Legume and Nitrogen Fertilization Affect Animal Performance and Enteric Methane Emission of Nellore Heifers

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Legume and nitrogen fertilization affect animal performance and enteric methane emission of Nellore heifers

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Key words: *Arachis pintoi*; beef cattle; *Brachiaria;* greenhouse gas; warm-season legume

Abstract

Methane emission from livestock operation is an important source of greenhouse gas and contributes to global warming. Forage legume secondary compounds may mitigate methane emissions by reducing methanogenic population in the rumen. This study evaluated animal performance and methane emission from beef cattle grazing either a mixed pasture [*Brachiaria brizantha* cv. Marandu (palisadegrass) and *Arachis pintoi* (forage peanut) cv. BRS Mandobi] or a palisadegrass monoculture with or without nitrogen (N) fertilisation. A 2.5-yr continuous stocking experiment was carried out in southeast Brazil, on a randomized complete block design with three treatments and four replicates. Two Nellore heifers were used as tester animals and additional put-and-takes were used to keep canopy height at 20-25 cm. The treatments comprised three pasture types: 1) palisadegrass-forage peanut mixed pasture **(GRASS+LEGUME)**; 2) palisadegrass + 150 kg N/ha/year **(GRASS+N)**; 3) palisadegrass without N fertilization **(GRASS)**. Response variables included average daily gain (ADG), forage intake, and methane emission. Methane emission was estimated by the sulphur hexafluoride (SF_6) tracer technique. There was no difference between grazing systems for the ADG ($P = 0.439$) and DMI ($P = 0.394$; averages of 0.433 kg/d and 2.10 %BW/d, respectively). In the GRASS+LEGUME, there was a decrease of 11.7% in methane emission per animal (148 vs. 170 and 165 g/day for GRASS+N and GRASS, respectively; $P = 0.001$). Grazing systems including legume reduced methane emission per unit of ADG (365 vs. 428 and 398 g/kg for GRASS and GRASS+N, respectively; $P = 0.061$) and per carcass gain (656 vs. 800 g of methane/kg carcass for GRASS; $P = 0.022$). Intake of condensed tannins was greater for GRASS+LEGUME (0.61 vs. 0.17 %BW/d, $P < 0.001$). Forage peanut decreased enteric methane emission intensity, reducing carbon footprint of livestock systems in Southeast Brazil.

Introduction

Climatic changes due to anthropogenic greenhouse gas emissions are a crucial threat to future food production. Ruminant animals are the largest cause of agricultural externalities in terms of land use and emissions of greenhouse gas, such as methane (CH_4; Oliveira Silva et al. 2016). The utilization of appropriate management practices in grass-livestock operations systems constitutes an important mitigation option, as about twenty billion animals make use of 30% of the world's lands for grazing (Steinfeld et al. 2006). The mitigation of ruminant CH_4 emissions is particularly important in Brazil, which has the largest commercial herd of cattle in the world (USDA 2019). Among the various CH_4 mitigation options and technologies, the introduction of legumes into pastures systems is considered to be a favourable alternative (Boddey et al. 2020). The presence of tannins makes legumes potential mitigators of enteric CH_4. Therefore, the aim of this study was to investigate animal performance and CH_4 emission responses of mixed pasture of Marandu palisadegrass and forage peanut compared to Marandu palisadegrass in monoculture, fertilised or not with N.

Methods and Study Site

The study was carried out on the Experimental Farm of the Federal University of Lavras, Brazil (21°14′S, 44°58′W; 918 m above sea level). The soil in the area is a Ferralsol (WRB/FAO classification) with clayey texture. The treatments comprised three types of pastures, namely: 1) Marandu palisadegrass and forage peanut mixed pasture without N fertiliser application **(GRASS+LEGUME)**; 2) Marandu palisadegrass monoculture fertilised with 150 kg N/ha/year **(GRASS+N)**; and 3) Marandu palisadegrass monoculture without N fertiliser application **(GRASS)**. The whole experimental area was seeded with Marandu palisadegrass at a rate of 6.0 kg/ha of pure live seeds. The 12-ha experimental area was limed (2,500 kg dolomitic lime/ha) 60 d before grass seeding and 52 kg of P/ha and 41 kg of K/ha were applied during grass seeding. The whole experimental area was divided into four paddocks with three hectares each (blocks). Afterwards, the blocks were divided into three paddocks where pasture types were randomly allocated. The GRASS+N, GRASS+LEGUME and GRASS paddocks size were 0.7, 1.0 and 1.3 ha, respectively. The GRASS+LEGUME paddocks were seeded with forage peanut into a previously established Marandu palisadegrass pasture with a seeding rate of 10 kg/ha of pure live seeds.

From December 2016 to January 2019, eight seasons were evaluated over time. Continuous stocking with a variable stocking rate was used to maintain the canopy height between 20 and 25 cm. Two Nellore heifers (234 ± 36 kg of BW and 12 ± 1.3 months of age) were used as tester animal in each paddock. When it was necessary to adjust the canopy height, put-and-take animals were added. Water and commercial mineral supplementation were supplied *ad libitum.* Average canopy height was measured weekly using a sward stick (Barthram 1985) at 100 random points per paddock. Annually, in the spring (between November and December), all paddocks were fertilised by application of 22 kg/ha of P and 41 kg/ha of K. In the GRASS+N, the N fertiliser application was divided into three applications per year (50 kg N/ha each in November, January, March), all applied as urea.

Cattle were weighed in the morning, every 28 d throughout each season, without food or water restriction. The values obtained in each weighing were submitted to individual analyses of regression per season ($y = ax + b$), being the resultant a linear equation ($y = ax + b$). In this equation, the individual initial weight in each season was the intercept, and the average daily gain (ADG) was the slope.

Forage intake (DMI) was estimated from fecal excretion and indigestible neutral detergent fiber (iNDF) once per season. Fecal production was estimated using titanium dioxide (10 g/animal/day) as an external marker (Titgemeyer et al. 2001) during eleven consecutive days, ten for adaptation and five for collection. Spot fecal samples were collected once a day and a composite sample was performed for each animal. Fecal samples were oven-dried at 55 °C for 72 h and grinded for determination of the titanium dioxide concentration (Myers et al. 2004). Fecal and hand-plucked forage samples were incubated in the rumen for 288 hours to determine iNDF (Huhtanen et al. 1994). In the GRASS+LEGUME pasture treatment, the proportion of grass and legume in the forage intake was estimated using $\delta^{13}C$ isotopes in the iNDF residual fecal samples (Lopes de Sá, 2017). Condensed tannin was determined according for Porter et al. (1985).

Emissions of enteric methane were measured using the sulfur hexafluoride (SF_6) tracer gas technique (Johnson et al. 1994), whereby each of the 24 animals (two in each paddock) were assessed daily for 24 h for five consecutive days once per season. The animals were equipped with gas collection halters attached to pre-evacuated polyvinyl chloride (PVC) sampling canisters, made to hold 50% filling in 24 h. Methane emission was expressed as g CH_4/animal/day. Other forms to express CH_4 emission were: g CH_4/kg DMI, g CH_4/ha/day (emission per hectare), g CH4/kg ADG (which is referred herein as CH_4 emission intensity) and g CH_4/kg carcass gain.

All variables were averaged per experimental unit before analysis, for each season. The experimental design was randomized complete blocks with three treatments (pasture type; GRASS+N, GRASS, and GRASS+LEGUME), four replications, and repeated measurements over time (seasons of the year). Data were analysed by fitting mixed models, using the MIXED procedure os SAS. The effects of pasture type, seasons, and their interactions were considered fixed and the effect of block and year as a random effect. The averages were estimated using the LSMEANS statement, and comparations were made using Fisher's protected least significant difference (LSD) test at 10% probability.

Results

There was no difference between pasture type for the ADG ($P = 0.439$) and DMI ($P = 0.394$; averages of 0.433 kg/d and 2.10 %BW/d, respectively; Table 1). Greatest condensed tannin intake was recorded in the GRASS+LEGUME pasture ($P < 0.001$; Table 1).

Regarding methane emission variables, GRASS+LEGUME pasture had the lowest average daily CH_4 emission per animal, per body weight and per metabolic body weight ($P = 0.001$, $P = 0.018$, and $P = 0.009$, respectively; Table 3). In the GRASS+N and GRASS+LEGUME pasture presented lower values of CH_4/kg DMI compared to GRASS pasture (P = 0.044; Table 1). The lowest CH_4/kg ADG was recorded for the GRASS+LEGUME pasture (P = 0.061; a decrease of 14.7 and 8.3% compared to GRASS and GRASS+N pasture, respectively; Table 1). The CH_4 emission per kg of carcass gain was lower in the GRASS+LEGUME pasture compared to GRASS pasture (P = 0.022; Table 1).

Table 1. Animal performance, intake, and methane emissions of Nellore heifers on Marandu palisadegrass pastures with or without N application, and mixed with forage peanut.

Variables	**Pasture type**			**SEM**[*]	***P - Value***
	GRASS	**GRASS+N**	**GRASS+LEGUME**		
Animal performance					
ADG[†], kg/d	0.398	0.468	0.434	0.040	0.439
DMI, %BW/d	2.00	2.21	2.09	0.17	0.394
CT intake, %BW/d	0.16b	0.19b	0.61a	0.06	<0.001
Methane emissions					
CH_4/animal/d	165a	170a	148b	4.23	0.001
CH_4/d/kg BW	0.525a	0.513a	0.481b	0.040	0.018
CH_4/d/kg $BW^{0.75}$	2.21a	2.18a	2.01b	0.12	0.009
CH_4/kg DMI	30.2a	26.7b	26.8b	1.15	0.044
CH_4/kg ADG	428a	398a	365b	44.3	0.061
CH_4/kg carcass gain	800a	712ab	656b	45.8	0.022

[*] Standard error means.

[†] ADG: average daily gain; DMI: dry matter intake; CT: Condensed tannin; CH_4: methane; BW: body weight.

Discussion

In forage diets, canopy structural characteristics of tropical forages are relatively more important than nutritional factors in terms of the regulation forage intake (Poppi et al. 1987). Nitrogen input in grazing systems promotes an increase of the leaf density in the canopy stratum, which could enhance the forage intake due to easier formation and realization of the bite (Mezzalira et al. 2014). However, even with N input in the GRASS+N and GRASS+LEGUME pastures, canopies managed under similar canopy height have the same forage intake, probably due to changes in the ingestive behaviour to maintain a steady intake (Table 1). Therefore, the same ADG between pasture systems may be due to similar forage intake, provided that the maximization of animal performance is linked to the capacity of intake by grazing animals (Table 1).

Lower daily CH_4 emissions (g CH_4/animal/day; Table 1) in the GRASS+LEGUME pasture could be associated with the condensed tannin intake in this treatment. The inclusion of temperate and tropical legumes that contain secondary metabolites can reduce methanogenesis (Andrade et al. 2016). Secondary compounds of tropical legumes may inhibit, by bactericidal or bacteriostatic action, the growth or activity of methanogenic archaea, probably by binding to microbial cell proteins and enzymes (Tavendale et al. 2005). In addition, tannins may inhibit some ruminal protozoa and indirectly affect methanogenic archaea (Gerber et al. 2013).

Despite the potential benefits of well-managed GRASS+LEGUME pasture for reducing CH_4 emissions, it is important to highlight that the impacts on all greenhouse gas together should be considered to better estimate the outcome of the action, which requires further studies. In summary, the experimental evidence suggests that the secondary metabolites in forage peanut may decrease the amount of enteric methane produced by ruminants, reducing carbon footprint of livestock systems in Southeast Brazil.

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