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Influence of the diet structure on ruminal biohydrogenation and milk fatty acid composition of cows fed extruded linseed

Q.C. Dang Van[a,*], M. Focant[a,b], E. Mignolet[a], C. Turu[a], E. Froidmont[c], Y. Larondelle[a]

[a] Institut des Sciences de la Vie, Université catholique de Louvain, 2/8 Croix du Sud, B-1348 Louvain-la-Neuve, Belgium
[b] Institut supérieur industriel agronomique, Haute Ecole Charlemagne, 3 rue Saint Victor, B-4500 Huy, Belgium
[c] Unité Nutrition animale et Durabilité, Département Production et Filières, Centre wallon de Recherches agronomiques, 8 rue de Liroux, B-5030 Gembloux, Belgium

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ABSTRACT

This experiment studied the influence of the diet structure value (SV) on ruminal biohydrogenation and milk fatty acid (FA) responses in cows fed heterogeneous basal diets equally supplemented with FA. Eight lactating Holstein cows were used in a replicated 4 × 4 Latin square design with four dietary treatments and four 21-day periods. The iso-fat, iso-18:2 *n* – 6 and iso-18:3 *n* – 3 diets were formulated to display three different SV, using different sources and proportions of forages, energy and nitrogen concentrates. The four diets contained maize silage as the main forage (SV1.2 diet), grass hay as the main forage (SV2.0 diet), maize silage and grass hay in a 4:1 ratio (SV1.6 M diet) or maize silage and grass hay in a 1:1 ratio (SV1.6H diet). The diets also contained soya bean meal and/or urea as additional sources of nitrogen, sugar beet pulp and barley in a 1:1 ratio as additional source of energy, extruded linseed as supplemental 18:3 *n* – 3, a mineral and vitamin mix and a vitamin E preparation. Wheat straw was added to the diets as additional structure source, except for the SV2.0 diet. Soya bean oil was added to the diets as supplemental 18:2 *n* – 6 to adjust the diets for this FA, except for the SV1.2 diet. The diets were distributed as a restricted total mixed ration. The various C18 FA expressed as 100 g of total C18 FA in milk fat are relevant indicators of ruminal biohydrogenation since duodenal concentrations of C18 FA follow similar changes as those in milk fat, and since these ratios only take into account FA involved in ruminal biohydrogenation. All the various C18 FA to total C18 FA in milk fat differed among diets ($P < 0.05$). Milk 18:2 *n* – 6 + 18:3 *n* – 3/total C18 FA and total trans-C18 FA/total C18 FA decreased from SV1.2 to SV2.0 diets, whereas 18:0/total C18 FA increased from SV1.2 to SV2.0 diets. Subsequently, transfer efficiencies of 18:2 *n* – 6 and 18:3 *n* – 3 from diet to milk were higher for the SV1.2 diet than for the other diets ($P < 0.05$). These results confirm the hypothesis that ruminal biohydrogenation is more complete with higher diet SV, which is consistent with results from other published experiments where high forage diets or grass silage compared to maize silage-based diets were used. This experiment showed that the concept of diet SV is a valid tool characterizing heterogeneous basal diets differing in sources and proportions of forages and concentrates.

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; BW, body weight; CLA, conjugated linoleic acid; CP, crude protein; DM, dry matter; FA, fatty acid; FAME, fatty acid methyl ester; F:C, forage-to-concentrate; GLC, gas–liquid chromatography; OM, organic matter; SMCFA, short and medium-chain fatty acid; SV, structure value; UFA, unsaturated fatty acid; MilkFU, Milk Forage Unit.

* Corresponding author. Tel.: +32 1047 3722; fax: +32 1047 3728.
E-mail address: quynh.dangvan@uclouvain.be (Q.C. Dang Van).

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1. Introduction

There has been growing interest in modulating the bovine milk fatty acid (FA) composition because some milk FA have well-known or potential positive effects on human health (mainly 18:3 $n-3$, c9-18:1 and c9,t11-conjugated linoleic acid (CLA)), whereas others have negative effects when consumed in excess (mainly 12:0, 14:0, 16:0 and some trans FA) (Mensink et al., 2003; Parodi, 2005).

Extensive research has examined the impact of unsaturated FA (UFA) supplements on bovine milk FA composition. In addition to the amount and type of UFA supplements, it is well established that the composition of the basal diet also influences the ruminal lipid metabolism and the milk FA response to lipid supplements (Chilliard and Ferlay, 2004; Dewhurst et al., 2006). Numerous studies have indeed examined the impact of characteristics of the basal diet, such as the forage-to-concentrate (F:C) ratio, types of forages and concentrates, dietary fibre level and forage particle length, on the milk FA response to FA supplements. In these studies however, the characteristics of the basal diet have hardly been considered as a whole, which makes comparisons difficult.

The concept of diet structure value (SV) takes into account several characteristics of the basal diet and allows to characterize and compare diets in terms of rumen buffering through saliva, acidotic effect (pH decrease), particle size and physical form of the feeds (De Brabander et al., 1999). In a previous paper, we demonstrated that even a modest increase in SV in a diet supplemented with extruded linseed decreased milk concentrations of c9,t11-CLA and t11-18:1 and increased that of 18:0 (Dang Van et al., 2008). We hypothesized that ruminal biohydrogenation may have been more complete for the diet with higher SV, going all the way to 18:0, thus allowing fewer intermediates to escape (Dang Van et al., 2008). However, in the previous experiment, the increase in diet SV was made by adding only wheat straw to the same basal diet, thus essentially increasing the intake of fibre. Therefore, the objective of the present experiment was to verify the influence of the diet SV on ruminal biohydrogenation and milk FA responses in cows fed heterogeneous basal diets equally supplemented with FA. To achieve this, different sources and proportions of forages (maize silage, grass hay and wheat straw), energy (sugar beet pulp and barley) and nitrogen (soya bean meal and urea) concentrates were used.

2. Materials and methods

2.1. Experimental design, animals and management

Eight lactating Holstein cows were blocked according to their milk yield and assigned to four dietary treatments in a replicated 4×4 Latin square design. Each experimental period lasted 21 days. At the onset of the experiment, cows averaged 3 ± 0.8 lactations, 91 ± 34.5 days in milk, 651 ± 48.8 kg of body weight (BW) and yielded 29.9 ± 4.87 kg of milk/day (mean $\pm$ standard deviation). Cows were housed in individual stalls. The floor of the stalls was bedded with mats and sawdust and cleaned twice daily. Cows were fed individually twice daily at 0830 and 1730 h and had free access to water and mineral blocks. Cows were milked twice daily at 0730 and 1630 h. The experiment was carried out at the Centre wallon de Recherches agronomiques (Gembloux, Belgium) from February to April 2008 and was in accordance with the recommendations on care and use of laboratory animals of the Université catholique de Louvain.

2.2. Experimental diets

Four heterogeneous diets were formulated to display three different SV, using different sources and proportions of forages, energy and nitrogen concentrates. The diets were formulated to be iso-fat, iso-18:2 $n-6$ and iso-18:3 $n-3$, to remove the effect of the FA composition of the forages. The four diets contained maize silage as the main forage (SV1.2 diet), grass hay as the main forage (SV2.0 diet), maize silage and grass hay in a 4:1 ratio (SV1.6 M diet) or maize silage and grass hay in a 1:1 ratio (SV1.6H diet). The diets also contained soya bean meal and/or urea as additional sources of nitrogen, sugar beet pulp and barley in a 1:1 ratio as additional source of energy, extruded linseed as supplemental 18:3 $n-3$ to favour UFA production in milk, a mineral and vitamin mix and a vitamin E preparation to avoid milk oxidation (Focant et al., 1998). Wheat straw was added to the diets as additional structure source, except for the SV2.0 diet. Soya bean oil was added to the diets as supplemental 18:2 $n-6$ to adjust the diets for this FA, except for the SV1.2 diet. Extruded linseed, supplied as Nutex Compact® (Dumoulin, Seilles, Belgium), consisted of an extruded mixture of linseed, wheat, sunflower cake, field beans, peas, BHT and salt (584, 150, 140, 51, 50, 20 and 5 g/kg total raw materials, respectively). The ingredient composition of the experimental diets as fed is given in Table 1. The dry matter (DM) content and chemical composition of individual feedstuffs are given in Table 2. The diets formulated in this way displayed two different ranges of F:C ratio (63:37 and 64:36 *versus* 48:52 and 50:50). The SV1.6M and SV1.6H diets had a similar SV but different F:C ratios. 18:3 $n-3$ was the predominant FA in the extruded linseed mixture and accounted for 52 g/100 g identified FA, whereas 18:2 $n-6$ was the predominant FA in soya bean oil and accounted for 55 g/100 g identified FA. The diets were formulated to meet energy and protein requirements of cows according to the French Milk Forage Unit (MilkFU) and Digestible Proteins Entering the Small Intestine systems using the INRAtion software (version 3.21; Institut National de la Recherche Agronomique, Paris, France) for a cow of 650 kg yielding 30 kg of milk/day. The amounts of the diets were distributed as a restricted total mixed ration in order to control the intakes and were adapted to the individual milk production of cows.

Table 1
Ingredient composition of the experimental diets as fed.

	Diet[a]			
	SV1.2	SV1.6M (g/kg DM)	SV1.6H (g/kg DM)	SV2.0 (g/kg DM)
	(g/kg DM)			
Maize silage	610	480	230	0
Grass hay	0	120	240	480
Wheat straw	20	40	30	0
Soya bean meal	160	160	80	40
Urea[b]	0	0	10	14
Barley	35	29	132	158
Sugar beet pulp	35	29	132	158
Extruded linseed mixture[c]	130	130	130	130
Soya bean oil	0	3	7	11
Mineral and vitamin mix[d]	10	10	10	10
Forage:concentrate ratio	63:37	64:36	50:50	48:52

DM, dry matter; SV, structure value.

[a] The four experimental diets received a vitamin E preparation distributed in the form of a powder containing 50% of all-rac-α-tocopheryl acetate (Dumoulin, Seilles, Belgium) added to the total mixed ration; the amount was calculated to deliver 10,000 IU/d of unprotected vitamin E to the diet.

[b] 980 g/kg fresh matter and 2875 g/kg DM (INRA, 2007).

[c] Extruded commercial concentrate (Dumoulin, Seilles, Belgium) made of linseed, wheat, sunflower cake, field beans, peas, BHT and salt (584, 150, 140, 51, 50, 20 and 5 g/kg total raw materials, respectively).

[d] Declared contents: 150 g/kg Ca, 50 g/kg Mg, 50 g/kg P, 10 g/kg Na, 7750 mg/kg Zn, 3300 mg/kg Mn, 1250 mg/kg Cu, 100 mg/kg I, 50 mg/kg Co, 20 mg/kg Se, 1,200,000 IU/kg vitamin A, 200,000 IU/kg vitamin D3, 15,000 mg/kg choline, 3000 mg/kg vitamin E, 500 mg/kg vitamin B1, 255 mg/kg vitamin B3, 75 mg/kg vitamin B2, 15 mg/kg vitamin B6, 15 mg/kg vitamin K (Dumoulin, Seilles, Belgium).

2.3. Recordings, sampling and analytical procedures

Individual feedstuffs were sampled at the end of each period and analysed for DM and chemical composition. Individual cow refusals were collected during the last week of each period and analysed for DM. Individual cow intakes during the last week of each period were calculated for each ingredient as the amounts offered minus refusals, on a DM basis. Means of individual cow intakes calculated during the last week of each period were included in the statistical analysis. Individual cow milk yield was recorded during each milking. Means of individual cow milk yields recorded during the last week of each period were included in the statistical analysis. Milk samples from individual cows were collected on d19 and d21 of each period at the morning and evening milkings. The morning and evening milk samples of each of the two days from individual cows were mixed. These samples were used for the statistical analysis. Cows were weighed at the onset and at the end of the experiment.

The following analytical procedures were adapted from Dang Van et al. (2008). Fresh maize silage and grass hay samples were analysed for DM by oven-drying at 105 °C for 48 h. The chemical composition of maize silage was determined using lyophilised samples. All feed samples were ground in a mill (1-mm mesh, Retsch, Dusseldorf, Germany; adapted from method 950.02; Association of Official Analytical Chemists, 1995) and stored at 4 °C in hermetic boxes until analysis. They were then analysed for DM by oven-drying at 105 °C for 16 h (adapted from methods 967.03 and 930.15; AOAC, 1995), crude

Table 2
Dry matter content and chemical composition of the individual feedstuffs.

	Maize silage	Grass hay	Wheat straw	Soya bean meal	Barley	Sugar beet pulp	Extruded linseed mixture	Soya bean oil	Mineral and vitamin mix
DM (g/kg fresh matter)	283	811	929	903	887	906	921	1000	944
	Chemical composition (g/kg DM)								
Crude ash	49	79	55	71	21	97	48	0	585
CP	80	83	40	505	124	86	228	0	61
aNDFom	414	671	839	105	241	483	275	0	266
ADFom	226	350	526	47	55	235	166	0	40
Starch	325	0	11	34	618	0	155	0	18
Crude fat	51	35	19	42	35	31	268	1000	50
Total fatty acids	23	6	3	23	18	10	222	950	18
6:0–14:0	1	0	0	0	0	0	1	4	1
16:0	4	2	1	4	4	2	13	107	5
18:0	0	0	0	1	0	0	8	39	1
c9-18:1	5	0	1	3	2	1	44	189	6
18:2 $n-6$	10	1	1	12	10	5	38	518	3
18:3 $n-3$	2	2	0	2	1	1	115	75	0
MilkFU (per kg DM)	0.96	0.61	0.42	1.21	1.09	0.99	1.55	2.73	0

ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; MilkFU, Milk Forage Unit.

ash by ashing at 550 °C for 16 h (adapted from methods 923.03, 967.04 and 942.05; AOAC, 1995), crude protein (CP) by the Kjeldahl method (*N**6.25) (adapted from methods 981.10 and 991.20; AOAC, 1995), crude fat (adapted from Directive 98/64/EC; Commission of the European Communities, 1998), starch (Commission of the European Communities, 2009), neutral detergent fibre (aNDFom) (Van Soest et al., 1991) and acid detergent fibre (ADFom) (adapted from method 973.18; AOAC, 1995). aNDFom and ADFom were determined sequentially and were expressed without residual ash. aNDFom was analysed with the addition of α-amylase and without sodium sulfite. For the determination of FA profiles, the lipids were extracted following the method of Folch modified by Christie (1982) and 13:0 was used as an internal standard. Methylation and gas–liquid chromatography (GLC) analyses were performed as described below for milk samples.

Milk samples were refrigerated at 4 °C. A portion of each sample was used for fat and CP analyses by the mid-infrared spectroscopic method (adapted from method 972.16; AOAC, 1995) using the MilkoScan FT6000 spectrophotometer (Foss Analytical, Hilleroed, Denmark) at the Milk Control Station (Battice, Belgium), and the rest of each sample was used for fat extraction.

The lipids from milk samples were extracted following the method described by Hara and Radin (1978). Milk samples were centrifuged at 17 800 × *g* at 4 °C for 30 min, and 300–350 mg of fat cake were collected. Lipid extraction from milk fat was performed with 7.2 ml of a hexane/isopropanol (3:2, v:v) solution followed by 4.8 ml of a Na_2SO_4 solution (67 g/L). The upper phase was removed and dried with 1 g of anhydrous Na_2SO_4. It was then transferred again and brought to dryness under a continuous stream of N_2. Fatty acids were methylated in a solution of KOH in methanol (0.1 mol/L) at 70 °C for 60 min, then in a solution of HCl in methanol (1.2 mol/L) at 70 °C for 20 min, and finally extracted with hexane.

Fatty acid methyl esters (FAME) were separated and quantified with a gas–liquid chromatograph (GC Trace ThermoQuest, ThermoFinnigan, Milan, Italy) equipped with a flame ionisation detector, an automatic injector and a fused silica capillary column (100 m * 0.25 mm internal diameter) coated with a 0.2-μm film of biscyanopropyl polysiloxane (Rt-2560, Restek, Bellefonte, PA, USA). The system used H_2 as the carrier gas operated at a constant pressure of 200 kPa. Injection was on column so as to inject the entire sample into the column head. This injection mode was preferred to the split injection mode because it minimizes the risk of discrimination between milk FA with very different volatility. The initial oven temperature was 80 °C; it increased at 25 °C/min to 175 °C (held for 25 min), then increased at 10 °C/min to 205 °C (held for 4 min), then increased at 10 °C/min to 225 °C (held for 20 min) and finally decreased at 20 °C/min to 80 °C. The temperature of the flame ionisation detector was maintained at 255 °C. Hydrogen flow to the detector was 35 ml/min and air flow was 350 ml/min. Each peak, except that of t10-18:1, was identified and quantified by comparison of retention times with pure FAME standards (Alltech Associates, Deerfield, IL, USA; except CLA isomers from Nu-Chek Prep, Inc., Elysian, MN, USA). Because no commercial standard was available for t10-18:1, the concentration of the corresponding peak was calculated by comparison with the t11-18:1 peak area (by multiplying the concentration of t11-18:1 by the t10 peak area-to-t11 peak area ratio). The validity of this method was verified by applying it to the peak of t9-18:1 and comparing this calculated concentration with the t9-18:1 concentration determined through the use of the appropriate standard. Each FA was expressed in g/100 g of all identified FA. The identified FA accounted for 92.0 ± 1.10% (mean ± standard deviation) of the total measured FA, as calculated by the ratio between the total area of identified peaks and the total area of identified and unidentified peaks. The analysis method did not allow quantification of 4:0, which is present in milk fat at ±3 g/100 g of the total theoretical FA (Jensen, 2002). Therefore, the identified FA would account for ±89% of the total theoretical FA.

The analysis of the distribution of CLA isomers was performed on a Ag^+-high pressure liquid chromatography (Gilson, Villiers-le-bel, France) with three columns coupled in series (250 mm * 4.6 mm internal diameter * 5-μm particle size, Ag^+-impregnated, Chromspher Lipids, Chrompack, Middelburg, The Netherlands) and ultraviolet detection at 233 nm. The carrier liquid was acetonitrile and hexane (0.1:99.9, v:v); the flow was 1.06 ml/min and the oven temperature was 25 °C. Isomers were identified by comparison of the elution order and retention times with data from the literature (Sehat et al., 1998; Kramer et al., 1999). Each CLA isomer was expressed in g/100 g of total CLA isomers. Concentrations of c9,t11-CLA were based on GLC analysis. However, according to the literature, the c9,t11-CLA GLC peak contained c9,t11-CLA and minor proportions of t7,c9 and t8,c10-CLA (Cruz-Hernandez et al., 2006).

2.4. Calculations

The SV (per kg of DM) of the maize silage and grass hay were estimated from their aNDFom content using the following regression equations (De Brabander et al., 1999):

$$\text{SV of maize silage} = -0.57 + 0.006 \times \text{aNDFom}$$

$$\text{SV of grass hay} = 0.15 + 0.006 \times \text{aNDFom}$$

where aNDFom is expressed in g/kg of DM.

The SV of concentrates were estimated from their NDF, starch and sugar contents, using a regression equation (De Brabander et al., 1999). In this way, the SV of unmolassed sugar beet pulp, barley and soya bean meal were estimated at 0.42, 0.02 and 0.18/kg of DM, respectively. The SV of straw is 4.30/kg of DM (De Brabander et al., 1999). From De Brabander et al. (1999) and from the composition of the extruded linseed mixture, we set the SV of the extruded linseed mixture, urea, mineral and vitamin mix, soya bean oil and vitamin E preparation at 0.

The SV of the diet is the sum of the SV of the balanced ingredients.

Individual milk FA yield (g/d) was calculated as:

$$\text{Individual milk FA yield} = \frac{\text{milk fat yield} \times \text{individual milk FA\%}}{100 \times 0.933 \times 0.89}$$

where 0.933 is the mean proportion of FA in milk fat estimated by Glasser et al. (2007a), 0.89 is the proportion of the FA reported in the total theoretical FA, and milk fat yield is expressed in g/d.

2.5. Statistical analyses

Data on milk composition, milk FA composition and milk CLA isomer composition were averaged over d19 and d21 of each period before statistical analyses. All data from the experiment are reported as least-squares means ± standard error and were analysed as a replicated Latin square using the MIXED procedure of SAS (version 9.1.3, SAS Institute Inc., Cary, NC, USA). The statistical model included period, cow, diet and random error. Fixed effects included period and diet. Cow was the random effect. Overall differences between treatment means were considered to be significant when $P < 0.05$. When a significant diet effect was observed, the TUKEY option was used to compare least-squares means.

3. Results

3.1.1. Feed intake and milk production

Individual cow refusals throughout the experiment varied from 2 to 6% of offered DM. Since all individual cow refusals collected for each period were grass hay, refusals were only analysed for DM, as described in the Materials and methods section. The nutrient intake and chemical composition of the four experimental diets as consumed is summarized in Table 3. The MilkFU contents were calculated using the INRAtion software (version 3.21; INRA, Paris, France), according to the chemical composition of the ingredients and amounts of individual ingredients consumed by the cows. The MilkFU contents decreased from SV1.2 to SV2.0 diets. This observation might be related to the increasing inclusions of grass hay in the diets (0, 120, 240 and 480 g/kg DM, respectively). In order to cover energy needs of the cows, DM intakes increased with decreasing MilkFU contents, except for the SV2.0 diet where grass hay refusals were more important. Crude fat, total FA, total C18 FA and 18:3 $n-3$ contents are similar between diets ($P > 0.05$), their intakes thus vary proportionally to the variations of DM intakes. From SV1.2 to SV2.0 diets, the diets as consumed displayed three increasing SV, four increasing aNDFom and ADFom contents and four decreasing starch contents. Therefore, the starch:aNDFom ratio decreased from SV1.2 to SV2.0 diets. The SV1.6M and SV1.6H diets had a similar SV but different aNDFom, ADFom and starch contents. The organic matter (OM) and CP contents were rather similar among diets, despite statistical significances. For all diets, the predominant FA were 18:3 $n-3$, 18:2 $n-6$ and c9-18:1, which accounted for 36, 29 and 19 g/100 g of total FA intake, respectively (means of the four diets). The cows did not lose weight during the experiment (651 ± 48.8 *versus* 658 ± 46.6 kg of BW, respectively at the onset and at the end of the experiment).

Milk yield and composition of cows fed the four experimental diets are shown in Table 4. Milk yield decreased from SV1.2 to SV2.0 diets. Protein yield and percentage differed among diets ($P < 0.05$), whereas fat yield and percentage did not ($P > 0.05$).

3.2. Milk fatty acid composition

Milk FA composition of cows fed the four experimental diets are shown in Table 5. Milk concentrations of 6:0, 8:0, 10:0, 12:0, 14:0, 16:0 and 6:0–14:0 decreased from SV1.2 to SV2.0 diets ($P < 0.05$), whereas those of iso-14:0, 15:0, anteiso-15:0, iso-16:0, 18:0, c9-18:1, total C18 FA and total UFA increased from SV1.2 to SV2.0 diets ($P < 0.05$). Milk concentrations of 18:2 $n-6$ and 18:3 $n-3$ were affected by the diets ($P < 0.05$). However, no linear relation could be observed among diets for 18:2 $n-6$ and 18:3 $n-3$. Milk concentrations of t11-18:1 and c9,t11-CLA were not affected by the diets ($P > 0.05$). All the various C18 FA to total C18 FA in milk fat differed among diets ($P < 0.05$). Milk 18:2 $n-6$ + 18:3 $n-3$/total C18 FA and total trans-C18 FA/total C18 FA decreased from SV1.2 to SV2.0 diets, whereas 18:0/total C18 FA increased from SV1.2 to SV2.0 diets.

Milk FA yields of cows fed the four experimental diets are shown in Table 6. Milk yields of 6:0–14:0 and 16:0 decreased from SV1.2 to SV2.0 diets ($P < 0.05$), whereas those of 18:0, c9-18:1 and total C18 FA increased from SV1.2 to SV2.0 diets ($P < 0.05$). The milk yield of total FA did not differ among diets ($P > 0.05$) and amounted to 926 g/d (means of the four diets). Transfer efficiencies of 18:2 $n-6$ and 18:3 $n-3$ from diet to milk could be estimated by dividing the milk 18:2 $n-6$ or 18:3 $n-3$ yield (g/d) by its dietary intake (g/d). In this way, transfer efficiencies of 18:2 $n-6$ and 18:3 $n-3$ decreased from SV1.2 to SV2.0 diets ($P < 0.05$).

Milk CLA isomer composition of cows fed the four experimental diets are shown in Table 7. Milk concentrations of t11,c13 and t12,t14-CLA increased from SV1.2 to SV2.0 diets, whereas those of the other CLA isomers were not affected by the diets ($P > 0.05$).

Table 3
Nutrient intake and chemical composition of the four experimental diets as consumed.

	Diet				SED	P
	SV1.2	SV1.6M	SV1.6H	SV2.0		
	Nutrient intake (kg/d)					
DM	20.1^{b}	21.1^{b}	23.2^{a}	22.6^{a}	0.42	<0.0001
OM	18.9^{b}	19.7^{b}	21.6^{a}	21.0^{a}	0.38	<0.0001
CP	3.5^{c}	3.7^{bc}	3.9^{a}	3.8^{ab}	0.07	0.0002
aNDFom	6.9^{d}	8.1^{c}	9.7^{b}	10.6^{a}	0.23	<0.0001
ADFom	3.7^{d}	4.3^{c}	4.9^{b}	5.3^{a}	0.12	<0.0001
Starch	4.8^{a}	4.0^{b}	4.1^{b}	2.8^{c}	0.06	<0.0001
Crude fat	1.6^{b}	1.6^{b}	1.7^{a}	1.7^{a}	0.03	<0.0001
	Fatty acid intake (g/d)					
Total fatty acids	1000^{b}	1050^{b}	1128^{a}	1116^{a}	20.4	<0.0001
Total C18 fatty acids	867^{b}	912^{b}	982^{a}	974^{a}	18.0	<0.0001
6:0-14:0	11^{a}	11^{a}	10^{b}	9^{c}	0.3	<0.0001
16:0	105^{c}	108^{bc}	115^{a}	111^{ab}	2.0	0.0003
18:0	32^{c}	35^{b}	38^{a}	39^{a}	0.7	<0.0001
c9-18:1	198^{b}	205^{ab}	213^{a}	205^{ab}	3.8	0.0116
18:2 $n-6$	282^{b}	298^{b}	330^{a}	330^{a}	6.2	<0.0001
18:3 $n-3$	355^{b}	374^{b}	401^{a}	400^{a}	7.8	<0.0001
	Chemical composition (g/kg DM intake)					
OM	939^{a}	935^{b}	933^{b}	928^{c}	1.0	<0.0001
CP	176^{a}	175^{a}	170^{b}	168^{c}	0.8	<0.0001
aNDFom	343^{d}	384^{c}	420^{b}	470^{a}	3.4	<0.0001
ADFom	184^{d}	206^{c}	213^{b}	232^{a}	1.9	<0.0001
Starch	237^{a}	190^{b}	176^{c}	122^{d}	1.3	<0.0001
Crude fat	78	78	76	77	0.8	ns
Total fatty acids	50	50	49	49	0.4	ns
Total C18 fatty acids	43	43	42	43	0.4	ns
6:0–14:0	1^{a}	1^{a}	0^{b}	0^{c}	0.0	<0.0001
16:0	5^{a}	5^{b}	5^{c}	5^{c}	0.0	<0.0001
18:0	2^{c}	2^{b}	2^{b}	2^{a}	0.0	<0.0001
c9-18:1	10^{a}	10^{a}	9^{b}	9^{b}	0.1	<0.0001
18:2 $n-6$	14^{b}	14^{b}	14^{ab}	15^{a}	0.1	0.0027
18:3 $n-3$	18	18	17	18	0.2	ns
Starch:aNDFom ratio	0.7^{a}	0.5^{b}	0.4^{c}	0.3^{d}	0.00	<0.0001
MilkFU (per kg DM intake)	1.1^{a}	1.0^{b}	1.0^{c}	0.9^{d}	0.00	<0.0001
SV (per kg DM intake)	1.2^{c}	1.6^{b}	1.6^{b}	2.0^{a}	0.03	<0.0001

ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; OM, organic matter; SED, standard error of the difference; SV, structure value; MilkFU, Milk Forage Unit. ns = not significant ($P > 0.05$). ^{a,b,c,d}Values within a row with different superscripts differ (diet effect). Values with no superscript do not differ significantly.

4. Discussion

Consistent with the hypothesis that ruminal biohydrogenation is more complete with higher diet SV (Dang Van et al., 2008), the milk concentration of 18:0 increased with increasing diet SV. Interestingly enough, Vlaeminck et al. (2006) reported that milk iso-14:0 was positively related to 18:0. Besides, Borreani et al. (2003) found that milk iso-14:0 concentration was highly correlated with diet and was related to the level of concentrate, it was particularly high in alpine milk and cheese from grass hay or silage based-diets, with concentrations two times higher than those commonly found in lowland cheeses from maize silage-based diets. In the present experiment, increasing proportions of grass hay and decreasing

Table 4
Milk yield and milk composition of cows fed the four experimental diets.

	Diet				SED	P
	SV1.2	SV1.6M	SV1.6H	SV2.0		
	(kg/d)					
Milk	32.3^{a}	30.0^{b}	30.9^{b}	27.8^{c}	0.46	<0.0001
Fat	1.14	1.10	1.10	1.06	0.049	ns
Protein	0.99^{a}	0.92^{b}	0.96^{a}	0.83^{c}	0.015	<0.0001
	(g/100 g)					
Fat	3.59	3.68	3.60	3.86	0.142	ns
Protein	3.11^{ab}	3.12^{ab}	3.13^{a}	3.03^{b}	0.033	0.0377

DM, dry matter; SED, standard error of the difference; SV, structure value. ns = not significant ($P > 0.05$). ^{a,b,c,d}Values within a row with different superscripts differ (diet effect). Values with no superscript do not differ significantly.

Table 5
Milk fatty acid composition of cows fed the four experimental diets.

	Diet				SED	P
	SV1.2	SV1.6M	SV1.6H	SV2.0		
	(g/100 g identified fatty acids)					
6:0	3.00[a]	2.94[ab]	2.83[b]	2.52[c]	0.048	0.0001
8:0	1.64[a]	1.58[a]	1.49[b]	1.19[c]	0.032	<0.0001
10:0	3.33[a]	3.14[ab]	2.92[b]	2.21[c]	0.085	<0.0001
12:0	3.49[a]	3.30[ab]	3.03[b]	2.27[c]	0.102	<0.0001
14:0	12.06[a]	11.70[a]	10.96[b]	9.24[c]	0.171	<0.0001
iso-14:0	0.08[b]	0.09[b]	0.09[b]	0.12[a]	0.005	<0.0001
c9-14:1	0.96[a]	0.96[a]	0.90[a]	0.73[b]	0.054	0.0010
15:0	0.84[b]	0.85[b]	0.88[ab]	0.92[a]	0.018	0.0014
iso-15:0	0.24[bc]	0.27[a]	0.24[b]	0.26[ac]	0.006	0.0001
anteiso-15:0	0.47[c]	0.49[bc]	0.55[ab]	0.57[a]	0.022	0.0004
16:0	28.35[a]	27.22[a]	25.03[b]	22.27[c]	0.693	<0.0001
iso-16:0	0.26[b]	0.26[ab]	0.27[ab]	0.33[a]	0.014	0.0003
c9-16:1	1.15[ab]	1.19[a]	1.07[ab]	0.95[b]	0.079	0.0384
t9-16:1 + iso-17:0	0.58	0.55	0.58	0.60	0.017	ns
17:0	0.54	0.54	0.56	0.57	0.015	ns
anteiso-17:0	0.53	0.44	0.58	0.60	0.076	ns
18:0	12.65[c]	13.99[bc]	15.71[b]	18.88[a]	0.683	<0.0001
c9-18:1	18.38[d]	20.42[c]	21.70[b]	25.20[a]	0.294	<0.0001
c11-18:1	0.42	0.42	0.42	0.44	0.011	ns
t9-18:1	0.46[ab]	0.44[b]	0.46[ab]	0.49[a]	0.014	0.0440
t10-18:1	0.78	0.56	0.61	0.57	0.114	ns
t11-18:1	4.37	3.80	4.00	4.06	0.387	ns
18:2 $n-6$	1.93[a]	1.71[b]	1.88[ab]	1.83[ab]	0.070	0.0326
c9,t11-CLA[e]	1.63	1.47	1.52	1.56	0.130	ns
18:3 $n-3$	1.45[a]	1.23[b]	1.32[ab]	1.24[b]	0.059	0.0047
20:0	0.12[c]	0.14[bc]	0.15[b]	0.19[a]	0.006	<0.0001
c11,c14,c17-20:3	0.00[b]	0.01[ab]	0.00[b]	0.02[a]	0.006	0.0291
c5,c8,c11,c14-20:4	0.09[ab]	0.10[a]	0.09[ab]	0.09[b]	0.003	0.0201
c5,c8,c11,c14,c17-20:5	0.07	0.08	0.07	0.07	0.004	ns
c7,c10,c13,c16,c19-22:5	0.10[a]	0.10[a]	0.09[ab]	0.08[b]	0.006	0.0010
6:0–14:0	23.52[a]	22.67[a]	21.23[b]	17.42[c]	0.354	<0.0001
Total trans-C18 fatty acids	7.24	6.27	6.59	6.65	0.446	ns
Total C18 fatty acids	42.08[c]	44.04[c]	47.61[b]	54.24[a]	0.911	<0.0001
Total saturated fatty acids	67.61[a]	66.96[a]	65.29[b]	62.10[c]	0.487	<0.0001
Total unsaturated fatty acids	32.39[c]	33.04[c]	34.71[b]	37.90[a]	0.487	<0.0001
	(g/100 g total C18 fatty acids)					
18:2 $n-6$ + 18:3 $n-3$	8.04[a]	6.69[b]	6.71[b]	5.65[c]	0.250	<0.0001
Total trans-C18 fatty acids	17.09[a]	14.25[b]	13.79[b]	12.22[b]	0.934	0.0006
18:0	30.01[c]	31.63[bc]	32.91[ab]	34.65[a]	0.953	0.0010

CLA, conjugated linoleic acid; SED, standard error of the difference; SV, structure value. ns = not significant ($P > 0.05$). [a,b,c,d]Values within a row with different superscripts differ (diet effect). Values with no superscript do not differ significantly. [e]The gas–liquid chromatography analysis was unable to separate the minor quantities of t7,c9 and t8,c10-CLA isomers from the main c9,t11-CLA isomer.

proportions of maize silage were used to formulate diets with increasing diet SV. Consistent with observations of Borreani et al. (2003) and Vlaeminck et al. (2006), milk iso-14:0 increased with increasing diet SV and was positively related to that of 18:0 and therefore to ruminal biohydrogenation.

The various C18 FA expressed as g/100 g of total C18 FA in milk fat are more valid indicators of ruminal biohydrogenation than C18 FA expressed as g/100 g of identified FA, since duodenal concentrations of C18 FA follow similar changes as those in milk fat (Loor et al., 2005; Glasser et al., 2007b), and since these values only take into account FA involved in ruminal biohydrogenation and not FA synthesized *de novo* in the mammary gland. Milk 18:0/total C18 FA increased with increasing diet SV, whereas milk total trans-C18 FA/total C18 FA and 18:2 $n-6$ + 18:3 $n-3$/total C18 FA decreased with increasing diet SV. These results cannot be attributed to the increased intake of C18 FA since transfer efficiencies of 18:2 $n-6$ and 18:3 $n-3$ from diet to milk decreased from SV1.2 to SV2.0 diets. Therefore, these results confirm the hypothesis that ruminal biohydrogenation is more complete with higher diet SV, going all the way to 18:0, thus allowing fewer intermediates to escape (Dang Van et al., 2008). In this experiment, the influence of the diet SV on ruminal biohydrogenation and on milk FA composition has been extended to heterogeneous basal diets differing in sources and proportions of forages (maize silage, grass hay and wheat straw), energy (sugar beet pulp and barley) and nitrogen (soya bean meal and urea) concentrates, resulting in variations of dietary intakes and contents of most nutrients.

Kalscheur et al. (1997) suggested that altered ruminal biohydrogenation resulting from low ruminal pH for cows fed a low forage diet (250 *versus* 600 g/kg of DM) and no buffer was responsible for the accumulation of trans-18:1 FA at the expense of 18:0 in the rumen. Indeed, high concentrate diets containing high amounts of non structural carbohydrates are quickly fermented by ruminal microbes, resulting in a decline in ruminal pH (Kalscheur et al., 1997). These results were

Table 6
Milk fatty acid yields of cows fed the four experimental diets.

	Diet				SED	P
	SV1.2	SV1.6M	SV1.6H	SV2.0		
	(g/d)					
6:0–14:0	224[a]	207[ab]	194[b]	153[c]	8.5	<0.0001
16:0	268[a]	246[ab]	228[b]	194[c]	11.3	<0.0001
18:0	121[b]	127[b]	144[ab]	167[a]	10.4	0.0015
c9-18:1	174[b]	185[b]	198[ab]	222[a]	8.8	0.0003
t11-18:1	43	36	37	37	4.9	ns
18:2 $n-6$	18	16	17	16	0.9	ns
c9,t11-CLA[e]	16	14	14	14	1.6	ns
18:3 $n-3$	14[a]	11[b]	12[ab]	11[b]	0.8	0.0121
Total trans-C18 fatty acids	71	58	61	60	6.1	ns
Total C18 fatty acids	402[b]	401[b]	436[ab]	479[a]	24.1	0.0138
Total saturated fatty acids	641[a]	609[ab]	597[ab]	545[b]	26.4	0.0143
Total unsaturated fatty acids	309	301	318	335	15.7	ns
Total fatty acids	951	910	915	879	40.7	ns
Transfer efficiency from diet to milk	(as fraction of intake)					
18:2 $n-6$	0.064[a]	0.053[b]	0.052[b]	0.048[b]	0.0028	0.0001
18:3 $n-3$	0.039[a]	0.030[b]	0.030[b]	0.027[b]	0.0019	<0.0001

CLA, conjugated linoleic acid; SED, standard error of the difference; SV, structure value. ns = not significant ($P > 0.05$). [a,b,c,d]Values within a row with different superscripts differ (diet effect). Values with no superscript do not differ significantly. [e]The gas–liquid chromatography analysis was unable to separate the minor quantities of t7,c9 and t8,c10-CLA isomers from the main c9,t11-CLA isomer.

confirmed by those of Flachowsky et al. (2006) for a low forage diet (300 *versus* 700 g/kg DM) supplemented with linseed oil. Besides, Kucuk et al. (2001) studied the effect of five dietary forage concentrations (184, 322, 458, 594 and 729 g/kg of DM) on ruminal digestion and duodenal flow of FA in ewes. They showed that duodenal flow of 18:0 increased linearly with dietary forage, suggesting that biohydrogenation increased as dietary forage increased, which was concomitant with increased ruminal pH, due to an expected lower rate of fermentation of fibre compared with starch (Kucuk et al., 2001). Moreover, biohydrogenation changes were gradual with diet, suggesting a gradual shift in ruminal microbial populations with increasing forage (Kucuk et al., 2001). By contrast, duodenal flow of 18:2 $n-6$ decreased with dietary forage, despite an increased dietary intake, indicating once again that biohydrogenation was greater as dietary forage increased (Kucuk et al., 2001). In the present experiment, the starch:aNDFom ratio decreased with higher diet SV, suggesting an increase in ruminal pH, which was in agreement with a gradual increase of milk 18:0. However, the dietary forage concentration decreased from 630 to 480 g/kg of DM with increasing diet SV, whereas biohydrogenation was more complete, indicating that the F:C ratio did not account for changes in ruminal pH with increasing diet SV. Indeed, the F:C ratio was accurate to predict ruminal pH changes in the studies of Kalscheur et al. (1997), Kucuk et al. (2001) and Flachowsky et al. (2006) because these authors used only one or two forage sources with similar ratios. Therefore the forage fibre contents were correlated with the F:C ratios. By contrast, in this experiment, three forage sources with different ratios were used, invalidating the use of the F:C ratio as a predictor for ruminal pH changes.

Rather than increasing the F:C ratio, increasing proportions of grass hay and decreasing proportions of maize silage were used in this experiment to formulate diets with increasing diet SV, resulting in biohydrogenation being more complete. Our

Table 7
Milk conjugated linoleic acid (CLA) isomer composition of cows fed the four experimental diets.

	Diet				SED	P
	SV1.2	SV1.6M (g/100 g total CLA isomers)	SV1.6H (g/100 g total CLA isomers)	SV2.0 (g/100 g total CLA isomers)		
	(g/100 g total CLA isomers)					
t7,c9	5.22	5.01	4.92	4.43	0.382	ns
t8,c10	0.31	0.29	0.25	0.26	0.042	ns
c9,t11	81.66	81.07	80.28	77.98	1.324	ns
t10,c12	0.25	0.13	0.17	0.11	0.103	ns
t11,c13	4.64[b]	4.86[b]	5.31[b]	7.08[a]	0.386	<0.0001
c12,t14	1.22	1.28	1.41	1.61	0.245	ns
t7,t9	0.36	0.32	0.41	0.34	0.038	ns
t8,t10	0.09	0.08	0.06	0.07	0.015	ns
t9,t11	1.15	1.22	1.29	1.24	0.076	ns
t10,t12	0.24	0.26	0.26	0.27	0.034	ns
t11,t13	2.73	3.04	3.03	3.53	0.411	ns
t12,t14	2.13[b]	2.43[ab]	2.62[ab]	3.08[a]	0.277	0.0224

CLA, conjugated linoleic acid; SED, standard error of the difference; SV, structure value. ns = not significant ($P > 0.05$). [a,b,c,d]Values within a row with different superscripts differ (diet effect). Values with no superscript do not differ significantly.

results are consistent with those of other groups who showed that grass silage compared to maize silage-based diets with or without supplemental UFA stimulated complete biohydrogenation of C18 UFA to 18:0 (Shingfield et al., 2005; Nielsen et al., 2006). This can also be reflected by lower transfer efficiencies of 18:3 $n-3$ (and 18:2 $n-6$) from diet to milk in this experiment for higher diet SV and in that of Shingfield et al. (2005) for grass silage compared to maize silage-based diets. Moreover, when starch was replaced by fibre, or when the starch:aNDFom ratio decreased, lipolysis and biohydrogenation rates increased, leading to higher production of rumen 18:0 at the expense of biohydrogenation intermediates (Gerson et al., 1985), which was observed in this experiment for higher diet SV and in that of Shingfield et al. (2005) for grass silage compared to maize silage-based diets. Changes attributed to the forage source may be even more pronounced when FA supplements are added to the diets (Kliem et al., 2008). Chilliard et al. (2007) reported a study where milk 18:0 and c9-18:1 concentrations increased and those of c9,t11-CLA, t10+t11-18:1 and PUFA decreased when adding linseed oil to a grass silage diet compared to a maize silage diet. These authors suggested that ruminal biohydrogenation was less complete with maize silage, likely due to either a lower ruminal pH or changes in microbial population with the maize silage diet.

The ruminal pH has often been suggested as a critical factor altering biohydrogenation. More specifically, two hypotheses have been developed in Dang Van et al. (2008) for the more complete biohydrogenation with higher diet SV. On the one hand, likely higher pH with higher diet SV may have enhanced the adhesion of cellulolytic bacteria to cellulose, boosting cellulose digestion and, hence, the growth of cellulolytic bacteria. Therefore, ruminal biohydrogenation, mainly carried out by cellulolytic bacteria (Harfoot and Hazlewood, 1997), may have been more complete with higher diet SV. On the other hand, higher diet SV probably provided more particles as biohydrogenation sites thus putatively increasing the number of cellulolytic bacteria adhering to them. According to the hypothesis of an amplified growth of cellulolytic bacteria, milk concentrations of iso-14:0 and anteiso-15:0, odd- and branched-chain FA that are largely derived from bacteria leaving the rumen (Vlaeminck et al., 2006), were higher in this experiment.

The increase in DM intakes from SV1.2 to SV2.0 diets resulted in an increase in intakes of most nutrients, especially total C18 FA, which in turn, may have increased milk concentrations and yields of total C18 FA, 18:0 and c9-18:1. This explanation is in agreement with that of Glasser et al. (2008), who showed a positive relationship between duodenal C18 FA flow after dietary lipid supplementation and their yield in milk. Besides, the increase in milk C18 FA yield after dietary lipid supplementation was concomitant with a decrease in milk short and medium-chain FA ($\leq$C16, SMCFA) yield (Glasser et al., 2008). Although part of 16:0 is derived from mammary uptake, Glasser et al. (2008) showed that the yield of 16:0 was highly correlated with that of 6:0–14:0, indicating that the yield of 16:0 was probably mainly under the same regulation as that of 6:0–14:0. Accordingly, in the present experiment, milk concentrations and yields of 6:0–14:0 and 16:0 varied similarly from SV1.2 to SV2.0 diets. Moreover, in this experiment, milk 6:0–16:0 varied inversely to C18 FA, underlining the interdependence between these two FA groups, as already reported by Glasser et al. (2008). The SMCFA are synthesized *de novo* in the mammary gland using acetate and, to a lesser extent, β-hydroxybutyrate taken up from the circulatory system (Bauman and Griinari, 2003). An increase in dietary fibre content at the expense of dietary starch content generally alters the proportions of volatile FA towards more acetate (and less propionate) supply to the mammary gland, leading to an increase in milk 6:0–16:0 (Bauman and Griinari, 2003). As an example, in the experiment of Dang Van et al. (2008), an inclusion of straw in the diet, essentially increasing the intake of fibre, was associated with an increase in milk concentration of 6:0–16:0. In this experiment, the increase in DM intakes from SV1.2 to SV2.0 diets resulted in an increase in intakes of most nutrients, especially total C18 FA, but also aNDFom. However, the milk 6:0–16:0 decreased with increasing dietary aNDFom content. This suggests that the effects on milk 6:0–16:0 of an increase in dietary aNDFom content were counteracted by those of an increase in C18 FA intake.

In the present experiment and consistent with the literature (for a review see Bauman et al., 2003), c9,t11-CLA was the most abundant CLA isomer in milk fat, followed by t7,c9 and t11,c13-CLA. The increase in milk concentrations of t11,c13 and t12,t14-CLA from SV1.2 to SV2.0 diets is more likely due to a higher dietary intake of 18:3 $n-3$ than to variations of diet SV. Indeed, t11,c13 and t12,t14-CLA are intermediates of ruminal 18:3 $n-3$ metabolism (Collomb et al., 2004; Shingfield et al., 2005; Chilliard et al., 2007).

5. Conclusion

This experiment confirms the hypothesis that ruminal biohydrogenation was more complete with higher diet SV, leading to higher concentration of 18:0 and subsequently lower transfer efficiencies of 18:2 $n-6$ and 18:3 $n-3$ from diet to milk, which is consistent with results from other published experiments where high forage diets or grass silage compared to maize silage-based diets were used. This experiment showed that the concept of diet SV is a valid tool characterizing heterogeneous basal diets differing in sources and proportions of forages and concentrates. Besides, further investigations on rumen samples from ruminally cannulated cows to determine FA composition and ruminal pH would be a good addition to support the present findings.

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