Animal (2008), **2:10**, pp 1538–1547 © The Animal Consortium 2008
doi:10.1017/S1751731108002620

animal

Influence of an increase in diet structure on milk conjugated linoleic acid content of cows fed extruded linseed

Q. C. Dang Van[1], M. Focant[1], D. Deswysen[1], E. Mignolet[1], C. Turu[1], J. Pottier[1], E. Froidmont[2] and Y. Larondelle[1†]

[1]*Unité de Biochimie de la Nutrition, Faculté d'Ingénierie Biologique, Agronomique et Environnementale, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium;* [2]*Département de Productions et Nutrition Animales, Centre Wallon de Recherches Agronomiques, B-5030 Gembloux, Belgium*

(Received 29 November 2007; Accepted 15 May 2008; First published online 15 July 2008)

This experiment studied the effect of a modest difference in diet structure value (SV) on milk conjugated linoleic acid (CLA) contents of cows fed diets supplemented with extruded linseed, in situations where the diets provided enough SV and therefore did not induce milk fat depression. Six lactating Holstein cows were used in a crossover design with two treatments ('SV 1.50' and 'SV 1.73') and two periods of 21 days. The 'SV 1.50' diet contained 59% maize silage, 13% soya bean meal, 13% sugar beet pulp and 14% Nutex Compact (containing 56% extruded linseed) (dry matter (DM) basis) and was offered as a restricted total mixed ration. For the 'SV 1.73' diet, 8% wheat straw (DM basis) was added to the 'SV 1.50' diet as an additional structure source. The two diets had a forage-to-concentrate ratio of 59 : 41 and 62 : 38. The inclusion of straw in the diet resulted in an additional intake of NDF (+1110 g/day), which accounted for 90% of the additional intake of OM, whereas additional intakes of the other nutrients were minor. Milk yield and composition did not differ among treatments. The inclusion of straw in the diet did not affect the milk levels of t*10–18:1, 18:2n-6,* c*9-16:1,* c*9-18:1,* c*11-18:1, 6:0, 8:0, 20:4 and 20:5. It decreased the milk levels of* c*9,*t*11-CLA (2.13% v. 3.03% of fatty acids (FA) reported,* $P < 0.001$*),* t*11-18:1 (4.99% v. 7.10% of FA reported,* $P < 0.001$*), 18:3n-3,* t*9-16:1 and* t*9-18:1, while it increased the milk levels of 6:0–14:0 (20.90% v. 19.69% of FA reported,* $P < 0.01$*), 16:0 (26.55% v. 25.25% of FA reported,* $P < 0.01$*), 18:0 (13.54% v. 12.59% of FA reported,* $P < 0.001$*), 17:0, 20:0 and 22:5. Regarding the ratio between FA, the inclusion of straw increased the 18:0/total C18 FA ratio (37.74% v. 32.07%,* $P < 0.001$*), whereas it decreased the total* trans*-C18 FA/total C18 FA ratio (15.46% v. 20.34%,* $P < 0.001$*), the* t*11-18:1/total C18 FA ratio (13.70% v. 17.95%,* $P < 0.01$*) and the* c*9,*t*11-CLA/total C18 FA ratio (5.82% v. 7.64%,* $P < 0.001$*). We conclude from this experiment that even a modest increase in SV to a diet supplemented with extruded linseed, yet already providing enough SV, alters the rumen lipid metabolism and, hence, CLA levels in milk fat.*

Keywords: conjugated linoleic acid, extruded linseed, milk fat, diet structure value

Introduction

The most common nutritional means to enhance the milk conjugated linoleic acid (CLA) content is to use oils or oilseeds rich in polyunsaturated fatty acids (PUFA) in the diet of dairy cows and it is well known that both the amount and type of PUFA supplements influence the ruminal lipid metabolism and the milk CLA production (Dhiman *et al.*, 2000; Bu *et al.*, 2007). However, there are still important variations in milk CLA responses to lipid supplements, suggesting that, in addition to the amount and type of PUFA supplements, the composition of the basal diet also influences the ruminal lipid metabolism and milk CLA responses to lipid supplements (Chilliard and Ferlay, 2004; Dewhurst *et al.*, 2006).

Numerous studies have compared milk fatty acid (FA) composition in response to variations in forage-to-concentrate (F : C) ratio with various lipid supplements but fewer with supplemental 18:3n-3 (Loor *et al.*, 2005; Flachowsky *et al.*, 2006). In these studies, combinations of the F : C ratio were marked, 65 : 35 *v.* 35 : 65 and 70 : 30 *v.* 30 : 70, and a decrease in milk fat yield was observed for high-concentrate diets supplemented with linseed oil, indicating that milk fat depression (MFD) occurred. Increases in milk *c*9,*t*11-CLA content were observed after PUFA supplementation and were higher for high-concentrate compared to low-concentrate diets (Loor *et al.*, 2005). Besides the F : C ratio parameter, different forage particle lengths (Soita *et al.*, 2005) as well as

† E-mail: yvan.larondelle@uclouvain.be

different energy sources (maize, wheat, barley, rye or sugar beet pulp) in the concentrate mixture (Loor *et al*., 2005; Flachowsky *et al*., 2006; Roy *et al*., 2006) have been used, making comparisons difficult between various milk CLA responses to lipid supplements. Furthermore, the relative amounts of fermentable carbohydrate and fibre in the diet have been demonstrated to alter biohydrogenation pathways and the formation of specific *trans*-18:1 isomers, therefore altering the milk CLA content (Griinari and Bauman, 1999).

In a preliminary experiment, we obtained higher CLA contents with a maize silage-based diet than with a grass silage-based diet (M Focant, unpublished results). Both diets had the same F : C ratio and were supplemented with PUFA and vitamin E. They were formulated to be iso-energy, iso-starch, iso-fat and iso-FA. However, one of the few differences was the fibre content of the forage.

In order to characterise and compare our experimental diets in terms of rumen buffering through saliva, acidotic effect (pH decrease), particle size and physical form of the feeds, we adopted the structure evaluation system developed by De Brabander *et al*. (1999). These authors considered the structure value (SV) as 'an expression of the extent to which a feedstuff, through its content and properties of the carbohydrates, contributes to an optimum and stable rumen function' (De Brabander *et al*., 1999). Therefore, the SV may significantly influence the rumen biohydrogenation and, hence, the CLA content in milk.

Numerous studies have examined the impact of various marked differences in the F : C ratio on FA biohydrogenation and milk fat composition (Loor *et al*., 2005; Shingfield *et al*., 2005; Flachowsky *et al*., 2006). To our knowledge, the present study is however the first one aiming at studying the milk CLA responses to extruded linseed in cows fed two diets with a modest variation in SV, in situations where both diets provided enough SV and therefore did not induce MFD.

Material and methods

Experimental design, animals and management

Six lactating Holstein cows were blocked for milk yield in two groups of three cows and randomly assigned to a crossover design with two treatments ('SV 1.50' and 'SV 1.73') and two periods of 21 days. At the onset of the experiment, cows were all in their second lactation, averaged 607 $\pm$ 62.4 kg of BW, 186 $\pm$ 25.8 days in milk and yielded 21.7 $\pm$ 8.71 kg/day of milk (mean $\pm$ s.d.). None of the cows was pregnant during the experiment. Cows were housed in individual tie stalls in order to avoid mixing of diets. They were fed individually twice daily at 0830 and 1730 h and had free access to water. Milking took place twice daily at 0730 and 1630 h. The experiment was carried out at the Alphonse de Marbaix research centre (Corroy-le-Grand, Belgium) from February to March 2005 and was approved by the Commission d'Ethique de l'Expérimentation animale of the Université catholique de Louvain.

Experimental diets

Before the experiment, cows were fed a balanced herd diet according to their milk production and specific maintenance needs, according to INRA standards (Institut National de la Recherche Agronomique, 1988).

The 'SV 1.50' diet contained maize silage, soya bean meal and urea as additional sources of protein, sugar beet pulp as additional source of energy, extruded linseed as supplemental 18:3n-3 to favour CLA production, a mineral and vitamin mix and a vitamin E preparation to avoid the milk oxidation (Focant *et al*., 1998). For the 'SV 1.73' diet, wheat straw was added to the 'SV 1.50' diet as an additional structure source. Extruded linseed was provided as Nutex Compact, an extruded commercial concentrate (Dumoulin, Seilles, Belgium) made of linseed, wheat, sunflower cake, field beans, BHT, linseed oil and salt (56.0%, 21.0%, 15.0%, 4.5%, 2.0%, 1.0% and 0.5% of total raw materials, respectively).

The chemical composition of the diets was calculated from the chemical composition of the ingredients (Table 1). The diets were formulated to cover animal needs according to INRA standards (INRA, 1988) for a cow of 650 kg yielding 25 kg/day of milk. The amounts of the diets were distributed as a restricted total mixed ration in order to control the intakes and were adapted according to the individual milk production of cows.

Recordings, sampling and analytical procedures

Individual feedstuffs were sampled at the end of each period and analysed for dry matter (DM) and chemical composition. Individual cow intakes during the last week of each period were included in the statistical analysis. Individual cow milk yield was recorded daily during each milking. Means of individual cow milk yields recorded on day 19, day 20 and day 21 of each period were included in the statistical analysis. Milk samples from individual cows were collected on day 19 and day 21 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 statistical analysis.

The following analytical procedures were adapted from Pottier *et al*. (2006).

Milk samples were refrigerated at 4°C. A portion of each sample was used for fat (Gerber, 1938) and CP (N $\times$ 6.38) analyses (Commission des Communautés européennes, 1985), and the rest of each sample was processed into butter and anhydrous milk fat to allow long-term storage. To obtain anhydrous milk fat, butter was frozen overnight and then melted and centrifuged at 400 $\times$ **g** at 50°C for 10 min. The upper phase was passed through filters (filter type 595; Schleicher and Schuell, Dassel, Germany) filled with anhydrous Na_2SO_4 as a desiccant at 45°C. The extracted fat was stored at -20°C under N_2 until analysis of FA. FA 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.

Table 1 *Dry matter (DM) content and chemical composition of individual feedstuffs*

Ingredient	Maize silage	Wheat straw	Soya bean meal	Sugar beet pulp	Nutex Compact	Mineral and vitamin mix*	Urea**
DM (%)	33.27	92.55	88.70	90.77	92.78	94.93	98.00
				Chemical composition (% of DM)			
Crude ash	4.35	6.12	6.73	6.77	4.27	62.29	0
CP	5.57	2.57	47.40	10.04	20.77	4.62	287.5
NDF	49.69	86.03	20.42	52.46	30.83	0	0
ADF	25.33	62.64	8.34	25.61	12.20	0	0
Crude fat	4.17	1.60	5.80	2.90	25.71	5.18	0
Total fatty acids	2.03	0.84	3.80	1.06	20.17	4.71	0
16:0	0.32	0.16	0.47	0.24	1.20	0.67	0
18:0	0.05	0.03	0.14	0.01	0.69	0.17	0
*c*9-18:1	0.47	0.11	0.73	0.11	3.75	1.03	0
18:2n-6	0.99	0.31	1.35	0.56	3.62	2.43	0
18:3n-3	0.12	0.17	0.99	0.10	10.63	0.29	0

*Declared contents: 21.0% Ca, 7.0% P, 5.0% Mg, 4.0% Na, 0.6% Zn, 0.3% Mn, 0.3% Fe, 0.13% Cu, 600 IU/g of vitamin A, 120 IU/g of vitamin D_3 and 0.5 mg/g of vitamin E (Dumoulin, Seilles, Belgium).
**Values from INRA (2007).

Fatty acid methyl esters (FAME) were quantified by a gas–liquid chromatograph (GC Trace ThermoQuest, Thermo-Finnigan, Milan, Italy) equipped with a flame ionisation detector, automatic injector and a fused silica capillary column (100 m $\times$ 0.25 mm i.d.) coated with a 0.2-μm film of cyanopropyl polysiloxane (CP-Sil 88; Chrompack, Middelburg, The Netherlands) using 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 minimises the risk of discrimination between very different volatile milk-FA. The initial oven temperature was 80°C, 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 airflow was 350 ml/min. Each peak, except that of *t*10-18:1, 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 *t*10-18:1, the concentration of the corresponding peak was calculated by comparison with the *t*11-18:1 peak area (by multiplying the concentration of *t*11-18:1 by the *t*10 peak area-to-*t*11 peak area ratio). The validity of this method was verified by applying it to the peak of *t*9-18:1 and comparing this calculated concentration with the *t*9-18:1 concentration determined through the use of the appropriate standard. Each FA was expressed as a percentage of FAME reported. The FAME reported (identified) accounted for 91.0 $\pm$ 1.42% (mean $\pm$ s.d.) of the total measured FAME, 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 $\pm$3% of the total theoretical FAME (Jensen, 2002). Therefore, the FAME reported would account for $\pm$88% of the total theoretical FAME.

The analysis of the distribution of CLA isomers was performed on a Ag^+-HPLC (Gilson, Villiers-le-bel, France) with three columns coupled in series (250 mm $\times$ 4.6 mm i.d. $\times$5-μm particle size, Ag^+-impregnated; Chromspher Lipids, Chrompack, Middelburg, The Netherlands) and UV detection at 233 nm. The carrier liquid was acetonitrile and hexane (0.1 : 99.9, vol : vol) ; 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 of CLA isomers reported in the literature (Sehat *et al.*, 1998; Kramer *et al.*, 1999). Each CLA isomer was expressed as a relative percentage of total CLA isomers. Concentrations of *c*9,*t*11-CLA were based on GLC analysis. However, according to the literature, the *c*9,*t*11-CLA GLC peak contained *c*9,*t*11-CLA and minor proportions of *t*7,*c*9 and *t*8,*c*10-CLA (Cruz-Hernandez *et al.*, 2006).

Fresh maize silage samples were analysed for DM and lyophilised before analysis of chemical composition. Partial DM of the maize silage was determined by oven-drying at 55°C for 16 to 24 h, analytical DM was then determined on the same maize silage samples. Total DM of the maize silage was calculated by multiplying partial DM by analytical DM. The chemical composition (except for DM) was determined using lyophilised samples. All feed samples were ground in a mill (1-mm screen; Retsch, Dusseldorf, Germany) 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; Association of Official Analytical Chemists, 1995), crude ash by ashing at 550°C for 16 h (adapted from methods 923.03, 967.04 and 942.05; AOAC, 1995), CP by the Kjeldahl method (N $\times$ 6.25) (adapted from methods 981.10 and 991.20; AOAC, 1995), crude fat (Commission des Communautés européennes, 1985), NDF and ADF (Goering and

Van Soest, 1970). NDF and ADF were determined sequentially and were expressed without residual ash. NDF was analysed without the addition of α-amylase and sodium sulphite. Lipids were extracted following the method of Folch modified by Christie (1982). Methylation and GLC analyses were performed as described for milk samples.

Calculations of structure values

The SV of the maize silage was estimated from its NDF content using the following regression equation (De Brabander *et al.*, 1999):

$$SV = -0.57 + 0.006 \times NDF.$$

SV of concentrates and supplements were estimated from their NDF and undegradable starch (USt) contents, and their sugar (SU) and degradable starch (DSt) contents, using the following regression equation (De Brabander *et al.*, 1999):

$$SV = 0.175 + 0.00082 \times NDF + 0.00047 \times USt - 0.001 \times (SU + a \times DSt),$$

where $a = 0.90$ to $1.3 \times$ starch resistance, and NDF, USt, DSt and SU are expressed in g/kg of DM. De Brabander (2006) calculated the SV for some commonly used concentrates and supplements using the regression equation above. In this way, the SV of soya bean meal and unmolassed sugar beet pulp were estimated at 0.18 and 0.42/kg of DM, respectively. The SV of straw is 4.30 (De Brabander *et al.*, 1999; De Brabander, 2006). From De Brabander *et al.* (1999) and De Brabander (2006) and from the composition of Nutex Compact, we set the SV of Nutex Compact, urea, mineral and vitamin mix and vitamin E preparation at 0.

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

Statistical analysis

Data for nutrient intake, milk yield and composition, milk FA composition and milk CLA isomer composition are reported as least squares means ± s.e. Data were analysed as a crossover using the mixed procedure of SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA). The statistical model included period, group, cow and diet. The fixed effects included period, group and diet. Cow was the random effect. Overall differences between treatment means were considered to be significant when $P < 0.05$. The model used was

$$Y_{ijk} = \mu + \text{cow}_{ij} + \text{period}_k + \text{diet}_{ik} + e_{ijk},$$

where Y_{ijk} = performance during the kth period of the jth cow in the ith group; μ = overall mean effect; cow_{ij} = effect of the jth cow on the ith group ($i = 1, 2$ and $j = 1, 2, \ldots, 6$), cow_{ij} $N(0, \sigma^2_{\text{cow}})$; period_k = effect of the kth period ($k = 1, 2$); diet_{ik} = effect of the treatment in the kth period in the ith group and e_{ijk} = random error, e_{ijk} $N(0, \sigma^2_e)$.

Results

Experimental diets

The DM content and chemical composition of individual feedstuffs are presented in Table 1. 18:2n-6 was the predominant C18 FA in the maize silage and accounted for 49% of the total FA, whereas 18:3n-3 was the predominant C18 FA in Nutex Compact and accounted for 53% of the total FA.

The ingredients and chemical composition of the two experimental diets are given in Table 2. The inclusion of straw in the diet (8.31% of DM) consequently modified the proportion of maize silage (−4.88% of DM), soya bean meal (−1.05% of DM), sugar beet pulp (−1.08% of DM) and Nutex Compact (−1.15% of DM) in the diet, therefore modifying the F : C ratio from 59 : 41 to 62 : 38 (Table 2). The NDF content of the 'SV 1.73' diet was slightly higher than that for the 'SV 1.50' diet (46.45% *v.* 42.86% of DM, respectively), whereas the total FA content was quite similar for both diets (4.35% and 4.67% of DM, respectively, for the 'SV 1.73' and 'SV 1.50' diets).

Nutrient intake

The daily nutrient intake of cows fed the two experimental diets is summarised in Table 2. The total FA intakes amounted to 651 and 664 g/day for the 'SV 1.50' and 'SV 1.73' diets, respectively. For both diets, the predominant FA were 18:3n-3, 18:2n-6 and *c*9-18:1, which accounted for 36%, 29% and 20% of the total FA intake, respectively.

The inclusion of straw in the 'SV 1.73' diet depressed the protein and fat contents of the diet but the fibre and ash contents were increased (Table 2). The intake of each of the nutrients for the 'SV 1.73' diet was higher ($P < 0.01$) than that for the 'SV 1.50' diet. However, the additional intakes were low for crude ash, CP, crude fat and total FA (+80, +40, +30 and +13 g/day, respectively) and could be considered as having no biological significance in spite of having statistical significance. By contrast, the additional intake of NDF was more important (+1110 g/day) and accounted for 90% of the additional intake of OM (+1230 g/day). The choice of straw as an additional structure source was thus appropriate since it essentially provided fibre. As a consequence, the SV of the 'SV 1.73' diet was higher than that of the 'SV 1.50' diet ($P < 0.001$) without being dramatically different and both SV were higher than the minimum required (1/kg of DM) to avoid MFD, as set out in the objective of this experiment. The calculated energy balance of the cows was evaluated by using INRAtion software (version 3.21; INRA, Paris, France) according to cow parity, BW and stage of lactation, milk yield and composition, chemical analyses of the individual feedstuffs and amounts of individual feedstuffs really consumed by the cows. For the 'SV 1.50' and 'SV 1.73' diets, respectively, 95% and 100% of energy needs were covered. Consistent with the calculated energy balance, the cows did not lose weight during the experiment (616 ± 67.9 *v.* 607 ± 62.4 kg of BW, respectively, at the end and at the onset of the experiment).

Table 2 *Ingredients, chemical composition and daily nutrient intake of cows fed the two experimental diets**

	Diet					
	SV 1.50		SV 1.73		s.e.	*P*
	Ingredients					
	(g of DM)	(% of DM)	(g of DM)	(% of DM)		
Maize silage	8195	58.73	8219	53.85		
Wheat straw	0	0	1269	8.31		
Soya bean meal	1772	12.70	1778	11.65		
Sugar beet pulp	1813	13.00	1819	11.92		
Nutex Compact	1931	13.84	1938	12.69		
Mineral and vitamin mix	197	1.41	198	1.29		
Urea	24	0.17	24	0.16		
Vitamin E preparation**	20	0.14	20	0.13		
Forage : concentrate ratio		59 : 41		62 : 38		
	Daily nutrient intake and chemical composition					
	(kg/day)	(% of DM)	(kg/day)	(% of DM)		
DM	13.93^{b}	100.00	15.24^{a}	100.00	1.019	< 0.0001
OM	13.13^{b}	94.23	14.36^{a}	94.20	0.961	< 0.0001
Crude ash	0.80^{b}	5.76	0.88^{a}	5.79	0.059	< 0.0001
CP	1.96^{b}	14.03	2.00^{a}	13.08	0.144	0.0009
Crude fat	1.00^{b}	7.19	1.03^{a}	6.73	0.074	0.0003
NDF	5.98^{b}	42.86	7.09^{a}	46.45	0.436	< 0.0001
ADF	2.92^{b}	20.95	3.73^{a}	24.41	0.212	< 0.0001
Fatty acid	(g/day)	(% of DM)	(g/day)	(% of DM)		
Total	651.39^{b}	4.67	664.11^{a}	4.35	47.723	0.0009
6:0–14:0	5.05^{b}	0.04	5.46^{a}	0.04	0.370	< 0.0001
16:0	63.54^{b}	0.46	65.78^{a}	0.43	4.661	< 0.0001
*c*9-16:1	0.72^{b}	0.01	0.77^{a}	0.01	0.052	< 0.0001
17:0	0.82^{b}	0.01	0.86^{a}	0.01	0.060	< 0.0001
18:0	20.77^{b}	0.15	21.19^{a}	0.14	1.520	0.0008
*c*9-18:1	127.97^{b}	0.92	129.75^{a}	0.85	9.392	0.0051
*c*11-18:1	5.46^{b}	0.04	5.57^{a}	0.04	0.400	0.0010
18:2n-6	189.99^{b}	1.36	194.57^{a}	1.27	13.961	0.0002
18:3n-3	235.20^{b}	1.69	238.17^{a}	1.56	17.171	0.0099
20:0	1.88^{b}	0.01	2.00^{a}	0.01	0.138	<0.0001
	(per kg of DM)					
Structure value	1.50^{b}		1.73^{a}		0.016	0.0005

DM = dry matter; OM = organic matter.
[a,b]Values within a row with different superscripts differ.
*Diets offered to the cows were entirely consumed (no refusals).
**Distributed in the form of a powder containing 50% of all-rac-α-tocopheryl acetate (Roche Co., Brussels, Belgium) added to the concentrates; the amount was calculated to deliver 10 000 IU/day of unprotected vitamin E to the diet.

Milk yield and composition

Milk yield and composition of cows fed the two experimental diets are shown in Table 3. Milk, fat and protein yields did not differ among treatments ($P > 0.05$) and averaged 21.10, 0.82 and 0.66 kg/day, respectively. The additional intake of NDF in the 'SV 1.73' diet was provided by straw, which is not well digested (apparent digestibility of NDF of 47%; INRA, 2007). This could explain why milk yield did not differ. Fat and protein contents did not differ among treatments ($P > 0.05$) and averaged 4.05% and 3.10%, respectively. These two diets were thus not MFD-inducing even though they were supplemented with 4.35% and 4.67% of FA (for the 'SV 1.73' and 'SV 1.50' diets, respectively, DM basis). On the contrary, Loor *et al.* (2005) reported a drop in milk fat concentration for 35 : 65 compared to 65 : 35 F : C ratio diets supplemented with 3% linseed oil (DM basis).

Milk fatty acid composition

Milk FA profiles of cows fed the two experimental diets are shown in Table 4. The highest concentration of *c*9,*t*11-CLA in milk fat was obtained with the 'SV 1.50' diet and amounted to 3.03% of FA reported. The inclusion of straw in this diet decreased the concentrations of *c*9,*t*11-CLA (2.13%

Table 3 *Milk yield and composition of cows fed the two experimental diets*

	Diet			
	SV 1.50	SV 1.73	s.e.	*P*
Yield	(kg/day)			
Milk	21.43	20.77	3.632	ns
4% FCM	20.71	20.59	2.982	ns
Fat	0.81	0.82	0.112	ns
Protein	0.66	0.66	0.100	ns
Composition	(%)			
Fat	3.97	4.12	0.359	ns
Protein	3.10	3.09	0.123	ns

ns = non-significant ($P > 0.05$).

Table 4 *Milk fatty acid composition of cows fed the two experimental diets*

	Diet			
	SV 1.50	SV 1.73	s.e.	*P*
	(% of FA reported)			
6:0	2.22	2.30	0.071	ns
8:0	1.23	1.29	0.061	ns
10:0	2.60[b]	2.78[a]	0.136	0.0415
12:0	2.77[b]	2.95[a]	0.149	0.0272
14:0	10.87[b]	11.59[a]	0.334	0.0022
16:0	25.25[b]	26.55[a]	1.913	0.0020
*c*9-16:1	1.32	1.26	0.186	ns
*t*9-16:1	0.77[a]	0.62[b]	0.055	0.0005
17:0	0.55[b]	0.63[a]	0.018	<0.0001
18:0	12.59[b]	13.54[a]	0.613	0.0008
*c*9-18:1	23.98	24.37	0.968	ns
*t*9-18:1	0.55[a]	0.47[b]	0.043	0.0020
*t*10-18:1	0.41	0.15	0.227	ns
*c*11-18:1	0.65	0.62	0.038	ns
*t*11-18:1	7.10[a]	4.99[b]	0.679	0.0001
*c*9,*t*11-CLA*	3.03[a]	2.13[b]	0.343	<0.0001
18:2n-6	2.17	2.02	0.119	ns
18:3n-3	1.56[a]	1.33[b]	0.085	0.0011
20:0	0.14[b]	0.17[a]	0.009	0.0001
20:4	0.06	0.06	0.008	ns
20:5	0.08	0.08	0.004	ns
22:5	0.10[b]	0.11[a]	0.011	0.0449
6:0–14:0	19.69[b]	20.90[a]	0.687	0.0079
trans-UFA**	11.86[a]	8.37[b]	0.931	<0.0001
Total C18 FA	39.45[a]	36.08[b]	1.787	<0.0001
	(%)			
18:0/total C18 FA	32.07[b]	37.74[a]	1.526	<0.0001
*t*11–18:1/total C18 FA	17.95[a]	13.70[b]	1.376	0.0017
*c*9,*t*11-CLA/total C18 FA	7.64[a]	5.82[b]	0.393	<0.0001
Total *trans*-C18 FA/total C18 FA	20.34[a]	15.46[b]	1.256	0.0002

CLA = conjugated linoleic acid; FA = fatty acid.
[a,b]Values within a row with different superscripts differ. Values with no superscript do not differ significantly.
ns = non-significant ($P > 0.05$).
*The GC analysis was unable to separate the minor quantities of *t*7,*c*9 and *t*8,*c*10-CLA isomers from the main *c*9,*t*11-CLA isomer.
**_trans_-UFA = *t*9-16:1 + *t*9-18:1 + *t*10-18:1 + *t*11-18:1 + *c*9,*t*11-CLA.

Table 5 *Milk conjugated linoleic acid (CLA) isomer composition of cows fed the two experimental diets*

	Diet			
	SV 1.50	SV 1.73	s.e.	*P*
	(% of total CLA isomers)			
*t*7,*c*9	3.81[b]	4.21[a]	0.159	0.0169
*t*8,*c*10	0.29	0.29	0.057	ns
*c*9,*t*11	84.58	84.16	0.479	ns
*t*10,*c*12	0.05	0.10	0.035	ns
*t*11,*c*13	6.91[a]	5.97[b]	0.484	0.0319
*c*12,*t*14	0.39[b]	0.56[a]	0.058	0.0083
*t*7,*t*9	0.35	0.38	0.016	ns
*t*8,*t*10	0.09[b]	0.10[a]	0.008	0.0161
*t*9,*t*11	0.89	0.98	0.039	ns
*t*10,*t*12	0.13	0.17	0.018	ns
*t*11,*t*13	1.41[b]	1.69[a]	0.111	0.0412
*t*12,*t*14	1.10[b]	1.38[a]	0.079	0.0056

[a,b]Values within a row with different superscripts differ. Values with no superscript do not differ significantly.
ns = non-significant ($P > 0.05$).

v. 3.03% of FA reported, $P < 0.001$), *t*11-18:1 (4.99% *v.* 7.10% of FA reported, $P < 0.001$) and the total *trans*-unsaturated fatty acids (UFA) (8.37% *v.* 11.86% of FA reported, $P < 0.001$) in milk fat. The same observation could be made for 18:3n-3 (1.33% *v.* 1.56% of FA reported, $P < 0.01$), *t*9-16:1 (0.62% *v.* 0.77% of FA reported, $P < 0.001$) and *t*9-18:1 (0.47% *v.* 0.55% of FA reported, $P < 0.01$). By contrast, the inclusion of straw in the diet increased the concentrations of 6:0–14:0 (20.90% *v.* 19.69% of FA reported, $P < 0.01$), 16:0 (26.55% *v.* 25.25% of FA reported, $P < 0.01$), 18:0 (13.54% *v.* 12.59% of FA reported, $P < 0.001$), 17:0 (0.63% *v.* 0.55% of FA reported, $P < 0.001$), 20:0 (0.17% *v.* 0.14% of FA reported, $P < 0.001$) and 22:5 (0.11% *v.* 0.10% of FA reported, $P < 0.05$) in milk fat. The inclusion of straw in the diet did not affect ($P > 0.05$) the levels of *t*10-18:1, 18:2n-6, *c*9-16:1, *c*9-18:1, *c*11-18:1, 6:0, 8:0, 20:4 and 20:5 in milk fat.

Table 5 shows the milk CLA isomer profiles of cows fed the two experimental diets. The inclusion of straw in the diet decreased the concentration of *t*11,*c*13-CLA (5.97% *v.* 6.91% of total CLA isomers, $P < 0.05$) in milk fat CLA. By contrast, inclusion of straw increased the concentrations of *t*7,*c*9 (4.21% *v.* 3.81% of total CLA isomers, $P < 0.05$), *c*12,*t*14 (0.56% *v.* 0.39% of total CLA isomers, $P < 0.01$); *t*8,*t*10 (0.10% *v.* 0.09% of total CLA isomers, $P < 0.05$); *t*11,*t*13 (1.69% *v.* 1.41% of total CLA isomers, $P < 0.05$) and *t*12,*t*14-CLA (1.38% *v.* 1.10% of total CLA isomers, $P < 0.01$) in milk fat CLA. The inclusion of straw in the diet did not affect ($P > 0.05$) the levels of *t*8,*c*10; *c*9,*t*11; *t*10,*c*12; *t*7,*t*9; *t*9,*t*11 and *t*10,*t*12-CLA in milk fat CLA.

Discussion

The concentrations of *c*9,*t*11-CLA and *t*11-18:1 in milk fat obtained with the 'SV 1.50' diet (3.03% and 7.10% of FA reported, respectively) were relatively high compared to those obtained so far in other studies aiming at increasing

the CLA content in milk fat (Kelly *et al*., 1998; Dhiman *et al*., 2000; Loor *et al*., 2005). However, we should bear in mind that FA values were overestimated since they were expressed as a percentage of FA reported and not as a percentage of total FA. Also, factors other than diets (e.g. stage of lactation) may explain minor differences among studies. According to our previous experiments, when there is no MFD, experimental periods of 21 days are sufficient to obtain a complete view of the milk FA responses to lipid supplements and basal diet changes. Bell *et al*. (2006) (values taken over an 8-week treatment period) and Roy *et al*. (2006) (values taken 18 days after the start of the lipid supplementation) also obtained in their experiments high concentrations of *c*9,*t*11-CLA (2.80% and 2.89% of total FA, respectively) and *t*11-18:1 (6.67% and 7.49% of total FA, respectively) associated with low levels of *t*10-18:1 (0.63% and 0.70% of FA, respectively). Interestingly enough, the 'SV 1.50' diet in the present experiment and the 'FLAX/E' diet in that of Bell *et al*. (2006) used a similar F : C ratio (59 : 41 and 60 : 40, respectively) and dietary NDF content (42.86% and 45.30% of DM, respectively) and both diets were supplemented with vitamin E and a source of 18:3n-3 (7.75% extruded linseed and 6.00% linseed oil, respectively, DM basis). In order to take into account differences in the forage and energy sources, the SV of the 'FLAX/E' diet of Bell *et al*. (2006) was estimated (based on De Brabander, 2006 and INRA feed tables of INRA, 2007) and amounted to 1.56/kg of DM, which was very similar to the SV of the 'SV 1.50' diet in the present experiment. Although the F : C ratio in the 'H-L' diet in the experiment of Roy *et al*. (2006) was higher than that in the present experiment (64 : 36 *v*. 59 : 41, respectively), their dietary NDF content was close to that of the 'SV 1.50' diet in the present experiment (40.70% *v*. 42.86% of DM, respectively) and the 'H-L' diet was also supplemented with a high level of 18:3n-3 (5.20% linseed oil, DM basis) as in the present experiment. However, we were not able precisely to estimate the SV of the 'H-L' diet in the experiment of Roy *et al*. (2006) because the grass hay NDF content was not available.

Much lower concentrations of CLA were reported in the experiments of Pottier *et al*. (2006) (values taken on day 21 of each period), Dhiman *et al*. (2000) (mean values taken from weeks 2 to 5) and Kelly *et al*. (1998) (mean values taken from day 11 to day 14). These concentrations amounted, respectively, to 1.14% (*c*9,*t*11-CLA), 1.63% (CLA) and 1.67% (CLA) of FA and were associated with MFD, except for the 'linseed + vitamin E' diet in Pottier *et al*. (2006) where vitamin E was added. All these diets had a similar low dietary NDF content (26.62%, 28.30% and 29.10% of DM, respectively) and were supplemented with a source of 18:3n-3 (5.83% extruded linseed + 0.99% linseed oil, 4.4% linseed oil and 5.3% linseed oil, respectively, DM basis). Besides, Sauvant *et al*. (1999) suggested that diets should contain a minimum of 35% NDF (DM basis) to avoid rumen acidosis. The SV of the 'linseed + vitamin E' diet in Pottier *et al*. (2006) was calculated precisely, the NDF content of the maize silage being available (403 g/kg of DM, unpublished data), and amounted to 1.01/kg of DM, which was very close to the minimum required SV of 1/kg of DM. Here again, we were not able to precisely estimate the SV of the diets in the experiments of Dhiman *et al*. (2000) and Kelly *et al*. (1998). However, we can reasonably assume that the lower dietary NDF contents in the experiments of Dhiman *et al*. (2000) and Kelly *et al*. (1998), as compared to those in the present experiment and in that of Bell *et al*. (2006) (28.30% and 29.10% *v*. 42.86% and 45.30% of DM, respectively) contributed to lower the SV.

Loor *et al*. (2005) reported a decrease in milk *c*9,*t*11-CLA concentration with a 65 : 35 F : C diet compared to a 35 : 65 F : C diet. Both diets were supplemented with 3% linseed oil and contained 43.30% and 32.80% NDF, respectively (DM basis). Similarly, in the present experiment, a decrease in milk *c*9,*t*11-CLA concentration was observed for the higher F:C diet. However, in contrast to the experiment of Loor *et al*. (2005), milk fat yield was not affected in our experiment. Interestingly enough, the dietary NDF content of the lower F : C diet in Loor *et al*. (2005) was slightly below the minimum of 35% suggested by Sauvant *et al*. (1999) to avoid rumen acidosis.

In our experiment, both SV were higher than the minimum required to avoid acidosis and MFD. Although we did not measure the rumen pH, we can reasonably assume that the inclusion of 8.31% of straw (DM basis) in the 'SV 1.73' diet, which resulted in higher dietary NDF and ADF contents (46.45% and 24.41% *v*. 42.86% and 20.95% of DM, respectively, for the 'SV 1.73' and 'SV 1.50' diets) and therefore higher SV, may have led to an increase in chewing activity, which in turn may have raised the rumen pH. Higher rumen pH has been demonstrated to be linearly and positively associated with higher dietary NDF or ADF, although this relationship was rather poor (Le Ruyet *et al*., 1992; Mertens, 1997; Kolver and de Veth, 2002). A stronger and positive relationship between the rumen pH and dietary physically effective NDF, a more specific measure of effective fibre, has been reported ($r^2 = 0.71$, Mertens, 1997; $r^2 = 0.67$, Zebeli *et al*., 2006). Besides, De Brabander (2006) showed that the replacement of maize silage with 7% straw (DM basis) in a maize silage-based diet had the same effect as an addition of 400 g/day sodium bicarbonate to the same diet in maintaining the rumen pH. Choi *et al*. (2005) showed that the pH had a marked influence on the biohydrogenation by rumen bacteria. Adhesion of rumen cellulolytic bacteria to cellulose is a necessity for subsequent cellulose digestion and is reduced by even modest declines in pH, whereas, under neutral pH, cellulose digestion is optimal and the growth of cellulolytic bacteria is maximum (Miron *et al*., 2001; Mouriño *et al*., 2001; Choi *et al*., 2005). According to this mechanism, the likely higher pH with the 'SV 1.73' diet may have enhanced the adhesion of cellulolytic bacteria to cellulose, boosting cellulose digestion and, hence, the growth of cellulolytic bacteria. According to the hypothesis of an amplified growth of cellulolytic bacteria in the 'SV 1.73' diet, the milk concentration of 17 : 0, an odd-chain FA derived from bacteria

leaving the rumen, was 15% higher (0.63% *v.* 0.55% of FA reported, $P < 0.001$). Increasing the dietary NDF has already been reported to result in a higher proportion of milk odd- and branched-chain FA (Sauvant and Bas, 2001; Vlaeminck *et al.*, 2006). Since the main rumen biohydrogenating bacteria are cellulolytic (Harfoot and Hazlewood, 1997), rumen biohydrogenation may have been more complete following the inclusion of additional dietary NDF. This may have resulted in rumen biohydrogenation going all the way to the 18:0, thus allowing fewer intermediates to escape.

Besides the pH hypothesis, Gerson *et al.* (1985) found that decreasing the dietary fibre content and increasing that of starch led to a reduction in the biohydrogenation rate and to an increased concentration of *t*11-18:1 at the expense of 18:0 in the sheep rumen digesta. Gerson *et al.* (1988) suggested that the biohydrogenation rate increased when increasing the particle surface area and subsequently the number of cellulolytic bacteria adhering to it. Similarly, in our experiment, the inclusion of 8.31% of straw (DM basis) in the 'SV 1.73' diet probably provided more particles as biohydrogenation sites, thus putatively increasing the number of cellulolytic bacteria adhering to them.

The pH and particles hypotheses described above may be the explanations for the decreased milk levels of *c*9,*t*11-CLA and *t*11-18:1 and the increased milk level of 18:0 in the 'SV 1.73' diet compared to the 'SV 1.50' diet.

The 'HLO 70' diet of Flachowsky *et al.* (2006) used an F : C ratio of 70 : 30 with a dietary NDF content of 49.38% of DM and 1.5% linseed oil supplementation (DM basis). The SV of the 'HLO 70' diet was estimated based on De Brabander (2006) and amounted to 2.69/kg of DM, which was much higher than that of the 'SV 1.73' diet in the present experiment. The milk CLA content of the 'HLO 70' diet of Flachowsky *et al.* (2006) (values taken in the third week of each period) was very low (0.49% of fat) and may have been the result of a very high diet SV associated with a low 18:3n-3 supplementation.

The various ratios of C18 FA to the total C18 FA in milk fat also reflected the difference in rumen biohydrogenation for the two diets of the present experiment (Table 4). The 18:0/total C18 FA ratio was higher for the 'SV 1.73' diet (37.74% *v.* 32.07%, $P < 0.001$). By contrast, the total *trans*-C18 FA/total C18 FA ratio was lower for the 'SV 1.73' diet (15.46% *v.* 20.34%, $P < 0.001$) and so were the *t*11-18:1/total C18 FA ratio (13.70% *v.* 17.95%, $P < 0.01$) and, hence, the *c*9,*t*11-CLA/total C18 FA ratio (5.82% *v.* 7.64%, $P < 0.001$). This approach can be considered valid since duodenal concentrations of C18 FA followed similar changes as those in milk fat (Loor *et al.*, 2005; Glasser *et al.*, 2007). All these results strongly suggest that the additional intake of NDF (+1110 g/day) due to straw inclusion resulted in rumen biohydrogenation being more complete.

The inclusion of straw in the diet was associated with an increase in short- and medium-chain FA in milk. The NDF content of the diet increased from 42.86% to 46.45% of DM and may have orientated the bacterial fermentations towards the production of acetate, which was further utilised for *de novo* synthesis of 6:0–16:0 (Bauman and Griinari, 2003). Accordingly, Le Ruyet *et al.* (1992) showed that a 21% dietary ADF content increased rumen acetate compared to a 16% dietary ADF content. Similarly, Loor *et al.* (2005) reported a decrease in plasma acetate and milk 8:0–16:0 yield with high-concentrate diets, regardless of the oil supplementation.

In the present experiment and consistent with the literature (for a review see Bauman *et al.*, 2003), *c*9,*t*11-CLA was the most abundant CLA isomer in milk fat and accounted for more than 84% of total CLA isomers, regardless of the diet (Table 5). The second most prevalent CLA isomer in milk fat is usually *t*7,*c*9-CLA (Corl *et al.*, 2002; Piperova *et al.*, 2002; Bauman *et al.*, 2003), whereas in our experiment *t*11,*c*13-CLA was the second, regardless of the diet. The *t*7,*c*9 CLA isomer is also derived almost exclusively from endogenous synthesis via the Δ9-desaturase using rumen *t*7-18:1 as a substrate (which explains its classical second position among CLA isomers), whereas the other CLA isomers are produced in the rumen (Piperova *et al.*, 2002; Bauman *et al.*, 2003). Interestingly enough, *t*11,*c*13-CLA is also the second most important CLA in milk fat from cows grazing mountain pasture, which is rich in 18:3n-3 (Kraft *et al.*, 2003; Collomb *et al.*, 2004) and in milk fat from cows receiving hay and linseed oil (Roy *et al.*, 2006). Moreover, a high correlation was found between the intake of 18:3n-3 and *t*11,*c*13-CLA (Collomb *et al.*, 2004). Similarly, in our experiment, the second position of *t*11,*c*13-CLA may be related to high amounts of 18:3n-3 provided by extruded linseed.

The means whereby the inclusion of straw differently affected the concentrations of *t*11,*c*13; *t*7,*c*9; *c*12,*t*14; *t*8,*t*10; *t*11,*t*13 and *t*12,*t*14-CLA isomers remain unclear.

Conclusion

The concept of diet SV, which includes rumen buffering and acidotic effect, was used as a tool for studying milk CLA responses in cows fed different basal diets supplemented with PUFA. More specifically, the present study showed that even a modest increase in SV to a diet supplemented with extruded linseed, yet already providing enough SV to ensure normal rumen function and therefore with no MFD effect, led to decreases in milk *c*9,*t*11-CLA and *t*11-18:1 contents, whereas milk fat yield and milk *t*10-18:1 content were not affected. This suggests a more complete rumen biohydrogenation going all the way to the 18:0, thus allowing fewer intermediates to escape. All this leads us to hypothesise that rumen lipid metabolism can be differently affected depending on the diet SV. On the one hand, if the diet SV is too low to ensure normal rumen function, rumen biohydrogenation pathways are altered and induce MFD. On the other hand, our study has shown that, in a non-MFD situation, a modest increase in SV can result in rumen biohydrogenation being more complete. This suggests that there may be an optimal range of diet SV enhancing the milk CLA content, for a given supplementation of PUFA.

However, further research is needed to confirm this hypothesis.

Acknowledgments

The authors acknowledge the General Directorate of Agriculture of the Ministry of the Walloon Region of Belgium (Project D31-1069A) for their financial support. We also acknowledge the Institute of Statistics of the Université catholique de Louvain for their advice and Dr. Roger A. Clegg for his careful reading of the manuscript.

References

Association of Official Analytical Chemists 1995. Official methods of analysis, vol. 2, 16th edition. AOAC International, Arlington, VA, USA.

Bauman DE and Griinari JM 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23, 203–227.

Bauman DE, Corl BA and Peterson DG 2003. The biology of conjugated linoleic acids in ruminants. In Advances in conjugated linoleic acid research (ed. JL Sébédio, WW Christie and R Adlof), vol. 2, pp. 146–173. AOCS Press, Champaign, IL, USA.

Bell JA, Griinari JM and Kennelly JJ 2006. Effect of safflower oil, flaxseed oil, monensin and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. Journal of Dairy Science 89, 733–748.

Bu DP, Wang JQ, Dhiman TR and Liu SJ 2007. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. Journal of Dairy Science 90, 998–1007.

Chilliard Y and Ferlay A 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reproduction, Nutrition, Development 44, 467–492.

Choi NJ, Imm JY, Oh S, Kim BC, Hwang HJ and Kim YJ 2005. Effect of pH and oxygen on conjugated linoleic acid (CLA) production by mixed rumen bacteria from cows fed high concentrate and high forage diets. Animal Feed Science and Technology 123–124, 643–653.

Christie WW 1982. Lipid analysis, 2nd edition. Pergamon Press, Oxford, UK.

Collomb M, Sieber R and Butikofer U 2004. CLA isomers in milk fat from cows fed diets with high levels of unsaturated fatty acids. Lipids 39, 355–364.

Commission des Communautés européennes 1985. Recueil des actes agricoles, Harmonisation des législations, Aliments des animaux, tome VI/4. Office des publications officielles des Communautés européennes. Grand-Duché de Luxembourg, Luxembourg.

Corl BA, Baumgard LH, Griinari JM, Delmonte P, Morehouse KM, Yurawecz MP and Bauman DE 2002. Trans-7, cis-9 CLA is synthesized endogenously by delta 9-desaturase in dairy cows. Lipids 37, 681–688.

Cruz-Hernandez C, Kramer JKG, Kraft J, Santercole V, Or-Rashid M, Deng Z, Dugan MER, Delmonte P and Yurawecz MP 2006. Systematic analysis of trans and conjugated linoleic acids in the milk and meat of ruminants. In Advances in conjugated linoleic acid research (ed. MP Yurawecz, JKG Kramer, O Gudmundsen, MW Pariza and S Banni), vol. 3, pp. 45–93. AOCS Press, Champaign, IL, USA.

De Brabander DL, De Boever JL, Vanacker JM, Boucqué CV and Botterman SM 1999. Evaluation of physical structure in dairy cattle nutrition. In Recent developments in ruminant nutrition 4 (ed. PC Garnsworthy and DJA Cole), pp. 47–80. Nottingham University Press, Nottingham, UK.

De Brabander DL 2006. Système d'évaluation de la structure physique pour l'alimentation des vaches laitières. Publication BicarZ, Paris, France.

Dewhurst RJ, Shingfield KJ, Lee MRF and Scollan ND 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Animal Feed Science and Technology 131, 168–206.

Dhiman TR, Satter LD, Pariza MW, Galli MP, Albright K and Tolosa MX 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. Journal of Dairy Science 83, 1016–1027.

Flachowsky G, Erdmann K, Hüther L, Jahreis G, Möckel P and Lebzien P 2006. Influence of roughage/concentrate ratio and linseed oil on the concentration of trans-fatty acids and conjugated linoleic acid in duodenal chyme and milk fat of late lactating cows. Archives of Animal Nutrition 60, 501–511.

Focant M, Mignolet E, Marique M, Clabots F, Breyne T, Dalemans D and Larondelle Y 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. Journal of Dairy Science 81, 1095–1101.

Gerber N 1938. Die praktische milch-prüfung, Einschliessend die kontrolle von molkereiprodukten. Wyss, Bern, Switzerland.

Gerson T, John A and King ASD 1985. The effects of dietary starch and fibre on the in vitro rates of lipolysis and hydrogenation by sheep rumen digesta. Journal of Agricultural Science 105, 27–30.

Gerson T, King ASD, Kelly KE and Kelly WJ 1988. Influence of particle size and surface area on in vitro rates of gas production, lipolysis of triacylglycerol and hydrogenation of linoleic acid by sheep rumen digesta or *Ruminococcus flavefaciens*. Journal of Agricultural Science 110, 31–37.

Glasser F, Doreau M, Ferlay A, Loor JJ and Chilliard Y 2007. Milk fatty acids: mammary synthesis could limit transfer from duodenum in cows. European Journal of Lipid Science and Technology 109, 817–827.

Goering HK and Van Soest PJ 1970. Forage fiber analyses: apparatus, reagents, procedure and some applications. Agriculture handbook no. 379. USDA, Washington, DC, USA.

Griinari JM and Bauman DE 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In Advances in conjugated linoleic acid research (ed. MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson), vol. 1, pp. 180–200. AOCS Press, Champaign, IL, USA.

Harfoot CG and Hazlewood GP 1997. Lipid metabolism in the rumen. In The rumen microbial ecosystem (ed. PN Hobson and CS Stewart), pp. 382–426. Springer, New York, NY, USA.

Institut National de la Recherche Agronomique 1988. Alimentation des bovins, ovins et caprins. INRA, Paris, France.

Institut National de la Recherche Agronomique 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux – Valeurs des aliments. INRA, Paris, France.

Jensen RG 2002. Invited review. The composition of bovine milk lipids: January 1995 to December 2000. Journal of Dairy Science 85, 295–350.

Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, Van Amburgh ME and Bauman DE 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. Journal of Nutrition 128, 881–885.

Kolver ES and de Veth MJ 2002. Prediction of ruminal pH from pasture-based diets. Journal of Dairy Science 85, 1255–1266.

Kraft J, Collomb M, Mockel P, Sieber R and Jahreis G 2003. Differences in CLA isomer distribution of cow's milk lipids. Lipids 38, 657–664.

Kramer JKG, Sehat N, Fritsche J, Mossoba MM, Eulitz K, Yurawecz MP and Ku Y 1999. Separation of conjugated fatty acid isomers. In Advances in conjugated linoleic acid research (ed. MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson), vol. 1, pp. 83–109. AOCS Press, Champaign, IL, USA.

Le Ruyet P, Tucker WB, Hogue JF, Aslam M, Lema M, Shin IS, Miller TP and Adams GD 1992. Influence of dietary fiber and buffer value index on the ruminal milieu of lactating dairy cows. Journal of Dairy Science 75, 2394–2408.

Loor JJ, Ferlay A, Ollier A, Doreau M and Chilliard Y 2005. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. Journal of Dairy Science 88, 726–740.

Mertens DR 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science 80, 1463–1481.

Miron J, Ben-Ghedalia D and Morrison M 2001. Invited review: adhesion mechanisms of rumen cellulolytic bacteria. Journal of Dairy Science 84, 1294–1309.

Mouriño F, Akkarawongsa R and Weimer PJ 2001. Initial pH as a determinant of cellulose digestion rate by mixed ruminal microorganisms in vitro. Journal of Dairy Science 84, 848–859.

Piperova LS, Sampugna J, Teter BB, Kalscheur KF, Yurawecz MP, Ku Y, Morehouse KM and Erdman RA 2002. Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive

synthesis is the predominant source of cis-9-containing CLA in lactating dairy cows. Journal of Nutrition 132, 1235–1241.

Pottier J, Focant M, Debier C, De Buysser G, Goffe C, Mignolet E, Froidmont E and Larondelle Y 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. Journal of Dairy Science 89, 685–692.

Roy A, Ferlay A, Shingfield KJ and Chilliard Y 2006. Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on trans-C18:1 fatty acids and isomers of conjugated linoleic acid. Animal Science 82, 479–492.

Sauvant D and Bas P 2001. La digestion des lipides chez le ruminant. Productions Animales 14, 303–310.

Sauvant D, Meschy F and Mertens D 1999. Les composantes de l'acidose ruminale et les effets acidogènes des rations. Productions Animales 12, 49–60.

Sehat N, Kramer JKG, Mossoba MM, Yurawecz MP, Roach JAG, Eulitz K, Morehouse KM and Ku Y 1998. Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. Lipids 33, 963–971.

Shingfield KJ, Reynolds CK, Lupoli B, Toivonen V, Yurawecz MP, Delmonte P, Griinari JM, Grandison AS and Beever DE 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. Animal Science 80, 225–238.

Soita HW, Fehr M, Christensen DA and Mutsvangwa T 2005. Effects of corn particle length and forage : concentrate ratio on milk fatty acid composition in dairy cows fed supplemental flaxseed. Journal of Dairy Science 88, 2813–2819.

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM and Dewhurst RJ 2006. Factors affecting odd- and branched-chain fatty acids in milk: a review. Animal Feed Science and Technology 131, 389–417.

Zebeli Q, Tafaj M, Steingass H, Metzler B and Drochner W 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. Journal of Dairy Science 89, 651–668.