The effect of rapeseed oil and a combination of linseed and fish oils in the diets for sheep on milk fatty acid profile

MAŁGORZATA SZUMACHER-STRABEL, A. CIEŚLAK, AGNIESZKA NOWAKOWSKA and A. POTKAŃSKI

1 Introduction

There is still increasing interest in improving unsaturated fatty acids concentrations in animals' products. Ruminants' milk, because of its components that can only come from these species, is being recognised as health promoting products. Biologically active substances present in milk include, among others, lipid components: conjugated isomers of oleic and linoleic acids. Milk from sheep has one of the highest levels of conjugated isomers among ruminants but there are large variations in milk fatty acids concentrations depending on breed and type of feeding. Conjugated linoleic acid is a collective term for geometric and positional isomers of linoleic acid. The primary CLA isomer c9 t11 C18:2 (CLA) is a potential anticarcinogen in animal models (Ip et al., 1999), and has also antiatherosclerotic properties (Lee et al., 1994 in Whitlock et al., 2002), whereas t10 c12 C18:2 has, among others, antiobesity action (West et al., 2000 in Whitlock et al., 2002). Isomer r11 C18:1 (VA; vaccenic acid) is an intermediate in rumen biohydrogenation of linoleic (LA) and linolenic (LNA) acids, and, is a substrate for CLA (c9 t11) tissue synthesis by the Δ⁹desaturase enzyme (AbuGhazaleh and Holmes, 2007). Cieślak et al. (2001) pointed that extent of biohydrogenation may be depended on source of fat added to the diets that determinates number of unsaturated bonds in fat and hence, intensity of biohydrogenation. Khanal and Olson (2004) suggested that milk fat CLA content can be elevated not only by delivering LA and LNA but by feed sources that can inhibit rumen biohydrogenation of these acids. Long-chain fatty acids (LCFA) can inhibit rumen biohydrogenation, hence fish oil as a source of LCFA is often used as dietary supplement. Some authors also stated that supplementation of combination of fish oil with the other being the source of LA and/or LNA may intensify conjugated linoleic acid formation in the rumen and tissue, and, as a consequence, concentration in milk (Whitlock et al., 2002). Rapeseed oil is also used as supplement to ruminants' diets and can change milk fatty acid composition.

The objective of this study was to examine the effects of rapeseed oil and the combination of fish and linseed oils supplemented to milking sheep rations on milk fatty acid profile, including conjugated isomers of C18:1 and C18:2. Rapeseed oil was used because of big scale of rape seeds production in Poland, as well as linseed oil. Fish oil was used as a potent biohydrogenation inhibitor.

2 Material and methods

Animals, animal management and milk composition

Fifteen milking ewes crossbred of Polish Merino and East Fresian (50 ± 3 kg) at 130 to 160 days of lactation were randomly allocated to three groups (n = 5) and fed basal diet composed of forage (60%) and concentrate (40%). Dietary treatments consisted
of: 0% supplemental oil (control group), the control group with supplementation of 5% of rapeseed oil (RSO) and the control group with 5% of blend of linseed and fish oils (n6:n3 = 2:1; LSO/FO). Control diet (Table 1) was formulated to contain 186 PDIN – protein truly digestible in the small intestine depends on NH3 – N amount, 204 PDIE – protein truly digestible in the small intestine depends on energy amount and 1,60 UFL (1UFL = 1700 kcal EN) INRA (IZ-INRA, 1993). Program INWAR version 1.0 and INRA- tion version 2.63 (1998) were used for calculation. Average daily milk yield was equal in all groups, 1.5 ± 0.2 kg per day per animal, whereas average feed intake was 2.0 kg of dietary dry matter per day per animal. No refusals were stated.

Animals were fed twice daily. Amounts of concentrate and forage offered and refused were recorded daily. Samples of particular feed ingredients were collected weekly and analyzed (AOAC, 1997). The experiment lasted 26 days. The last 5 days were devoted to milk samples collections. Sheep were machine milked twice daily at 0530 and 1630. Individual milk weights were recorded at each milking. Daily composites were prepared by using a proportion of morning (AM) and evening (PM) milking. Composites were prepared in two portions for analyses. One part was refrigerated at 4°C and analyzed for milk constituents. Separate aliquots were stored at –20°C, lyophilized and analyzed for fatty acids composition (Cieślak et al., 2006).

Calculations and statistical analyses

Ratios of fatty acids illustrating Δ9desaturase activity were calculated according to Lock and Garnsworthy (2003). Calculations included: C14:1 to C14:0, C16:1 to C16:0, c9 C18:1 to C18:0 and c9 t11 C18:2 (CLA) to t11 C18:1 (VA). Also atherogenicity index was calculated according to Chilliard et al. (2003), as follows: (C12:0 + 4x C14:0 + C16:0)/ (monounsaturated + polyunsaturated fatty acids).

The obtained data was subjected for variance analysis using general linear model (GLM) procedure of SAS (SAS Inst., Inc., Cary, NC). Significance was determined at P<0.05. Means among treatments were analyzed using Duncan test.

3 Results and discussion

There was no effect (P<0.05) of treatment on total MUFA, although the statistically significant differences were observed in two fatty acids: total C18:1 cis and c9 C18:1. Increase of its concentration was observed in sheep fed RSO diet in comparison with control and LSO/FO groups. Concentrations of these fatty acids increased by 66% and
by 70%, for total C18:1 \textit{cis} and c9 C18:1, respectively. Feeds rich in unsaturated fatty acids can increase the level of t11 C18:1 through biohydrogenation in the rumen. Jenkins (2000) working with dairy cows observed an increase up to 145% in milk \textit{trans} vaccenic acid when animals were fed canola oil diet, rich in oleic acid (78%). In this experiment t11 C18:1 was not affected by treatments. The RSO diet, in which rapeseed oil with 65% oleic acid was used, did not increase level of described fatty acid. This situation could be attributed to low biohydrogenation efficiency, which can be supported by the lowest level of C18:0 in LSO/FO group in comparison to control and RSO groups. The type of forage fed could also be a reason for the low t11 C18:1 increase. In the described experiment meadow grass was fed as a basic dry matter source. Effect of forage was evaluated by Shingfield et al. (2005) when supplemental sunflower and fish oils were added to a ration based on grass vs. corn silage. Corn silage was more effective in t11 C18:1 improvement. Similarly Reynolds et al. (2006) tested alfalfa pellets and corn silage in their experiments with soybean and marine algal oil on milk fatty acid composition of ewes. Again the response to oil was greater for the corn silage diet.

It has also been observed that a decrease in the vaccenic acid concentration in milk i.e. under restricted fiber diet correlates directly with the concentration of CLA (Martin et al., 2007), because VA is a substrate to endogenous synthesis (by delta-9-desaturase enzyme) of CLA in the mammary gland. CLA concentration in sheep milk is found to be around 1% of total fatty acids (Luna et al., 2005) and is the highest in comparison to dairy cows and goats (Jahreis, 1999). It seems to be important to increase milk concentration of conjugated isomers like c9 t11 and t10 c12, which in fact may act as chemopreventive agents for many diseases. Consuming such enriched products lowers the risk of obesity, cancer, diabetes, and cardiovascular diseases. Because breeding for

\begin{table}
\centering
\begin{tabular}{lccc}
\hline
FATTY ACIDS & RSO & LSO & FO \\
\hline
C14:0 & - & - & 3,9 \\
C16:0 & 4,0 & 4,5 & 13,1 \\
C16:1 & - & - & 5,1 \\
C18:0 & 1,5 & 4,0 & 2,8 \\
C18:1 & 65,2 & 22,5 & 25,3 \\
C18:2 & 19,3 & 17,6 & 14,9 \\
C18:3 & 8,4 & 50,4 & 6,0 \\
C20:0 & - & - & 0,3 \\
C20:1 & - & - & 0,3 \\
C21:0 & - & - & 1,6 \\
C20:4 & - & - & 7,5 \\
C20:5 & - & - & 2,1 \\
C22:6 & - & - & 9,2 \\
SFA & 5,9 & 8,9 & 23,2 \\
MUFA & 66,3 & 22,8 & 31,1 \\
PUFA & 27,8 & 68,3 & 45,7 \\
Sum & 98,4 & 99,0 & 92,1 \\
Other** & 1,6 & 1,0 & 7,9 \\
\hline
\end{tabular}
\caption{Fatty acid composition of supplemented oils (FAME* %)}
\end{table}

*FAME – fatty acids methyl esters
1saturated fatty acids
2monounsaturated fatty acids
3polyunsaturated fatty acids
**less than 0.02 FAME %
milk quality is a long-term process and there is always the risk that market demand will have changed by the time the target milk quality has been met, therefore manipulating milk quality through nutritional changes is the best option. AbuGhazaleh et al. (2003) concluded that supplementing dairy cows’ diets with the combination of FO and oils rich in linoleic acid is the most effective dietary way to increase \( c_9 t_{11} \) in milk. Also linolenic acid in the diet can be an intermediate in CLA formation by delivering \( t_{11} C_{18}:1 \) as a substrate to \( c_9 t_{11} \) de novo synthesis in mammary gland. In the presented experiments, a blend of fish oil and linseed oil, rich in, first of all, \( C_{18}:3 \) and, in the second experimental group, rapeseed oil were tested. The purpose to use LSO and RSO instead of soybean or sunflower oils (rich in \( C_{18}:2 \)) was to evaluate effect of products grown in Poland on the big scale. However, as observed by Addis et al. (2005) in spring, sheep milk rumenic acid (\( c_9 t_{11} \)) content showed a positive relationship with the intake of linoleic acid, whereas a negative relationship was found with linolenic acid intake. In our experiment, despite lack of high level of \( C_{18}:2 \) in diet composed mostly of green meadow grass (spring feeding), statistically significant increase (\( P<0.05 \)) either in \( c_9 t_{11} \) or in \( t_{10} c_{12} \) was stated in LSO/FO group. Concentrations of \( c_9 t_{11} \) increased by 76% and \( t_{10} c_{12} \) by 84% in comparison to the control group. Similar results were obtained by Lawless et al. (1998), who found that supplementation of grazing cows with rapeseed increased milk CLA by 61%, relative to a control group and by Luna et al. (2005a), who stated an increase in total CLA in ewes’ milk fat after feeding an enriched alpha-linoleate diet. In experiments carried out previously by Szumacher-Strabel et al. (2001), rapeseed oil added to sheep diets at 0, 4, 8 or 10% in dry matter had no influence on CLA level in milk. The basal diet consisted of meadow hay and concentrate (60:40). In this experiment the supplementation of linseed oil significantly (\( P<0.05 \)) affected the CLA concentration from 0.42 to 2.72 \( \mu g/g \) of lyophilized milk samples in experimental group with 10% of oil supplement.

There is the hypothesis that transfer of EPA and DHA to milk depends on species and the milking sheep seems to be the most effective, but still further investigation is needed (Kitessa et al., 2003). Protected tuna oil used in their research enriched sheep milk in \( \omega-3 \) fatty acids, including EPA and DHA. Milk from sheep fed diets supplemented with LSO/FO was significantly enriched in EPA in comparison to group with RSO supplement, whereas differences in DHA concentration were not statistically significant. Concentration of EPA increased by 82%. PUFA \( \omega-3 \) concentration was statistically higher in LSO/FO group but PUFA \( \omega-6 \) was not affected by dietary treatment. The ratio of total \( \omega-6 \) to \( \omega-3 \) was significantly lower in group with LSO/FO supplement. Rapeseed oil, as a source mostly of \( C_{18}:1 \) and \( C_{18}:2 \), increased (\( P<0.05 \)) \( \omega-6 \) to \( \omega-3 \) ratio in milk. Kitessa et al. (2003) obtained comparable results when protected tuna oil was fed to lactating sheep. Although according to Bourre (2005) the efficiency of \( \omega-3 \) improvement by feeding sheep with fish extracts or algae (oils) is extremely low, up to 2-fold, and protected tuna oil increased \( \omega-3 \) level more than 4-fold.

According to Grinari et al. (2000), and as mentioned earlier, most \( c_9 t_{11} C_{18}:2 \) (CLA) that is present in milk fat is formed during endogenous synthesis in the mammary gland by \( \Delta^9 \)desaturase activity. Even at supplementing the diet with \( \Delta^9 \)desaturase inhibitors, more than 64% \( CLA \) comes from endogenous synthesis. \( \Delta^9 \)desaturase activity may be defined as product:substrate ratio. There are four main products formed by \( \Delta^9 \)desaturase: \( C_{14}:1, C_{16}:1, c_9 C_{18}:1 \) and \( c_9 t_{11} C_{18}:2 \) (CLA), being formed of \( C_{14}:0, C_{16}:0, C_{18}:0 \) and \( t_{11} C_{18}:1 \) (VA) (Lock and Garnsworthy, 2003). The more adequate index is \( C_{14}:1 \) to \( C_{14}:0 \), because all \( C_{14}:0 \), that is present in milk fat, is produced de novo in the mammary gland. Feeding diets supplemented either with rapeseed oil or with linseed oil and fish oil blend had no effect on \( C_{14}:1 \) to \( C_{14}:0 \) ratio. Statistically significant differences (\( P<0.05 \)) were found in calculated \( c_9 C_{18}:1 \) to \( C_{18}:0 \) ratio. Higher values were found in both experimental groups in comparison to control one. A higher value of calculated ratio means higher \( \Delta^9 \)desaturase activity. In previously realized
Tab 3. Milk composition of sheep milk (%)

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>RSO</th>
<th>LSO/FO</th>
<th>ALL</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>6.64</td>
<td>6.90</td>
<td>7.84</td>
<td>7.15</td>
<td>0.65</td>
</tr>
<tr>
<td>Protein</td>
<td>6.93</td>
<td>7.88</td>
<td>7.85</td>
<td>7.63</td>
<td>0.37</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.47</td>
<td>3.67</td>
<td>3.64</td>
<td>3.86</td>
<td>0.27</td>
</tr>
<tr>
<td>Total solids</td>
<td>18.74</td>
<td>19.16</td>
<td>20.03</td>
<td>19.34</td>
<td>0.74</td>
</tr>
<tr>
<td>SNF</td>
<td>12.10</td>
<td>12.25</td>
<td>12.18</td>
<td>12.19</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Tab 4. Fatty acid composition of sheep milk (% of total fatty acids, division according to Collomb et al., 2002).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control</th>
<th>RSO</th>
<th>LSO/FO</th>
<th>ALL</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>∑ saturated¹</td>
<td>72.5</td>
<td>68.7</td>
<td>71.5</td>
<td>70.6</td>
<td>2.00</td>
</tr>
<tr>
<td>∑ short chain²</td>
<td>13.1</td>
<td>12.5</td>
<td>12.2</td>
<td>12.5</td>
<td>1.08</td>
</tr>
<tr>
<td>∑ medium chain³</td>
<td>58.5</td>
<td>54.8</td>
<td>59.0</td>
<td>57.1</td>
<td>1.72</td>
</tr>
<tr>
<td>Sat. C12. C14 and C16</td>
<td>54.6</td>
<td>51.4</td>
<td>55.4</td>
<td>53.5</td>
<td>1.60</td>
</tr>
<tr>
<td>C16:0</td>
<td>29.4</td>
<td>29.3</td>
<td>32.8</td>
<td>30.5</td>
<td>3.23</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.5</td>
<td>2.4</td>
<td>1.6</td>
<td>2.6</td>
<td>2.03</td>
</tr>
<tr>
<td>C18:1</td>
<td>17.6</td>
<td>21.4</td>
<td>16.5</td>
<td>18.8</td>
<td>0.02</td>
</tr>
<tr>
<td>∑ C18:1 cis</td>
<td>8.0ᵇ</td>
<td>13.4ᵃ</td>
<td>8.7ᵇ</td>
<td>10.5</td>
<td>1.54</td>
</tr>
<tr>
<td>∑ C18:1 trans</td>
<td>9.5</td>
<td>8.0</td>
<td>7.7</td>
<td>8.3</td>
<td>1.06</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>7.0ᵇ</td>
<td>12.0ᵃ</td>
<td>7.7ᵇ</td>
<td>9.4</td>
<td>0.14</td>
</tr>
<tr>
<td>C18:1 t11 VA</td>
<td>2.6</td>
<td>1.1</td>
<td>3.1</td>
<td>2.1</td>
<td>1.03</td>
</tr>
<tr>
<td>∑ C18:2</td>
<td>3.8</td>
<td>4.3</td>
<td>3.9</td>
<td>4.0</td>
<td>0.39</td>
</tr>
<tr>
<td>C18:2 t10 c12</td>
<td>0.1ᵇ</td>
<td>0.1ᵇ</td>
<td>0.2ᵃ</td>
<td>0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>C18:2 c9 t11 CLA</td>
<td>0.4ᵇ</td>
<td>0.5ᵃᵇ</td>
<td>0.8ᵃ</td>
<td>0.6</td>
<td>0.56</td>
</tr>
<tr>
<td>∑ C18:3</td>
<td>1.1ᵇ</td>
<td>0.7ᵇ</td>
<td>1.7ᵃ</td>
<td>1.2</td>
<td>0.57</td>
</tr>
<tr>
<td>∑ monounsaturated⁴</td>
<td>20.7</td>
<td>24.6</td>
<td>20.2</td>
<td>22.2</td>
<td>1.60</td>
</tr>
<tr>
<td>∑ polyunsaturated⁵</td>
<td>6.6</td>
<td>6.6</td>
<td>8.2</td>
<td>7.1</td>
<td>1.07</td>
</tr>
<tr>
<td>∑ ω-6ᵇ</td>
<td>3.1</td>
<td>3.7</td>
<td>3.1</td>
<td>3.1</td>
<td>0.08</td>
</tr>
<tr>
<td>ω-6 / ω-3</td>
<td>1.4ᵇ</td>
<td>1.6ᵃ</td>
<td>0.96ᶜ</td>
<td>1.4</td>
<td>0.37</td>
</tr>
<tr>
<td>C20:5 (EPA)</td>
<td>0.2⁹ᵇ</td>
<td>0.2³ᵇ</td>
<td>0.4²ᵃ</td>
<td>0.3¹ᵇ</td>
<td>0.08</td>
</tr>
<tr>
<td>C22:6 (DHA)</td>
<td>0.3⁰</td>
<td>0.2⁸</td>
<td>0.5¹ᵇ</td>
<td>0.3⁶</td>
<td>0.65</td>
</tr>
<tr>
<td>C14:1/C14:0</td>
<td>0.03³</td>
<td>0.02³ᵇ</td>
<td>0.02⁸</td>
<td>0.02⁶</td>
<td>0.01</td>
</tr>
<tr>
<td>C16:1/C16:0</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:1 cis 9/C18:0</td>
<td>3.3³ᵇ</td>
<td>5.1⁴ᵃ</td>
<td>4.9⁵ᵃ</td>
<td>4.6²ᵇ</td>
<td>0.4¹</td>
</tr>
<tr>
<td>c9 t11 CLA/VA</td>
<td>0.07</td>
<td>0.1⁷</td>
<td>0.0⁶</td>
<td>0.1¹</td>
<td>0.04</td>
</tr>
<tr>
<td>Atherogenicity index</td>
<td>3.9</td>
<td>3.3</td>
<td>3.7</td>
<td>3.6</td>
<td>0.3⁹</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different; a,b – P < 0.05

   C20:1 c9
experiments statistically significant differences were found in C16:1 to C16:0 and in CLA/VA ratios (Szumacher-Strabel, 2006). Supplementing sheep diets with low linolenic linseed oil resulted in higher values of the mentioned ratios.

It was found out in experiment with bovine that conjugated t10 c12 C18:2 isomer is acting as a potent inhibitor of milk fat synthesis (Sinclair et al., 2007). Lock et al. (2006) stated that lipid-encapsulated supplements containing t10 c12 reduced milk fat synthesis in sheep to a similar extent as in dairy cows. No changes (P<0.05) either in fat concentration or in other basic milk components were found in the presented experiment (Table 3), although concentration of t10 c12 was improved. The tendency to increase fat content was observed in both experimental groups, relative to the control group. The reason for that effect may be the relatively restricted level of supplemented fat. Also protein concentration was stable under experimental conditions. According to some authors fat supplementation of sheep’ rations produces only a slight fall in the content of milk protein or no change at all (Sanz Sampelayo et al., 2007), which can confirm results obtained in the presented experiment. Supplementation of the diets with plant and fish oils was not reflected in atherogenicity index, although a slight, non significant decrease was observed in both experimental groups. Decreased value of atherogenicity index in milk that is mass-consumption product is demanded effect from a public health point of view.

The feeding regimen based on forage supplemented with fish and linseed oil blend seems to be more effective in improving unsaturated fatty acids concentration in sheep milk in comparison to rapeseed oil. Rapeseed oil can be used as a substrate to elevate primarily monounsaturated fatty acid concentration and indirectly CLA concentration in milk. Based on these results it can be concluded that sheep fatty acids profile can be modified by supplemented fat rich in unsaturated fatty acids.

Summary

The objective of this study was to investigate the effects of plant and fish oil on unsaturated fatty acids concentration in sheep milk. Fifteen milking ewes were used in the experiment. Dietary treatments consisted of either 0 % supplemental oil (control group), the experimental group with supplementation of 5 % of rapeseed oil (RSO) and the experimental group with 5 % of blend of linseed and fish oils (n6:n3 = 2:1; LSO/FO). Fatty acid composition and basic components were determined in milk samples. Conjugated isomers of linoleic acid, either c9 t11 or t10 c12 were improved (P<0.05) when LSO/FO was supplemented. Sheep fed LSO/FO had also higher (P<0.05) level of EPA in milk. RSO supplements increased ∑ C18:1 cis and c9 C18:1. Compared with rapeseed oil, fish and linseed oils blend seems to be more effective in increasing unsaturated fatty acids concentration in sheep milk.

Key words: fatty acids, CLA, sheep, milk, oil

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Martin, C. A., M. C. Milinsk, J. V. Visentainer, M. Matsushita and N. E. De-Souza


Einfluss eines Zusatzes von Rapsöl und der Mischung aus Lein- und Fischöl in den Futtergaben der Schafe auf Fettsäurenmuster der Milch

By Małgorzata Szymacher-Strabel, A. Ciesłak, Agnieszka Nowakowska und A. Potkański

Das Ziel der Untersuchung war die Ermittlung des Einflusses von Pflanzenöl und Fischöl in der Futterration auf die Konzentration der ungesättigten Fettsäuren in der Schafmilch. Der Versuch wurde auf fünfzehn Mutterschafen durchgeführt. Die Tiere wurden in drei Gruppen unterteilt: in der ersten Gruppe bekamen das Futter ohne Zusatz von Öl (Kontrollgruppe) und in der zwei Versuchsgruppen – Futter mit 5% Zusatz von Rapsöl (RSO) und Futter mit 5% Zusatz einer Mischung aus Lein- und Fischöl (n6:n3 Verhältnis – 2:1; LSO/FO). In den entnommenen Milchproben wurden die Milchzusammensetzung und das Fettsäurenmuster bestimmt. Es wurde ein Anstieg (P < 0,05) der Konzentration der konjugierten Isomere der Linolensäure in der Milch, sowie c9 t11 als auch t10 c12 in der Gruppe LSO/FO festgestellt (P < 0,05). In der Milch der Mutterschafe bei denen das Futter mit der Mischung aus Lein- und Fischöl LSO/FO zugegeben wurde, konnte ein höherer Gehalt an EPA festgestellt werden (P < 0,05). Die Zugabe von RSO erhöhte dagegen die Konzentration von Σ C18:1 cis und c9 C18:1. Vergleichend die Steigerung des Gehaltes an ungesättigten Fettsäuren in der Milch, scheint die Zugabe der Mischung aus den Ölen effektiver als Zusatz von Rapsöl zu sein.

Schlüsselwörter: Fettsäuren, CLA, Schaf, Milch, Öl