



Enteric methane emission from grazing dairy cows receiving corn silage or ground corn supplementation

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ABSTRACT

This study assessed the effects of corn silage or ground corn supplementation on CH₄ emission, milk production and total dry matter (DM) intake by dairy cows under lenient grazing conditions. Twelve Holstein × Jersey dairy cows were divided into 6 homogeneous groups and randomly distributed among the experimental treatments, which were compared using a replicated 3 × 3 Latin square design over three periods of 17 days (evaluation period of 5 days). The dairy cows strip-grazed a mixture of annual ryegrass (*Lolium multiflorum*) and oat (*Avena sativa*) grass. The treatments consisted of three feeding strategies: one without supplementation (the WS strategy), one supplemented with ground corn (the GC strategy; 3.2 kg as DM basis), and one supplemented with corn silage (the CS strategy; 4.2 kg as DM basis). The post-grazing sward height (average = 8.8 cm) was held constant between treatments by reducing the herbage allowance (HA) in the supplemented treatments. The HA values were 41.3, 30.8 and 34.6 kg of DM/cow/day for the WS, CS, and GC treatments, respectively. Daily enteric CH₄ emission was measured using the sulfur hexafluoride (SF₆) tracer gas technique. The CH₄ yield (g/kg of DM intake) decreased in the GC treatment group compared to the WS and CS treatments, although the CH₄ emission (g/day) and CH₄ intensity (g/kg of milk) did not differ between treatments, averaging 337 g/day and 15.1 g/kg milk, respectively. The total DM intake increased by 2.9 kg/day, and milk production increased by 1.4 kg/day in the GC treatment group compared to the WS group, whereas the total DM intake increased by 1.5 kg/day, and milk production did not differ between the CS and WS treatment groups. In conclusion, it was possible to reduce the HA and maintain or increase the total DM intake with corn silage or ground corn supplementation, respectively. In dairy cows grazing temperate grass under lenient grazing conditions, corn silage and ground corn supplementation were effective for reducing CH₄ yield but not CH₄ intensity.

1. Introduction

Enteric methane (CH₄) is a normal product of a ruminant's digestive process, and its emission represents approximately 44% of the total greenhouse gas (GHG) emissions by the livestock sector (Gerber et al., 2013). The synthesis of enteric CH₄ is directly associated with the hydrogen (H₂) concentration in the ruminal environment microbial metabolism (Janssen, 2010). Hence, the dry matter (DM) intake and diet composition may affect the formation of enteric CH₄ due to differences in the amount and characteristics

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of the substrate available for ruminal fermentation.

The use of corn silage and concentrate supplementation has been proposed as a strategy for CH₄ mitigation in grazing dairy cows through increased starch intake (Beauchemin et al., 2008; Hristov et al., 2013; Martin et al., 2010). Reports have shown that the emission of enteric CH₄ is reduced when the concentrate supplement ranges from 350 to 400 g/kg of DM in the total diet (Sauvant and Nozière, 2015). Moreover, unlike other conserved forages, corn silage provided at up to 500 g/kg of DM is sufficient to decrease the production of enteric CH₄ (Hassanat et al., 2013; Van Gastelen et al., 2015). However, the impacts of different energy supplementation regimens on CH₄ intensity (kg CH₄/kg milk) in grazing dairy cows remain unclear and warrant further investigation.

The use of supplements is known to cause a greater reduction in DM herbage intake and a smaller change in milk production (kg milk increased/kg DM supplement intake) in dairy cows under non-restrictive grazing conditions than under restrictive grazing conditions (Bargo et al., 2002; Pérez-Prieto et al., 2011). This phenomenon may be related to the similarity between the energy supplied by grazed herbage and the energy requirements of non-restrictive grazing conditions. A theoretical model published by Delagarde et al. (2011) has shown that the impact of conserved-forage supplementation on herbage intake is more pronounced than that of concentrate supplementation due to a greater substitution rate (kg herbage DM intake decreased per kg DM supplement intake) in the former case. In practical conditions, the substitution rate allows the herbage allowance (HA) to be reduced in order to avoid herbage waste. In this case, the grazing strategy may target post-grazing sward heights similar to those left by unsupplemented dairy cows. However, there have been very few studies using grazing management protocols with similar sward heights (both before and after grazing) for unsupplemented and supplemented dairy cows.

Highlighting the major influence of supplementation type on herbage intake and milk production response, this work aimed to evaluate the effects of corn silage or ground corn supplementation on CH₄ emission, milk production and total DM intake by dairy cows under lenient grazing conditions. We hypothesized that under lenient grazing conditions, energy supplementation would increase milk production per cow, daily CH₄ emission would depend on total DM intake, and CH₄ intensity would decrease when the cow diet are supplemented with starch.

2. Materials and methods

The experiment was performed from August to November 2016 in Lages, SC, Brazil (50.18 °W, 27.47 °S; 920 m altitude) in accordance with the regulations of the Santa Catarina State University Ethical Committee (Protocol no. 43.73.09.08.16). During the study, the average daily maximum and minimum temperatures were 20.8 and 8.6 °C, respectively, and the total rainfall was 161 mm. The average maximum and minimum temperatures and rainfall over 10 years were 20.5 °C, 9.9 °C and 183 mm, respectively.

2.1. Animals, experimental design and treatments

Twelve multiparous Holstein × Jersey cows were separated into 6 groups with comparable milk production (23.3 ± 6.9 kg/day), days in milk (101 ± 57.6 day), body weight (BW, 492 ± 76.8 kg) and parity (2.3 ± 1.5). Each group was randomly assigned to treatments according to a replicated 3×3 Latin square design. Each experimental period lasted 17 days and consisted of 12 days of adaptation and 5 days of measurement. A 12-day adaptation period was used because it has been shown that when the proportion of supplement is lower than 5 kg/day or 30% of total DM intake, eight days is sufficient to stabilize the milk response after a treatment change (Delaby et al., 1995; Pérez-Ramírez et al., 2012).

The treatments consisted of access to annual ryegrass and oat grass without supplementation (WS) or with either of two energy supplements: ground corn (GC) or corn silage (CS). The quantity of corn silage was calculated to offer the same net energy for lactation (NE_L) provided by the ground corn supplementation. The NE_L of each supplement was estimated using the equations published by the (INRA, 2007) in which the net energy values are calculated from organic matter (OM) digestibility; in the present study, OM digestibility was predicted based on OM, neutral detergent fibre (aNDF) and crude protein (CP) content. The quantities offered were 3.2 and 4.2 kg (on a DM basis) of ground corn and corn silage, respectively. The supplements were offered individually twice daily after morning and afternoon milking for 60 min in the GC and CS treatments, whereas in the WS treatment, the cows were returned immediately to the pasture after milking. Any supplement that remained following the afternoon feeding was considered ort. Water and minerals were continually available at the grazing sites and indoors. The chemical compositions and nutritive values of the supplements are presented in Table 1.

2.2. Grazing management and pasture

Ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) pastures were sown in May 2016 on a total area of 6.5 ha. The area was split into six paddocks, two per treatment, and the same paddock was grazed by the same two cows during each period. The length of the average grazing cycle was 32 days (one day of grazing and 31 days of rest), and the ratio of ryegrass to oat was 0.55: 0.45 on a DM basis. After each period, the entire area was mowed to standardize the herbage regrowth between treatments. Thirty days before the start of the experiment and immediately after each experimental period, the experimental area was fertilized with 50 kg N/ha supplied as urea. In the intervals (15 days on average) between the experimental periods, the cows grazed as a single group in a non-experimental pasture predominated by tall fescue and white clover and received 4 kg/day of ground corn, offered individually, twice daily, after the morning and afternoon milking.

The grazing method selected for this study was strip grazing, and the area allocated daily to each treatment group was calculated by estimating the pre-grazing herbage mass (HM). In the treatment groups that were not supplemented, the HA above ground level

Table 1
Chemical composition and nutritive value of the supplements (corn silage and ground corn).

Item	Corn silage	Ground corn
DM, g/kg	281	911
<i>Chemical composition, g/kg DM</i>		
Organic matter	963	984
Crude protein	61	85
aNDF	404	159
ADF	240	31
<i>Nutritive value¹</i>		
NE _L , Mcal/kg DM	1.53	1.82
PDIN, g/kg DM	37	67
PDIE, g /kg DM	65	87

¹ (INRA, 2007); NE_L, net energy for lactation; PDIN, truly digestible protein when degradable nitrogen limits microbial growth; and PDIE, truly digestible protein when available energy limits microbial growth.

was 35 kg DM/cow. In the supplemented treatment groups (GC and CS), the HA was adjusted to obtain the same post-grazing sward height as the unsupplemented cows. The HA for the supplemented treatment groups was adjusted over the first days of adaptation in the first experimental period, and no changes were necessary for the rest of the experiment because the post-grazing height targets were achieved.

2.3. Animal measurements

The cows were milked twice daily (7 a.m. and 3:30 p.m.). Over the last 5 days of each period, individual milk production was recorded daily using an electronic milk meter (Waikato Milking Systems, New Zealand) approved by the ICAR (International Committee for Animal Recording), and milk samples were collected to determine the composition. The milk fat and milk protein percentages were determined by infrared spectrophotometry (Dairy Spect FT, Bentley Instruments Inc., Chaska, Minnesota, USA). The energy-corrected milk production (ECM), standardized to 4.0% fat and 3.3% protein, was calculated according to the equation proposed by Tyrrell and Reid (1965):

$$\text{ECM (kg/cow per day)} = \text{milk production kg} \times (376 \times \text{fat\%} + 209 \times \text{protein\%} + 948) / 3,138$$

The herbage DM intake was estimated in each group by determining the difference between the pre- and post-grazing HM values (Lantinga et al., 2004). For details on the pre- and post-grazing HM estimations, see section 2.4 below. The individual supplement intake was quantified daily as the difference between the quantity supplied and the quantity of orts.

The NE_L requirements for lactation and maintenance were calculated based on 4% fat-corrected milk (FCM) production and BW as proposed by (INRA, 2007). The NE_L and metabolizable protein (MP) supply were calculated from the herbage, corn silage and ground corn DM intake and the NE_L and MP concentrations of each feed. The energy balance was calculated as the ratio of the NE_L supply to the NE_L requirement. The energy balance was calculated because the experimental periods were too short to measure any changes in body condition score or live weight. The rumen-degradable protein (RDP) balance was calculated from the difference between the supply of truly digestible protein when degradable nitrogen limits microbial growth (PDIN) and the supply of truly digestible protein when the available energy limits microbial growth (PDIE).

The daily pattern of grazing time of each individual was determined through visual observations performed every 5 min (Penning and Rutter, 2004). No behaviour was recorded indoors or at night. The herbage intake rate (g DM/min) was calculated by dividing the daily herbage intake by the mean daily grazing time.

Daily CH₄ emissions were measured individually using the sulfur hexafluoride (SF₆) tracer technique described by Johnson et al. (1994). Each cow received one SF₆ capsule 15 days before collection of the expired air, and the average release rate was 2.44 ± 0.11 mg/day. The gas samples were collected on the last 5 days of each period. The air-sampling devices consisted of stainless steel cylinders (volume of 0.5 L) with the sample flow regulated by a brass ball bearing (Gere and Gratton, 2010). The cylinders were cleaned with high-purity nitrogen gas (N₂) and pre-evacuated prior to each sample collection. The flow regulators were calibrated to allow a remaining vacuum of approximately 500 mb (which represents half of the total cylinder volume) in the cylinder at the end of the sample collection period (five consecutive days). In addition to breath samples, an identical apparatus was placed 2 m above the soil in the experimental paddocks and indoors to allow the measurement of background CH₄ and SF₆ levels in air. During the last 5 days of each experimental period, three background samples were taken outdoors, and two samples were taken indoors (a location expected to have high background levels of both gases).

The concentrations of CH₄ (ppm) and SF₆ (ppt) were determined using a GC-2014 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector (FID) at 250 °C and a 1/8" Shimalite Q packed column (0.7 m, 80/100 mesh) for CH₄ and an electron capture detector (ECD) at 325 °C and a 1/8" Porapak N packed column (1.5 m, 100/180 mesh) for SF₆. A mixture of 5% CH₄ and argon was used as the make-up gas in SF₆ analysis (ECD). The gas chromatography column was maintained at 80 °C during the analysis, and N₂ was used as the gas carrier, with a flow of 25 cm³/min. Calibration curves were established using certified standards (White Martins Development Laboratory), with CH₄ concentrations of 2.41, 5, 10.42 and 203.20 ppm and SF₆

concentrations of 11, 30, 98 and 1000 ppt. The minimum detection limits were 0.15 ppm for CH₄ and 5.2 ppt for SF₆.

The methane emissions (g/day) were calculated in relation to the known release rate of SF₆ by subtracting the background concentrations of CH₄ and SF₆ (Berndt et al., 2014) as follows:

$$R_{CH_4} = R_{SF_6} \frac{[CH_4]_M - [CH_4]_{BG}}{[SF_6]_M - [SF_6]_{BG}} \times \frac{MW_{CH_4}}{MW_{SF_6}} \times 1000$$

where R_{CH_4} is enteric CH₄ (g/cow/day), R_{SF_6} is the release rate of SF₆, MW_{CH_4} is the molecular weight of CH₄ (16), and MW_{SF_6} is the molecular mass of SF₆ (146). $[CH_4]_{BG}$ and $[SF_6]_{BG}$ are the background concentrations of CH₄ and SF₆, respectively. The background CH₄ and SF₆ concentrations were calculated by weighting the predicted indoor and outdoor background concentrations according to the duration of time the animals spent in each environment.

2.4. Feed and sward measurements

Samples of the silage and ground corn were collected twice daily from day 12 to day 17 of each period. These samples were tested as a composite for each period. Samples of the orts left by each cow were collected during the last 5 days of each period and were used to create a composite sample for each group and period. All samples were dried in an oven for 72 h at 60 °C and ground (Solab SL-31, Piracicaba, Brazil) to pass through a 1-mm screen for subsequent chemical analyses.

The pre- and post-grazing HM values above ground level were estimated using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand), which was calibrated as a function of the DM content under the plate area (0.1 m²; Mannetje and Jones, 2000). For calibration, during each experimental period, samples from 10 points were cut with scissors at ground level before and after grazing and then dried in an oven for 72 h at 60 °C. At the end of the experiment, the HM was recalculated with the equations used to estimate the pre- and post-grazing HM values.

The pre- and post-grazing herbage heights were measured by averaging 60 readings taken randomly throughout the area allocated for grazing by each group using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand). The pre-grazing extended heights of the leaf blade and the highest sheath were measured on 100 tillers at random on days 14 and 16. The post-grazing extended leaf and sheath heights were measured on day 16 and day 18 on 100 tillers per treatment.

The morphological and chemical compositions of the sward were determined on day 14 and day 16. In each paddock, twenty handfuls of randomly selected herbage (~800 g fresh) were cut at ground level. This herbage sample was separated into two subsamples. One subsample was used to estimate the chemical composition of the herbage selected by grazing. For that purpose, the post-grazing sward height measured with a sward stick was considered to cut the herbage sample. Subsequently, the cut portion was dried in an oven for 72 h at 60 °C with forced ventilation and stored for chemical analysis. The other subsample was used for morphological separation. The ryegrass and oat were separated into leaf blades, pseudostems + stems, and dead tissue. Each component was dried in an oven for 72 h at 60 °C to determine the morphological composition of the herbage.

2.5. 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 based on the mass difference. The total N was assayed using the Kjeldahl method (Method 984.13; AOAC, 1997). The aNDF concentration was assessed according to Mertens (2002) except that the samples were weighed in filter bags and treated with neutral detergent in an ANKOM A220 system (ANKOM Technology, Macedon, NY, USA). This analysis included alpha-amylase and residual ash but did not include sodium sulfite. The concentration of acid detergent fibre (ADF) was analysed according to Method 973.18 of the AOAC (AOAC, 1997).

2.6. Statistical analysis

The dependent variables were subjected to an analysis of variance using the function PROC MIXED in the software SAS (version 9.3, SAS Institute, Cary, NC, USA). The animal variables, which were averaged per cow and period (n = 36), were analysed using the following model:

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

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

The pasture variables were averaged per paddock and period (n = 18) and analysed using the following model:

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

where Y_{jk} , μ , paddock_i , period_j , treatment_k and e_{jk} represent the analysed variable, the overall mean, the random effect of paddock, the random effect of period, the fixed effect of the treatment and the residual error, respectively.

Table 2

Pre- and post-grazing herbage characteristics of annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) grass grazed by dairy cows with or without corn silage or ground corn supplementation.

Item	Treatments ¹			SEM	P-value
	WS	CS	GC		
Offered area, m ² /cow per day	172 ^a	137 ^b	149 ^b	9.45	0.007
<i>Pre-grazing</i>					
Herbage mass, kg DM/ha	2595	2440	2566	106.4	0.564
Rising plate meter, cm	13.8	13.1	14.1	0.64	0.504
Extended tiller height, cm	38.9	37.0	38.7	1.39	0.587
Extended sheath height, cm	15.8	15.4	16.3	0.71	0.675
Extended lamina height, cm	23.1	21.5	22.4	0.81	0.444
<i>Post-grazing</i>					
Herbage mass, kg DM/ha	1811 ^a	1572 ^b	1702 ^{ab}	58.3	0.041
Rising plate meter, cm	9.3	8.2	9.0	0.37	0.147
Extended tiller height, cm	17.3 ^a	14.7 ^b	17.7 ^a	1.16	0.041
Extended sheath height, cm	12.3	10.6	11.9	0.71	0.221
Extended lamina height, cm	7.23	6.47	7.48	0.330	0.118
<i>HA, kg DM/day</i>					
Above ground level	41.3 ^a	30.8 ^c	34.6 ^b	0.86	< 0.001
Green material	39.8 ^a	30.1 ^c	32.5 ^b	0.79	< 0.001
Live lamina	23.1 ^a	18.3 ^b	18.7 ^b	0.60	< 0.001
<i>Chemical composition, g/kg DM</i>					
DM, g/kg	170	166	170	4.03	0.509
Organic matter	928	925	928	4.47	0.762
CP	219	204	213	6.25	0.277
aNDF	452	450	439	9.27	0.601
ADF	236	240	231	4.50	0.350
<i>Nutritive value</i>					
OM digestibility ²	0.79	0.78	0.79	0.005	0.321
NE _L , Mcal/kg DM ³	1.67	1.65	1.69	0.019	0.369
PDIN, g/kg DM ⁴	141	133	139	3.59	0.305
PDIE, g/kg DM ⁵	107	105	108	1.13	0.217

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

³ Net energy for lactation.

⁴ Truly digestible protein when degradable nitrogen limits microbial growth.

⁵ Truly digestible protein when available energy limits microbial growth.

3. Results

The pre-grazing HM and pre-grazing sward heights were similar between treatments and averaged 2534 kg DM/ha and 13.7 cm, respectively (Table 2). The daily offered area per cow decreased in the supplemented treatment groups compared to the un-supplemented group (-17%; $P < 0.01$). The CP, aNDF and ADF contents of the herbage did not differ between the treatments and averaged 212, 447 and 236 g/kg DM, respectively. The OM digestibility, NE_L, and truly digestible protein in the small intestine were, on average, 0.79, 1.67 Mcal/kg DM and 107 g/kg DM, respectively.

The CH₄ emission and CH₄ intensity (per kg milk production and ECM) did not differ between treatments, averaging 337 g/day, 15.1 g/kg milk and 14.6 g/kg ECM, respectively (Table 3). The CH₄ yield was decreased ($P < 0.01$) by 7.3 and 4.6 g/kg DM intake in the GC treatment relative to that in the WS and CS treatments, respectively.

The total DM intake increased ($P < 0.001$) by 2.9 and 1.3 kg/day in the GC and CS treatments, respectively, compared to the WS treatment (Table 4). However, the herbage DM intake decreased (-1.8 kg/day) in the CS group compared with the WS group but did

Table 3

Methane emissions of dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) grass without supplementation or supplemented with corn silage or ground corn.

Item	Treatment ¹			SEM	P-value
	WS	CS	GC		
Methane g/day	334	356	321	14.1	0.290
g/kg DM intake	27.7 ^a	25.0 ^b	20.4 ^c	1.32	0.008
g/kg milk	15.3	15.6	14.3	0.59	0.352
g/kg ECM ²	15.0	15.1	13.8	0.57	0.291
% GE intake	8.0	8.1	7.2	0.53	0.482

² ECM = Energy-corrected milk production.

Table 4

Dry matter intake, energy balance and grazing behaviour of dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) grass without supplementation or supplemented with corn silage or ground corn.

Item	Treatment ¹			SEM	P-value
	WS	CS	GC		
DM intake, kg/d					
Herbage	11.9 ^a	10.1 ^b	11.2 ^{ab}	0.18	< 0.001
Corn silage	–	3.1	–	–	–
Ground corn	–	–	3.6	–	–
Total	11.9 ^c	13.2 ^b	14.8 ^a	0.19	< 0.001
aNDF intake, g/kg DM intake	452 ^a	443 ^a	371 ^b	3.78	< 0.001
NE _L supply, MJ/d ²	82.8 ^b	80.6 ^b	106.5 ^a	1.80	< 0.001
PDIN supply, g/day ³	1682 ^b	1387 ^c	1800 ^a	37.21	< 0.001
PDIE supply, g/day ⁴	1278 ^b	1170 ^c	1518 ^a	25.35	< 0.001
NE _L balance	0.79 ^b	0.75 ^b	0.97 ^a	0.015	< 0.001
RDP balance, g/day ⁵	404 ^a	217 ^c	282 ^b	14.56	< 0.001
Grazing time, min/d	408 ^a	355 ^c	392 ^b	5.3	< 0.001
Proportion of time spent grazing	0.67	0.64	0.66	0.010	0.081
Herbage DM intake rate, g/min	29.7	28.6	28.7	0.43	0.160

² Net energy for lactation.

³ Truly digestible protein when degradable nitrogen limits microbial growth.

⁴ Truly digestible protein when available energy limits microbial growth.

⁵ Rumen-degradable protein balance.

not differ between the GC and WS treatments ($P < 0.001$). The grazing times of CS and GC were 53 min/day and 16 min/day shorter than that of WS ($P < 0.001$), respectively. The proportion of time spent grazing (0.66) and the herbage DM intake rate (29 g DM/min) were similar between the treatments.

Milk production (+1.4 kg/day; $P = 0.03$) and milk protein production (+60 g/day; $P < 0.001$) increased in the cows supplemented with ground corn compared to those supplemented with corn silage and those in the unsupplemented group (Table 5). The milk fat concentration was similar between treatments.

4. Discussion

The current study aimed to evaluate the effects of different energy supplements (corn silage or ground corn) on dairy cows grazing the upper sward layer of an annual temperate pasture. The goal of grazing management was successfully achieved because the same sward height proportion was obtained between treatments and averaged 40% of the pre-grazing sward height. Additionally, with the same post-grazing sward height, the results showed that by using corn silage and ground corn supplementation, the HA can be reduced by 25 and 16%, respectively.

4.1. Methane emissions

The average CH₄ emission was consistent with the values reported for dairy cows grazing temperate grasses (O'Neill et al., 2012; Robertson and Waghorn, 2002; Wims et al., 2010) and the predicted daily CH₄ emissions of 208–461 g CH₄/day (Ellis et al., 2010). Additionally, despite the strong relationship between total DM intake and enteric CH₄ emission reported previously ($R^2 = 0.86$; Hristov et al., 2013), our results agree with those other authors (Jiao et al., 2014; van Wyngaard et al., 2018) in showing no difference in daily CH₄ emission between supplemented and unsupplemented dairy cows. This result may be explained by the

Table 5

Milk production, milk composition and plasma urea nitrogen of dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) grass without supplementation or supplemented with corn silage or ground corn supplement.

Item	Treatments ¹			SEM	P-value
	WS	CS	GC		
Milk production, kg/day	21.7 ^b	21.8 ^b	23.1 ^a	0.38	0.030
4% FCM, kg/day ²	22.7 ^b	22.8 ^b	23.8 ^a	0.31	0.029
ECM, kg/day ³	22.2 ^b	22.2 ^b	23.5 ^a	0.31	0.011
Milk fat, %	4.34	4.36	4.25	0.053	0.312
Milk protein, %	3.13 ^b	3.07 ^c	3.21 ^a	0.020	< 0.001
Milk fat, g/day	931 ^b	938 ^{ab}	974 ^a	12.7	0.056
Milk protein, g/day	673 ^b	662 ^b	733 ^a	11.2	< 0.001

² FCM = fat-corrected milk production.

³ ECM = energy-corrected milk production.

increase in CH₄ emission expected in supplemented cows (which had greater DM intake than did unsupplemented cows) being offset by the decrease in CH₄ yield (in g/kg DM intake) due to the inclusion of rapidly fermentable carbohydrates (starch) in the diet (Jiao et al., 2014). Indeed, in the current study, the CH₄ yield decreased as the total DM intake increased.

When we included dietary NDF concentration as predictor variable, CH₄ yield (g/kg DM intake) was on average 16% greater than the average values calculated by those calculated from the average proposed by Charmley et al. (2016) and the yield values calculated from prediction equations developed by (Niu et al., 2018). However, the results were close to the maximum values obtained by Charmley et al. (2016; n = 220, maximum = 25 g/kg DM intake) and within the range of the global database collected by (Niu et al., 2018); n = 2566, maximum = 30 g/kg DMI). The reduction in CH₄ yield in supplemented cows might be explained, at least partially, by the starch supply. Although CH₄ formation may be related to the H₂ available in the rumen, non-structural carbohydrate fermentation increases propionate formation. This reaction decreases the availability of H₂ for CH₄ production by *Archaea*. Unfortunately, the dietary starch concentration was not measured in the current study, but it is well known that herbage starch content is negligible, whereas corn silage and ground corn may have starch contents of 250 and 740 g/kg DM, respectively (INRA, 2007a). Sauvante et al. (2011) found no reduction in methane yield until the amount of concentrate inclusion exceeded 300 g/kg DM; however, the response was highly dependent of DMI level (% BW), with a strong effect when the DMI level was greater than 2.5% BW due to digestive interactions. In the current work, the dietary concentrate inclusion was not greater than 250 g/kg DM, but DM intake increased from 2.6% BW in the WS treatment to 2.8 and 3.1% BW in the CS and GC treatments, respectively, which helps explain the effects of supplements on CH₄ yield.

The lack of a reduction in CH₄ intensity in the GC group relative to the unsupplemented group was a consequence of the milk production response, which did not exceed 7.0% in the dairy cows receiving 4 kg of ground corn (230 g/kg DM of ground corn). Muñoz et al. (2015), using similar concentrate intake levels, did not find any improvement in CH₄ intensity relative to the intensity at 50 g/kg DM concentrate intake. According to O'Neill et al. (2012), for dairy cows grazing pastures with an OM digestibility greater than 0.80, the ability of supplementary feeding to increase milk production may be limited. In the current study, the average OM digestibility was 0.79. To our knowledge, previous studies have not compared the three feeding strategies used in this work; however, improvements in individual milk production and the absence of an effect on CH₄ emissions per unit of milk product were observed when the concentrate inclusion level of the diets were increased (Lovett et al., 2005; Muñoz et al., 2015) or when a partially mixed ration supplementation regimen based on corn silage was used (O'Neill et al., 2012).

4.2. Dry matter intake and grazing behaviour

The DM herbage intake decreased only in the cows supplemented with corn silage, indicating that the effects of supplementation on the total and herbage intake may be directly linked to the supplement type. It is well known that corn silage supplementation has a greater effect than concentrate supplementation on herbage DM intake (Bargo et al., 2003; Delagarde et al., 2011), which may be at least partially explained by the superior effect of corn silage supplementation on ruminal filling capacity (INRA, 2007). Moreover, the effect of concentrate supplementation on herbage intake (substitution rate) decreases to the extent that the energy supply from herbage is in excess of the cow's energy requirements (INRA, 2007). In the present study, the NE_L balance (NE_L supply/NE_L requirement) was 0.79 for the WS group and 0.96 for the GC group.

The effect of substitution rate on the CS group and the lack of reduction of herbage intake on the GC group affected grazing time but not herbage DM intake rate, which averaged 29.1 g/min. In the current work, the grazing time decreased to 16.5 min. kg DM corn silage intake. These results are supported, at least partially, by previous work in which increased corn silage supplementation levels in dairy cows were found to decrease grazing time (Miguel et al., 2014). Additionally, reductions in grazing time to 11.4 and 15.1 min/kg DM corn silage intake were recorded at low and high HA values, respectively (Pérez-Prieto et al., 2011).

4.3. Milk production and milk composition

The increased milk production in dairy cows supplemented with ground corn was a consequence of increased total DM intake and NE_L supply. In contrast, the absence of a milk production response following corn silage supplementation was likely a consequence of the low energy value of this supplement relative to that of the grazed herbage (1.53 vs 1.7 Mcal/kg DM), which is consistent with previous reports (Burke et al., 2008). Thus, milk production was clearly affected by NE_L supply because dairy cows receiving ground corn supplementation had 0.97 of their energy requirements supplied, whereas the WS and CS groups had less than 0.80 of their energy requirements supplied.

The increased energy intake observed in dairy cows receiving ground corn supplementation may also be associated with the slightly increased milk protein production and concentration in this treatment, and similar responses were observed in other works using concentrate (Bargo et al., 2002) or corn silage supplementation (Miguel et al., 2014). Moreover, the lack of variation in the concentration of milk fat between treatments may be associated with the slight milk production responses to supplementation and the aNDF content of the diets. The aNDF content (in total DM) ranged from 377 to 455 g/kg DM. According to the values proposed by Stockdale (1997), the milk fat concentration is not affected when the aNDF content in the total diet is between 250 and 400 g/kg DM. Additionally, it is well known that in feeding practice conditions, increasing the dietary fat percentage increases the milk fat concentration by almost 0.4% per kg of dietary fat added (Palmquist et al., 1993; R² = 0.40). Although dietary milk fat content was not measured in the present study, it is logical to assume that corn silage and ground corn have mean fat contents of approximately 30 and 40 g/kg DM, respectively (INRA, 2007). Hence, the amount of dietary fat added in CS and GC treatments was unlikely to have exceeded 0.100 and 0.150 kg, respectively, and was thus unlikely to have increased the milk fat content.

5. Conclusions

The ground corn and corn silage supplementation strategies implemented allowed for reductions in the herbage allowance without compromising milk production or total DM intake. Under these conditions, a moderate level of corn silage or ground corn supplementation for dairy cows grazing herbage with good nutritive value was an effective feeding strategy to reduce the CH₄ yield, but it was not effective in reducing the CH₄ intensity.

Conflict of interest statement

The authors of manuscript entitled “**Energetic supplementation is not effective in the mitigation of enteric methane intensity in dairy cows grazing temperate grass**” declare that there is not any actual or potential conflict of interest with other people or organizations that could inappropriately influence their work.

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