Enhancing unsaturated fatty acids in ewe's milk by feeding rapeseed or linseed oil

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ABSTRACT: The aim of our research was to evaluate whether rapeseed and linseed oils used as feed additives to ewe's diets allow to achieve the higher secretion of unsaturated fatty acids into milk with extended emphasis on the conjugated linoleic acid concentration. Two experiments were carried out on 6 lactating ewes in two independent 3×3 Latin square designs. In each feeding cycle the animals were subjected to one of the treatments: (1) without oil supplementation, (2) with 3.5% of rapeseed oil (RS) or linseed oil (LS), (3) with 7.0% of rapeseed oil or linseed oil. Feeding diets rich in mono- and polyunsaturated fatty acids to lactating ewes under our experimental conditions resulted in demanded changes in milk fatty acid content without causing milk fat depression or modification of other milk constituents. Milk fat was improved in unsaturated fatty acids including C18:2 *c9 t*11 CLA isomer without changing the milk fat concentration. The C18:2 *c9 t*11 concentration increased from 0.03 (FAME, %) in the control group to 0.21 and 0.12 (FAME, %) in groups receiving 3.5 and 7% RS, respectively. The diet with 7% LS also elevated the C18:2 *c9 t*11 level in milk from 0.04 (FAME, %) in the control group to 0.11 (FAME, %). Hence a conclusion is drawn that plant oils may be an important source of monoenic and also polyenic fatty acids in milk, however, our research and analysis of literature data indicate that results are strictly dietary and supplement-dependent.

Keywords: unsaturated fatty acids; milk; ewe; linseed oil; rapeseed oil

Rapeseed and linseed are products of commonly grown oil plants in Poland. The area of rapeseed cultivation is especially high and amounts up to 800 000 ha, whereas the area under linseed is still increasing. Rapeseed and linseed oils may be used as components improving dietary energy density for ruminants. Because of a high concentration of unsaturated fatty acids, these oils may also modulate rumen fermentation and, as an effect, fatty acid composition of animal product. Rapeseed oil is rich in oleic and linoleic acids, whereas linseed oil is rich in linoleic and linolenic acids. Supplementation of unsaturated fatty acids to ruminant diets may result first of all in the increased level of monounsaturated fatty acids and also of polyunsaturated fatty acids to a lesser extent, like vaccenic acid (VA) and conjugated isomers of linoleic acids (CLA), respectively (Czauderna et al., 2004; Niedźwiedzka et al., 2008). However, there are also other factors affecting the conjugated isomer concentration, e.g. basic diet composition and, as the result, the rumen microbial population (Cieślak et al., 2009;

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Frelich et al., 2009; Jalč et al., 2009; Samková et al., 2009). Antongiovanni et al. (2004) and Addis et al. (2005) indicated that up to 15% of the total C18:1 in sheep milk is *trans* configuration and VA was found to be the principal acid (Sanz Sampelayo et al., 2007). It should also be stressed that ewe's milk contains a higher concentration of bioactive components in comparison with milk of other animal species. The term 'milk bioactive components' is mainly related to conjugated isomers of linoleic acid (CLA) and to vaccenic acid (VA). CLA are a series of positional and geometric isomers of linoleic acid demonstrating beneficial effects for milk consumers. The higher concentration of CLA and VA in ewe's milk is determined by the composition of the extensive diet consumed (Bauman and Griinari, 2001; Collomb et al., 2002). The improvement of milk quality by increasing the unsaturated fatty acid concentration is the answer to a higher demand for milk-derived bioactive components with special consideration of their effects on the human health. Although CLA is the best known for its anti-cancer properties, researchers have also found, among others, that the C18:2 c9 t11 form of CLA can reduce the risk of cardiovascular diseases and help fight inflammation (Tricon et al., 2004; Zulet et al., 2005). VA is a precursor of CLA synthesis in mammary gland, and hence dietary VA may contribute to the amount of CLA available to the human body. There is also an evidence that dietary VA favourably alters the pro-inflammatory tendency of mesenteric lymphocytes from JCR:LA-cp rats (Blewett et al., 2009). VA was also shown to increase the serum level of C18:2 c9 t11 CLA in humans (Salminen et al., 1998; Turpeinen et al., 2002).

The research hypothesis is that rapeseed and linseed oils used as feed additives to ewe's diets allow to achieve the higher secretion of unsaturated fatty acids (UFA) into milk with extended emphasis on the conjugated linoleic acid concentration.

MATERIAL AND METHODS

Diets, experimental procedure and sampling

Two experiments were carried out. Six lactating ewes (Polish Merino breed) of 50 ± 5 kg body weight were assigned to two independent 3×3 Latin square designs. In both experiments dietary Original Paper

Fatty acid	RS	LS
C16:0	4.7	5.0
C18:0	1.8	3.6
C18:1 cis9	61.7	20.6
C18:2 cis9, cis12	19.4	16.8
C18:3 cis9, cis12, cis15	8.4	53.2
Other	4.0	0.8
SFA	8.5	8.7
MUFA	63.7	21.3
PUFA	27.8	70.0

*FAME – fatty acid methyl esters; SFA – saturated fatty acids (C14:0, C16:0, C18:0); MUFA – monounsaturated fatty acids (C16:1, C18:1 *cis*-9, C18:1 *cis* 11); PUFA – polyunsaturated fatty acids (C18:2 *cis*9, *cis*12, C18:3 *cis*9, *cis*12, *cis*15); others (C14:0, C16:1, C18:1 *cis*11)

treatments consisted of 0% supplemental oil (control group), the control group with supplementation of 3.5 or 7% rapeseed (RS) or linseed (LS) oil (Table 1), respectively, for the first (RS) and second (LS) experiment; dietary DM basis. Each experiment lasted 75 days, comprised 11 weeks of lactation from 4th to 14th week and consisted of three 25-day periods: 21 days for the animal and rumen microflora adaptation to the corresponding experimental diet and 4-day sample collection period. The control diet (Table 2) was formulated with forage and concentrate (60:40) and contained 160 PDIN, 184 PDIE and 1.60 UFL INRA (IZ-INRA, 1993). The programs INWAR version 1.0 and INRAtion version 2.63 (1998) were used for calculation. Animals were fed twice a day and the concentrate was fed at milking. Amounts of concentrate and forage fed and refused were recorded daily. Dry matter intake (DMI) per day was calculated.

Sheep were milked twice a day at approximately 8.00 a.m. and 5.00 p.m. and daily milk yields were recorded. Daily composites of milk were prepared 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 acid composition (Czauderna and Kowalczyk, 2001).

Ingredient	Control (0%)	Oil (3.5%)	Oil (7%)
Meadow hay	63.0	60.5	58.5
Wheat meal	30.0	28.9	27.7
Rapeseed meal	5.0	4.9	4.7
Mineral-vitamin premix*	2.0	2.2	2.1
Oil**	0.0	3.5	7.0

Table 2. Ingredient composition (% of DM) of the experimental diets

*1 kg polfamix OK contains: vitamin A 300 000 IU; vitamin D_3 30 000 IU; vitamin E 1.5 g; Fe 0,5 g; Zn 2.5 g; Mg 65.0 g; Co 0.015 g; Mn 3.0 g; J 0.01 g; Se 0.003 g; Na 60 g; Ca 240 g; P 120 g

**1st experiment – rapeseed oil; 2nd experiment – linseed oil

Scheme of the 3×3 Latin square designs used in both experiments:

	А	В	С	
1	0	3.5	7	
2	3.5	7	0	
3	7	0	3.5	
where:				
А, В, С	= consecutive animal			
1, 2, 3	= experimental cycle			
0; 3.5; 7	= percentage of oil in dietary dry matter			

Calculations and statistical analyses

Atherogenicity index (AI) was calculated according to Chilliard et al. (2003) as follows:

 $(C12:0 + 4 \times C14:0 + C16:0)/(monounsaturated + polyunsaturated fatty acids)$

whereas thrombogenic index (TI) was calculated:

(14:0 + 16:0 + 18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA (n-3 PUFA/n-6 PUFA)

in accordance with Ulbricht and Southgate's proposition (1991).

The acquired data (separately for each experiment) were subjected to one-way analysis of variance using the general linear model (GLM) procedure of the SAS (2006). Multiple comparisons among means were carried out by Duncan's test. Significant differences were declared at P < 0.05.

RESULTS

Fatty acid composition of oils used in experiments and ingredient composition of experimental

Table 3. Effect of rapeseed oil (RS) on milk yield and its composition

<u>I</u> 4		Diets	
Item	control (mean ± SD)	RS 3.5 (mean ± SD)	RS 7 (mean ± SD)
DMI, kg/d	1.6 ± 0.15	1.7 ± 0.20	1.7 ± 0.25
Milk yield, g/d	$1\ 250.0^{\mathrm{b}} \pm\ 10.7$	$1\ 312.5^{\mathrm{ab}}\pm17.9$	$1\ 362.5^{a}\pm 17.9$
Fat (g/day)	$80.8^{b}\pm0.85$	$84.6^{a} \pm 1.18$	$85.0^{a} \pm 2.15$
Protein (g/day)	$69.6^{b} \pm 0.10$	$71.5^{ab} \pm 0.25$	$73.9^{a} \pm 0.24$
Total solids (g/day)	$208.9^{b} \pm 1.35$	$214.5^{a} \pm 1.11$	$219.9^{a} \pm 1.18$
Composition (%)			
Fat	6.5 ± 0.13	6.4 ± 0.21	6.2 ± 0.09
Protein	5.6 ± 0.08	5.5 ± 0.21	5.4 ± 0.15
Total solids	16.7 ± 0.26	16.3 ± 0.36	16.1 ± 0.07

means with the same letter are not significantly different ${}^{\rm a,b,c}P < 0.05$

It		Diets	
Item	control (mean ± SD)	LS 3.5 (mean ± SD)	LS 7 (mean ± SD)
DMI (kg/day)	1.6 ± 0.10	1.7 ± 0.15	1.7 ± 0.30
Milk yield (g/day)	$1\ 275.0^{\rm b}\pm 16.5$	$1\ 337.5^{a}\pm25.0$	$1\ 387.5^{a}\pm 17.0$
Fat (g/day)	$83.7^{b} \pm 0.64$	$86.7^{a} \pm 1.07$	$89.8^{a} \pm 1.01$
Protein (g/day)	$71.0^{b} \pm 1.15$	$74.2^{ab}\pm0.84$	$75.7^{a} \pm 1.03$
Total solids (g/day)	$212.7^{b} \pm 1.19$	$219.0^{ab} \pm 1.04$	$225.6^{a} \pm 1.02$
Composition (%)			
Fat	6.6 ± 0.04	6.5 ± 0.07	6.5 ± 0.07
Protein	5.6 ± 0.15	5.5 ± 0.13	5.5 ± 0.15
Total solids	16.7 ± 0.19	16.4 ± 0.04	16.3 ± 0.12

Table 4. Effect of linseed oil (LS) on milk	yield and its composition
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means with the same letter are not significantly different $^{\rm a,b,c}P < 0.05$

Table 5. Effect of rapeseed oil (RS) supplementation on the fatty a	acid composition of sheep milk ((FAME, %)
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Item –		Diets	
nem	control (mean ± SD)	RS 3.5 (mean ± SD)	RS 7 (mean ± SD)
Σ saturated ¹	$62.7^{a} \pm 6.87$	$36.0^{b} \pm 3.16$	$47.1^{b} \pm 1.74$
Σ short chain ²	$23.5^{a} \pm 3.18$	$11.2^{b} \pm 4.29$	$12.1^{b} \pm 4.45$
Sat C12 \times C14 and C16	$26.7^{a} \pm 2.64$	$17.2^{b} \pm 6.50$	$22.2^{ab} \pm 2.61$
C18:0	$12.5^{a} \pm 2.14$	$7.5^{b} \pm 3.78$	$12.8^{a} \pm 2.90$
$\Sigma \text{ C18:1}^3$	$16.3^{\circ} \pm 5.76$	$50.8^{b} \pm 2.74$	$38.0^{a} \pm 2.55$
Σ C18:1 <i>cis</i> ⁴	$16.1^{\circ} \pm 5.74$	$48.9^{b} \pm 2.19$	$37.7^{a} \pm 2.98$
Σ C18:1 <i>trans</i> ⁵	$0.3^{b} \pm 0.38$	$2.9^{a} \pm 0.50$	$2.1^{a} \pm 0.77$
C18:2 <i>c</i> 9 <i>c</i> 12	$15.5^{a} \pm 3.29$	$5.8^{b} \pm 4.39$	$7.0^{b} \pm 3.28$
C18:2 <i>c</i> 9 <i>t</i> 11; CLA	$0.03^{\circ} \pm 0.04$	$0.21^{a} \pm 0.24$	$0.12^b\pm0.02$
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	5.3 ± 2.00	4.0 ± 3.66	5.0 ± 2.57
$\Sigma \omega$ -3 ⁶	5.3 ± 2.00	4.0 ± 3.66	5.0 ± 2.57
$\Sigma \omega$ -6 ⁷	$15.6^{a} \pm 2.37$	$7.0^{b} \pm 2.21$	$7.4^{b} \pm 3.51$
ω-6/ω-3	3.6 ± 2.34	2.3 ± 0.87	1.6 ± 0.32
Σ monounsaturated ⁸	$16.4^{b} \pm 5.78$	$51.8^{a} \pm 2.73$	$39.8^{a} \pm 2.37$
Σ polyunsaturated ⁹	21.0 ± 3.42	12.2 ± 3.98	13.1 ± 4.91
Atherogenic index ¹⁰	$1.3^{a} \pm 0.40$	$0.5^{b} \pm 0.34$	$0.8^{b} \pm 0.07$
Thrombogenic index ¹¹	$0.8^{a} \pm 0.10$	$0.3^{c} \pm 0.34$	$0.7^{b} \pm 0.09$

 $\label{eq:FAME-fatty acids methyl esters; LS-linseed oil; {}^{1}C8 \times C10 \times C12 \times C14 \times C16 \times C18; {}^{2}C8 \times C10; {}^{3}C18:1 \ t6 \times C18:1 \ t7 \times C18:1 \ t9 \times C18:1 \ c6 \times C18:1 \ c9; {}^{4}C18:1 \ c6 \times C18:1 \ c9; {}^{5}C18:1 \ t6 \times C18:1 \ t7 \times C18:1 \ t9; {}^{6}C18:3 \ c9c12c15 \ (\omega-3); {}^{7}C18:2 \ c9 \ c12 \ (\omega-6) \times C18:3 \ c9c12c12 \ (\omega-6); {}^{8}C16:1 \times C18:1 \ t6 \times C18:1 \ t7 \times C18:1 \ t9 \times C18:1 \ c9; {}^{9}C18:2 \ c9 \ c12 \times C18:2 \ c9 \ t11 \times C18:3 \ c9c12c12 \times C18:3 \ c9c12c12; {}^{10}(12:0 + 4 \times 14:0 + 16:0)/(MUFA + PUFA); {}^{11}(C14:0 + C16:0 + C18:0)/(0.5 \times MUFA + 0.5 \times PUFAn-6 + 3 \times PUFAn-3 + UFAn-3/PUFAn-6), means with the same letter are not significantly different; {}^{a,b,c}P < 0.05$

diets are shown in Table 1 and 2, respectively. Tables 3 and 4 show milk yield and milk composition of ewes fed RS and LS supplements, respectively. The fatty acid profile of milk fat from ewes fed the control diet and rapeseed oil-supplemented rations is presented in Table 5 while Table 6 documents the effect of control diet and linseed oil-supplemented rations on the same characteristic.

During the experiments animals consumed all concentrate and forage. No refusals were recorded. Compared with the control treatment, the supplementation of plant oils tended to increase milk yield. The improvement was statistically significant, except for the group with 3.5% RS supplementation. The increase in milk yield was followed by a tendency to increase milk solids expressed in grams per day, whereas neither RS nor LS supplements modified the percentage of milk constituents. Dietary supplementation of plant oils generally resulted in a decreased concentration of saturated fatty acids including short- and medium-chain ones and it elevated the total amount of monounsaturated fatty acids in milk compared with the control diet during the experimental period. Feeding RS characterized by high MUFA contents significantly increased total C18:1 and both *cis* and *trans* forms whereas LS enlarged total C18:1, *cis* and *trans* forms, except when 7% LS was supplemented. 3.5% of RS caused a higher decrease in saturated and a remarkably high increase in monoenes in comparison with 7% supplementation. The adverse relation was found when LS was added.

The C18:2 *c*9 *t*11 CLA increased as an effect of 3.5 and 7% RS supplementation and also 7% LS addition. The highest statistically significant increase

Item		Diets	
item	control (mean ± SD)	LS 3.5 (mean ± SD)	LS 7 (mean ± SD)
Σ saturated ¹	$62.2^{a} \pm 7.76$	$42.3^{ab} \pm 20.02$	$29.9^{b} \pm 8.45$
Σ short chain ²	$22.7^{a} \pm 6.66$	$12.3^{b} \pm 6.89$	$7.1^{b} \pm 3.38$
Sat C12 \times C14 and C16	$28.0^{a} \pm 0.09$	$19.9^{ab} \pm 11.35$	$16.8^{b} \pm 2.17$
C18:0	$11.5^{a} \pm 1.00$	$10.0^{a} \pm 1.11$	$6.1^{b} \pm 1.33$
Σ C18:1 ³	$19.1^{\rm c} \pm 2.09$	$43.8^{b} \pm 2.65$	$62.7^{a} \pm 1.59$
Σ C18:1 <i>cis</i> ⁴	$19.0^{\circ} \pm 2.09$	$43.2^{\rm b} \pm 2.37$	$62.5^{a} \pm 1.38$
Σ C18:1 <i>trans</i> ⁵	$0.4^{\mathrm{b}} \pm 0.47$	$1.04^{a} \pm 0.41$	$0.4^{b} \pm 0.48$
C18:2 <i>c</i> 9 <i>c</i> 12	$10.9^{\rm a} \pm 0.59$	$7.7^{ab} \pm 5.25$	$4.1^{b} \pm 2.00$
C18:2 <i>c</i> 9 <i>t</i> 11; CLA	$0.04^{\rm b} \pm 0.03$	$0.06^{ab} \pm 0.05$	$0.11^{a} \pm 0.02$
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	$6.7^{a} \pm 0.30$	$3.5^{b} \pm 2.36$	$2.8^{b} \pm 1.79$
$\Sigma \omega$ -3 ⁶	$6.7^{a} \pm 0.30$	$3.5^{b} \pm 2.36$	$2.8^{b} \pm 1.79$
$\Sigma \omega$ -6 ⁷	$11.6^{a} \pm 0.59$	$7.8^{ab} \pm 5.19$	$4.2^{b} \pm 1.83$
ω-6/ω-3	1.7 ± 0.25	2.2 ± 1.05	1.7 ± 0.50
Σ monounsaturated ⁸	$19.4^{\circ} \pm 2.43$	$44.2^{b} \pm 2.98$	$62.9^{a} \pm 1.82$
Σ polyunsaturated ⁹	$18.4^{a} \pm 0.33$	$13.5^{\rm b} \pm 4.44$	$7.1^{b} \pm 3.52$
Atherogenic index ¹⁰	$1.4^{\rm a} \pm 0.30$	$0.7^{\rm b} \pm 0.47$	$0.5^{b} \pm 0.11$
Thrombogenic index ¹¹	$0.8^{a} \pm 0.23$	$0.9^{a} \pm 0.31$	$0.4^{b} \pm 0.11$

Table 6. Effect of linseed oil (LS) supplementation on the fatty acid composition of sheep milk (FAME, %)

 $\begin{array}{l} {\rm FAME-fatty\ acids\ methyl\ esters;\ LS-linseed\ oil;\ ^1C8\times C10\times C12\times C14\times C16\times C18;\ ^2C8\times C10;\ ^3C18:1\ t6\times C18:1\ t7\times C18:1\ t9\times C18:1\ c6\times C18:1\ c9;\ ^4C18:1\ c6\times C18:1\ c9;\ ^5C18:1\ t6\times C18:1\ t7\times C18:1\ t9;\ ^6C18:3\ c9c12c15\ (\omega-3);\ ^7C18:2\ c9\ c12\ (\omega-6)\times C18:3\ c9c12c12\ (\omega-6);\ ^8C16:1\times C18:1\ t6\times C18:1\ t7\times C18:1\ t9\times C18:1\ c6\times C18:1\ c9;\ ^9C18:2\ c9\ c12\times C18:2\ c9\ t11\times C18:3\ c9c12c12\times C18:3\ c9c12c12;\ ^{10}(12:0\ +\ 4\times 14:0\ +16:0)/(MUFA\ +PUFA);\ ^{11}(C14:0\ +\ C16:0\ +\ C18:0)/(0.5\times MUFA\ +\ 0.5\times PUFAn\ -6\ +\ 3\times PUFAn\ -3\ +PUFAn\ -3\ /PUFAn\ -6\);\ means\ with\ the\ same\ letter\ are\ not\ significantly\ different;\ ^{a,b,c}P\ <\ 0.05 \end{array} }$

was observed when 3.5% of rapeseed oil was fed to animals.

Plant oils reduced the total concentration of polyunsaturated fatty acids, but only LS caused significant differences (P < 0.05). None of the plant oils used affected the ω -6/ ω -3 ratio that tended to decrease with increasing RS concentration and to increase when 3.5% of LS was supplemented. The values of atherogenic index were significantly decreased in both experiments in all experimental groups, however, a higher decline was obtained when 3.5% of RS and 7% LS were supplemented to the diets. Supplementation of RS decreased the values of thrombogenic index in both experimental groups whereas only 7% of LS reduced TI significantly.

DISCUSSION

The milk fat composition reflects the effect of the environment in terms of both the specific feeding system and more generally the type of rearing system, hence many factors such as season, feeding practices and breed may change the fat content and the milk fatty acid profile (Scintu and Piredda, 2007). The experimental factors that could change milk parameters used in this research were plant oils differing in C18:1 *c*9 and C18:3 *c*9 *c*12 *c*15 fatty acids.

The observed increase in milk yield was probably caused by the higher energy content of the oil-supplemented diet and not by the higher DM intake. A similar interdependence was also observed by Gómez-Cortés et al. (2008) when olive oil was supplemented to dairy ewe diets. Absence of changes in the percentage of milk constituents and some changes in their concentration expressed were also determined in experiments carried out by these authors.

The results of the present work confirmed that both rapeseed oil and linseed oil supplemented to sheep's diet may significantly ($P \le 0.05$) affect the milk fatty acid composition. Milk fat of supplemented diets for ewes had a lower saturated and a higher content of monoenic fatty acids. According to Harfoot and Hazlewood (1988) an increased concentration of dietary oleic acid results in an increased ruminal level of stearic acid, but other authors reported the conversion of oleic acid to a great number of *trans* monoenes (AbuGhazaleh et al., 2005). The study of Mosley et al. (2002) demonstrated the ability of mixed ruminal microbes to convert oleic acid to a multitude of trans positional isomers during the process of oleic acid biohydrogenation. A decrease in or stable concentration of stearic acid in milk samples of the experimental group is the evidence for not complete rumen biohydrogenation. The observed increase in the level of C18:1 trans fatty acids, especially when RS was supplemented, might be an effect of C18:1 *t*11 formation. Vaccenic acid, C18:1 t11, the most important oleic acid isomer serves as a precursor for the synthesis of saturated fatty acids in the rumen and of CLA at the tissue level (Mosley et al., 2002). During the metabolic process a part of vaccenic acid that escapes from the rumen is absorbed in the intestine and finally it is $\Delta 9$ -desaturated in the mammary gland into rumenic acid (C18:2 *c*9 *t*11), the major (more than 90% of the total) CLA isomer in milk (Griinari and Bauman, 1999). This isomer was also shown to have antioxidant and anticarcinogenic properties (Mc Donald, 2000; Mosley et al., 2002). The concentration of Σ C18:1 *trans* was significantly affected by RS supplementation, whereas only 3.5% of LS increased trans C18:1 acid content. Our earlier experiments on sheep (Szumacher-Strabel et al., 2008) also indicated that rapeseed oil (5% supplementation to the diet) may increase Σ C18:1 *cis* and C18:1 *c*9 in milk.

Oil supplements that were used in presented experiments are also a rich source of polyunsaturated fatty acids like linoleic (C18:2 c9 c12) and linolenic (C18:3 c9 c12 c15) acid. These are predominant polyunsaturated fatty acids in forages and oil seeds and are major substrates for biohydrogenation by rumen microorganisms. The biohydrogenation pathway for linoleic acid involves an initial isomerization step that results in the formation of c9 t11 CLA. Subsequent sequential reduction steps may convert CLA to C18:1 t11 and C18:0 (Carriquiry et al., 2008). Supplementation of RS led to a significantly increased content of C18:2 c9 t11 CLA in fat in comparison with the control diet, and also 7% of LS increased the C18:2 c9 t11 CLA level almost three times in comparison with the control. The more evident increase when RS was supplemented might result from its higher linoleic acid content in comparison with LS. Also in experiments by Castro et al. (2009) the supplementation of soybean oil, rich in linoleic acid, to ewe diets increased the C18:2 c9 t11 CLA content by 29% in comparison with the control diet. Milk from ewes fed the sunflower seed diet contained more

C18:2 *c*9 *t*11 CLA and less C18:3 *c*9 *c*12 *c*15 than milk from ewes fed the flaxseed diet (source of linolenic acid) (Zhang et al., 2006). In our research a significantly decreased content of linoleic acid was determined when linseed oil was added, whereas no changes were observed when RS, a rich source of oleic acids, was supplied. Production of improved ewe milk is of economic and health importance. In comparison with cow milk, which contains 0.6% CLA isomers with C18:2 *c*9 *t*11 CLA representing ~80% of this percentage, the CLA concentration in ewe milk was found to be around 1% of total fatty acids (Luna et al., 2005).

The supplementation of plant oils affected also the content of saturated fatty acids in milk, which confirms the potential for decreasing saturated fatty acids with lipid supplementation and thus, when the bioavailability of C18 fatty acids increases as a result of increased dietary intake, C10:0 to C16:0 de novo synthesis decreases as does their concentration in milk (Gómez-Cortés et al., 2008). This relation may be explained by the ruminal biohydrogenation of polyunsaturated fatty acids into e.g. C18:1 trans, which is one of the inhibitors of de novo fatty acid synthesis, mainly C8:0 to C16:0 (Chilliard et al., 2003). Both plant oils used as feed ingredients decreased the total content of saturated fatty acids in milk except 3.5% of LS that did not affect the content of this group of milk fatty acids.

Milk fat is usually considered to be proatherogenic, mainly because of the presence of a large amount of saturated fatty acids (mainly lauric, myristic and palmitic acid) (Valeille et al., 2006). We decided to test whether the dietary supplementation of plant oils may affect the milk atherogenic index (AI). The atherogenic index is a criterion for the level and interrelation of some fatty acids that may have atherogenic properties. We determined advantageous changes in milk AI when either RS or LS were supplemented. This may be related e.g. to the increased concentration of C18:2 c9 t11 CLA, which is now described as having antiatherogenic properties. The study of Valeille et al. (2006) suggested that feeding practices that improve the rumenic acid concentration in milk should be encouraged in the context of antiatherogenic action.

According to Ulbricht and Southgate (1991) C14:0, C16:0 and C18:0 fatty acids are thrombogenic. The inclusion of either RS or LS in ewe diets allows to decrease the concentration of saturated fatty acids and as an effect the value of TI. Huang et al. (2008) also showed the beneficial effect of dietary plant oil supplementation and stated that the addition of soy oil (source of C18:1 *c*9 and C18:2 *c*9 *c*12) decreased the thrombogenic (and atherogenic) index of dairy milk (P < 0.05).

Feeding diets rich in mono- and polyunsaturated fatty acids to lactating ewes under our experimental conditions resulted in demanded changes in milk fatty acid content without causing milk fat depression or modification of other milk constituents. Milk fat was improved in unsaturated fatty acids including C18:2 *c*9 *t*11 CLA isomer without changing the milk fat concentration. Hence a conclusion is drawn that plant oils may be an important source of monoenic and also polyenic fatty acids in milk, however our research and analysis of literature data indicate that results are strictly dietary and supplement-dependent.

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