THE INFLUENCE OF BY-PASS FATS USED IN EWES' DIET ON THE PRODUCTIVE PERFORMANCES AND ON THE FATTY ACIDS PROFILE FROM MILK

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Abstract

Diet has sovereign effects on milk yield and on fatty acids profile from milk fat, influencing the concentration of n-3 polyunsaturated fatty acids (n-3 FA) and conjugated linoleic acid (CLA), which turned out to be beneficial for human health. However, the effects of by-pass fats on production and on fatty acids profile of ewe milk have received little attention. The aim of study was to evaluate the effects of dietary supplementation of lactating ewes with sunflower oil treated with calcium salts (saponified fats or calcium soap - CS) at a rate of 6% (% of concentrate), thus the biohydrogenation processes of fatty acids were reduced in the rumen. It has been studied: a) milk production and chemical composition of milk; b) milk fat content of fatty acids with special reference to n-3 FA and CLA. 24 Ţurcană ewes (2-4 lactation) were fed using two diets (12 ewes/ diet): the control diet (no CS supplementation) and the experimental diet, which was supplemented with CS, for 14 weeks. Milk production, its chemical composition and feed intake were recorded weekly. Milk fat content of fatty acids was determined by gas chromatography using a Shimadzu GC-17A gas chromatograph, equipped with a Chrompack capillary column. Ewes' diet supplementation with CS resulted in a decrease in daily DM intake by 16.9%, but increased milk production on test day with 8.02% and milk fat content by 2.62%, while the protein level (N x 6.38) and lactose did not registered statistically insured change ($p > 0.05$). Ewes receiving saponified fats (SC) showed a tendency to produce milk with a lower content of saturated FA, especially capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0) and higher in polyunsaturated FA (with 12.5%), mainly in CLA *cis*-9, *trans*-11 and α - linolenic acid (ALA) (C18: 3), as the main representative of n-3 FA family. Atherogenic index was lower ($p < 0.05$) in milk fat from ewes for which it was used CS (2.20 vs. 2.57) in the food; fats with lower atherogenic index are less harmful to human health. The results seem to indicate that increasing CLA cis-9, trans-11, in fat milk following the introduction of CS in food, is due to increased feed intake of VA (C18:1 *trans*-11) in rumen and, also, to the intensified activity of Δ^9 – Desaturase enzyme.

Key words: **c**alcium soaps, dairy ewes, performance, fatty acids profile from milk

It is widely recognized that alimentary factors are sovereign in the modulation of fatty acid profile of milk from cows (Kay, J.Ket al., 2005), goats (Chilliard, Y. Et al., 2003, Andrade, P.V.D., Schmidely, Ph., 2006) and sheep (Addis, M., et al., 2005, Gomez-Cortes, P. et al., 2008, De La Fuente, L.F. et al., 2009) By manipulating the diet, milk fat content in the n-3 FA and CLA can be changed up to five times (Bauman, D.E et al., 2006, Gomez-Cortes, et al., 2008, Gomez-Cortes, P. Et al., 2009, De La Fuente, et al., 2009). The largest increases of n-3 FA and CLA concentrations in ewe milk fat is obtained through the use of fresh grass in food , which is rich in polyunsaturated fatty acids (PUFA) and it forms a larger amount of intermediates as VA (C18: 1 trans-11) (Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Piris,i A., Molle, G., 2005, Mikolayunas, C. M., Thomas, D. L., Albrecht, K. A., Combs, D.K., Berger, Y. M., Eckerman, S. R., 2008, Hervas, G., Gomez-Cortes, P., De La Fuente, M.A. Mantencon, A.R., Juarez, M., Giraldez, F.J., Frutos, P., 2009) through rumenal biohydrogenation or by supplementing the diet with fat (Mele, M., Serra, A., Conte, G., Pollicardo, A., Del Viva, M., Secchiari, P., 2007, Mele, M., Buccioni, A., Petacchi, F., Serra, A., Banni, S., Antongivanni, M., Secchiari P., 2006, Gomez-Cortes, P., Frutos, P., Mantecon, A.R., Juarez, M., De La Fuente, M.A., Hervas, G., 2008). By-pass fats, protected against rumenal fermentation, most frequently by treatment with calcium salts of fatty acids (calcium soap), are used in the diet of diary ewes to enlarge the concentrations of dietary energy in early lactation when appetite is low (Mele, M., Buccioni, A., Petacchi, F., Serra, A., Banni, S., Antongivanni, M., Secchiari P., 2006) or to improve the milk fatty acid profile by increasing the share of n-3 FA and CLA (conjugated linoleic acid), which have

beneficial effects on human health. The aim of this study was to evaluate the effects of dietary supplementation of lactating ewes with sunflower oil treated with calcium salts (saponified fats or calcium soap - CS) at a rate of 6% (% of concentrates mixture), respectively 48g of calcium salt/head/day, thus the biohydrogenation processes of fatty acids were reduced in the rumen. There has been followed: a) milk production and chemical composition of milk; b) milk fat content of fatty acids with special reference to n-3 FA and CLA.

MATERIAL AND METHOD

24 Ţurcană ewes were used (43.12 ± 1.31 kg), multiparous (2-4lactation), randomizly distributed into two groups and assigned to two diets (12 sheep per diet): the control diet (without addition of CS) and the experimental diet (CS), which was supplemented with 6% CS (% of concentrates mixture). Feeding was done *ad libitum* with alfalfa hay and pasture hay, then added concentrated in quantity of 800 g/day *(tab. 1)*, thus providing for ewes in the experimental group a supplement of 48 g of saponified fat/head/day.

Table 1

Ingredients and chemical composition of the experimental diets

	Diets[1]	
	Control	CS
Alfalfa hay	*ad libitum*	*ad libitum*
Mowing grass	*ad libitum*	*ad libitum*
Concentrate mixture (g/day):	800	800
-Structure of concentrates mixture (%):		
Corn	37.0	30.0
Triticales	18.0	18.0
Barley	18.0	18.0
Soy oil cakes	26.0	27.0
Saponified fat	-	6.0
Premix vit.- mineral	1.0	1.0
-Mixture of concentrates nutritional characteristics:		
Crud protein (CP) - %	18.26	18.15
Ether extract - %	2.60	8.32

[1] *Control diet (without addition of saponified fat); CS diet supplemented with 6% saponified fat (6% of concentrates mixture)*

The study was conducted after weaning lambs in April-August 2010. The experiment lasted 14 weeks, from witch the first three were to accommodate the digestive microflora to the new diet. After the induction period, it was daily recorded the DM intake and milk production and weekly milk samples were collected to determinate the chemical composition and the fatty acid profile (FA) from milk fat. For the analysis there were used milk samples consisting of milk milked in the morning and in the evening, at the constant ratio of 60:40 (am: pm). Milk samples for FA analysis were immediately frozen at – 20°C until analysis, and milk samples for analysis of chemical components were preserved with 2-bromo-2-nitropropane-1-2-diol and stored at 4°C. From the collected milk samples there was determined the fat (Gerber method), the total nitrogen (Kjeldahl method), the lactose (infrared method, Combifoss 4000 FOSS, Hillerød, Denmark) and the FA content of milk fat.

Milk samples collected for FA analysis were thawed in a water bath at 35°C. Total milk fat was extracted using the method recommended by Luna et. al. (2005). Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides according to ISO-IDF. Separation of FAME was performed using a gas chromatograph SHIMADZU GC-17A equipped with a capillary column CHROMPACK length of 25 m and diameter 0.25 mm, stationary phase (a derivative of polyethylene glycol) was deposited inside the column as a 0.2 mm thin film. A FID detector was used and the mobile phase was 99.9% pure helium.

Operating parameters of gas chromatograph were as follows: injector and detector temperature – 260°C, column temperature - a platter of 5 minutes at the initial temperature of 150°C and a temperature gradient of 4°C per minute until 235°C, carrier gas flow rate - 1.9 ml per minute and a split ratio of 1:19. After gas chromatography reached the programmed operating parameters, there was manually injected 0.5 ml solution of fatty acid methyl esters using a Hamilton syringe. Individual FA were identified by comparing the retention times to those of standards (Sigma-Aldrich, St. Louis, USA) and expressed in g/100 g of total fatty acid methyl esters.

Data on DM intake, milk production, the chemical composition and the FA content of milk fat were processed by the GLM procedure of SAS (version 8.0, SAS Inst., Inc., Cary, NC) for repeated measurements. Differences determined by supplementing the ewes' diet with fat saponified were considered significant at $p < 0.05$. The values presented are least square means followed by standard error of mean.

RESULTS AND DISCUSSIONS

Average results for DM intake, milk production and its chemical composition are presented in *table 2*.

Table 2

Dry matter intake, and milk yield and composition in ewes fed the experimental diets

	Treatment[1]		SED[2]	p-value
	Control	CS		
Dry matter intake, g/d	2576	2141	112.7	*
Yield, g/d:				
- Milk	542.7	586.3	134.0	*
- Protein (N x 6.38)	33.10	36.53	5.62	NS
- Fat	37.12	41.16	6.80	*
- Lactose	26.43	27.91	4.91	NS
Composition, g/100 g raw milk:				
- Protein (N x 6.38)	6.10	6.23	0.081	NS
- Fat	6.84	7.02	0.212	*
- Lactose	4.87	4.76	0.114	NS

[1] *Control diet – with no oil supplementation; CS – diet supplementation on 6% oil by-pass (42 g/d on oil by-pass).*
[2] *SED – standard error of the difference.*

Ewes' diet supplementation with CS resulted in a decrease in daily DM intake by 16.9%, but increased milk production on test days with 8.02% (542.7 ml/d vs. 586.3 ml/d) and milk fat content by 2.62 %, while the level of protein (N x 6.38) and lactose did not registered statistically insured change ($p > 0.05$). The current study results are consistent with those found by Gomez-Cortes et. al., 2008 in terms of DM intake and milk production, noting that ewes' diet supplementation with olive oil led to lower proportion of protein, in contrast to our study that found no significant changes in this respect. These differences are probably determined by the quantity of olive oil used in food, 6% of DM (Gomez-Cortes et. al., 2008), which led to disturbances in the process of microbial protein synthesis in the rumen.

Ewes receiving saponified fat (CS) in food showed a tendency to produce milk with a lower content of saturated FA, especially capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0) and higher in polyunsaturated FA (6.88 vs. 7.74 g/100 g of total fatty acid methyl esters), mainly in the CLA cis-9, trans-11 and α - linolenic acid (ALA) (C18:3) as the main representative of the n-3 FA family (*table 3*). Short and medium chain FA (C4:0 - C14:0 and approximately half of C16: 0) are the result of de novo synthesis in the mammary gland, based on acetic acid and ß - hydroxybutyrate resulted in rumen fermentation, being influenced by the energy balance of food and also by specific rumenal microflora (Chilliard, Y., et al, 2003), Addis et al., 2005 found that ewes which have a higher milk production also have a higher concentration of SCFA and MCFA in milk. This conclusion was not confirmed in our studies, where the highest level of SCFA and MCFA in milk was associated with lowest milk production. Long chain fatty acids (LCFA) in milk fat had an opposite SCFA and MCFA tendency. This group of fatty acids was significantly influenced by the saponified fat food supplement, the highest value being recorded in ewes' milk from the experimental group (CS) ($p < 0.01$).

Increasing global concentrations of total PUFA in milk fat from ewes in whom food saponified fat was introduced 12.5%, is considered beneficial to human health.

Milk fat content in the n-3 FA was influenced by the food saponified fat supplement, these FA have alimentary origins, and their concentration in milk is dependent on their amount in the rumen. Values obtained in this study for milk fat content in n-3 FA and especially ALA (C18: 3) are comparable to those reported in previous studies (Dimitrov, et al., 2001, Mihaylova, G., et al, 2004, Mele, M. et al, 2006, Federica, S., et al, 2008, Gerchev, G., et al., 2009, De La Fuente, L.F., et al., 2009, Sanchez, J.P., et al., 2010).

n-6/n-3 FA ratio was lower in milk fat in ewes receiving the saponified fat (CS) in food (1.09/1 vs. 1.60/1), being closer to the suggested optimal human diet 1: 1 (Simopoulos, A.P., 2008**).** Thus, it has been confirmed the findings of numerous previous studies [5, 9] that have shown that ewe milk is an important source of n-3 FA in humans.

Food supplementation by saponified fat significantly influenced the concentration of milk fat in CLA cis-9, trans-11, the highest values being registered in CS ewes ($p < 0.05$). We found a close relationship, first between VA (C18: 1 trans-11) and CLA cis-9, trans-11 from milk fat and on the

other hand between the CLA cis-9, trans- 11 and Δ^9 – Desaturase system (*tab. 3*), these relationships are in agreement with those determined by Kay et. al., in cow milk fat (r = 0.16-0.63). The results seem to indicate that increasing CLA cis-9, trans-11, in fat milk following the introduction of CS in food, is due to increased feed intake of VA (C18:1 *trans*-11) in rumen and, also, to the intensified activity of Δ^9 – Desaturase enzyme.

Table 3

Effect of saponified fat (CS) supplement in food on the fat content of fatty acids in ewe milk (g/100 g of total fatty acid methyl esters)

	Diets[1]		SEM	p-value
	Control	CS		
C4:0	4.93	3.11	0.17	*
C6:0	2.78	2.31	0.22	*
C8:0	2.05	2.40	0.13	NS
C10:0	6.57	6.07	0.73	NS
C12:0	3.57	2.62	0.17	*
C14:0	11.14	10.24	0.33	*
C15:0	1.56	1.44	0.07	NS
C16:0	24.62	26.70	0.40	**
C17:0	0.96	0.92	0.07	NS
C18:0	13.52	12.45	0.24	*
C14:1	0.22	0.17	0.03	NS
C16:1	0.54	0.78	0.02	*
C18:1 trans-9	0.33	0.49	0.01	**
C18:1 trans-11 (VA)	6.15	8.88	0.41	**
C18:1 cis-9	13.76	13.19	0.55	NS
C18:1 cis-11	0.42	0.49	0.07	NS
C18:2 n-6 trans	0.44	0.34	0.06	NS
C18:2 n-6 cis	2.30	2.08	0.07	NS
C18:2 c-9, t-11 CLA	2.15	2.84	0.05	*
C18:3 n-3	1.42	1.85	0.04	*
C20:4 n-6	0.17	0.14	0.01	NS
C20:5 n-3, EPA	0.09	0.12	0.01	NS
C22:3 n-3	0.07	0.08	0.00	NS
C22:5 n-3, DPA	0.15	0.20	0.01	*
C:22:6 n-3, DHA	0.09	0.09	0.00	NS
SCFA	16.33	13.89	1.02	**
MCFA	56.13	55.32	0.64	NS
LCFA	27.54	30.79	1.12	**
SFA	71.70	68.17	0.97	*
MUFA	21.42	24.09	0.68	**
PUFA	6.88	7.74	0.11	*
n -3 FA	1.82	2.34	0.08	*
n - 6 FA	2.91	2.56	0.07	NS
n – 6/n - 3	1.60	1.09	0.03	*
Atherogenic index[2]	2.57	2.20	0.14	*
Δ^9 – Desaturase ratios[3]				
C14	0.019	0.016	0.002	*
C16	0.021	0.028	0.002	*
C18	0.60	0.64	0.03	NS
CLA	0.24	0.26	0.015	NS
Index Δ^9 – Desaturase[4]	0.30	0.32	0.018	*

*[1] Control diet – with no oil supplementation; CS – diet supplementation on 6% oil by-pass (42 g/d on oil by-pass). SCFA = short-chain fatty acids (C4 – C11); MCFA = medium-chain fatty acids (C12 – C16); LCFA = long-chain fatty acids (C17 – C24) (Addis et. al. 2007); SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; [a, b, c]: means with different superscripts differ significantly; SEM = standard error of mean; NS p>0.05, *p<0.05, **p<0.01, ***p<0.001; [2] Atherogenic index was calculated according to Chilliard et. al., (2003), as follows: (C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA); [3] Calculated for each pair of FA according to Kelsey et. al., (2003) as: (product of Δ^9 – desaturase)/ (product of Δ^9 – desaturase + substrate of Δ^9 – desaturase); ie: C14: C14:1/ (C14:1 + C14:0); [4] Calculated according to Kay et. al., (2005), as follows: (C14:1 + C16:1 + C18:1 cis-9 + CLA cis-9, trans-11)/(C14:0 + C16:0 + C18:0 + C18:1 trans-11 + C14:1 + C16:1 + C18:1 cis-9 + CLA cis-9, trans-11).*

Atherogenic index, which characterizes the food fats in terms of impact on human health, was lower (p <0.05) in milk fat from CS ewes compared to the control group (2.20 vs. 2.57); there is a direct correlation between the saturated FA content and the value of this index. Fats that have a higher atherogenic index are harmful to human health.

CONCLUSIONS

Ewes' diet supplementation with by-pass fats resulted in a decrease in daily DM intake by 16.9%, but increased milk production on test days with 8.02% and milk fat content by 2.62%, while the level of protein (N x 6.38) and lactose did not registered statistically insured change ($p > 0.05$).

Ewes receiving saponified fat (CS) in food showed a tendency to produce milk with a lower content of saturated FA and higher in polyunsaturated FA (by 12.5%), mainly in CLA cis-9, trans-11 and α - linolenic acid (ALA) (C18:3) as the main representative of the n-3 FA family.

Atherogenic index was lower ($p < 0.05$) in ewes' milk fat in whom food CS (2.20 vs. 2.57) was used; fats that have a lower atherogenic index are less harmful to human health.

The results seem to indicate that increasing CLA cis-9, trans-11, in fat milk following the introduction of CS in food, is due to increased feed intake of VA (C18:1 *trans*-11) in rumen and, also, to the intensified activity of Δ^9 – Desaturase enzyme.

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