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Effects of concentrate supplementation on enteric methane emissions and milk production of grazing dairy cows

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ABSTRACT

Although concentrate supplements in ruminant diets have been recognised as an effective enteric methane mitigation strategy, very few studies have examined the effects of concentrate supplementation on enteric methane emissions under grazing conditions. Twenty four multiparous Holstein Friesian cows were used in a crossover design study to investigate the effects of two concentrate feeding levels across two periods on enteric methane emissions and milk production of grazing dairy cows. Each period had a duration of four weeks (three weeks for diet adaptation and one week for measurements) and no interval in between them. Dietary treatments consisted of two concentrate feeding levels per cow (1 vs. 5 kg; as-fed basis) offered daily in equal meals during milking. Enteric methane emissions from cows grazing perennial ryegrass pasture were measured during the final week of each period using the sulphur hexafluoride tracer technique. Milk yield and liveweight were determined daily during each methane measurement period, whereas milk composition and body condition score (BCS) were determined weekly. Daily herbage intake by individual cows during methane measurement weeks was estimated using an energy requirement model and animal records and diet composition. In period 1, cows receiving 5 kg concentrate supplement were estimated to reduce herbage intake by 1.8 kg DM/d compared to cows receiving 1 kg of concentrate, whereas in period 2 cows receiving the 5 kg concentrate supplementation were estimated to reduce herbage intake by 4.4 kg DM/d, compared to cows receiving 1 kg of concentrate. In both periods, milk yield increased with increasing concentrate level, with an average milk response to concentrate supplementation of 0.68 kg milk DM/kg concentrate DM over the two periods. Concentrate feeding level had no effect on milk fat, protein or total solids contents. In period 2, lactose content increased in cows offered 5 kg/d concentrate. Increasing concentrate feeding level increased liveweight and BCS in period 1, but not in period 2. Feeding 5 kg of concentrate supplement increased enteric methane emission by 34 g/d in period 1 (323 vs. 357 g/d) and 41 g/d in period 2 (349 vs. 390 g/d) compared to 1 kg of concentrate supplement. However, enteric methane emission per unit of estimated feed intake (dry matter or gross energy) or milk output (gross or energy corrected) was not affected by level of concentrate supplementation. It was concluded that under generous grazing conditions (high allowance of good quality herbage) a moderate increase in concentrate supplementation resulted in a simultaneous increase in milk yield and enteric methane emission, so that enteric methane emission per unit of milk yield was

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unaffected. Thus, a moderate level of concentrate supplementation of dairy cows grazing pastures of high digestibility would not be an effective enteric methane mitigation strategy.

1. Introduction

Enteric methane (CH_4) is an end product of fermentation in the rumen. It represents a production inefficiency for cattle, as between 2 and 12% of the gross energy ingested through the diet is lost as CH_4 (Johnson and Johnson, 1995). Additionally, CH_4 is a potent greenhouse gas and there is growing concern for the contribution of CH_4 emissions from ruminants to global warming.

Concentrate supplements in ruminant diets have been recognised as an effective CH_4 mitigation strategy (Boadi et al., 2004; Beauchemin et al., 2008; Martin et al., 2010; Hristov et al., 2013). Evidence found with diets based on conserved forages indicates that increasing the proportion of concentrate in the diet will lower CH_4 emissions per unit of feed intake and animal product (Hristov et al., 2013). However, very few studies have examined the effects of concentrate supplementation on CH_4 emissions under grazing conditions. Lovett et al. (2005), when comparing the effects of two levels (1 vs. 6 kg, as-fed basis) of a high fibre concentrate, reported that while the daily production of CH_4 increased with increased concentrate level, so did the DMI and milk production, resulting in no effect of concentrate supplementation on CH_4 emission per unit of feed intake or milk yield. However, when CH_4 was related to fat-corrected milk yield, a decreasing tendency was identified with increased concentrate supplementation. O'Neill et al. (2012) reported higher CH_4 emissions from grazing cows supplemented with a partial mixed ration (4 kg DM) than unsupplemented cows, with no differences between treatments in CH_4 emission per unit of feed intake or milk yield. A more recent study (Jiao et al., 2014) with grazing dairy cows offered a range of concentrate feeding levels (2, 4, 6 and 8 kg as-fed basis) reported that daily CH_4 emissions were not affected, but CH_4 emissions per unit of feed intake and energy-corrected milk yield decreased with increased concentrate supplementation.

Until now, CH_4 emissions from livestock systems in southern Chile had not been determined. These systems are based on grazing and strategic supplementation with concentrates, conserved forages and fodder crops when the quantity or quality of forage decreases. We hypothesised that increasing concentrate feeding level in the diet of grazing dairy cows would decrease the proportion of dietary energy converted to CH_4 and CH_4 emissions per unit of feed intake and milk yield. The aim of the present study was to evaluate the effects of two concentrate feeding levels on CH_4 emissions and milk production of dairy cows under the grazing conditions of the south of Chile.

2. Materials and methods

The work described in this paper was conducted at Instituto de Investigaciones Agropecuarias (INIA), research farm Remehue (40°31′LS; 73°03′LW y 65 mamsl, Osorno, Chile), in accordance with the requirements of the Chilean Law 20380 on Animal Protection and with the approval of INIA Bioethics Committee.

2.1. Animals, experimental design and treatments

Twenty-four multiparous (mean parity 3.4 ± 1.3) Holstein Friesian dairy cows were used in the study. At the beginning of the study the cows averaged 70 ± 23 days in milk and 494 ± 44 kg liveweight.

The study involved a balanced crossover design (2 concentrate levels × 2 periods), with 4 wk/period, which included 21 d of adaptation to diet followed by 7 d of CH_4 and animal data collection. There was no interval between periods. Before the commencement of the study, all cows were grazing a perennial ryegrass-based sward and received 2.5 kg/d of a commercial concentrate feeding. The ingredient composition of the pelleted commercial concentrate mixture offered (12.32®, Concentrados Cisternas, Osorno, Chile) was as follows (g/kg, as-fed basis): steam-rolled corn (350), ground corn (220), rolled barley (150), wheat bran (140), dried distillers grains with solubles (50), ground beans (50) and rice bran (40). Cows were blocked into pairs according to calving date and milk yield, and within each block were allocated to 1 of 2 dietary treatments, so, that there were equal number of animals per treatment. Treatments consisted of 2 concentrate feeding levels: 1 vs. 5 kg/d of concentrate per animal (as-fed basis). The commercial concentrate used was the same as previously described and was offered in 2 equal meals by automatic parlour feeders during milking. Feed transitions were introduced gradually over 5 days during the first week of each period.

2.2. Pasture and grazing management

The study was conducted in the spring of 2012 during the months of October and November, on 2 experimental paddocks grazed successively resulting in 1 round of rotational grazing of both paddocks per period. The smaller paddock (2.15 ha) was subdivided into 4 sub-paddocks and the largest one (3.82 ha) was subdivided into 6 sub-paddocks. Each treatment group grazed adjacent sub -paddocks under strip grazing using temporary electric fencing. Paddocks were of predominantly perennial ryegrass (*Lolium perenne*) pasture sown the previous year and had not been grazed for 23 d prior to the study. Pre-grazing herbage mass was similar for both treatment groups. For both treatments, target post-grazing residuals were 5.5 cm compressed herbage height, measured using a rising plate meter (Farmworks, New Zealand). This residual sward height was initially targeted by providing daily herbage allowances of approximately 29 and 25 kg DM/cow for concentrate treatments 1 and 5 kg/d, respectively. However, these herbage allowances were reduced as the study progressed and adjusted for each treatment so as to maintain target post-grazing sward heights. The herbage strip area allocated daily to each treatment group was adjusted by daily measurements of the pre-grazing herbage mass. Consequently, the area grazed per cow per day fluctuated throughout the experiment. Fresh grazing pasture strip was offered after each milking, with grazing areas being back-fenced. Daily herbage allocation was split 40 and 60% between the day and night-time allowances, respectively. All cows had free access to water for the duration of the study.

2.3. Herbage and concentrate measurements

Pre and post grazing compressed herbage heights were measured daily in each treatment using a rising platemeter (Farmworks, New Zealand) with 60 measurements per treatment made at random while traversing in a zigzag across each grazing area. Herbage mass was estimated above ground level from compressed herbage heights by a linear equation developed for naturalized and sown pastures in southern Chile (Canseco et al., 2007) as follows: Herbage mass (kg DM/ha)=[sward height (cm) × 100]+ 400. During the last week of each period, for determination of chemical composition, herbage samples representative of herbage grazed were cut by hand for each treatment twice weekly at the post-grazing height of the previous day. Concentrate DMI was recorded through parlour feeding. During the last week of each period, two samples of concentrates were taken daily and bulked weekly for determination of chemical composition. Herbage substitution rates were calculated as the ratio between the decrease in estimated herbage intakes and the difference in concentrate intake between treatments.

2.4. Animal measurements

Throughout the study cows were milked twice daily at around 0530 and 1600 h, with individual milk yields recorded automatically at each milking. Milk composition (protein, fat, lactose and urea) was determined weekly from samples taken at 2 consecutive milkings using a MilkoScan instrument (Foss Electric, Denmark). Milk yield and composition reported correspond to the last week of each period. Energy-corrected milk yield (ECMY) was calculated using Eqs. (1) and (2) (Tyrrell and Reid, 1965):

$$\text{ECMY (kg/d)} = \text{milk yield (kg/d)} \times \text{milk energy content (MJ/kg)}/3.1 \quad (1)$$

$$\text{Milk energy content (MJ/kg)} = [0.0384 \times \text{fat}] + [0.0223 \times \text{crude protein}] + [0.0199 \times \text{lactose}] - 0.108 \quad (2)$$

where 3.1 is the energy content (MJ/kg) of a standard milk for Holstein Friesian cows with 4% fat, 3.2% crude protein and 4.8% lactose.

Milk response to concentrate supplementation was calculated as the ratio between the increase in milk production and the difference in concentrate intake between treatments. Animal liveweight was recorded automatically twice daily after every milking, with mean liveweight calculated for each week. Body condition score was assessed weekly using a five-point condition scoring system (Edmonson et al., 1989).

Individual herbage intakes for the last week of each period were estimated through a process of back calculation according to the Eqs. (3)–(6), for which energy requirement models (AFRC, 1993; Agnew et al., 2004), estimated herbage and concentrate ME contents (Garrido and Mann, 1981) and animal data obtained in the present study were used:

$$\text{Herbage DMI (kg/d)} = \frac{\text{Herbage ME supply (MJ/d)}}{\text{herbage ME content (MJ/kg DM)}} \quad (3)$$

$$\text{Herbage ME supply (MJ/d)} = \text{Total ME requirement (MJ/d)} - \text{concentrate ME supply (MJ/d)} \quad (4)$$

$$\text{Total ME requirement (MJ/d)} = \text{sum of ME requirements for maintenance, milk production, liveweight change and pregnancy (Agnew et al., 2004), plus ME requirement for grazing activity allowance (AFRC, 1993)} \quad (5)$$

$$\text{Concentrate ME supply (MJ/d)} = \text{concentrate DMI (kg/d)} \times \text{concentrate ME content (MJ/kg)} \quad (6)$$

2.5. Enteric CH_4 emissions

During the last 7 days of each period, enteric CH_4 emissions from individual animals were estimated using the sulphur hexafluoride (SF_6) tracer gas technique (Johnson et al., 2007). One week prior to the beginning of the study, cows were dosed orally with a brass permeation tube containing SF_6 gas (National Institute of Water & Atmospheric Research NIWA, Wellington, New Zealand). Prior to the beginning of the study and to their deployment into the rumen, the SF_6 permeation rate of each tube was measured during 10 weeks of serial weighing at 39 °C to produce a linear regression curve ($R^2 > 0.999$). Tubes were blocked by measured SF_6 permeation rate for their assignment to treatments. The permeation rates of SF_6 tubes ranged from 4.05 to 5.85 mg/d, with a mean of 5.00 ± 0.62 mg/d.

During CH_4 measurement days, a modified headcollar was placed on each cow sustaining a sample line (nylon tubing) running from just above the animal's nostrils to a pre-evacuated gas collection canister. The sample line tubing connected successively a Y-shaped inlet, a 15-μm filter, a short capillary tube housed within the filter as a sample flow restrictor, and an airline (3.175-mm diameter), with an evacuated polyvinyl chloride (PVC) V-shaped canister (2.5 L). The capillary tube was adjusted (by crimping a small section) to restrict the sample flow to allow around 55.0 kPa pressure in the canister at the end of a 24-h sampling period. Canisters with pressures that ranged between 45.0 and 70.0 kPa were deemed acceptable; canisters with pressures outside of this range were discarded. Canisters with samples were then over-pressurized with nitrogen (N_2) gas to approximately 162 kPa and a subsample from each canister was transferred to a glass vial (22 mL), previous to gas concentration analysis. For measuring background SF_6 and CH_4 gases, two types of background collectors were used, the traditional fixed background collectors (Johnson et al., 2007) and mobile background collectors (Berndt et al., 2014). Two fixed canisters, one per treatment, located outside the grazing sub-paddocks were used as fixed background collectors. For mobile background collectors, two additional cows per treatment group that did not have an SF_6 permeation tube in their rumen and with the inlet of their sample line tubing located behind their heads towards their neck, were used.

Concentrations of SF_6 and CH_4 in the canisters were determined by GC (Perkin Elmer Clarus 600; Waltham, MA, USA). Samples were manually injected through a 1-mL sample loop at a flow rate of 1 mL/min using He as carrier gas. All samples were analysed in duplicate. The CH_4 column was an Elite-PLOT Q, 30 m × 0.32 mm ID × 10 μm (Perkin Elmer), and a fused silica PLOT column RT®-Msieve 5 A, 15 m × 0.25 mm ID × 50 μm (Restek, Bellefonte, PA, USA) was used for SF_6 analysis. The operating temperatures of the injector and the oven were 100 and 50 °C, respectively. A flame ionization detector was used for the detection of CH_4 (250 °C), and SF_6 concentration was measured using an electron capture detector (300 °C). The GC was calibrated using 3 gas standards of known concentrations (Scott-Marrin Inc., Riverside, CA).

Enteric CH_4 emissions (CH_4Q; g/d) were calculated from the measured SF_6 and CH_4 concentrations sampled by the canisters (SF_6C and CH_4C), the background SF_6 and CH_4 concentrations (SF_6B and CH_4B) sampled both by fixed and mobile collectors, the predetermined permeation rate of SF_6 from the permeation tubes (SF_6Q), and the molar mass (MM) of the gases according to the following equation:

$$CH_4Q = [SF_6Q] \times [(CH_4C - CH_4B)/(SF_6C - SF_6B)] \times [CH_4MM/SF_6MM] \quad (7)$$

2.6. Chemical analyses

All chemical analyses were performed at Laboratorio de Nutrición Animal y Ambiente, INIA Remehue (Nch-ISO 17025), with the exception of gross energy which was determined at Laboratorio de Nutrición Animal, Universidad Austral de Chile. Herbage and concentrate samples were thoroughly homogenised, dried at 60 °C for 48 h, ground through a 1 mm sieve and then stored in plastic bags at room temperature until chemical analyses. Samples were analysed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash contents according to AOAC (1995). Dry matter digestibility was determined in vitro as by Goering and Van Soest (1970). Gross energy was determined by oxygen bomb calorimetry (Bateman, 1970). All samples were analysed in duplicates.

2.7. Statistical analyses

Sward characteristics, herbage chemical composition and estimated feed intakes for the last week of each period were analysed as responses to fixed effects of treatment, period and their interaction. Milk yield and milk composition, for the last week of each period were analysed with a mixed model including the fixed effects of treatment, period and their interaction, and the random effect of the cow. Changes in liveweight and body condition score were analysed as described for the milk variables. Lactation number and days in milk were included in the models when appropriate as co-variables and eliminated if not significant ($P > 0.10$).

Measured CH_4 emission data presented variation and the extreme values were unphysiological. To address this, first outliers for CH_4 emission were identified as those observations whose studentized residuals fell out of a 95% CI of a normal distribution, and eliminated. Second, CH_4 data with post-sampling canister pressures outside the 95% interpercentile range were eliminated. Milk production and CH_4 emission data were analysed by REML repeated measures including the fixed effects of treatment, period, measurement day and double interactions, and the random effect of cow by period nested within treatment. For each variable, the random effects of headcollar and canister, and the fixed effects of canister pressure pre-sampling, post-sampling and after N pressurization and SF_6 permeation rates were initially used as covariates. Background CH_4 emission data were analysed including the fixed effect of type of background collector (mobile or fixed), treatment, day of measurement and interactions. Non-significant ($P > 0.10$) covariates and interactions were removed from the models. Significance for main effects and interactions was declared at $P \leq 0.05$.

All statistical analyses were conducted using JMP® 11.0.0 (SAS Institute Inc.).

3. Results

3.1. Feeds chemical composition

There were no interaction effects between concentrate feeding level and period on the chemical composition of grazed herbage ($P > 0.05$). There were no differences between treatments for herbage chemical composition throughout the study ($P > 0.05$; not shown). The chemical composition of the concentrate and grazed herbages across both periods are presented in Table 1. Herbage DM content was higher in period 2 than in period 1 ($P = 0.02$). The CP, GE, ash and ME contents, and in vitro DM digestibilities of the herbages were similar for both

Table 1
Dry matter (DM) content and chemical composition (g/kg DM unless otherwise stated) of dietary components.

	Grazed pasture ($n=8$)[a]				Concentrate ($n=4$)
	Period 1	Period 2	SE	*P* value	
DM (g/kg)	135	179	7.6	0.015	888
Crude protein	211	215	8.6	0.74	127
Gross energy (MJ/kg DM)	16.9	16.4	0.28	0.26	16.2
Neutral detergent fibre	473	420	6.5	0.011	201
Acid detergent fibre	283	251	3.1	0.006	–
Ash	121	113	6.4	0.41	97
Estimated ME (MJ/kg DM)	11.3	11.4	0.06	0.13	12.0
In vitro DM digestibility	839	844	4.9	0.50	877

[a] Grazing strips allocated to each treatment did not differ in herbage chemical composition ($P>0.05$).

periods ($P>0.05$). Herbage NDF and ADF contents were higher in period 1 than in period 2 ($P=0.01$).

3.2. Pasture measurements

There were no interaction effects ($P>0.05$) between concentrate feeding level and period on any of the sward measurements or herbage allowances (Table 2). Pre-grazing sward height and herbage mass did not differ ($P>0.05$) between treatments. Mean post-grazing residual height achieved was 5.6 cm and did not differ between treatments ($P=0.60$). Cows with a concentrate feeding level of 1 kg were offered a higher daily herbage allowance ($P<0.001$), required a greater grazing area per day ($P<0.001$), and removed more herbage per day ($P<0.001$), than cows with a concentrate feeding level of 5 kg.

The first period of the study had a higher pre-grazing height and pre-grazing herbage mass than the second period ($P<0.001$). Accordingly, the grazing area ($P<0.001$) and herbage allowance ($P=0.01$) in the first period were lower than in the second period. The herbage removed per cow per day was greater in period 1 than in period 2 ($P=0.03$). There were no differences in post grazing residual heights between periods ($P=0.26$).

3.3. Estimated feed intake

There were interaction effects between concentrate feeding level and period on estimated herbage and total DMI ($P<0.001$; Table 3). Cows supplemented with a concentrate level of 5 kg had a lower estimated herbage intake compared to their counterparts supplemented with a concentrate level of 1 kg in both periods, but the difference was of greater magnitude in period 2 (-1.8 vs. -4.4 kg/d; $P\leq 0.001$). Consequently, in period 1, cows receiving 1 kg of concentrate supplement had a lower estimated total DMI than their counterparts receiving 5 kg of concentrate supplement, but in period 2, the contrary seemed to occur. The calculated herbage substitution rates were 0.51 and 1.24 kg of herbage DM/kg concentrate DM, for periods 1 and 2, respectively.

3.4. Animal performance

Animal performance variables measured across both periods are shown in Table 4. Milk yield was higher for cows offered a concentrate level of 5 kg than cows offered a concentrate level of 1 kg (+2.4 kg/d; $P=0.01$) and tended to be higher in period 1 than 2 (+1.7 kg/d; $P=0.07$). Concentrate supplementation at grazing resulted in an average response of 0.68 kg milk per kg of concentrate supplemented. Concentrate supplementation did not affect the content of milk fat ($P=0.15$), protein ($P=0.27$) or total solids ($P=0.96$). Milk solids content tended to be lower in period 1 than period 2 ($P=0.08$). There was an interaction effect between concentrate feeding level and period on milk lactose ($P=0.04$): cows with a concentrate feeding level of 1 kg tended to increase lactose by 1.0 g/kg in period 1 ($P=0.01$) and to reduce lactose by 1.2 g/kg in period 2 ($P=0.05$), compared to cows with a concentrate feeding level of 5 kg. There also tended to be an interaction effect between concentrate feeding level and period on milk urea content ($P=0.10$), with a level of 5 kg of concentrate supplement increasing milk urea content in period 1 but decreasing it in period 2, compared to 1 kg of concentrate supplement. There were no effects of concentrate feeding level ($P=0.99$) or period ($P=0.11$) on energy-corrected milk yield.

There was an interaction effect between concentrate feeding level and period on liveweight change, where, in period 1, cows receiving a level of 1 kg concentrate had a liveweight loss compared to their counterparts receiving 5 kg of concentrate, but in period 2, both treatments gained liveweight ($P=0.02$). Similarly, cows receiving 5 kg of concentrate improved their BCS in period 1, compared to their counterparts receiving 1 kg of concentrate, but in period 2, both groups lost BCS ($P=0.05$).

3.5. Enteric CH_4 emissions

Out of a total of 336 enteric CH_4 samples (24 cows × 7 d × 2 periods), 3 samples were lost due to the breakage of the canister, 24 due to breakages of the sampling line, 34 due to disconnections between the sampling line and canister and 6 during sample over-pressurization. A further 12 samples were eliminated due to post sampling pressure being outside the established range. Finally, 9 outliers were identified and eliminated and 19 samples in the 2.5 upper and lower post-sampling canister pressure percentile were further eliminated. Thus, reported results are based on a final total of 229 samples.

Emissions of SF_6 were not affected by concentrate supplementation ($P=0.93$; not shown) or period ($P=0.28$; not shown). There were no interaction effects between concentrate level and period on any of the CH_4 variables studied ($P>0.05$; Table 5). A concentrate level of 5 kg increased total CH_4 emissions by 37 g/d, compared to a concentrate level of 1 kg ($P=0.02$). However, there were no effects of concentrate feeding level when CH_4 was expressed per unit of feed intake ($P=0.31$), milk yield ($P=0.73$) or energy-corrected milk yield ($P=0.32$). There

Table 2
Effect of concentrate supplementation level on sward measurements and herbage allowance.

	Period 1		Period 2		SE	P value		
	1 kg Concentrate	5 kg Concentrate	1 kg Concentrate	5 kg Concentrate		Treatment	Period	Interaction
Sward height (cm)								
Pre-grazing	15.8	16.2	13.1	12.4	0.59	0.79	< 0.001	0.29
Post-grazing	5.6	5.8	5.6	5.6	0.14	0.60	0.26	0.46
Herbage mass (kg DM/ha[a])								
Pre-grazing	3556	3645	3027	2888	118.7	0.79	< 0.001	0.29
Post-grazing	1525	1557	1518	1512	28.8	0.57	0.26	0.47
Herbage allowance (kg DM/cow per day[a])	24.3	19.9	26.1	21.6	0.69	< 0.001	0.013	0.92
Daily grazed area (m^2/cow)	71.1	56.9	92.5	76.3	2.81	< 0.001	< 0.001	0.73
Herbage removed (kg DM/cow per day)	13.7	11.2	12.6	10.3	0.44	< 0.001	0.032	0.78

[a] Estimated above ground level using a rising plate meter.

Table 3
Effect of concentrate supplementation level of on daily intake (kg DM/cow) of herbage, concentrate and total feed.

	Period 1		Period 2		SE	P value		
	1 kg Concentrate	5 kg Concentrate	1 kg Concentrate	5 kg Concentrate		Treatment	Period	Interaction
Herbage	16.2	14.4	19.2	14.8	0.33	< 0.001	< 0.001	< 0.001
Concentrate	0.89	4.45	0.89	4.44	–	–	–	–
Total	17.0	18.9	20.1	19.2	0.33	0.16	< 0.001	0.001

Table 4
Effect of concentrate supplementation level on daily milk yield, milk composition, and changes in liveweight and body condition score of cows.

	Period 1		Period 2		SE	P value		
	1 kg Concentrate	5 kg Concentrate	1 kg Concentrate	5 kg Concentrate		Treatment	Period	Interaction
Milk yield (kg/d)	26.4	28.5	24.4	27.1	0.91	0.011	0.070	0.75
Milk fat (g/kg)	35.5	33.9	41.9	36.8	3.03	0.15	0.15	0.61
Milk protein (g/kg)	30.9	31.0	30.4	30.9	0.71	0.27	0.62	0.79
Milk lactose (g/kg)	48.9	47.9	46.3	47.5	0.44	0.78	0.001	0.040
Milk solids (g/kg)	125.8	122.5	130.4	134.0	4.44	0.96	0.084	0.49
Milk urea (mg/100 mL)	34.0	37.5	34.9	31.5	1.91	0.97	0.23	0.098
Energy-corrected milk yield (kg/d)	23.7	26.5	24.4	25.8	1.29	0.989	0.109	0.581
Liveweight change (kg/d)	−0.374	0.236	0.802	0.551	0.17	0.27	< 0.001	0.021
BCS change	−0.040	0.117	−0.117	−0.154	0.08	0.59	0.12	0.050

were no differences between concentrate feeding levels on CH_4 conversion rate factor (enteric CH_4 energy as a percent of GE intake; Y_m) with an average value of 6.3% ($P=0.13$).

Total CH_4 emissions ($P=0.06$) and CH_4 per unit of energy-corrected milk yield ($P=0.07$) tended to increase in the second period of the study, while CH_4 per unit of milk yield significantly increased in period 2 ($P=0.02$) compared to period 1. Methane emissions per unit of feed intake ($P=0.99$) and Y_m ($P=0.67$) were not affected by period.

There were no interaction effects between type of background collector (fixed or mobile) and period or treatment on background SF_6 and CH_4 emissions (not shown). Background SF_6 emissions ranged from 5.4 to 52.4 ppt, with a mean of 20.0 ± 9.9 ppt. Background CH_4 emissions ranged from 4.1 to 67.5 ppm, with a mean of 28.7 ± 15.2 ppt. Background CH_4 emissions varied with day of measurement ($P=0.014$) and were higher in the mobile background collectors than in the fixed background collectors (34.1 vs. 23.9 ppm, respectively; SE = 1.77 ppm; $P=0.003$). Additionally, background CH_4 emissions tended to by higher in the 5 kg treatment background canisters than the 1 kg treatment background canisters (32.5 vs. 25.5 ppm, respectively; SE = 1.81 ppm; $P=0.09$). For background emissions, out of a total of 84 samples (4 mobile and 2 fixed background collectors × 7 d × 2 periods), 1 sample was loss due to the breakage of a canister, 7 due to sampling line breakages, 5 due to disconnections between the sampling line and the canister and 5 were outside the established final pressure range. All lost samples were from mobile background collectors.

Table 5
Effect of concentrate supplementation level on enteric methane emissions of cows.

	Period 1		Period 2		SE	P value		
	1 kg Concentrate	5 kg Concentrate	1 kg Concentrate	5 kg Concentrate		Treatment	Period	Interaction
CH_4 g/d	323	357	349	390	14.6	0.015	0.057	0.81
CH_4 g/kg DMI	19.4	19.0	17.9	20.5	1.06	0.31	0.99	0.18
CH_4 g/kg MY	12.5	12.7	14.4	14.7	0.75	0.73	0.015	0.95
CH_4 g/kg ECMY[a]	12.6	13.4	14.3	15.4	1.06	0.32	0.067	0.86
Y_m[b]	6.4	6.4	5.7	6.7	0.35	0.13	0.67	0.15

[a] ECMY: energy-corrected milk yield.
[b] CH_4 conversion rate factor: enteric CH_4 energy as percent of GE intake.

4. Discussion

4.1. Herbage characteristics

The present experiment was conducted in early- to mid-spring with pasture in a vegetative stage throughout the study. Lower NDF and ADF concentrations were found in herbage in period 2, compared to period 1. This finding was likely related to the lower sward height and herbage mass of the second period of the study, as a result of the two rounds of rotational grazing used (one per period). In general, a lower herbage mass contains a higher proportion of green leaves, and digestible nutrients, and a lower proportion of stems and senescent materials (Hoogendoorn et al., 1992). The differences in fibre content between periods did not reflect on DM digestibility, which probably indicates increased fibre digestibility in period 1 compared to period 2, likely related to lower lignification. Overall, the lack of effect of period on in vitro DM digestibility suggests that pasture quality was similar between periods.

4.2. Feed intake

In the present study, herbage intake decreased with the increased concentrate feeding level across both periods, although the magnitude of the difference was greater in period 2. This was most likely a result of the substitution of herbage by the supplement fed; i.e. the reduction in herbage intake due to the supplement intake (Doyle et al., 2005). In addition, due to the residual grazing management target imposed in the study, cows with the higher concentrate feeding level were offered a lower herbage allowance and required a smaller grazing area per day, than cows on the lower concentrate feeding level. This suggests that in addition to herbage substitution, herbage allowance was also a factor contributing to the decreased herbage intake, as in general, there is a positive relationship between herbage allowance and DMI (Wales et al., 1999). Herbage intake was most likely less affected by the herbage quality component of the diet, first because of the good quality of herbage offered to both treatments, and second, due to the experimental set up of the present study, where a relatively high fixed post-grazing height was imposed to both treatment groups. This target was chosen so that herbage intakes would not be restricted and grazing severity would be similar between treatments.

The accurate determination of grazed herbage intakes by individual animals when evaluating production efficiency and CH_4 emissions is a major challenge for grazing studies. There are a number of approaches to estimate grazed herbage intake, e.g., calculation of herbage removal by animals based on grazed herbage mass disappearance, the use of indirect markers such as the *n*-alkane technique and back-calculations from energy requirements. Each technique has its strengths and weaknesses. Individual grazed herbage intake in the present study was estimated using back calculations of total ME requirements for different functions divided by the estimated ME content in grazed herbage. The major weakness of this method is that it does not account for animal-to-animal variation in ME requirements and inefficiencies of energy metabolism, neither does it account for effects of the environment surrounding a grazing animal (e.g. weather, social interaction, etc.), as all energy feeding systems have been developed for average dairy cow under highly controlled conditions. However, this technique has been previously accepted for estimating grazed herbage intake for the evaluation of the production efficiency and CH_4 emissions of cattle (e.g., Jiao et al., 2014; McBride et al., 2014).

In the present study, the interaction effect found between treatment and period on herbage intake estimated through back calculations was not present when calculated based on removed herbage (difference between pre- and post-grazing herbages masses), although the main effect of concentrate feeding level was similar. However, period effects differed. Herbage intake was lower in period 1 than in period 2 when estimated through back calculation, while the contrary seemed to occur when using herbage removal calculation. As there is a positive effect of herbage mass on herbage intake when estimated at ground level (Pérez-Prieto et al., 2012), a greater herbage intake would have been expected in period 1.

4.3. Milk yield and composition

The increase in milk yield observed in the present study with increasing concentrate feeding level is consistent with previous reports (Bargo et al., 2003), with an average response of 0.68 kg of milk/kg of concentrate. Yet, there were no effects of concentrate feeding level on energy-corrected milk yield. Responses of 1 kg of milk/kg of concentrate have been reported in high genetic merit Holstein cows up to a concentrate supplementation level of 6 kg/d (Peyraud and Delagarde, 2013). However, milk responses to supplementary feeds are highly variable as they depend on a wide range of factors such as cow genetic potential, stage of lactation, pasture availability and quality and management systems (Baudracco et al., 2010). The response in milk yield obtained in the present study with the increased concentrate feeding level seems associated to the increase in lactose concentration, at least in the second period. Holmes et al. (1987) indicated that in diets with a higher proportion of concentrate, high milk yields reflect the increased availability of precursors for lactose synthesis. Diets with a high proportion of concentrate and low proportion of roughage are typically associated with low fat concentrations (Sutton, 1980). The lack of effect of concentrate feeding level on fat content and energy-corrected milk yield obtained in the present study probably reflects the moderate concentrate feeding levels used. Nevertheless, the milk constituents responses obtained are in agreement with Lovett et al. (2005) who used similar concentrate feeding levels at grazing (0.9 vs. 5.2 kg/d concentrate DM).

4.4. Enteric CH_4 emissions

This study constitutes the first report of measured enteric CH_4 emissions from grazing dairy cattle in Chile. The average CH_4 emission per unit of estimated feed intake (19.1 g of CH_4/kg DMI) was similar to average values reported in the literature for grazing dairy cows: 19.3 g/kg DMI (Wims et al., 2010), 19.2 g/kg DMI (O'Neill et al., 2011) and 19.5 g/kg DMI (Grainger et al., 2009), although there have been lower (17.2 g/kg DMI, Ulyatt et al., 2002) and higher (20.6 g/kg DMI, Lassey et al., 1997) values reported. The average CH_4 emission per unit of milk yield (MY) in the present study was 13.6 g/kg MY. Other average values reported include 15.7 g/kg MY (Wims et al., 2010), 18.6 g/kg MY (Lassey et al., 1997) and 19.4 g/kg MY (Lovett et al., 2005), which reflects the higher milk yield obtained in the present study. These studies differ in the range of milk yields and herbage intakes and qualities, all of which can affect measured CH_4 response. Although, it is recognized that the SF_6 technique is inherently associated with greater variation than chamber measurements, the SF_6 technique can provide reasonably accurate estimations of enteric CH_4 emissions of dairy cows, comparable with direct measurements in respiration chambers (Muñoz et al., 2012).

From a technical perspective, the measurement of background emissions using both fixed and mobile background collectors produced large variation for both CH_4 and SF_6 background emissions. For the purpose of the SF_6 tracer technique, "background air" can be defined as the concentration of CH_4 and SF_6 within the sample that has a source other than the animal being sampled i.e. trace quantities in the atmosphere but also emission from nearby animals (Berndt et al., 2014). From the results obtained in this study, it seems that the use of spare cows for measuring background CH_4 and SF_6 concentrations may be more accurate, although less precise, than traditional stationary measurements, as they should be more representative of the air surrounding the animals throughout the sampling period. Background samples measured in this manner account for differences between treatments and also, for emissions at locations other than the grazing paddock such as the dairy parlour or crush. Although there are labour and cost implications involved, it is recommended in future research initiatives to consider including mobile background collectors for measuring background emissions.

The increase in total CH_4 emissions with increasing concentrate feeding level, observed in the present study, is in agreement with previous grazing studies (Lovett et al., 2005; O'Neill et al., 2012). However, in those studies, higher CH_4 emission was associated with higher feed intake. There is a strong relationship between DMI and ruminal CH_4 emission (Hristov et al., 2013). In contrast, another grazing study with a range of concentrate feeding levels (2, 4, 6 and 8 kg/cow per day; fresh basis) reported an increase in estimated DMI, but no effect on daily CH_4 emissions, of increasing concentrate feeding level (Jiao et al., 2014). These contrasting reports stress the importance of having reliable herbage intake estimations in grazing studies as it is a major challenge and a key component in the interpretation of CH_4 emissions.

Based on studies with cows in confinement, it has been widely reported that increasing the proportion of concentrate in the diet lowers CH_4 emissions per unit of feed intake and animal product if production remains the same or is increased (Hristov et al., 2013). However, in the present study energy-corrected milk yield did not respond to concentrate feeding level, and decreased CH_4 emissions were not observed. There are several mechanisms involved in the reduction of CH_4 emissions when concentrate feeding level is increased. The first is based on the stoichiometry of ruminal fermentation, as fermentation of digested starch produces less hydrogen and consequently less CH_4 compared to fibre due to lower acetate to propionate ratio (Johnson and Johnson, 1995). Another mechanism involves the methanogens and protozoa, which are inhibited with high levels of concentrate due to decreased pH in the rumen (Iqbal et al., 2008); methanogenesis in the rumen is favoured by protozoa because some methanogens are ecto- and endosymbiotically-associated with protozoa (Dohme et al., 1999). A further mechanism for reduced CH_4 emissions per unit of product as a consequence of high concentrate supplementation is the occurrence of dilution of the maintenance requirements over greater milk production units. It appears that the latter mechanism is only important when milk production increases are greater than what was observed in the present study.

At the concentrate inclusion levels used in the present study (estimated 5 and 23% of total DM), concentrate feeding level had no effect on Y_m. Sauvant and Giger-Reverdin (2009) concluded in their meta-analysis that a marked decrease in Y_m can be expected beyond 35 to 40% inclusion of grain in the diet and that this is dependent on the level of feed intake. Therefore, moderate variation in dietary concentrate proportion, as in the present study, is unlikely to alter rumen function and lead to reduced CH_4 emission. The concentrate feeding levels selected in the present study are representative of common spring management practices in dairy grazing systems in the south of Chile. Apart from the relatively small proportion of concentrate in the diet, the lack of response in Y_m to concentrate feeding level was also most likely affected by the quality of the grazed herbage used in the present study. Throughout the experiment, the cows grazed spring pastures of high DM digestibility, similar to the DM digestibility of the concentrate offered. O'Neill et al. (2011) reported that cows grazing perennial ryegrass in spring produced less CH_4 emissions per cow, per unit of feed intake or per unit of fat or protein yield, than cows offered standard TMR. This was explained in that study by the fact that the herbage offered to the cows was highly digestible, even higher than the TMR.

5. Conclusion

The higher concentrate feeding level (5 kg) used in this study reflect a common farming practice in grazing dairy systems in the south of Chile. This concentrate feeding level, in comparison to the lower concentrate feeding level (1 kg), reduced herbage intake and increased both daily milk yield and gross enteric CH_4 emissions, resulting in no differences in enteric CH_4 emissions per unit of feed intake or per unit of milk yield. These results suggest that moderately increasing concentrate feeding level of dairy cows grazing pastures of high digestibility is not enough to reduce enteric CH_4 emissions per unit of milk yield.

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