Effects of carbohydrate type or bicarbonate addition to grass silage-based diets on enteric methane emissions and milk fatty acid composition in dairy cows

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ABSTRACT

The aim of the study was to compare the effect of fiber- or starch-rich diets based on grass silage, supplemented or not with bicarbonate, on CH₄ emissions and milk fatty acid (FA) profile in dairy cows. The experiment was conducted as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement: carbohydrate type [starch- or fiber-rich diets with dietary starch level of 23.1 and 5.9% on a dry matter basis, respectively], without or with bicarbonate addition [0 and 1% of the dry matter intake, respectively]. Four multiparous lactating Holstein cows were fed 4 diets with 42% grass silage, 8% hay, and 50% concentrate in 4 consecutive 4-wk periods: (1) starch-rich diet, (2) starch-rich diet with bicarbonate, (3) fiber-rich diet, and (4) fiber-rich diet with bicarbonate. Intake and milk production were measured daily and milk composition was measured weekly; CH₄ emission and total-tract digestibility were measured simultaneously (5 d, wk 4) when animals were in open-circuit respiration chambers. Sensors continuously monitored rumen pH (3 d, wk 4), and fermentation parameters were analyzed from rumen fluid samples taken before feeding (1 d, wk 3). Cows fed starch-rich diets had less CH₄ emissions (on average, −18% in g/d; −15% in g/kg of dry matter intake; −19% in g/kg of milk) compared with fiber-rich diets. Carbohydrate type did not affect digestion of nutrients, except starch, which increased with starch-rich diets. The decrease in rumen protozoa number (−36%) and the shift in rumen fermentation toward propionate at the expense of butyrate for cows fed the starch-rich diets may be the main factor in reducing CH₄ emissions. Milk of cows fed starch-rich diets had lower concentrations in *trans*-11 C18:1, sum of *cis*-C18, *cis*-9,*trans*-11 conju-

gated linoleic acid (CLA), and sum of CLA, along with greater concentration of some minor isomers of CLA and saturated FA in comparison to the fiber-rich diet. Bicarbonate addition did not influence CH₄ emissions or nutrient digestibility regardless of the carbohydrate type in the diet. Rumen pH increased with bicarbonate addition, whereas other rumen parameters and milk FA composition were almost comparable between diets. Feeding dairy cows a starch-rich diet based on grass silage helps to limit the negative environmental effect of ruminants, but does not lead to greater milk nutritional value because milk saturated FA content is increased.

Key words: bicarbonate, carbohydrate type, dairy cow, methane emission, milk fatty acid

INTRODUCTION

Livestock farming is a great contributor to total greenhouse gas emissions via CH₄ production by cattle. Dairy cow CH₄ emissions account for 46% of the total greenhouse gas emissions in dairy supply chains, when expressed as CO₂-equivalents (Gerber et al., 2013), and also lead to significant energy losses ranging between 2 to 12% of the gross energy (GE) intake by animals (Johnson and Johnson, 1995). Livestock competitiveness needs to reduce enteric CH₄ emissions without altering animal performance to improve feed efficiency and to reduce the carbon footprint of the dairy cattle sector.

Among the different dietary strategies tested worldwide, increasing the proportion of concentrate to above 30 to 40% in the diet is known to mitigate CH₄ emissions in ruminants (Martin et al., 2010). Limited information is available on the effect of carbohydrate type in the diet on methanogenesis, though Hindrichsen et al. (2005) studied the effects of concentrates (50% of the diet) providing different carbohydrates type on enteric CH₄ emissions in dairy cows. These authors reported similar CH₄ emissions (g/d and g/kg of DMI) with fiber-rich concentrate (containing soybean hulls) as compared with starch-rich concentrate (containing wheat).

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Moe and Tyrrell (1979) reported that CH₄ production in dairy cows is reduced further as the carbohydrate digestion rate is high. In addition, diets rich in readily fermentable carbohydrates such as starch are known to modify the rumen environment greatly through a decrease in pH and, consequently, to increase the risk of SARA (Krause and Oetzel, 2006). To limit these rumen disorders associated with high-starch diets, fiber can replace starch in carbohydrate diets because it is fermented more slowly, or sodium bicarbonate can be added to diets as a digestive regulator to reduce the risk of SARA (Solorzano et al., 1989). To the best of our knowledge, the effect of buffer addition to the diet of dairy cows on methanogenesis has been studied by very few authors. Hellwing et al. (2012) reported no effect of bicarbonate addition (9.5 g/kg of DM) to a grass-clover silage-based diet rich in molasses on CH₄ emissions (g/d and g/kg of DMI) in dairy cows when compared with a diet rich in wheat.

In addition to CH₄ mitigation, high-starch diets may decrease milk fat content and modify milk fatty acid (FA) composition in dairy cows (Cabrita et al., 2007; Shingfield et al., 2008). Such diets may influence rumen biohydrogenation (RBH) of PUFA (Bauman and Griinari, 2003), resulting in a shift from the *trans*-11 C18:1 to the *trans*-10 C18:1 pathway. High-starch diets also modify the activity or number of bacteria implicated in the synthesis of odd- and branched-chain FA (Vlaeminck et al., 2006; Pirondini et al., 2015). Nevertheless, to the best of our knowledge, no authors have studied the effect of carbohydrate type on milk FA composition in dairy cows fed grass silage-based diets.

The aim with the study was to test the effects of (1) the carbohydrate type in diets [fiber-rich diets (F) or starch-rich diets (S)], (2) the addition of bicarbonate to diets, and (3) the interaction between the carbohydrate type and bicarbonate addition on digestive process, more particularly on CH₄ emissions, and on milk FA composition in lactating dairy cows fed grass silage-based diets.

MATERIALS AND METHODS

The experiment was conducted at the animal experimental facilities of INRA Theix (Saint-Genès-Champagne, France) from February to June 2015. Procedures involving animals were performed in accordance with the French Ministry of Agriculture guidelines for animal research and the applicable European Union guidelines and regulations on animal experiments. The Auvergne Regional Ethics Committee on Animal Experimentation C2EA-02 approved the experiment with the reference number 821–2015060811534198.

Cows, Diets, and Experimental Design

Four multiparous lactating Holstein cows (mean $\pm$ SD, average BW of 639 $\pm$ 62 kg, DIM of 61 $\pm$ 12.5, lactation rank of 2.8 $\pm$ 0.4, and milk yield of 31.5 $\pm$ 4.6 kg/d at the start of the experiment) were used in the experiment which was conducted as a 4 $\times$ 4 Latin square design with a 2 $\times$ 2 factorial arrangement. Each experimental period lasted 4 wk (28 d). From d 1 to 20, cows were housed together in a free-stall barn and received the experimental ad libitum concentrates and forages. From d 21 to 26, cows were moved to individual open-circuit respiration chambers for measurement of CH₄ emissions and total-tract digestibility, and were fed 95% of individual voluntary feed intake (determined during d 1 to 20) to ensure complete consumption of the feed. Changes from one diet to another were achieved with 6-d transition at the beginning of each 28-d period. From d 27 to 28, cows returned to the freestall barn and were fed the same diet than from d 1 to 26.

The 4 dietary treatments aimed at evaluating the main effects of the type of carbohydrates (fiber vs. starch), addition of bicarbonate, and their interaction and were (1) high-fiber diet (F), (2) high-fiber diet with bicarbonate addition (F+b), (3) high-starch diet (S), and (4) high-starch diet with bicarbonate addition (S+b). Diets contained a 50:50 forage-to-concentrate ratio, on a DM basis, 45% grass silage (natural grassland, first cut), 5% hay (natural grassland, first cut), and 50% pelleted concentrates and were formulated to meet individual energy and protein requirements for lactation and maintenance (INRA, 2007). In the F+b and S+b diets, bicarbonate was weighed and mixed every day with the concentrate and given all together with the grass silage at the level of 1% of the DMI. The chemical composition of the different dietary ingredients and diets are reported in Table 1. Diets were iso-energy and iso-protein and were adjusted daily to maintain the forage-to-concentrate ratio as close as possible to the 50:50 ratio targeted. Hay was given once daily (0730 h); the grass silage and concentrates were mixed together by hand as a partial mixed ration (PMR) and were given twice a day (66% at 0900 h and 34% at 1600 h). Cows had free access to water throughout the experiment.

Measurements, Sampling, and Chemical Analyses

Feed Intake and Composition. Feed intake was weighted and recorded on 4 d in wk 1, 2, and 3 and on 5 d in wk 4 of each experimental period to estimate DMI as the difference between DM offered and refused. The DM content of feed was determined (103°C for 24 h)

Table 1. Chemical composition of the dietary ingredients and diets

Item ¹	Ingredient				Diet ²	
	Grass silage ³	Hay	Fiber-rich concentrate ⁴	Starch-rich concentrate ⁵	F	S
OM	890	924	919	944	906	918
CP	140	73	153	158	141	144
NDF	395	636	404	160	419	297
ADF	232	365	223	55	238	154
Starch	—	—	117	462	58.5	231
Ether extract	21.0	15.3	36.3	36.5	28.0	28.5
Gross energy, MJ/kg of DM	19.0	17.9	18.2	18.3	18.5	18.6
Fatty acids (FA), mg/g of DM						
C14:0	0.11	0.07	0.03	0.03	0.07	0.07
C16:0	3.11	1.58	5.38	4.88	4.17	3.92
C16:1 <i>cis</i> -9	0.04	0.06	0.08	0.06	0.06	0.05
C18:0	0.29	0.16	0.84	0.45	0.56	0.37
C18:1 <i>cis</i> -9	0.65	0.34	8.11	6.16	4.37	3.39
C18:1 <i>cis</i> -11	0.08	0.06	0.40	0.30	0.23	0.19
C18:2n-6	4.19	1.21	17.41	16.71	0.02	0.02
C18:3n-3	9.51	1.98	1.23	1.04	4.99	4.90
Other ⁶	2.65	1.93	0.96	0.76	6.80	6.60

¹Chemical composition expressed as grams per kilogram of DM unless stated otherwise.

²F = fiber-rich diets; S = starch-rich diets.

³Fermentation characteristics of fresh silage juice: pH = 4.3; lactic acid = 53.1 g/kg of DM; N-NH₃ = 8.0% of total N.

⁴Composition (% on DM basis) of the fiber-rich concentrate: beet pulp (22.3), soybean hull (22.2), distillers dried corn grains (15.5), wheat bran (10.9), corn grain (7.5), hay (5.4), corn gluten 60 (0.9), molasses (1.5), and vitamins and minerals (3.6).

⁵Composition (% on DM basis) of the starch-rich concentrate: wheat (30.1), corn grain (25.5), wheat middling (9.7), wheat starch (6.8), beet pulp (6.3), corn gluten 60 (4.8), molasses (1.5), and vitamins and minerals (4.2).

⁶Sum of all the other FA analyzed.

on samples (100 g) taken twice a week for grass silage and once a week for hay and concentrates (on wk 1, 2, and 3), and for 5 consecutive days in wk 4. If there were refusals in wk 4, the DM content of each refused feedstuffs was measured (103°C for 24 h). In addition, samples of each feedstuff (100 g) were taken twice in wk 4, pooled to provide 1 sample per period, and stored at 4°C (concentrates, hay) or at −20°C (grass silage). At the end of the experiment, all feedstuff samples were freeze-dried and ground (1-mm screen, ZM 200 Retsch Mill) for chemical composition determination (In Vivo Labs, Chierry, France) including fermentation parameters from fresh grass silage. Organic matter was determined by ashing at 550°C for 6 h (method 942.05; AOAC International, 2005). Total N was analyzed by combustion according to the Dumas method (method 968.06; AOAC International, 2005), and CP content was calculated as N content × 6.25. Fiber (NDF and ADF) was determined by sequential procedures (Van Soest et al., 1991) after pretreatment with amylase, and expressed exclusive of residual ash. Starch was analyzed using an enzymatic method (Faisant et al., 1995). Gross energy was analyzed by isoperibolic calorimetry (model C200, IKA, Staufen, Germany). Ether extract content was determined after acid hydrolysis (method 954.02; AOAC International, 2005). The pH of fresh grass si-

lage juice as well as lactate, VFA, and ammonia-N concentrations were determined as described in Guyader et al. (2016). Fatty acid profile was analyzed according to Sukhija and Palmquist (1988) in all samples for grass silage (1 sample per period) and in a pooled sample for hay and for concentrates for the whole experiment. Total lipids from each diet ingredient were extracted with chloroform:methanol (1:3) along with an internal standard (C23:0, tricosanoic acid). For each ingredient, lipids were methylated with methanolic HCl and each individual FA amount and concentration was determined relative to the response factors for a known amount of the internal standard. Because refusals were negligible (<1%, data not shown), chemical composition of refusals was considered similar to that of the composition of the diet offered.

Digestibility. Total feces and urine collection was performed in individual boxes for 5 consecutive days on wk 4 when cows were housed in the open-circuit respiration chambers. Each morning, after weighing and mixing of feces and urine, one aliquot (1%) was used for DM determination (103°C for 24 h) and another aliquot (0.5%) was pooled per week and per animal before being frozen (−20°C). At the end of the experiment, samples were thawed, freeze-dried, and ground (1-mm screen, ZM 200 Retsch mill) for chemical composition

determination of DM, OM, NDF, ADF, starch, and GE as previously described.

Body Weight, Energy Balance, and Milk Production and Composition. Cows were weighted at the beginning of the experiment and on wk 3 for each period. The energy balance (EB) was calculated as the difference between the energy intake and the energy requirement for lactation and maintenance (INRA, 2007). Cows were milked twice daily at 0730 and 1530 h, and milk yield was individually recorded at each milking. Milk samples (30 mL) were collected for each cow on d 22 to 24 (wk 4) before storage at +4°C with Bronopol (2–2-nitropropane-1,3-diol) as a preservative for milk component analysis. Fat, protein, lactose, and urea nitrogen contents were determined using MilkoScan 4000 (Foss Electric A/S, Hillerød, Denmark; Lial, Aurillac, France). Fat- and protein-corrected milk yield was calculated according to Gerber et al. (2011). For milk FA composition, another individual milk sample (3 mL) was taken on d 24 (wk 4) and frozen (–20°C) without preservative after each milking. All samples were freeze-dried and then composited per day based on am and pm milk yields. The milk FA composition was determined as described by Ferlay et al. (2013). The composition of FAME of CLA isomers in milk fat was analyzed according to Lerch et al. (2012), with some modifications. Briefly, the FAME of CLA isomers were determined using an HPLC system (Agilent, 1200 series) equipped with 3 silver-impregnated silica columns (ChromSpher 5 Lipids, 250 × 4.6 mm, 5-μm particle size; Chromoptic, Courtaboeuf, France) coupled in series. Methyl esters of CLA were separated under isocratic conditions at 22°C using 0.1% (vol/vol) acetonitrile in n-heptane at a flow rate of 1 mL/min and monitoring effluent at 233 and 210 nm. The CLA isomers were identified based on retention time comparisons with a mixture of authentic standards (O5632, Sigma-Aldrich, St.-Quentin-Fallavier, France). Concentrations of CLA isomers were calculated from the proportionate peak area responses determined by HPLC and the sum of concentrations of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA [with the following minor CLA isomers also taken into account in the sum: *trans*-11,*cis*-13 CLA (coeluted with *cis*-9,*cis*-11), *trans*-11,*trans*-13 CLA, *trans*-10,*trans*-12 CLA, *trans*-9,*trans*-11 CLA, and *trans*-8,*trans*-10 CLA] weight percentage determined by GC analysis.

Methane Emissions. Cows were housed in open-circuit respiration chambers (16.6 m³ each) from d 21 (0730 h) to d 26 (0730 h) in wk 4, during which the total CH₄ emissions of each cow were measured continuously. The design and associated analytical equipment

of the open-circuit respiration chambers are detailed in Guyader et al. (2015). Open-circuit respiration chambers operated at a slight negative pressure, with an airflow of 421 ± 12 m³/h on average (approximately 45 air changes per h). The open-circuit respiration chambers were flushed with ambient air for 3 d before each measurement period.

The front and rear doors were never opened simultaneously to avoid an air stream into the open-circuit respiration chambers. Rear doors were opened twice daily: in the morning for milking and to remove recovery boxes for feces and urine collection, and in the afternoon for milking. When the rear doors were closed, the front doors were opened for morning feeding (0830 h for hay and 0900 h for the PMR) and afternoon feeding (1600 h for the PMR). Missing data were estimated as being similar to the last measurement data before open-circuit respiration chambers disturbance. Methane emissions were calculated as the difference between open-circuit respiration chambers and ambient CH₄ concentrations multiplied by the airflow corrected for environmental parameters (temperature, relative humidity, and pressure) according to Pinares-Patiño et al. (2012).

Plasma Parameters, Rumen pH, and Fermentation Parameters. Blood samples were collected from the coccygeal vein using tubes containing EDTA (2.1 mg/mL) after morning milking and before feeding on d 18 (wk 3). Blood samples were kept on ice after sampling and plasma was separated within 1 h, by centrifugation at 1,700 × *g* for 20 min at 4°C, and frozen at –20°C until it was analyzed for nonesterified FA (NEFA), BHB, acetate (C2), and glucose concentrations. Plasma NEFA, glucose, and acetate concentrations were determined by spectrophotometry using glucose dehydrogenase (Glucose RTU kit; BioMérieux, Lyon, France), acyl-CoA synthetase (Wako NEFA HR2 kit, Oxoid, Dardilly, France), and L-malate dehydrogenase/citrate synthase-acyl-CoA synthetase methods (Enzyplus EZA 811 + kit, Biocontrol Systems, Lyon, France), respectively. The BHB concentration was determined as described by Brashear and Cook (1983).

Rumen pH was monitored continuously using a commercial sensor (eBolus, eCow, Exeter, UK) over 3 d in wk 4 when animals were in open-circuit respiration chambers. At the start of the experiment, 1 calibrated sensor per cow was introduced permanently in the rumen through the esophagus by using a dedicated balling gun. Each sensor was set up to record mean pH over 15 min (96 data points per day) with an accuracy of ±0.1. Data were downloaded every 15 d using an eCow handset (smartphone + antenna) with an eCow Android application.

Rumen fluid samples (500 mL) were collected by stomach tubing (Shen et al., 2012) before the morning feeding on d 18 (wk 3) of each experimental period. Samples were strained through a polyester monofilament fabric (250 µm pore size) and the filtrate was subsampled for VFA concentration and protozoa counting. For VFA, 0.8 mL of filtrate was mixed for 2 h at 4°C with 0.5 mL of a 0.5 M HCl solution containing 2% (wt/vol) metaphosphoric acid and 0.4% (wt/vol), and then stored at -20°C. The VFA concentration was analyzed by GC with a flame ionization detector (Morgavi et al., 2008). For protozoa counting, 1-mL aliquot of rumen filtrate was mixed with 1 mL of methyl green formalin-saline solution, and stored at room temperature in darkness. Protozoa counts were done by microscopy and categorized as either small (<100 µm) or large (>100 µm) entodiniomorphs, or as holotrichs (*Dasytricha* or *Isotricha*; Williams and Coleman, 1992). Data for protozoa were log₁₀-transformed for statistical analysis.

Statistical Analysis

Data were analyzed using mixed effect models with the lme4 package (version 1.1–10) in R statistical software (version 0.98.1102, R Foundation for Statistical Computing, Vienna, Austria). Fixed effects of period, carbohydrate type (F and F+b vs. S and S+b), bicarbonate addition (F+b and S+b vs. F and S), and the random effect of cow were tested with the following model:

$$Y_{ijk} = \mu + P_i + A_j + B_k + C_l + (C_l \times B_k) + \varepsilon_{ijkl},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the period ($i = 1$ to 4), A_j is the animal ($j = 1$ to 4), B_k is the bicarbonate addition ($k = 1$ and 2), C_l is the type of carbohydrate ($l = 1$ and 2), $C_l \times B_k$ is the interaction between carbohydrate type and bicarbonate addition, and ε_{ijkl} is the random residual error.

Data measured for several days in wk 4 (intake, milk production and composition, CH₄ emissions, rumen pH, total-tract digestibility) were averaged before being included in the statistical analyses. In period 1, the cow fed the diet S was taken out of the experiment because of health problems. It was replaced by another cow, fed diet S+b in period 1, and was used for the following 3 periods. In addition, it was not possible to download the pH data for one cow during the experiment because the sensor was not responding.

Least squares means were reported with the pooled standard error of the mean derived from the model. Effects were considered significant at a probability of $P \leq 0.05$.

RESULTS

Total DMI, Milk Production and Composition, and Plasma Metabolites

Total DMI was significantly reduced for the starch-rich compared with the fiber-rich diets (-750 g; $P = 0.02$) because of a reduced intake of grass silage and concentrates (Table 2). The type of carbohydrate had no effect on milk production and composition, as well as on feed efficiency, BW, and EB (Table 2). Plasma NEFA, glucose, and acetate concentrations were not influenced by the carbohydrate type (Table 3). However, plasma BHB concentration was reduced ($P < 0.001$) and urea concentration was greater ($P = 0.003$) for starch-rich diets than for fiber-rich diets, respectively.

Bicarbonate addition did not change total DMI, BW, EB, milk yield, milk content and yields of fat, protein and lactose, milk urea content, and feed efficiency. Bicarbonate addition to both starch- and fiber-rich diets reduced plasma NEFA concentration ($P = 0.03$; Table 3), whereas the other plasma metabolites were unchanged.

Nutrients Intake and Diet Digestibility

Intakes of OM did not differ among diets (Table 4). Due to the experimental design, fiber intake was greater (on average +2.9 and +2.0 kg/d for NDF and ADF, respectively; $P < 0.01$), and starch intake was lesser (on average -3.7 kg/d; $P < 0.01$) for fiber- than for starch-rich diets, respectively. Gross energy intake was also increased in the fiber-rich diet as compared with the starch-rich diets (on average +12.4 MJ/d; $P = 0.03$). Bicarbonate addition had no effect on the different nutrient intakes for the F and S diets.

Total-tract apparent digestibility of DM and OM was significantly greater for the starch-rich than for the fiber-rich diets (on average +5.4 and +5.0%, respectively; $P < 0.001$; Table 4). Fiber (NDF and ADF) digestibility did not differ among diets, whereas starch and GE digestibility was also greater for starch-rich than for fiber-rich diets (on average +2.7 and +3.9%, respectively; $P < 0.01$). Bicarbonate addition did not change total-tract diet digestibility of nutrients for F and S diets.

Methane Emissions

The carbohydrate type affected CH₄ emissions (Table 5). Compared with fiber-rich diets, CH₄ emissions (g/d) decreased by -18% with starch-rich diets ($P = 0.007$), by roughly -15% when expressed per kilogram of DMI, kg of OM intake, or % of GE intake ($P = 0.02$).

Table 2. Feed intake, milk yield, and composition in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item ¹	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
Feed intake, kg of DM/d								
Grass silage + concentrate	21.5	21.9	20.9	21.0	1.57	0.02	0.31	0.55
Hay	1.51	1.50	1.49	1.50	0.007	0.23	0.96	0.79
Total DMI	23.0	23.4	22.4	22.4	1.57	0.02	0.09	0.53
Forage:concentrate ratio	1.04	1.07	1.06	1.04	0.271	0.91	0.83	0.29
Milk production, kg/d								
Milk yield	29.6	30.4	29.6	29.5	3.34	0.22	0.57	0.24
FPCM ⁴	30.2	29.8	29.1	30.7	2.60	0.94	0.72	0.50
Milk composition, %								
Fat	4.41	4.34	4.08	4.55	0.322	0.72	0.27	0.16
Protein	3.13	3.25	3.29	3.27	0.191	0.15	0.24	0.12
Lactose	5.28	5.30	5.22	5.19	0.059	0.10	0.26	0.54
Milk urea, mg/L	185	164	181	176	10.7	0.71	0.12	0.24
Milk yield, kg/d								
Fat yield	1.28	1.32	1.20	1.29	0.113	0.410	0.30	0.66
Protein yield	0.88	0.97	0.95	0.96	0.076	0.36	0.21	0.27
Lactose yield	1.56	1.61	1.56	1.54	0.182	0.48	0.66	0.42
Feed efficiency ⁵	1.29	1.32	1.31	1.37	0.042	0.41	0.26	0.61
Energy balance, ⁶ kJ/d	-5.14	-0.46	-1.06	0.97	4.570	0.50	0.41	0.74
BW, kg	645	657	663	648	40.2	0.58	0.86	0.13

¹Means of measurements on wk 4: 5 d for feed intake and milk production and 3 d for milk composition.

²F = diet rich in fiber; S = diet rich in starch; F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.

³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).

⁴FPCM, fat- and protein-corrected milk = milk yield (kg/d) × [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] according to Gerber et al. (2011).

⁵Feed efficiency = FPCM/DMI.

⁶Energy balance was estimated as the difference between energy intake and total energy requirements for lactation and maintenance (INRA, 2007).

Expressed per kilogram of NDF intake or kilogram of NDF digested, CH₄ emissions were greater for starch than for fiber diets by +19% ($P < 0.05$). Emissions of CH₄ per kilogram of milk were significantly reduced with starch-rich diets in comparison to fiber-rich diets (-19%, $P < 0.001$). Irrespective of the unit used, bicarbonate addition in the diets did not affect CH₄ emissions in dairy cows.

Rumen pH and Fermentation Parameters

Mean rumen pH was not affected by the carbohydrate type, but minimum pH was reduced with starch-rich diets as compared with fiber-rich diets (on average -0.14 pH unit, $P < 0.01$; Table 6). Bicarbonate addition in F and S diets increased the mean pH (+0.14 pH unit; $P = 0.006$) and minimum pH (+0.06 pH unit, $P \leq 0.05$).

Table 3. Plasma metabolites concentration in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item ¹	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
NEFA, mmol/L	0.26	0.09	0.25	0.17	0.045	0.48	0.03	0.40
BHB, mmol/L	0.72	0.74	0.51	0.42	0.042	<0.001	0.26	0.12
Glucose, g/L	0.72	0.71	0.74	0.74	0.024	0.23	0.96	0.73
Acetate, mmol/L	1.0	0.85	0.72	0.64	0.148	0.16	0.49	0.82
Urea, g/L	0.13	0.14	0.18	0.17	0.016	0.003	0.76	0.22

¹Measurements taken on wk 3. NEFA = nonesterified fatty acids.

²F = diet rich in fiber; S = diet rich in starch; F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.

³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).

Table 4. Daily nutrient intake and total-tract apparent digestibility in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item ¹	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
Daily nutrient intake, kg/d (unless stated otherwise)								
OM	20.9	21.2	20.6	20.7	1.43	0.12	0.32	0.61
NDF	9.6	9.7	6.8	6.7	0.53	<0.001	0.81	0.13
ADF	5.4	5.5	3.6	3.5	0.30	<0.001	0.97	0.15
Starch	1.3	1.3	4.9	5.1	0.28	<0.001	0.48	0.49
Gross energy, MJ/d	426.6	433.8	416.8	418.8	29.1	0.03	0.31	0.55
Apparent digestibility, %								
DM	62.2	62.4	68.4	67.0	0.78	<0.001	0.48	0.35
OM	68.7	69.2	74.4	73.4	0.82	<0.001	0.74	0.38
NDF	63.5	62.7	61.6	61.7	1.37	0.31	0.81	0.76
ADF	62.1	61.0	60.3	61.3	2.02	0.66	0.96	0.54
Starch	95.7	95.3	98.1	98.2	0.42	<0.001	0.68	0.51
Gross energy	63.5	64.9	68.1	68.1	0.75	0.003	0.41	0.41

¹Average data of the 5-d measurement period in wk 4.²F = diet rich in fiber; S = diet rich in starch; F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).

Total VFA concentration was reduced with starch-rich than with fiber-rich diets (-13.5 mM; $P = 0.02$; Table 6). The proportion of C2 in total VFA was unaffected, whereas the proportions of propionate (C3) and valerate were increased ($P = 0.003$) and that of butyrate (C4) was decreased ($P = 0.002$), as were C2:C3 and (C2+C4):C3 ratios ($P < 0.002$), by starch- compared with fiber-rich diets. Bicarbonate addition increased the proportion of C3 in total VFA and decreased the C2:C3 and (C2+C4):C3 ratios ($P \leq 0.05$), whatever the diet.

Total protozoa concentration was on average 2.8 times greater for fiber-rich compared with starch-rich diets ($P = 0.01$, Table 6). Type of carbohydrate and bicarbonate addition did not modify the rumen concen-

trations of the total and different species of protozoa, irrespective of diet.

Milk Fatty Acid Composition

The starch-rich diets induced a greater concentration of SFA (72.1 vs. 67.6% of total FA for starch- and fiber-rich diets, respectively; Table 7, $P \leq 0.05$) and of short- and medium-chain FA (sum FA <16 C, 30.0 vs. 26.1% of total FA for starch- and fiber-rich diets, respectively; Table 7, $P \leq 0.05$) than with the fiber-rich diet. However, starch-rich diets induced less MUFA concentration as compared with fiber diets (21.3 vs. 25.5% of total FA, respectively; $P = 0.03$). The C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0,

Table 5. Methane emissions in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item ¹	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
CH ₄ , g/d	470	495	408	381	34.8	0.007	0.82	0.36
CH ₄ , g/kg of DMI	20.9	21.3	18.5	17.3	1.55	0.02	0.73	0.44
CH ₄ , g/kg of OM intake	23.0	23.5	20.1	18.8	1.68	0.01	0.73	0.44
CH ₄ , g/kg of NDF intake	50.2	51.3	61.8	58.6	4.99	0.04	0.76	0.55
CH ₄ , % of gross energy intake	6.2	6.3	5.5	5.2	0.46	0.02	0.63	0.45
CH ₄ , g/kg of OM digested	33.5	34.0	27.2	25.8	2.39	0.009	0.80	0.63
CH ₄ , g/kg of NDF digested	79.1	81.8	100.1	96.2	8.71	0.06	0.93	0.67
CH ₄ , g/kg of milk	16.9	17.0	14.0	13.2	1.91	<0.001	0.49	0.38
CH ₄ , ⁴ g/kg of FPCM	16.4	18.7	14.2	12.7	2.08	0.06	0.83	0.31

¹Average data of 5-d measurement period in wk 4.²F = diet rich in fiber; S = diet rich in starch; F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).⁴FPCM, fat- and protein-corrected milk = milk yield (kg/d) × [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] according to Gerber et al. (2011).

C15:0, C16:0, C17:0, *cis*-9 C12:1, *cis*-9 C14:1, *trans*-9 C14:1 concentrations were greater for starch-rich than for fiber-rich diets ($P \leq 0.05$; Table 7 and Supplemental Table S1; <https://doi.org/10.3168/jds.2017-14041>).

Cows fed starch-rich diets had less milk concentration of total C18 FA than those fed fiber-rich diets (31.0 vs. 37.2% of total FA, respectively; $P \leq 0.05$; Table 6), but greater concentration of several isomers of CLA (i.e., *trans*-10,*trans*-12, *trans*-7,*cis*-9 + *trans*-8,*cis*-10, and *cis*-11 *trans*-13; Table 8) and *trans*-9,*cis*-12 C18:2, *cis*-11 C18:1, and *cis*-13 C18:1 (Table 8). However, concentrations of the sum of *cis* isomers of C18:1, *cis*-9,*trans*-11 CLA, and the sum of CLA were reduced with starch-rich than with fiber-rich diets ($P \leq 0.05$; Table 8).

Bicarbonate addition had a slight effect on milk FA composition. Milk concentrations of C4:0, *iso* C18:0, and some isomers of CLA (*trans*-10,*trans*-12, *trans*-7,*trans*-9, and *trans*-12,*cis*-14) were reduced by bicarbonate addition, whereas milk concentrations of *cis*-9,*trans*-11 CLA, *cis*-9 20:1 ($P \leq 0.05$; Table 8), total PUFA, and total CLA were increased ($P \leq 0.05$; Table 7).

DISCUSSION

Methane Emissions and Other Digestive Processes

Effect of Carbohydrate Type. In our study, cows fed the starch-rich diets had less CH₄ emissions (on average, −18% in g/d; −15% in g/kg of DMI; −14% in % of GE intake) than fiber-rich diets. Using a modeling approach, Benchaar et al. (2001) also observed a 14% decrease in CH₄ emissions (g/d) in cows when 10% NDF from beet pulp was replaced by starch from barley in a 70% alfalfa hay-based diet. To the best of our knowledge, very few authors have compared the concentrate carbohydrate type with grass silage based-diet on CH₄ emissions. Hindrichsen et al. (2005) studied the effects of concentrates (50% of the diet) containing different carbohydrates types on enteric CH₄ emissions in dairy cows fed a mixture of forages (22% corn silage, 45% grass silage, 33% hay on a DM basis). Contrary to our results, these authors did not report effect of the carbohydrate types on CH₄ emissions (g/d and g/kg of DMI) between fiber-rich (containing soybean hulls) and starch-rich diets (containing wheat). However, less CH₄

Table 6. Rumen pH¹ and fermentation characteristics² in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item	Diet ³				SEM	P-value ⁴		
	F	F+b	S	S+b		C	b	C × b
Rumen pH								
Mean pH	6.38	6.52	6.40	6.54	0.071	0.75	0.006	0.47
Minimum pH	5.93	5.99	5.79	5.85	0.082	<0.001	0.05	0.89
Total VFA, mM	61.3	60.6	41.8	53.1	4.38	0.02	0.29	0.23
VFA composition, % of total VFA								
Acetate (C2)	70.4	69.5	70.2	70.1	0.55	0.65	0.25	0.43
Propionate (C3)	15.1	15.8	17.1	18.6	0.59	0.003	0.05	0.44
Butyrate (C4)	11.5	11.8	9.3	8.1	0.92	0.002	0.38	0.20
Valerate	1.0	1.0	1.4	1.5	0.07	0.002	0.55	0.66
Caproate	0.4	0.4	0.4	0.3	0.03	0.55	0.11	0.05
Branched-VFA ⁵	1.6	1.6	1.8	1.6	0.17	0.57	0.41	0.67
C2:C3	4.7	4.4	4.1	3.8	0.13	0.002	0.03	0.80
(C2+C4):C3	5.5	5.2	4.7	4.3	0.19	0.001	0.04	0.62
Total protozoa, ⁶ 10 ³ cells/mL	227.1	218.9	101.1	57.9	54.1	0.04	0.66	0.77
Entodiniomorphs, 10 ³ cells/mL	221.6	216.7	101.0	57.1	52.6	0.04	0.67	0.74
Small (<100 μm)	214.7	212.1	98.3	54.3	51.0	0.03	0.68	0.71
Large (>100 μm)	6.95	4.58	2.64	2.85	3.51	0.44	0.78	0.74
Holotrichs, 10 ³ cells/mL	5.48	2.22	1.25	0.77	1.855	0.13	0.28	0.41
<i>Isotricha</i> spp.	0.50	0.24	0.32	0.15	0.208	0.53	0.33	0.82
<i>Dasytricha</i> spp.	4.98	1.98	0.87	0.62	1.734	0.12	0.32	0.40

¹Average data of 3 d when animals were in open-circuit respiration chambers (wk 4). Data were not recovered for 1 cow throughout the duration of the experiment and for another cow during 1 experimental period (n = 11).

²Data from rumen juice sampled before morning feeding on d 18.

³F = diet rich in fiber; S = diet rich in starch; F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.

⁴C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).

⁵Sum of isobutyrate and isovalerate.

⁶For protozoa data, statistical analyses were done with log₁₀ values.

Table 7. Milk fatty acid (FA) composition¹ in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item, % of total FA	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
C4:0	3.48	3.38	3.32	3.26	0.053	0.006	0.05	0.59
C6:0	2.52	2.52	2.73	2.58	0.048	0.03	0.16	0.15
C8:0	1.45	1.48	1.77	1.60	0.058	0.008	0.28	0.14
C10:0	3.10	3.20	4.31	3.72	0.215	0.006	0.25	0.12
C12:0	3.25	3.38	4.82	4.02	0.296	0.008	0.26	0.13
C13:0	0.08	0.09	0.16	0.13	0.013	0.003	0.25	0.13
C14:0	10.9	11.0	12.9	11.9	0.252	<0.001	0.14	0.09
<i>cis</i> -9 14:1	0.80	0.82	1.01	0.99	0.078	0.03	0.99	0.74
C16:0	29.7	29.9	32.8	31.7	0.562	0.003	0.49	0.35
<i>cis</i> -9 16:1	1.18	1.11	1.29	1.42	0.107	0.01	0.58	0.11
C18:0	11.0	10.8	7.4	7.7	0.912	0.002	0.98	0.69
C18:3n-6	0.03	0.03	0.03	0.03	0.005	0.31	0.50	0.80
C18:3n-3	0.67	0.67	0.63	0.68	0.042	0.29	0.20	0.24
ΣSFA ⁴	69.0	69.4	73.5	70.1	1.24	0.05	0.19	0.12
ΣMUFA ⁵	24.3	23.9	20.1	22.9	1.16	0.04	0.25	0.14
Σ <i>cis</i> MUFA ⁶	21.0	20.6	18.0	18.6	0.777	0.02	0.91	0.52
Σ <i>trans</i> MUFA ⁷	2.72	2.78	2.20	3.75	0.774	0.77	0.31	0.35
ΣPUFA ⁸	5.03	5.13	4.76	5.22	0.301	0.34	0.02	0.08
ΣFA <i>trans</i>	7.63	7.72	6.63	8.46	0.796	0.87	0.23	0.27
ΣCLA	0.53	0.58	0.39	0.49	0.047	0.005	0.02	0.34
ΣOBFA ⁹	3.56	3.63	3.88	3.69	0.137	0.07	0.48	0.16
ΣFA <16C	25.9	26.2	31.3	28.5	0.862	0.006	0.22	0.13
ΣFA 16C	29.7	29.9	32.7	31.7	0.562	0.003	0.49	0.35
ΣFA >16C	40.0	39.5	31.3	34.7	1.12	<0.001	0.26	0.13
ΣC18:1 <i>cis</i>	19.3	18.8	15.5	16.3	0.761	0.004	0.85	0.46
ΣC18:1 <i>trans</i>	3.09	3.14	2.52	3.89	0.696	0.89	0.31	0.34
ΣFA C18	37.5	36.8	28.8	32.1	1.10	<0.001	0.27	0.14

¹Measurement taken on d 24 in wk 4.²F = diet rich in fiber; S = diet rich in starch, F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).⁴Sum SFA = odd-chain fatty acids + branched-chain fatty acids + even fatty acids, including fatty acids from 4 to 26 carbon atoms.⁵Sum of MUFA from 10 to 22 carbon atoms.⁶Sum of MUFA from 10 to 22 carbon atoms with the *cis* configuration.⁷Sum of MUFA from 10 to 22 carbon atoms with the *trans* configuration.⁸Sum of PUFA from 18 to 26 carbon atoms.⁹OBFA = sum of odd-chain and branched-chain fatty acids.

emissions were measured for the fiber-rich diet when expressed per unit of NDF, which is in agreement with our results.

The observed CH₄ abatement with starch-rich diets appears to be linked to pre-prandial modifications in rumen fermentation and microbial population. The reduced total VFA concentration in the rumen with starch-rich diets may be due to the more limited DMI. Carbohydrate type in diets also affected the rumen VFA profile, with greater C3 and reduced C4 proportions in starch-rich diets. It is well known that increasing starch level in the diet, at the expense of fiber carbohydrates (NDF and ADF), leads to a shift in rumen fermentation in favor of the propionate pathway (Bannink et al., 2006), creating an alternative H₂ sink to methanogenesis (Martin et al., 2010).

The total count of protozoa, specifically entodino-morphs, was reduced for starch-rich than for fiber-rich diets, leading to less C4 proportion, as protozoa are C4 producers (Morgavi et al., 2012). Hassanat et al. (2013) also reported a decrease in protozoa population and in C4 proportion in the rumen of cows fed high-starch diets based on corn silage. In addition, the observed decrease in protozoa population may have led to a reduction of H₂ transfer from protozoa to methanogens and consequently to reduced methanogenesis. A meta-analysis of 28 experiments and 91 treatments indicated a significant linear relationship between CH₄ emissions and protozoa population ($r = 0.96$) in the rumen (Guyader et al., 2014). Also, the reduced protozoa population with starch-rich diets led to faster bacterial rumen fermentation and a reduced minimum

Table 8. Milk C18:1, C18:2, and CLA composition¹ in dairy cows fed diets containing concentrates rich in fiber or starch, with or without bicarbonate addition

Item, % of total fatty acids	Diet ²				SEM	P-value ³		
	F	F+b	S	S+b		C	b	C × b
<i>trans</i> -4 C18:1	0.03	0.03	0.02	0.03	0.002	0.09	0.21	0.76
<i>trans</i> -5 C18:1	0.02	0.02	0.02	0.03	0.002	0.61	0.07	0.19
<i>trans</i> 6–8 C18:1	0.29	0.28	0.23	0.29	0.039	0.52	0.42	0.41
<i>trans</i> -9 C18:1	0.27	0.27	0.23	0.28	0.031	0.44	0.39	0.27
<i>trans</i> -10 C18:1	0.31	0.44	0.64	1.50	0.502	0.19	0.33	0.46
<i>trans</i> -11 C18:1	1.31	1.27	0.75	1.01	0.149	0.03	0.47	0.34
<i>trans</i> -16 C18:1 ⁴	0.44	0.41	0.31	0.36	0.022	0.005	0.61	0.14
<i>cis</i> -9 C18:1	17.4	17.0	13.8	14.2	0.736	0.004	0.98	0.64
<i>cis</i> -11 C18:1	0.44	0.39	0.45	0.50	0.031	0.05	0.85	0.11
<i>cis</i> -12 C18:1	0.35	0.34	0.28	0.29	0.024	0.03	0.93	0.60
<i>cis</i> -13 C18:1	0.09	0.08	0.09	0.10	0.006	0.04	0.82	0.08
<i>cis</i> -15 C18:1 ⁵	0.19	0.20	0.20	0.22	0.014	0.43	0.39	0.45
<i>cis</i> -16 C18:1	0.10	0.09	0.08	0.09	0.005	0.10	0.59	0.05
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.86	2.88	2.66	2.82	0.257	0.03	0.08	0.16
<i>cis</i> -9, <i>trans</i> -13 C18:2	0.22	0.21	0.20	0.23	0.027	0.90	0.18	0.09
<i>trans</i> -9, <i>trans</i> -12 C18:2	0.02	0.02	0.02	0.03	0.009	0.19	0.65	0.70
<i>trans</i> -9, <i>cis</i> -12 C18:2 ⁶	0.02	0.02	0.04	0.03	0.004	0.02	0.12	0.68
<i>trans</i> -11, <i>cis</i> -15 C18:2	0.12	0.14	0.13	0.20	0.043	0.39	0.27	0.48
<i>cis</i> -9, <i>cis</i> -11 CLA	0.02	0.03	0.03	0.02	0.004	0.74	0.68	0.15
<i>trans</i> -9, <i>trans</i> -11 CLA	0.02	0.02	0.02	0.01	0.002	0.21	0.05	0.05
<i>trans</i> -12, <i>trans</i> -14 CLA	0.01	0.01	0.01	0.01	0.001	0.49	0.23	0.54
<i>trans</i> -11, <i>trans</i> -13 CLA	0.01	0.01	0.02	0.01	0.002	0.26	0.51	0.74
<i>trans</i> -10, <i>trans</i> -12 CLA	0.004	0.004	0.005	0.004	0.0003	0.01	0.01	0.31
<i>trans</i> -8, <i>trans</i> -10 CLA	0.003	0.002	0.003	0.002	0.0001	0.07	0.01	0.18
<i>trans</i> -7, <i>trans</i> -9 CLA	0.008	0.008	0.007	0.005	0.001	0.004	0.03	0.37
<i>trans</i> -12, <i>cis</i> -14 CLA	0.006	0.004	0.006	0.005	0.0007	0.28	0.02	0.77
<i>trans</i> -11, <i>cis</i> -13 CLA	0.01	0.01	0.01	0.01	0.001	0.07	0.73	0.28
<i>cis</i> -11, <i>trans</i> -13 CLA	0.0007	0.0006	0.001	0.001	0.0001	0.01	0.19	0.49
<i>cis</i> -9, <i>trans</i> -11 CLA ⁷	0.38	0.39	0.37	0.38	0.006	0.03	0.40	0.40
<i>trans</i> -7, <i>cis</i> -9 CLA ⁸	0.04	0.04	0.05	0.05	0.003	0.009	0.34	0.63

¹Measurement taken on d 24 in wk 4.²F = diet rich in fiber; S = diet rich in starch, F+b and S+b = F and S diets supplemented with bicarbonate at 1% of DMI.³C = carbohydrate type effect (F and F+b vs. S and S+b); b = bicarbonate supplementation effect (F and S vs. F+b and S+b), and their interaction (C × b).⁴Coelution with *cis*-14 C18:1.⁵Coelution with C19:0.⁶Coelution with *trans*-10 C19:1.⁷Coelution with *trans*-10,*cis*-12 CLA and *trans*-9,*cis*-11 CLA.⁸Coelution with *trans*-8,*cis*-10 CLA.

pH (Guyader et al., 2014), which may have impaired methanogen activity when pH is less than 6.0 (Van Kessel and Russell, 1996).

Effect of Bicarbonate Addition. To the best of our knowledge, the effect of dietary buffer addition on CH₄ production in ruminants has poorly been studied. Our experiment showed that the addition of 1% bicarbonate had no effect on methanogenesis in dairy cows, regardless of the type of carbohydrate in diets based on grass silage, which is in agreement with Hellwing et al. (2012). Indeed, these authors reported no effect of 0.95% bicarbonate addition to a grass-clover silage-based diet rich in molasses. Furthermore, the addition of bicarbonate to diets did not influence nutrient digestibility, which confirms previous data in dairy cows fed a diet based on alfalfa and corn silage supplemented

with 0.8% bicarbonate (Pereira and Armentano, 2000). Meschy et al. (2004) found by a meta-analysis approach (42 diets, 40 studies) that adding buffer at concentrations ranging from 0.5 to 2.5% of DMI had no effect on DM digestibility, but improved fiber digestibility.

Rumen pH measured thanks to commercial sensors positioned permanently in the reticulum of animals, could explain the relative high minimum pH values reported in our study whatever the diet. Reticulum pH has been reported to be higher than in the ventral and dorsal sacs of the rumen of cows fed diets of hay or mixed with 60% barley (Martin et al., 1999). As expected, bicarbonate addition acted as a rumen digestive regulator by increasing rumen pH (on average +0.14 units for mean pH and +0.06 units for minimum pH). Meschy et al. (2004) observed a similar effect of

bicarbonate addition on the mean rumen pH, but to a lesser extent (+0.07 units for pH mean per % added buffer). Other rumen characteristics (VFA and protozoa) measured before the morning feeding were unaffected by addition of bicarbonate (only C3 proportions and ratios were slightly modified) regardless of the type of carbohydrates in diets. This was consistent with the absence of effect of bicarbonate addition on methanogenesis and on nutrient digestibility whatever the diet.

Milk Fatty Acid Composition

Effect of Carbohydrate Type. We reported that short- and medium-chain FA (C6:0, C8:0, C12:0, C14:0, and C16:0), as well as the sum of SFA, were enhanced in milk fat from dairy cows fed starch-rich diets. As described in a review paper (Kalač and Samkova, 2010), high starch intake is associated with more milk SFA arising from a great level of de novo mammary synthesis. The milk C4:0 concentration was reduced with cows fed the starch-rich diets, and this could be explained by the reduced numerical plasma C2 concentration, as well as the reduced rumen C4 concentration because C2 and C4 are precursors of de novo FA synthesis (Chilliard et al., 2007). Another explanation concerning the specific variation of C4:0 compared with other short-chain FA could be due to the fact that this FA is synthesized in part by non-malonyl CoA mechanisms (not involving acetyl-CoA carboxylase; Chilliard et al., 2000).

Cows fed the starch-rich diets produced milk fat with greater C15:0 and C17:0 concentrations than fiber-rich diets. Our results are in agreement with those of Vlaeminck et al. (2006) and Cabrita et al. (2007) who reported a positive relationship between odd-chain FA (C15:0 and C17:0) and dietary starch content. The increased milk concentration of C15:0 and C17:0 with more degradable dietary carbohydrate (i.e., starch-rich diets) could be due to a greater population of amylolytic bacteria, which produce and contain relatively large concentrations of C15:0 and C17:0 (Minato et al., 1988). A lower milk *anteiso* C15:0 concentration was found with cows fed starch-rich diets, which agrees with previous data on cows fed concentrate rich in corn grain compared with concentrate rich in citrus pulp (Cabrita et al., 2007).

Cows fed the starch-rich diets had less milk concentrations of total CLA, several major isomers of CLA, and C18:0. Our results suggest that RBH was reduced when cows received starch-rich diets. The lesser C18:2 n-6 and C18:3 n-3 intakes, due to the limited DMI in starch-rich diets, may explain the reduced RBH because these 2 FA are the main precursors of RBH. Moreover, the lower minimum pH in the rumen observed with starch-rich diets could have modified the RBH in cows

fed the starch-rich diets in comparison to fiber-rich diets, by increasing the milk *trans*-10 C18:1 concentration, leading only to a shift from *trans*-11 to *trans*-10 C18:1 for the S+b diet, as shown by Bauman and Griinari (2003). In our study, it seems that the RBH pathway from C18:2 n-6 to C18:0, including the intermediates as *trans*-10, *trans*-12 and *trans*-8, *cis*-10 CLA, and *trans*-10 C18:1, has been favored for starch-rich diets.

Effect of Bicarbonate Addition. Bicarbonate addition decreased the concentration of some RBH intermediates, whereas we observed a greater concentration of the sum of CLA and *cis*-9, *trans*-11 CLA. These variations in milk FA composition suggest modifications in RBH pathways with bicarbonate addition, favoring the production of *cis*-9, *trans*-11 CLA. In contrast, Cabrita et al. (2009) observed a decrease in almost all RBH intermediate concentrations and a greater C18:0 concentration, suggesting a more complete RBH with dietary buffer addition. Kennelly et al. (1999) observed a decrease in *trans*-10 C18:1 when buffer was added to a diet based on 75:25 concentrate-to-forage ratio. These authors suggested that the mechanism by which dietary buffers promote the completeness of RBH is due to an increase in rumen pH, which modifies bacterial activity. In our study, buffer addition stabilized the rumen pH, as hypothesized, and could have increased bacterial activity or number involved in the production of *cis*-9, *trans*-11 CLA. We observed a greater content of some *trans* FA, which was not expected at first. Further studies are needed to improve understanding of the mechanism by which dietary buffers modify RBH.

CONCLUSIONS

This study shows that for a 50:50 forage:concentrate ratio in grass silage-based diets, the dietary carbohydrate type had an effect on CH₄ emissions in dairy cows as expected, but not on milk yield or on fat- or protein-corrected milk yield. Replacement of fiber by starch in diets decreased the energetic loss as CH₄ emissions without improving feed efficiency of diets. The decrease in rumen protozoa number and the shift in rumen fermentation toward propionate for cows fed the starch-rich diets may be the main factors for reduced methanogenesis. Furthermore, starch-rich diets increased milk saturated short- and medium-chain FA and decreased the completeness of the biohydrogenation pathways of PUFA in the rumen, resulting in greater CLA content than in fiber-rich diet. The addition of 1% bicarbonate to diets induced rumen environment changes, through the increase of pH in starch-rich diets. Furthermore, no effects on CH₄ emissions or nutrient digestibility were observed with bicarbonate addition, regardless of the type of carbohydrate in diets. Milk FA

composition was slightly different between diets with or without bicarbonate, but bicarbonate addition did not prevent *trans* FA increase in milk as hypothesized. Reducing CH₄ emissions in dairy cows fed starch-rich diets based on grass silage help to limit the negative environmental effect of ruminant livestock. However, milk nutritional value was depressed as saturated milk FA were present at greater concentration with starch-rich diets than with fiber-rich diets.

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