Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets

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ABSTRACT

The objective of this study was to determine the effects of replacing grass silage (GS) with corn silage (CS) in dairy cow diets on enteric methane (CH₄) production, rumen volatile fatty acid concentrations, and milk fatty acid (FA) composition. A completely randomized block design experiment was conducted with 32 multiparous lactating Holstein-Friesian cows. Four dietary treatments were used, all having a roughage-to-concentrate ratio of 80:20 based on dry matter (DM). The roughage consisted of either 100% GS, 67% GS and 33% CS, 33% GS and 67% CS, or 100% CS (all DM basis). Feed intake was restricted (95% of ad libitum DM intake) to avoid confounding effects of DM intake on CH₄ production. Nutrient intake, apparent digestibility, milk production and composition, nitrogen (N) and energy balance, and CH₄ production were measured during a 5-d period in climate respiration chambers after adaptation to the diet for 12 d. Increasing CS proportion linearly decreased neutral detergent fiber and crude protein intake and linearly increased starch intake. Milk production and milk fat content (on average 23.4 kg/d and 4.68%, respectively) were not affected by increasing CS inclusion, whereas milk protein content increased quadratically. Rumen variables were unaffected by increasing CS inclusion, except the molar proportion of butyrate, which increased linearly. Methane production (expressed as grams per day, grams per kilogram of fat- and protein-corrected milk, and as a percent of gross energy intake) decreased quadratically with increasing CS inclusion, and decreased linearly when expressed as grams of CH₄ per kilogram of DM intake. In comparison with 100% GS, CH₄ production was 11 and 8% reduced for the 100% CS diet when expressed per unit of DM intake and per unit fat- and

protein-corrected milk, respectively. Nitrogen efficiency increased linearly with increased inclusion of CS. The concentration of *trans* C18:1 FA, C18:1 *cis*-12, and total CLA increased quadratically, and *iso* C16:0, C18:1 *cis*-13, and C18:2n-6 increased linearly, whereas the concentration of C15:0, *iso* C15:0, C17:0, and C18:3n-3 decreased linearly with increasing inclusion of CS. No differences were found in short- and medium-straight, even-chain FA concentrations, with the exception of C4:0 which increased linearly with increased inclusion of CS. Replacing GS with CS in a common forage-based diet for dairy cattle offers an effective strategy to decrease enteric CH₄ production without negatively affecting dairy cow performance, although a critical level of starch in the diet seems to be needed.

Key words: dairy cow, enteric methane production, grass silage, corn silage

INTRODUCTION

Developing strategies to reduce enteric methane (CH₄) emissions from ruminants has received increasing interest recently, as it reduces the ecological footprint of milk production and potentially improves feed efficiency. Dietary manipulation seems to be the most direct and effective approach for reducing CH₄ production from ruminants (Beauchemin et al., 2009) because CH₄ production depends greatly on the level of feed intake and dietary composition, in particular the type of carbohydrates (Beauchemin et al., 2008; Ellis et al., 2008). Including various inhibitors or electron receptors in ruminant diets can reduce CH₄ production up to 50%, but in view of effectiveness and safety issues (e.g., issues with nitrates include potential toxicity from intermediate products), reductions of 10 to 30% are more likely in commercial practice (Hristov et al., 2013). Roughage represents the major component in dairy cow diets and, therefore, it is interesting to investigate the reduction of CH₄ production using roughage-based diets.

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Replacing fiber-rich roughage with starch-rich roughage has potential to reduce CH₄ emissions (Brask et al., 2013; Hassanat et al., 2013). Fermentation of starch favors the ruminal production of propionate at the expense of acetate and decreases rumen pH, which reduces hydrogen availability and activity of rumen methanogens (Van Kessel and Russell, 1996; Hook et al., 2011). The scientific evidence for this particular dietary replacement strategy is limited and does not always reflect diets used in practice. Staerfl et al. (2012) investigated this strategy, but the corn silage (CS) used had a net energy content some 10% lower than that of the grass silage (GS), which is uncommon in many countries. Brask et al. (2013) also investigated the effect of this dietary strategy, but the CS used had a starch content of only 150 g/kg of DM, which is low and uncommon compared with the reported starch content of CS at comparable DM contents (Sutton et al., 2000; Mc Geough et al., 2010a); therefore, the difference in starch content between the CS- (141 g/kg of DM) and the GS-diet (43 g/kg of DM) was not large.

When manipulating dairy cow diet for CH₄ reduction, one should be aware that the composition of milk can also change. Several studies observed that changes in dietary proportion of GS and CS can alter milk FA composition (Ferlay et al., 2006; Nielsen et al., 2006; Kliem et al., 2008). These studies were mainly interested in altering milk FA from a human health perspective, because milk and dairy products are an important source of fat and specific FA in the human diet (van Valenberg et al., 2013). In terms of dietary CH₄ mitigation strategies, differences in milk FA are interesting because they reflect the variations in the amount and composition of carbohydrate between GS and CS (Nielsen et al., 2006), which influences both rumen environment and biohydrogenation of unsaturated FA (Kliem et al., 2008). Consequently, milk FA composition has been suggested as a method to predict enteric CH₄ output in lactating dairy cattle (Dijkstra et al., 2011).

Roughages are nutritionally and economically important (Hassanat et al., 2013). Therefore, it is imperative to investigate dietary strategies using roughage-based diets to mitigate CH₄ production and to determine its effect on milk FA composition. Although GS and CS represent the major conserved roughages and are commonly used in dairy production (Wilkinson et al., 1996), to the best of our knowledge no study has investigated the effect of replacing GS with CS on enteric CH₄ production, rumen VFA concentrations, and milk production and composition, including milk FA composition together. Thus, the objectives of our study were (1) to gain more scientific evidence for the

CH₄ mitigation strategy of replacing fiber-rich GS with starch-rich CS, (2) to examine the changes in ruminal VFA concentration and pH when replacing GS with CS, and (3) to determine the effects of replacing GS with CS on milk production and milk FA composition.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted from October to December 2012 in accordance with Dutch law and approved by the Animal Care and Use Committee of Wageningen University. The experiment followed a completely randomized block design with 4 dietary treatments and 32 multiparous lactating Holstein-Friesian cows with an average milk production of 34.0 ± 5.71 kg/d and 192 ± 87 DIM at the start of the experiment. Cows were blocked in groups of 4 according to lactation stage, parity, milk production, and presence of a rumen cannula (12 cows), and within each block cows were randomly assigned to 1 of 4 dietary treatments; treatment periods, 8 in total, lasted 17 d.

Diets and Feeding

All dietary treatments had a roughage-to-concentrate ratio of 80:20 based on DM content. The composition of the compound feed was the same for all 4 treatments, whereas the roughage was GS, CS, or a mixture of both. The ingredient and chemical composition of the 4 diets are presented in Table 1. Dietary treatments were (ingredient as percentage of the total amount of roughage in the diet; DM basis): (1) 100% GS (**GS100**); (2) 67% GS and 33% CS (**GS67**); (3) 33% GS and 67% CS (**GS33**); and (4) 100% CS (**GS0**).

Cows were fed individually and feed refusals collected to determine DMI throughout the experiment. The cows received their feed twice daily in equal portions before milking, with compound feed supplied on top of the roughage. The cows were fed ad libitum during the first 7 d of the adaptation period in the tiestalls. From d 8 to 17 [i.e., last 5 d of the adaptation period and the 5-d period in the climate respiration chambers (**CRC**)], feed intake was restricted to 95% of the ad libitum DMI of the cow within a block consuming the lowest amount of feed during d 5 to 8, as described previously by van Zijderveld et al. (2011a).

Samples of GS, CS, and compound feed were obtained when fresh feed was prepared (i.e., twice a week). These samples were subsequently pooled per period and subsampled for analyses. Orts, when present during the 5-d period in the CRC, were collected and pooled per cow

Table 1. Ingredient and chemical composition (g/kg of DM, unless otherwise noted) of experimental diets

Item	Treatment ¹			
	GS100	GS67	GS33	GS0
Ingredient				
Grass silage ²	800	533	267	—
Corn silage ³	—	267	533	800
Concentrate ⁴	200	200	200	200
Chemical composition				
OM	924	931	938	945
CP	192	182	172	163
Crude fat	22	22	21	21
Gross energy (MJ/kg of DM)	18.8	18.7	18.6	18.5
NDF	431	396	360	325
ADF	233	219	204	190
ADL	14	14	15	15
Starch	5	91	177	262
Reducing sugars	130	98	66	34

¹Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all treatments. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0.

²Dry matter = 471 g/kg, chemical composition (g/kg of DM): CP = 112, fat = 22, ash = 68, gross energy = 18.8 MJ/kg of DM, NE_L = 6.5 MJ/kg of DM, NDF = 510, sugar = 133, ensiling characteristics (g/kg of DM): acetic acid = 22, lactic acid = 47, ammonia = 2, and pH = 4.8.

³Dry matter = 320 g/kg, chemical composition (g/kg of DM): CP = 76, fat = 20, ash = 42, gross energy = 18.5 MJ/kg of DM, NE_L = 6.7 MJ/kg of DM, NDF = 377, starch = 322, sugar = 14, ensiling characteristics (g/kg of DM): ammonia = 1, and pH = 3.8.

⁴Contained (g/kg of DM): solvent-extracted soybean meal = 502, formaldehyde treated soybean meal = 300, citrus pulp = 80, molasses = 50, urea = 30, CaCO₃ = 15, NaCl = 8, trace mineral and vitamin premix = 8, and MgO = 7; DM = 882 g/kg; chemical composition (g/kg of DM): CP = 510, fat = 23, ash = 109, gross energy = 18.6 MJ/kg of DM, NE_L = 7.4 MJ/kg of DM, NDF = 116, starch = 24, sugar = 116.

and a representative sample was collected. The samples of GS, CS, compound feed, and orts were stored at −20°C until further analyses.

Housing and CRC

In each treatment period, 4 cows of 1 block were individually housed in tiestalls for a 12-d period to become accustomed to the diet and restriction in movement. After the adaptation period, the cows were housed in identical CRC for a 5-d period to determine gaseous exchange, energy and nitrogen (N) balance, and apparent digestibility. Clean drinking water was ad libitum provided and cows were milked and fed twice daily at 0600 and 1600 h during the entire experiment. Cows were exposed to 16 h of light per day (from 0530 to 2130 h).

Two large CRC were used, each containing 2 individual airtight compartments. The CRC were equipped with thin walls with windows, to ensure cows could see and hear each other to minimize the effect of social isolation on cow behavior and performance. The principles of the CRC are described in detail by Verstegen et al. (1987). The ventilation rate within the CRC was 42 m³/h per compartment. Each compartment had an area of 11.8 m² and a volume of 34.5 m³, and the relative hu-

midity was maintained at 70% and temperature at 16°C by 2 computer-controlled air conditioning units. The relative humidity was monitored by 1 relative humidity sensor (Novasina Hygrodat100, Novasina AG, Lachen, Switzerland), and the temperature was monitored by 5 PT100 temperature sensors (Sensor Data BV, Rijswijk, the Netherlands) evenly distributed over the chamber at animal height.

Air from outside was pumped into each compartment via a gas volume meter (Itron Delta 2080 G100, Itron GmbH, Karlsruhe, Germany). Exhaust air exited through a duct with an iris valve controlling the pressure inside the compartment. Within each compartment, a positive pressure of 120 Pa was maintained. The inlet and exhaust air of each compartment was sampled for gas analysis (CH₄, O₂, and CO₂). Gas analyzers (ABB Advance Optima AO2000 systems, ABB, Berlin, Germany) were setup in series with analysis of CO₂ and CH₄ concentration using a nondispersive infrared method, and O₂ concentration using a paramagnetic method.

The volumes of inlet and exhaust air of the CRC were corrected for pressure, temperature, and humidity to arrive at standard temperature pressure dewpoint volumes. Inlet and exhaust volumes of CH₄, O₂, and CO₂ were calculated by multiplying the respective gas

concentrations with the standard temperature pressure dewpoint volumes of inlet and exhaust air. Production of CO₂ and CH₄ and consumption of O₂ was calculated from the difference between inlet and exhaust gas volumes. This was measured with 10-min intervals because the 4 compartments share 1 gas analysis system. Computer-controlled valves direct the air sample from the 4 compartments in sequence (i.e., inlet air, exhaust air compartment A, B, C, and D) to the gas analysis system. Sampled air was flushed through the gas analysis system for 120 s and the average gas concentration of the last 30 s was stored in a computer database. After 120 s the air valves switched to the next compartment.

Once a day, calibration gasses were sampled for gas analysis instead of the inlet air. The analyzed and actual values of these calibration gasses were used to correct the measured gas concentrations from the inlet air and exhaust air of the 4 compartments. In addition, before the experiment started, compartments were checked by releasing known amounts of CO₂ in each compartment and comparing these values with the data from the gas analysis system to calculate the recovery. The recovered amounts of CO₂ were between 98 and 100%.

Staff entered each CRC compartment twice daily at 0600 and 1600 h for approximately 30 min for milking and feeding. The gas measurements during the opening of the CRC were not used for data analysis; CH₄ and CO₂ production and O₂ consumption during these periods was assumed to be linear between the last data point before opening and the first data point after closing the CRC.

For the CH₄ and CO₂ production and O₂ consumption, 3 full 24-h periods were used (i.e., starting at 0800 h of d 14 until 0800 h of d 17). For N and energy balance, manure of each cow of the complete measuring period in the CRC (i.e., starting at 1500 h on d 13 until 0900 h on d 17) was quantitatively collected in the CRC, weighed, mixed, sampled for analyses, and stored at -20°C until further analyses. Cows were weighed immediately after entering and before leaving the CRC. All data presented in the current paper refer to the period the cows were in the CRC. In contrast to other experiments previously performed in the CRC of Wageningen University where 2 cows were housed in 1 large CRC (e.g., van Zijderveld et al., 2011b), in the present experiment the experimental unit is the individual cow because cows were individually housed in the compartments of the CRC.

Milk Yield and Composition

Milk yield was recorded during each milking. In the CRC, a milk sample (10 mL) of each milking was col-

lected in a tube containing sodium azide (5 µL) for preservation. These samples, 8 in total per cow, were analyzed for fat, protein, and lactose content by mid-infrared spectroscopy, and for MUN using the pH difference technique (ISO 14637; ISO, 2004) at Qlip (Zutphen, the Netherlands). Milk composition was corrected for differences in milk yield between individual milkings and the average was used for data analysis.

A representative sample (5 g/kg of milk production) was obtained at each milking from each cow, pooled per cow for the entire period in the CRC, and stored at -20°C pending analyses for gross energy (GE) and N. For milk FA composition, another representative sample was obtained (5 g/kg of milk production at each milking from each cow). Sodium azide (0.05% wt/wt) was added afterward to the pooled sample of the first 4 milkings, followed by the same procedure for milking 5 to 8 in a separate bottle. Both bottles were stored at 5°C. After the last milking, these 2 subsamples (milkings 1–4 and 5–8) were pooled and stored at -40°C until FA composition analysis.

Rumen Fermentation Parameters and VFA

Samples of rumen fluid were taken from the rumen cannulated cows on d 10 and 11 to determine VFA concentration and pH levels. Rumen fluid samples (approximately 200 mL) were collected 1 h before, and 1, 2, 4, 6, and 8 h after morning feeding on both days. Rumen fluid samples were obtained as described by van Zijderveld et al. (2011b), and collected in 3 equal amounts from the front and middle of the ventral sac and from the cranial sac of the rumen. After collection, the collected rumen fluid was thoroughly mixed, pH was measured using an electronic pH meter (HI9024C, Hanna Instruments, IJsselstein, the Netherlands), and 2 rumen fluid samples (600 µL each) were taken and acidified with an equal volume of 0.85% *M* orthophosphoric acid containing 19.68 mM isocaproic acid as internal standard. These 2 rumen fluid samples were directly frozen (-20°C) to stop microbial fermentation and stored at -20°C until VFA analysis.

Analytical Procedures

Prior to analyses, GS, CS, and compound feed samples were thawed at room temperature, air-dried at 60°C, ground to pass a 1-mm screen using a Wiley mill (Peppink 100AN, Olst, the Netherlands), and analyzed for DM, ash, crude fat, starch (except for GS samples), reducing sugars (all carbohydrates with reducing properties and soluble in 40% ethanol), NDF, ADF, ADL, GE, and N. Orts were analyzed for DM, ash, GE, and N.

The manure samples (i.e., feces plus urine combined) were analyzed for DM, ash, N, NDF, GE, crude fat, and starch. Prior to analyses, these samples were thawed at room temperature, air-dried at 60°C, and ground to pass a 1-mm screen. In addition, to determine the N balance, the total amount of condensed water (i.e., collected from the heat exchanger) produced, and the increase in 25% sulfuric acid solution (wt/wt; i.e., through which the outflowing air was led to trap aerial ammonia) of each CRC compartment was measured (both in grams). Samples of both condensed water and 25% sulfuric acid solution were analyzed for N.

Ash, DM, N, crude fat, starch, reducing sugars (all carbohydrates with reducing properties and soluble in 40% ethanol), NDF, ADF, and ADL of feed and manure samples were analyzed as described by Abrahamse et al. (2008a). Bomb calorimetry (ISO 9831; ISO, 1998) was used to determine GE. Crude protein was calculated as $N \times 6.25$. Starch, NDF, and crude fat were assumed to be absent in urine, allowing for calculation of apparent digestibility of these components from analysis of starch, NDF, and crude fat in the combined mixture of feces and urine and in feed.

Milk FA composition was analyzed through gas chromatograph analysis by Qlip. Milk fat was extracted from the milk samples and FAME were prepared from fat fractions (ISO 15884; ISO, 2002a). Methyl esters were analyzed (ISO 15885; ISO, 2002b) on a TRACE Ultra gas chromatograph (Thermo Electron Corporation, Waltham, MA) with a split/splitless injector operated in split mode (split ratio 1:100), at a temperature of 270°C, using a Varian WCOT fused silica column with CP-select CB for FAME as stationary phase (100 m $\times$ 0.25 mm i.d.; Varian Inc., Palo Alto, CA) and hydrogen as carrier gas, and fitted to a flame ionization detector (FID; 250°C). The initial temperature was held at 65°C for 1 min, increased to 225°C at 3°C/min, and held at 225°C for 5 min. A volume of 1 μ L was injected. Peaks were identified and quantified using pure methyl esters (Sigma-Aldrich, Zwijndrecht, the Netherlands; Larodan, Malmö, Sweden; Lipidox). Results of FA were corrected for FID response and expressed as grams per 100 g of total FA.

For determination of VFA, the rumen fluid samples were thawed and centrifuged for 5 min at $14,000 \times g$ at room temperature. The clear supernatant (1 μ L) was injected onto a gas chromatograph (Fisons HRGC Mega 2, CE Instruments, Milan, Italy) with a split/splitless injector operated in split mode (split ratio 1:10), at a temperature of 225°C, using a capillary column (EC-1000, Alltech, Deerfield, IL; 30 m, i.d. = 0.53 mm, film thickness = 1 μ m) and helium as carrier gas, and fitted to an FID. The initial temperature of the column was held at 110°C for 2 min, increased to 200°C at 18°C/

min, and held at 200°C for 2 min. Identification and quantification was conducted with a chemical standard solution (0.85% *M* ortho-phosphoric acid), including an internal standard (19.681 mM isocaproic acid) for correction.

Statistical Analysis

All parameters related to feed, milk production, and milk composition while cows were housed in the CRC were averaged over a 4-d period. The parameters related to energy and N balance were expressed per kilogram of metabolic body weight ($BW^{0.75}$) per day. One cow (receiving diet GS0) was excluded from the experiment because large feed residuals while housed in the CRC resulted in much lower DMI compared with the last 4 d in the tiestall and the other cows in that block. Cow was considered the experimental unit for all parameters. Data were analyzed using the MIXED procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC).

The model included dietary treatment as a fixed effect and period (which is equal to block) as a random effect. For all analyses, the fixed effect of CRC was initially included in the model, but was removed because it was found not significant. Autoregressive 1, variance component, compound symmetry, and unstructured covariance structures were tested for each analysis, and the covariance structure with the lowest overall Akaike's information criterion values (i.e., variance component) was selected. The rumen variables were averaged per time point per cow and subjected to repeated-measures ANOVA to take repeated samples within the same animal into account. This model included cow and period as random effects and diet, time of sampling, and the interaction of diet and time of sampling as fixed effects. To take the unbalanced sampling time intervals into account, the spatial covariance structure was selected.

Both models had unequal variances, therefore, the Kenward-Roger option was used to estimate the denominator degrees of freedom. Orthogonal polynomial contrasts (linear and quadratic) were used to examine treatment effect on response variables. A significant effect of treatment on least squares means was declared when $P \leq 0.05$.

RESULTS

Feed Intake and Digestibility

Increasing the proportion of CS in the diet resulted in a linear increase of DMI (Table 2). No difference was found when comparing the DMI of the cows housed in the CRC with the DMI of the cows housed in the

Table 2. Intake and apparent total-tract digestibility of nutrients in lactating dairy cows fed different proportions of grass silage in the diet

Item	Treatment ¹				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
Intake (kg/d, unless otherwise noted)							
DM	16.2	16.7	16.6	17.5	0.41	0.001	0.858
OM	14.8	15.4	15.4	16.4	0.47	0.020	0.776
CP	3.11	3.05	2.90	2.86	0.101	0.002	0.790
Crude fat	0.36	0.36	0.35	0.36	0.013	0.324	0.915
Gross energy (MJ/d)	303.7	311.2	308.7	322.5	9.34	0.222	0.863
NDF	6.97	6.58	5.97	5.68	0.202	<0.001	0.926
ADF	3.78	3.64	3.39	3.32	0.114	<0.001	0.873
ADL	0.23	0.24	0.24	0.26	0.013	0.159	0.831
Starch	0.08	1.51	2.92	4.59	0.070	<0.001	0.105
Reducing sugars	2.11	1.65	1.10	0.61	0.130	<0.001	0.776
Apparent digestibility (% of intake)							
Fat	48.9	56.4	61.8	68.4	1.43	<0.001	<0.001
NDF	73.1	71.4	62.6	48.4	1.42	<0.001	<0.001
Starch	13.8	94.4	97.5	98.6	1.52	<0.001	0.716

¹Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all treatments. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

tiestall during the 4 d before entering the CRC ($P = 0.153$, data not shown). Intakes of CP, NDF, ADF, and reducing sugars declined linearly ($P < 0.002$), whereas intake of starch increased linearly ($P < 0.001$) as the dietary proportion of CS increased. Apparent total-tract digestibility of NDF decreased quadratically ($P < 0.001$), whereas apparent total-tract digestibility of fat increased quadratically ($P < 0.001$) and of starch linearly ($P < 0.001$) as the CS proportion in the diet increased.

Milk Production and Composition

Milk production, fat- and protein-corrected milk production (**FPCM**), milk fat content, and fat yield were unaffected by increasing proportion of CS in the diet (Table 3). A quadratic increase was observed for milk

protein and milk lactose content ($P < 0.001$) when CS proportion of diet increased. Protein yield increased linearly ($P < 0.02$), whereas MUN decreased linearly ($P < 0.001$) with an increasing proportion of CS in the diet.

Methane Production

Methane (g/kg of DMI) decreased linearly ($P = 0.01$) with increasing dietary CS proportion (Table 4). A quadratic decrease ($P < 0.001$) was observed for CH₄ when expressed as grams per day, grams per kilogram of FPCM, and as a percent of GE intake (**GEI**). The total decrease in CH₄ production observed when the dietary GS proportion was replaced with CS was 11, 8, and 7% ($P < 0.05$) when CH₄ production was expressed as grams per kilogram of DMI, grams per kilogram of FPCM, and as a percent of GEI, respectively.

Table 3. Milk production and milk composition of lactating dairy cows fed different proportions of grass silage in the diet

Item	Treatment ¹				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
Milk production (kg/d)	22.6	23.2	24.2	23.6	1.19	0.457	0.185
FPCM ² (kg/d)	24.0	24.9	25.7	25.6	0.93	0.125	0.297
Milk fat content (%)	4.61	4.77	4.72	4.62	0.148	0.885	0.317
Milk protein content (%)	3.44	3.49	3.34	3.67	0.104	0.003	<0.001
Milk lactose content (%)	4.39	4.55	4.60	4.61	0.041	<0.001	<0.001
Fat yield (g/d)	1,019	1,069	1,106	1,080	40.0	0.165	0.190
Protein yield (g/d)	771	782	781	833	19.0	0.019	0.245
MUN (mg/dL)	14.6	11.9	11.5	10.3	0.80	<0.001	0.288

¹Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all treatments. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

²Fat- and protein-corrected milk = $(0.337 + 0.116 \times \text{fat \%} + 0.06 \times \text{protein \%}) \times \text{milk yield (kg/d)}$.

Table 4. Methane production of lactating dairy cows fed different proportions of grass silage in the diet

CH ₄	Treatment ¹				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
g/d	399	414	411	387	12.8	0.028	<0.001
g/kg of DMI	24.6	25.0	24.5	22.0	0.38	0.010	0.107
g/kg of FPCM ²	16.6	17.0	16.2	15.3	0.50	<0.001	<0.001
% of GEI ³	6.96	7.17	7.11	6.45	0.107	<0.001	<0.001

¹Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all diets. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

²Fat- and protein-corrected milk.

³Gross energy intake.

Rumen VFA Concentrations and pH

All rumen variables were affected by time of rumen sampling ($P \leq 0.002$) and the interaction between diet and time of rumen sampling ($P < 0.001$; data not shown). In general, rumen pH initially decreased after morning feeding and increased again several hours later, whereas VFA concentration showed the opposite pattern. Butyrate molar proportions increased linearly ($P = 0.006$) when the dietary proportion of CS increased (Table 5). No other rumen variables responded linearly or quadratically upon increasing the dietary CS proportion.

Energy and Nitrogen Balance

All parameters related to the energy balance and expressed per kilograms of BW^{0.75} per day [i.e., GEI, CH₄ production, metabolizable energy intake (**MEI**), heat production, energy retention (**ER**) total, ER protein, ER fat, and energy output in milk] were unaffected by increasing the proportion of CS in the diet (Table 6). Nitrogen intake and N output in manure decreased

linearly ($P < 0.001$) with increasing dietary CS proportion (Table 6), whereas N output in milk and the N balance were unaffected by dietary CS proportion. A linear increase was observed for N efficiency ($P < 0.001$).

Milk FA Composition

The total SFA, total MUFA, and total PUFA concentrations in milk fat were unaffected by dietary CS proportion (Table 7). Concentrations of C18:3n-3 decreased linearly ($P < 0.001$), whereas C18:2n-6 concentration increased linearly ($P = 0.003$) and the n-6-to-n-3 ratio increased quadratically ($P = 0.005$) with an increasing proportion of CS in the diet. Concentration of C18:1 *cis*-13 increased linearly ($P = 0.01$), and the concentrations of C18:1 *cis*-12, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, and total CLA increased quadratically ($P < 0.026$) with increasing proportion of dietary CS. A quadratic response was observed for C18:0 ($P = 0.022$).

Most of the short- and medium-chain milk FA were unaffected, whereas some odd- and branched-chain FA

Table 5. Rumen pH, total VFA concentration, and VFA molar proportions of rumen cannulated lactating dairy cows fed different proportions of grass silage in the diet¹

Item	Treatment ²				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
pH	6.77	6.74	6.73	6.72	0.100	0.671	0.917
Total VFA (mM)	103	100	98	98	6.6	0.580	0.804
VFA (% of total VFA)							
Acetate	65.6	66.0	65.8	63.6	1.23	0.126	0.127
Propionate	18.9	17.8	17.7	17.1	1.10	0.141	0.590
Butyrate	11.7	12.5	13.0	15.2	0.41	0.006	0.577
Isobutyrate	1.06	1.05	0.96	1.05	0.033	0.756	0.138
Valerate	1.54	1.33	1.28	1.37	0.138	0.338	0.227
Isovalerate	1.20	1.32	1.30	1.73	0.069	0.061	0.313
Acetate:propionate	3.55	3.83	3.97	3.78	0.318	0.426	0.241

¹Data shown are the mean of values on d 10 and 11.

²Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all diets. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

Table 6. Energy balance and nitrogen balance of lactating dairy cows fed different proportions of grass silage in the diet

Item	Treatment ¹				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
Metabolic BW ² (kg ^{0.75})	123	128	126	126	1.1	0.124	0.035
Energy balance (kJ/kg of BW ^{0.75} per day, unless otherwise noted)							
GEI ³	2,587	2,515	2,551	2,644	79.2	0.671	0.297
CH ₄ production	180	179	181	171	6.5	0.110	0.172
MEI ⁴	1,647	1,638	1,634	1,651	45.8	0.923	0.834
MEI:GEI ratio (%)	63.8	65.1	64.1	62.5	0.74	0.154	0.057
Heat production	896	876	893	922	21.0	0.265	0.113
Energy in milk	590	622	629	620	30.8	0.507	0.435
ER total ⁵	161	138	113	110	39.7	0.261	0.738
ER protein ⁶	52	60	67	51	10.5	0.989	0.175
ER fat ⁷	109	78	45	59	39.0	0.217	0.415
Nitrogen balance (mg/kg of BW ^{0.75} per day)							
N intake	4,155	3,942	3,827	3,694	141.0	<0.001	0.971
N manure	2,748	2,487	2,330	2,251	108.0	<0.001	0.410
N milk	993	1,000	987	1,059	44.6	0.370	0.479
N condense + acid	59	45	53	38	8.2	0.044	0.857
N balance	355	409	457	347	71.1	0.989	0.175
N efficiency ⁸	0.24	0.25	0.26	0.29	0.008	<0.001	0.380

¹Treatments had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all diets. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

²The mean BW per cow per balance period was used to calculate metabolic BW (BW^{0.75}).

³GEI = Gross energy intake.

⁴MEI (metabolizable energy intake) = GEI – methane production – energy in feces + urine.

⁵Energy retention total = MEI – heat production – energy in milk.

⁶Energy retention protein = protein gain (N × 6.25) × 23.6 kJ/g (energetic value of body protein).

⁷Energy retention fat = energy retention total – energy retention protein.

⁸N efficiency = N milk/N feed.

were affected by dietary treatment (Table 7). Concentration of *iso* C15:0 decreased linearly ($P < 0.001$), whereas *iso* C16:0 concentrations increased linearly ($P < 0.001$) with increasing dietary CS proportion. A quadratic response was observed for *iso* C14:0 concentrations ($P = 0.011$). Concentrations of C15:0 and C17:0 decreased linearly ($P < 0.001$) and *anteiso* C15:0 concentration decreased quadratically ($P = 0.027$) with increasing dietary CS proportion.

DISCUSSION

Milk Production and Composition

Replacing GS with CS resulted in a lower CP content in the diets and a lower CP intake, but did not affect milk production. Law et al. (2009) observed that increasing dietary protein content from 14.4 to 17.3% has beneficial effects on milk production for cows in early lactation, but not for cows in late lactation. The cows in the current experiment were in mid lactation (average 192 DIM); the effect of replacing GS with CS on milk production in the present study (CP content of 19.2 and 16.3%, respectively) may have been different (i.e.,

lower milk production) for cows in early lactation. For the current experiment, protein intake was sufficient because MUN decreased linearly with increasing CS inclusion but remained within the range of MUN values commonly observed in practice (5.0–15.0 mg/dL) and above a minimum value (10.0 mg/dL) considered to indicate possible shortage of protein (Spek et al., 2013).

Milk fat content and milk fat yield did not change when GS was replaced by CS, which is in agreement with Brask et al. (2013). A decrease in milk fat content was expected, because an increase in starch intake coupled with a decrease in NDF intake is, in general, associated with a decrease in milk fat content (Nielsen et al., 2006; Abrahamse et al., 2008b). However, feeding CS- compared with GS-based diets is not always associated with a significant reduction of milk fat content (e.g., Fitzgerald and Murphy, 1999; Kliem et al., 2008). The differences in results can probably be ascribed to variations in chemical composition of the CS, especially NDF and starch content; physical characteristics of the silages (i.e., particle size) may also be important (Griinari and Bauman, 2006).

Both milk protein content and milk protein yield increased with increasing CS proportion at the expense of

Table 7. Milk FA composition of lactating dairy cows fed different proportions of grass silage in the diet

FA (g/100 g of FA)	Treatment ¹				SEM	P-value	
	GS100	GS67	GS33	GS0		Linear	Quadratic
C4:0	3.22	3.30	3.44	3.53	0.108	0.033	0.967
C6:0	2.18	2.19	2.20	2.27	0.050	0.247	0.619
C8:0	1.21	1.18	1.18	1.22	0.040	0.967	0.408
C10:0	2.80	2.68	2.62	2.69	0.136	0.498	0.478
C12:0	3.37	3.17	3.07	3.19	0.193	0.404	0.426
C14:0	11.57	10.89	11.13	11.22	0.335	0.594	0.256
<i>iso</i> C14:0	0.09	0.08	0.08	0.09	0.005	0.260	0.011
C14:1 <i>cis</i> -9	1.23	1.18	1.11	1.27	0.107	0.965	0.297
C15:0	1.23	1.05	0.93	0.91	0.061	<0.001	0.221
<i>iso</i> C15:0	0.30	0.24	0.24	0.21	0.009	<0.001	0.183
<i>anteiso</i> C15:0	0.45	0.39	0.39	0.41	0.017	0.255	0.027
C16:0	35.73	35.50	34.40	34.86	1.107	0.398	0.755
<i>iso</i> C16:0	0.16	0.15	0.18	0.19	0.006	<0.001	0.057
C16:1 <i>cis</i> -9	2.11	1.98	1.87	1.97	0.112	0.246	0.205
C16:1 <i>trans</i> -9	0.19	0.20	0.22	0.23	0.012	0.012	0.825
C17:0	0.70	0.66	0.59	0.52	0.021	<0.001	0.396
<i>iso</i> C17:0	0.38	0.37	0.37	0.37	0.012	0.499	0.530
<i>anteiso</i> C17:0	0.41	0.40	0.40	0.42	0.018	0.776	0.292
C17:1 <i>cis</i> -9	0.29	0.30	0.28	0.26	0.014	0.036	0.378
C18:0	7.10	8.04	8.16	7.16	0.438	0.781	0.022
C18:1 <i>cis</i> -9 ²	17.38	18.49	19.06	17.66	1.178	0.740	0.272
C18:1 <i>cis</i> -12	0.12	0.13	0.20	0.34	0.050	<0.001	<0.001
C18:1 <i>cis</i> -13	0.10	0.11	0.11	0.13	0.007	0.010	0.155
C18:1 <i>trans</i> -9	0.11	0.12	0.14	0.19	0.009	<0.001	0.026
C18:1 <i>trans</i> -10	0.12	0.13	0.20	0.45	0.021	<0.001	<0.001
C18:1 <i>trans</i> -11	0.72	0.74	0.77	1.18	0.098	0.001	0.025
C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	0.52	0.56	0.64	0.69	0.040	0.001	0.870
Total CLA ³	0.38	0.35	0.37	0.64	0.059	0.004	0.012
C18:2n-6	1.35	1.37	1.55	1.65	0.085	0.003	0.553
C18:3n-3	0.54	0.44	0.38	0.23	0.073	<0.001	0.210
C18:3n-6	0.07	0.07	0.08	0.09	0.003	0.001	0.115
C20:0	0.13	0.13	0.13	0.10	0.004	<0.001	0.006
C20:1 <i>cis</i> -11	0.04	0.05	0.05	0.04	0.003	0.242	0.008
C20:2n-6	0.05	0.05	0.05	0.04	0.002	0.140	0.462
C20:3n-6	0.09	0.08	0.09	0.10	0.006	0.269	0.028
C20:4n-3	0.08	0.06	0.07	0.07	0.014	0.484	0.529
C20:4n-6	0.13	0.12	0.13	0.15	0.009	0.063	0.069
C20:5n-3	0.08	0.06	0.06	0.05	0.004	<0.001	0.533
C22:0	0.09	0.07	0.06	0.04	0.004	<0.001	0.104
C22:5n-3	0.09	0.09	0.09	0.09	0.006	0.685	0.536
C24:0	0.06	0.05	0.05	0.03	0.004	<0.001	0.117
SFA ⁴	71.20	70.59	69.62	69.43	1.232	0.238	0.876
MUFA ⁵	22.70	23.65	24.22	23.94	1.096	0.370	0.580
PUFA ⁶	2.86	2.68	2.86	3.10	0.139	0.126	0.107
n-6 to n-3 ratio ⁷	2.16	2.58	3.22	4.83	0.210	<0.001	0.005

¹Treatment had a roughage-to-concentrate ratio of 80:20 (DM basis). Concentrate was similar for all diets. Roughage consisted of (all DM basis) 100% grass silage for GS100; 67% grass silage and 33% corn silage for GS67; 33% grass silage and 67% corn silage for GS33; 100% corn silage for GS0 (n = 8 for GS100, GS67, and GS33; n = 7 for GS0).

²C18:1 *cis*-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible, as this FA is always present in small amounts.

³Total CLA consists mainly of C18:2 *cis*-9,*trans*-11.

⁴Sum of all SFA reported in this table.

⁵Sum of all MUFA reported in this table.

⁶Sum of all PUFA reported in this table.

⁷Ratio between the sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, and C20:4n-6 and the sum of C18:3n-3, C20:4n-3, C20:5n-3, and C22:5n-3.

GS. Other studies have also reported increases in milk protein content when GS was replaced by CS (Abrahamse et al., 2008b; Kliem et al., 2008). An increase in milk protein concentration may be attributed to microbial protein synthesis being energetically more efficient

on CS- rather than GS-based diets (Givens and Rulquin, 2004). In addition, in contrast to GS, CS supplies rumen-resistant starch that is digested postruminally; this results in glucose absorption, which is associated with an increase in milk protein concentration likely

mediated through changes in arterial insulin concentrations (Rius et al., 2010).

Energy and Nitrogen Balance

Replacing GS with CS did not affect any of the energy balance parameters. The ratio of MEI to GEI varied between 62.5 and 65.1%, which reflects the high quality of the silages used. Replacing GS with CS resulted in a higher DMI, whereas GEI and MEI were unaffected. This explains why milk production did not increase with the increasing DMI. Total ER was positive, which was expected given that cows were, on average, in midlactation. The mean N balance was 49 g/d and unaffected by dietary treatment. This positive N balance is in line with the average N balance (39 g of N/d) reported by Spanghero and Kowalski (1997) in a review on dairy cattle N balance trials. Replacing GS with CS decreased N intake; this, in combination with an unaffected milk N secretion, resulted in a greater efficiency of dietary N utilization for milk N production. This increased N efficiency with increased starch content and decreased protein content in the diet is in agreement with Hassanat et al. (2013) and Benchaar et al. (2014).

Milk FA Composition

In the present study, replacing GS with CS did not affect total concentration of SFA, MUFA, and PUFA contents in milk fat. This is in agreement with Chilliard et al. (2001), who indicated that PUFA content in milk of cows fed either GS or MS does not differ to a large extent; this is also in agreement with Kliem et al. (2008), who did not find an difference in SFA content in the milk when replacing GS with CS.

Replacing GS with CS in the present study resulted in an increase of C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, and total CLA, suggesting that rumen biohydrogenation was less complete. During biohydrogenation in the rumen, unsaturated FA are converted to C18:0, with an array of *trans* C18:1 isomers and CLA as major intermediates. The isomer profile formed during biohydrogenation can influence milk FA profile. Previous studies (Ferlay et al., 2006; Nielsen et al., 2006; Chilliard et al., 2007) have reported that CS-based diets increase milk fat CLA content compared with GS-based diets. Nielsen et al. (2006) also found increased C18:1 *trans*-10 and C18:1 *trans*-11 and Kliem et al. (2008) reported an increase in C18:1 *trans*-10 and total *trans* C18:1 FA when replacing GS with CS.

Replacing GS with CS did not affect short- and medium-chain FA contents (defined here as straight, even-chain FA up to 16 carbon chain length) in milk

fat, suggesting that de novo synthesis of milk FA in the mammary gland was unaffected, which is in line with the results found for acetate. Acetate is the major carbon source for de novo synthesized FA (Bauman and Griinari, 2003) and was unaffected in the present study by replacing GS with CS. Only C4:0 in milk fat increased linearly when CS dietary proportions increased.

The presence of odd- and branched-chain FA in milk can be used to identify shifts in the rumen microbial population, as most of them are of bacterial origin (Kliem et al., 2008). In the present study, *iso* C15:0 concentration decreased linearly, whereas *iso* C14:0 and *iso* C16:0 concentrations increased (quadratically and linearly, respectively) when GS was replaced by CS. The results for *iso* C14:0 and *iso* C16:0 were unexpected, because Vlaeminck et al. (2006) reported that diets rich in starch decrease *iso* C14:0, *iso* C15:0, and *iso* C16:0 in milk fat. Shingfield et al. (2005) observed similar shifts as Vlaeminck et al. (2006) when replacing GS with CS, except for *iso* C16:0, suggesting *iso* C14:0 and *iso* C15:0 originate from rumen fibrolytic bacteria. However, Kliem et al. (2008) reported that replacing GS with CS did not affect the proportion of *iso* C14:0 and *iso* C16:0 and showed a quadratic relationship for *iso* C15:0, with the lowest *iso* C15:0 content in the diet with GS only.

In the present study, C15:0, *anteiso* C15:0, and C17:0 decreased when GS was replaced by CS. These results are in contrast with Vlaeminck et al. (2006), who suggested that amylolytic bacteria contain high amounts of linear odd-chain FA and *anteiso* FA. Higher starch content in the diet enhances the growth of amylolytic bacteria, potentially leading to an increase in linear odd-chain FA and *anteiso* FA leaving the rumen. Thus, one might expect that with increasing CS inclusion, milk C15:0, *anteiso* C15:0, C17:0, and *anteiso* C17:0 would increase. The results of C15:0 and C17:0 in the present study are, however, in agreement with Kliem et al. (2008), who found a linear decrease in milk C15:0 and C17:0 content when replacing GS with CS, and with Dijkstra et al. (2011), who found an decrease in these linear odd-chain FA when CH₄ decreased.

Methane Production and Ruminal VFA Concentrations

Dry matter intake is a major determinant for CH₄ production (Ellis et al., 2008). Despite restricted feeding with the aim of similar DMI for all treatments, DMI increased linearly when GS was replaced by CS, which was caused by 2 reasons. First, there was a difference in the DM content of silages measured in a sample of silages before the start of the experiment (used to calculate the amounts of silage to be mixed in the total

diet fed to the cows) and the DM content of silages actually fed during the experiment (determined after the trial was finished). Second, there was a difference in the amount of feed refusals between the treatments (data not shown). Feed refusals were not expected, as the cows were fed a restricted diet. Despite the linear increase in DMI in the present study, CH₄ production (g/d) decreased quadratically when GS was replaced by CS. This decline in CH₄ production might be partially explained by passage rate of digesta in the gastrointestinal tract. The rumen residence time decreases with increased feed intake, thereby reducing the extent of the rumen fermentation and shifting digestion from the rumen to the small intestine (Aluwong et al., 2011). If the cows in the current study were fed ad libitum, results may have differed. According to Abrahamse et al. (2008b), DMI is higher for CS- compared with GS-based diets, and higher DMI has been associated with a higher absolute CH₄ production (Ellis et al., 2008). When fed ad libitum, increasing the inclusion of CS in the diet would result in a higher DMI and, therefore, may not have reduced CH₄ production (g/d). However, when CH₄ production would be expressed per unit GEI or DMI it would again be lower for the CS- compared with the GS-based diet.

Replacing GS with CS resulted in decreased CH₄ production (in g/d, g/kg of DMI, g/kg of FPCM, and % of GEI), which is in agreement with Staerfl et al. (2012) and Brask et al. (2013). In general, decreased CH₄ production is associated with decreased rumen acetate proportion and increased propionate proportion (Johnson and Johnson, 1995). In the present study, however, acetate and propionate proportions in the rumen were unaffected when replacing GS by CS. A decrease in acetate proportion when replacing GS with CS was expected because NDF intake and apparent total-tract digestibility of NDF decreased and because acetate is a major end product of NDF fermentation (Bannink et al., 2008). In addition, an increase in propionate proportion when replacing GS with CS was expected because starch intake and apparent total-tract digestibility of starch increased and diets with high starch content are often associated with increased propionate in the rumen (Ellis et al., 2008). Overall, total VFA concentration was unaffected when replacing GS with CS, which is consistent with the absence of difference in rumen pH. However, ruminal pH measurements in the current study seemed high. According to Dijkstra et al. (2012), pH is expected to be around 6.2 with a VFA concentration of 100 mM. In the present study the pH ranged between 6.72 and 6.77 with a VFA concentration of approximately 100 mM. Although pH seems high, in combination with the roughage-based diets used, it helps explain the absence of an effect when replacing GS

with CS on ruminal propionate proportion. According to Bannink et al. (2008), with roughage-based diets and high rumen pH, the proportion of starch fermented to propionic acid per unit glucose fermented is only marginally higher than the proportion of fiber fermented to propionic acid per unit glucose fermented. Hence, with the present high-roughage diets, no or small differences in molar proportion of propionic acid may be expected.

The increased butyrate proportion when replacing GS with CS was unexpected. In general, fermentation of fiber favors the production of acetate and butyrate (Johnson et al., 1996). However, Benchaar et al. (2014) also found an increase in butyrate proportions and a decline in CH₄ production when barley silage was replaced with CS. This increase in butyrate proportion was accompanied by a linear increase in protozoa numbers in the rumen, which is consistent with protozoa being associated with butyrate production (Morgavi et al., 2012).

The quadratic decrease in CH₄ production observed suggests that a critical dietary concentration of starch is required to decrease CH₄ production. A similar response in daily CH₄ output (g/d) was observed by Mc Geough et al. (2010b) in beef cattle fed whole-crop wheat silages with increasing grain content ($P = 0.004$, quadratic response), by Hassanat et al. (2013) in dairy cattle fed diets in which alfalfa silage was replaced with CS ($P < 0.01$, quadratic response), and by Benchaar et al. (2014) in dairy cattle fed diets where barley silage was replaced with CS ($P = 0.07$, quadratic response).

It appears that CH₄ mitigation with roughage-based diets is more difficult than grain-based diets. Mc Geough et al. (2010a) showed that increasing the starch content of roughage can decrease CH₄ production, but CH₄ production of roughage-fed cattle is still considerably higher than for concentrate-fed cattle. Opportunities to use compound feed to lower CH₄ production from the dairy sector is limited, as milk quality is negatively affected once compound feed exceeds approximately 50% of the diet (Beauchemin et al., 2008), and it ignores the importance of ruminants in converting fibrous feeds, unsuitable for direct human consumption, to the high-quality protein source milk (Gill et al., 2010). Roughages represent a major source of ingredients in dairy cow diets and are nutritionally and economically important (Hassanat et al., 2013). Therefore, it is important to investigate dietary strategies to mitigate CH₄ production using roughage-based diets.

CONCLUSIONS

Our results showed that replacing GS with CS in a common roughage-based diet for dairy cattle can be an effective strategy to decrease enteric CH₄ production,

without negative consequences for milk production and milk composition, and improve N efficiency. A critical dietary concentration of starch seems to be required to decrease CH₄ production.

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