N32.15

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EFFECT OF FEEDING RAPESEED AND LINSEED TO NURSING EWES ON DIURNAL CHANGES IN THE LIPID PROFILE OF MILK

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Recently intensified research has shown that rational use of oilseeds in the nutrition of ruminants, including sheep, can be an effective way of improving the quality of animal food products, particularly meat and milk. As regards milk, little is known about the highly complex processes of fat metabolism in ruminants (Jenkins 2004, Szumacher-Strabel 2005), and still less is known about the dynamic of changes in the lipid profile of milk according to frequency and time of its collection.

The aim of the present study was to make a preliminary determination of the effect of feeding rapeseed and linseed during the lamb suckling period on milk lipid profile and the dynamic of its diurnal changes.

Material and methods

A total of 12 nursing Kołuda ewes of prolific and milk variety were investigated during the 5th week of lactation. Sheep were kept indoors in groups and fed in accordance with the Nutrient Requirements of Cattle, Sheep and Goats (Normy Żywienia Bydła, Owiec i Kóz 2001). The level of experimental feeding to 5 months of pregnancy was consistent with physiological condition and litter size. Feeds contained grass and lucerne forage, ensiled sugar-beet pulp, grass hay and a concentrate mixture. Treatment was the composition of concentrate mixture. In the control group (C), animals received a standard diet based on cereals and rapeseed meal. In the experimental group (RL), sheep additionally received whole rapeseed and linseed at 100 and 50 g/animal/day. The components and nutritive value of the feeds given to groups C and RL are given in Borys et al. (2006b).

The entire daily ration was given to sheep at one feeding at 7 am. After eating the entire concentrate mixture, sheep were given the other feeds.

The fat content and fat lipid profile of milk produced by the ewes was analysed in 4 series at 6, 12, 18 and 24 hours postprandial. Lambs were allowed to suckle their mothers for 15 min per hour before every sampling of milk for analysis. The samples were taken from both udder halves of each ewe.

The feeds were analysed for fat content according to the method of Soxhlet and for fatty acid profile using gas chromatography according to Kramer et al. (1997) with modifications introduced by the Meat and Fat Research Institute in Warsaw (Borys et al. 1999). Fat extraction from the feeds for fatty acid determination was carried out using hexane in an ultrasound bath. Fatty acid content was determined using a Hewlett Packard model 6890 gas chromatograph with a flame-ionization detector and a 105 m × 0.25 mm × 20 μm Rtx-2330 column. Cholesterol content was determined using a Hewlett Packard 5890 sII with a flame-ionization detector and a 25 m × 0.20 mm × 0.11 μm HP-1 column.

Fat in milk was determined using a MilkoScan 133B analyser.

Milk fat was analysed for the composition of fatty acids, including c9t11 conjugated linoleic acid (CLA), and for total cholesterol content. Fat extraction from milk was carried out using the procedures of Folch et al. (1957), and fatty acids were determined using the same method

as for feeds.
Cholesterol content was determined using the method of Thompson and Merol (1993) with modifications introduced by the Meat and Fat Research Institute in Warsaw (Borys et al. 1999). Analysis was performed using a HP 6890 gas chromatograph with a flame-ionization detector and a 25 m × 0.20 mm × 0.11 μm HP-1 column. Operating conditions were as follows: injector 300°C, column 250°C, (4 min.) - 5°/min. - 300°C (5 min.), detector 310°C, carrier gas helium (100 kPa), splitter 25:1. Cholestan was used as the internal standard, which was added prior to extraction. Before sample analysis, the time of cholestan and cholesterol retention was determined by way of chromatographic analysis of the standard solution of these compounds after derivatization.
The results were analysed statistically using the ANOVA procedure of the Statistica 6PL packet for factorial designs: a two-factorial design (ewe feeding and series of observations) with interactions (Statistica - Przewodnik, 2000). Significant differences between the series of observations were estimated using Duncan's multiple range test.

Results and discussion

Fatty acid profile of the feeds - Table 1

Adding rapeseed and linseed to the RL concentrate diet increased the fat content almost two-fold compared to the C (control) diet. Fat of the RL diet differed markedly in the level of fatty acids in relation to the C diet. The proportion of oilseeds caused a clear decrease in the level of all the saturated fatty acids (SFA) determined except stearic acid (C18), the level of which was 23.8% higher in the RL diet than in the C diet. However, the total SFA content of RL fat was 36.4% lower than in C.

Table 1. Fat content and fatty acid profile in compounded diets

Item	Compounded diets			
	C		RL	
	- g in 100g of fat	- g in 100g of diet DM	- g in 100g of fat	- g in 100g of diet DM
Tłuszcz		2,20		4,27
Kwasy tłuszczowe:				
C 14:0	0,60	0,013	0,20	0,008
C 16:0	16,90	0,372	9,50	0,406
C 18:0	2,10	0,046	2,60	0,111
C 18:1	20,50	0,451	36,50	1,558
C 18:2	34,00	0,748	24,10	1,029
C 18:3	22,20	0,488	22,40	0,956
C 20:0	0,60	0,013	0,60	0,026
C 20:1	0,90	0,020	0,90	0,038
C 22:0	0,60	0,013	0,40	0,017
C 24:0	0,50	0,011	0,30	0,013
SFA	21,70	0,477	13,80	0,589
MUFA	21,90	0,482	38,40	1,640
PUFA	56,20	1,236	47,50	2,028
UFA:SFA	3,599		6,225	
PUFA:SFA	2,590		3,442	
PUFA:MUFA	2,566		1,237	

C - control, RL - experimental, with rapeseed and linseed, DM - dry matter
SFA = Σ: C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0,
MUFA = Σ: C16:1, C18:1, C20:1, C24:1, PUFA = Σ: C18:2, C 18:3; UFA = MUFA + PUFA

The total level of unsaturated fatty acids in RL fat was 10% higher than in C fat, but this was

due to a markedly (75.3%) higher level of monounsaturated fatty acids (MUFA), with a 15.5% lower level of polyunsaturated fatty acids (PUFA). These differences were nonexistent for gadoleic acid (C20:1) and linolenic acid (C18:3).
Due to the almost twice higher content of fat in the RL diet than in the C diet, the absolute amount of individual fatty acids and whole groups of fatty acids in feeds was different from their fat content. Only the level of myristic acid (C14:0) was lower in the RL diet than in the C diet (by 38.5%), while the level of margaric (C17:0) and lignoceric acids (C24:0) was similar. The RL diet, however, was a considerably richer source of all the other acids. Overall differences for SFA, MUFA and PUFA were 23.5, 240.2 and 64.1%, respectively. Such a large increase in the absolute MUFA content of the RL diet was due to the fact that oleic acid (C18:1) is the dominant fatty acid in rapeseed fat (Borys et al. 2006a), and linolenic acid (C18:3) is the dominant acid in linseed fat (Borowiec et al. 2001). Because a smaller linseed than rapeseed supplement was used in the experimental diet, the C18:3 content of dietary fat did not increase in relation to the C diet, nor did it decrease as in the second major PUFA acid, i.e. linoleic acid C18:2 (a 29.1% decrease).
Compared to the control diet, the experimental diet was characterized by much more favourable MUFA:SFA and PUFA:SFA ratios (higher by 73.0 and 32.9%, respectively), and less favourable PUFA:MUFA ratio (lower by 51.8%).
Overall, adding 100 g of rapeseed and 50 g of linseed to the feed caused significant changes in the structure (profile) of fatty acids in dietary fat and a general increase in the amount of acids (mainly MUFA and PUFA) taken by RL sheep in the daily ration.

Effect of feeding rapeseed and linseed on the fatty acid profile of milk fat

Feeding rapeseed and linseed caused a significant decrease in the content of almost all saturated fatty acids in milk fat (Table 2). The decrease was lower in the RL group than in the C group by 3.2% for C4:0 and by 33.6% for C10:0. One exception was stearic acid (C18:0), which was more abundant (by 54.4%) in the milk fat of RL ewes ($P \leq 0.01$). In general, however, RL milk fat contained 4.7% less SFA ($P \leq 0.01$). The generally lower SFA content of milk fat from ewes given oilseed was due to their lower supply in the RL diet than in the C diet. At the same time, however, the significantly higher content of stearic acid (C18:0) can be partly attributed to the higher supply of this acid in the experimental diet than in the control diet (by 141.3%; Table 1), and partly to the more intense processes of bacterial biohydrogenation of 18-carbon unsaturated acids (C18:1, C18:2 and C18:3) in relation to C18:0 acid in the rumen of RL ewes (Jenkins 2004, Szumacher-Strabel 2006).
Rapeseed and linseed in the RL diet had a significant effect on the increase in the total level of unsaturated fatty acids (UFA) in the milk fat of RL sheep, which was 8.7% higher than in the C group ($P \leq 0.01$) (Table 3). This difference resulted from the higher total MUFA content in the RL group (by 9.9%, $P \leq 0.01$), whereas the total PUFA content of the milk of both feeding groups was practically the same. Both in the group of MUFA and PUFA acids, relationships in the content of single fatty acids between RL and C groups were largely different. As regards MUFA, feeding rapeseed and linseed caused an increase in the level of the dominant oleic acid C18:1 (by 11.9%) and a decrease in C16:1 and C18:1T acids (by 15.8 and 12.1%, respectively). As regards PUFA, there was a significant increase in the level of linolenic acid and sum of Ω3 acids (by 38.8 and 30.3%), and a decrease in linoleic acid and sum of Ω6 – by 7.0 and 8.2%, respectively (Table 3). These differences were reflected in a generally more beneficial UFA:SFA ratio (higher by 13.2%, $P \leq 0.01$) and Ω6:Ω3 PUFA (lower by 30.4%, $P \leq 0.01$) in the milk fat of RL ewes, with similar PUFA:SFA ratios (Table 4).
The CLA content of milk fat from both ewe groups was very similar, but with a much higher fat content of milk from ewes fed rapeseed and linseed (6.90% in group RL vs. 5.58% in

group C; Borys et al. 2006b), with the absolute CLA content in the milk of RL ewes being 21.8% higher than in the milk of the control ewes (Table 4). The increase in the CLA content of ewe milk as a result of using rapeseed and linseed is reflected in the results of the majority of ruminant studies in this field (Reklewska and Bernatowicz 2002, Szumacher-Strabel 2005), while quantitatively the results obtained were intermediate in relation to those obtained earlier in our studies (Borys and Mroczkowski 2002, Borys et al. 2006a).

Table 2. Fatty acid content in sheep's milk fat; g/100 g

Trait	Feeding		Time after feeding [h] - Series of observation				SEM
	C	RL	6	12	18	24	
No of simples	24	24	12	12	12	12	
Σ SFA	63,82A	60,83A	59,75AB	63,82B	64,22A	61,51	0,586
C 4:0	2,49	2,41	2,70Aa	2,57B	2,42Ca	2,12ABC	0,048
C 6:0	2,16A	1,73A	1,77Aa	2,02a	2,07A	1,92	0,047
C 8:0	2,22A	1,58A	1,62ABC	1,96B	2,08A	1,93C	0,065
C 10:0	6,76A	4,49A	4,53ABC	5,87B	6,39A	5,71C	0,232
C 12:0	3,68A	2,52A	2,55ABC	3,14C	3,47A	3,25B	0,122
C 14:0	8,58A	7,50A	6,80ABa	8,42B	8,90A	8,03a	0,214
C 15:0	1,82A	1,67A	1,60Aa	1,77a	1,86A	1,74	0,031
C 16:0	23,36A	21,02A	20,78AB	22,82B	23,12A	22,04	0,327
C 17:0	2,52A	2,15A	2,48ab	2,24b	2,23a	2,38	0,042
C 18:0	9,80A	15,13A	14,38ABC	12,47Ca	11,14Aa	11,87B	0,461
Σ UFA	35,70A	38,81A	39,81AB	35,82B	35,39A	37,97	0,582
Σ MUFA	31,64A	34,76A	35,95AB	31,89B	31,28A	33,67	0,555
C 16:1	1,14A	0,96A	1,15A	0,95A	1,02	1,07	0,026
C 18:1T	2,40	2,11	2,18	2,17	2,25	2,41	0,080
C 18:1	29,13A	32,61A	33,57AB	29,71B	28,91A	31,29	0,556
Σ PUFA	4,06	4,05	3,87	3,95	4,10	4,30	0,068
C 18:2	2,56	2,38	2,38	2,38	2,48	2,62	0,048
C 18:3	0,49A	0,68A	0,52Aa	0,57b	0,59a	0,64Ab	0,018
Σ Ω6	2,81a	2,58a	2,60	2,62	2,71	2,82	0,052
Σ Ω3	0,76A	0,99A	0,79	0,87	0,90	0,93	0,026
CLA;							
- in fat; g	0,49	0,48	0,48	0,47	0,49	0,51	0,010
- in milk; mg	27,5A	33,5A	28,7	29,7	30,2	33,4	0,975
UFA:SFA	0,567A	0,642A	0,670AB	0,566B	0,556A	0,625	0,015
PUFA:SFA	0,064	0,067	0,065	0,062	0,064	0,071	0,001
PUFA:MUFA	0,130A	0,117A	0,108ABC	0,125C	0,132A	0,129B	0,002
PUFA Ω6:Ω3	3,804A	2,649A	3,414	3,179	3,138	3,174	0,120
Cholesterol*; mg/100 g milk	12,9	13,5	11,0A	15,4Aa	12,5a	13,7	0,548

C - control group, RL - experimental group; SEM - standard error of means
AA, BB, CC - P≤0,01; aa, bb - P≤0,05; * - interaction feeding x series significant at P≤0,05
SFA = Σ: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0,
MUFA = Σ: C10:1, C12:1, C14:1, C15:1, C16:1, C17:1, C18:1T, C18:1, C20:1,
PUFA = Σ: C18:2, C 18:3, C20:4, C20:5, C22:5, C22:6; UFA = MUFA + PUFA

In general, changes in the fatty acid profile of fatty acids in the milk of rapeseed- and linseed-fed ewes were similar to those obtained in our earlier studies (Borys and Mroczkowski 2002, Borys et al. 2005a) using the same oilseeds. However, the scale of results obtained in relation

to the control groups differed markedly in all these experiments. Large variation in the results of modifying the sheep's milk fatty acid profile using different sources of plant oils and different feeding conditions was shown by many studies (e.g. Antongiovanni et al. 2004, Borys et al. 2005b, Marciński et al. 2003, Szumacher-Strabel 2005) and review studies (Bencini and Pulina 1997, Borys 2006, Haenlein 1996).

Diurnal changes in the fatty acid profile of milk

Significant changes were observed in the fatty acid content of sheep's milk fat produced at 6, 12, 18 and 24 h postprandial (Tables 2-4).

The least SFA acids were found in milk fat at 6 h postprandial. In the next two series, their content increased significantly (by 6.8 and 7.5%, respectively, $P \leq 0.01$), and at 24 h they decreased to a level only 2.9% higher than at 6 h. For most of the individual acids from this group (from C6:0 to C16:0), these relationships were similar. The highest level of butyric acid (C4:0) was found in milk fat at 6 h, followed by a decrease in the next two series and a 21.5% lower value at 24 h than at 6 h ($P \leq 0.01$). Differences in the level of SFA with the longest carbon chains (C17:0 and C18:0) were of a similar nature. They were the most abundant in milk fat at 6 h, 7.9% higher on average than in the other series for C17:0, and 17.8% higher for C18:0.

In principle, diurnal changes in the total UFA and MUFA content corresponded to changes in the dominant oleic acid (C18:1) in UFA (Table 3). Oleic acid was the most abundant in milk fat at 6 h postprandial. There was a decrease at 12 and 18 h (by 12.7% on average, $P \leq 0.01$), and an increase at 24 h to a lower level than in the first series of observations. Differences for the other MUFA acids were less distinct (C16:1) or non-significant (C14:1 and C18:1T).

There were no statistically significant changes in PUFA content according to the time after feeding (Table 3). However, there was a general upward tendency in the successive series of observations, for the content of both individual and all PUFA acids (including Ω6 and Ω3 PUFA). Differences for linolenic acid C18:3 proved mostly significant, with a difference of 23.1% between the extreme series "6" and "24" ($P \leq 0.01$).

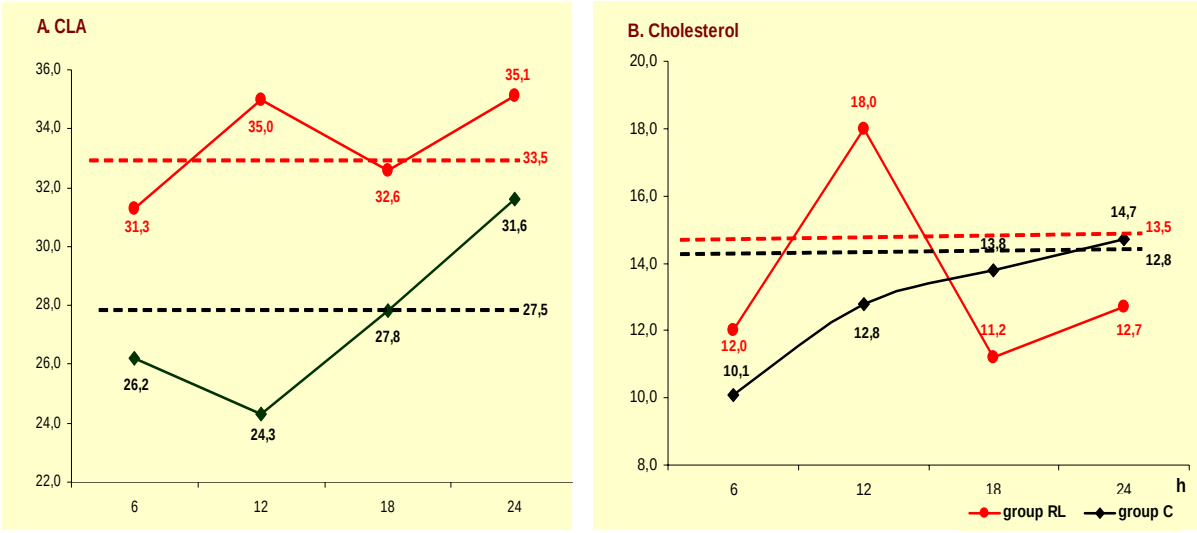
There were no significant differences in the CLA content of milk fat or milk depending on the time after feeding (Table 4). A more distinct difference between milk at 24 and 6 h (16.4%) resulted from a higher fat content (6.62 vs. 5.97%, respectively) (Borys et al. 2006b).

The above changes in the SFA and MUFA content in successive series of observations caused significant changes in UFA:SFA and PUFA:MUFA ratios, with a similar PUFA:SFA ratio (Table 4). The highest UFA:SFA ratio was found in milk at 6 h postprandial, with a significant decrease at 12 and 18 h (by 16.2% on average, $P \leq 0.01$) and an increase at 24 h postprandial to a 6.7% lower level (NS) than in the "6" series. In contrast, the PUFA:MUFA ratio in milk at 6 h was significantly lower than in the other, similar series of observations by 16.3% on average (all differences significant at $P \leq 0.01$).

With regard to the analysed fatty acids and parameters of fatty acid profile, there were no statistically significant feeding × series of observation interactions. Curves for diurnal changes in the milk of ewes of both feeding groups showed different levels but were of a similar nature. One non-significant exception was the CLA content (Fig. 1A). Differences in the CLA content of milk from RL and C ewes at 6, 18 and 24 h postprandial were similar and followed a linear pattern, with an average of 15.9% higher level in the RL group. At 12 h postprandial, there was an unevenly greater increase in the CLA content in the group fed rapeseed and linseed and a concurrent decrease in group C, which resulted in a difference of 44% in CLA content.

The lack of comparable literature data regarding the dynamic of diurnal changes in the fatty acid content of milk of sheep and other ruminants prevents a discussion of the results obtained.

Figure 1. Diurnal changes of CLA and cholesterol content in milk; mg/100g



Effect of feeding rapeseed and linseed on diurnal changes in milk cholesterol

The diets used were found to have no great effect on the cholesterol content of sheep's milk. There were significant differences in cholesterol level depending on the series of observations. The observed differences were non-linear (Table 4). The highest cholesterol level was found in milk at 12 h and the lowest at 6 h postprandial (a difference of 40.0%, $P \leq 0.01$).

For cholesterol, there was a significant feeding group × series of observation interaction, resulting from the fact that while the cholesterol content of milk of the control (C) ewes increased linearly from 6 to 24 h postprandial (a difference of 45.5% between these series), the changes were uneven in the RL group (Figure 1B). At 6, 18 and 24 h, fluctuations in the cholesterol content of RL milk were relatively lower and cholesterol level was not markedly different from the level in the milk of ewes from the C group (12.0 vs. 12.9 mg/100 g on average), while at 12 h postprandial the cholesterol content of RL milk was clearly the highest (40.1% higher than in group C).

In our earlier studies (Borys and Mroczkowski 2002, Borys et al. 2005a) using the same proportions and amounts of rapeseed and linseed, no significant differences in the content sheep's milk cholesterol were found. The available literature contains no data on diurnal changes in the cholesterol content of sheep's milk.

Conclusions

1. Adding rapeseed and linseed to the diets of ewes (group RL) at 100 and 50 g/animal/day, respectively, caused significant changes in the fatty acid profile of dietary fat and a general increase in the amount of fatty acids (mainly MUFA and PUFA) taken by RL sheep in daily ration.
2. Feeding the RL diet compared to the control diet had a clear and generally favourable effect on the fatty acid content of milk fat and its health parameters.
3. Differences were observed in the dynamic of diurnal changes in the milk fat of sheep according to the time after feeding. A more distinct effect of feeding rapeseed and linseed on the dynamic of these changes was observed for the CLA and cholesterol content of milk.

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