Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows

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ABSTRACT

The objective of this study was to investigate the effects of starch varying in rate of fermentation and level of inclusion in the diet in exchange for fiber on methane (CH₄) production of dairy cows. Forty Holstein-Friesian lactating dairy cows of which 16 were rumen cannulated were grouped in 10 blocks of 4 cows each. Cows received diets consisting of 60% grass silage and 40% concentrate (dry matter basis). Cows within block were randomly assigned to 1 of 4 different diets composed of concentrates that varied in rate of starch fermentation [slowly (S) vs. rapidly (R) rumen fermentable; native vs. gelatinized corn grain] and level of starch (low vs. high; 270 vs. 530 g/kg of concentrate dry matter). Results of rumen in situ incubations confirmed that the fractional rate of degradation of starch was higher for R than S starch. Effective rumen degradability of organic matter was higher for high than low starch and also higher for R than S starch. Increased level of starch, but not starch fermentability, decreased dry matter intake and daily CH₄ production. Milk yield (mean 24.0 ± 1.02 kg/d), milk fat content (mean 5.05 ± 0.16%), and milk protein content (mean 3.64 ± 0.05%) did not differ between diets. Methane expressed per kilogram of fat- and protein-corrected milk, per kilogram of dry matter intake, or as a fraction of gross energy intake did not differ between diets. Methane expressed per kilogram of estimated rumen-fermentable organic matter (eRFOM) was higher for S than R starch-based diets (47.4 vs. 42.6 g/kg of eRFOM) and for low than high starch-based diets (46.9 vs. 43.1 g/kg of eRFOM). Apparent total-tract digestibility of neutral detergent fiber and crude protein were not affected by diets, but starch digestibility was higher for diets based on R starch (97.2%) compared with S starch (95.5%). Both total volatile fatty acid concentration (109.2 vs. 97.5 mM) and propionate proportion (16.5 vs. 15.8 mol/100

mol) were higher for R starch– compared with S starch–based diets but unaffected by the level of starch. Total N excretion in feces plus urine and N retained were unaffected by dietary treatments, and similarly energy intake and output of energy in milk expressed per unit of metabolic body weight were not affected by treatments. In conclusion, an increased rate of starch fermentation and increased level of starch in the diet of dairy cattle reduced CH₄ produced per unit of eRFOM but did not affect CH₄ production per unit of feed dry matter intake or per unit of milk produced.

Key words: methane, starch fermentability, starch level, dairy cow

INTRODUCTION

Starch is a major source of glucogenic energy for high-yielding dairy cows and a source of fermentable energy for rumen microorganisms (Koenig et al., 2003). In addition to carbon dioxide, microbial matter, and VFA production, fermentation of feeds in the rumen results in release of hydrogen, which is used by methanogenic archaea to reduce carbon dioxide and produce methane, a potent greenhouse gas that has 25 times more global-warming potential than carbon dioxide (IPCC, 2007). The production of methane (CH₄) is influenced by dietary factors, such as type and amount of feed, and various dietary strategies have been suggested to reduce enteric CH₄ production (Hristov et al., 2013; Knapp et al., 2014). Compared with dietary fiber, starch fermentation in the rumen may result in reduced enteric CH₄ production because fermentation of starch favors production of propionate (Bannink et al., 2006), creating an alternative hydrogen sink to methanogenesis. Moreover, unlike fiber and sugar, a substantial fraction of potentially fermentable starch may escape from rumen fermentation to be digested enzymatically in the small intestine, adding to the energy supply of the animal without associated losses of energy with CH₄ production (Dijkstra et al., 2011).

The use of starch versus fiber as well as increasing dietary starch content in the concentrate are potential

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options to reduce ruminal CH_4 production, relative to total energy supply to the animal (Hristov et al., 2013). Using a modeling approach, Benchaar et al. (2001) estimated that the use of less ruminally fermentable starch and replacing fibrous concentrate with starchy concentrate reduces CH_4 emissions by 17 and 22% in ruminants, respectively. Compared with rapidly fermentable starch sources such as barley or wheat, the use of a slowly fermentable starch such as corn may result in a reduction of CH_4 production (Mills et al., 1999, 2001), mainly attributed to a shift in starch digestion from the rumen to the small intestine. Similarly, the substitution of sugar in the concentrate with a rapidly fermentable starch source such as barley or wheat was estimated to reduce CH_4 production in ruminants (Mills et al., 2001), due to lower ratio of acetate and butyrate to propionate production from starch fermentation (Benchaar et al., 2001; Mills et al., 2001) and the subsequent increase in ME supply to the dairy cow (Mills et al., 2001). However, increasing the amount of rapidly fermentable starch in the diet at the expense of forage fiber can increase the production of VFA beyond the buffering and absorptive capacity of the rumen, leading to a decreased ruminal pH that has negative consequences on fiber degradation and production of dairy cows (Dijkstra et al., 2012). Moreover, the level of abatement of enteric CH_4 production achievable in dairy cattle with grass silage-based diets in which sources and levels of starch in the concentrate vary is largely lacking.

The objective of this study was to evaluate the effects of starch varying in rate of fermentation and in level of inclusion in concentrate that accounted for 40% of the TMR DM on CH_4 production of dairy cows. We hypothesized that increasing the inclusion of ruminally fermentable starch in the diet at the expense of fiber enhances propionate production, and that decreasing rate of fermentation of the starch in the rumen shifts starch digestion from the rumen to the small intestine, both expected to decrease CH_4 production expressed per unit of feed or milk.

MATERIALS AND METHODS

This experiment was conducted as a complete randomized block design at the animal research facility of Wageningen University (Wageningen, the Netherlands). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Wageningen University and carried out under the Dutch Law on Animal Experimentation.

Cows, Experimental Design, and Diets

Forty multiparous lactating Holstein-Friesian dairy cows were selected and grouped in 10 blocks based on

parity (2.9 ± 1.1 ; mean $\pm$ SD), DIM (215 ± 89 d), fat- and protein-corrected milk (**FPCM**; 35.9 ± 9.5 kg/d) at the start of the experiment, and presence or absence of rumen cannula. Sixteen cows were rumen cannulated and used to evaluate the effects of dietary treatments on rumen fermentation characteristics (pH and VFA concentration). Cows within a block were randomly assigned to 1 of 4 dietary treatments. Treatments were 1) 270 g of slowly fermentable starch per kilogram of concentrate DM, 2) 530 g of slowly fermentable starch per kilogram of concentrate DM, 3) 270 g of rapidly fermentable starch per kilogram of concentrate DM, and 4) 530 g of rapidly fermentable starch per kilogram of concentrate DM. Cows were fed a total mixed diet composed of grass silage and concentrate mixed at a 60:40 ratio (DM basis). Diets were offered individually and in equal portions during a.m. and p.m. (0600 and 1600 h) feedings. The concentrates were in meal form and mixed with the forage portion manually when fed.

The primary starch sources in the concentrate were native corn grain, which is slowly fermentable (**S**), and gelatinized corn grain, which is rapidly fermentable (**R**), and each source was included at 2 levels: a low (**L**, 270 g of starch/kg of concentrate DM) and a high (**H**, 530 g of starch/kg of concentrate DM) level. Increasing the level of starch in concentrate was achieved by exchanging either ground native corn grain or ground gelatinized corn grain with beet pulp and palm kernel expeller on DM basis. The ingredient composition of the concentrates is shown in Table 1. Both native and gelatinized corn grain were obtained from a single batch of corn. These 2 starch sources were chosen to create a large difference in rate of starch fermentation.

The experiment was conducted in 10 successive periods of 17 d each. In each period cows were individually housed in tie-stalls for 12 d as an adaptation period and to determine individual daily feed intake. Diets were supplied ad libitum for the first 8 d in the tie-stalls (approximately at 110% of expected voluntary intake). From d 9 to 17, feed intake was restricted per block to 95% of the ad libitum feed intake of the animal consuming the lowest amount of feed during d 3 to 8 within a block to avoid the potential confounding effect of feed intake level on CH_4 measurements. After the end of the adaptation period, cows were housed for 5 d in 1 of the 2 identical climate-controlled respiration chambers for the measurement of CH_4 production. In addition, digestibility measurements and a complete N and energy balance were performed. Because 2 chambers were available, measurements were obtained in 10 periods, staggered in time in an incomplete randomized block design, as described previously by Van Zijderveld et al. (2011a). Within each period, 2 cows receiving the same treatment were housed in one chamber, and 2 cows re-

Table 1. Ingredients and nutrient composition (g/kg of DM, unless otherwise stated) of concentrates fed during the experiment

Item	Slowly fermentable		Rapidly fermentable	
	Low level	High level	Low level	High level
Ingredient				
Native corn grain	412.9	816.1	—	—
Gelatinized corn grain	—	—	412.9	816.1
Palm kernel expeller	266.8	119.8	266.8	119.8
Beet pulp (low sugar)	282.7	—	282.7	—
Soybean meal	18.7	—	18.7	—
Soybean meal, formaldehyde treated	—	13.5	—	13.5
Urea	13.5	23.4	13.5	23.4
Limestone	0.9	14.0	0.9	14.0
Magnesium oxide	—	5.0	—	5.0
Salt	3.1	3.7	3.1	3.7
Sodium bicarbonate	—	2.5	—	2.5
Vitamin-mineral premix ¹	1.5	1.5	1.5	1.5
Nutrient composition				
DM (g/kg of product as fed)	884	889	887	882
Ash	54	50	47	48
CP	169	170	167	186
NDF	311	170	308	153
ADF	187	90	181	80
ADL	41	21	41	23
Crude fat	43	34	47	41
Starch	275	518	303	542
Sugar	36	22	33	22
Gross energy (MJ/kg of DM)	18.5	18.4	18.7	18.3

¹Contained per kilogram of premix: 4,000,000 IU of vitamin A; 833,000 IU of vitamin D; 10,000 mg of vitamin E; 10,000 mg of Cu; 23,333 mg of Zn; 18,467 mg of Mn; 500 mg of Co; 667 mg of I, and 200 mg of Se.

ceiving a different treatment were housed in the other chamber. Within each chamber, the 2 cows originated from different blocks and each dietary treatment was not paired with the other dietary treatments in equal number of periods. The experimental unit for data measured in the respiration chambers (in particular gaseous exchange, N and energy balance parameters) therefore consisted of a pair of cows. The respiration chambers have been described in detail by Verstegen et al. (1987) and Van Zijderveld et al. (2011b). Cows had free access to drinking water throughout the experiment.

Ruminal pH and Concentration of VFA

On d 10 and 11 of each experimental period, equal volumes of rumen fluid from rumen-cannulated cows were collected to determine rumen pH and VFA concentration. Rumen fluid for each cow was collected from the front ventral, middle ventral, and cranial dorsal sac of the rumen (Abrahamse et al., 2008) at 0 (just before the 0600 h feeding), 1, 2, 3, 4, 6, and 8 h after a.m. feeding. Rumen fluid was collected by suction method using a solid plastic tube (0.85 m long and 2.5 cm in diameter) perforated at the end. Ruminal pH was determined immediately after sampling using a portable pH meter (Hanna Instruments Model HI 9024, IJsselstein, the Netherlands). A subsample (0.75 mL)

of ruminal fluid was mixed with an equal volume of meta-phosphoric acid and immediately stored at -20°C pending VFA analysis.

Feed Intake, Nutrients Digestibility, Nitrogen and Energy Balance

Samples of grass silage and concentrates were collected when feeds were prepared. During the CH_4 measurement period, orts (when present) were weighed daily and stored at 4°C . At the end of each period, daily orts were composited per cow and mixed and a subsample was retained and stored at -20°C . Apparent total-tract digestibility of nutrients was determined using chromium oxide (Cr_2O_3) as an external marker included in the concentrate. The marker (1.5 g of Cr_2O_3 /kg of concentrate DM) was supplied for each cow starting on d 1 of the experimental period. During the CH_4 measurement period grab samples of feces (ca. 300 g) were collected daily during milking and stored at -20°C . Prior to freeze drying, the samples were pooled per cow and mixed and a subsample was taken.

The BW of cows was taken on the first (d 13) and the last day (d 17) of the measurement period. The animals in the chamber were tethered in individual stalls complete with slatted floor fitted for collection of the manure (mixture of feces and urine). For com-

plete N-balance determination, the manure produced by the 2 cows in a chamber during the 5-d period was quantitatively collected, weighed, mixed thoroughly, subsampled, and stored at -20°C pending analysis. Also, N volatilized in the form of ammonia that may result from mixing of feces and urine was obtained from samples of condensed water (i.e., collected from the heat exchanger) and 25% sulfuric acid solution wt/wt (i.e., through which the outflowing air was led to trap aerial ammonia) of each chamber.

Milk Yield and Milk Composition

Cows were milked twice daily (0600 and 1600 h), and milk yield was recorded during the 5 d of CH₄ measurement period. During each milking samples were collected in duplicate. Morning and afternoon samples were collected separately into tubes containing sodium azide and stored no longer than 4 d at $+4^{\circ}\text{C}$ pending fat-, CP-, and lactose-contents analysis and determination of SCC concentration. The second sample was pooled per cow based on a weight basis proportional to milk yield (5 g/kg of milk) and stored at -20°C until analysis for urea, energy, and N content.

In Situ Rumen Degradation of Diets

Ruminal degradability of starch, OM, and N in concentrate was determined in a separate experiment using 3 rumen-cannulated lactating Holstein-Friesian dairy cows. The cows were 387.0 ± 7.8 DIM and producing 22.8 ± 3.9 kg/d of milk. Cows were fed ad libitum a mixed ration of 50% grass silage (CP, 104 g/kg of DM; NDF, 516 g/kg of DM) and 50% maize silage (CP, 72 g/kg of DM; NDF, 397 g/kg of DM, starch, 374 g/kg of DM) and a commercial concentrate (160 g/kg of DM starch, 200 g/kg of DM CP, 38 g/kg of DM crude fat, and 80 g/kg of DM ash) according to milk production up to a maximum of 8 kg/d. Only concentrate samples were incubated in the rumen. The effective rumen degradability (ED) of OM in the grass silage was estimated by near infrared spectroscopy analysis (BLGG AgroXpertus, Wageningen, the Netherlands). Nylon bags were prepared according to the Dutch in situ protocol (Tas et al., 2006). Briefly, nylon bags with an inner size of 10×8 cm, a pore size of 40 μm , and porosity of 0.30 (PA 40/30, Nybolt, Switzerland) were filled with approximately 5 g (DM basis) of concentrate ground to pass a 3-mm sieve. Three bags per incubation time for each concentrate were incubated for 2, 4, 6, 8, 12, 24, and 48 h in the rumen of each cow using the all-in, all-out procedure. After incubation, bags were immediately placed in ice water for approximately 5 min to stop fermentation, and rinsed with

tap water. The bags were then washed in a washing machine (AEG Turnamat SL, Nuremberg, Germany) for 40 min in cold water (gentle wool-wash program without centrifuging) and freeze-dried. Dried samples were weighed, pooled to one sample per incubation time per cow; ground through a 1-mm sieve (Peppink 100AN, Olst, the Netherlands); and analyzed for DM, ash, N, and starch.

To determine the rumen degradation characteristics of OM, N, and starch in the concentrates, residues per incubation time per cow were fitted to a first-order nonlinear model: $Y(t) = U + D \times e^{-k_d \times t}$, where $Y(t)$ = proportion of total residue present at time t (g/kg); U = the truly undegradable fraction (g/kg; for OM and N only); D = the potentially degradable fraction (g/kg); t = time of incubation (h); and k_d = the fractional rate of degradation of the D fraction (per hour). The NLIN procedure of SAS (SAS Institute Inc., 2010) was used to estimate the parameter values, with D , U , and k_d constrained to be positive. The ED of starch, OM, and N in the concentrates was calculated as described by Ørskov and McDonald (1979) using the formula $\text{ED} = W + (D \times k_d)/(k_d + k_p)$ and assuming a fractional ruminal outflow rate (k_p) of 0.06 per hour for concentrates (Tamminga et al., 1994). The washout (W) fraction (g/kg), which is assumed to be rapidly degradable, was calculated as $1,000 - D - U$. The estimated rumen-fermentable OM (**eRFOM**) in the total mixed diets was calculated using the ED of OM of concentrates obtained from the rumen incubations and the estimated ED of OM of grass silage, by taking into account the OM content of grass silage and each concentrate, and the proportion of these diet ingredients.

Analytical Procedures

All samples of feeds, orts, feces, and manure were freeze-dried and ground to pass through a 1-mm sieve using a Wiley mill (Peppink 100AN) before analysis, except for N analysis in manure and $\text{NH}_3\text{-N}$ analysis in the silage for which fresh samples were used. Feed and feces samples were analyzed for DM (ISO 6496, 1999), ash (ISO 5984, 2002), N (ISO 5983, 2005), crude fat (ISO 6492, 1999), starch (ISO 15914, 2004), and gross energy (**GE**; ISO 9831, 1998). Crude protein content was calculated as $\text{N} \times 6.25$. Neutral detergent fiber was analyzed according to Van Soest et al. (1991) after pre-treatment with amylase. Acid detergent fiber and ADL were determined according to Van Soest (1973). Determination of sugar content in the feed was based on the method described by Van Vuuren et al. (1993). Concentrate and feces samples were analyzed for chromium using atomic absorption spectrophotometry (Williams et al., 1962). Milk protein, fat, and lactose contents

and SCC concentration were determined according to ISO 9622 (1999) at VVB (VVB, Doetinchem, the Netherlands), and milk urea was determined using the pH difference technique (ISO 14637, 2004).

For determination of the concentration of VFA, the frozen ruminal fluid samples were thawed and centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatant was transferred to a gas-chromatography vial for analysis of VFA concentration using gas chromatography (Fisons HRGC MEGA2, CI instruments, Milan, Italy) according to the method described by Taweel et al. (2005).

Statistical Analysis

In general, statistical analysis were carried out by ANOVA using the PROC MIXED procedure in SAS (SAS Institute Inc., 2010) using various models. The data for 2 cows fed on the diet consisting of 270 g of rapidly fermentable starch per kilogram of concentrate DM in one respiration chamber had to be excluded from all analysis because of malfunctioning of the chamber and unreliable data. Daily data were averaged per experimental unit per period before statistical analysis. Cow was considered as the experimental unit for all measurements except for CH_4 -production parameters and N and energy balance traits, for which a pair of cows housed in the same respiration chamber was considered to be the experimental unit. For CH_4 -production parameters and N and energy balance traits, the model included the fixed effects of respiration chamber, starch source, level of starch, and source $\times$ level interaction, and a random effect of period. Block was not included in the model, because the 2 cows housed within the same chamber originated from different blocks. For DMI, fecal digestibility, and milk characteristics the model included respiration chamber, block, starch source, level of starch, and source $\times$ level interaction as fixed effects, and period as a random factor. For all analysis, the fixed effect of respiration chamber was initially included in the model but was removed from the model because it was not significant. Because of unequal variances, the Kenward-Roger option was used to estimate the denominator degrees of freedom. Autoregressive 1, compound symmetry, and unstructured covariance structures were tested for each analysis. Depending on the characteristics of analysis, the covariance structure with the lowest Akaike's information criterion was selected (Littell et al., 1998), which in most cases was the compound-symmetry covariance structure.

Similarly, data for ruminal pH and VFA concentration were analyzed using the PROC MIXED procedure in SAS with block, starch source, level of starch, time,

source $\times$ level, source $\times$ time, and level $\times$ time interactions as fixed effects and cow as a random effect using the repeated measures procedure with time as repeated measures. The relationship between measurements was assumed to be linear. The covariance structure was defined in the model as being spatial power for unequally spaced measurement times.

Data on in situ rumen degradation of concentrates and diets were similarly analyzed using the PROC MIXED procedure in SAS with starch source, level of starch, and source $\times$ level interaction as fixed effects and cow as random effect.

All results are reported as least squares means. Significance of effect was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Chemical Composition of Concentrates and Diets

The chemical composition of concentrates, grass silage, and total mixed diets is presented in Table 1 and Table 2. The nutrient composition in the concentrates had slight differences, in particular in starch content. Starch content in concentrates with rapidly fermentable (R) starch had a 26 g/kg of DM higher starch content than concentrates with slowly fermentable (S) starch, resulting in an average 11 g/kg of DM higher starch content in total mixed diets containing R starch compared with S starch. The analyzed GE content of grass silage was somewhat higher than expected and higher than that of concentrates. Because the proportion of grass silage in the total diet did not differ between treatments, the high GE content of the silage did not affect differences between treatments.

In Situ Rumen-Degradation Characteristics

Starch source affected all degradation characteristics of starch, N, and OM (Table 3). Concentrates composed of R starch had about 3 times higher ($P < 0.001$) k_d of starch than concentrates based on S starch (average $k_d = 0.155/\text{h}$ vs. $0.054/\text{h}$, respectively) and a much higher W fraction (average 315 vs. 64 g/kg, respectively; $P < 0.001$), leading to a 59% higher ED of starch. A significant interaction between source and level of starch indicated that the effect of source of starch on W , D , k_d , and ED of starch was more pronounced with low starch levels than with high starch levels in the concentrate. The effects of starch resulted in a lower ED of OM and N for S starch- compared with R starch-based concentrates and a higher ED with H compared with L. These effects were less pronounced with L compared with H for ED of starch. The calculated eRFOM in

Table 2. Analyzed chemical composition of grass silage and calculated chemical composition of total mixed diets (g/kg of DM, unless otherwise stated)

Item	Grass silage	Total mixed diet ¹			
		SL	SH	RL	RH
DM (g/kg of product as fed)	512	664	666	663	659
Ash	88	74	72	71	72
CP	148	156	157	156	163
NDF	528	441	385	440	378
ADF	296	252	214	250	210
ADL	20	29	21	29	21
Crude fat	38	40	36	42	39
Starch	NA ²	110	207	121	217
Sugar	79	62	56	61	56
Gross energy (MJ/kg of DM)	19.1	18.8	18.8	18.9	18.7

¹Calculated based on analyzed chemical composition of grass silage and concentrate and mixed at a 60:40 ratio (on DM basis); SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²Not analyzed.

a total mixed diet was lower for S starch than for R starch, and lower for L starch than for H starch ($P < 0.001$), but a significant interaction between source and level of starch indicated that the effects are not additive.

Ruminal pH and Concentration of VFA

The effects of source and level of starch in the diet on rumen pH, VFA concentration, and VFA molar proportions are presented in Table 4. Mean ruminal pH was not affected by either starch rate of fermentation or by level of starch in the diets, whereas total VFA concentration was higher for diets based on R starch than on S starch (109.2 vs. 97.5 mM, $P = 0.002$). Both source and level of starch in the diet did not affect VFA molar proportions, except for higher propionate proportions ($P = 0.046$) and a trend for lower isobutyrate proportions ($P = 0.051$) and acetate:propionate ratio ($P = 0.054$) with the R starch-based diets compared with the S starch-based diets. On average for all dietary treatments, the pH decreased from the prefeeding value of 6.73 to 6.41 and 6.33 at 3 and 4 h after a.m. feeding, respectively (Figure 1). Rumen total VFA concentrations (Figure 2) and acetate:propionate ratio (Figure 3) varied with time of sampling relative to a.m. feeding. The interactions between time and source or level of starch were not significant, except for rumen pH ($P < 0.001$).

Feed Intake and Nutrient Digestibility

Dry matter intake and apparent total-tract nutrient digestibility are presented in Table 5. Dry matter intake was 0.8 kg/d lower with the high level of starch

($P = 0.022$) but was unaffected by source of starch in the diet. Dry matter intake in the chambers (mean 19.0 kg/d) did not differ from DMI during the last 3 d of the adaptation period in the tie-stalls (mean 18.9 kg/d). Apparent total-tract digestibility of NDF and CP was not affected by dietary treatments. However, for both DM and OM digestibility as well as for crude fat and GE digestibility, significant interactions existed between source and level of starch in the diet, showing a higher digestibility with increased starch level of R starch but a lower digestibility with increased starch level for S starch. Diets based on R starch had a 1.7% higher starch digestibility compared with S starch-based diets ($P = 0.006$). The level of starch in the diet had no effect on starch digestibility.

Milk Yield and Milk Composition

Daily milk yield (average 24.0 kg/d) and content of fat (5.05%), protein (3.64%), lactose (4.53%), and milk urea (3.51 mmol/L) were not influenced by either source or level of starch in diet (Table 6). Similarly SCC concentration was not affected by either source or level of starch in the diet. The FPCM and milk fat yield showed a tendency ($P = 0.092$ and $P = 0.077$, respectively) to be higher for diets containing a low level of starch. Milk protein yield showed a tendency ($P = 0.088$) to be higher for cows fed diets based on R starch compared with S starch.

Dietary Treatments and Methane Production

Daily CH₄ production was not affected by source of starch but was higher at low starch levels than at a high

Table 3. In situ rumen degradation characteristics of the concentrates used in the experiment and estimated effective rumen degradability of OM in the total mixed diet

Item	Concentrate ¹				SED ²	P-value		
	SL	SH	RL	RH		Source (S)	Level (L)	S × L
Concentrate								
Starch								
<i>W</i> ³	38	89	343	286	21.2	<0.001	0.861	0.011
<i>D</i> ⁴	963	911	657	714	21.2	<0.001	0.861	0.011
<i>k_d</i> ⁵	0.054	0.054	0.173	0.137	0.0104	<0.001	0.037	0.039
ED ⁶	495	521	830	782	12.1	<0.001	0.238	0.005
Organic matter								
<i>W</i>	116	167	187	224	8.7	<0.001	<0.001	0.280
<i>D</i>	831	818	667	622	24.0	<0.001	0.139	0.375
<i>U</i> ⁷	54	15	146	154	21.7	<0.001	0.351	0.175
<i>k_d</i>	0.043	0.041	0.078	0.139	0.0066	<0.001	0.001	0.001
ED	459	500	564	660	9.4	<0.001	<0.001	0.005
Nitrogen								
<i>W</i>	299	471	363	536	12.3	0.001	<0.001	0.938
<i>D</i>	701	529	637	464	12.3	0.001	<0.001	0.938
<i>k_d</i>	0.025	0.024	0.025	0.028	0.0039	0.033	0.197	0.049
ED	504	623	552	681	4.2	<0.001	<0.001	0.135
Total mixed diet								
eRFOM ⁸	494	510	536	575	3.9	<0.001	<0.001	<0.001

¹SL = concentrate containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = concentrate containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = concentrate containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = concentrate containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

³W = washable fraction (g/kg of respective nutrient).

⁴D = potentially degradable fraction (g/kg of respective nutrient).

⁵k_d = fractional degradation rate (per hour) of potential degradable fraction.

⁶ED = effective rumen degradability (g/kg of respective nutrient).

⁷U = undegradable fraction (g/kg of respective nutrient) and estimated to be zero for starch and N.

⁸eRFOM = estimated rumen-fermentable OM in the total mixed diets (g/kg of OM) was calculated using the ED of OM of concentrates obtained from the rumen incubations and the estimated ED of OM in the grass silage estimated by near-infrared spectroscopy analysis.

starch levels (432 vs. 399 g/d, respectively; $P = 0.017$; Table 7). Methane production per kilogram of milk, per kilogram of FPCM, per kilogram of DMI, per kilogram of digested DM, and as percentage of GE intake was not influenced by dietary treatments. However, CH₄

expressed per kilogram of eRFOM was higher for diets based on S starch than R starch (47.4 vs. 42.6 g/kg of eRFOM, respectively; $P < 0.001$), and for diets based on L than H (46.9 vs. 43.1 g/kg of eRFOM, respectively; $P = 0.002$).

Table 4. Rumen fluid pH and concentration of VFA of lactating dairy cows fed diets that differed in starch rate of fermentation and level of inclusion in concentrate

Item	Diet ¹				SED ²	P-value		
	SL	SH	RL	RH		Source (S)	Level (L)	S × L
Rumen pH	6.53	6.49	6.51	6.53	0.088	0.872	0.889	0.650
Total VFA (mM)	101.1	93.9	110.9	107.4	4.39	0.002	0.107	0.555
VFA (mol/100 mol)								
Acetate (A)	68.3	69.1	68.6	67.9	0.55	0.272	0.859	0.069
Propionate (P)	15.9	15.6	16.2	16.8	0.46	0.046	0.684	0.172
Butyrate	11.5	11.2	11.1	11.8	0.32	0.791	0.358	0.052
Isobutyrate	1.12	0.88	0.80	0.66	0.185	0.051	0.164	0.691
Valerate	1.54	1.54	1.54	1.63	0.106	0.550	0.580	0.520
Isovalerate	1.69	1.64	1.82	1.29	0.311	0.637	0.215	0.281
A:P	4.31	4.45	4.27	4.06	0.146	0.054	0.725	0.107

¹n = 4 for all diets except for RL for which n = 3. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

Figure 1. Effects of source and level of starch in the diet of lactating dairy cows on rumen pH as a function of time after a.m. feeding. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM. Effect of time ($P < 0.001$) and interactions of source of starch with time ($P = 0.143$) and level of starch with time ($P = 0.027$). Error bars represent the standard error of the difference.

Nitrogen and Energy Balance

Nitrogen and energy balance data are presented in Table 8. Daily N intake was not affected by source and level of starch. Total N excretion in manure (feces and

Figure 2. Effects of source and level of starch in the diet of lactating dairy cows on rumen total VFA concentration as a function of time after a.m. feeding. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM. Effect of time ($P < 0.001$) and interactions of source of starch with time ($P = 0.725$) and level of starch with time ($P = 0.592$). Error bars represent the standard error of the difference.

Figure 3. Effects of source and level of starch in the diet of lactating dairy cows on rumen acetate:propionate ratio as a function of time after a.m. feeding. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM. Effect of time ($P = 0.002$) and interactions of source of starch with time ($P = 0.928$) and level of starch with time ($P = 0.147$). Error bars represent the standard error of the difference.

urine mixture) tended ($P = 0.064$) to be higher with diets based on R starch as compared with S starch. Diets based on R starch showed a tendency ($P = 0.060$) of higher secretion of N in milk as compared with diets based on S starch. There was a tendency ($P = 0.088$) of a lower secretion of N in milk for H compared with L in the diet. Nitrogen retention was not affected by level or source of starch in the diet. Also, no significant differences were observed for GE intake, ME intake, heat production, milk energy output, and total energy retention (all expressed per unit metabolic BW) between treatments.

DISCUSSION

This study investigated the effects of starch varying in rate of fermentation and level of inclusion in the diet in exchange for fiber on CH_4 production in lactating dairy cows. Our hypothesis was that increasing the inclusion of ruminally fermentable starch in the diet at the expense of fiber would increase propionate in the rumen, and that decreasing rate of fermentation of starch would shift digestion from the rumen to the small intestine, both expected to decrease CH_4 production expressed per unit of feed or milk. Rumen propionate molar proportion was higher with R starch (16.5 mol/100 mol) than with S starch (15.8 mol/100 mol), but level of starch did not influence rumen molar

Table 5. Dry matter intake and apparent total-tract digestibility of nutrients in lactating dairy cows fed diets that differed in starch rate of fermentation and level of inclusion in concentrate

Item	Diet ¹				SED ²	P-value		
	SL	SH	RL	RH		Source (S)	Level (L)	S × L
DMI (kg/d)	19.4	18.5	19.4	18.6	0.51	0.970	0.022	0.791
Digestibility (%)								
DM	72.9	69.7	67.2	72.7	1.82	0.302	0.377	0.002
OM	74.9	71.1	68.9	74.4	1.81	0.299	0.509	0.001
CP	63.0	63.0	59.0	64.3	2.02	0.348	0.074	0.074
NDF	72.8	71.0	69.9	69.5	2.05	0.140	0.456	0.631
Crude fat	63.9	56.4	61.3	64.7	2.21	0.081	0.203	0.002
Starch	95.6	95.4	96.7	97.6	0.01	0.006	0.491	0.237
Gross energy	69.5	67.5	65.6	69.8	1.75	0.523	0.381	0.019

¹n = 10 for SL, SH, and RH, and n = 8 for RL. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

Table 6. Milk yield and milk composition of dairy cows fed diets that differed in starch rate of fermentation and level of inclusion in concentrate

Item	Diet ¹				SED ²	P-value		
	SL	SH	RL	RH		Source (S)	Level (L)	S × L
Milk yield (kg/d)	24.5	22.5	24.4	24.7	1.34	0.276	0.390	0.238
FPCM ³ (kg/d)	27.9	25.2	27.1	26.9	1.17	0.614	0.092	0.146
Milk composition								
Fat (%)	5.24	5.11	4.97	4.88	0.313	0.271	0.602	0.930
Protein (%)	3.61	3.58	3.68	3.68	0.154	0.428	0.870	0.866
Lactose (%)	4.53	4.55	4.47	4.55	0.065	0.486	0.236	0.486
Milk urea (mmol/L)	3.60	3.49	3.46	3.50	0.185	0.619	0.809	0.587
SCC × 1,000 (cells/mL)	203	165	237	195	84.7	0.597	0.519	0.975
Milk solids yield (kg/d)								
Fat	1.25	1.11	1.17	1.15	0.061	0.643	0.077	0.223
Protein	0.87	0.79	0.88	0.88	0.039	0.088	0.155	0.202
Lactose	1.11	1.03	1.09	1.13	0.063	0.393	0.710	0.203

¹n = 10 for SL, SH, and RH, and n = 8 for RL. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

³Fat- and protein-corrected milk = [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] × milk yield (kg/d).

Table 7. Methane (CH₄) production of lactating dairy cows fed diets that differed in starch rate of fermentation and level of inclusion in concentrate

Item	Diet ¹				SED ²	P-value		
	SL	SH	RL	RH		Source (S)	Level (L)	S × L
CH ₄ (g/d)	436	397	427	401	16.3	0.862	0.017	0.606
CH ₄ (g/kg of DMI)	22.4	21.5	22.2	21.6	0.65	0.852	0.131	0.732
CH ₄ (g/kg of milk)	17.8	17.9	17.8	16.6	0.95	0.346	0.389	0.345
CH ₄ (g/kg of FPCM ³)	15.7	15.9	15.9	15.0	0.59	0.450	0.476	0.196
CH ₄ (g/kg of digested DM)	30.5	30.7	33.0	29.9	1.24	0.339	0.119	0.090
CH ₄ (g/kg of eRFOM ⁴)	49.1	45.6	44.6	40.5	1.31	<0.001	0.002	0.770
CH ₄ (% of gross energy intake)	6.6	6.3	6.5	6.4	0.20	0.898	0.112	0.587

¹n = 5 for SL, SH, and RH, and n = 4 for RL. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

³FPCM = fat- and protein-corrected milk.

⁴eRFOM = estimated rumen-fermentable OM.

Table 8. Nitrogen and energy balance in lactating dairy cows fed diets that differed in starch rate of fermentation and level of inclusion in concentrate

Item	Diet ¹				P-value			
	SL	SH	RL	RH	SED ²	Source (S)	Level (L)	S × L
Metabolic BW (kg of BW ^{0.75})	126.4	124.6	129.2	123.9	4.15	0.739	0.247	0.554
Gross energy intake (kJ/kg of BW ^{0.75} per day)	2,908	2,824	2,817	2,841	126.3	0.687	0.739	0.559
ME intake ³ (kJ/kg of BW ^{0.75} per day)	1,740	1,656	1,630	1,653	79.7	0.333	0.596	0.360
Methane production (kJ/kg of BW ^{0.75} per day)	192	178	184	181	7.9	0.670	0.140	0.360
Heat production (kJ/kg of BW ^{0.75} per day)	969	927	961	950	23.1	0.645	0.133	0.366
N intake (g/d)	489.1	474.6	479.6	484.0	14.93	0.993	0.643	0.389
N in manure (g/d)	322.7	325.2	341.7	352.3	14.39	0.064	0.411	0.556
N in milk (g/d)	136.3	123.8	138.4	137.1	5.23	0.060	0.088	0.159
N retained ⁴ (g/d)	25.5	21.0	-6.4	-10.9	26.75	0.112	0.815	1.000
Energy in milk (kJ/kg of BW ^{0.75} per day)	692	625	657	678	32.7	0.776	0.390	0.072
Energy retention total ⁵ (kJ/kg of BW ^{0.75} per day)	80	104	12	25	85.7	0.243	0.763	0.932
Energy retention protein ⁶ (kJ/kg of BW ^{0.75} per day)	29	25	-9	-14	31.8	0.110	0.838	0.990

¹n = 5 for SL, SH, and RH, and n = 4 for RL. SL = diet containing 270 g of slowly fermentable starch per kilogram of concentrate DM; SH = diet containing 530 g of slowly fermentable starch per kilogram of concentrate DM; RL = diet containing 270 g of rapidly fermentable starch per kilogram of concentrate DM; RH = diet containing 530 g of rapidly fermentable starch per kilogram of concentrate DM.

²SED = SE of the difference of means.

³ME intake = Gross energy intake – methane production – energy in manure.

⁴N retained = N intake – N in manure – N in milk – N in condensate collected from heat exchanger – N trapped from the outflowing air.

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

⁶Energy retention protein = protein gain × 23.7 kJ/g of protein.

proportion of propionate. The in situ ruminal starch degradation rates of the 2 starch sources differed almost by a factor of 3 (0.054 vs. 0.155 per hour; S vs. R starch), and the estimated amount of rumen-degraded starch was 508 versus 806 g/kg of dietary starch for S versus R starch. The in situ characteristics were in agreement with our aim to select the starch sources that represent a wide range of starch fermentation rate. Methane expressed per unit of eRFOM was 10% lower for R than S starch and 8% lower for H- than L-based diets. However, CH₄ production per kilogram of milk, per kilogram of FPCM, per kilogram of DMI, or as percentage of GE intake was not influenced by dietary treatments.

Effects on Ruminal pH and Concentration of VFA

In the current study, the contrast in starch content of the diet was achieved by replacement of fiber-rich beet pulp and palm kernel expeller with corn grain. Much of the nonfiber carbohydrate in beet pulp is pectin and has a tendency for a rumen fermentation profile with more acetate and butyrate (Voelker and Allen, 2003a). Similarly, in a study in which barley was partially substituted with beet pulp in the concentrate, molar proportion of acetate increased and that of propionate and total VFA concentration in the rumen contents decreased (Bodas et al., 2007). Bannink et al. (2006) analyzed VFA data from 182 diets and found higher molar proportions of propionic acid to occur upon fermentation of starch compared with fermentation of cellulose or hemicellulose. In contrast with these findings, rumen acetate and propionate molar proportions were not affected by level of starch, although the source of starch (R vs. S) did significantly affect propionate molar proportion. The relatively high mean daily rumen pH recorded with all treatments may have been the reason for the absence of a substantial response in propionic acid production. Bannink et al. (2008) estimated that with elevated rumen pH the fraction of starch fermented to propionic acid rather than acetic and butyric acid decreases, with the minimum reached at pH values above 6.5. At this pH, the minimum fraction of starch fermented to propionic acid is just slightly higher than the fraction of cellulose or hemicellulose fermented to propionic acid on roughage-type diets (more than 50% roughage on DM basis).

In the present study, starch content increased from on average 116 (low starch level) to 212 (high starch level) g/kg of DM. In a study with diets containing 152, 192, 218, and 224 g/kg of DM of starch from oats, barley, corn, and wheat as the primary source of carbohydrate, respectively, ruminal pH and VFA concentrations were unaffected (Gozho and Mutsvangwa, 2008). In another

study, substitution of beet pulp for high-moisture corn up to 243 g/kg of DM also did not affect daily mean or minimum ruminal pH (Voelker and Allen, 2003a). In the present study, the bicarbonate added as a buffer to the high starch concentrates may have prevented a pronounced drop in rumen pH, which is expected to increase rumen propionate proportions (Bannink et al., 2008). This may have contributed to the absence of effect of level of starch on VFA molar proportions in the rumen.

Effects on DMI, Nutrient Digestibility, and Milk Production

Cows fed H starch-based diets consumed less feed compared with those fed L starch-based diets, whereas source of starch did not affect DMI. The effects of source and level of starch on feed intake are inconsistent among studies reported in the literature. In agreement with our results, a reduced DMI on a high-starch concentrate diet compared with a high-NDF concentrate diet was reported by Miron et al. (2004). Conversely, in other experiments the type of carbohydrate in concentrate mixture (starch vs. cell wall constituents) in total mixed diets did not affect DMI (De Visser et al., 1990; Abrahamse et al., 2008), whereas Beckman and Weiss (2005) reported a tendency of higher DMI as NDF in the diet increased and starch decreased. Other studies reported an increased DMI by cows fed slowly fermentable corn starch compared with rapidly fermentable barley starch (Casper and Schingoethe, 1989; McCarthy et al., 1989). Intake can be affected by numerous factors such as rate of fermentation of starch and fiber, meal patterns, metabolic fuel absorbed, and ruminal patterns of fermentation and pH (Allen, 2000; Voelker and Allen, 2003b; Reynolds, 2006). In the present study, the ED of OM in the rumen was 13% higher for cows fed the H starch diet compared with L starch diet, and this higher ED may be associated with reduced feed intake. With high-starch diets resulting in high propionic acid production in the rumen, DMI may decrease because of the hepatic oxidation of propionate affecting feed intake (Allen et al., 2009). Propionate uptake by liver could have been altered but was not measured. The supply could have been changed as suggested by Sutton et al. (2003).

A significantly higher apparent total-tract starch digestibility for R starch compared with S starch diets was consistent with rumen ED of starch. However, the difference in total-tract digestibility was much smaller (972 vs. 955 g of digested starch/kg of dietary starch; R vs. S starch-based diets) than the difference in ED of starch (806 vs. 508 g of rumen-degraded starch/kg of dietary starch; R vs. S starch-based concentrates).

These values indicate that a much higher fraction of dietary starch could have been digested postruminally with S starch than with R starch diets. In agreement with our results, Gozho and Mutsvangwa (2008) reported apparent total-tract digestibility of starch in cows fed rapidly fermentable starch (oats-based diet) to be higher than in cows fed slowly fermentable starch (corn- and wheat-based diets), with no differences observed for DM, OM, and NDF digestibility. In contrast, Ferraretto et al. (2013) in their meta-analysis reported that increased dietary starch levels typically decreased ruminal and total-tract NDF digestibility when cows are fed high-starch diets. The potential for the negative associative effects of high level of fermentable starch on ruminal fiber digestion (Firkins et al., 2001; Ferraretto et al., 2013) probably did not occur in the present study with pH remaining at levels high enough not to impair activity of fibrolytic bacteria.

Milk characteristics were not affected by dietary treatments. The lack of effect of starch source on milk fat in the present study is in agreement with Silveira et al. (2007), when cows were fed on wheat-, barley-, or corn-based diets. In contrast, Voelker and Allen (2003b) observed a reduced FCM yield and fat yield when lactating dairy cows were fed a diet with 18% starch compared with 27 and 31% starch diets. The tendency in higher milk protein yield observed with R starch-based diets may be explained by the higher eRFOM, because a rise in OM degraded may stimulate microbial protein synthesis leading to an increased absorption of amino acids in the intestine.

Effects on Methane Production

Based on differences in propionate molar proportion between treatments, a reduction in CH₄ production could be expected with R starch compared with S starch, because high propionic acid levels are associated with reduced methanogenesis (Benchaar et al., 2001). However, in the present study the treatment differences for propionate molar proportion were either not present (level of starch) or rather small in size (source of starch), and this may have contributed to the absence of reduction of CH₄ production per kilogram of DMI, as percentage of GE intake, or per kilogram of FPCM. In contrast, lower CH₄ emissions per kilogram of DMI and as a percentage of GE for cattle fed corn compared with barley were reported by Beauchemin and McGinn (2005). Rapidly fermentable starch showed a tendency to decrease the acetate:propionate ratio. However, Martin et al. (2010) suggested that low acetate:propionate ratio and depressed CH₄ production may not always be linked in high concentrate-fed animals. One of the possible explanations for the lack of relationship between

CH₄ emission and rumen VFA pattern may be due, in part, to the observation that the molar proportions of ruminal VFA do not necessarily reflect the proportion in which they are produced (Sutton et al., 2003) but rather reflect the balance between production and absorption (Dijkstra et al., 1993). This balance can be influenced by many factors including level of DMI, rumen volume, rumen absorptive capacity, and rumen pH (Bannink et al., 2008). Also, all dietary treatments in the current study were rather high in NDF (378 to 441 g/kg of DM), which could promote chewing, increase salivation rate, and have contributed to rumen buffering.

In contrast to the absence of effects of dietary treatments on CH₄ expressed per kilogram of DMI, CH₄ production expressed per unit of eRFOM was lower for R than S starch-based diets. Such an effect was not established when CH₄ production was expressed per kilogram of digested OM, or per kilogram of FPCM, in agreement with data from other studies (Mills et al., 1999; Firkins et al., 2001) because large differences in rumen ED of starch were almost completely compensated by digestion of bypass starch in the intestine. In partial agreement with our results, Hassanat et al. (2013) also found no effect on CH₄ production per unit of feed intake, GE intake, and milk upon increasing dietary starch content from 170 to 228 g/kg of DM in dairy cattle, but a further increase to 300 g/kg of DM did reduce CH₄ production. This might be an indication that higher starch levels than the highest level of 217 g/kg of DM tested in the present study are required to reduce enteric CH₄ production. In a recent review on CH₄ mitigation options, Hristov et al. (2013) concluded that inclusion of starch-rich concentrates below 350 to 400 g/kg of total diet DM influences CH₄ production to a minor extent only, and CH₄ emission intensity decreased particularly with levels greater than 400 g/kg of DM. Because the starch content in the present study remained well below 400 g/kg of DM and the contrast tested was relatively small (100 g/kg of DM), the absence of starch level on CH₄ production per kilogram DMI or per kilogram of FPCM is in line with these findings.

Daily CH₄ production (g/d) was reduced mainly because of a higher level of starch and associated lower DMI but not due to source of starch in the diet. Similar responses in daily CH₄ output were also observed by Mc Geough et al. (2010) in beef cattle fed whole-crop wheat silages with increasing dietary starch content. The 8% reduction in daily CH₄ production due to high dietary level of starch observed in the present study though was for a large part due to the 4% lower DMI rather than due to the level of starch in the diet. Studies reported in literature confirm the direct relationship

between CH₄ production and DMI (Benchaar et al., 2001; Hristov et al., 2013).

Effects on Nitrogen Balance

For better assessment of the overall environmental impact of including different sources and levels of starch in the diet of dairy cows to reduce enteric CH₄ production, emissions of other greenhouse gases such as N₂O need to be accounted for. The amount and form of excreted N has a major effect on emissions of N₂O (Dijkstra et al., 2011), and therefore in the present study we also evaluated the effects of the dietary treatments on N balance. Daily N intake and N retention remained unaffected by treatments. The tendency of higher N output in milk in cows fed R starch suggests that rumen-digested starch was stimulatory for milk protein synthesis and was associated with a numerical net mobilization of N, compared with a numerical net retention of N with S starch that have been digested relatively more in the small intestine (Mills et al., 2001). In contrast, Ferraretto et al. (2013) reported a tendency of lower N secreted in milk in cows fed rapidly fermentable starch such as a wheat-based diet compared with cows fed slowly fermentable starch sources such as barley- or corn- based diets.

The positive N retention on S starch-based diets and the small negative N retention on R starch-based diets were not in line with the actual BW change recorded (−0.6 vs. −1.3 kg/d, S vs. R). Inherent errors associated with N-balance studies in lactating dairy cows (such as volatile N losses from manure during collection) likely result in overestimating the true N retention (as reviewed by Spanghero and Kowalski, 1997; Benchaar et al., 2013; Spek et al., 2013). However, in our study, all major N sources emitted were captured with the setup of our respiration chambers. In addition, across all treatments the N retention was quite close to zero, indicating no such problems of losses has occurred, whereas in a review on dairy cattle N balance trials, Spanghero and Kowalski (1997) calculated an average N balance of 39 g/d.

CONCLUSIONS

Results from this study show that both increasing the level of starch in the diet at the expense of fiber (beet pulp and palm kernel expeller) and increasing the rate of fermentation of starch did not affect CH₄ emissions expressed per unit of DMI, per unit of OM digested, per unit of GE intake, or per unit of milk produced but does reduce enteric CH₄ emissions of dairy cattle expressed per unit of eRFOM. Rapidly fermentable starch, but not starch level in the diet, increased the

propionate molar proportion and tended to decrease the acetate:propionate ratio in the rumen.

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