DIETARY EFFECTS ON MILK YIELD, RUMEN FERMENTATION AND MILK FATTY ACID COMPOSITION IN THE MILK OF TURCANA EWES

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Abstract

The primary obiective of this trial was to evaluate the effect of energy and protein content in the diet on the FA profile of milk fat from ewes, especially polyunsaturated fatty acids Omega 3 (C18:3, EPA, C22:3, C22:5 and DHA) and CLA (conjugated linoleic acid C18:2 n-9c, 11t). A secondary obiective was to effect with different level energy and protein on diet of caracteristics fermentation ruminal, yield and quality of sheep milk. Consequently, experimental diets were constituted by a 2 x 2 factorial arrangement of energy level in diets (Low – 0,90 UFL/kg DM vs. High – 0,97 UFL/kg DM) and protein level (Low – 14% CP vs. High – 16% CP). On a dry matter (DM) basis, forage : concentrate ratios were 73 : 27 (low energy diets) and 57 : 43 (high energy diets). Protein level diet were given either with soybean meal (5,5% of DM) or without. Increased levels of protein and energy of diet in lactating sheep, influenced positively the quantitative and qualitative production of milk and, in particular, the profile of fatty acids. So, the average daily production of milk increased up to 14.7% ($p \leq 0.05$) and, also, fat, protein and lactose content, but it decreased the casein content(which could reduce the processing performance of milk in cheese) and nonproteic nitrogen. After feeding, ewes fed the high energy and protein diets had higher proportion of propionate and lower proportion of acetate and acetate/propionate ratio than those fed the low energy and protein diets. Sheep fed the high energy and high protein diets had lower NH_3-N in ruminal fluid, than those fed the low diets ($p \leq 0,05$). Nutritional quality of sheep milk has been improved as a result of declining the degree of fats saturation, by reducing the proportion SFA which are responsible for the occurrence of cardiovascular diseases in humans, and increasing share of PUFA and especially those of Omega 3 type (C18:3, EPA, C22:3, C22:5 and DHA) and CLA (conjugated linoleic acid – C18:2 n-9 cis, 11 trans), which have an important role in preventing and combating cardiovascular disease, and CLA has anticancerous and antidiabetic effect. The highest milk quality, analyzed through the influence on human health, determined by a high content of Omega 3 fatty acids and CLA, was obtained from sheep fed with a ration of high energy and protein level (0,97 UFL/kg and 16% CP of DM). At the same lot it has been also recorded the best proportion PUFA n-6/n–3 and the lowest atherogenicity index of milk fats.

Key words: PUFA Omega 3, CLA- conjugated linoleic acid, milk fat, energy and protein in the diet, dairy ewes.

INTRODUCTION

The nutritional quality of animal products is a very important parameter, especially with regard to the links between food and health, a vital research field today. Modifying feeding practices for dairy animals aims to increase the amount of unsaturated fatty acids in milk fat. The international medical-scientific world now holds saturated fatty acids partially responsible for some diseases, especially those of a cardio-vascular nature, and ewes milk is particularly rich in these fatty acids (Ney, 1991; Strata, 2000; Martni, 2004).

The type and proportion of forages and concentrates in the diet may also have a significant effect on the yield and composition of the milk given by dairy ewes (Kukuk et al., 2001; Mele et al., 2006; Cannas et al., 1998; Guitard et al., 1996; Bocouier et al. 2001; Bencini et al., 1997). The concentration of fat in the milk is correlated positively with the concentration of fibre in the diet. Bencini et al. (1997) have calculated the relationship between milk fat and NDF in the diet (fat% = 4,59 + 0,05 NDF; r = 0,48). The protein content of the diet affects the quantity and the partition of nitrogenous substances in the milk; that milk protein was significantly reduced if ewes were fed a protein deficient diet. Milk yield and concentration of milk fat can be increased by increasing the protein content of the diet (Bencini et al. 1997). Little research has been done on the composition of fatty acids of sheep milk, but the diet seems important on this regard. Gargouri, 2005, raported that sheep milk fat contains high procentages of palmitic and oleic acid, while essential fatty acids, linoleic acid and linolenic acid are low. If the ewes are underfed and mobilize their body reserves, the proportions of high molecular weight fatty acids are increased (Bocquier et al., 2001).

The primary obiective of this trial was to evaluate the effect of energy and protein content in the diet on the FA profile of milk fat from ewes, especially polyunsaturated fatty acids Omega 3 (C18:3, EPA, C22:3, C22:5 and DHA) and CLA (conjugated linoleic acid C18:2 n-9c, 11t). A secondary obiective was to effect with different level energy and protein on diet of caracteristics fermentation ruminal, yield and quality of sheep milk.

MATERIAL AND METHODS

Researches were conducted on 48 lactating Ţurcană sheep (with a live weight of 46,4 ± 4,2 kg and milk yield 0,61±0,12 kg), being at lactation 3 and 4, respectively, months 2-4 of lactation. The sheep were divided into 4 groups of 12 sheep/lot in a preexperimental period of 14 days, when feeding was made with a basic portion and it was followed the average milk yield per lot, so it does not differ significantly from a lot to another. The animals were kept in collective boxes (one box for a lot) on the permanent straw bedding, and food was administered twice daily (at 7.00 and 18.00), ad libitum.

The analytical composition of the diet (table 1) have also been reported. The 4 experimental diets differed in terms of level energy and protein, of forage/concentrate ratio respectively (diet composition expressed as the percentage of dry matter):

- A diet with low energy and low protein (forage/concentrate ratio – 73 : 27), made up of forage (73%), corn grein (18,8%) and triticale meal (7,2%);
- A diet with low energy and hig protein (forage/concentrate ratio – 73 : 27), made up of forage (73%), corn grein (13,3%), triticale meal (7,2%) and soybean meal (5,5%);
- A diet with high energy and low protein (forage/concentrate ratio – 57 : 43), made up of forage (57%), corn grein (32,6%) and triticale meal (9,4%);
- A diet with high energy and high protein (forage/concentrate ratio – 57 : 43), made up of forage (57%), corn grein (29,2%), triticale meal (7,3%) and soybean meal (5,5%).

Table 1

Feed ingredients of the experimental diets of dairy ewes (% DM)

Energy lelel	**Low** – 0,90 UFL		**High** – 0,97 UFL	
Protein level	14% CP	16% CP	14% CP	16% CP
Grass hay	13,8	13,8	8,9	8,9
Mixed grass pasture	28,6	28,6	18,5	18,5
Alfalfa grass	30,7	30,7	29,8	29,8
Corn grein	18,8	13,3	32,6	29,2
Triticale meal	7,2	7,2	9,4	7,3
Soybean meal	-	5,5	-	5,5
Minerals and vitamins[1]	1,0	1,0	1,0	1,0
Forage : concentrate	***73 : 27***	***73 : 27***	***57 : 43***	***57 : 43***

1 : Contains (g/kg): I, 1,22; Mn, 103; Zn, 110; Fe, 137; Cu, 16; Co, 0,35; Se, 0,31; (IU/kg): vitamin A, 11000; vitamin D, 3200; vitamin E, 56.

Forage (grass hay, mixed grass pasture and lucerne grass) was fed separately twice a day, controlling the actual intakes of both forage and concentrate.

The chemical composition of the diet was determined according to AOAC methods (AOAC, 1990). Fiber fractions (NDF and ADF) were analyzed according to the method described by Van Soest et al. (1991). Non structural carbohidrates (NSC) were calculated according to Van Soest et al. (1991). Net energy for lactation (NE_L) and intestinal digestible protein

(PDIN and PDIE) were estimated from INRA tables (1989). Extraction of lipid from the diets was performed according to Folch et al. (1957).

Experimental diets were constituted by a 2 x 2 factorial arrangement of level energy in diets (Low – 0,90 UFL/kg DM vs. High – 0,97 UFL/kg DM) and level protein (Low – 14% CP vs. High – 16% CP). After a 10-day adaptation period to the diets experiments, meant to let the rumen microbes get accustomed to the changed diet.

The animals were milked twice daily (at 06:00 h and 18:00 h). The two samples were gathered in a single sample according to the morning and afternoon yield. Milk samples were then analyzed to determine fat matter (Gerber method), total nitrogen (Kjeldahl method, N x 6,38 = total protein), lactose (infrared method, Combifoss 4000 FOSS, Hillerod, Denmark), and fatty acids composition. On week 6 experiments, ruminal fluid was colected before and 2 h after the morning feeding. Following immediate determination of pH, ruminal fluid was acidified with 25% (wt/vol.) H_3PO_4 and frazen until analysis of ammonia (Wheatherburn, 1967) and VFA (Kristensen et al. 2000).

Milk fat was extracted using ethanol and hexane as extraction solvent mixture, and fatty acid methyl esters were obtained according to Chin et al. (1992). Separation and quantification of the methyl esters were carried aut using a Perkin Elmer Auto System gas chromatography fitted with Omega Was 320 capillary column, FID type detector, and helium as gas carrier, according to Secchiari et al., 2001.

A mixture of fatty acids standards (Sigma-Aldrich) was used for the calibration and identification of single peaks according to the relative retention times; quantified with respect to the following internal standards: C5:0 (C4:0 – C8:0), C13:0 (C10:0 – C17:0) and C18:0. Since the gas chromatographic analysis with a 30-m length column did not allow the separation of cis and trans isomers, the sum of positional and geometrical isomers of C18:1, C18:2 (CLA) was evaluated.

Ruminal samples obtained before and 2 h after feeding were analyzed separately with a model including the fixed effects of energy level, protein level diet and their interaction. All other data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., 1999). The statistical model included energy level, protein level, energy x protein interaction and residual error. Data are reported as least squares means ± SEM. Overall diferences between treatment means and interaction for level of energy leve land protein level were considered to be significant when $p \leq 0,05$.

RESULTS AND DISCUSSION

Portions with high energy value (0.97 UFL / kg DM) are characterized by a lower content of fibers (NDF and ADF) and a higher content of ether extract (EE) and nonstructural carbohidrates (NSC), comparing to low energy value portions(table 2). Increasing the energy value of forage ratios, attracted by itself an improvement of the profile of fatty acids from food by increasing the share of polyunsaturated fatty acids (PUFA) to the detriment of saturated fatty acids (SFA) (table 3).

The increase of food protein level from 14% CP to 16% CP, by introducing soybean meal (5.5% of DM), regardless of energy level, it determines the increase of ether extract (EE) share and the decrease of non-structural carbohidrates (NSC) proportion.

Table 2

Chemical composition and nutritional values of experimental diets (% DM)[1]

Energy lelel	**Low** – 0,90 UFL		**High** – 0,97 UFL	
Protein level	14% CP	16% CP	14% CP	16% CP
CP (crude protein)	14,18	16,10	13,87	16,17
NDF	44,10	44,0	35,4	35,1
ADF	26,90	26,60	24,70	24,40
NSC	16,4	14,2	34,6	31,4
EE	1,72	2,17	2,52	3,08
PDIE (g/kg DM)[2]	90,1	98,6	94,3	112,8
PDIN (g/kg DM)[2]	86,1	102,4	89,3	108,7
NE_L (kcal/kg DM)[3]	1547	1561	1664	1678

[1] Data presented are least square means (n = 4 samples per diet). [2]PDIN and PDIE = digestible CP in the intestine from microbial protein synthesis when availability of fermentable N in the rumen is limiting, and from microbial protein synthesis when availability of energy in the rumen is limiting, respectivively (INRA, 1989). [3] Calculated values (INRA, 1989).

Table 3

Fatty acids composition of the four experimental diets (% of total fatty acid)

Energy lelel	**Low** – 0,90 UFL		**High** – 0,97 UFL	
Protein level	14% CP	16% CP	14% CP	16% CP
C 14:0	0,79	0,39	6,46	0,21
C 16:0	17,89	9,95	16,39	8,45
C 16:1	0,40	0,32	0,27	0,32
C 18:0	1,90	1,69	3,87	1,62
C 18:1	35,1	38,16	30,51	34,96
C 18:2	19,50	23,40	24,41	32,16
CLA – C 18:2 n-9cis, 11trans	0,07	0,17	0,12	0,21
C 18:3	20,90	23,36	15,45	19,56

Data presented are least square means (n = 3 samples per diet).

Ruminal pH, ruminal ammonia-N and VFA (acetate, propionate and acetate/propionate ratio) were affected by the dietary factors or by their interaction (table 4). Sheep fed the high energy diets to have lower acetate and higher butyrate molar proportion before feeding than those fed the low energy diets. After feeding, ewes fed the high energy and protein diets had higher proportion of propionate and lower proportion of acetate and acetate/propionate ratio than those fed the low energy and protein diets. Sheep fed the high energy and high protein diets had lower NH_3-N in ruminal fluid, than those fed the low diets ($p \leq 0,05$).

Table 4

Fermentation characteristics in the ruminal fluid collected of 2 h after feeding in dairy ewes fed low - or high – energy and protein diets[1].

Energy lelel	**Low** – 0,90 UFL		**High** – 0,97 UFL		SEM	p values of effects[2]		
Protein level	14% CP	16% CP	14% CP	16% CP		E	CP	ExCP
pH	6,70	6,82	6,30	6,21	0,05	*	NS	NS
VFA, mol/100 mol								
- Acetate	68,4	67,5	64,0	65,3	0,50	***	NS	*
- Propionate	16,7	18,2	19,0	21,0	0,47	***	*	NS
- Butyrate	12,2	11,9	13,3	11,4	0,31	NS	*	NS
- Isobutyrate	1,82	1,40	2,31	1,34	0,07	NS	*	NS
- Valerate	0,77	0,90	0,96	0,87	0,03	NS	NS	*
- Isovalerate	0,11	0,10	0,43	0,09	0,05	NS	NS	*
Acetate/propionate	4,09	3,71	3,36	3,11	0,11	***	*	NS
Ammonia – N, mM	3,38	4,30	3,13	3,01	0,45	NS	NS	*

[1] Data presented are least square means – n = 4 ewes per group – 2 h after feeding
[2] E = effect of energy level, CP = effect of protein level, ExCP = interaction between energy and protein level diet. *** : $p \leq 0,001$; ** : $p \leq 0,01$; * : $p \leq 0,05$.

The composition of the diets had a marked effect on the fatty acid profile of the milk fat produced (table 5). Increased feeding energy level resulted in lower degree of saturation of milk fat, mainly, on account of declining share of acids C10: 0, C14: 0, C16: 0 and C18: 0, the results being in agreement with those published by Rotunno et al. (1998), Dhiman et al., (1999), Martini et al (2004), Mele et al. (2006), Andrade et al. (2006) and Mele et al. (2008), this did not occur in the study of Antongiovanni et al. (2002). The most important changes in the profile of milk fatty acids were recorded in the polyunsaturates fatty acids (PUFA) whose weight was significantly influenced by the level of food energy ($p \leq 0.001$), protein level ($p \leq 0.05$), and, also, by the interaction of the two factors ($p \leq 0.01$).

Table 5

.Effects of energy and protein content in the diet on milk fatty acid profile in dairy ewes (*g/100 g FAME*)

Energy level	Low – 0,90 UFL		High – 0,97 UFL		Sem.	P values of effects[2]		
Protein level	14% CP	16% CP	14% CP	16% CP		E	CP	ExCP
C 4:0	2,57	2,65	3,51	2,94	0,095	*	*	NS
C 6:0	1,60	2,11	2,31	1,08	0,04	**	*	NS
C 8:0	2,56	2,70	2,40	2,81	0,32	NS	NS	NS
C 10:0	9,18	9,21	8,07	7,69	0,31	**	**	NS
C 12:0	2,41	5,43	3,12	3,60	0,12	**	**	NS
C 14:0	14,19	10,16	12,15	8,76	0,01	**	**	NS
C 15:0	0,45	0,40	0,44	0,29	0,014	**	NS	NS
C 16:0	34,21	32,05	26,70	25,11	0,67	**	NS	NS
C 17:0	0,61	0,49	0,42	0,37	0,02	**	*	NS
C 18:0	9,15	9,61	11,45	10,80	0,21	**	NS	NS
C 14:1	0,28	0,21	0,16	0,09	0,02	**	*	NS
C 16:1	0,72	0,97	0,48	0,59	0,04	**	NS	NS
C 17:1	0,18	0,16	0,10	0,11	0,01	***	*	NS
C 18:1, n-9 trans	0,16	0,18	0,29	0,53	0,04	***	**	**
C 18:1, n-11 trans	0,85	0,93	1,24	3,19	0,17	***	**	*
C 18:1, n-9 cis	14,78	16,21	18,83	22,58	1,34	**	NS	NS
C 18:1, n-11 cis	0,35	0,30	0,49	0,71	0,31	***	*	*
C 18:2, n-6 trans	0,22	0,33	0,34	0,42	0,01	**	**	NS
C 18:2, n-6 cis	2,56	2,70	2,88	3,21	0,05	**	*	*
C 18:2, n-9c, 11t (CLA)	0,79	1,22	2,34	2,77	0,311	***	*	**
C 18:3, n-3	1,60	1,41	1,65	1,67	0,02	***	**	**
C 20:4, n-6	0,17	0,13	0,14	0,11	0,014	***	NS	NS
C 20:5, n-3 EPA	0,11	0,10	0,12	0,14	0,01	**	*	*
C 22:3, n-3	0,08	0,07	0,08	0,10	0,01	*	NS	*
C 22:5, n-3	0,15	0,18	0,20	0,21	0,01	***	**	**
C 22:6, n-3 DHA	0,07	0,09	0,09	0,12	0,01	***	*	*
Saturated FA	76,93	74,81	70,57	63,43	1,62	***	*	*
Unsaturated FA	23,07	25,19	29,43	36,55	1,31	***	NS	*
MUFA	17,32	18,96	21,59	27,80	1,40	**	*	NS
PUFA	5,75	6,23	7,84	8,75	0,92	***	*	**
SFA/MUFA	4,44	3,95	3,27	2,28	0,08	***	*	**
SFA/PUFA	13,38	12,00	9,00	7,25	0,12	***	*	**
MUFA/PUFA	3,01	3,04	2,75	3,18	0,11	**	NS	*
PUFA, n-6	2,95	3,16	3,36	3,74	0,17	**	*	*
PUFA, n-3	2,01	1,85	2,14	2,24	0,14	***	*	*
n-6/n-3	1,467	1,708	1,570	1,700	0,10	**	*	NS
AI[3]	4,05	3,10	2,66	1,75	0,21	***	NS	**

[1] Data presented are least square means – n = 8 per group; [2] E = effect of energy level, CP = effect of protein level, ExCP = interaction between energy and protein level diet. *** : $p \leq 0,001$; ** : $p \leq 0,01$; * : $p \leq 0,05$.

[3] AI = Atherogenicity Index : (C12 + 4 x C14 + C16) / (MUFA + PUFA) (Kelsey et al., 2003)

The greatest part of PUFA in the milk fat was recorded in sheep fed with a ratio of high energy and protein level (0,97 UFL and 16% CP of DM). Thus, the share of PUFA in milk increased up to 52.1%, while the share of SFA decreased up to 21.2%. Of the two isomers of linoleic acid, C18: 2 cis n-6 is better represented in the milk of sheep fed with the rations with high energy and protein level(2,56 g/100 g FAME – fatty acid methyl esters).

Increased PUFA n - 3 share, also called Omega 3 (C18: 3, EPA, C22: 3, C22: 5 and DHA) in the milk fat, with the increasing energy and protein level of ratios, leads to improvement of milk nutritional value, since Omega 3 fatty acids helps to reduce blood cholesterol levels, prevent and, even, combat cardiovascular diseases, obesity and associated diseases (Jensen et al., 1990, Hornstra, 1999). The increased energy and protein level of food has led to increasing part of conjugated linoleic acid (CLA = C18: 2 n-9cis, 11trans) in the fatty acids in milk up to 250% (0.79 g/100 g FAME - in the case of sheep fed with low protein and energy level diet, 2.77 g/100 g FAME in the case of sheep fed with a diet of high energy and protein level). Foods enriched in CLA are considered - functional food - because of beneficial effects they have on human health; CLA has, among others, and antidiabetic and anticancerous effect (Chin et al., 1992).

CONCLUSIONS

Increased energy level of lactating sheep food(0.90 UFL / kg DM vs. 0.97 UFL / kg DM) by increasing the share of concentrates in the ration (from 27% to 43% in DM) and protein level (from 14% CP to 16% CP of DM) by introducing soybean groats (5.5% of DM), positively influenced the quantitive and qualitative production of milk and, in particular, the profile of fatty acids. This way, the average daily production of milk increased up to 14.7% ($p \leq 0.05$) and, also, fat content, protein and lactose content increased, but decreased the casein content (which could reduce the performance of processing milk in cheese) and non protein nitrogen.

It hes been improved the nutritional quality of sheep milk as a result of the declining degree of saturation of fats, through reducing the proportion of SFA which are responsible for the occurrence of cardiovascular diseases in humans, and through increasing the part of PUFA and, especialy those of Omega 3 type (C18: 3, EPA, C22: 3, C22: 5 and DHA) and CLA (conjugated linoleic acid - C18: 2 n-9 cis, 11 trans), which play an important role in preventing and combating cardiovascular disease; CLA has an anticancerous and antidiabetic effect (Chin et al., 1992). The highest quality milk, analyzed through the influence on human health, determined by a high content of Omega 3 fatty acids and CLA, was obtained from sheep fed with high energy and protein level(0,97 UFL/kg and 16% CP of DM).

At the same lot was also recorded the best PUFA n - 3 and 6/n-propportion and the lowest atherogenicity index of milk fat.

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