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Forage peanut legume as a strategy for improving beef production without increasing livestock greenhouse gas emissions

B.G.C. Homem [a,b], L.P.C. Borges [a], I.B.G. de Lima [a], B.C. Guimarães [a], P.P. Spasiani [a], I.M. Ferreira [a], P. Meo-Filho [c], A. Berndt [c], B.J.R. Alves [b], S. Urquiaga [b], R.M. Boddey [d], D.R. Casagrande [a,*]

[a] Department of Animal Sciences, Federal University of Lavras, UFLA, Lavras, MG 37200-900, Brazil
[b] Embrapa Agrobiologia, Rodovia BR-465, km 7, Seropédica 23897-970 RJ, Brazil
[c] Embrapa Southeast Livestock, Rodovia Washington Luiz, km 234, Sao Carlos, SP 13560-970, Brazil
[d] Department of Soil Science, Federal Rural University of Rio de Janeiro, Rodovia BR 465, km 7, Seropédica, RJ 23897-000, Brazil

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ABSTRACT

The transformation of pastures from a degraded state to sustainable productivity is a major challenge in tropical livestock production. Stoloniferous forage legumes such as *Arachis pintoi* (forage peanut) are one of the most promising alternatives for intensifying pasture-based beef livestock operations with reduced greenhouse gas (**GHG**) emissions. This 2-year study assessed beef cattle performance, nutrient intake and digestibility, and balance of GHG emissions in three pasture types (**PT**): (1) mixed Palisade grass – *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster (syn. *Brachiaria brizantha* Stapf cv. Marandu) and forage peanut (*A. pintoi* Krapov. & W.C. Greg. cv. BRS Mandobi) pastures (Mixed), (2) monoculture Palisade grass pastures with 150 kg of N/ha per year (Fertilised), and (3) monoculture Palisade grass without N fertiliser (Control). Continuous stocking with a variable stocking rate was used in a randomised complete block design, with four replicates per treatment. The average daily gain and carcass gain were not influenced by the PT ($P = 0.439$ and $P = 0.100$, respectively) and were, on average, 0.433 kg/animal per day and 83.4 kg/animal, respectively. Fertilised and Mixed pastures increased by 102 and 31.5%, respectively, the liveweight gain per area (kg/ha/yr) compared to the Control pasture ($P < 0.001$). The heifers in the Mixed pasture had lower CH_4 emissions (g/animal per day; $P = 0.009$), achieving a reduction of 12.6 and 10.1% when compared to the Fertilised and Control pastures, respectively. Annual (N_2O) emissions (g/animal) and per kg carcass weight gain were 59.8 and 63.1% lower, respectively, in the Mixed pasture compared to the Fertilised pasture ($P < 0.001$). Mixed pasture mitigated approximately 23% of kg CO_2eq/kg of carcass when substituting 150 kg of N/ha per year via fertiliser. Mixed pastures with forage peanut are a promising solution to recover degraded tropical pastures by providing increased animal production with lower GHG emissions.

Implications

Areas of degraded pasture represent a major opportunity for decreasing the carbon footprint of tropical livestock production. Degradation is normally related to a lack of fertiliser application, especially nitrogen. However, nitrogen fertiliser has high cost and increases greenhouse gas emissions. An alternative strategy is to provide nitrogen from biological N_2 fixation into tropical pastures via the introduction of forage peanut (*Arachis pintoi*). Mixed pasture with forage peanut mitigated 23.0% of CO_2eq per kg of carcass yield compared to the application of 150 kg N/ha per year via fertiliser.

Introduction

Brazil has the largest commercial cattle herd in the world, estimated at 203 million head, with 82% of total production depending exclusively on pasture, and has an important contribution to gross domestic product (ABIEC, 2023). The great challenge at present is to increase animal performance and production and, at the same time, reduce the environmental impact of the activity, especially greenhouse gas (**GHG**) emissions (Cardoso et al., 2020).

The Agriculture sector in Brazil is responsible for the greatest contributions of CH_4 and N_2O to the national GHG inventory.

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* Corresponding author at: Current address: Department of Animal Sciences, Federal University of Lavras, Campus Universitario, Caixa Postal 3037, 37200-900 Lavras, MG, Brazil.

E-mail address: danielcasagrande@ufla.br (D.R. Casagrande).

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Approximately, three-quarters of all CH_4 emissions, with over 80% originating from enteric fermentation, are attributed to this sector. Additionally, N_2O emissions from agriculture make up almost 90% of the country's total emissions of this gas (SIRENE MCTI, 2023). Therefore, it becomes a priority that crop and livestock systems are managed to reduce the emissions of these gases.

Although the performance of livestock farming in tropical regions has evolved considerably in recent decades, the average productivity of pastures is still far below their potential (Strassburg et al., 2014). For example, it is estimated that up to 50 million ha of pasture lands in Brazil are in advanced stages of degradation (Feltran-Barbieri and Féres, 2021). Recovery of these areas back into production and increasing their productivity are key approaches to improving land use in tropical regions. At the same time, these strategies reduce the pressure for new expansion over natural vegetation. Nitrogen fertilisation as a form of intensification promotes an increase in pasture productivity (Carvalho et al., 2018; Marques et al., 2017) causing a positive effect on weight gain per animal and per area (Delevatti et al., 2019). However, the manufacturing process for nitrogen fertiliser, which relies on fossil fuel (Haber-Bosch system) results in GHG emissions. Additionally, there are GHG emissions derived from the transport and application of the fertiliser. Considering these factors, it is estimated that one kg of fertiliser N is equivalent to 4.5 kg of fossil CO_2 emissions (Robertson and Grace, 2004). Worldwide, it is estimated that the N fertiliser supply chain is responsible for 10.6% of agricultural emissions and 2.1% of global anthropogenic GHG emissions (Menegat et al., 2022).

An alternative strategy to provide N into tropical pastures would be via the introduction of a forage legume (Muir et al., 2014) with an efficient biological N_2 fixation (**BNF**) system. This source of N avoids entirely the GHG emissions that are associated with the synthesis, transport, and application of N fertiliser. Recent research has shown that with appropriate management, stoloniferous forage legumes such as *Desmodium ovalifolium* or *Arachis pintoi* (forage peanut) are able to persist for many years in mixed pastures with *Urochloa* spp. and to increase animal productivity (Boddey et al., 2020; dos Santos et al., 2023; Homem et al., 2021a; Pereira et al., 2020). Recent studies showed that emissions of N_2O from a mixed pasture of *U. brizantha* (cv. Marandu) with forage peanut were lower than from an N fertilised pasture (Guimarães et al., 2022). The presence of higher concentrations of condensed tannins in the diet derived from the legume indicates that there is a potential for the reduction in enteric methane emissions from the cattle grazing the mixed pasture (Archimède et al., 2011; Jayanegara et al., 2012).

It was hypothesised that the use of N sources, whether from industrial synthesis or biological fixation, could accelerate forage production and increase animal productivity. Additionally, the use of legumes in conjunction with grasses may reduce GHG emissions. Therefore, in this study, the objective was to evaluate forage and animal performance and the balance of GHG emissions in different production systems of Nellore heifers in *U. brizantha* cv. Marandu with or without N fertiliser or mixed with *Arachis pintoi* cv. BRS Mandobi.

Material and methods

Location of the experiment and treatments

The experiment was carried out at the Department of Animal Science at the Federal University of Lavras, Brazil (21° 14′S, 44° 58′W; 918 m altitude). Meteorological data were obtained from a station located 1 000 m from the experimental area. The average air temperature and annual precipitation during the experimental period were 21.0 °C and 1 191 mm, respectively. Additional information about the meteorological data throughout the experiment is given by Homem (2020) and Guimarães (2020).

The soil in the experimental area is classified as a Rhodic Ferralsol (WRB/FAO classification) with clayey texture and uniform clay content down the profile (563–574 g of clay/kg soil 0–0.40 m). At the beginning of the experimental period, the soil (0–0.20 m) had the following characteristics: $pH_{(H2O)}$ = 5.9; exchangeable Al, Ca, Mg of 0.07, 2.4 and 0.7 $cmolc/dm^3$, respectively; Available P (Mehlich-I) = 7.6 mg/dm^3, exchangeable K = 82.8 mg/dm^3, and organic matter = 31.0 g/kg (Guimarães, 2020).

Three pasture types (**PTs**) were evaluated: (1) Palisade grass pasture (*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster [syn. *Brachiaria brizantha* Stapf] cv. Marandu) mixed with forage peanut (*Arachis pintoi* Krapov. & W.C. Greg. cv. BRS Mandobi) without N fertiliser (Mixed); (2) Palisade grass monoculture fertilised with 150 kg N/ha per year (Fertilised); and 3) Palisade grass monoculture without N fertilisation (Control).

Pasture and treatment establishment

Initially, in November 2013, 2 500 kg of dolomitic lime was applied to the entire experimental area (12 ha). In January 2014, Palisade grass was established (6.0 kg/ha of pure viable seeds), and 52 kg P (single super phosphate) together with 41 kg K/ha (as potassium chloride) were applied.

In December 2015, the experimental area was divided into four blocks, where the Fertilised, Mixed and Control treatments were randomly allocated to paddocks with an area of 0.7, 1.0 and 1.3 ha, respectively, within each block (Homem et al., 2021a). The experiment consisted of four replications (blocks), totalling 12 experimental units. After allocating the treatments to the relevant areas, forage peanut was established in the Mixed paddocks, using 10 kg of pure viable seeds/ha. Additional information about the establishment of this experiment is given by Homem et al. (2021a).

Annually, in spring (between November and December), all paddocks were fertilised with single super phosphate (22 kg P/ha) and potassium chloride (41 kg K/ha). In the Fertilised treatment, N fertilisation was divided into three applications per year (50 kg/N per ha in November, January, and March) in the form of urea.

The experimental period was from December 2016 to January 2019, being divided into years and seasons: 22 December to 21 March (Summer), 22 March to 21 July (Autumn), 22 July to 21 September (Winter), and 22 September to 21 December (Spring). The stocking system used was continuous with a variable stocking rate. Two Nellore heifers per experimental unit (234 ± 36 kg initial BW and 12 ± 1.3 months of age) were used as tester animals to maintain a target canopy height of 0.15 m during winter and 0.20–0.25 m for the remainder of the year (Guimarães, 2020; Homem et al., 2021a). To maintain the target canopy heights, additional regulatory animals ('put and take') were used (Allen et al., 2011). The average canopy height was measured weekly using a graduated sward stick (Barthram, 1985) at 100 random points per experimental unit and the stocking rate was adjusted as necessary.

During the experimental period, animals received water and commercial mineral supplementation [see Homem et al. (2021b) for commercial mineral composition] *ad libitum*. At the end of the 1st year of experimental evaluation, the animals were replaced by a new batch.

Experimental evaluations

Herbage mass and forage nutritive value

Herbage mass was measured by cutting at ground level, all material contained inside rectangular frames measuring 0.5 × 1.0

m, every 30 days. After harvesting the forage, the botanical and morphological components were separated manually. The different fractions were weighed and dried in an oven with air circulation at 55 °C for 72 h. The herbage mass was considered as the above-ground biomass (mass of grass or grass and legume) without dead material.

For forage nutritive value analysis, forage samples were collected manually using the simulated grazing method ("hand-plucking") during the intake evaluation. In the Mixed treatment, grasses and legumes were collected and later separated. A composite sample of each species was made for each experimental unit. The forage samples were dried in an oven at 55 °C for 72 h and then ground in a Cyclotec mill (Tecator, Herndon, VA) with 2- and 1-mm sieves.

The forage samples were analysed for DM, CP concentration (CP = total N × 6.25), and ash according to AOAC (2000) and NDF free of ash and proteins (Pell and Schofield, 1993). Condensed tannin was extracted using methanol, acetone, and ascorbic acid solution; the Fe reagent and n-butanol-HCl were added to the tannin extract, which was then heated at 95 °C (Porter et al., 1985). Detailed information of HM and forage nutritive value assessments is given by Homem et al. (2021a,2021b).

Animal performance

Heifers were weighed every 28 days, throughout the experimental period, without food and water restrictions, always early in the morning. Average daily gain (**ADG**) was estimated using linear regression in each season. In this equation, the individual initial weight for each season was the intercept and the ADG was the slope. The stocking rate was calculated by summing the weights of all animals grazing per day in each paddock, divided by the number of days in the period and the area of each paddock. The stocking rate was expressed in AU/ha, considering an Animal Unit (**AU**) as 500 kg (Allen et al., 2011). Liveweight gain per area was calculated by multiplying the ADG by the stocking rate in animals/ha. Variables were averaged per experimental unit for each season before analysis.

Comparative slaughter of animals

Prior to the experimental period, three heifers of weight and age similar to those used at the beginning of each experimental year were slaughtered as a reference for initial carcass weight. At the end of each experimental year, the two tester heifers from each paddock were slaughtered to estimate the final carcass weight.

Heifers were weighed at the end of each study period to determine the shrunk final BW (after 16 h of feed and water withdrawal). The heifers were moved to a commercial slaughterhouse (Supremo Carnes, Campo Belo, MG, Brazil) that was 59 km away from the research centre the next day after being loaded onto trucks. Animals were brought to the slaughterhouse and placed under humanitarian slaughter in accordance with Brazilian RIISPOA (Regulation of Industrial and Sanitary of Animal Products), following standard procedures of Brazilian Federal Inspection, after being housed in resting pens for 18 h (with unrestricted access to water). The hot carcass weight was recorded. The equations derived with the animals slaughtered at the start of the experiment were used to determine the initial carcass weight for calculating the carcass increase. Gain yield was calculated by dividing carcass gain by BW gain.

Forage intake

Forage intake was estimated once for each season of the year (totalling eight measurements during the experiment) from faecal production and indigestible neutral detergent fibre (**iNDF**). Faecal production was estimated using the external indicator titanium dioxide (Titgemeyer et al., 2001). The indicator was supplied daily to the animals in the amount of 10 g/animal per day, for 11 consecutive days (6 days of adaptation and 5 days of faeces collection). Faecal samples were collected directly from the rectum, once a day, at the same time (noon), for 5 consecutive days. The faecal samples were dried in a forced air oven at 55 °C for 72 h and processed in a Wiley-type mill, with a 2- and 1-mm mesh sieve. Subsequently, individual samples were transformed into composite samples to determine the concentration of TiO_2 in faeces, using colorimetric absorption spectroscopy as described by Myers et al. (2004). Faecal production (kg/DM per day) was estimated based on the ratio between the amount of indicator administered to the animal and its concentration in faeces.

Samples of forage from hand-plucked samples and faeces were incubated in the rumen of two cannulated heifers (fed a diet composed of Palisade grass and forage peanut or Palisade grass monoculture) for 288 h to determine iNDF (Huhtanen et al., 1994). Daily faecal production represents the amount of indigestible fraction excreted daily. Therefore, it was possible to estimate how much iNDF was ingested per day from the daily excretion. Then, with the iNDF value of the forage (hand-plucking), the forage intake was estimated.

For the Fertilised and Control treatments, forage intake was calculated using the following equation:

$$\mathrm{DMI}\,(kg/dia) = (\mathrm{Faecal\,production} \times \%\mathrm{iNDFfaeces})\,/\,\%\mathrm{iNDFhand\,plucked\,sample}$$

For the Mixed pasture treatment, the proportion of grass and legume in forage intake was estimated using the $\delta^{13}C$ natural abundance technique, from the equation:

$$\%\mathrm{legume} = 100 \times (\delta^{13}\mathrm{CG} - \delta^{13}\mathrm{CS})\,/\,(\delta^{13}\mathrm{CG} - \delta^{13}\mathrm{CL})$$

where %legume is the proportion of legume carbon in the iNDF incubation residue of faeces (Lopes de Sá, 2017); and $\delta^{13}CG$ (grass), $\delta^{13}CL$ (legume), and $\delta^{13}CS$ (samples) are the $\delta^{13}C$ abundance values of the iNDF residue in grass (−11.9 ‰), of the legume (−27.4 ‰), and the iNDF residue in faecal samples, respectively. To determine the ^{13}C abundance, samples were ground to a fine powder (Arnold and Schepers, 2004), subsampled (subsamples containing between 300 and 500 μg C), and subsequently analysed for total C and ^{13}C abundance using an automated continuous-flow isotope-ratio mass spectrometer (Finnigan DeltaV mass spectrometer coupled to the output of a Costech [model ECS4010] total C and N analyser Finnigan MAT, Bremen, Germany) in the "John Day Stable Isotope Laboratory" at Embrapa Agrobiologia (Guimarães, 2020; Homem, 2020).

The intake of the organic matter (**OM**) and CP were calculated. The nutrient concentration in the diet was calculated by nutrient intake divided by the forage intake. The coefficients of apparent digestibility of the DM, OM, CP, and NDF in the total digestive tract were determined through faecal excretion of the external titanium dioxide indicator (Myers and Robbins, 1991). Furthermore, DM, OM, CP, and NDF concentrations of faecal samples were determined in the same way as described for the hand-plucked samples. Total digestibility (g/kg) was calculated as (% DM and nutrients in the diet − % DM and nutrients in faeces)/(% DM and nutrients in diet). The apparent digestibility coefficients were calculated to DM, OM, CP, and NDF (Guimarães, 2020; Homem, 2020).

Enteric methane emission from animals

During periods of assessment of forage intake, the emission of enteric CH_4 from grazing animals was evaluated. For each treatment, CH_4 emissions were evaluated from eight animals. The SF_6 tracer gas technique (Johnson and Johnson, 1995) was used for measuring enteric CH_4 emissions from rumination, eructation, and breathing. Thirty days before gas sampling, the animals were fitted with gas collection halters to allow acclimatisation in an adaptation period. Seventy-two hours prior to the first sampling period, a small brass permeation tube was placed in the rumen allowing the tracer gas to equilibrate in the ruminal environment. Each animal was sampled daily (24 h) for 5 consecutive days. The gas samples were obtained continuously through a capillary tube connected to a collecting container placed on the neck of the animal (Johnson and Johnson, 1995). A halter with a 0.127 mm stainless steel capillary tube and a 15 μm in-line filter was placed on the animal's head and connected to an evacuated sampling vessel (Johnson et al., 2007). Before the experiment, collection canisters made of polyvinyl chloride (**PVC**) were attached to a vacuum pump in the laboratory to create a negative pressure (approximately − 13.15 psi). As the vacuum in the sampling vessel slowly dissipated, the negative pressure continuously drew in the air sample around the animal's mouth and nose.

Additional PVC canisters were placed near the experimental pastures to monitor the ambient daily concentration ("basal concentration") of CH_4 and SF_6 during each sampling period. Sampling was performed daily at 0700 h when the animals were removed from the paddocks and transferred to the working facilities of the Federal University of Lavras. After gas sampling, pure nitrogen was added (approximate pressure of 1.5 psi above ambient was carried out with synthetic N_2 5.0) to the canisters and then, CH_4 and SF_6 were measured using gas chromatography (Agilent HP-6890, Wilmington, DE, USA; or Shimadzu® GC-2014, Kyoto, Japan). Both chromatographs were equipped with a flame ionisation detector and megabore column (0.53 mm, 30 m) Plot HP-Al/M (for CH_4) and an electron capture detector (μ-ECD) and megabore column HP- MolSiv (for SF_6), with two 0.5 cm^3 loops coupled to two six-way valves.

The calibration curves were established using gas standards certified by White Martins (Praxair), with concentrations in ppt (54 ± 9, 97 ± 9 and 954 ± 98 ppt) for SF_6 and in ppm (0.996; 4.98; 10.36, and 51.57 ppm) for CH_4, according to Westberg et al. (1998). The CH_4 flux released by the animal was calculated from the SF_6 flux, correlating the results to the known rate of tracer release in the rumen (Westberg et al., 1998), following the equation:

$$\boldsymbol{CH_4 emission} = \boldsymbol{QSF_6} \times \left(\frac{[(\boldsymbol{CH_4})\boldsymbol{y} - (\boldsymbol{CH_4})\boldsymbol{b}]}{[(\boldsymbol{SF_6})\boldsymbol{y} - (\boldsymbol{SF_6})\boldsymbol{b}]} \right)$$

where CH_4 emission = CH_4 emission rate per animal; QSF_6 = known SF_6 emission rate from the capsule in the rumen; $(CH_4)y$ = CH_4 concentrations in the collection device; $(CH_4)b$ = basal concentration of CH_4; $(SF_6)y$ = SF_6 concentration in the collection device; and $(SF_6)b$ = basal SF_6 concentration.

The CH_4 emission was calculated per unit of BW and metabolic weight ($BW^{0.75}$). By relating daily emissions to daily DM intake, emissions per kg intake and digestible organic matter intake were obtained. In a similar manner, the CH_4 emissions per kg BW gain, per kg of carcass weight gain and kg per hectare were estimated.

Nitrogen excretion and emission factors for N2O from livestock excreta

Urinary and faecal N excretions (g of N/day) were evaluated. Nitrogen concentrations in livestock excreta (faeces and urine) were evaluated using the semi-micro Kjeldahl (method 920.87 AOAC, 2000). Faecal N excretion was assessed by the concentration of N in faeces and multiplied by total faecal production (see section *Forage intake*). Urine samples were obtained by vulvar stimulation on the same day and times as faecal collection. Urinary volume was estimated from the concentration of creatinine in the urine using the method and equation of Silva et al. (2012). The total N excreted in urine (g of N/day) was calculated from the urine volume and the N concentration. Details of the collection of samples and the calculations are given by Homem et al. (2021b).

The total excretion of N in faeces and urine per ha was estimated by multiplying the excretion per animal per day and the stocking rate in animals/ha. The ratio of N excreted in urine/N excreted in faeces was also calculated.

The emission factors for N_2O and NH_3 were determined in this field experiment by Guimarães et al. (2022). Using the results reported in this publication, the emissions of N_2O and NH_3 in g per animal per day were calculated by multiplying the respective emission factors (**EFs**) by N excretions in urine and faeces. The rainy season EFs were considered for the Spring, Summer, and Autumn seasons, and dry season EFs were used for the Winter season calculations. The N_2O and NH_3 emissions per animal and per ha were calculated. The N_2O and NH_3 emissions relationships to intake and carcass yield variables were made in the same manner as for the CH_4 emissions. There were considered direct and indirect N_2O emissions. The N_2O emissions from forage residues were not included in the current analysis.

Balance of greenhouse gas emissions in each pasture type

Total GHG emissions were calculated in CO_2eq using global warming potential (**GWP**) conversion factors of 27.2 and 273 for enteric CH_4 and N_2O, respectively. The GWPs used were extracted from the IPCC sixth assessment cycle (AR6), which covers the latest scientific information on the physical state of the global climate made by the Intergovernmental Panel on Climate Change in the years 2021–2022 (IPCC, 2022).

For the fertilised system, the potential N_2O emissions from the fertiliser were considered in the system's emissions balance. Thus, the emission factor of N_2O and NH_3 of N derived from urea fertiliser were assumed as 1.1 and 14.5%, respectively, as described in the 2019 Refinement of the 2006 IPCC Guidelines for National Greenhouse Gas Inventories (IPCC, 2019). Additionally, the GHG emissions derived from the synthesis, transport, and application of the fertiliser were considered as 4.5 kg of fossil CO_2 for one kg of N fertiliser applied (Robertson and Grace, 2004). In summary, the scope of the study was heifers from 12 to 24 months of age (backgrounding and finishing). The sources of GHG were enteric CH_4 measured, direct and indirect N_2O emission from faeces and urine measured, direct and indirect N_2O emission from N fertiliser estimated according to IPCC (2019) and CO_2 emission from synthesis, transport and application from N fertiliser estimated according to Robertson and Grace (2004).

Statistical analyses

The experimental design was in randomised blocks with three treatments (Pasture types [PT]: Fertilised, Control, and Mixed), four replications and repeated measurements over time (seasons). The paddocks were considered as experimental units in the statistical analysis. Data were analysed by mixed models (Littell et al., 2000) using the SAS MIXED procedure (SAS Institute, Cary NC). The effects of PT and seasons were considered fixed and the effects of block and year as random effects. The Akaike's information criterion was used to choose the best (co)variance structure (Akaike, 1974). All variance components were estimated using the restricted maximum likelihood method. The averages were estimated using the LSMEANS statement, and comparisons were made between treatments using Fisher's protected LSD test. Significance was declared at $P < 0.05$ and tendencies at $0.05 < P < 0.10$. The statistical model for data analysis was as follows:

$$Y_{ijkz} = \mu + B_i + PT_j + y_{ij} + Y_k + S_z + (PT * S)_{jz} + \varepsilon_{ijkz}$$

where Y_{ijkz} = value observed in the *i*th block of the *j*th PT of the *k*th year of the *z*th season; μ = overall average; B*i* = random effect associated with the *i*th block, *i* = 1, 2, 3, 4; PT*j* = fixed effect associated with jth pasture types, j = 1, 2, 3; γ_{ij} = random error associated with the ith block in the jth PT. Y_k = random effect associated with kth year, k = 1, 2; S_z = fixed effect associated with zth season, z = 1, 2,. . ., 8; $(PT \times S)_{jz}$ = fixed effect of interaction jth PT with the zth season. ε_{ijkz} = random error associated with the ith block, the jth PT, the kth year, and the zth season.

The effect of the N excreted in urine/N excreted in faeces ratio and total N_2O emissions per animal were analysed using regression analysis with a P of 5% using PROC REG from SAS (SAS Institute). Further details on the statistical analysis are provided in Supplementary Material S1.

Results

Forage and animal performance

The canopy height of all three PTs was similar, ranging from 0.20 to 0.25 m in the spring, summer, and autumn and from 0.15 to 0.20 m during the winter (dry season; Fig. 1). The variables related to herbage mass were influenced by the PTs (Fig. 1). The Fertilised and Mixed pastures presented greater herbage mass (P = 0.047) during the summer and spring seasons. For the autumn and winter, the Fertilised pasture presented the greatest herbage mass. Furthermore, Fertilised pasture also had the greatest grass mass (P < 0.001) in all seasons evaluated. The average legume mass during the two experimental years was approximately 1 183 kg/ha, accounting for 30.2% of the botanical composition (legume proportion) of the canopy in the Mixed pasture treatment.

The Fertilised and Mixed pastures resulted in the lowest NDF values in the animals' diet (P < 0.001; Table 1). Regarding the concentration of CP in the diet, the Fertilised pasture registered the greatest values, followed by the Mixed, and the lowest for the Control (P < 0.001). Lower NDF concentrations were found in the spring, and higher CP concentrations were observed in spring and summer (P < 0.001). There was an interaction between PT and seasons for the concentrations of condensed tannins in the animals' diet (P = 0.016). In all seasons, the condensed tannin concentration in the diet was greatest in the Mixed pasture. Regarding apparent digestibility, the Fertilised pasture had the greatest DM, OM, CP and NDF apparent digestibility, which was also observed for all PTs during the Spring when seasons were compared ($P \leq$ 0.010).

There was a tendency for the greatest DM and NDF intakes in the Fertilised pasture (P = 0.056 and P = 0.094, respectively; Table 2). Similarly, the digestible organic matter intake (**dOMI**) was greatest for the Fertilised pasture (P = 0.027). The greater CP intake was recorded for Fertilised pasture, followed by the Mixed pasture and the lowest was recorded in the Control (P < 0.001). The ADG was not influenced by the PT (P = 0.439) and was on average 0.433 kg/day. On the other hand, the stocking rate (AU/ha) was

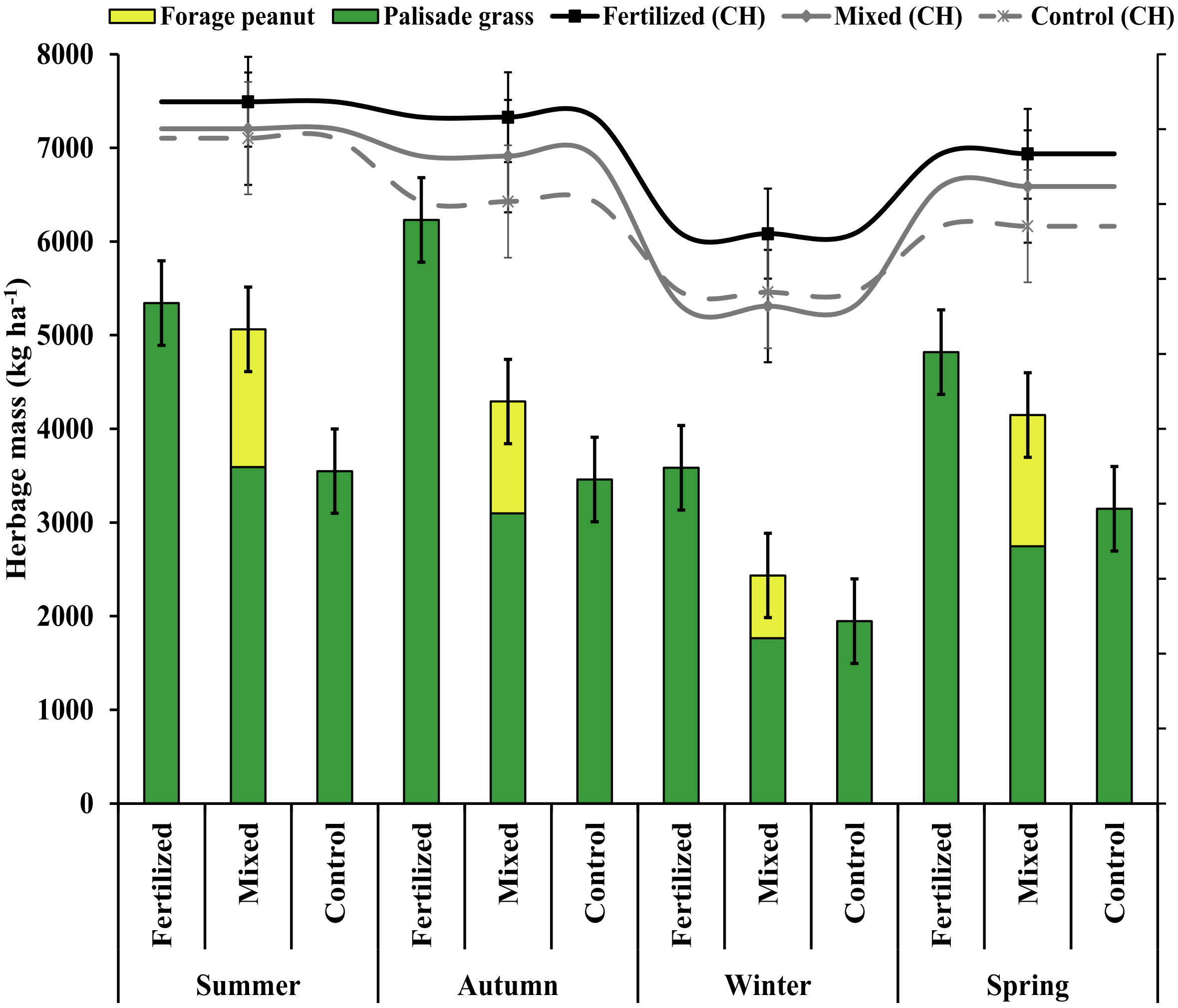

Fig. 1. Herbage, grass, and legume mass (bars ± SEM), and canopy height (**CH;** lines ± SEM) of Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped (Mixed) with forage peanut grazed by Nellore heifers throughout the seasons of the year.

Table 1
Diet composition and nutrients apparent digestibility of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped (Mixed) with forage peanut.

Variables	Pasture type (PT)			Seasons (S)				SEM	P value		
	Fertilised	Mixed	Control	Summer	Autumn	Winter	Spring		PT	S	PT*S
Diet composition, g/kg											
NDF	578^b	584^b	621^a	601^A	609^A	602^A	566^B	6.15	<0.001	<0.001	0.216
CP	125^a	105^b	82.2^c	122^A	97.3^B	74.0^C	123^A	4.69	<0.001	<0.001	0.213
CT	18.2^b	54.9^a	14.5^b	36.6^A	25.7^{BC}	22.3^C	32.4^{AB}	0.63	<0.001	0.007	0.160
Apparent digestibility, g/kg											
DM	545^a	493^b	470^b	535^B	456^C	365^D	655^A	16.2	0.004	<0.001	0.155
OM	583^a	551^b	527^b	583^B	514^C	427^D	690^A	14.1	0.010	<0.001	0.249
NDF	559^a	501^b	511^b	563^B	484^C	377^D	670^A	15.7	0.009	<0.001	0.327
CP	533^a	473^b	379^c	530^B	404^C	267^D	645^A	23.4	<0.001	<0.001	0.271

Note: Least squares means within a row with different lowercase for PTs and uppercase letters for Seasons differ at $P \leq 0.05$.
Abbreviations: CTs = Condensed tannins; OM = organic matter.

greater for the Fertilised pasture (P < 0.001), being on average 51.4% greater than the other treatments. In relation to the seasons, the animals presented greater DM, dOM, NDF and CP intakes (P < 0.001) in the spring when compared to the other seasons. The ADG were greater during spring (P < 0.001); however, the stocking rate was greater in the summer (P < 0.001).

Regarding CH_4 emissions of the heifers in the Mixed treatment, the emissions were approximately 11.6% lower when expressed as g CH_4/animal per day (P = 0.001), 7.3% lower as g CH_4/d per kg BW (P = 0.018), and 8.4% lower as g CH_4/d per kg MW (P = 0.009) in relation to the average of the other PTs (Table 2). The N input into the systems, whether in the form of fertiliser or biological fixation, resulted in CH_4 emissions 11.4% lower when expressed as a function of DMI (g/kg DMI) when compared to the Control pasture (P = 0.044). More CH_4 was emitted by heifers in relation to dOM intake for the Control treatment (P = 0.008). Regarding the seasons, there was no significant effect ($P \geq 0.386$) for CH_4 emission as a function of BW and MW. Daily emissions per animal were lower in summer compared to other seasons (P < 0.001). The CH_4 emissions in relation to DM and dMO intakes were greatest in the winter season (P < 0.001).

Nitrogen excretion and emissions of N_2O and NH_3 from faeces and urine

Animals grazing in the Fertilised pasture showed an average of 38% more total N excretion and 57% more N excreted in urine when compared to the Control and Mixed pastures (P < 0.001; Table 3). The N excretion in the faeces (P = 0.039) and the ratio of N excreted in urine/N excreted in faeces (P = 0.001) were greater for the Fertilised pasture, followed by the Mixed pasture and lowest for the Control pasture. More total N and urine N were excreted during the spring season (P < 0.001). On average, the N excretion per kg of BW was 0.37, 0.31 and 0.26 g for the Fertilised, Mixed and Control pastures, respectively (P = 0.032; data not shown). In the faeces, N excretion was greater during the summer, autumn and spring (P < 0.001). There was no difference among seasons for the ratio of N excreted in urine/N excreted in faeces (P = 0.110).

The total N_2O emissions from animal excreta (g/animal per day) were 67% greater in the Fertilised pasture than in the Mixed pasture and 137% greater than in the Control (P < 0.001). The N_2O emissions from the urine of animals grazing on the Mixed pasture were 38% lower than in the Fertilised system but 84% higher than in the Control treatment (P < 0.001). Animals in the Mixed pasture had lower N_2O emissions from faeces (P < 0.001) compared to the Fertilised (53% less) and Control (20% less) pastures. Total N_2O emissions per animal varied according to the ratio of N excreted in urine/N excreted in faeces (P < 0.001), presenting a positive linear relationship (Fig. 2).

The total volatilisation loss of NH_3 from excreta (g/animal per day) in the Fertilised treatment was on average 6.6 times greater, and the NH_3 loss from urine 7.5 times greater, than the losses from the Control and Mixed pastures (P < 0.001) (Table 4). The NH_3 losses from faeces were also greater in the Fertilised pasture, fol-

Table 2
Forage and nutrient intake, and enteric methane emissions of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped (Mixed) with forage peanut.

Variables	Pasture type (PT)			Season (S)				SEM	*P*-value		
	Fertilised	Mixed	Control	Summer	Autumn	Winter	Spring		PT	S	PT*S
Animal performance											
DMI, kg/d	7.53	6.55	6.34	7.16^B	5.88^C	4.67^D	9.52^A	0.372	0.056	<0.001	0.529
dOMI, kg/d	4.24^a	3.51^b	3.31^b	3.91^B	2.85^C	1.86^D	6.13^A	0.250	0.027	<0.001	0.281
NDFI, kg/d	4.33	3.77	3.91	4.92^B	3.58^C	2.79^D	5.37^A	0.221	0.094	<0.001	0.562
CPI, g/d	986^a	739^b	563^c	893^B	576^C	368^D	$1\ 215^A$	67.4	<0.001	<0.001	0.391
ADG, kg/d	0.468	0.434	0.398	0.597^B	0.420^C	0.01^D	0.713^A	0.032	0.439	<0.001	0.432
SR, AU/ha	3.12^a	2.23^b	1.90^b	3.36^A	2.70^B	1.09^C	2.50^B	0.21	<0.001	<0.001	0.613
Methane emissions											
CH_4 g/animal per day	170^a	148^b	165^a	145^C	156^B	162^B	181^A	4.62	0.001	<0.001	0.801
CH4, g/d per kg BW	0.513^a	0.481^b	0.525^a	0.515	0.505	0.495	0.510	0.040	0.018	0.663	0.786
CH_4 g/d per kg MW	2.18^a	2.01^b	2.21^a	2.10	2.12	2.10	2.21	0.12	0.009	0.386	0.803
$CH_{4,}$ g/kg DMI	26.7^b	26.8^b	30.2^a	23.1^C	29.0^B	38.1^A	21.4^C	1.43	0.044	<0.001	0.247
$CH_{4,}$ g/kg dOMI	51.3^b	56.9^b	66.5^a	42.7^C	59.3^B	98.4^A	32.6^C	4.05	0.008	<0.001	0.127

Note: Least squares means within a row with different lowercase for PTs and uppercase letters for Seasons differ at $P \leq 0.05$.
Abbreviations: DMI = DM intake; dOMI = digestible organic matter intake, NDFI = NDF intake; CPI = CP intake; ADG = Average daily gain; SR = Stocking rate; AU = Animal unit (500 kg BW); MW = Metabolic weight ($BW^{0.75}$).

Table 3
Nitrogen excretion and N_2O and NH_3 emissions from faeces and urine of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped with forage peanut (Mixed) during the different seasons of the year.

Variables	Pasture type (PT)			Season (S)				SEM	*P*-value		
	Fertilised	Mixed	Control	Summer	Autumn	Winter	Spring		PT	S	PT*S
N excreted in the livestock excreta											
Total Nexc.,g/animal per day	122.3^{a}	96.3^{b}	80.7^{b}	103.1^{B}	97.4^{B}	72.0^{C}	127.0^{A}	5.8	<0.001	<0.001	0.602
Urine Nexc.,g/animal per day	70.6^{a}	50.3^{b}	39.6^{b}	51.0^{B}	53.7^{B}	35.8^{C}	73.4^{A}	4.8	<0.001	<0.001	0.189
Faeces Nexc.,g/animal per day	51.7^{a}	46.0^{ab}	41.3^{b}	52.0^{A}	43.7^{AB}	36.1^{B}	53.5^{A}	3.3	0.039	<0.001	0.960
Nexc. urine/Nexc. faeces ratio	1.50^{a}	1.20^{ab}	0.99^{b}	1.02	1.29	1.15	1.45	0.16	0.001	0.110	0.476
Nitrous oxide emissions, g/animal per day											
N_2O total	0.936^{a}	0.559^{b}	0.394^{b}	0.480^{B}	0.833^{A}	0.568^{B}	0.637^{B}	0.056	<0.001	<0.001	0.290
N_2O from urine	0.767^{a}	0.479^{b}	0.259^{c}	0.346^{B}	0.704^{A}	0.459^{B}	0.499^{B}	0.058	<0.001	<0.001	0.300
N_2O from faeces	0.169^{a}	0.079^{c}	0.135^{b}	0.134^{AB}	0.130^{AB}	0.109^{B}	0.138^{A}	0.009	<0.001	0.038	0.740
Ammonia volatilised, g/animal per day											
NH_3 total	10.50^{a}	2.96^{b}	2.16^{b}	1.65^{C}	10.39^{A}	6.52^{B}	2.26^{C}	0.81	<0.001	<0.001	0.101
NH_3 from urine	10.11^{a}	2.62^{b}	1.85^{b}	1.27^{C}	10.06^{A}	6.25^{B}	1.87^{C}	0.82	<0.001	<0.001	0.121
NH_3 from faeces	0.383^{a}	0.341^{ab}	0.306^{b}	0.385^{A}	0.324^{AB}	0.267^{B}	0.396^{A}	0.024	0.039	<0.001	0.959

Note: Least squares means within a row with different lowercase for PTs and uppercase letters for Seasons differ at $P \leq 0.05$.
Abbreviations: Nexc. = Nitrogen excretion; N_2O = Nitrous oxide; NH_3 = Ammonia.

lowed by the Mixed, and lower for the Control pasture ($P = 0.039$). In relation to the seasons, the greatest total and urine N_2O and NH_3 emissions were recorded for the autumn ($P < 0.001$). For the N_2O and NH_3 emissions from the faeces, more emissions were recorded for the spring, summer, and autumn ($P = 0.038$ and $P < 0.001$, respectively).

The total and urine N excretion per hectare were on average 95.0 and 67.0% greater in the Fertilised pasture when compared to the other pasture types ($P < 0.001$; Table 4). The N excreted in faeces was also greater for the Fertilised pasture, followed by the Mixed and Control pastures ($P = 0.003$). N_2O emissions per hectare from the Fertilised pasture were 184, 201 and 129% greater for the total, urine, and faeces N_2O emissions, respectively, from the Mixed and Control PTs ($P < 0.001$). Total and urine NH_3 emissions were 76 and 78% lower for the Control and Mixed pastures, respectively, when compared to the Fertilised pasture ($P < 0.001$). The NH_3 emissions per hectare from faeces were greater for the Fertilised pasture, followed by the Mixed pasture and lowest for the Control ($P = 0.003$). In relation to the seasons, total and faecal N excretion per hectare were greater during the summer ($P < 0.001$). Urine N excretion per hectare was lower in winter compared to the other seasons ($P < 0.001$). Total and urine N_2O and NH_3 emissions per hectare were greatest in the autumn ($P < 0.001$). On the other hand, faecal emissions per hectare of N_2O and NH_3 were greater during summer ($P < 0.001$).

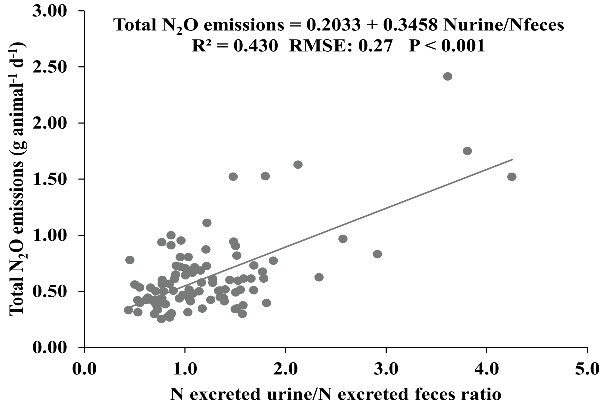

Fig. 2. Direct and indirect N_2O emissions (g/animal per day) as a function of the N excreted from urine/N excreted from faecal ratio of Nellore heifers grazing different pasture types. (Total N_2O emissions = 0.2033 + 0.3458 × Nurine/Nfaeces ratio; $P < 0.001$ and $P < 0.001$ for intercept and slope, respectively; $R^2 = 0.430$).

The N_2O emissions for mg/d per kg BW, mg/d per kg MW and g/kg DMI were greatest for the Fertilised pasture ($P < 0.001$; Table 5). More N_2O kg/dOMI was recorded for the Fertilised pasture, followed by the Mixed pasture, and with a lower emission for the Control pasture ($P = 0.006$). There was no statistically significant effect of the PT on N_2O emissions per kg of CPI ($P = 0.167$). All relationships between NH_3 emissions from faeces and urine and animals and diets were higher for the Fertilised pasture ($P < 0.001$) when compared to the other PTs.

Regarding the seasons, the N_2O and NH_3 emissions as a function of body and metabolic weight (g/d per kg BW and g/d per kg MW, respectively) were greatest for the autumn ($P < 0.001$). The N_2O and NH_3 emissions as a function of forage and nutrient intake (g/kg DMI, g/kg dOMI and g/kg CPI) increased in the autumn and winter seasons compared to summer and spring ($P < 0.001$).

Greenhouse gas emissions on different pasture types

There was no difference between PTs for the final and initial BW, carcass weight, and carcass weight gain ($P \geq 0.100$; with an average of 234, 387, 187, and 83.4 kg, respectively; Table 6). The PT with nitrogen fertilisation (Fertilised) showed the greatest liveweight gain per area ($P < 0.001$), with an increase of 103% when compared to the Control pasture. In the Mixed pasture, the liveweight gain per area was 31.6% greater than in the Control pasture. For annual CH_4 emissions, the animals in the Mixed pasture had lower emissions ($P = 0.009$) by 12.6 and 10.1%, respectively, when compared to the Fertilised and Control pastures. There was a tendency for greater CH_4 emissions by animals per kg of BW gain in the Control pasture ($P = 0.069$). A similar result was recorded for CH_4 per kg carcass weight gain, in which animals in the Control pasture showed higher CH_4 emissions ($P = 0.005$). The greatest CH_4 emission per hectare was recorded for the Fertilised pasture ($P < 0.001$).

For annual N_2O emissions and per kg carcass weight gain, the values were 40 and 37% lower, respectively, in the Mixed treatment compared to the Fertilised pasture ($P < 0.001$). However, the annual N_2O emission and the N_2O emission per kg carcass weight gain of the Mixed pasture were 62 and 31% greater, respectively, when compared to the Control pasture. The N_2O emission from the Fertilised pasture was 82% (g of N_2O/kg BW gain) and 185% (g of N_2O/ha per year) higher per BW gain and per ha when compared to the Control and Mixed pastures ($P < 0.001$).

More CO_2eq emissions per animal were recorded for the Fertilised pasture, where emissions were 16.5 and 6.0% greater than

Table 4
Nitrogen excretion and emissions of N_2O and NH_3 from faeces and urine per hectare of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped with forage peanut (Mixed) during the different seasons of the year.

Variables	Pasture type (PT)			Season (S)				SEM	P-value		
	Fertilised	Mixed	Control	Summer	Autumn	Winter	Spring		PT	S	PT*S
Excretion of N, kg/ha per season											
Total Nexc.	55.7^{a}	33.4^{b}	23.8^{b}	57.6^{A}	40.7^{B}	12.1^{C}	40.0^{B}	5.1	<0.001	<0.001	0.117
Urine Nexc.	32.0^{a}	16.9^{b}	11.5^{b}	29.0^{A}	23.2^{A}	5.1^{B}	23.2^{A}	2.7	<0.001	<0.001	0.244
Faeces Nexc.	23.7^{a}	16.5^{ab}	12.2^{b}	28.5^{A}	17.5^{B}	7.1^{C}	16.8^{B}	2.7	0.003	<0.001	0.630
Nitrous oxide emissions, g/ha per season											
N_2O total	262.2^{a}	113.9^{b}	70.3^{b}	175.6^{AB}	232.6^{A}	55.7^{C}	131.3^{B}	24.2	<0.001	<0.001	0.110
N_2O from urine	213.2^{a}	96.3^{b}	45.1^{b}	128.4^{B}	199.1^{A}	41.8^{C}	103.5^{B}	19.7	<0.001	<0.001	0.098
N_2O from faeces	49.0^{a}	17.6^{b}	25.2^{b}	47.3^{A}	33.5^{B}	13.8^{C}	27.8^{B}	5.1	<0.001	<0.001	0.084
Ammonia volatilised, g/ha per season											
NH_3 total	3 619.2^{a}	667.7^{b}	434.9^{b}	840.1^{B}	3 976.7^{A}	807.3^{B}	671.7^{B}	394.5	<0.001	<0.001	0.078
NH_3 from urine	3 474.5^{a}	567.1^{b}	360.1^{b}	666.1^{B}	3 869.6^{A}	764.0^{B}	569.2^{B}	381.5	<0.001	<0.001	0.068
NH_3 from faeces	144.7^{a}	100.6^{ab}	74.8^{b}	174.0^{A}	107.1^{B}	43.3^{C}	102.4^{B}	16.6	0.003	<0.001	0.630

Note: Least squares means within a row with different lowercase for PTs and uppercase letters for Seasons differ at $P \leq 0.05$.
Abbreviations: Nexc. = Nitrogen excretion; N_2O = Nitrous oxide; NH_3 = Ammonia.

Table 5
Relationship between N_2O and NH_3 emissions from faeces and urine per animal with animals and diet characteristics of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped with forage peanut (Mixed) during the different seasons of the year.

Variables	Pasture type (PT)			Season (S)				SEM	P-value		
	Fertilised	Mixed	Control	Summer	Autumn	Winter	Spring		PT	S	PT*S
Nitrous oxide emissions											
N_2O, mg/d per kg BW	2.81^{a}	1.80^{b}	1.26^{c}	1.71^{B}	2.64^{A}	1.70^{B}	1.77^{B}	0.14	<0.001	<0.001	0.093
N_2O, mg/d per kg MW	12.0^{a}	7.54^{b}	5.29^{c}	6.98^{B}	11.1^{A}	7.28^{B}	7.72^{B}	0.59	<0.001	<0.001	0.094
N_2O, g/kg DMI	0.148^{a}	0.098^{b}	0.069^{b}	0.071^{B}	0.148^{A}	0.131^{A}	0.070^{B}	0.014	<0.001	<0.001	0.066
N_2O, g/kg dOMI	0.301^{a}	0.223^{ab}	0.159^{b}	0.140^{B}	0.314^{A}	0.346^{A}	0.110^{B}	0.037	0.006	<0.001	0.148
N_2O, g/kg CPI	1.34	1.11	0.98	0.59^{B}	1.49^{A}	1.90^{A}	0.59^{B}	0.24	0.167	<0.001	0.870
Ammonia volatilised											
NH_3, g/d per kg BW	31.50^{a}	9.52^{b}	6.91^{b}	5.81^{C}	32.64^{A}	19.27^{B}	6.17^{C}	2.05	<0.001	<0.001	0.104
NH_3, g/d per kg MW	134.4^{a}	39.9^{b}	29.0^{b}	23.9^{C}	137.7^{A}	82.6^{B}	27.0^{C}	8.9	<0.001	<0.001	0.105
NH_3, g/kg DMI	1.80^{a}	0.61^{b}	0.44^{b}	0.25^{B}	1.84^{A}	1.48^{A}	0.24^{B}	0.21	<0.001	<0.001	0.080
NH_3, g/kg dOMI	3.81^{a}	1.51^{b}	1.09^{b}	0.48^{B}	3.87^{A}	3.82^{A}	0.37^{B}	0.56	<0.001	<0.001	0.106
NH_3, g/kg CPI	17.11^{a}	7.61^{b}	7.03^{b}	1.89^{B}	17.28^{A}	21.25^{A}	1.92^{B}	3.43	<0.001	<0.001	0.174

Note: Least squares means within a row with different lowercase for PTs and uppercase letters for Seasons differ at $P \leq 0.05$.
Abbreviations: MW = Metabolic weight (=BW$^{0.75}$); DMI = dry matter intake; dOMI = digestible organic matter intake; CPI = Crude protein intake.

for the Mixed and Control pastures, respectively (P = 0.004). There was a tendency for more CO_2eq emissions by animals per kg of BW gain in the Control pasture (P = 0.096). A similar result was recorded for CO_2eq per kg carcass weight gain, in which animals in the Control pasture showed greater CO_2eq emissions (P = 0.005). The Fertilised pasture had 54.6 and 63.8% greater total CO_2eq emissions per ha than the Control and Mixed pastures (P < 0.001).

The total GHG emissions of the Mixed pasture were 44.9% lower than those from the Fertilised pasture (P < 0.001), but 5.9% higher than the Control pasture (Fig. 3A). In the Fertilised pasture, the GHG emissions from N fertiliser application corresponded to 0.800 Mg CO_2eq/ha or 8.0% of the total emission in this PT. Likewise, the N fertiliser synthesis and transport contributed to 0.675 Mg CO_2eq/ha or 6.7% of the total emission for the Fertilised pasture. The Mixed pasture was 18.8% lower in total GHG emissions per kg carcass yield (P < 0.001) than the Control pasture (Fig. 3B). When compared to Fertilised pasture, the Mixed pasture had total GHG emissions per kg carcass yield 22.8% lower.

Discussion

How does N input (via fertiliser or biological N fixation) affect animal productivity?

Animal performance and stocking rate are the main metrics responsible for improving animal productivity in pasture systems (McCarthy et al., 2016; Pereira et al., 2020). The ADG of grazing animals is directly related to the forage intake capacity and the nutritive value of the diet (Mertens, 1994). Despite the difference in the forage nutritive value between PTs, in which the diet of the animals in the Fertilised pasture presented a greater CP and lower NDF concentrations (Homem et al., 2021a), the similarity of ADG between treatments suggests that the canopy structure prevails over the forage nutritive value (Farias et al., 2020).

In the current study, the same grazing management criteria were adopted (similar canopy height target), which ensured that canopies in all PTs had a similar structure with minimal variation (Homem et al., 2021b). Thus, the animals had the same DMI owing to minimal differences in the canopy structure between PTs, which probably was compensated for by changes in the animal's ingestive behaviour resulting in similar ADG. In a different way, Homem et al. (2021b) found that animals on pasture with either N fertilisation or legume N_2 fixation showed higher ADG than animals on Palisade grass in monoculture without N addition. Homem et al. (2021b) estimated that the ADG of heifers for the Fertilised and Mixed pastures was, respectively, 92 and 67 g/day greater than for the Control treatment. However, in this study, the experimental period was only in three seasons (spring, summer, and autumn), in which the highest temperatures and accumulated rainfall are found, being the best period for animal performance. In the present study, all four seasons of the year were evaluated. Thus, the winter period was included, where the animals had practically zero weight gain (~0.01 kg/day).

Table 6
Carcass characteristics, liveweight gain per area, and enteric CH_4, N_2O, and CO_2eq emissions per year of Nellore heifers grazing Palisade grass with (Fertilised) or without (Control) N fertilisation or intercropped with forage peanut (Mixed).

Variables	Pasture types			SEM	*P*-value
	Fertilised	Mixed	Control		
Animal performance					
BW initial, kg	237	230	234	33.4	0.596
BW final, kg	397	383	380	23.0	0.384
Carcass weight, kg	192	186	182	15.7	0.196
Carcass weight gain, kg/animal	88.3	84.4	77.4	4.08	0.100
BW gain, kg/ha per year	648^{a}	421^{b}	320^{c}	55.7	<0.001
Methane emissions					
CH_4, kg/animal per year	61.9^{a}	54.1^{b}	60.2^{a}	1.65	0.009
CH_4, g/kg BW gain	391	362	422	37.9	0.069
CH_4, g/kg carcass weight gain	702^{ab}	650^{b}	788^{a}	35.3	0.005
CH_4, kg/ha per year	297^{a}	196^{b}	187^{b}	19	<0.001
Nitrous oxide emissions					
N_2O, g/animal per year	341^{a}	204^{b}	144^{c}	20	<0.001
N_2O, g/kg BW gain	2.17^{a}	1.37^{b}	1.00^{b}	0.22	<0.001
N_2O, g/kg carcass weight gain	3.88^{a}	2.45^{b}	1.87^{c}	0.28	<0.001
N_2O, g/ha per year	$1\,049^{a}$	456^{b}	281^{b}	91	<0.001
CO_2eq emissions[†]					
CO_2eq, kg/animal per year	$1\,778^{a}$	$1\,526^{b}$	$1\,677^{a}$	45	0.004
CO_2eq, kg/kg BW gain	11.2	10.2	11.7	1.1	0.096
CO_2eq, kg/kg carcass weight gain	20.3^{ab}	18.4^{b}	22.7^{a}	1.1	0.008
CO_2eq, kg/ha per year	$8\,544^{a}$	$5\,525^{b}$	$5\,216^{b}$	571	<0.001

Note: Least squares means within a row with different lowercase letters differ at $P \le 0.05$.
Abbreviations: CH_4 = Enteric methane; N_2O = Nitrous oxide from livestock excreta.
[†] Carbon Dioxide Equivalent (CO_2eq) emissions associated with enteric CH_4 and, direct and indirect N_2O emissions from faeces and urine.

The Fertilised pasture increased the stocking rate by an average of 51% when compared to the other PTs, which was due to greater forage production (Delevatti et al., 2019; Pereira et al., 2015). The N input into the system directly influences the plant dynamics, which alters its structural characteristics by increasing the morphogenetic rhythm, density, and tiller appearance rate, due to the faster generation of new leaves and axillary buds (Paiva et al., 2012; 2015; Yasuoka et al., 2018). Thus, as the same grazing management target was used in the current study, the increase in the canopy growth rate necessitated more animals in the area, resulting in an increase in the stocking rate. This increase in stocking rate for the Fertilised pasture increased the liveweight gain per area by 103 and 54% compared to the Control and Mixed pastures, respectively.

It was expected that the Mixed pasture would present a greater stocking rate than the Control pasture without N fertilisation since legumes have the capacity for BNF (Moreira and Siqueira, 2006). However, the lack of a large difference in stocking rate between the Control and Mixed pastures was partially linked to the seasonality of forage peanut production in the Cerrado/Atlantic Forest transition biome. During the dry period, there was a drastic reduction in legume production. Homem et al. (2021c) reported a reduction of 50.9% in the forage peanut mass for the winter compared to other seasons. Furthermore, the transfer of N fixed by the legume to the companion grass is gradual and initially has much less impact than the N fertiliser application (Liu et al., 2017).

In a long-term experiment (9 years) carried out in the Atlantic Forest biome, it was found that the stocking rates increased significantly to maintain the same grazing management targets on a Palisade grass/forage peanut mixed pasture or Palisade grass monoculture fertilised with 120 kg of N/ha per year (Pereira et al., 2020). In this study, the annual liveweight gain per area was 789 kg/ha for the Mixed pasture compared to 655 kg/ha for the Fertilised pasture (Pereira et al., 2020). Thus, it has been observed that after 2 or 3 years following mixed pasture establishment, there is significant N transfer from the legume to the companion grass and this is registered as an increase in grass N concentration (dos Santos et al., 2023; Monteiro, 2020). As the present study was carried out shortly after the establishment of the Mixed pasture, less of the N fixed by forage peanut was cycled into the system and did not cause such a large increase in productivity as in the Fertilised system (Homem et al., 2021c).

However, when comparing the effect of legumes introduction to the system, the Mixed pasture increased the liveweight gain per area by 31.5% when compared to monoculture Palisade grass without N addition. Thus, these results demonstrate that legumes may be a sustainable alternative, as they introduce N into the system via biological fixation and improve the diet's nutritive value, resulting in greater animal productivity even shortly after establishment.

How does N input (via fertiliser or biological N fixation) affect enteric CH_4 emissions?

Livestock farming faces a major challenge today, which consists of reconciling animal productivity with sustainability in order to reduce GHG emissions. Enteric CH_4 is the predominant source of GHG emissions in livestock systems due to the GWP in the atmosphere compared to CO_2 (Crosson et al., 2011; Guerci et al., 2013).

Enteric CH_4 production is directly affected by diet quality and rumen fermentation conditions (Ruggieri et al., 2020). In the present study, the use of forage peanut reduced CH_4 emissions (in g/animal, g/kg BW, g/kg DMI, and kg/animal per year) in relation to animals grazing in the Palisade grass pastures with or without N application. This can be explained by the lower fibre content, higher passage rate and the presence of secondary compounds, such as tannins and saponins in the legumes when compared to tropical grasses (Beauchemin et al., 2008).

The C_4 grasses have a higher fibre concentration with a lower digestibility compared to most forage legumes (Gomes et al., 2018; Homem et al., 2021a). The lower amount of fibre in legumes may be partially responsible for the decrease in CH_4 production by animals grazing Mixed pasture (Pinares-Patiño et al., 2003). With C_4 grasses, the fibre is retained in the rumen for a longer time and exposed to rumen microorganisms, increasing acetate production, and consequently, resulting in greater CH_4 production (Archimède et al., 2018). The presence of secondary metabolites

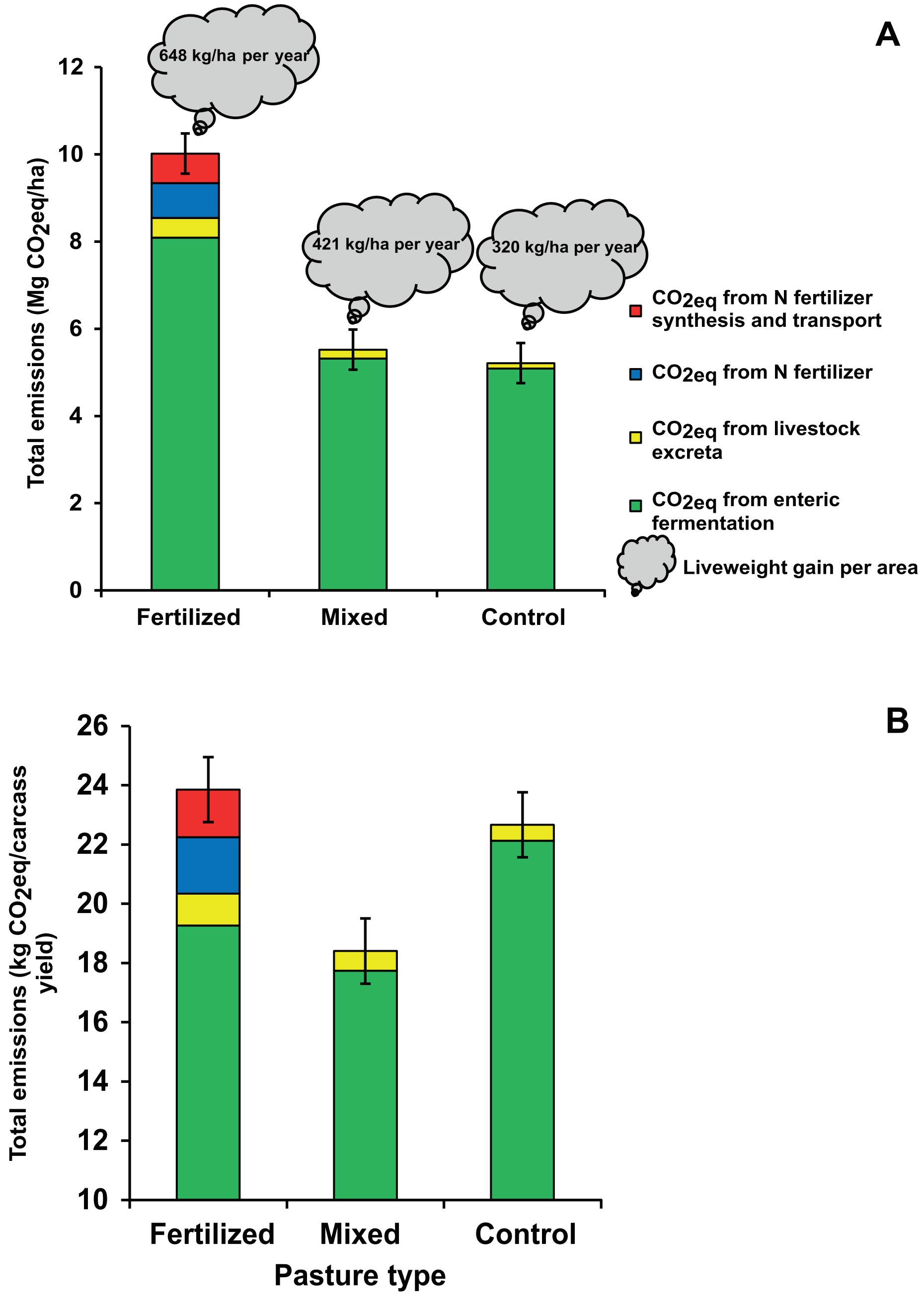

Fig. 3. Total emissions by area (A; Mg CO_2eq/ha) and in CO_2 equivalents per kg of carcass yield of Nellore heifers (B; kg CO_2eq/kg carcass yield) from Palisade grass pastures with (Fertilised) or without (Control) N fertilisation or intercropped (Mixed) with forage peanut. Error bars represent ± SEM.

in legumes such as condensed tannins can inhibit ruminal methanogenesis (Andrade et al., 2016; Jayanegara et al., 2012) through bactericidal or bacteriostatic action, inhibiting the multiplication or activity of methanogenic *Archaea* in the rumen (Aboagye and Beauchemin, 2019). In the present study, the heifers in the Mixed pasture had an average condensed tannin intake of 42 g/day versus no condensed tannin intake for heifers in the monoculture Palisade grass pastures.

Conversely, animals on Palisade grass monocultures, with or without N fertilisation, showed higher CH_4 emissions per animal. However, only the Fertilised pasture had greater annual CH_4 emissions per hectare, which can be explained by the greatest stocking rate of this PT. The great advantage of intensifying grass-fed beef operations with the use of N fertiliser is not directly associated with enteric CH_4 emissions, but rather in the reduction of the area necessary to produce the same amount of product, the so-called “land-saving effect” (Cardoso et al., 2016). Thus, although N fertilisation increases animal productivity and promotes greater liveweight gain per unit area, the use of N fertilisers can increase GHG emissions (Grassmann et al., 2020; Raposo et al., 2020). However, the use of legumes, such as forage peanut, promotes an increase in meat production without increasing CH_4 emissions, although requiring somewhat more land for the same production target, in some circumstances.

How does N input (via fertiliser or biological N fixation) affect N excretions and N_2O and NH_3 emissions?

The use of N fertiliser leads to an increase in the non-protein N proportion of the plant, which has high degradability in the rumen

(Abbasi et al., 2012; Peyraud and Astigarraga, 1998). The large supply of readily available N in the rumen increases losses mainly via urinary excretion (Homem et al., 2021b, 2021c). Thus, heifers in the Fertilised pasture showed 95 and 125% greater total and urinary N excretion compared to other PTs, respectively. More N excreted from urine in the Fertilised pasture is linked to the greatest CP intake. Furthermore, greater stocking rates in the Fertilised pasture increased the N excreted per hectare.

The heifers in the Mixed pasture had greater N intake than heifers in the Control pasture; however, there were no differences in the total and urinary N excretion between these pastures, demonstrating that the animals had greater efficiency of N use in the Mixed pasture. In the forage peanut, a large part of the protein fractionation is found in the fraction linked to potentially digestible fibre (Gomes et al., 2018). In this way, this N from forage peanut will be released into the rumen gradually, which will enhance synergism with carbohydrate degradability and consequently, increase microbial protein synthesis (Homem et al., 2021b). Another advantage of forage peanut shown in this experiment is the reduction in the ratio of N excreted in urine to N excreted in faeces. As demonstrated in several studies (Guimarães et al., 2022; Lessa et al., 2014; Van der Weerden et al., 2011), urine has a greater N_2O emission factor than faeces. Furthermore, N_2O emissions are directly linked to the N-urine/N-faeces ratio, showing a positive linear relationship. Condensed tannins present in the legume can bind to protein, and thus reduce the degradable protein and increase the non-degradable protein in the rumen (Castro-Montoya et al., 2018). Less N being made available in the rumen causes a smaller supply of N in the ruminal environment, causing less N excretion in the urine. Furthermore, part of this complexed N with tannins makes faecal N more recalcitrant and less available, which allows the release of mineral N into the soil to occur slowly (Guimarães et al., 2022).

The presence of legumes increases N cycling (Homem et al., 2021c), which could increase N_2O emissions from forage residues (litter) after N mineralisation (Boddey et al., 2020). However, in the present study, N excretions and N_2O emissions were lower for the Mixed pasture compared to the Fertilised pasture, showing that the inclusion of forage peanut is a viable strategy for introducing N into the system with lower N_2O emissions. A further very important advantage of using forage legumes instead of N fertiliser is that the emissions of fossil CO_2 incurred in the manufacture, transport, and application of N fertiliser are completely avoided (Jensen et al., 2012).

Regarding NH_3 emissions, as mentioned previously, the use of N fertiliser increases the concentration of non-protein N in the plant, which is quickly converted into N-NH_3 by ruminal microorganisms, favouring microbial protein synthesis and efficiency of N use (Mota et al., 2022). However, when the ruminal degradation rate of protein is greater than the assimilation capacity for microbial synthesis, excess NH_3 is converted into urea in the liver and excreted in the urine (Getahun et al., 2019). Thus, the Fertilised system presented a greater emission of total NH_3 from urine compared to the other PTs, mainly due to the greater N contribution in urea being added to the soil via urine (Guimarães et al., 2022). Conversely, as previously discussed, animals grazing Mixed pasture will have N released into the rumen gradually, which will enhance synergism with carbohydrate degradability and, consequently, decrease urinary N excretion and NH_3 emissions.

How does N input (via fertiliser or biological N fixation) affect total emissions from pasture types?

Within grass-fed beef operations, enteric CH_4 represents the greenhouse gas with the largest share of emissions (Crosson et al., 2011; Guerci et al., 2013). In the present study, enteric CH_4 accounted for more than 80% of the total CO_2eq emitted for the Fertilised pasture and more than 95% for the Mixed and Control pastures. Thus, even though N_2O has a GWP around 10 times that of CH_4 (273 vs 27.2 GWP, respectively), mitigation strategies that act to reduce enteric CH_4 emissions will have a greater impact on GHG mitigation in pasture-based livestock production systems.

The Fertilised pasture increased total emissions per hectare by around 84.2% compared to the other PTs. In addition to the animals in this pasture presenting greater CH_4 and N_2O emissions, CO_2eq emissions from the N fertiliser application were responsible for increasing total emissions by 8.0%. Furthermore, if the expenditure of fossil energy associated with the Haber-Bosch process of N fertiliser synthesis and its transport is included, there is a further increase of 6.7% of the total emissions for the Fertilised pasture per hectare (2.25 kg CO_2eq/kg N urea; Robertson and Grace, 2004).

Even though the Fertilised pasture had greater animal production capacity, and consequently, greater carcass yield per area, its emissions were also greater per kg of carcass yield when compared to the Mixed pasture. Therefore, the use of N fertilisation in pasture systems leads principally to mitigation through the ‘‘land saving” effect. Conversely, Mazzetto et al. (2015) and Cardoso et al. (2016) showed that emission intensity (emissions per kg product) of beef production in Brazil could be reduced by 40% or more with the application of N fertiliser compared to degraded pastures. The results of the current experiment deviate from these studies due to differences in grazing management. In the current study, all treatments were managed under the same grazing management target, which implied similar forage intake and animal performance. Thus, improved grazing management stands as the initial step in improving the sustainability of pasture-based beef livestock operations.

Palisade grass/forage peanut mixed pasture provided a total CO_2eq emission similar to the Palisade grass system in monoculture without N fertilisation (Control). With the Mixed pasture, there was an increase of 31.5% in the liveweight gain per area without increasing total GHG emissions per hectare compared to the Control pasture. A more promising result is observed when we evaluate emissions per kg of carcass yield, in which with the Mixed pasture, there was a mitigation of 18.8% when compared to the Control pasture.

When we compared the N replacement via fertilisation with the BNF, there was a decrease of 4.5 Mg CO_2eq/ha, which represented a reduction of 44.9% of CO_2eq com BNF. In the Fertilised pasture, the stocking rate was greater than the Mixed pasture (3.12 vs 2.23 AU/ha, respectively), which directly impacted greater total emissions per area. However, the magnitude of the increase in the stocking rate (28.5%) for the Fertilised pasture in relation to the Mixed pasture was less than the increase in emissions.

The mitigation advantages of using forage peanut become clearer when we evaluate the emissions per kg of carcass yield. The animals in the Mixed pasture had the same carcass gain as the animals in the Fertilised pasture. However, there was a CO_2eq mitigation of approximately 22.8% when we compared the use of forage peanut to replace 150 kg N/ha per year via fertiliser. The magnitude of the results with the use of forage peanut obtained in this work will probably increase over the years, given that the present study was carried out shortly after its establishment. Homem et al. (2021c) reported that the forage peanut establishment is slow, and a large part of their photoassimilates will initially be used to colonise the area. Furthermore, the transfer of N fixed by forage peanut to the accompanying grass is much slower compared to the application of N fertiliser, which has an immediate impact on the grass yield. However, the use of forage peanut shows great promise in the recovery of degraded pastures in tropical regions, as it is a highly viable alternative for many regions by increasing animal production (Pereira et al., 2020; Homem et al., 2021b), improving soil quality (Homem et al., 2021c; Souza et al., 2023) and mitigating GHG emissions.

There are several feed additives that can reduce enteric methane emissions without reducing animal performance or feed intake. These range from single compounds such as 3-nitrooxypropanol (3-NOP) to tannins and extracts of seaweed, such as *Asparagopsis taxiformis* or *A. armata* (Cardoso-Gutierrez et al., 2021; Hristov et al., 2022; Roque et al., 2021). However, all these options come at a financial cost and will not be adopted if consumers are unwilling to pay a higher price for animal products with a decreased carbon footprint. Results from our studies show that after 2–3 years, the stoloniferous legumes *Arachis pintoi* and *Desmodium ovalifolium* will produce the same liveweight gains as annual additions of 120–150 kg N/ha (Pereira et al., 2020; dos Santos et al., 2023). Furthermore, forage peanut would produce 28% less CO_2eq emissions per hectare when compared to applying N fertiliser. Thus, the cost of introduction of forage legumes to tropical pastures with management that ensures their persistence is compensated within a few years by the savings on N fertiliser. In the face of this scenario, public policies in tropical regions should adopt pasture-based beef livestock operations with forage peanut in the face of the challenge of recovering degraded pastures as proposed during Conference of the Parties (COP) 28.

Conclusions

It is clear from this study that the adoption of pastures combined with the use of forage peanut in tropical regions is one of the most promising alternatives for intensifying pasture-based beef cattle operations with a reduction in GHG emissions per kg of carcass yield, the so-called carbon footprint. The use of forage peanut to replace the N fertiliser application resulted in a GHG mitigation of 23% per kg of product. Given the global scenario of climate change and the tropical region's challenge in the recovery of degraded pastures, pastures mixed with forage peanut or other stoloniferous legumes are a promising solution by providing improved animal production without increasing greenhouse gas emissions in a sustainable way.

Supplementary material

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Ethics approval

The experimental procedures for this study were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras (protocol number 064/2015).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings and models are available from authors upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

Bruno G. C. Homem: https://orcid.org/0000-0001-7787-0133.
Lucas P. C. Borges: https://orcid.org/0000-0002-2719-789X.
Italo Braz G. de Lima: https://orcid.org/0000-0002-4026-5260.
B.C. Guimarães: https://orcid.org/0000-0003-3098-0567.
Paola P. Spasiani: https://orcid.org/0000-0001-6078-5303.
Igor M. Ferreira: https://orcid.org/0000-0003-3389-7806.
Paulo Meo-Filho: https://orcid.org/0000-0002-4292-6210.
Alexandre Berndt: https://orcid.org/0000-0002-8976-2399.
Bruno J. R. Alves: https://orcid.org/0000-0002-5356-4032.
Segundo Urquiaga: https://orcid.org/0000-0002-3601-1233.
Robert M. Boddey: https://orcid.org/0000-0003-3648-9859.
D. R. Casagrande: https://orcid.org/0000-0003-0732-6196.

CRediT authorship contribution statement

B.G.C. Homem: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **L.P.C. Borges:** Methodology, Investigation. **I.B.G. de Lima:** Methodology, Investigation. **B.C. Guimarães:** Methodology, Investigation. **P.P. Spasiani:** Methodology, Investigation. **I.M. Ferreira:** Methodology, Investigation. **P. Meo-Filho:** Methodology, Investigation. **A. Berndt:** Writing – review & editing, Resources, Methodology, Conceptualization. **B.J. R. Alves:** Writing – review & editing, Resources, Methodology, Conceptualization. **S. Urquiaga:** Writing – review & editing, Resources, Methodology. **R.M. Boddey:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **D.R. Casagrande:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of interest

None.

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