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Ryegrass pasture combined with partial total mixed ration reduces enteric methane emissions and maintains the performance of dairy cows during mid to late lactation

Aline C. Dall-Orsoletta,* João Gabriel R. Almeida,* Paulo C. F. Carvalho,† Jean V. Savian,† and Henrique M. N. Ribeiro-Filho*[1]
*Departamento de Produção Animal e Alimentos, Universidade do Estado de Santa Catarina, Av. Luiz de Camões, 2090, Lages, SC, Brazil, 88520-000
†Departamento de Plantas Forrageiras e Agrometeorologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 7712, Porto Alegre, RS, Brazil, 91540-000

ABSTRACT

The inclusion of grazed pasture in dairy feeding systems based on a total mixed ration (TMR) reduces feed costs, benefits herd health, and reduces environmental impact. The present study aimed to evaluate the effect of ryegrass pasture combined with a partial TMR on enteric methane emissions, dry matter intake (DMI), and performance of dairy cows from mid to late lactation. The experimental treatments included 100% TMR (control), partial TMR + 6 h of continuous grazing (0900–1500 h), and partial TMR + 6 h of grazing that was divided into 2 periods of 3 h each that took place after milking (0900–1200 h; 1530–1830 h). Twelve F_1 cows (Holstein × Jersey; 132 ± 44 DIM) were divided into 6 lots and distributed in a 3 × 3 Latin square design with 3 periods of 21 d (15 d of adaptation and 6 d of evaluation). Ryegrass (*Lolium multiflorum* Lam.) pasture was used, and the TMR was composed of 80% corn silage, 18% soybean meal, and 2% mineral and vitamin mixture, based on dry matter. The same mixture was used for cows with access to pasture. The total DMI, milk production, and 4% fat-corrected milk were similar for all cows; however, the pasture DMI (7.4 vs. 6.0 kg/d) and grazing period (+ 40 min/d) were higher in cows that had access to pasture for 2 periods of 3 h compared with those that grazed for a continuous 6-h period. Methane emission was higher (656 vs. 547 g/d) in confined cows than in those that received partial TMR + pasture. The inclusion of annual ryegrass pasture in the diet of dairy cows maintained animal performance and reduced enteric methane emissions. The percentage of grazed forage in the cows' diet increased when access to pasture was provided in 2 periods after the morning and afternoon milking.

Key words: dairy cow, grazing, methane, pasture dry matter intake

INTRODUCTION

The inclusion of grazed pasture in dairy feeding systems based on TMR can reduce feed costs (Soriano et al., 2001; Tozer et al., 2003) and benefit herd health. The milk production of cows that received supplementation with a partial TMR in pasture-based systems was increased compared with that of cows that consumed solely a pasture diet (O'Neill et al., 2012; Miguel et al., 2014). Further per-cow advantages have also been found during periods of low pasture accumulation rate or in areas with low grazing availability (Wales et al., 2013). Additionally, a mixed diet, including grazed pasture + partial TMR, improved the DMI and milk production of high-producing dairy cows compared with supplementation with concentrate (Bargo et al., 2002). However, comparisons of the effects of a TMR and a mixed diet, such as annual ryegrass pasture and a partial TMR, on dairy cows with low energy requirements, such as during mid to late lactation, are scarce.

In terms of environmental impact, cows that grazed high-quality pastures in early lactation produced similar or lower methane emissions per kilogram of DMI compared with cows that exclusively received a TMR (Robertson and Waghorn, 2002; O'Neill et al., 2011). Similarly, compared with cows that solely consumed a pasture diet, cows that grazed high-quality pastures during mid to late lactation did not have lower enteric methane emissions per unit of DMI when they received TMR supplementation (O'Neill et al., 2012). Additionally, the daily amount of time spent at pasture and in certain grazing sections (after a.m. or p.m. milking) affected the proportion of pasture in total DMI (Orr et al., 2001). This effect occurs because ruminants

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[1] Corresponding author: henrique.ribeiro@udesc.br

change their ingestive behavior when the time available to graze is restricted (Chilibroste et al., 2007); under these conditions, ruminants increase the proportion of time spent grazing and the intake rate (Pérez-Ramírez et al., 2008, 2009). However, the way in which these effects influence enteric methane emissions has not been sufficiently studied.

Thus, the aim of this work was to compare the enteric methane emissions and milk production of dairy cows in mid to late lactation that grazed pastures at different times of day and that received partial TMR or TMR only. We hypothesized that a mixed diet (graze pasture + partial TMR) would maintain milk production and mitigate methane emissions compared with TMR. We further hypothesized that access to pasture after a.m. and p.m. milking would increase the proportion of pasture in total DMI and mitigate methane emissions.

MATERIALS AND METHODS

Treatments, Experimental Design, and Animals

All procedures were approved by the Santa Catarina State University Ethical Committee, protocol no. 01.77.14. The treatments included the following: 100% TMR; 6 h of continuous access to pasture between morning and afternoon milking (0900 to 1500 h) + supplementation with partial TMR (**6h** treatment); and 6 h of access to pasture divided into 2 periods of 3 h after each milking (0900 to 1200 h and 1530 to 1830 h) + supplementation with partial TMR (**3+3h** treatment). The TMR was a mixture based on the DM of corn silage and soybean meal and was balanced for net energy and protein as recommended by INRA (2007). The same mixture was used in all treatments. The chemical composition and energy value of the supplements are presented in Table 1.

The mixture was offered twice daily in amounts 20% greater than the amount consumed the previous day. Cows received TMR after the morning and afternoon milking. The cows in the 6h treatment group received partial TMR after the afternoon milking, whereas those in the 3+3h group received it after each grazing event. The TMR and partial TMR were offered in a covered feed trough in an outdoor facility with 2 cows/pen. The remaining mixture was considered refused and removed from the barn once daily (0700 h). Water and minerals were continually available indoors and during grazing.

The treatments were compared according to a 3 × 3 Latin square design that was replicated twice. Each experimental period lasted 21 d, with a 15-d adaptation period and a 6-d measurement period. Twelve multiparous Holstein × Jersey F_1 cows were separated into 6 homogeneous groups (experimental unit) according to milk production (19.4 ± 2.3 kg/d), lactation stage (132 ± 44 d), and BW (501 ± 52 kg) 1 wk before the start of the experiment.

Pasture and Grazing Management

The study was performed in Lages, SC, Brazil (50.18°W, 27.47°S; 920 m altitude) and was conducted from August 26 to November 5, 2014. An area that contained 6.5 ha of annual ryegrass (*Lolium multiflorum* Lam.) pasture was seeded in April 2014, after the corn crop (*Zea mays*) harvest. Thirty days before the start of the experiment and immediately after each experimental period, the experimental area was fertilized with 50 kg of N/ha supplied as urea.

The grazing method was strip grazing, and the area allocated daily to each treatment group was calculated from a daily estimate of pregrazing pasture mass (**PM**; see pasture measurements) to give each cow 35 kg of DM/d. One uniform 2.0-ha paddock was split into four 0.5-ha paddocks; 2 were assigned to the 6h treatment group, and 2 were assigned to the 3+3h treatment group. The same paddock was grazed 3 times, once per period. After each period, the entire area was mowed to standardize pasture regrowth between treatments. Between the period intervals (2 periods of 15 d), the cows grazed ryegrass pastures and were supplemented daily with 10 kg of DM of partial TMR. During the experiment, the mean temperature was 17.0°C, and the total rainfall was 147 mm. The 10-yr climatic averages of temperature and rainfall were 14.5°C and 161 mm, respectively.

Table 1. Chemical and nutritive value of the corn silage and TMR offered to dairy cows with or without access to annual ryegrass pastures

Item	Corn silage	TMR[1]
DM, g/kg fresh	250	275
Chemical composition, g/kg of DM		
OM	950	928
CP	83	157
NDF	504	425
ADF	257	224
Nutritive value[2]		
NE_L, Mcal/kg of DM	1.58	1.67
PDIN, g/kg of DM	52.0	98.3
PDIE, g/kg of DM	71.0	86.7

[1] TMR composition: 800 g/kg of DM corn silage; 180 g/kg of DM soybean meal; and 20 g/kg of a DM mineral and vitamin supplement that contained 21% Ca, 6% P, 2% S, 7% Na, 3.5% K, 15 mg of Co/kg, 700 mg of Cu/kg, 200,000 IU of vitamin A/kg, 1,500 IU of vitamin E/kg, and 50,000 IU of vitamin D/kg.

[2] PDIN: protein truly digested in the intestine when nitrogen is limiting for microbial synthesis in the rumen; PDIE: protein truly digested in the intestine when energy is limiting for microbial synthesis in the rumen (INRA, 2007).

Animal Measurements

Milk production was recorded for each cow at each milking. Milk composition (fat, protein, and MUN content) was measured on each of the last 6 d of each period by infrared spectrophotometry (International IDF Standard 141C:2000; IDF, 2000). The BW was measured at the beginning and end of each experimental period.

The cows' pasture intake was estimated as the difference between the total biomass at pregrazing and postgrazing (Lantinga et al., 2004) on each of the last 6 d of each period. Their TMR and partial TMR intake were quantified daily as the difference between the quantity supplied and the orts on each of the last 6 d of each period and averaged.

Individual balances for NE_L and protein truly digested in the intestine with energy-limiting microbial synthesis in the rumen (**PDIE**) were calculated according to the INRA (2007). Theoretical NE_L requirements were calculated from the preexperimental BW and the expected 4% FCM production during the experiment. The calculation of theoretical PDIE requirements included these 2 factors and the preexperimental milk protein concentration. The expected 4% FCM production during the experiment was calculated by applying a monthly persistency of 90% from the preexperimental 4% FCM production. Net energy and PDIE supplies were calculated from the intake of pasture, corn silage, and soybean meal and from the concentrations of NE_L and PDIE, respectively. The digestive interactions between forage and concentrates were considered in these calculations by assuming a negative effect of the percentage of concentrate on the energy digestibility (INRA, 2007). Energy balance was calculated because the experimental periods were too short to measure changes in BCS or BW.

The daily pattern of grazing time was analyzed individually for each cow through visual observations that were made every 5 min. The rumination time was quantified with the same methodology and at the same time as grazing time when the cows were at pasture (Penning and Rutter, 2004). No behavior was recorded outdoors when the cows were milked or fed the supplement. The pasture intake rate (g of DM/min) was calculated by dividing the mean daily pasture intake per group by the mean of daily grazing time per group.

Daily methane emission was measured individually using the sulfur hexafluoride (SF_6) tracer technique described by Johnson et al. (1994). Of the data from 36 individual measurements, 14% were considered outliers and were not used in the statistical analysis. The SF_6 permeation tubes used in the experiment had an average permeation rate (**PR**) of 4.93 ± 0.36 mg/d at the time of their deployment in the reticulum, and breath samples were collected after 21 d of tube deployment. The SF_6 PR was measured by placing permeation tubes in 39°C water bath and measuring the daily weight loss during 6 wk. The air sampling system used stainless steel cylinders (0.5 L volume) as the sample collection devices, and sample flow was regulated by a brass ball bearing (Gere and Gratton, 2010). The cylinders were cleaned with high-purity nitrogen gas (N_2) and preevacuated (<0.5 mb) before each sample collection. The flow regulators were calibrated to allow a remaining vacuum in the canister of approximately 500 mb at the end of the sample collection period (5 d). The inflow restrictor was located just above the animal's nostrils and included a double filter to protect against water and dust. In addition to the breath samples, 4 background air samples (2 at paddock and 2 indoors) were collected in each period using a sampling system that was placed approximately 2 m above the ground. The periods of gas collection were selected to match the evaluation period for herbage DMI during the last 5 d of each period.

After the collection period, the pressure in each tube was measured. Each sample was then diluted with N_2, and the pressure was measured again to obtain the final pressure in the tube. Breath and background samples were analyzed for concentrations of methane (ppm, parts per million by volume) and SF_6 (ppt, parts per trillion by volume) by gas chromatography (2010, Shimadzu, Kyoto, Japan) using flame ionization (250°C) and electron capture (350°C) detectors, respectively. Three standards of methane and SF_6 mixtures were used to calibrate the gas chromatograph and to track its performance over a range of 5 to 20 ppm and 30 to 1,000 ppt for methane and SF_6, respectively.

Estimates of methane emissions over the sample collection period were calculated using the tracer technique. The specific PR of SF_6 and the methane/SF_6 ratio for mixed ratios (vol/vol) in breath samples were used after correcting for background gas concentrations (Johnson et al., 1994). For this purpose, the PR of SF_6 was expressed per day; consequently, the emission estimates corresponded to daily emissions.

Feed and Pasture Measurements

Samples of the offered TMR and partial TMR were collected twice daily from d 15 to 20 in each period. These samples were combined to create a composite sample for each period. Samples of the orts for each lot were collected during the last 6 d of each period and were used to create composite samples for the lot and period. All samples were dried in an oven for 72 h at 60°C and ground (Solab SL-31, Piracicaba, Brazil) through a 1-mm screen for subsequent chemical analyses.

The pre- and postgrazing PM above ground level was estimated using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand) that was calibrated based on DM content, taking into account the plate area (0.1 m^2; t'Mannetje and Jones, 2000). For calibration, during each experimental period, samples from 12 points were cut with scissors at ground level before and after grazing. The samples were dried in an oven for 72 h at 60°C. At the end of experiment, PM was recalculated using one equation to estimate the pregrazing PM and one equation to estimate the postgrazing PM:

$$\text{pregrazing PM (kg of DM/ha)} =$$
$$88.594 + 355.73 \text{ pregrazing sward height (cm)}$$
$$(n = 36, R^2 = 0.8146);$$
$$\text{postgrazing PM (kg of DM/ha)} =$$
$$91.111 + 230.12 \text{ postgrazing sward height (cm)}$$
$$(n = 36\ R^2 = 0.8325).$$

The pregrazing extended height of the tallest leaf blade and sheath were measured on 100 randomly selected tillers on d 16, 18, and 20. The postgrazing leaf and sheath extended heights were measured on d 17, 19, and 21 on 100 tillers per treatment.

The morphological and chemical compositions of the sward were determined on d 16, 18, and 20. Twenty handfuls of randomly selected herbage (~800 g fresh) were cut at the average postgrazing extended tiller height. This pasture was considered to represent the pasture selected by grazing cows and was separated into 2 smaller subsamples. One subsample was dried in an oven for 72 h at 60°C with forced ventilation and stored for chemical analyses. The chemical composition of the selected pasture was estimated based on the postgraze tiller height, as proposed by Delagarde et al. (2000). The other subsample was used for morphological classification (ryegrass only). The ryegrass was separated into leaf blades, pseudostems, stems, flowers, and dead tissue, if any was present. Each constituent was dried in an oven for 72 h at 60°C to determine the morphological composition of the pasture on a DM basis.

Chemical Analyses

The DM content was determined by drying the samples at 105°C for 24 h. The ash was quantified by combustion in a muffle furnace at 550°C for 4 h, and the OM was quantified by mass difference. The total N was assayed using the Kjeldahl method (method 984.13; AOAC International, 1998). The NDF concentration was assessed according to Mertens et al. (2002), except that the samples were weighed in filter bags and treated with neutral detergent in Ankom A220 equipment (Ankom Technology, Macedon, NY). This analysis included α-amylase but did not include sodium sulfite because sodium sulfite also destroys lignin, thereby reducing its recovery (Van Soest, 1994). The concentrations of ADF and ADL were analyzed according to AOAC International (1998).

Statistical Analyses

The data were subjected to variance analysis using PROC MIXED of SAS software (1999, version 9.3, SAS Institute, Cary, NC). The animal variables, averaged per group and period (n = 18), were analyzed using the following model:

$$Y_{ijk} = \mu + \text{group}_i + \text{period}_j + \text{treatment}_k + e_{ijk},$$

where Y_{ijk}, μ, group_i, period_j, treatment_k and e_{ijk} represent the analyzed variable, the overall mean, the random effect of the group, the random effect of the period, the fixed effect of the treatment and the residual error, respectively.

The pasture variables were averaged per treatment and period (n = 12) and analyzed using the following model:

$$Y_{jk} = \mu + \text{period}_j + \text{treatment}_k + e_{jk}.$$

The effects of the treatments were tested by orthogonal contrasts that compared the TMR treatment versus the average of the 6h and 3+3h treatments and the 6h treatment versus the 3+3h treatment. Values of $P <$ 0.05 were considered significantly different, and values between 0.05 and 0.10 were considered to have a tendency to differ.

RESULTS

The pregrazing PM, pregrazing sward height, and pasture allowance (**PA**) were similar between the treatments that included ryegrass pasture (Table 2). The NDF and ADF content did not differ in the offered ryegrass pasture, but the CP content was lower ($P <$ 0.05) in the pastures that were offered to cows for a continuous 6-h period compared with that in pastures that were offered in 2 sessions, each 3 h. The postgrazing PM, postgrazing sward height, and NDF, ADF,

Table 2. Pregrazing pasture characteristics and composition of offered pasture swards when the pasture was grazed by dairy cows during 2 time periods throughout the day

Item	Treatment[1]		SEM	*P*-value
	6h	3+3h		
Pregrazing				
Pasture mass, kg of DM/ha	2,706	2,696	43.4	0.937
Pregrazing sward height				
Rising plate meter	26.8	26.7	0.48	0.937
Extended tiller, cm	42.6	42.3	0.89	0.812
Extended sheath, cm	21.7	20.7	0.52	0.202
Extended lamina, cm	20.9	21.6	0.48	0.320
Offered area, m^2/cow per d	124	125	3.58	0.843
Pasture allowance, kg of DM/d				
Above ground level	33.3	33.4	0.05	0.449
Green material	30.3	29.6	0.13	0.012
Live lamina	8.3	8.7	0.35	0.515
Offered pasture				
Chemical composition, g/kg of DM				
DM, g/kg	154	145	2.88	0.063
OM	911	909	1.86	0.417
CP	145	162	4.11	0.022
NDF	547	536	5.63	0.194
ADF	277	283	6.08	0.540

[1] Treatments: 6h = access to annual ryegrass pastures after morning milking (between 0900 and 1500 h); 3+3h = access to annual ryegrass pastures after morning and afternoon milking (between 0900 and 1200 h and between 1530 and 1830 h).

and CP content of the selected pasture did not differ between the ryegrass pasture treatments (Table 3). The energetic value of the selected pasture and protein truly digested in the intestine were similar between treatments, averaging 1.54 Mcal of NE_L/kg of DM and 96 g/kg of DM, respectively.

The cows' milk production, FCM, milk fat, and milk protein production did not differ between treatments

Table 3. Postgrazing pasture characteristics and nutritive value of selected pasture when the pasture was grazed by dairy cows during 2 time periods throughout the day

Item	Treatment[1]		SEM	*P*-value
	6h	3+3h		
Postgrazing pasture mass, kg of DM/ha	2,098	2,020	30.49	0.115
Postgrazing sward height				
Rising plate meter	20.0	19.2	0.33	0.115
Extended tiller, cm	26.7	25.1	0.67	0.154
Extended sheath, cm	16.1	15.1	0.47	0.211
Extended lamina, cm	10.6	10.0	0.44	0.373
Selected pasture				
Chemical composition, g/kg of DM				
CP	171	169	0.65	0.120
NDF	354	348	4.61	0.411
ADF	135	122	6.54	0.181
Nutritive value				
OM digestibility[2]	0.73	0.74	0.003	0.891
NE_L, Mcal/kg of DM	1.54	1.55	0.002	<0.001
PDIN,[3] g/kg of DM	111	110	0.49	0.273
PDIE,[4] g/kg of DM	96	96	0.15	0.046

[1] Treatments: 6h = access to annual ryegrass pastures after morning milking (between 0900 and 1500 h); 3+3h = access to annual ryegrass pastures after morning and afternoon milkings (between 0900 and 1200 h and between 1530 and 1830 h).

[2] OM digestibility was estimated as a function of the CP and ADF content of selected pasture (INRA, 2007).

[3] PDIN = protein truly digested in the intestine when nitrogen is limiting for microbial synthesis in the rumen (INRA, 2007).

[4] PDIE = protein truly digested in the intestine when energy is limiting for microbial synthesis in the rumen (INRA, 2007).

Table 4. Milk production and composition in dairy cows that received TMR exclusively or partial TMR with annual ryegrass (*Lolium multiflorum* Lam.) strip-grazing during 2 time periods throughout the day

	Treatment[1]				Contrast (P-value)	
Item	TMR	6h	3+3h	SEM	TMR vs. grazing	6 vs. 3+3h
Milk production, kg/d	19.5	20.0	20.2	0.39	0.192	0.728
4% FCM production, kg/d	20.5	20.2	20.5	0.35	0.859	0.579
Milk fat production, g/d	838	805	819	16.9	0.249	0.557
Milk protein production, g/d	649	654	665	11.0	0.455	0.525
Milk fat concentration, %	4.34	4.04	4.08	0.09	0.042	0.782
Milk protein concentration, %	3.35	3.28	3.30	0.01	0.024	0.396
MUN, mg/dL	22.8	18.9	19.5	0.48	<0.001	0.408
SCC, $\times 10^3$/mL	168	125	124	17.8	0.084	0.965
BW, kg	525	519	516	6.85	0.396	0.793

[1] Treatments: TMR = total mixed ration exclusively; 6h = partial mixed ration + access to annual ryegrass pastures after morning milking (between 0900 and 1500 h); 3+3h = partial mixed ration + access to annual ryegrass pastures after morning and afternoon milkings (between 0900 and 1200 h and between 1530 and 1830 h); grazing = 6h and 3+3h access to annual ryegrass pasture.

(Table 4). The milk fat concentration ($P < 0.05$), milk protein concentration ($P < 0.05$), and MUN concentration decreased ($P < 0.001$) in cows that had access to ryegrass pasture compared with cows that received TMR exclusively. However, these parameters did not differ between cows that had access to pasture for a continuous 6-h period and those that were offered pasture after each milking. The total DMI was similar between treatments, averaging 16.1 kg/d, but the pasture DMI increased (+1.4 kg of DM/d; $P < 0.05$) and the DMI of partial TMR decreased (0.9 kg of DM/d; $P < 0.01$) when cows had access to ryegrass pasture after each milking compared with when cows had access to pasture for a continuous 6-h period (Table 5). The total grazing time and the proportion of time spent grazing increased ($P < 0.001$) by 40 min/d and 11%, respectively, in cows that had access to ryegrass pasture after each milking compared with cows that grazed ryegrass pasture after the morning milking only. The pasture DMI rate was similar between ryegrass pasture treatments, averaging 26 g of DM/min.

The daily enteric methane emissions (−110 g/d; $P < 0.05$), the enteric methane emission per kilogram DMI ($P < 0.05$) and NDF consumed ($P = 0.05$), and the enteric methane emission per kilogram of milk yield ($P < 0.05$) and the proportion of gross energy intake ($P < 0.05$) decreased in cows with access to ryegrass pasture + partial TMR compared with cows that received TMR exclusively (Table 6).

DISCUSSION

This study aimed to compare dairy cows during mid to late lactation; one group received TMR, whereas the second group grazed high-quality pasture under grazing management conditions that did not restrict pasture

Table 5. Dry matter intake, energy balance, and grazing behavior of dairy cows that received TMR or partial TMR with annual ryegrass (*Lolium multiflorum* Lam.) strip-grazing during 2 periods throughout the day

	Treatment[1]				Contrast (P-value)	
Item	TMR	6h	3+3h	SEM	TMR vs. grazing	6 vs. 3+3h
DMI, kg/d						
Pasture	—	6.0	7.4	0.25	—	0.036
TMR	16.4	9.7	8.8	0.18	<0.001	0.007
Total	16.4	15.7	16.2	0.29	0.292	0.413
NDF intake, kg/d	6.6	6.0	6.0	0.12	0.010	0.995
OM intake, kg/d	15.2	14.6	14.8	0.27	0.176	0.497
NE_L supply,[2] Mcal/d	27.8	25.9	26.2	0.47	0.017	0.669
NE_L balance, Mcal/d	4.2	2.5	2.6	0.39	0.009	0.844
Grazing time, min/d	—	240	280	3.96	—	<0.001
Ruminating time, min/d	—	76	43	2.24	—	<0.001
Proportion of time spent grazing	—	0.66	0.77	0.01	—	<0.001
Pasture DMI rate, g/min	—	25.7	26.2	0.66	—	0.655

[1] Treatments: TMR = total mixed ration exclusively; 6h = partial mixed ration + access to annual ryegrass pastures after morning milking (between 0900 and 1500 h); 3+3h = partial mixed ration + access to annual ryegrass pastures after morning and afternoon milkings (between 0900 and 1200 h and between 1530 and 1830 h); grazing = 6h and 3+3h access to annual ryegrass pasture.

[2] Net energy for lactation estimated according to INRA (2007).

Table 6. Enteric methane production of lactating dairy cows that received TMR or partial TMR with annual ryegrass (*Lolium multiflorum* Lam.) strip-grazing during 2 time periods throughout the day

Methane	Treatment[1] TMR	6h	3+3h	SEM	Contrast[1] (*P*-value) TMR vs. grazing	6 vs. 3+3h
g/d	656	590	503	33.8	0.049	0.110
g/kg of DMI	41.7	37.4	31.2	2.00	0.024	0.054
g/kg of NDF	104.5	97.3	82.9	5.41	0.058	0.086
g/kg of milk yield	34.2	30.0	25.3	1.93	0.045	0.138
% gross energy intake	11.3	9.8	8.6	0.56	0.013	0.140

[1] Treatments: TMR = total mixed ration exclusively; 6h = partial mixed ration + access to annual ryegrass pastures after morning milking (between 0900 and 1500 h); 3+3h = partial mixed ration + access to annual ryegrass pastures after morning and afternoon milkings (between 0900 and 1200 h and between 1530 and 1830 h); grazing = 6 h and 3+3 h of access to annual ryegrass pasture.

intake and were supplemented with partial TMR. We cannot explain the lower CP content in the 6h treatment pasture compared with the 3+3h treatment pasture. However, the selected pasture for each treatment displayed similar CP content, approximately 17% on average, which is sufficient to avoid N restrictions to microbial growth (Peyraud and Delagarde, 2013). The NDF averaged less than 40%, with an energy concentration greater than 1.5 Mcal of NE_L/kg of DM, consistent with the typical characteristics of pasture with good energetic value (Peyraud and Delagarde, 2013). Similarly, under grazing management conditions without restrictions on pasture intake, annual ryegrass must have a pregrazing herbage mass greater than 2,000 kg of DM/ha (Miguel et al., 2014) and a postgrazing leaf lamina height of no less than 10 cm (Ribeiro Filho et al., 2011). In the current study, the pregrazing herbage mass, postgrazing herbage mass, and postgrazing leaf lamina height were, on average, 2,700 kg of DM/ha, 2,060 kg of DM/ha and 10.3 cm, respectively.

DMI and Grazing Behavior

The maintenance of the total DMI when animals had access to pasture can be explained, at least in part, by the energy value, structural characteristics, and management conditions of the grazed forage. Feed supplementation with preserved forage has been shown to negatively influence pasture intake in cows with access to pasture in the early growing season (organic matter digestibility, **OMD** = 0.71); however, when cows had access to pasture in the late growing season (OMD = 0.65), silage supplementation had a positive effect on the total DMI and ME (Bargo et al., 2003). In this study, the average OMD of ingested forage was 0.73, demonstrating the potential of pasture with OMD >0.7 to maintain DMI and milk production in cows during mid lactation.

The absence of constraints due to the structural characteristics of the forage or the management of forage consumption was reflected in the herbage intake rate and pasture intake. Under severe grazing conditions, some studies have observed an herbage intake rate close to 16 g of DM/min, whereas values >20 g of DM/min are typical of better quality, freely grazed pasture (Rook et al., 1994; Pérez-Prieto and Delagarde, 2012). In this study, the average herbage intake rate was >25 g of DM/min.

The pasture DMI was 20% lower in animals that had access to pasture for a continuous 6-h period compared with those that received the 3+3h treatment. This result may be explained by differences in substitution rate (**SR**) and behavioral factors. It is well known that using concentrate supplementation SR is caused by negative associative effects in the rumen or grazing time (Bargo et al., 2003). Moreover, the relationship between SR and corn silage supplementation is dependent on PA; SR increases as PA increases (Phillips, 1988). In the current study, the concentrate content and PA were similar between the 6h and 3+3h treatments, averaging 10.5% of DMI and 33 kg of DM/d, respectively. Thus, the consumption of grazed forage seemed to be directly associated with the ingestion period of the main daily meals, during which consumption can reach 60 to 80% of the total daily intake (Baumont et al., 2000). During grazing, pasture intake is associated with environmental factors (dawn and dusk) and with stimuli such as milking (Gregorini et al., 2006). Therefore, access to pasture after milking may have encouraged the animals to graze for a longer period, thereby increasing the grazing efficiency from 1.0 to 1.2 kg of DM/h; this increase could have then contributed to the higher consumption of grazed forage. Shorter grazing periods can increase grazing efficiency (DM consumed per hour of access to pasture) due to the increased period devoted to grazing activity (Kennedy et al., 2009; Pérez-Ramirez et al., 2009).

Milk Production and Milk Composition. The maintenance of milk production with the inclusion of pasture in the diet is closely related not only to the total DMI but also to the stage of lactation and the production potential of the animals. Bargo et al. (2002) evaluated highly productive cows (44.9 ± 7.5 kg/d) and observed that confined animals produced 19% more milk than animals that received partial TMR + pasture due to the lower energy consumption and higher energy expenditure of grazing animals (Kolver and Muller, 1998). In our study, even with the lowest consumption of NE_L, cows that had access to pasture did not exhibit lower milk production than cows that were fed exclusively with TMR. In addition, the energy balance was positive for all treatments, indicating that energy consumption among animals that had access to pasture was sufficient to meet the maintenance and production requirements of this stage of lactation and for this level of production.

Although the milk fat concentration of the control cows were higher than those of the cows that had access to pasture, the mean values in all cows were greater than 4.0%. The fat level can be at least partially explained by the relatively low levels of concentrate (approximately 13.0% of the total DMI) in all of the experimental diets. The decreased milk fat content in the grazing lactating cows that were supplemented with partial TMR compared with those that received exclusively TMR may be associated with the level of NDF in the diet (Elgersma et al., 2004; Morales-Almaráz et al., 2010). In this study, the percentage of NDF decreased from 41 to 37% of the total DM in animals that had access to pasture compared with animals that were fed with TMR. At an average of 20.3 mg/dL, the MUN levels were higher than the level of variation (9.1–17.1 mg/dL) reported by other authors (Bargo et al., 2002; Fontaneli et al., 2005; Vibart et al., 2008). This result does not have a satisfactory explanation, but the approach of Faverdin and Vérite (1998) to estimate the MUN concentration was based on the balance of degradable nitrogen in the rumen (relationship between degradable protein and nitrogen required for microbial growth in the rumen). The difference between the intake of degradable nitrogen and the amount of nitrogen required for microbial growth in the rumen was positive in all treatments, with an average of 300 g/d.

Enteric Methane Emissions. The higher daily production of methane in animals that were fed TMR compared with those that received the other treatments cannot be attributed to differences in the daily intake of DM because DMI was similar between treatments; this result differs from observations made in previous studies (O'Neill et al., 2011, 2012; Hassanat et al., 2013). Thus, in the present study, the higher emission of methane in animals that were fed the TMR compared with those that received the partial TMR treatments was correlated with the NDF content and ADF:NDF relationship.

The NDF intake was greater in the TMR compared with the partial TMR treatments (6.6 vs. 6.0 kg/d). Aguerre et al. (2011) observed that dairy cows that ingested an average of 6.0 kg NDF/d emitted 593 g of methane per day, with a positive correlation between methane emission and NDF consumption. In the present study, the average NDF consumption was 6.2 kg/d, and the average daily emission of methane was 583 g/d. The ADF:NDF relationship was greater in the TMR diet compared with that of the pasture + partial TMR diet (0.52 vs. 0.46). These results indicate that the ryegrass pasture contained more rapidly degradable cell wall carbohydrates than the corn silage. Increasing the dietary fiber content is known to decrease the molar ratio of propionate and to increase the release of hydrogen for methanogenic Archaea (Benchaar et al., 2001). Additionally, the higher daily emission of methane of the animals that received the TMR resulted in higher energy loss relative to the energy consumed. These results agree with a comprehensive review published by Boadi et al. (2004). Compared with the fermentation of soluble sugars, the fermentation of structural carbohydrates results in the loss of a considerable amount of gross energy in the form of methane. According to Johnson and Johnson (1995), this loss varies between 2 and 12% of the gross energy consumed. In this study, these values ranged between 8.6 and 11.3%.

CONCLUSIONS

Providing mid- to late-lactation dairy cows that were receiving a TMR access to annual ryegrass pasture for 6 h/d enabled the consumption of 42% of the total DMI from pasture, maintained milk production, and mitigated enteric methane emissions. Providing access to pasture after morning and afternoon milking is a method to increase the proportion of pasture in the total DMI.

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REFERENCES

Aguerre, M. J., M. A. Wattiaux, J. M. Powell, G. A. Broderick, and C. Arndt. 2011. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. J. Dairy Sci. 94:3081–3093. http://dx.doi.org/10.3168/jds.2010-4011.

AOAC International. 1998. Official Methods of Analysis. CD-ROM. AOAC International, Rockville, MD.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. J. Dairy Sci. 85:2948–2963. http://dx.doi.org/10.3168/jds.S0022-0302(02)74381-6.

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. *Invited review:* Production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1–42. http://dx.doi.org/10.3168/jds.S0022-0302(03)73581-4.

Baumont, R., S. Prache, M. Meuret, and P. Morand-Fehr. 2000. How forage characteristics influence behaviour and intake in small ruminants: A review. Livest. Prod. Sci. 64:15–28. http://dx.doi.org/10.1016/S0301-6226(00)00172-X.

Benchaar, C., C. Pomar, and J. Chiquette. 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. Can. J. Anim. Sci. 81:563–574. http://dx.doi.org/10.4141/A00-119.

Boadi, D., C. Benchaar, J. Chiquette, and D. Massé. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84:319–335. http://dx.doi.org/10.4141/A03-109.

Chilibroste, P., P. Soca, D. A. Mattiauda, O. Bentancur, and P. H. Robinson. 2007. Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. Aust. J. Exp. Agric. 47:1075–1084. http://dx.doi.org/10.1071/EA06130.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin–cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. Anim. Feed Sci. Technol. 84:49–68. http://dx.doi.org/10.1016/S0377-8401(00)00114-0.

Elgersma, A., G. Ellen, H. Van Der Horst, H. Boer, P. R. Dekker, and S. Tamminga. 2004. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. Anim. Feed Sci. Technol. 117:13–27. http://dx.doi.org/10.1016/j.anifeedsci.2004.08.003.

Faverdin, P., and R. Vérite. 1998. Utilisation de la teneur en urée du lait comme indicateur de la nutrition protéique et des rejets azotés chez la vache laitière. Ren. Rech. Rumin. 5:209–212.

Fontaneli, R. S., L. E. Sollenberger, R. C. Littell, and C. R. Staples. 2005. Performance of lactating dairy cows managed on pasture-based or in freestall barn-feeding systems. J. Dairy Sci. 88:1264–1276. http://dx.doi.org/10.3168/jds.S0022-0302(05)72793-4.

Gere, J. I., and R. Gratton. 2010. Simple, low-cost flow controllers for time averaged atmospheric sampling and other applications. Lat. Am. Appl. Res. 40:377–381.

Gregorini, P., S. Tamminga, and S. A. Gunter. 2006. Review: Behavior and daily grazing of cattle. Prof. Anim. Sci. 22:201–209.

Hassanat, F., R. Gervais, C. Julien, D. I. Massé, A. Lettat, P. Y. Chouinard, H. V. Petit, and C. Benchaar. 2013. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, N balance, and milk production. J. Dairy Sci. 96:4553–4567. http://dx.doi.org/10.3168/jds.2012-6480.

IDF. 2000. Determination of milk fat, protein and lactose content. Guidance on the operation of mid-infrared instruments. IDF Standard 141C:2000 Int. Dairy Fed., Brussels, Belgium.

INRA. 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux-valeurs des aliments. 4th ed. INRA, Institut National de Recherche Agronomique, Paris, France.

Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. Environ. Sci. Technol. 28:359–362. http://dx.doi.org/10.1021/es00051a025.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.

Kennedy, E., M. McEvoy, J. P. Murphy, and M. O'Donovan. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. J. Dairy Sci. 92:168–176. http://dx.doi.org/10.3168/jds.2008-1091.

Kolver, E. S., and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403–1411. http://dx.doi.org/10.3168/jds.S0022-0302(98)75704-2.

Lantinga, E. A., J. H. Neuteboom, and J. A. C. Meijs. 2004. Sward methods. Pages 1–191 in Herbage Intake Handbook. P. D. Penning, ed. The British Grassland Society, Maidenhead, UK.

Mertens, D. R., M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C. S. Jeon, D. Jones, D. Kaplan, G. N. Kim, S. Kobata, D. Main, X. Moua, B. Paul, J. Robertson, D. Taysom, N. Thiex, J. Williams, and M. Wolf. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.

Miguel, M. F., H. M. N. Ribeiro-Filho, E. de Andrade, T. C. M. Genro, and R. Delagarde. 2014. Pasture intake and milk production of dairy cows grazing annual ryegrass with or without corn silage supplementation. Anim. Prod. Sci. 54:1810–1816.

Morales-Almaráz, E., A. Soldado, A. González, A. Martínez-Fernández, I. Domínguez-Vara, B. de la Roza-Delgado, and F. Vicente. 2010. Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. J. Dairy Res. 77:225–230. http://dx.doi.org/10.1017/S002202991000004X.

O'Neill, B. F., M. H. Deighton, B. M. O'Loughlin, N. Galvin, M. O'Donovan, and E. Lewis. 2012. The effects of supplementing grazing dairy cows with partial mixed ration on enteric methane emissions and milk production during mid to late lactation. J. Dairy Sci. 95:6582–6590. http://dx.doi.org/10.3168/jds.2011-5257.

O'Neill, B. F., M. H. Deighton, B. M. O'Loughlin, F. J. Mulligan, T. M. Boland, M. O'Donovan, and E. Lewis. 2011. Effects of a perennial ryegrass diet or total mixed ration diet offered to spring-calving Holstein-Friesian dairy cows on methane emissions, dry matter intake, and milk production. J. Dairy Sci. 94:1941–1951. http://dx.doi.org/10.3168/jds.2010-3361.

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass Forage Sci. 56:352–361. http://dx.doi.org/10.1046/j.1365-2494.2001.00284.x.

Penning, P. D., and S. M. Rutter. 2004. Ingestive behaviour. Pages 1–191 in Herbage Intake Handbook. P. D. Penning, ed. British Grassland Society, Maidenhead, UK.

Pérez-Prieto, L. A., and R. Delagarde. 2012. Meta-analysis of the effect of pregrazing pasture mass on pasture intake, milk production, and grazing behavior of dairy cows strip-grazing temperate grasslands. J. Dairy Sci. 95:5317–5330. http://dx.doi.org/10.3168/jds.2012-5609.

Pérez-Ramírez, E., R. Delagarde, and L. Delaby. 2008. Herbage intake and behavioural adaptation of grazing dairy cows by restricting time at pasture under two feeding regimes. Animal 2:1384–1392. http://dx.doi.org/10.1017/S1751731108002486.

Pérez-Ramírez, E., J. L. Peyraud, and R. Delagarde. 2009. Restricting daily time at pasture at low and high pasture allowance: Effects on pasture intake and behavioral adaptation of lactating dairy cows. J. Dairy Sci. 92:3331–3340. http://dx.doi.org/10.3168/jds.2008-1951.

Peyraud, J. L., and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply under grazing. Animal 7:57–67. http://dx.doi.org/10.1017/S1751731111002394.

Phillips, C. J. C. 1988. The use of conserved forage as a supplement for grazing dairy cows. Grass Forage Sci. 43:215–230.

Ribeiro Filho, H. M. N., E. A. Setelich, S. Crestani, K. M. Dias, C. Mantovani, and J. Valenti. 2011. Relationship between diurnal grazing time and herbage intake in dairy cows in rotational grazing. Ciênc. Rural 41:2010–2013. http://dx.doi.org/10.1590/S0103-84782011005000140.

Robertson, L. J., and G. C. Waghorn. 2002. Dairy industry perspectives on methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. Pages 213–218 in Proc. New Zealand Soc. Anim. Prod. Vol. 62.

Rook, A. J., C. A. Huckle, and P. D. Penning. 1994. Effects of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. Appl. Anim. Behav. Sci. 40:101–112. http://dx.doi.org/10.1016/0168-1591(94)90075-2.

Soriano, F. D., C. E. Polan, and C. N. Miller. 2001. Supplementing pasture to lactating Holsteins fed a total mixed ration diet. J. Dairy Sci. 84:2460–2468. http://dx.doi.org/10.3168/jds.S0022-0302(01)74696-6.

t'Mannetje, L., and R. M. Jones. 2000. Field and Laboratory Methods for Grassland and Animal Production. CABI Publishing, Wallingford, UK.

Tozer, P. R., F. Bargo, and L. D. Muller. 2003. Economic analyses of feeding systems combining pasture and total mixed ration. J. Dairy Sci. 86:808–818. http://dx.doi.org/10.3168/jds.S0022-0302(03)73663-7.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Vibart, R. E., V. Fellner, J. C. Burns, G. B. Huntington, and J. T. Green. 2008. Performance of lactating dairy cows fed varying levels of total mixed ration and pasture. J. Dairy Res. 75:471–480. http://dx.doi.org/10.1017/S0022029908003361.

Wales, W. J., L. C. Marett, J. S. Greenwood, M. M. Wright, J. B. Thornhill, J. L. Jacobs, C. K. M. Ho, and M. J. Auldist. 2013. Use of partial mixed rations in pasture-based dairying in temperate regions of Australia. Anim. Prod. Sci. 53:1167–1178. http://dx.doi.org/10.1071/AN13207.