252	0.8091	**0.0283**	0.3421	0.8371	0.2783	0.2025
Season			**<.0001**	**<.0001**	**0.0001**	**<.0001**	0.2538	**<.0001**	**<.0001**	**0.0003**	**0.0007**	**<.0001**
Grazing × N source			0.9183	0.9176	0.6942	0.6814	0.6853	0.3933	0.9976	0.2565	0.5330	0.6273
Grazing × Season			0.5581	0.1529	0.3072	0.2819	0.0447	0.7228	0.2141	0.2347	0.5492	0.5855
N source × Season			0.1689	0.2545	0.2015	0.7984	0.3626	0.2684	0.1146	0.5235	0.9761	0.4737
Grazing × N Source × Season			0.2961	0.7495	0.3313	0.8256	0.8779	0.9894	0.1673	0.2515	0.7565	0.0529

[1] N Source: nitrogen source. [2] CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; LIG: Lignin; EE: Ether Extract; MM: Mineral Matter; IVDMD: In Vitro Dry Matter Digestibility; NFC: Non-fiber carbohydrates; CE: Crude energy; TDN: Total digestive nutrients.

4.4. Animal performance

The individual performance was evaluated by the heifer’s average daily gain (ADG), obtained by dividing the body weight (BW) difference between two successive weighing and by the interval of days between measurements, according to the equation:

$$ADG = (BW_F - BW_I)/IW$$

Where: ADG = Average daily gain (kg); BW_F = Final BW, most current weight (kg); BW_I = initial BW, weight from previous weighing (kg); IW = Interval between weighing (days).

The animals were weighed at the beginning of the experiment and later at regular intervals of approximately 28 days, using a digital scale with 0.1 kg of precision, and in the first and last weighing of the experiment, the animals were weighed after 16 hours of fasting.

4.5. Dry matter intake

Indirect methods with makers were used for estimating dry matter intake. Titanium dioxide (TiO_2) was used as an external marker of fecal production, indigestible neutral detergent fiber (iNDF) as an internal marker to determine forage digestibility and to estimate supplement intake, the Cr_2O_3 was used as an external indicator of the fecal output.

Two heifers from each treatment were used to measure intake. For the supply of TiO_2, the heifers were taken to the corral, and the dosing method was manual, where 15 g of TiO_2 wrapped in paper was provided and deposited directly in the oral cavity of each heifer daily at 8:00 am. The sampling period was carried out in the middle of each year's season (totaling 8 samplings over 2 years).

The dosing period was 10 days (five days for adaptation and the last five days for stool collection). Feces were collected directly from the animal's rectum while TiO_2 was given, except on the last day, when only feces were collected. The collected feces were stored in a freezer (at -20ºC) until further analysis according to the methodology described by Myers et al. (2004). The samples were thawed, dried in a forced air circulation oven at 65°C for 96 hours and ground into Willey type mill using 2 mm mesh screen, to determine the concentration of

TiO_2 and chromium oxide in dry feces, through atomic absorption spectrophotometry technique described by Myers et al. (2004). That sample was also used for iNDF analysis.

To determine dry matter forage intake, first we determined the fecal excretion by means of a known amount of external marker administered (kg/day) and that recovered in feces as follows:

Fecal excretion = TiO_2 diet (kg/day) / TiO_2 feces (kg)

In which: TiO_2 diet: Titanium oxide administered; TiO_2 feces: Titanium oxide recoverd in feces (kg)

Subsequently, the forage dry matter intake (DMI) was calculated by means of the iNDF as internal marker concentration (%) from pastures and feces using the following equation:

$$\text{Forage DMI (kg/day)} = [(\text{Fecal excretion}) \times (\%\ \text{iNDF on feces})] / (\%\ \text{iNDF on forage})$$

To estimate supplement intake, Cr_2O_3 was mixed with the supplement, in the proportion of 10 and 7.5%, in the dry and rainy seasons, respectively, and supplied in the same period of administration of TiO_2.

To determine supplement DMI, we used the following equation:

$$\text{Supplement DMI (kg/day)} = (\text{Fecal excretion} \times Cr_2O_3\ \text{on feces}) / Cr_2O_3\ \text{on supplement}$$

4.5.1. Grazing simulation (hand-plucking technique)

The hand-plucking technique (SOLLENBERGER & CHERNEY, 1995) seeks to simulate what the animal’s graze to get an accurate estimate of diet nutritive value. The pasture was sampled manually, simulating the forage consumed by the animals. For the sampling to be like the forage consumed by the animals, heifers were observed for a few minutes and followed during grazing. It is taken approximately 10 meters’ distance from where animal’s grazing takes place, and then, by clipping a hand full of forage at the locations where animals were grazing the samples are done up to attain approximately 500 g of material.

This procedure was also adopted in the continuously stocked pasture, and, to better represent the quality of the forage that animals were grazing at the week, in which the other parameters were also being taken, hand plucking method was performed on day 1, 4 and 7 of the rotational periods. Each experimental unit had a sample composed of 3 days of sampling and the grazing simulation was carried out once each year season, totaling 4 samples per year.

The samples were dried in a forced air circulation oven at 65°C for 72 hours and ground into Willey type mill using 1- and 2-mm mesh screens to determine the chemical composition and for iNDF analysis, respectively.

4.5.2. Indigestible neutral detergent fiber (iFDN)

The internal marker iNDF was used to determine the digestibility of feeds. The samples of forage (from hand-plucking), feces, and supplements were placed in 100 g / m^2 TNT filter bags and incubated for 288 hours in the rumen of cannulated animals fed pasture. After removing the TNT bags from the rumen, it was washed in a stream until completely cleared and dried in a forced air circulation oven at 65°C for 72 hours to determine neutral detergent fiber (NDF) content, according to the method described by Van Soest et al. (1991). The remaining residue was considered as iNDF content. The final indigestibility of the feed was determined by the iNDF of the feed divided by the iNDF of the animal's feces.

4.6. Ruminal methane measurements

The method used to measure the CH_4 eructed by the animals was the sulfur hexafluoride (SF_6) tracer gas, in accordance with the recommendations proposed by Primavesi et al. (2004). Small brass capsules (permeation tube), with a known SF_6 permeation rate, were deposited in the animals' reticulum at the beginning of the experiment to allow the tracer gas to equilibrate in the rumen. The sampling apparatus, called canister (storage-collector), was composed of a PVC tube, closed, and molded to fit the neck of the animals. Connected to the canister was a halter that had a silicone tube attached to capture the CH_4 expelled through the animal's nostrils and mouth. The gas captured by the silicone tube was transported by a capillary tube and deposited in the storage-collector. Before each sampling, the canisters were subjected to vacuum and had their pressures (initial and final) recorded. Two heifers from each experimental unit were used, which went through a five days adaptation before the sampling period. The animals were taken to the stockyard daily, for five consecutive days and the sampling was carried out once per season (winter-July; spring-October; summer-January; autumn-April). Collections were made between 7:30 and 8:00 am and the same order of animals was respected to avoid large differences in collection times from one day to the next, ensuring 24 hours of gas collection. To complete five recommended collections, animals that had a broken canister, or if there was a problem with the capillary gas collector, had their canister replaced and the collection was extended for one more day. Throughout the collection period, two systems of capillary tubes coupled to a canister were prepared, placed on the fences, to collect the gas present in the environment in which the animals were submitted. These canisters were called "blank" and contained the basal CH_4 concentration of the environment.

After each sampling period (season of the year), the samples were sent for analysis using gas chromatographs (Agilent HP-6890, Delaware, USA; and Shimadzu GC-2014, Columbia, MD, USA) at Embrapa Meio Ambiente, in Jaguariúna, SP, Brazil. The CH_4 flux was calculated according to Westberg et al. (1998), using the following equation:

$$QCH_4 = QSF_6[(CH_4)y-(CH_4)b]/[(SF_6)y-(SF_6)b]$$

Where: QCH_4 = CH_4 emission rate per animal; QSF_6 = known rate of SF_6 emission from the capsule in the rumen; $(CH_4)y$ = CH_4 concentrations in the collecting apparatus; $(CH_4)b$ = baseline CH_4 concentration; $(SF_6)y$ = SF_6 concentration in the collection device and $(SF_6)b$ = baseline SF_6 concentration in room air.

The percentage of gross energy intake converted in CH_4 (Y_m%) was calculated by dividing the daily methane output of each animal by gross energy daily intake during the methane sampling.

4.7. Carcass and non-carcass traits

At the end of each year the animals were slaughtered in the teaching slaughterhouse of the University of São Paulo in Pirassununga-SP, Brazil, supervised by the State Inspection Service. Before slaughter, the animals were fasted for solids for 18 hours, receiving only water *ad libitum* and then were transported according to the blocks, with no batch mixing in the transport or in the slaughterhouse pens. The animals were stunned by brain concussion and exsanguinated through the jugular vein. Carcasses were hung by the Achilles tendon. Heads, feet, hides, and visceral organs were removed.

At the end of the slaughter line, the carcasses were weighed to obtain the hot carcass weight (HCW) to calculate each animal's carcass yield (%). The dressing percentage (or hot carcass yield, CY) was calculated as the ratio between the hot carcass weight and the live weight of the animals according to the equation:

$$CY\ (\%) = \left(\frac{Hot\ carcass\ weight\ (kg)}{Live\ carcass\ weight\ (kg)}\right) \text{ x } 100$$

Subsequently, the carcasses were carried to a cold room at 0 to 2 °C for 24 hours. Half-carcasses were divided into forequarters (with five ribs), hindquarters and spare ribs (BARROS; VIANNI, 1979). After chilling, the carcass halves were weighed to obtain the cold carcass weight (CCW).

On the deboning, the left halves of the carcasses were cut between the 12th and 13th ribs to measure pH with a digital pH meter (Hanna Instruments Inc®, Model HI 99163, Woonsocket, RI, EUA). The rib eye area (cm^2) was measured using specific squared ruler, with scale in cm^2 by the point quadrant method, fat thickness (mm) was determined using a digital caliper (Amatools®, Model ZAAS Precision), and the marbling score (scale of slight; small; modest; moderate; slight abundant; moderately abundant) followed the methodology describe by AMSA (2001). After that, the carcasses were cooled between 0 and 2ºC for 24 h and the *Longissimus thoracis* muscle (LT) was removed. Then, 2.5 cm thick steaks were taken between the 12th and 13th ribs and vacuum-packed individually. All vacuum-packed samples were identified and samples from Time 0 were frozen at -18ºC, while samples from Time 14 were taken to the aging chamber at 2ºC. After the maturation period, samples Time 14 were frozen at -18°C, along with Time 0 samples, further analyses.

Cold carcass cuts correspond to the edible carcass portion (CEP) and were expressed as kilograms and as a percentage of CCW (CEP%). The CEP was calculated as the sum of edible portions of the Brazilian primal cuts (YOKOO et al., 2003): hindquarter, forequarter, and spare ribs. The hindquarter and forequarter edible portions (HEP, FEP) and the spare ribs were also expressed in kilograms and as a percentage of CCW. The HEP and FEP were calculated as the sum of the edible portions of retail cuts: HEP - sirloin, tenderloin, rump, knuckle, topside, flat, eye of round, cap and tail, and shank; FEP - shoulder clod, hump, chuck, and brisket. Hindquarter fat trimmings (HFT) and forequarter fat trimmings (HFT), with the standardization of about 3 mm of fat on the retail beefs, were expressed in kilograms and as a percentage of CCW - HFT% and HFT%, respectively. These traits were be considered representative of fat carcass content. Bones are non-edible components were also be expressed in kilograms and as a percentage of CCW.

4.8. Beef quality

For qualitative analyses, four LT muscle steaks (2.54 cm thick) were taken between the 12th and 13th ribs for the analyses of cooking loss, shear force and sensory traits (after 14 days of aging at 2ºC. Another two steaks were obtained to determine beef color and fatty acid profile. The determination of the color of the beef was carried out 30 minutes after the cut of the steak in the deboning of the slaughterhouse. The analysis was performed as described by Houben et al. (2000), in three locations of each steak (LT muscle) sample after a 30 min bloom time at 4ºC, for oxygenation of myoglobin to occur. For this a portable colorimeter (Model MiniScan EZ, Hunter Lab®, Reston, Virginia, EUA) was used measuring lightness (L*), redness (a*), and yellowness (b*). The color aspects were assessed by the CIE L*a*b color system using 0º/45º and the unit was calibrated using a black and white standard plate.

For the analyses for cooking loss and shear force, the beef samples were cooked in a gas oven at 175ºC until they reach 72ºC at their geometric centers. The weights of the steaks before and after cooking were measured to calculate the cooking losses (CL), according to Honikel (1998). After 24 h cooling, six cores were removed from the steaks using a 2.5 cm diameter drawn punch. A Brookfield® CT-3 Texture Analyser (Brookfield, USA) measured the force necessary to transversally cut each core. The average cutting force was calculated, representing the shear force of each sample as described by Wheeler et al. (2001).

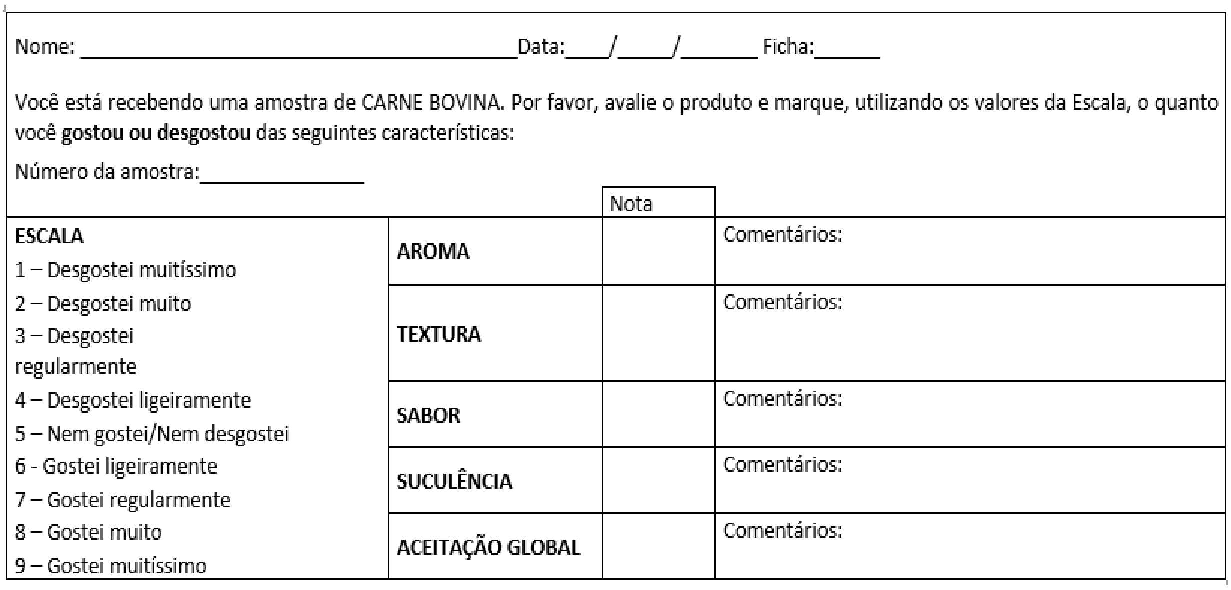
4.8.1. Sensory Analysis – Consumer’s acceptance test

One steak of the LT muscle (13th rib, 2.54 cm thick) was taken for the sensory analysis. Four samples of LT muscle were offered to each consumer, referring to the four treatments of the experiment (DG+U; RG+U; DG+AN; RG+AN), all aged for 14 days (2ºC). The samples were coded with a three-digit number and give one at a time to the consumers (FERREIRA et al., 2000). To minimize the effect of presentation in the judgments, the order of presentation of the samples was balanced among the consumers (AMSA, 2016).

For sensory evaluation of fresh beef, the samples were kept in a domestic refrigerator (7°C) for 24 hours for defrosting and cut to a standard size. The steaks were roasted in an oven at 175°C until reaching a temperature of 75°C in the geometric center, which was monitored by individual thermocouples. After this procedure, the beef was cut into cubes (2cm × 2cm), packed in foil paper, kept in a water bath to maintain the temperature, and served to a panel of untrained consumers in individual cabins, using a consumer's acceptance test (*n* = 127 consumers) (MEILGAARD et al., 1999).

The samples were offered sequentially to each consumer in coded plastic coffee cups, accompanied by a salt and water biscuit for residual taste removal and a cup of water to wash the palate. The attributes of aroma, tenderness, juiciness, flavor, and overall acceptability were evaluated according to the methodology described by AMSA (1995). The samples were evaluated by hedonic scale scores ranging from 1 to 9, with 1 being the minimum score and 9 being the maximum score (MEILGAARD et al., 1999).

Figure 2 - Form used by consumers to assign scores in the sensory analysis.



Nome: ____________________________________Data:____/____/______ Ficha:______

Você está recebendo uma amostra de CARNE BOVINA. Por favor, avalie o produto e marque, utilizando os valores da Escala, o quanto você **gostou ou desgostou** das seguintes características:

Número da amostra:______________

		Nota	
ESCALA 1 – Desgostei muitíssimo 2 – Desgostei muito 3 – Desgostei regularmente 4 – Desgostei ligeiramente 5 – Nem gostei/Nem desgostei 6 - Gostei ligeiramente 7 – Gostei regularmente 8 – Gostei muito 9 – Gostei muitíssimo	**AROMA**		Comentários:
	TEXTURA		Comentários:
	SABOR		Comentários:
	SUCULÊNCIA		Comentários:
	ACEITAÇÃO GLOBAL		Comentários:

5. STATISTICAL ANALYSIS

Heifers were considered the experimental units for data obtained per animal, while the consumers were considered the experimental units for the sensory panel. Data were statistically analyzed using the SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2013). Data were analyzed for the presence of disparate information ("outliers") and residuals' normality (Shapiro-Wilk). When the normality assumption was not accepted, the logarithmic or the square root transformation were tested. The mixed procedure (PROC MIXED) was used, and seasons of the year considered as repeated variable (split-plot in time), except for data post-slaughter. Among the 15 different covariance structures tested, the chosen one was based on the lowest value of Corrected Akaike Information Criterion (AICC) (Wang & Goonewardene, 2004).

The model included the effects grazing systems, nitrogen source, and seasons of the year (winter, spring, summer, and autumn) as fixed factors and the interaction between the fixed effects, as seen in the following statistical model. The block effect (replicate area) and year were considered random factors.

The following model was used for animal performance, dry matter intake and enteric CH_4 production data:

$$Y_{ijkl} = \mu + b_i + a_i + g_j + n_k + (gn)_{jk}\ e_{(A)ijk} + s_l + (sg)_{lj} +(sn)_{lk} + (sgn)_{ljk}\ e_{(B)ljk}$$

Where:

μ: overall average;
b_i: random block effect;
a_i: year random effect;
g_j: fixed effect of grazing system;
n_k: fixed effect of nitrogen source;
$(gn)_{jk}$: interaction effect of grazing system and nitrogen source;
$e_{(A)ijk}$: random residual error A;
s_l: fixed effect of season of the year;
$(sg)_{lj}$: interaction effect of season of the year and grazing system;
$(sn)_{lk}$: interaction effect of season of the year and nitrogen source;
$(sgn)_{ljk}$: interaction effect of season of the year, grazing system and nitrogen source; e(B)ljk: random residual error B.

To statistically analyze the data from post-slaughter, we used the following model:

$$Y_{ijkl} = \mu + b_i + a_i + g_j + n_k + (gn)_{jk}\ e_{ijk}$$

Where:

μ: overall average;
b_i: random block effect;
a_i: year random effect;

g_j: fixed effect of grazing system;
n_k: fixed effect of nitrogen source;
$(gn)_{jk}$: interaction effect of grazing system and nitrogen source;
e_{ijk}: random residual error.

In the presence of interaction between fixed effects, the effects of one factor within the other were evaluated using the SLICE command of PROC MIXED. All means were presented as least squares means, and the PDIFF option of SAS separated the treatment effects. Effects were considered significant at $P \leq 0.05$.

6. RESULTS

6.1. Animal Performance and Dry Matter Intake

As reported in Table 3, an interaction effect between grazing system and season of the year was found for ADG (kg/d) and total DMI expressed in percentage of live body weight (DMI_{TLW}, %), which are decomposed and shown on the Figures 3 and 4.

Table 3 - Average daily gain and dry matter intake of Nellore heifers submitted to grazing systems and nitrogen sources during different seasons of the experimental period.

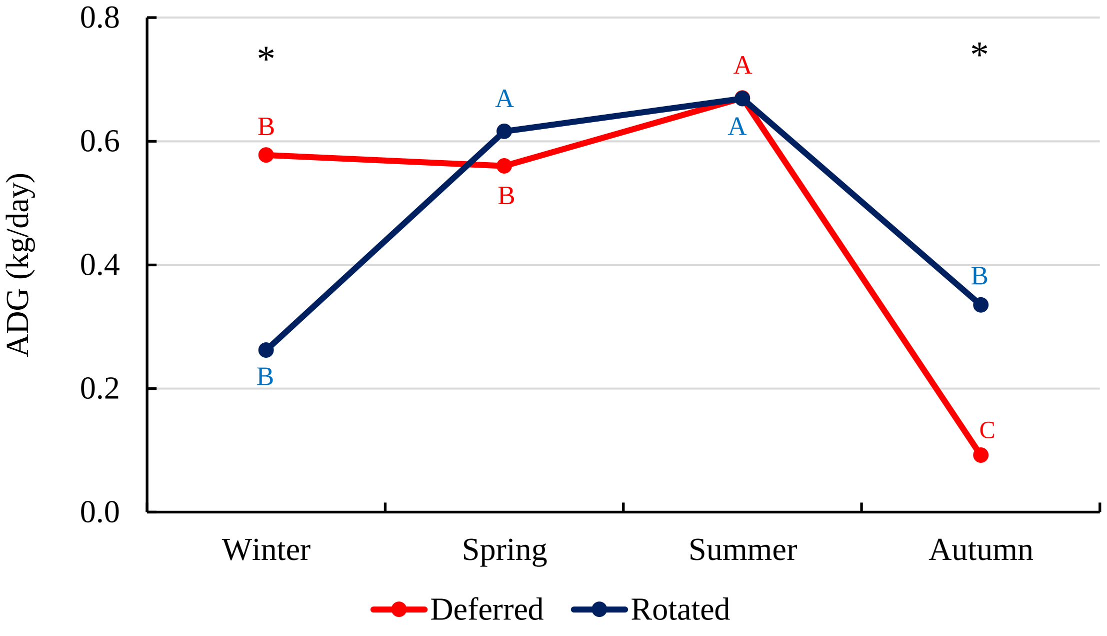
Fixed effects[1]			Variables[2]				
Grazing	**N Source**	**Season**	**ADG (kg/d)**	**DMI_F (kg/d)**	**DMI_S (kg/d)**	**DMI_T (kg/d)**	**DMI_{TLW} (%)**
Deferred			0.47	7.07	0.41	7.46	1.73
Rotated			0.47	6.91	0.41	7.30	1.69
	Nitrate		0.45	7.09	0.32	7.39	1.72
	Urea		0.50	6.90	0.50	7.37	1.70
		Winter	0.42	5.35^B	0.37	5.68^B	1.57
		Spring	0.59	5.21^B	0.32	5.53^B	1.42
		Summer	0.67	8.96^A	0.43	9.37^A	2.05
		Autumn	0.21	8.46^A	0.51	8.95^A	1.80
Average Data							
Average			0.47	7.00	0.41	7.40	1.71
SEM[3]			0.02	0.30	0.04	0.31	0.05
Statistics Probabilities							
Grazing			0.8471	0.6648	0.9724	0.7435	0.7968
N Source			**0.0246**	0.6087	0.0684	0.9752	0.7601
Season			**<.0001**	**<.0001**	0.3253	**<.0001**	**<.0001**
Grazing × N Source			0.0797	0.2923	0.5322	0.4001	0.1413
Grazing × Season			**<.0001**	0.0661	0.2381	0.0945	**0.0268**
N Source × Season			0.1676	0.4622	0.6321	0.4087	0.4202
Grazing × N Source × Season			0.8426	0.3193	0.6333	0.1616	0.2372

[1] N Source: Nitrogen source. [2]ADG: Average daily gain; DMI_F: Forage dry matter intake; DMI_S: Supplement dry matter intake; DMI_T: Total dry matter intake; DMI_{TLW}: Total dry matter intake in relation to live body weight. [3]SEM: Standard Error of Mean.

Analyzing the decomposition of the interaction between grazing system and season for ADG, it was possible to detect that there is difference when contrasting grazing systems within seasons. Heifers under deferred stocking grazing system had higher ADG in winter (0.578 kg) compared to those in the rotational stocking grazing system (0.261 kg), showing that animals under deferred stocking grazing system had ADG of 0.317 kg/d higher than that of rotational stocking systems. On the other hand, the opposite was found within autumn when heifers submitted to rotational stocking system (0.332 kg) displayed higher ADG when compared to deferred stocking system (0.092 kg; Figure 3).

It can also be observed that within grazing systems, animals in deferred stocking grazing had the highest ADG in summer (0.663 kg) as expected, while in winter and spring, they obtained intermediate ADG (0.577 kg and 0.542 kg, respectively), and the lowest ADG occurred in autumn (0.074 kg) (Figure 3). In the rotational stocking system, the lowest ADG values were found in winter and autumn (0.252 kg and 0.306 kg, respectively), while spring and summer showed higher gains (0.616 kg and 0.669 kg, respectively) as seen in Figure 3.

Figure 3 - Interaction between grazing systems and seasons for average daily gain of Nellore heifers in different grazing systems and seasons.



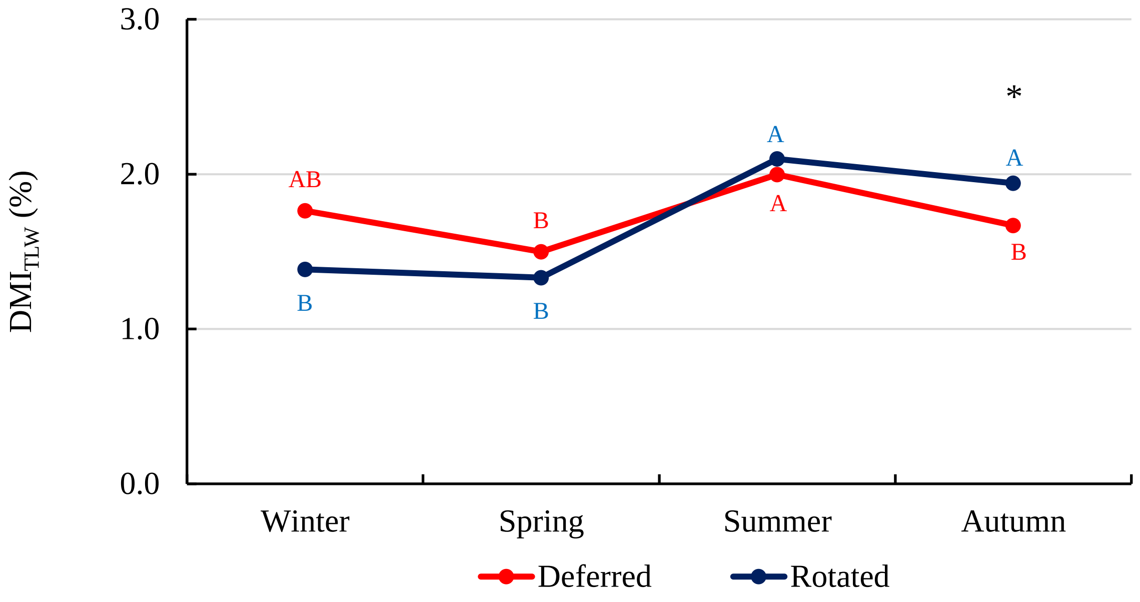
Capital letters within the same grazing differ for the season.
Asterisk (*) over the season indicates difference for grazing.

Besides the interactions found for the mentioned variables, season effects were found for forage and total DMI when expressed as kg per day ($P<0.05$) (Table 3).

It is possible to notice that heifers had higher intake of DMI_F in summer and autumn, while lower intake was found during the winter and spring. This probably influenced the DMI_T, which also showed higher values in summer and autumn. Regarding the supplement dry matter intake (DMI_S, kg/d), no treatment effect was found (Table 3).

In the interaction between grazing system and season of the year for the variable DMI_{TLW} (Figure 4), we found effect of grazing systems treatment within autumn. Rotational stocking grazing system had 13.91% higher DMI_{TLW} when compared to the deferred stocking grazing system. Within the grazing systems, the percentage of DMI_{TLW} was higher in summer and autumn (2.10% and 1.94%, respectively) and lower in winter and spring (1.38% and 1.33%, respectively) for the rotational stocking grazing system. The deferred stocking grazing system showed the highest percentage of DMI_{TLW} in the summer (2.0%) intermediate in the winter (1.76%) and the lowest percentages occurred in spring and autumn (1.50% and 1.67%) (Figure 4).

Figure 4 - Interaction between grazing systems and seasons for total dry matter intake in relation to live body weight of Nellore heifers in different grazing systems and seasons.



Capital letters within the same grazing differ for the season.

Asterisk (*) over the season indicates difference for grazing.

6.2. Enteric Methane Production

Significant season effect was found for daily CH_4 emission (kg/day) (P<.0001) and CH_4 per DMI_T (P=0.0018; Table 4). When expressed per ADG (kg/kg) interaction effect between grazing systems and seasons of the year was found (P=0.0068). In the same way, CH_4 emission per live body weight (LBW, g/kg) showed a significant interaction between grazing systems and season of the year (P=0.0410), which are shown in Figures 5 and 6, respectively. For gross energy spent in methane emission (Y_m, %), there was interaction effect between grazing system, nitrogen source and season of the year (P<0.05), as seen in Figure 7.

Effect of season of the year was found for CH_4 emission per DMI_T (g/kg) (P=0.0018). No significant effect was found for any of the variables of CH_4 emission by carcass traits, hot carcass weight (HCW, kg/kg) nor by carcass edible portion (CEP, kg/kg) (P>0.05).

Table 4 - Enteric methane production by average daily gain, dry matter intake and hot carcass weight and percentage of gross energy intake converted in CH_4 (Y_m) of Nellore heifers submitted to grazing systems and nitrogen sources during different seasons during two years.

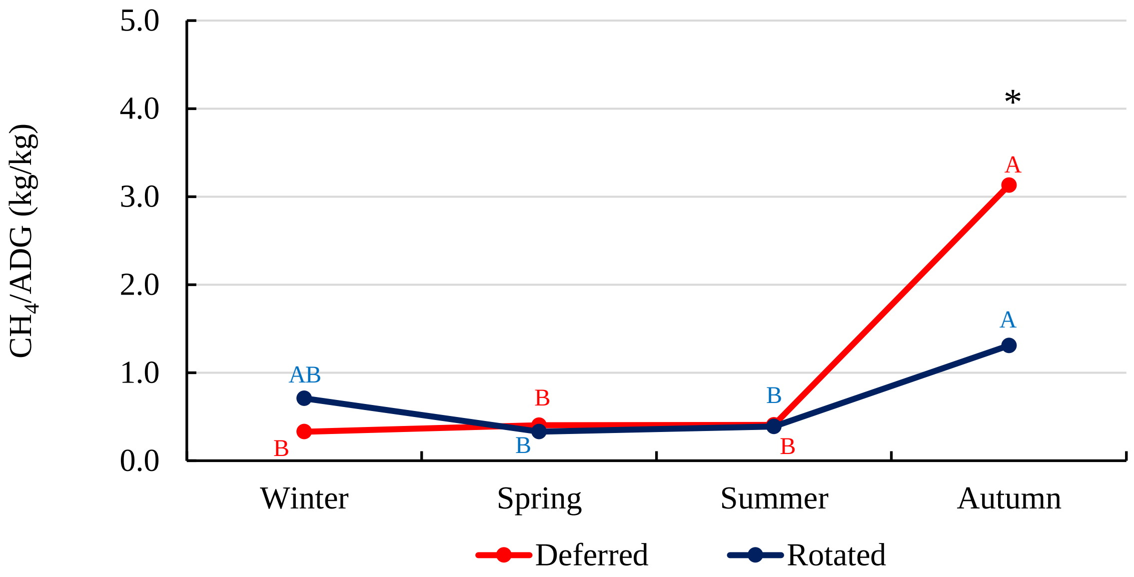
Fixed effects			**Variables[1]**						
Grazing	**N Source**	**Season**	**CH_4 (kg/day)**	**CH_4/ADG (kg/kg)**	**CH_4/LBW (g/kg)**	**CH_4/DMI_T (g/kg)**	**CH_4/HCW (kg/kg)**	**CH_4/CEP (kg/kg)**	**Y_m (%)**
Deferred			0.22	1.07	0.51	29.9	0.85	1.35	9.40
Rotated			0.22	0.68	0.51	32.4	0.89	1.41	9.93
	Nitrate		0.22	0.83	0.51	30.3	0.89	1.38	9.30
	Urea		0.22	0.92	0.51	32.0	0.85	1.39	10.03
		Winter	0.17^{C}	0.52	0.48	30.5^{B}	-	-	9.84
		Spring	0.21^{B}	0.37	0.53	36.8^{A}	-	-	11.03
		Summer	0.25^{A}	0.40	0.54	27.6^{B}	-	-	8.15
		Autumn	0.25^{A}	2.22	0.49	29.6^{B}	-	-	8.64
				Average Data					
Average			0.22	0.88	0.51	31.1	0.87	1.38	9.67
SEM[3]			0.004	0.13	0.01	0.82	0.01	0.02	0.15
				Statistics Probabilities					
Grazing			0.9317	0.1244	0.7540	0.4280	0.1831	0.5591	0.2008
N Source			0.7613	0.7227	0.9939	0.3147	0.1831	0.8520	**0.0077**
Season			**<.0001**	**0.0019**	**0.0003**	**0.0018**	**-**	-	**<.0001**
Grazing × N Source			0.3133	0.7508	0.5384	0.5159	0.0754	0.1153	**0.0357**
Grazing × Season			0.1018	**0.0068**	**0.0410**	0.8245	**-**	-	**0.0121**
N Source × Season			0.6860	0.6822	0.7137	0.2414	**-**	-	**0.0020**
Grazing × N Source × Season			0.6669	0.9589	0.7228	0.1499	**-**	-	**0.0014**

[1] N Source: Nitrogen source. [2]CH_4: methane emission per animal; ADG: Average Daily Gain; LBW: Live Body Weight; DMI_T: Dry matter intake total; HCW: Hot carcass weight; CEP: Carcass Edible Portion; Y_m: percentage of gross energy intake converted to CH_4. [3]SEM: Standard Error of Mean. (Own authorship).

As seen in Table 4, higher CH_4 emissions per animal (kg/d) were found in summer and autumn (0.25 kg/d), while moderate emission was found during the spring (0.21 kg/d) and lower in winter (0.17 kg/d). Therefore, emissions in winter were 32% lower when compared to the summer and autumn seasons.

A significant interaction between grazing system and season of the year were found for CH_4/ADG (kg/kg) and CH_4/LBW (g/kg). As seen in the decomposition unfolded in Figure 5, animals in the rotational stocking grazing system had 58.1% lower emissions when compared to those kept in the deferred stocking grazing system within the autumn season. It was found a constant CH_4 emission within deferred stocking grazing during winter (0.33 kg/kg), spring (0.40 kg/kg), and summer (0.41 kg/kg) seasons ($P>0.05$). For the rotational stocking grazing, CH_4 emission per ADG was higher in autumn (1.31 kg/kg), presented an intermediate value in winter (0.71 kg/kg), and was constant in spring and summer (0.33 kg/kg and 0.39 kg/kg, respectively) (Figure 5).

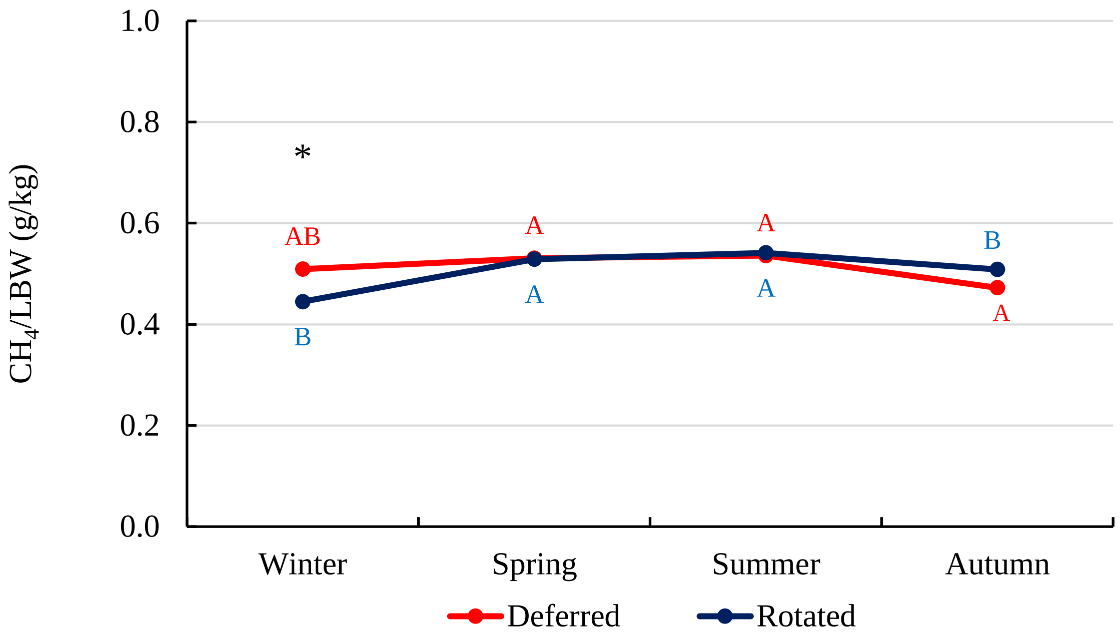
Figure 5 - Interaction between grazing and season for enteric CH_4 emission per ADG of Nellore heifers in different grazing systems and seasons.



Capital letters within the same grazing differ for the season.
Asterisk (*) over the season indicates difference for grazing.

When expressed per LBW (g/kg) during the winter, 11.76% higher CH4 emission was found for the heifers kept under deferred stocking grazing systems ($P<0.05$; Figure 6). For the rotational stocking grazing system, lower emissions were found during winter and autumn when compared to spring and summer seasons (P<0.05; Figure 6).

Figure 6 - Interaction between grazing and season for enteric CH_4 emission per LBW of Nellore heifers in different grazing systems and seasons.

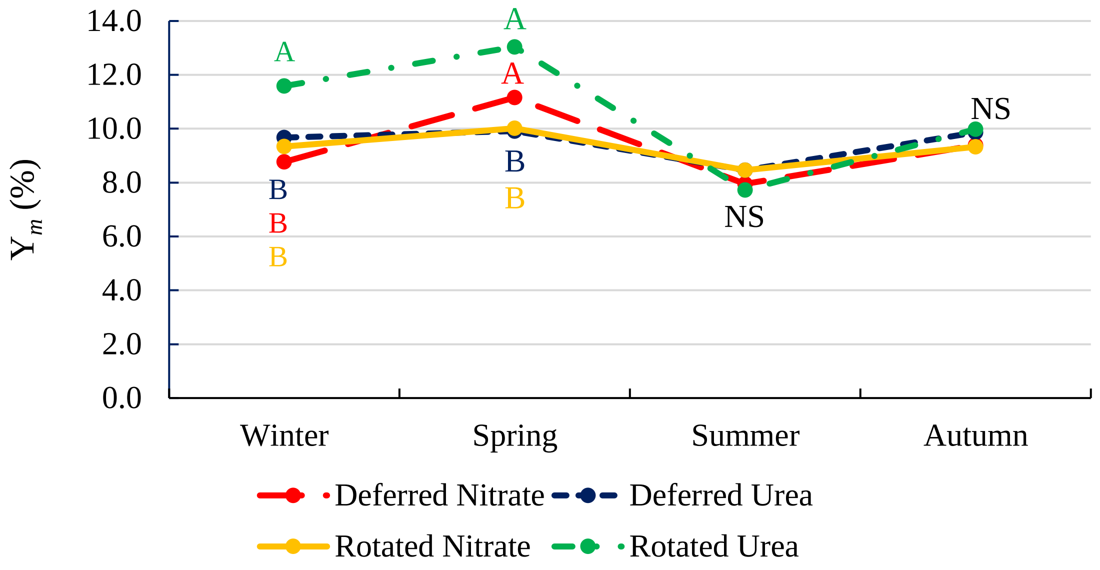


Capital letters within the same grazing differ for the season.

Asterisk (*) over the season indicates difference for grazing.

Considering the CH_4 conversion rate (Y_m), a significant interaction between grazing system × nitrogen source × season of the year was found (P=0.0014; Figure 7). During the winter season, heifers from the rotational stocking grazing system receiving urea presented higher values of Y_m (11,59%), while in the spring higher values of Y_m were found for animals in the deferred stocking grazing system supplemented with ammonium nitrate (11,16%) and those from the rotational stocking grazing system receiving urea (13,04%). In the other seasons, summer and autumn, similar values of Y_m were found among the treatments, whether heifers were under deferred or rotational grazing systems, receiving urea or ammonium nitrate (Figure 7).

Figure 7 - Interaction between grazing system, nitrogen source and season for the percentage of gross energy intake converted to enteric CH_4 emission of Nellore heifers in different grazing systems and seasons.



Capital letters within the same season differ for the treatments.

6.3. Carcass and Non-carcass Traits

Significant effect of the grazing system ($P<0.05$) for the variable HCW and dressing percentage (DP) showed that animals kept in a deferred stocking grazing system had higher carcass weight (279.2 kg) and yield (54.47%) than animals that were in the rotational stocking grazing system (268.5 kg and 53.68%, respectively) (Table 5).

The dressing percentage was also influenced by the source of nitrogen present in the supplement ($P<0.05$), indicating that heifers fed ammonium nitrate, as the main source of non-protein nitrogen, had higher dressing percentage than heifers that were supplemented with urea. No statistical effect was found for the variables initial live body weight (ILBW, kg), final live body weight (FLBW, kg), rib eye area (RYA, cm^2), fat thickness (FT, mm) and marbling score (MS) as shown on Table 5 ($P>0.05$).

Table 5 - Live body weight and carcass traits of Nellore heifers in different pasture systems receiving two sources of nitrogen during different seasons by two years.

Fixed effects		**Variables^1**						
Grazing	**N Source**	**ILBW (kg)**	**FLBW (kg)**	**HCW (kg)**	**DP (%)**	**RYA (cm^2)**	**FT (mm)**	**MS**
Deferred		349.20	526.01	279.21	54.47	68.15	8.66	4.96
Rotated		348.95	514.83	268.54	53.68	65.19	8.91	4.94
	Nitrate	350.31	513.55	271.96	54.46	67.01	8.66	4.99
	Urea	347.83	527.29	275.79	53.70	66.33	8.91	4.91
Average Data								
Average		349.29	521.92	274.34	54.00	66.69	8.80	4.93
SEM^2		3.08	5.15	2.67	0.23	0.92	0.40	0.08
Statistics Probabilities								
Grazing		0.9705	0.1926	**0.0323**	**0.0215**	0.1323	0.7438	0.9537
N Source		0.7060	0.1111	0.4307	**0.0259**	0.7263	0.7480	0.6780
Grazing × N Sourc		0.6751	0.5492	0.3222	0.0751	0.5615	0.7720	0.8197

[1] ILBW: Initial live body weight; FLBW: Final live body weight; HCW: Hot carcass weight; DP: dressing percentage; RYA: Rib eye area; FT: Fat thickness; MS: Marbling Score (Slight: 4.0 - 4.9; small: 5.0 - 5.9; modest: 6.0 - 6.9; moderate: 7.0 - 7.9; slight abundant: 8.0 - 8.9; moderately abundant: 9.0 - 9.9). [2] SEM: Standard error mean.

Left half-carcass and non-carcass components of Nellore heifers in different pasture systems, receiving two sources of nitrogen during different seasons for two years, expressed in kilograms, are presented in Table 6.

It was noticed a significant effect (P<0.05) of grazing systems for the variables weight of the left cold carcass weight (LCCW), carcass edible portion (CEP), spareribs (SR), and striploin. Heifers in the deferred stocking grazing system showed higher values for these variables than those kept in the rotational stocking grazing system. Other variables did not show significant differences (P>0.05; Table 6).

Table 6 - Left half carcass and non-carcass components of Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons for two years expressed as kilograms.

Fixed effects[1]		**Variables[2]**									
Grazing	**N Source**	**LCCW (kg)**	**CEP (kg)**	**HEP (kg)**	**FEP (kg)**	**SR (kg)**	**HFT (kg)**	**FFT (kg)**	**Bones (kg)**	**Tenderloin (kg)**	**Striploin (kg)**
Deferred		139.5	108.5	50.01	38.33	22.25	5.45	4.10	21.50	1.88	8.04
Rotated		134.4	103.9	48.26	37.54	20.84	5.08	4.12	21.82	1.82	7.51
	Nitrate	135.7	105.2	48.95	37.67	21.27	5.00	4.12	21.37	1.87	7.73
	Urea	138.2	107.2	49.32	38.20	21.82	5.53	4.09	21.94	1.83	7.82
Average Data											
Average		137.0	126.2	49.14	37.94	21.54	5.25	4.06	21.59	1.85	7.78
SEM[3]		1.80	1.40	0.50	0.44	0.51	0.74	0.15	0.29	0.03	0.12
Statistics Probabilities											
Grazing		**0.0392**	**0.0149**	0.0706	0.3265	**0.0426**	0.2440	0.9713	0.8600	0.3380	**0.0070**
N Source		0.3131	0.2916	0.6944	0.5071	0.4231	0.1031	0.8714	0.4496	0.5153	0.6140
Grazing × N Source		0.3452	0.2154	0.3112	0.1651	0.2971	0.1941	0.5776	0.4218	0.4680	0.3537

[1] N Source: Nitrogen source. [2]LLCW: Left cold carcass weight; CEP: Carcass edible portion; HEP: hindquarter edible portion; FEP: Forequarter edible portion; SR: Spareribs; HFT: Hindquarter fat trimmings; FFT: Forequarter fat trimmings. [3]SEM: Standard Error of Mean.

When analyzing the carcass and non-carcass components expressed in percentage, no significant effect was found for grazing, nitrogen source nor interaction effect of grazing and nitrogen source for the variables carcass edible portion, hindquarter edible portion (HEP), forequarter edible portion (FEP), spareribs, hindquarter fat trimmings (HFT), forequarter fat trimmings (FFT) and bones (P>0.05), as seen in the Table 7.

Table 7 - Left half carcass and non-carcass components of Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons for two years expressed as a percentage.

Fixed effects[1]		**Variables**[2]						
Grazing	**N Source**	**CEP (%)**	**HEP (%)**	**FEP (%)**	**SR (%)**	**HFT (%)**	**FFT (%)**	**Bones (%)**
Deferred		78.08	36.26	26.94	15.88	3.88	2.98	15.45
Rotated		76.96	36.24	27.46	15.44	3.77	3.00	16.21
	Nitrate	77.58	36.39	27.15	15.62	3.65	3.01	15.76
	Urea	77.46	36.09	27.25	15.70	3.99	2.97	15.91
Average Data								
Average		77.67	36.16	27.20	15.66	3.82	2.98	15.80
SEM[3]		0.18	0.30	0.14	0.21	0.10	0.03	0.17
Statistics Probabilities								
Grazing		0.1529	0.9731	0.0574	0.2344	0.5728	0.9222	0.4996
N Source		0.7801	0.4327	0.7125	0.8154	0.0972	0.6965	0.7121
Grazing × N Source		0.0534	0.2991	0.2193	0.5384	0.1806	0.8506	0.1361

[1] N Source: Nitrogen source. [2] CEP: Carcass edible portion; HEP: Hindquarter edible portion; FEP: Forequarter edible portion; SR: Spareribs; HFT: Hindquarter fat trimmings; FFT: Forequarter fat trimmings. [3] SEM: Standard Error of Mean.

6.4. Beef Quality

No significant effects were found for pH, shear force (0 and 14 days of aging), cooking loss (0 and 14 days of aging) and beef color (P>0.05) (Table 8).

Table 8 - Beef quality of Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons for two years.

Fixed effects[1]		**Variables**[2]							
Grazing	**N Source**	pH	SF_{T0} (N)	SF_{T14} (N)	CL_{T0} (%)	CL_{T14} (%)	**Beef Color** L*	a*	b*
Deferred		5.75	114.0	88.82	31.05	30.95	30.74	16.17	11.51
Rotated		5.71	112.0	87.75	29.59	31.80	33.32	16.32	11.97
	Nitrate	5.76	111.0	85.14	30.52	31.61	30.96	16.34	11.77
	Urea	5.70	115.0	91.43	30.12	31.14	33.10	16.15	11.71
Average Data									
Average		5.74	113.38	90.67	30.32	31.31	31.57	16.26	11.76
SEM[3]		0.02	3.48	3.42	0.46	0.36	0.60	0.37	0.41
Statistics Probabilities									
Grazing		0.6778	0.9370	0.9370	0.1056	0.7312	0.2318	0.8427	0.5966
N Source		0.3024	0.6926	0.3956	0.6488	0.5997	0.0734	0.8128	0.9359
Grazing × N Source		0.6349	0.6654	0.3621	0.0767	0.7617	0.7487	0.5606	0.4211

[1]N Source: Nitrogen source. [2]SF_{T0}: Shear Force (0 days of aging time); SF_{T14}: Shear force (14 days of aging time); CL_{T0}: Cooking Loss (0 days of aging time); CL_{T14}: Cooking Loss force (14 days of aging time); L*: Lightness; a*: Redness; b*: Yellowness. [3]SEM: Standard Error of Mean.

6.5. Sensory analysis

The attributes aroma, juiciness, and flavor, evaluated in the sensory analysis of the beef were affected by the different grazing systems ($P<0.05$). For the attributes tenderness and overall acceptance there was an interaction effect between grazing system and nitrogen source ($P<0.05$) (Table 9).

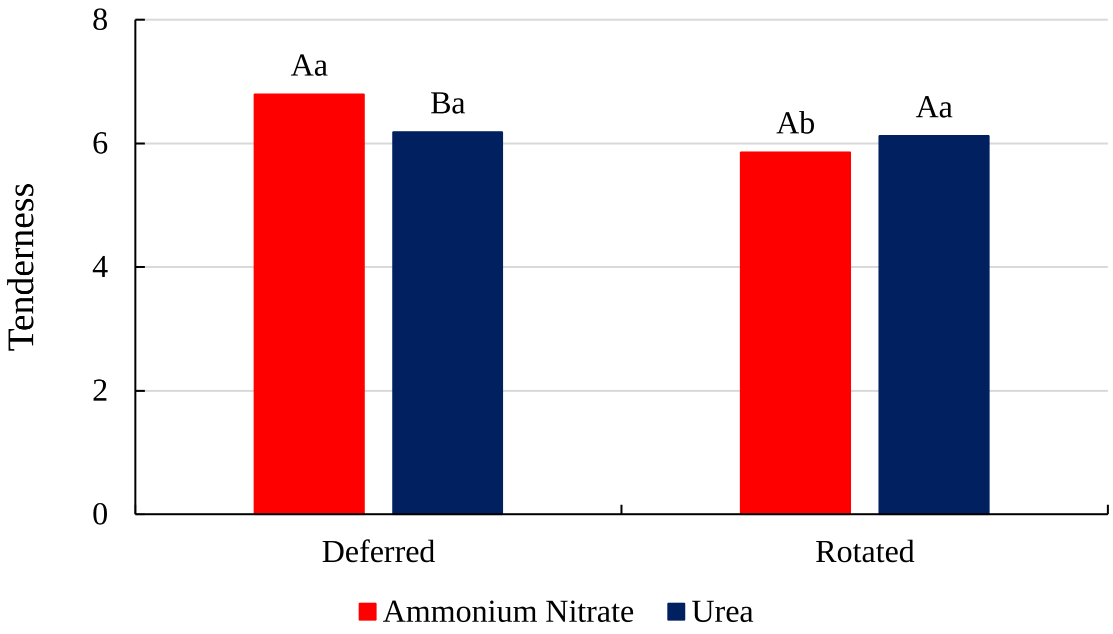
Table 9 - Characteristics evaluated in the consumer acceptance test of the beef at 14 days of aging of Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons for two years.

Fixed effects[1]		**Variables**[2]				
Grazing	**N Source**	Aroma	Tenderness	Juiciness	Flavor	OA
Deferred		7.1	6.5	6.7	6.9	6.9
Rotated		6.8	6.0	6.2	6.6	6.5
	Nitrate	6.9	6.3	6.5	6.8	6.7
	Urea	6.9	6.1	6.3	6.7	6.7
Average Data						
Average		6.9	6.2	6.4	6.8	6.7
SEM[3]		0.05	0.07	0.06	0.05	0.05
Statistics Probabilities						
Grazing		**<.0001**	**<.0001**	**<.0001**	**0.0002**	**<.0001**
N Source		0.5431	0.1120	0.0539	0.5066	0.5151
Grazing × N Source		0.7945	**<.0001**	0.0997	0.4919	**0.0007**

[1] N Source: Nitrogen source. [2] OA: Overall Acceptance. [3] SEM: Standard Error of mean.

In the interaction decomposition for the tenderness variable (Figure 8), the beef of heifers kept in the deferred stocking grazing system, fed ammonium nitrate as a nitrogen source, was more tender (6.79) than heifers in the same grazing system but received urea as nitrogen source (6.18). No difference was found for tenderness when heifers were grazing in a rotational stocking system supplemented with ammonium nitrate or urea (5.85 and 6.11, respectively). When the comparison is made between the sources of nitrogen, there was a difference between the tenderness of the beef of the heifers fed with ammonium nitrate, where the untrained sensory panel assigned scores of 6.79 to the deferred stocking grazing system and 5.85 for the rotational stocking grazing system (Figure 8).

Figure 8 - Interaction between grazing system and nitrogen source for sensory evaluation of Tenderness the *Longissimus thoracis* muscle from Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons for two years.

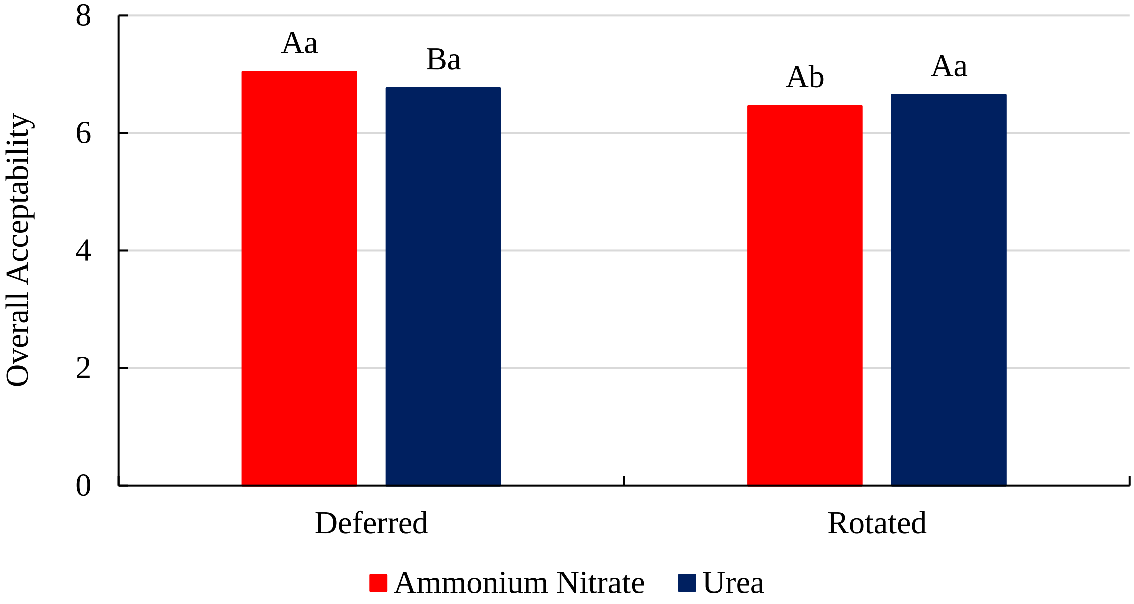


Capital letters within the same grazing system differ for the nitrogen source.

Small letters within the same nitrogen source differ for the grazing system.

The interaction between grazing and nitrogen source for the overall acceptance of fresh beef followed the same patter for tenderness. For the deferred stocking grazing system, the overall acceptance was 7.04 for beef from heifers fed with ammonium nitrate and 6.76 for beef from heifers fed with urea (Figure 9). There were no differences between the scores for the rotational stocking grazing system, which received scores of 6.46 for beef from heifers fed with ammonium nitrate and 6.65 for beef from heifers fed with urea. Comparing the nitrogen sources there was a difference in the overall acceptance of beef from heifers fed with ammonium nitrate, which had scores of 7.04 in the deferred stocking grazing system and 6.46 in the rotational stocking grazing system (Figure 9).

Figure 9 - Interaction between grazing system and nitrogen sources for sensory evaluation of Overall Acceptability the *Longissimus thoracis* muscle from Nellore heifers in different grazing systems receiving two sources of nitrogen during different seasons.



Capital letters within the same grazing system differ for the nitrogen source.
Small letters within the same nitrogen source differ for the grazing system.

7. DISCUSSION

7.1. Animal Performance and Dry Matter Intake

The different grazing methods affected ADG during the different seasons. As expected, deferred stocking grazing was able to promote higher weight gain than rotational stocking in winter, probably due to greater forage availability (Table 3). Lelis (2021) evaluating the forage production of this same study, found a higher production of dry forage mass in winter for deferred stocking grazing, with this production being 11368 kg ha^{-1} *vs.* 5807.56 kg ha^{-1} in the rotational stocking grazing system. The greater availability of forage in the deferred stocking grazing system at that time possibly made the animals able to select more leaves, the place on the plant that has the highest concentration of protein. Considering that the forage crude protein (CP) content, estimated through grazing simulation, was around 9% in winter and considering the supplementation provided to the animals, the nitrogen supply for ruminal microorganisms was not impaired, ensuring higher ADG for heifers in the deferred stocking grazing system, since according to Van Soest (1994) the ruminal microbiota is harmed when crude protein levels are below 7%.

An opposite effect was found during the autumn. The lowest ADG values were observed in this season, a fact that may be related to the growth of the animals. Animal growth is characterized by an allometric curve and tissue growth rates change throughout the animal's life stage (BERG; BUTTERFIELD, 1976). As the animal grows, feed intake increases to meet the needs of its body, but when they reach physiological maturity, muscle mass gain is practically nil, as it has already reached the maximum growth point and weight gain is now composed of the greatest adipose tissue deposition (OWENS et al., 1995).

The nitrogen sources used in the supplements had a significant effect on the ADG and the urea supplementation provided a higher ADG for the animals. Although nitrate supplementation showed lower ADG in the present study (Table 3), some authors have investigated the capabilities of nitrate to improve the productive performance of ruminants when included as a source of non-protein nitrogen in the diet (LEE; BEAUCHEMIN, 2014; HERGARTY et al., 2016). Nitrate has low palatability because it has a bitter taste, interfering with ingestion (LEE et al., 2014), this characteristic may have influenced the lower consumption of the supplement containing this nitrogen source by the animals. The nitrogen sources used in the supplements had no effect on DMI_F. Lee et al. (2015a; 2015b) also used nitrate or urea in bovine supplementation and did not find differences in DMI.

The highest percentage of DMI_{TLW} in summer and autumn may be related to the forage quality in these seasons, seeing that in these seasons’ higher CP content and lower NDF and ADF content were found (Table 3). Although NDF contents were lower in summer and autumn (64.20% and 62.15%), the values were slightly above the recommended, because NDF is negatively correlated with forage intake and above 55-60% can reduce the DMI (Van Soest, 1994). The ADF content is related to the NDF content (ARAUJO et al., 2002); however, the forage ADF content at these stations was below the 40% limit (REIS; DA SILVA; 2011) that could compromise the DMI.

7.2. Enteric Methane Production

When expressed per kg/d, summer and autumn were the seasons in which heifers emitted more CH_4 (0.25 kg/d for both seasons). As mentioned before, enteric CH_4 production is related to food consumption and the greater this consumption, the greater the production of this gas (LANCASTER et al., 2009; HEGARTY et al., 2007). Therefore, as the heifers in this experiment showed higher consumption in the summer and autumn seasons, there was probably a greater supply of substrate for rumen fermentation and, consequently, a greater supply of hydrogen for methanogenic *Archaea* (HEGARTY et al., 2007) resulting in greater production of CH_4 (Table 3). When it comes to CH_4 emission per total DMI (CH_4/DMI_T, g/kg), spring was the season with the highest CH_4/DMI_T emission, even though it was not the season in which the animals had the highest DMI_T, and neither higher ADG.

The higher CH_4 emission when expressed per ADG (CH_4/ADG, kg/kg) in autumn may be related to the production efficiency of heifers in this season, since, as seen previously, autumn was the season of the year that heifers had the lowest ADG. In a study evaluating four grazing systems, Sakamoto (2018) found that the lowest ADG in the degraded and rainfed pasture treatments with high stocking rate (0.392 kg/d and 0.483 kg/d, respectively), reflected in higher emission of enteric CH_4 per kg of ADG (478.4 and 484.5 g/kg, respectively).

Although the deferred stocking grazing system showed more forage availability and higher ADG in the winter, the forage quality probably affected CH_4 emissions per average live weight (CH_4/LBW, g/kg), because in this season the forage had the lowest CP content and highest NDF, ADF, and lignin values. In pasture production systems, CH_4 emissions can be affected by the nutritional composition of the diet (MORAES et al., 2014) and differences in chemical analyzes of *Urochloa brizantha* cv. Marandu and enteric CH_4 emissions, between seasons, were found by Demarchi et al. (2016).

The variables of CH_4 production expressed per hot carcass weight (CH_4/HCW, kg/kg) and edible carcass portion (CH_4/CEP, kg/kg) were not affected by grazing systems nor nitrogen sources (Table 3). However, Fernandes (2018), evaluating Nellore males, found that supplementing the animals with nitrate tended to reduce the CH_4 emission per kg of accumulated carcass by 18.02%.

None of the nitrogen sources influenced the variables related to enteric CH_4 production. Likewise, Tomkins et al. (2016) evaluating urea (32.5 g/day) and nitrate levels (4.6 g and 7.9 g of nitrate/kg DM), found no significant effects of using nitrate ($P>0.05$), while higher nitrate level tended ($P<0.07$) to reduce CH_4 emissions in *Bos indicus* cattle.

According to Niu et al. (2018), the CH_4 conversion factor (Y_m) it is widely used for national GHG emission inventories and global research on mitigation strategies. This factor indicates the proportion of the animal's gross energy intake (GEI) converted to enteric CH_4 energy and was introduced by the Intergovernmental Panel on Climate Change (IPCC). In a study Congio et al. (2022) compiled a dataset from individual beef cattle data for the Latin America and Caribbean region. The authors found values of Y_m with a mean of 6,42% for the high-forage subset, that accommodates grazing animals receiving some level of concentrate. In the present study, the average value found for Y_m was 9,67%, demonstrating a higher value when compared to the result obtained by Congio et al. (2022).

Values for the CH_4 conversion factor close to those found in our study were obtained by Kaewpila & Sommart (2016), who evaluated zebu beef cattle fed low-quality crops and by-products in tropical regions and found values of 4.8 to 13.7% for Y_m. While in tropical productions systems, Patra (2017) observed a mean of 5.84% for the Y_m.

7.3. Carcass and Non-carcass Traits

Even with no significant effect on the FLBW of heifers, there is a numerical difference of 11.18 kg in the FLBW, when grazing systems are compared, possibly influenced by the hot carcass weight of heifers, resulting in carcasses from the deferred stocking grazing system that are 10.67 kg heavier than those from the rotational grazing system. The minimum weight of HCW for the category of females, regulated for the standard of the Brazilian market, is 180.0 kg (BRASIL, 2004). Thus, the carcasses of heifers in this study reached values above the minimum required, with an average of 274.34 kg of HCW. This finding evidence positive results for the HCW, which is similar to the findings of Sanches et al. (2021), who evaluated Nellore heifers finished on pasture with feed and mineral supplementation, also reaching values above 180 kg for HCW (average of 202.10 kg of HCW).

The dressing percentage showed a significant effect for grazing systems and the nitrogen sources used in the supplement. This carcass characteristic is influenced by the diet, slaughter weight, and degree of finishing (DIMARCO et al., 2006). In addition, the dressing percentage is fundamental in the profitability of beef production in Brazil, as cattle ranchers are rewarded by the HCW (kg) and not by the live weight of the animals (MIGUEL et al., 2014). According to Lopes et al. (2012), many slaughterhouses that purchase animals based on live weight consider only 50% of the DP. Considering these factors, the DP achieved in the present study showed satisfactory values. However, the only variable that may have contributed to these values is the PCQ, since variables such as ADG, DMI, FT and RYA were not significantly influenced by grazing systems or nitrogen sources ($P>0.05$).

Treatments had no significant effect on RYA and FT measures. The beef composition of the carcass is represented by the RYA, which is the characteristic that helps evaluate the cuts yield with greater commercial value, as it is related to the total muscles in the carcass (VAN CLEEF et al., 2012). To be an indication of good yields of cuts in cattle, the RYA adjusted for 100 kg of carcass weight must be at least 29 cm^2/100 kg of the carcass (LUCHIARI FILHO, 2000). Accordingly, if we consider the average of 274.34 kg of carcass weight, the RYA should be approximately 79.0 cm^2, which is above the 66.69 cm^2 value found in this study (Table 4), equivalent to 24.30 cm^2/100 kg of carcass weight, indicating that the RYA is below the recommended. Oliveira et al. (2018), evaluating Nellore males in five different grazing systems over two years, also failed to reach the minimum value of 29 cm^2/100 kg of the carcass for RYA. Likewise, Fernandes (2018), Lee et al. (2017), and Hegarty et al. (2016) found no effect of nitrate use on RYA. The FT is a fundamental characteristic for determining the degree of carcass finishing, but fat deposition is different according to animal category, and females have greater potential for this deposition (LUCHIARI FILHO, 2000). In addition, the FT acts as a thermal insulator during the cooling of the carcasses, reducing fluid losses and, consequently, carcass dehydration and preserving the color of the beef (BRIDI; CONSTANTINO, 2009).

The Brazilian beef industry requires that the subcutaneous fat cover must be at least 3 mm (RIBEIRO et al., 2004) and according to Luchiari Filho (2000), the fat thickness must be between 5 and 7 mm, as when in excess, fat thickness has a high positive correlation with percentage of trimming fat, but negative correlation with the percentage of lean beef in the carcass. Therefore, the FT values found in this study (Table 5) are above the recommended values, which according to Coutinho Filho et al. (2006), may indicate an excess fat, which could result in waste and/or lower yield of edible portions.

The use of nitrate did not affect the subcutaneous FT, similar to the results found by Lee et al. (2017). However, Fernandes (2018) reported that the greater thickness of subcutaneous fat in the carcass of animals supplemented with nitrate in their study might be related to the increase in energy consumption of animals, which received energy supplementation in the finishing phase on pasture, since that the deposition of subcutaneous fat is directly related to the energy status of the animal. The marbling score (MS) was not affected by treatments (P>

0.05) and all beefs were considered *Select slight* (4.0 – 4.9), which is considered the lowest marbling score according to the description of the Meat Evaluation Handbook (2013). Intramuscular fat is the last fat to be deposited and, in cases of food restriction, it is the first to be mobilized (PACHECO et al., 2005). It is important to emphasize that females have a superior capacity to deposit fat compared to males, configuring higher marbling scores and carcass quality (TATUM et al., 2007), however, the Nellore breed (*Bos taurus indicus*) is known to have low values in percentage of marbling when comparing to *Bos taurus taurus* (DOS SANTOS, 2008). However, the marbling scores of heifers (Table 4) are remarkably close to 5.0, almost reaching *Choice small* (5.0 – 5.9) category, rarely seen in beefs from Nellore cattle kept in pasture systems. This may have occurred because heifers reached the age of slaughter at an earlier age, a fact that can be verified by the values of FT, showing that there was beginning of deposition of intramuscular fat.

The left cold carcass weight (LCCW) showed a significant effect of the grazing system ($P<0.05$), evidenced that even with no effect for the HCW ($P>0.05$), the carcasses from animals kept in the deferred stocking grazing system were heavier (Table 5), resulting in heavier left cold carcasses when compared to those from the rotational stocking grazing system (Table 5). This result probably influenced the weight of the edible carcass portion (CEP), which also had a significant effect for grazing systems ($P<0.05$), with the deferred stocking grazing system having higher CEP compared to the rotational stocking grazing system (Table 5). On the other hand, when the carcass portions were separated into edible hindquarter portion, forequarter edible portions, and spareribs, only the spareribs had a significant effect on the grazing systems ($P<0.05$). Following the pattern found for LCCW and CEP, the deferred stocking system showed higher spareribs weights (Table 5). In a study comparing grazing systems, Oliveira et al. (2018) working with Nellore males for two years, reported that there was an effect of grazing systems ($P<0.05$) on the weights of the hindquarters, forequarters and spareribs, pointing out that the animals that remained in the degraded grazing system had lower weight of these traits than animals kept in intensified grazing systems. The same authors concluded that to achieve higher slaughter weight, the intensification of pasture systems is necessary, as this will increase the carcass edible portions and reduce processing costs per unit of beef produced.

In beef cattle carcass, tenderloin and striploin represent economically important hindquarter cuts (OLIVEIRA et al., 2018) and therefore, it is recommended to seek higher hindquarter yield (SUGUISAWA et al., 2006). In our study, the deferred stocking grazing system had a positive effect on top quality cuts, as the striploin weight was higher in this type of pasture system ($P<0.05$) (Table 5).

The grazing systems did not affect hindquarters, forequarters and fat trimmings production, and none of the non-carcass components showed a significant effect (P>0.05) due to the different nitrogen sources (Table 5). No studies were found in the literature that verified the effect of supplementing nitrate on non-carcass traits of Nellore cattle.

7.4. Beef Quality

In view of the results presented, it is possible to observe that the final pH values of the beef remained in the range of 5.7 regardless of the grazing system and nitrogen source. Hegarty et al. (2016) also used nitrate in cattle feed and found no effect on the beef pH of animals fed with urea (pH = 5.48) or with nitrate (pH = 5.49), as well as Fernandes (2018), who used encapsulated nitrate in pasture raised beef cattle and found beef pH values with an average of 5.7.

For bovines, final pH beef values between 5.4 and 5.9 are considered normal (PRIETO et al., 2018). In this way, it was possible consider that the animals did went not underwent stress during the pre-slaughter management, because in these situations the final pH value of the beef could reach values above 6.0, resulting in beef of the DFD type ("dark, firm and dry") (HONIKEL, 2014).

The shear force (SF) values, both for unaged and 14 days aged beefs, were above the preconized by the American threshold for WBSF, which is 43.1 newtons (N) (ASTM, 2011). Therefore, the beef of the heifers in this experiment are consider tough, regardless of the aging period. On the other hand, Fernandes (2018) obtained mean values of 3.42 kgf (equivalent to 33.5 N) for the SF of Nellore males supplemented with encapsulated nitrate.

However, it is possible to notice that the average SF of beefs with a 14-day aging period presented a value 20.02% lower when compared to the average SF of unaged beefs. In the beef aging process, endogenous proteolytic enzymes act on the degradation of myofibrillar proteins in the beef, making the beef more tender (CONTRERAS-CASTILLO et al., 2016), which may explain the difference between the SF for the aging times of the beefs in this study. Even so, the average values for the SF of aged beef were above the established for WBSF (43.1 N) (ASTM, 2011), indicating a tough beef. Possibly, a longer period of aging would contribute to the tenderness of the beef, consequently, a reduction in the values of SF.

During beef cooking, the total losses of water, fat, proteins, and minerals occurs because of the contraction of myofibrillar proteins (actin and myosin), characterizing the total cooking losses (CL) (YU et al., 2005). According to Muchenje et al. (2009), the normal CL range for beef is 13.1% to 34.54%. Therefore, the CL values presented in this study are within the expected range regardless the aging times.

When considering beef color, only the luminosity component (L*) found in the beef from the rotational stocking grazing system (33.32) is within the variation patterns cited by Muchenje et al. (2009) for beef (L*: 33.2 to 41.0). This factor may be related to the low deposition of intramuscular fat in heifers, as intramuscular fat promotes higher luminosity values since it influences light reflection (FIEMS et al., 2000). The red intensity value (a*: 16.26) are within the variation patterns indicated by Muchenje et al. (2009) (a*: 11.10 to 23.60) and the yellow intensity values (b*: 11.76) presented values slightly above these standards (b*: 6.1 to 11.3). In a study carried out with sheep by El-Zaiat (2013), beef color variables were not affected by nitrate supplementation. Freire (2016) evaluated increasing levels of encapsulated nitrate in sheep diets and found no effect on a* or b* components, despite a quadratic effect on L*.

According to Fernandes (2018), nitrate could change the color of the beef due to its ability to oxidize myoglobin, oxymyoglobin and metamyoglobin, making it darker. However, in their study, supplementation of beef cattle with encapsulated nitrate during rearing and finishing did not affect the color of the beef, as the nitrite present in the beef was below detection levels and the nitrate residue did not differ between the supplements.

7.5. Sensory analysis

No studies were found in the literature that performed sensory analysis of consumer acceptance considering Nellore females in pasture-based grazing systems supplemented with different sources of nitrogen, with similar conditions to this study.

For aroma, juiciness and flavor characteristics, beef from heifers kept in the deferred stocking grazing system was better evaluated by the consumers when compared to rotational stocking grazing system ($P<.0001$). Juiciness, tenderness, and flavor determine attributes of beef palatability (VOGES et al., 2007). Although the results found in this study did not show a significant effect on CL, beef aged for 14 days showed a difference of 0.85% when comparing grazing systems. With this in mind, fresh beef from heifers under deferred stocking grazing lost less water during cooking, which may have affected the juiciness of the beef.

Tenderness, of all the sensorial characteristics, is the determining attribute in the acceptability of the beef by the consumer since it is the main attribute of palatability (WHIPPLE et al., 1990; LUCHIARI FILHO, 2000). Thus, the consumers scores for beef tenderness showed a preference for beef from heifers from the deferred stocking grazing system. In addition, supplementation with ammonium nitrate in the deferred stocking system also resulted in more tender beef based on the consumers' classification compared to the same supplementation in the rotational stocking grazing system. Although no significant effect was found for SFT_{14} for grazing or nitrogen source, it is possible to notice a difference of 6.29 N in SFT_{14} between the nitrogen sources used in supplementing heifers. Based on the study conducted by Miller et al. 1995, ASTM (2011) defined a threshold value of 4.9 in WBSF as what an average beef consumer can detect. Therefore, the difference obtained in the SFT_{14} for the nitrogen source is greater than the threshold value of 4.9 N, showing that the consumers could differentiate the tenderness of beef from two different nitrogen sources.

Overall acceptance refers to how much consumers liked or disliked the beef. The results obtained in the interaction for the overall consumer acceptance trend are similarly to those obtained for the tenderness interaction, reinforcing that tenderness is the determining attribute in consumer acceptability, as described above.

8. CONCLUSIONS

The deferred stocking grazing system was able to provide a higher amount of forage mass in the most critical period of the year (winter), when forage production is scarce, allowing higher ADG. In addition, at the end of the experiment, heifers finished in this pasture system had higher weight and dressing percentage, which provided a higher yield of commercially important cuts. Beef quality was not influenced by treatments.

The use of nitrate as a nitrogen source did not influence the production of CH_4 in this study. However, it presented performance, dry matter intake, and beef quality results similar to the use of urea, being a viable alternative as a source of nitrogen supplementation.

Finally, the deferred stocking proved to be an efficient intensification method, as the performance of the heifers was similar to the rotational stocking grazing system, however, the dressing percentage and carcass finishing were higher in this system, resulting in a greater carcass edible portion. Thus, the deferred stocking grazing system can be used associated with the rotational stocking grazing system, to provide forage for the animals in the dry seasons of the year.

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