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

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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 (1,110 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 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 < 0.01), 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 t11-18:1/total C18 FA ratio (13.70% v. 17.95%, P < 0.01) and the c9,t11-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 c9,t11-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...
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 feedstuffs, 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 situations where both diets provided enough SV and therefore did not induce MFD.

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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 ± 62.4 kg of BW, 186 ± 25.8 days in milk and yielded 21.7 ± 8.71 kg/day of milk (mean ± 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’Experimenteration 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 × 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 × 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 Na2SO4 as a desiccant at 45°C. The extracted fat was stored at −20°C under N2 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.
**Values from INRA (2007).**

delburg, The Netherlands) using H2 as the carrier gas.

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 (FID). The GC was 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 injec-
tion 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 main-
tained at 255°C. Hydrogen flow to the detector was 35 ml/min
and airflow was 350 ml/min. Each peak, except that of
10:18:1, was identified and quantified by comparison of retention times with pure FAME standards (Alltech Associ-
ates, Deerfield, IL, USA; except CLA isomers from Nu-Chek
Prep, Inc., Elysian, MN, USA). Because no commercial
standard was available for 10:18:1, the concentration of
the corresponding peak was calculated by comparison with the
11:18:1 peak area (by multiplying the concentration of
11:18:1 by the 10:18:1 peak area ratio). The validity of this method was verified by applying it to the peak of 9:18:1 and comparing this calculated concentra-
tion with the 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 (iden-
tified) accounted for 91.0 ± 1.42% (mean ± 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 ±3% of the total theoretical FAME (Jensen, 2002). Therefore, the FAME reported would account for ±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 × 4.6 mm
i.d. × 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
9,11-CLA were based on GLC analysis. However, according
to the literature, the 9,11-CLA GLC peak contained
9,11-CLA and minor proportions of 7,9 and 8,10-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 analy-
tical 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 anal-
ysis. 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 × 6.25) (adapted from methods 981.10 and
991.20; AOAC, 1995), crude fat (Commission des Commu-
nautés européennes, 1985), NDF and ADF (Goering and

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Maize silage</th>
<th>Wheat straw</th>
<th>Soya bean meal</th>
<th>Sugar beet pulp</th>
<th>Nutex Compact</th>
<th>Mineral and vitamin mix*</th>
<th>Urea**</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>33.27</td>
<td>92.55</td>
<td>88.70</td>
<td>90.77</td>
<td>92.78</td>
<td>94.93</td>
<td>98.00</td>
</tr>
</tbody>
</table>

Chemical composition (% of DM):

<table>
<thead>
<tr>
<th>Component</th>
<th>Maize silage</th>
<th>Wheat straw</th>
<th>Soya bean meal</th>
<th>Sugar beet pulp</th>
<th>Nutex Compact</th>
<th>Mineral and vitamin mix*</th>
<th>Urea**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude ash</td>
<td>4.35</td>
<td>6.12</td>
<td>6.73</td>
<td>6.77</td>
<td>4.27</td>
<td>62.29</td>
<td>0</td>
</tr>
<tr>
<td>CP</td>
<td>5.57</td>
<td>2.57</td>
<td>47.40</td>
<td>10.04</td>
<td>20.77</td>
<td>4.62</td>
<td>287.5</td>
</tr>
<tr>
<td>NDF</td>
<td>49.69</td>
<td>86.03</td>
<td>20.42</td>
<td>52.46</td>
<td>30.83</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADF</td>
<td>25.33</td>
<td>62.64</td>
<td>8.34</td>
<td>25.61</td>
<td>12.20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crude fat</td>
<td>4.17</td>
<td>1.60</td>
<td>5.80</td>
<td>2.90</td>
<td>25.71</td>
<td>5.18</td>
<td>0</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>2.03</td>
<td>0.84</td>
<td>3.80</td>
<td>1.06</td>
<td>20.17</td>
<td>4.71</td>
<td>0</td>
</tr>
<tr>
<td>16:0</td>
<td>0.32</td>
<td>0.16</td>
<td>0.47</td>
<td>0.24</td>
<td>1.20</td>
<td>0.67</td>
<td>0</td>
</tr>
<tr>
<td>18:0</td>
<td>0.05</td>
<td>0.03</td>
<td>0.14</td>
<td>0.01</td>
<td>0.69</td>
<td>0.17</td>
<td>0</td>
</tr>
<tr>
<td>c9-18:1</td>
<td>0.47</td>
<td>0.11</td>
<td>0.73</td>
<td>0.11</td>
<td>3.75</td>
<td>1.03</td>
<td>0</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.99</td>
<td>0.31</td>
<td>1.35</td>
<td>0.56</td>
<td>3.62</td>
<td>2.43</td>
<td>0</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.12</td>
<td>0.17</td>
<td>0.99</td>
<td>0.10</td>
<td>10.63</td>
<td>0.29</td>
<td>0</td>
</tr>
</tbody>
</table>

*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 D₃ and 0.5 mg/g of vitamin E (Dumoulin, Selles, Belgium).

**Values from INRA (2007).
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 \(a\) is 0.90 to 1.3 × 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 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, cow 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

\[
Y_{ijk} = \mu + cow_{ij} + period_{k} + diet_{ik} + e_{ijk},
\]

where \(Y_{ijk}\) is performance during the \(k\)th period of the \(j\)th cow in the \(i\)th group; \(\mu\) is overall mean effect; \(cow_{ij}\) is effect of the \(j\)th cow on the \(i\)th group; \(period_{k}\) is effect of the \(k\)th period; \(diet_{ik}\) is effect of the \(i\)th diet and \(e_{ijk}\) is 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 FA in the maize silage and accounted for 49% of the total FA, whereas 18:3n-3 was the predominant 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 c9-18:1, which accounted for 90% of the additional intake of OM having statistical significance. By contrast, the additional intake of NDF was more important (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).
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 c9,t11-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 c9,t11-CLA (2.13%...
Table 3  Milk yield and composition of cows fed the two experimental diets

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

ns = non-significant (P > 0.05).

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

<table>
<thead>
<tr>
<th>Diet</th>
<th>SV 1.50</th>
<th>SV 1.73</th>
<th>s.e.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA (% of FA reported)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:0</td>
<td>2.22</td>
<td>2.30</td>
<td>0.071</td>
<td>ns</td>
</tr>
<tr>
<td>8:0</td>
<td>1.23</td>
<td>1.29</td>
<td>0.061</td>
<td>ns</td>
</tr>
<tr>
<td>10:0</td>
<td>2.60b</td>
<td>2.78a</td>
<td>0.136</td>
<td>0.0415</td>
</tr>
<tr>
<td>12:0</td>
<td>2.77b</td>
<td>2.95a</td>
<td>0.149</td>
<td>0.0272</td>
</tr>
<tr>
<td>14:0</td>
<td>10.87b</td>
<td>11.59a</td>
<td>0.334</td>
<td>0.0022</td>
</tr>
<tr>
<td>16:0</td>
<td>25.25b</td>
<td>26.55a</td>
<td>1.913</td>
<td>0.0020</td>
</tr>
<tr>
<td>c9-16:1</td>
<td>1.32</td>
<td>1.26</td>
<td>0.186</td>
<td>ns</td>
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<tr>
<td>c9-16:1</td>
<td>0.77</td>
<td>0.62b</td>
<td>0.055</td>
<td>0.0005</td>
</tr>
<tr>
<td>17:0</td>
<td>1.63b</td>
<td>1.63a</td>
<td>0.018</td>
<td>0.0001</td>
</tr>
<tr>
<td>18:0</td>
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<td>13.54a</td>
<td>0.063</td>
<td>0.0008</td>
</tr>
<tr>
<td>c9-18:1</td>
<td>23.98</td>
<td>24.37</td>
<td>0.968</td>
<td>ns</td>
</tr>
<tr>
<td>c9-18:1</td>
<td>0.55</td>
<td>0.47b</td>
<td>0.043</td>
<td>0.0020</td>
</tr>
<tr>
<td>t9-18:1</td>
<td>0.41</td>
<td>0.15</td>
<td>0.227</td>
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</tr>
<tr>
<td>c11-18:1</td>
<td>0.65</td>
<td>0.62</td>
<td>0.038</td>
<td>ns</td>
</tr>
<tr>
<td>t11-18:1</td>
<td>7.10b</td>
<td>4.98a</td>
<td>0.679</td>
<td>0.0001</td>
</tr>
<tr>
<td>t9-11,CLA*</td>
<td>3.03</td>
<td>2.33</td>
<td>0.343</td>
<td>0.0001</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>2.17</td>
<td>2.02</td>
<td>0.119</td>
<td>ns</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>1.56</td>
<td>1.33b</td>
<td>0.085</td>
<td>0.0011</td>
</tr>
<tr>
<td>20:0</td>
<td>0.14b</td>
<td>0.17a</td>
<td>0.009</td>
<td>0.0001</td>
</tr>
<tr>
<td>20:4</td>
<td>0.06</td>
<td>0.06</td>
<td>0.008</td>
<td>ns</td>
</tr>
<tr>
<td>20:5</td>
<td>0.08</td>
<td>0.08</td>
<td>0.004</td>
<td>ns</td>
</tr>
<tr>
<td>22:5</td>
<td>0.10b</td>
<td>0.01b</td>
<td>0.011</td>
<td>0.00449</td>
</tr>
<tr>
<td>6:0–14:0</td>
<td>19.69b</td>
<td>20.90a</td>
<td>0.687</td>
<td>0.0079</td>
</tr>
<tr>
<td>trans-UFA**</td>
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<td>8.37</td>
<td>0.931</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total C18 FA</td>
<td>39.45</td>
<td>36.08</td>
<td>1.787</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(% of total CLA isomers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r7,c9</td>
<td>3.81b</td>
<td>4.21a</td>
<td>0.159</td>
<td>0.0169</td>
</tr>
<tr>
<td>r8,c10</td>
<td>0.29</td>
<td>0.29</td>
<td>0.057</td>
<td>ns</td>
</tr>
<tr>
<td>c9,r11</td>
<td>84.58</td>
<td>84.16</td>
<td>0.479</td>
<td>ns</td>
</tr>
<tr>
<td>t10,c12</td>
<td>0.05</td>
<td>0.10</td>
<td>0.035</td>
<td>ns</td>
</tr>
<tr>
<td>r11,c13</td>
<td>6.91b</td>
<td>5.97a</td>
<td>0.484</td>
<td>0.0319</td>
</tr>
<tr>
<td>c12,r14</td>
<td>0.09b</td>
<td>0.06a</td>
<td>0.058</td>
<td>0.0038</td>
</tr>
<tr>
<td>t7,r9</td>
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<td>0.38</td>
<td>0.016</td>
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</tr>
<tr>
<td>r8,r10</td>
<td>0.09b</td>
<td>0.10a</td>
<td>0.008</td>
<td>0.0161</td>
</tr>
<tr>
<td>r9,r11</td>
<td>0.89</td>
<td>0.98</td>
<td>0.039</td>
<td>ns</td>
</tr>
<tr>
<td>r10,r12</td>
<td>0.13</td>
<td>0.17a</td>
<td>0.018</td>
<td>ns</td>
</tr>
<tr>
<td>r11,r13</td>
<td>1.41b</td>
<td>1.69a</td>
<td>0.111</td>
<td>0.0412</td>
</tr>
<tr>
<td>r12,r14</td>
<td>1.10b</td>
<td>1.38a</td>
<td>0.079</td>
<td>0.0056</td>
</tr>
</tbody>
</table>

a,bValues within a row with different superscripts differ. Values with no superscript do not differ significantly.

Discussion

The concentrations of c9,r11-CLA and t11-18:1 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 same observation could be made for 18:3n-3 (1.33% v. 1.56% of FA reported, P < 0.001) and 18:2n-6 (0.62% v. 0.77% of FA reported, P < 0.001) and 18:1-9 (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 t10-18:1, 18:2n-6, c9-16:1, c9-18:1, c11-18:1, 6:0, 8:0, 20:4 and 20:5 in milk fat.

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

<table>
<thead>
<tr>
<th>Diet</th>
<th>SV 1.50</th>
<th>SV 1.73</th>
<th>s.e.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>t7,c9</td>
<td>3.81b</td>
<td>4.21a</td>
<td>0.159</td>
<td>0.0169</td>
</tr>
<tr>
<td>r8,c10</td>
<td>0.29</td>
<td>0.29</td>
<td>0.057</td>
<td>ns</td>
</tr>
<tr>
<td>c9,r11</td>
<td>84.58</td>
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<td>0.479</td>
<td>ns</td>
</tr>
<tr>
<td>t10,c12</td>
<td>0.05</td>
<td>0.10</td>
<td>0.035</td>
<td>ns</td>
</tr>
<tr>
<td>r11,c13</td>
<td>6.91b</td>
<td>5.97a</td>
<td>0.484</td>
<td>0.0319</td>
</tr>
<tr>
<td>c12,r14</td>
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<td>0.0038</td>
</tr>
<tr>
<td>t7,r9</td>
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<td>0.016</td>
<td>ns</td>
</tr>
<tr>
<td>r8,r10</td>
<td>0.09b</td>
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<td>0.008</td>
<td>0.0161</td>
</tr>
<tr>
<td>r9,r11</td>
<td>0.89</td>
<td>0.98</td>
<td>0.039</td>
<td>ns</td>
</tr>
<tr>
<td>r10,r12</td>
<td>0.13</td>
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<td>r11,r13</td>
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<td>0.111</td>
<td>0.0412</td>
</tr>
<tr>
<td>r12,r14</td>
<td>1.10b</td>
<td>1.38a</td>
<td>0.079</td>
<td>0.0056</td>
</tr>
</tbody>
</table>

- Values within a row with different superscripts differ. Values with no superscript do not differ significantly.

ns = non-significant (P > 0.05).

CLA = conjugated linoleic acid; FA = fatty acid.

*The GC analysis was unable to separate the minor quantities of t7,c9 and r8,c10-CLA isomers from the main c9,r11-CLA isomer.

**trans-UFA = t9-16:1 + t9-18:1 + r10-18:1 + r11-18:1 + c9,r11-CLA.
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 c9,t11-CLA (2.80% and 2.89% of total FA, respectively) and r11-18:1 (6.67% and 7.49% of total FA, respectively) associated with low levels of r10-18:1 (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% (c9,t11-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 c9,t11-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 c9,t11-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 (r² = 0.71, Mertens, 1997; r² = 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 (Mironet al., 2001; Mourriño 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 \( r_11-18:1 \) 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 \( c_9, r_{11}-CLA \) and \( r_{11}-18:1 \) 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 \( \Delta 9 \)-CLA/total C18 FA ratio was lower for the ‘SV 1.73’ diet (15.46% v. 20.34%, \( P < 0.001 \)) and so were the \( r_{11}-18:1/1 \)-total C18 FA ratio (13.70% v. 17.95%, \( P < 0.01 \)) and, hence, the \( c_9, r_{11}-CLA/1 \)-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 Grinari, 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), \( c_9, r_{11}-CLA \) 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 \( t_7, c_9-CLA \) (Cori et al., 2002; Piperova et al., 2002; Bauman et al., 2003), whereas in our experiment \( r_{11}, c_{13}-CLA \) was the second, regardless of the diet. The \( t_7, c_9 \) CLA isomer is also derived almost exclusively from endogenous synthesis via the \( \Delta 9 \)-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, \( r_{11}, c_{13}-CLA \) 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 \( r_{11}, c_{13}-CLA \) (Collomb et al., 2004). Similarly, in our experiment, the second position of \( r_{11}, c_{13}-CLA \) 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 \( r_{11}, c_{13} \); \( t_7, c_9 \); \( c_{12}, t_{14} \); \( t_8, t_{10} \); \( t_{11}, t_{13} \) and \( r_{12}, t_{14}-CLA \) 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 \( c_9, r_{11}-CLA \) and \( r_{11}-18:1 \) 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
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Influence of an increase in diet structure on milk conjugated linoleic acid content of cows fed extruded linseed