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Progressive inclusion of pearl millet herbage as a supplement for dairy cows fed mixed rations: Effects on methane emissions, dry matter intake, and milk production

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ABSTRACT

The inclusion of grazing in dairy feeding systems can improve animal welfare and reduce feed costs and labor for animal care and manure management. This work aimed to evaluate the effects of including pearl millet herbage (*Pennisetum glaucum* 'Campeiro') as a supplement for dairy cows fed total mixed rations (TMR). The treatments included 100% TMR offered ad libitum (control, TMR_{100}), 75% TMR ad libitum intake + access to grazing of a pearl millet pasture between the morning and afternoon milkings (7 h/d; $pTMR_{75}$), and 50% TMR ad libitum intake + access to grazing of a pearl millet pasture between the morning and afternoon milkings (7 h/d; $pTMR_{50}$). Nine multiparous Holstein and F_1 Jersey × Holstein cows were distributed in a replicated 3 × 3 Latin square design with 3 periods of 21 d (a 16-d adaptation period and a 5-d measurement period). Cows in the TMR_{75} and TMR_{50} groups strip-grazed a pearl millet pasture with pre- and postgrazing sward height targets of 60 and 30 cm, respectively. The herbage dry matter intake (DMI) increased with decreasing mixed ration supplies, and the total DMI decreased linearly from 19.0 kg/d in the TMR_{100} group to 18.0 kg/d in the $pTMR_{50}$ group. Milk production decreased linearly from 24.0 kg/d in the TMR_{100} group to 22.4 kg/d in the $pTMR_{50}$ group, and energy-corrected milk (ECM) production decreased linearly from 26.0 kg/d to 23.6 kg/d. Enteric methane (CH_4) emissions decreased linearly from 540 g/d in the TMR_{100} group to 436 g/d in the $pTMR_{50}$ group, and CH_4 yields (g/kg of DMI) tended to decrease linearly. The CH_4 intensity was similar between treatments, averaging 20 g of CH_4/kg of ECM. The inclusion of pearl millet herbage in the dairy cow diets decreased the total DMI and milk production to a small extent without affecting CH_4 intensity (g/kg of ECM).

Key words: dairy cow, grazing, *Pennisetum glaucum*, methane

INTRODUCTION

The use of grazing in dairy production systems can improve animal welfare and reduce health problems (Arnott et al., 2017) as well as reduce labor for animal care and manure management (White et al., 2002; Schingoethe, 2017). Grazing can also decrease feeding costs and improve income-over-feed costs (Soriano et al., 2001; White et al., 2002). In contrast, pastures alone are rarely able to meet the energy requirements of lactating dairy cows (Kolver and Muller, 1998; Delaby et al., 2001; O'Neill et al., 2011) and do not provide a constant herbage supply throughout the year (Wilkinson et al., 2020). Thus, mixed feeding systems involving grazing pastures and TMR have been proposed and used worldwide (Wales et al., 2013).

Investigations of dairy cow systems where cows are both grazing on temperate pastures and receiving a mixed ration have, in some studies, not affected total DMI and milk production (Vibart et al., 2008; Mendoza et al., 2016); in other studies, total DMI and milk production had been reduced up to 16% (Soriano et al., 2001; Bargo et al., 2002; White et al., 2002). Reductions in milk production have been observed in high-production cows (Bargo et al., 2002) and when the proportion of TMR is relatively low (Vibart et al., 2008; Mendoza et al., 2016). Relatively large reductions in DMI and milk production have also been observed

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when the quality of herbage decreases according to the season (White et al., 2002; Vibart et al., 2008). However, compared with investigations done on cows grazing temperate pastures, studies assessing the effect of including tropical herbage on diets of dairy cows receiving mixed rations are rare.

The effects of combining temperate pastures with mixed rations on enteric methane (CH_4) emissions have shown that CH_4 yields (g/kg of DMI) may be lower in dairy cows exclusively grazing a high-quality perennial grass in the spring than in dairy cows receiving a TMR diet (O'Neill et al., 2011). Therefore, CH_4 intensity (g/kg of ECM) did not change in cows exclusively grazing on a temperate pasture or receiving TMR supplementation, which was explained by the similarity between herbage and TMR quality (O'Neill et al., 2012). Additionally, CH_4 intensity decreased in cows receiving a mixture of corn silage and soybean meal with the inclusion of an annual temperate pasture because of the improved quality of herbage compared with that of corn silage (Dall-Orsoletta et al., 2016). However, the quality of herbage may be relatively low in tropical pastures such as those of pearl millet (*Pennisetum glaucum*), which has shown great potential for use in dairy cows. Compared with other tropical pastures, pearl millet pastures have been shown to increase DM yields from 10 to 20 t/ha and have both a high protein content and high digestibility (de Assis et al., 2018). Thus, the effects of including pearl millet pasture on CH_4 emissions from dairy cows receiving TMR warrant further study.

We hypothesized that, due to greater herbage and diet NDF content, a progressive inclusion of pear millet in dairy cow diets would decrease total DMI, milk production, and CH_4 emissions (g/d), but CH_4 yields (g/kg of DMI) and CH_4 intensity (g/kg of ECM) would increase. The aim of this work was to quantify the effects of partial TMR replacement by pearl millet on CH_4 emissions and production responses in dairy cows.

MATERIALS AND METHODS

The Ethics Committee of the University of Santa Catarina State (Brazil) approved all the procedures in this study under protocol number 4373090816.

Treatments, Experimental Design, and Animals

The experiment was conducted according to a 3 × 3 Latin square design replicated 3 times. Nine multiparous cows were divided into 3 homogeneous groups (squares) of 3 animals, having 1 Holstein and 2 Holstein × Jersey cows as similar as possible in terms of milk production. Each square was then assigned a different treatment sequence. The following variables were determined during the week before the beginning of the experiment (means ± SD): milk production (25.1 ± 3.93 kg/d), DIM (136 ± 40 d), BW (533 ± 41.7 kg), and number of lactations (2.6 ± 0.69). Each experimental period lasted 21 d, with a 16-d adaptation period and a 5-d measurement period.

The treatments were as follows: 100% TMR (**$\mathbf{TMR_{100}}$**) offered ad libitum (control), 75% TMR ad libitum intake + access to grazing of a pearl millet pasture (**$\mathbf{pTMR_{75}}$**), and 50% TMR ad libitum intake + access to grazing of a pearl millet pasture (**$\mathbf{pTMR_{50}}$**). All treatments received the same mixed ration, which was balanced after chemical analysis of the ingredients to meet the net energy and metabolizable protein (**PDI**) requirements of the control treatment, according to equations developed by the INRA (2007). The TMR was composed of corn silage and a concentrate (60:40 ratio on a DM basis). The ingredients, chemical composition, and nutritive value of the TMR are presented in Table 1.

The individual voluntary DM TMR intake was quantified before the experiment started in a 14-d preexperimental period where cows been housed and fed individually. The average DMI of the last 5 d was considered for calculating the amount of mixed ration to be offered in the $pTMR_{75}$ and $pTMR_{50}$ treatments for each cow throughout the experiment. During the experiment, the cows were housed individually, where

Table 1. Chemical composition and nutritive value of mixed rations offered to dairy cows[1]

Item	Content
Ingredient, g/kg of DM	
Corn silage	600
Ground corn	260
Soybean meal	140
Chemical composition, g/kg of DM	
DM, g/kg fresh	380
OM	962
CP	149
NDF	340
ADF	173
Nutritive value[2]	
Gross energy, MJ/kg of DM	18.7
OM digestibility	0.77
NE_L, MJ/kg of DM	7.02
PDIN, g/kg of DM	97.8
PDIE, g/kg of DM	99.0

[1] Mineral supplement composition available in the feeding area and paddocks (on natural basis): 150 g/kg of calcium, 78 g/kg of phosphorus; 26 g/kg of sulfur, 20 g/kg of magnesium, 114 g/kg of sodium, 100 mg/kg of cobalt, 1,500 mg/kg of copper, 30 mg/kg of chromium, 2,000 mg/kg of iron, 80 mg/kg of iodine, 2,300 mg/kg of manganese, 30 mg/kg of selenium, 5,000 mg/kg of zinc, and 780 mg/kg of fluorine.

[2] Estimated from chemical analysis and via equations proposed by INRA (2007); PDIN = metabolizable protein when N is limiting for microbial synthesis in the rumen; PDIE = metabolizable protein when energy is limiting for microbial synthesis in the rumen.

either TMR or pTMR were offered in covered outdoor feeders. The cows in the TMR_{100} group were fed twice a day after the morning and afternoon milkings; these cows received a daily quantity that was 20% greater than the voluntary DMI measured the prior day. In $pTMR_{75}$ and $pTMR_{50}$ treatments, cows received 75 and 50% of their TMR ad libitum intake measured during the preexperimental period, respectively. They also had access to a pasture between the morning and afternoon milkings (7 h/d of access to the pasture, from 0800 h to 1500 h) and received pTMR after the afternoon milking (13 h/d of access to pTMR, from 1700 h to 0600 h). The TMR and pTMR refusals were individually collected and weighed once per day during the morning milking. Water and mineral supplements (Bovigold, DSM Tortuga, São Paulo, Brazil) were continuously available in the feeding area and paddocks.

Pasture and Grazing Management

The experiment was performed in Lages, SC, Brazil (50.18° W, 27.47° S; 920 m above sea level), from January 25 to March 29, 2019. An area of 2 ha of pearl millet sown in 2017 was used. During the experimental period, the average temperature was 19.6°C, and the cumulative rainfall was 215 mm. The 10-yr climatic average temperature and rainfall during the months of the experiment were 14.5°C and 161 mm, respectively. Before the first grazing cycle (after pearl millet had developed the third-leaf stage) and after each experimental period, the experimental area was fertilized with 50 kg of N/ha, which was supplied as urea.

The area was divided into 2 paddocks, with 1-third and 2-thirds of the surface assigned to the $pTMR_{75}$ and $pTMR_{50}$ groups, respectively. This size ratio of the surface was chosen because the expected herbage DMI for the $pTMR_{75}$ and $pTMR_{50}$ groups were 25% and 50% of the ad libitum DMI, respectively, and thus the TMR_{75} group was expected to require half the area of the $pTMR_{50}$ group. The paddocks were strip-grazed with pre- and postgrazing sward height targets of 60 and 30 cm, respectively. To achieve these targets, the areas allocated daily to the $pTMR_{75}$ and $pTMR_{50}$ groups were 41 and 82 m^2/cow, respectively, which was defined on the basis of a 1-wk preexperimental period. As the actual pre- and postgrazing sward heights throughout the experiment were close to the pre- and postgrazing target heights, no other adjustments for area allocation were necessary. To minimize variations in herbage quality between periods, different areas were used during the last 14 d of each period. These areas were mowed 18 d before starting the measurement periods for controlling of pregrazing sward height and chemical composition. Grazing management processes aimed to ensure that animals in the $pTMR_{75}$ and $pTMR_{50}$ groups removed the same proportion of forage in relation to the pregrazing height and that this removal did not exceed 50% of the initial height. The aim of 50% was chosen because it is the threshold at which the grazing management and structural characteristics of the herbage at the end of the occupation period can impose restrictions on herbage intake (Zanini et al., 2012; Mezzalira et al., 2013).

Animal Measurements

Individual milk production values were recorded twice daily (at 0700 h and 1600 h), and milk samples were collected at each milking via an electronic milk meter (Waikato Milking Systems, Hamilton, New Zealand) approved by the International Committee for Animal Recording (ICAR). The milk composition (fat, milk CP, and MUN concentrations) was individually measured for samples collected during each milking of the last 5 d of each period via infrared spectrophotometry (IDF, 2013). The ECM production standardized to 4.0% fat and 3.3% protein was calculated according to the equation proposed by Tyrrell and Reid (1965) as follows: ECM (kg/d) = milk production kg × [37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948]/3,138.

The TMR and pTMR intake, as well OM, CP, NDF and ADF intakes, and diet concentration, were measured as the average difference between the supplied quantity and the remaining quantity from each of the last 5 d of each period, when the DM, OM, CP, NDF and ADF content of offered TMR, pTMR, and refusals were measured separately. The individual herbage intake was measured according to the n-alkane technique (Mayes et al., 1986) via the C_{31} (naturally present in the forage):C_{32} (supplied to the animals) ratio. Animals received cellulose pellets (Carl Roth, GmbH, Karlsruhe, Germany) containing 186 mg of C_{32} twice per day after each milking from d 8 to 21 of each experimental period. During the last 5 d of each experimental period, fecal grab samples were collected from each cow after each milking. The fecal samples were oven-dried at 60°C for at least 72 h, composited by period and cow, and then ground (Solab SL-31, Piracicaba, Brazil) to pass through a 1-mm screen for subsequent chemical analyses.

The daily grazing time in the $pTMR_{75}$ and $pTMR_{50}$ groups was measured individually via visual observations every 5 min between 0800 h and 1500 h during the last 5 d of each period. The cows were previously accustomed to humans, and the time spent watching each individual animal was no more than 10 s, during which grazing behavior or no grazing was recorded (Penning and Rutter, 2004). No behavior was recorded indoors when the cows were milked or received the TMR. The herbage intake rate (g of DM/min) was estimated per cow and period by dividing the average daily herbage intake by the average daily grazing time.

The gross energy (**GE**) of herbage was estimated as proposed by INRA (2018) as GE (kcal/kg of OM) = 4,531 + 1.731 × CP (g/kg of OM) − 71 (n = 166, $R^2 = 0.89$), whereas the GE for the mixed ration was calculated from tabulated values for the concentrates and corn silage (INRA, 2018). The OM digestibility was estimated from the chemical composition of forages according to specific equations for corn silage, herbage, and concentrates (INRA, 2018). The NE_L and PDI balances were estimated per cow and period according to the difference between the NE_L and PDI supply and requirements, according to the methods of the INRA (2007). The NE_L requirements were estimated considering BW (kg) and FCM (kg/d) as follows: NE_L requirements (Mcal/d) = $0.080 \times BW^{0.75} + FCM \times 0.7476$. The PDI requirements were estimated with consideration of the BW (kg), actual milk production (kg/d), and CP content (g/kg of milk) as follows: PDI requirements (g/d) = $3.25 \times BW^{0.75}$ + milk production × (CP × 0.93) × 10. The NE_L and PDI supplies were estimated considering herbage and TMR intakes and their NE_L and PDI contents, respectively.

Daily CH_4 emissions were measured individually according to the sulfur hexafluoride (SF_6) tracer gas technique described by Johnson et al. (1994). Each cow received 1 SF_6 capsule 21 d before beginning the experiment, with an average SF_6 release rate of 3.68 ± 0.10 mg/d. This average release rate was quantified by immersing the capsules in a 39°C water bath and then measuring weight loss during a period of 6 wk. The gas samples were collected on the last 5 d of each period, from the afternoon milking of d 16 to the afternoon milking of d 21, which was possible due to the calibration of flow regulators and storage capacity of the air-sampling devices (Pinares-Patiño et al., 2012). Thus, from 5 d of gas sampling, only a half-day was not concomitant with intake measurements; however, both variables were measured for 120 h consecutively.

Cows with or without access to pastures received the same kind of air-sampling devices concomitantly; each device was put on the head halters such that the sampling point was positioned above the nostrils. The air-sampling devices consisted of stainless-steel cylinders (0.5-L volume) with the sample flow regulated by a brass ball bearing (Gere and Gratton, 2010). The cylinders were cleaned with high-purity N gas and preevacuated before each sample collection. The flow regulators were calibrated to allow for an expected remaining vacuum of approximately 500 mbar (which represents half of the total cylinder volume) in the cylinder at the end of the sample collection period (5 consecutive days). In addition to breath samples, 2 identical apparatuses were placed 1.5 m above the soil in the paddocks, and 2 others were placed where the TMR was offered to measure the background concentrations of CH_4 and SF_6 in the environment.

To ensure the most successful individual gas samples, 2 gas-sampling cylinders were used simultaneously per animal. When 2 concomitant air samples per cow were collected successfully, the average was used. Operation of the gas-sampling apparatus was considered successful if the residual vacuum was between 350 and 650 mbar, which correspond to 37.6 and 69.8% of the initial vacuum, respectively (Pinares-Patiño et al., 2012). The average residual vacuum in the gas-sampling apparatus was similar between treatments, and overall collection of the 54 gas samples (2 gas-sampling cylinders × 9 cows × 3 periods) was 69% successful. In 4 situations, samples from both gas-sampling cylinders of the same cow within the same period were considered lost.

The CH_4 emissions (g/d) were calculated in relation to the known release rate of SF_6 by subtracting the background concentrations of CH_4 and SF_6 (Berndt et al., 2014) as follows:

$$R_{\mathrm{CH4}} = R_{\mathrm{SF6}} \frac{[\mathrm{CH_4}]_M - [\mathrm{CH_4}]_{BG}}{[\mathrm{SF_6}]_M - [\mathrm{SF_6}]_{BG}} \times \frac{MW_{\mathrm{CH4}}}{MW_{\mathrm{SF6}}} \times 1{,}000,$$

where R_{CH4} is the enteric CH_4 (g/cow/d), R_{SF6} is the release rate of SF_6 (mg/d), MW_{CH4} is the molecular mass of CH_4 (16 g), and MW_{SF6} is the molecular mass of SF_6 (146 g). $[\mathrm{CH_4}]_{BG}$ and $[\mathrm{SF_6}]_{BG}$ are the background concentrations of CH_4 (ppm) and SF_6 (ppt), respectively. The background CH_4 and SF_6 concentrations in the treatments with access to pearl millet pastures were calculated according to the weighted average of indoor and outdoor background concentrations, according to the length of time the animals spent in the pastures (7/24 h) or in confinement (17/24 h).

Feed and Pasture Measurements

Offered TMR and pTMR were sampled twice daily from d 15 to 20 of each period, and the samples were composited per period. Samples of the orts left by each cow were collected during the last 5 d of each period and were used to create a composite sample for each cow and period. All samples were dried in an oven for 72 h at 60°C and then ground (Solab SL-31, Piracicaba, Brazil) to pass through a 1-mm screen for subsequent chemical analyses.

The pregrazing herbage mass was measured at ground level by cutting four 1-m^2 squares of pearl millet with scissors per treatment every day during the last 5 d of each period. The herbage DM concentration was determined for each square from an 800-g subsample. The pre- and postgrazing sward heights were measured daily via a 1.0-m sward stick (Barthram, 1986) by averaging the first contact of 60 readings taken randomly throughout the area allocated for grazing by each group. Selected herbage samples were collected by the hand-plucked method daily during the last 5 d of each experimental period. The samples were dried in a forced-ventilation oven for 72 h at 60°C and then stored for chemical analyses. The morphological composition of the canopy was determined on d 18 and 20. In each treatment, 20 handfuls of randomly selected herbage were cut at ground level. These samples were separated into leaf (lamina + sheath), stem, and senescent material. Each component was oven-dried at 60°C for 72 h and subsequently weighed.

Chemical Analyses

After the samples were ground, their DM content was determined by drying at 105°C for 24 h. The ash content was quantified by combustion in a muffle furnace at 550°C for 4 h, and the OM was quantified on the basis of the mass difference. The total N content was measured according to the Dumas combustion method 968.06 (AOAC International, 1998) via a Leco FP 528 instrument (LC, Leco Corporation, Saint Joseph, MI). The CP content was calculated as N content multiplied by 6.25. The NDF concentration was assessed according to the methods of Mertens (2002), with the exception that the samples were weighed in filter bags and treated with a neutral detergent in an Ankom A220 system (Ankom Technology, Macedon, NY). This analysis included α-amylase and residual ash, but did not include sodium sulfite. The concentration of ADF was analyzed according to method 973.18 of the AOAC (AOAC International, 1998).

The n-alkane content was quantified on the basis of the protocol described by Dove and Mayes (2006), which was adapted for the use of the columns, as proposed by Oliveira and Tedeschi (2010). The n-alkane content was analyzed via gas chromatography by a Clarus 580 instrument (PerkinElmer, Inc., Waltham, MA) equipped with a flame ionization detector and capillary column (PerkinElmer Elite-1, 100% dimethyl polysiloxane; 30 m × 0.25 mm and a 0.25-μm film thickness).

The concentrations of CH_4 (ppm) and SF_6 (ppt) were determined via a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan). The chromatograph was equipped with a flame ionization detector at 250°C and a 1/8" Shimalite Q packed column (0.7 m, 80/100 mesh; Shinwa Chemical Industries Ltd., Kyoto, Japan) for the detection of CH_4, and equipped with an electron capture detector at 325°C and a 1/8" Porapak N packed column (1.5 m, 100/180 mesh) for the detection of SF_6. A mixture comprising 5% CH_4 and argon was used as the compositional gas in the SF_6 analysis (electron capture detector). The gas chromatograph column was maintained at 80°C during the analysis, and N gas was used as a carrier with a flow of 25 cm^3/min. Calibration curves were established by the use of certified standards (White Martins Development Laboratory, Osasco, Brazil), with CH_4 concentrations of 2.5, 5.0, 10, and 20 ppm, and SF_6 concentrations of 11, 30, and 100 ppt. The minimum detection limit, which is usually critical because of the low concentration of background CH_4 and SF_6, were 0.15 ppm and 5.2 ppt, respectively.

Statistical Analyses

The dependent variables were subjected to ANOVA via the PROC MIXED function of SAS software (version 9.4, SAS Institute, Cary, NC). The animal variables, which were averaged per cow and per period (n = 27), were analyzed using the following model:

$$Y_{ijk} = \mu + \text{square}_i + \text{period}_j + \text{treatment}_k + \text{square}_i \times \text{treatment}_k + \text{cow}_{l(i)} + e_{\text{ijk}},$$

where Y_{ijk}, μ, square_i, period_j, treatment_k, $\text{square}_i \times \text{treatment}_k$, $\text{cow}_{l(i)}$, and e_{ijkl} represent the analyzed variable, the overall mean, the fixed effects of the square, the fixed effects of period, the fixed effects of treatment, the fixed effects of square × treatment interaction, the random effect of cow nested in square, and the residual error, respectively. The fixed effect of treatment × period interaction was not significant for DMI, milk production, and methane variables, and thus was removed from the model.

The variables were tested via orthogonal polynomial contrasts to determine the linear and quadratic effects of the proportion of herbage inclusion in the diet. The least squares means were considered as significantly different if $P < 0.05$; P-values between 0.05 and 0.10 were considered trends, and standard errors of the mean were reported to describe variations.

RESULTS

The pre- and postgrazing sward heights and the pregrazing herbage mass of pearl millet averaged 62 cm, 32 cm, and 3,500 kg of DM/ha, respectively (Table 2). The CP, NDF, and ADF contents of the ingested pearl millet averaged 201, 625, and 306 g/kg of DM, respectively. The OM digestibility, energetic value, and

Table 2. Herbage characteristics and grazing management of a pearl millet pasture (*Pennisetum glaucum* 'Campeiro') grazed by dairy cows receiving mixed rations

Item	Treatment[1]	
	$pTMR_{75}$	$pTMR_{50}$
Herbage mass, kg of DM/ha	3,424	3,592
Pregrazing sward height, cm	62.9	61.0
Postgrazing sward height, cm	31.8	31.7
Daily offered area, m^2/cow	41	82
Herbage allowance, kg of DM/d		
Aboveground level	13.9	29.4
Living leaves	6.4	11.9
Pregrazing herbage morphological composition, g/kg of DM		
Leaves (lamina + sheath)	470	403
Stems	516	576
Dead material	13	17
Herbage chemical composition, g/kg of DM		
DM, g/kg	158	156
OM	915	917
CP	193	208
NDF	625	625
ADF	302	310
Herbage nutritive value[2]		
Gross energy, MJ/kg of DM	18.5	18.6
OM digestibility	0.69	0.70
NE_L, MJ/kg of DM	5.82	5.80
PDIN, g/kg of DM	128	134
PDIE, g/kg of DM	96	98

[1] pTMR75 = 75% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d); $pTMR_{50}$ = 50% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d).

[2] PDIN = metabolizable protein when N is limiting for microbial synthesis in the rumen (INRA. 2007); PDIE = metabolizable protein when energy is limiting for microbial synthesis in the rumen (INRA. 2007).

PDI content of selected herbage averaged 69.4%, 5.81 MJ of NE_L/kg of DM and 97 g/kg of DM, respectively. Throughout the experiment, CP content of ingested pearl millet herbage were 198, 215, and 188 g/kg of DM in periods 1, 2, and 3, respectively. The pearl millet NDF content was 611, 631, and 632 g/kg of DM, and OM digestibility was 0.70, 0.69, and 0.69 in periods 1, 2, and 3, respectively.

The CH_4 emissions (g/d) decreased linearly with the progressive inclusion of grazed herbage in the diet (linear effect: $P < 0.01$), and the CH_4 yield (g/kg of DMI) tended to decrease linearly ($P < 0.07$; Table 3). For each kilogram of pearl millet herbage inclusion, there was a reduction of 13.3 g/d of CH_4 production and a reduction of 0.2 g/kg of CH_4 yield. The CH_4 intensity was similar between treatments, averaging 20 g of CH_4/kg of ECM. The herbage DMI increased with decreasing mixed ration supply, and the total DMI decreased linearly (Table 4). The mixed ration DMI decreased quadratically with decreasing mixed ration supply, with a greater reduction occurring between TMR_{100} and $pTMR_{75}$ than between $pTMR_{75}$ and $pTMR_{50}$. The concentrate DMI decreased from 7.6 kg/d in the TMR_{100} group to 5.5 and 4.1 kg/d in the $pTMR_{75}$ and $pTMR_{50}$ groups, respectively. The grazing time (+48 min/d) and herbage DMI rate (+9.6 g of DM/min) were 22 and 42% greater ($P < 0.001$), respectively, in the $pTMR_{50}$ group than in the $pTMR_{75}$ group.

The milk production and ECM production decreased in cows with access to grazed herbage compared with that of cows in the TMR_{100} group (Table 5). For each kilogram of pearl millet herbage inclusion, there was a 0.2 kg of milk yield reduction. The milk fat and MUN concentrations were similar between treatments, and the milk protein content decreased linearly as the mixed ration supply was reduced.

DISCUSSION

Methane Emissions

The hypothesis concerning CH_4 production was confirmed in part because daily CH_4 emissions decreased linearly, but the CH_4 yield and CH_4 intensity did not increase with decreasing mixed ration supply. The linear reduction in daily CH_4 emissions with decreasing mixed ration intake and the inclusion of grazing herbage is consistent with the linear reduction in total DMI, which is well known as the main driver of enteric CH_4 emissions (Hristov et al., 2013). These results are also in agreement with those of other studies (O'Neill et al., 2011; Cameron et al., 2018) that show that reductions

Table 3. Enteric methane emissions by dairy cows receiving mixed rations and with or without grazing access to a pearl millet pasture[1]

	Treatment[2]				*P*-value		
Methane	TMR$_{100}$	pTMR$_{75}$	pTMR$_{50}$	SEM	ANOVA	Linear	Quadratic
g/d	540	481	436	16.3	0.005	0.001	0.59
g/kg of DMI	26.9	26.9	25.6	1.11	0.09	0.07	0.45
g/kg of ECM[3]	20.4	19.9	19.6	0.77	0.48	0.25	0.78
% gross energy intake	8.03	8.01	7.51	0.423	0.39	0.28	0.49

[1] Data from 23 observations.

[2] TMR$_{100}$ = total mixed ration ad libitum; pTMR$_{75}$ = 75% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% ad libitum intake TMR + grazing herbage after the morning milking (7 h/d).

[3] ECM calculated as follows: kg of milk production × [37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948]/3,138 (Tyrrell and Reid, 1965).

in daily CH_4 emissions occur because of reductions in DMI in dairy cows receiving fresh herbage plus pTMR compared with that of cows receiving TMR exclusively. Moreover, the CH_4 emission values reported in the present study are within the range of values observed when cows consumed TMR (Dall-Orsoletta et al., 2016) or grazed on a pearl millet pasture (Alves et al., 2017).

The tendency for linearly decreasing CH_4 yields with decreasing TMR supply was unexpected because decreasing the concentrate content may increase CH_4 emissions per unit of DMI (Hristov et al., 2013). However, both the NDF (Niu et al., 2018) and concentrate (INRA, 2018) contents of the diet have been shown to be strongly related to enteric CH_4 yields. Though the dietary NDF content was linear and positively related to CH_4 yields, the dietary concentrate content exhibited a curvilinear relationship, where the maximum methanogenesis per kilogram of OM intake occurred with the inclusion of 35% concentrate (Sauvant and Giger-Reverdin, 2009). In the present study, the diet concentrate content averaged 22, 30, and 40% in the TMR$_{50}$, TMR$_{75}$, and TMR$_{100}$ groups, respectively. Therefore, it is logical to assume that reductions in CH_4 yields due to a lower NDF content in the TMR$_{100}$ group compared with the other groups were offset by an increase in amount of ruminal fermentable OM. This probably occurred because the concentrate content in the TMR$_{100}$ diet was not high enough to affect the CH_4 yield compared with that in the other treatments.

The similarity in CH_4 intensity (g/kg of ECM) between treatments may be explained because the ECM production and DMI decreased linearly in similar proportions when the mixed ration supply decreased. The average value of CH_4 intensity (20 g/kg of ECM) was close to the values estimated by Moate et al. (2016) as emissions from the Australian dairy industry (19.9 g/kg of ECM) and values of studies assessing dairy cows receiving TMR or pTMR plus fresh temperate herbage grazing in Europe (16–24 g/kg of ECM; O'Neill et al., 2011, 2012). This is evidence that dairy cow diets in-

Table 4. Dry matter intake, behavior, and chemical composition of the diet of dairy cows receiving mixed rations with or without grazing access to a pearl millet pasture

	Treatment[1]				*P*-value		
Item	TMR$_{100}$	pTMR$_{75}$	pTMR$_{50}$	SEM	ANOVA	Linear	Quadratic
DMI, kg/d							
Total	19.0	18.4	18.0	0.27	0.04	0.02	0.51
Herbage	—	4.6	7.8	0.10	<0.001	—	—
TMR	19.0	13.8	10.2	0.19	<0.001	<0.001	0.007
Grazing time, min/d	—	216	264	6.6	0.03	—	—
Herbage DMI rate, g/min	—	23.0	32.6	2.34	0.001	—	—
Chemical composition, g/kg of DM							
OM	962	951	942	—	—	—	—
CP	155	169	175	—	—	—	—
NDF	348	429	463	—	—	—	—
ADF	177	215	232	—	—	—	—
NE$_L$ supply,[2] MJ/d	136	124	118	—	—	—	—
NE$_L$ balance,[3] MJ/d	20	13	10	—	—	—	—

[1] TMR$_{100}$ = total mixed ration ad libitum; pTMR$_{75}$ = 75% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d).

[2] Net energy for lactation supply.

[3] Net energy for lactation balance (NE$_L$ supply − NE$_L$ requirements).

Table 5. Milk production and milk composition of dairy cows receiving mixed rations and with or without grazing access to a pearl millet pasture

	Treatment[1]				*P*-value		
Item	TMR_{100}	TMR_{75}	TMR_{50}	SEM	ANOVA	Linear	Quadratic
Milk production, kg/d	24.0	22.7	22.4	0.83	<0.001	<0.001	0.07
4% FCM production, kg/d[2]	26.3	24.8	24.0	0.73	<0.001	<0.001	0.12
ECM, kg/d[3]	26.0	24.5	23.6	0.68	<0.001	<0.001	0.16
Milk fat, g/kg	46.9	46.8	45.4	2.53	0.19	0.11	0.37
Milk protein, g/kg	33.8	33.4	32.3	0.63	0.009	0.003	0.33
Milk fat production, g/d	1,112	1,049	1,008	12.7	<0.001	<0.001	0.34
Milk protein production, g/d	808	754	716	12.9	0.003	<0.001	0.50
MUN, mg/L	18.4	18.0	18.0	0.82	0.64	0.42	0.64

[1] TMR_{100} = total mixed ration ad libitum; $pTMR_{75}$ = 75% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d); $pTMR_{50}$ = 50% ad libitum TMR intake + grazing herbage after the morning milking (7 h/d).

[2] 4% fat-corrected milk production.

[3] ECM calculated as follows: kg of milk production × [37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948]/3,138 (Tyrrell and Reid, 1965).

cluding tropical herbage may have similar CH_4 intensities as do those of confined or temperate herbage-based diets.

Dry Matter Intake and Grazing Behavior

The linear reduction in total DMI in cows grazing on the pearl millet pasture compared with that in cows in the TMR_{100} group (−0.1 kg for each kilogram of pearl millet herbage inclusion) could be explained primarily by the reduction in mixed ration intake, and thus the reduction in the concentrate intake. The substitution rate between forages and concentrates is typically in the range of 0.0 to 0.7 at grazing (Delagarde et al., 2011), leading to a reduction in total intake when the concentrate supply is reduced within a large range of herbage quality and herbage allowance values (Bargo et al., 2003; Faverdin et al., 2011). In this study, this reduction was no larger than 6%, which could be explained by the forage-concentrate substitution increase with increasing concentrate intake (Faverdin et al., 2011), because high substitution rate values (approximately 1.0) have little or no effect on total DMI. Additionally, the average percentage of reduction in total DMI observed when grazing partly replaced the mixed ration is also in agreement with the variation range (−4 to −7% of total DMI) observed for dairy cows when the concentrate proportion in the diet decreased in a similar range of roughage (corn silage or grass silage):concentrate (high starch) ratios (60:40 to 80:20; Faverdin et al., 1991).

The similar reduction in total DMI in the $pTMR_{75}$ and $pTMR_{50}$ groups compared with the TMR_{100} groups may be explained by the reduction in the TMR supply from 75 to 50% of ad libitum intake being partly offset by an increase of 3.2 kg in herbage DMI. This increase was mediated through a greater grazing time (+48 min/d) and herbage intake rate (+9.6 g of DM/min) in the $pTMR_{50}$ group than in the $pTMR_{75}$ group. These results agree with those of other studies where dairy cows grazing on temperate herbage presented an increased herbage DMI as the feed supplement amount decreased (Pérez-Ramírez et al., 2008; Vibart et al., 2008). For instance, Pérez-Ramírez et al. (2008) reported that cows increased their herbage DMI (+3.0 kg/d) by increasing their daily grazing time (+36 min/d) and herbage intake rate (+7 g of DM/min) when the feed supplement (corn silage + soybean meal) was reduced from 10 to 5 kg of DM/d.

Milk Production and Milk Composition

The linear reduction in ECM production in the $pTMR_{75}$ and $pTMR_{50}$ groups compared with that in the TMR_{100} group (−0.3 kg for each kilogram of pearl millet herbage inclusion) was a consequence of the concomitant linear reductions in total DMI and in concentrate DMI, both of which reduced the net energy intake. When the reduction in milk production was calculated as a function of only the decrease in concentrate intake, the milk production decreased by only 0.5 kg/d for each kilogram of reduced concentrate intake. This reduction is lower than that for classic milk production responses to concentrate supplementation (1 kg of milk/kg of concentrate intake) described in the literature (Peyraud and Delaby, 2001; Delagarde et al., 2011), which can be explained by the reduction in NE_L supply from the TMR being partly offset by the NE_L supply from the herbage intake. Finally, cows with access to the pearl millet pasture presented an important reduction in concentrate intake, but produced more than 90% of the amount of milk produced by the TMR_{100} cows. However, owing to the potential of shifting herbage nutritive values throughout the growing season, long-term continuous studies with larger number of cows grazing both pearl millet and other tropical forage species are highly recommended.

The linear reduction in milk fat production with the progressive reduction in mixed ration was a consequence of milk production being lower than that of cows without access to grazing of the pearl millet pasture because the milk fat content was similar between the treatments. The high milk fat content observed in this study (46.2 g/kg) can be explained by the breed characteristics and agrees with the milk fat content reported in another study involving cows from the same herd (Dall-Orsoletta et al., 2019). The reduction in milk protein content in cows with access to the grazing herbage compared with that in cows in the TMR_{100} group is in good agreement with variations in the energy supply. The role of the energy supply, rather than the protein or AA supply, in improving milk protein content has already been demonstrated in 2 comprehensive literature reviews (Coulon and Rémond, 1991; Beever et al., 2001).

CONCLUSIONS

Including pearl millet in dairy cow diets decreased the total DMI and milk production, but even at the greatest level of herbage inclusion, cows were able to achieve more than 90% of the total DMI and milk production recorded for cows that were fed only TMR. As the relative reduction in milk production was similar to that of the DMI, CH_4 emissions (g/d) decreased, but the CH_4 intensity (g/kg of ECM) was unaffected by the progressive inclusion of herbage in the diet. Additional studies with dairy cows grazing tropical forages throughout the whole growing season are strongly encouraged.

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