Fatty acid profile in milk of Bovec sheep fed in the stable or grazed in different pastures

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FATTY ACID PROFILE IN MILK OF BOVEC SHEEP UNDER TRADITIONAL FEEDING MANAGEMENT

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Original scientific paper

SUMMARY

The fatty acid profile in the milk of Bovec sheep fed total mixed ratio (TMR) and grazed natural pastures in the lowland (480 m altitude) supplemented with the second harvest (L) as well as grazed different altitude mountain pastures; M1 (1100-1300 m altitude), M2 (1600-1700 m altitude), M3 (1800 m altitude), M4 (1900 m altitude), M5 (2200 m altitude) were determined. There was an important effect when ewes were turned from the stable to the pasture on all fatty acids. The percentage of α-linolenic acid (ALA), arachidonic acid (ARA) and docosahexaenoic acid (DHA) increased significantly (P<0.001) with the diet. In the milk from M5 grazing the percentage of ALA was 2.5 times higher than in milk from L and 2.6 times higher than in milk from TMR. The percentage of ARA and DHA in milk was the highest when ewes were grazing on the M5 pasture (0.21±0.02 wt. %; 0.22±0.02 wt. %) respectively. Total n-3PUFA and n-6PUFA increased significantly (P<0.001) by the diet. Therefore, the n-6/n-3PUFA ratio was the best (1.2) in milk produced in the highest mountain pasture (M5), in terms of nutritional requirements.

Key-words: ewe milk, fatty acid, stable, mountain grazing

INTRODUCTION

Diet is the most important factor affecting ruminant's milk composition, particularly fatty acid composition. Appropriate feeding systems were implemented to increase the content of beneficial fatty acids in the most studies (Biondi et al., 2006; Morand-Fehr et al., 2007; Ostrovský et al., 2009). The milk composition of the grazing ewes is believed to be relatively healthy due to their richness in polyunsaturated fatty acids, mainly α-linolenic acid (ALA) and conjugated linoleic acid (CLA) compared to milk of ewes fed indoors with total mixed rations (TMR). Only a few studies dealing with milk fat quality of ewes raised on the pasture were published (Cabiddu et al., 2005; Mel'uchová et al., 2008). In Slovenia, all ewes' milk is processed into cheese. The Bovec sheep breed is reared in the local area (northwest Slovenia) under traditional extensive conditions to produce milk for Bovec cheese. Bovec cheese production starts from the stable and lowland produced milk and continues with mountain grazing produced milk. The aim of this study was to monitor the fatty acid profile of Bovec sheep milk produced under traditional feeding management, to investigate the effect of the diet.

MATERIAL AND METHODS

Fifteen randomly assigned ewes of the autochthonous Bovec sheep from a larger flock were observed during the lactation period started in the stable and finished in mountain pastures. The farm was located in Bovec (northwest Slovenia) and the flock is included in the national Breeding program for Bovec sheep. The rearing technology used is traditional for that area, whereby sheep are housed during the winter in lowland farms and, during the vegetation period, they are on all-day mountain grazing. Ewes started milking in the stable on April 21st, and were fed *ad libitum* with the total mixed ratio (TMR), consisted of grass silage, corn grain and the second harvest. Thereafter, from April 26th until June 16th ewes were grazing in lowland (480 m) and were supplemented by the second harvest (L). Then, after June 16th they were gradually moved to mountain pastures at the following altitudes: M1 (1100-1300 m altitude), M2 (1600-1700 m altitude), M3 (1800 m altitude), M4

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(1900 m altitude) and, finally to M5 (2200 m altitude), on the mountain Mangart. The observed flock grazed in the mountain pastures from June 17th to September 4th. Ewes were milked twice per day, in the stable by machine and after moving to the mountain pastures by hand. The milk yield was changing during lactation period and reached on the average1.7 kg, 1.67 kg, 0.90 kg, 1.08 kg, 1.15 kg, 0.84 kg and 0.60 kg per ewe/day in the TMR, L, M1, M2, M3, M4 and M5, respectively. Milk was used for Bovec sheep cheese production.

The chemical composition of grass was determined from clipped samples cut from randomly selected places at all pasture locations. Grass samples were cut using hand grass shears and collected as a composite sample from each location: L, M1, M2, M3, M4 and M5. Grass was cut on the same days as milk samples were taken and it was packed in polyethylene bags and stored at -20°C. The chemical composition of TMR components were determined from randomly selected places of grass silage, corn and second harvest depository and were taken on the same days as milk samples were.

Milk sampling was performed by the ICAR guidelines for AT4 milk recording (ICAR, 2011), with the recommended first (month) sampling during the morning and second (next month) sampling during the evening milking etc. The 50 ml milk samples were taken from each ewe for further fatty acid analysis. Milk samples were immediately packed in a cold chamber and transported to the laboratory. In the laboratory milk samples were shaken to homogenize the milk and then distributed into aliquots of 1.5 ml and stored at -20ºC until the analysis started.

Milkoscan 6000FT was used for determining fat content in the milk. The method was based on the measurement of the absorption of mid-infrared radiation at wavelengths (International Standard, ISO 9622:1999). The fat content was expressed in percentages (g/100 g). Fatty acid methyl esters (FAME) from milk samples were prepared using the *in-situ* transesterification method of Park & Goins (1994). An Agilent 6890 series GC instrument equipped with an Agilent 7683 Automatic Liquid Sampler, a split injector, a flame-ionization detector (GC-FID) and a WCOT fused silica capillary column CP-select CB for FAME (Varian, 100 m x 0.25 mm i.d.) were used for separation of FAME. The Agilent GC ChemStation was used for data acquisition and processing. The Separated FAME were identified by retention time comparison and the results were quantified using response factors derived from chromatographic standards of the known composition (Nu Chek Prep, Nu Chek 85, Nu Chek 411, Nu Chek 68A). The fatty acid composition was expressed as a weight percentage (wt.%) of the total identified fatty acids. The accuracy and reliability of the method used was assessed with certified reference material (NIST 8435 whole milk powder). The repeatability of the method was tested with six injections of a composite milk sample. The detection limit was 0.005% and the limit of quantification was 0.05 wt.%. Fat content in the reference material was 21.3±2.4% for validating the material.

Data were analysed using the GLM procedure in the statistical package SAS/STAT (SAS Institute Inc., 2001) using the Model 1. All fatty acids with values above 0.01 wt.% of total fatty acids were included in the data processing.

$$y_{ij} = \mu + D_i + b_I (x_{ij} - \bar{x})^2 + e_{ij} \qquad \text{Model 1}$$

where: y_{ij}=trait; μ=mean; D_i=diet, i=total mixed ratio (TMR), lowland grazing supplemented with the second harvest (L), mountain grazing 1 (M1), mountain grazing 2 (M2), mountain grazing 3 (M3), mountain grazing 4 (M4), mountain grazing 5 (M5); x_{ij}=days in milk (DIM); b_I=regression coefficient, e_{ij}=residual

RESULTS AND DISCUSSION

The fatty acid composition in the TMR diet and grazing are shown in Table 1. Feed fatty acid analysis highlighted the difference between stable (TMR) and pasture (grazing) diet. Linoleic acid (LN) was predominant in the TMR. The percentage of LN was the highest in the corn (55.37 wt.%). Due to grass silage and the second harvest in TMR the percentages of α-linolenic acid (ALA) was high, 51.03 wt.% and 42.43 wt.% respectively. LN and ALA together accounted for more than 60% of fatty acids in the TMR diet. The ALA was predominant in grazing. The highest proportion was in grazing from the highest mountain pasture (M5; 61.08 wt.%).

Table 2 shows that the fatty acid composition in milk was changed with the diet transition from TMR to grazing. There was an interesting effect on all fatty acids when ewes were turned from the stable to the pasture. Saturated fatty acids as lauric (C12:0) and myristic (C14:0) were influenced (P<0.001) by the diet. When sheep started grazing in lowland the percentage of lauric acid decreased from 4.9 wt.% in milk from TMR to 3.8 wt.% in milk from lowland (L) grazing.

Table 1. Average fatty acid composition in total mixed ratio (TMR) and grazing (wt.%)

Fatty acid	TMR			L	M1	M2	M3	M4	M5
	Grass silage	Corn	Second harvest						
C12:0	0.28	/	0.44	0.30	0.22	0.73	0.57	0.32	0.61
C14:0	0.59	0.04	0.65	0.57	0.47	0.90	0.70	0.44	1.05
C16:0	16.91	12.47	20.74	15.56	15.33	16.69	17.33	13.82	13.69
C18:0	1.45	1.85	2.47	1.38	1.15	2.23	1.54	1.38	1.28
LN	20.35	55.37	19.15	20.30	17.33	16.71	17.27	13.27	11.48
ALA	51.03	1.66	42.43	54.69	57.89	47.41	48.53	60.66	61.08
DHA	/	/	/	/	/	0.32	0.25	0.05	/
SFA	24.50	15.21	31.34	22.29	21.08	27.69	26.86	21.49	22.55
MUFA	3.69	27.72	6.81	2.73	3.70	6.34	5.71	3.74	4.76
PUFA	71.81	57.07	61.85	74.99	75.22	65.97	67.43	74.77	72.69
n-6 PUFA	20.68	55.39	19.15	20.30	17.33	18.15	18.59	14.03	11.61
n-3 PUFA	51.12	1.66	42.70	54.69	57.89	47.82	48.84	60.73	61.08
n-6/n-3	0.40	33.40	0.45	0.37	0.30	0.38	0.38	0.23	0.19

LN: C18:2n-6 (linoleic acid); ALA: C18:3n-3 (α-linolenic acid); DHA: C22:6n-3; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6 PUFA: n-6 polyunsaturated fatty acids; n-3 PUFA: n-3 polyunsaturated fatty acids; n-6/n-3: n-6/n-3 PUFA ratio; TMR: total mixed ratio; L: lowland grazing supplemented with second harvest; M1–M5: mountain pastures

ALA remained constant until sheep were grazing on the mountain pasture M2. After ewes were moved from M2 to the highest mountain pasture (M5) ALA increased. In the milk from M5 grazing the percentage of ALA was 2.5 times higher than in milk from L and 2.6 times higher than in milk from TMR. The percentage of arachidonic acid (ARA) in milk was the highest when ewes were grazing on M5 pasture (0.21±0.02 wt.%) and differed significantly only from TMR milk (0.12±0.02 wt.%). DHA increased significantly ($P<0.001$) with the diet, the lowest value were in milk from TMR (0.01±0.02 wt.%) and the highest in milk from M5 grazing (0.22±0.02 wt.%). The CLA percentage decreased after the transition from TMR to L and remained stable when grazing occurred in mountain pastures. The differences were significant between TMR and M3, M4 and M5. The highest percentage of CLA was in milk from TMR. This was probably due to grass silage and the second harvest of good quality as the main components of TMR. Total n-3 PUFA and n-6 PUFA increased significantly ($P<0.001$) according to the pastures altitudes. Therefore, the n-6/n-3PUFA ratio was the best (1.2) in milk produced in the highest mountain pasture (M5), in terms of nutrition.

Beside the effect of the diet, the fatty acid composition was influenced by the stage of lactation (days in milk) as well. ALA, LN, ARA, n-6 PUFA ($P<0.05$), n-3 PUFA ($P<0.01$) and DHA ($P<0.001$) increased significantly with the lactation stage.

Biondi et al. (2008) confirmed the diet effect when switching from stall to pasture feeding for all fatty acids in sheep milk, except C4:0 and C6:0. They reported a 2-fold ALA increasing within the first 2 days of transition to pasture. Nevertheless, the increasing of ALA was even higher after the transition from stall to herbage diet. Ostrovský et al. (2009) investigated the effect of ewes' diets on the milk fatty acids from a TMR to a pasture diet. They found similarities to our results with increased ALA values from a TMR period throughout a transition period to the beginning of the grazing season. They also found out a decreased CLA contents in July, which increased again in the middle of September.

Table 2. Fat content (g/100g) and fatty acid profile (wt. %) in ewes' milk from different diet

Trait	LSM ± SE							p-value	
	TMR	L	M1	M2	M3	M4	M5	D	DIM
Milk fat	5.5±0.8[a]	8.3±0.4[b]	6.8±0.3[ab]	7.1±0.3[ab]	6.5±0.3[ab]	6.8±0.4[ab]	7.7±0.8[ab]	***	ns
C12:0	4.9±0.4	3.8±0.2[a]	3.1±0.1[ab]	2.9±0.1[b]	2.7±0.1[b]	2.8±0.2[ab]	2.0±0.4[b]	***	*
C14:0	12.4±0.7[a]	11.4±0.4[a]	10.4±0.3[ab]	9.5±0.2[bc]	9.2±0.3[bc]	9.7±0.3[ab]	7.0±0.8[bd]	**	***
C16:0	22.8±1.2[ab]	26.0±0.6[a]	24.0±0.5[ab]	23.0±0.4[b]	23.2±0.5[ab]	24.3±0.6[ab]	22.3±1.4[ab]	***	ns
C18:0	9.8±1.1[a]	11.4±0.5[ab]	12.6±0.4[ab]	13.4±0.4[ab]	13.8±0.4[b]	12.8±0.5[ab]	14.2±1.3[b]	**	*
LN	2.5±0.3[ab]	2.3±0.1[a]	3.0±0.1[b]	3.0±0.1[b]	3.4±0.1[b]	3.5±0.1[b]	4.00±0.3[b]	***	*
ALA	1.07±0.2[a]	1.1±0.1[a]	1.4±0.1[a]	1.9±0.05[b]	2.2±0.1[bc]	2.4±0.1[c]	2.8±0.2[c]	***	*
CLA	2.1±0.2[a]	1.6±0.1[ab]	1.6±0.1[ab]	1.7±0.1[ab]	1.5±0.1[b]	1.5±0.1[b]	1.5±0.2[b]	**	ns
ARA	0.12±0.02[a]	0.14±0.01[ab]	0.13±0.01[ab]	0.13±0.01[ab]	0.16±0.01[ab]	0.17±0.01[ab]	0.21±0.02[b]	**	*
EPA	0.18±0.01[a]	0.12±0.01[b]	0.15±0.01[a]	0.14±0.01[ab]	0.16±0.01[ac]	0.16±0.01[ab]	0.18±0.02[ab]	***	ns
DHA	0.01±0.02[a]	0.05±0.01[a]	0.09±0.01[b]	0.09±0.01[b]	0.10±0.01[bc]	0.14±0.01[c]	0.22±0.02[d]	***	***
SFA	66.6±2.1[a]	66.2±1.0[a]	62.8±0.8[ab]	60.7±0.7[b]	60.3±0.8[b]	61.9±0.9[ab]	55.9±2.3[ab]	**	*
MUFA	26.9±1.7[ab]	27.8±0.8[a]	29.9±0.6[ab]	31.5±0.6[b]	31.6±0.7[ab]	29.4±0.8[ab]	34.4±1.9[ab]	**	*
PUFA	6.5±0.5[ab]	5.9±0.2[b]	7.2±0.2[a]	7.7±0.2[ac]	8.0±0.2[ac]	8.6±0.2[c]	9.6±0.6[c]	***	ns
n-6PUFA	2.8±0.3[ab]	2.7±0.1[a]	3.5±0.1[b]	3.5±0.1[b]	3.9±0.1[b]	4.0±0.1[b]	4.6±0.3[b]	***	*
n-3PUFA	1.4±1.2[abc]	1.5±0.1[c]	2.0±0.1[ab]	2.4±0.1[d]	2.6±0.1[d]	3.1±0.1[d]	3.6±0.2[d]	***	**
n-6/n-3	1.8±0.1[a]	1.7±0.1[a]	1.7±0.03[a]	1.4±0.03[b]	1.5±0.03[a]	1.3±0.04[c]	1.2±0.1[c]	***	ns

Means within a row with different letters differ significant ($P<0.05$);*:$P<0.05$; **:$P<0.01$; ***:$P<0.001$; ns: not significant. LN: C18:2 n-6 (linoleic acid); ALA: C18:3n-3 (α-linolenic acid); CLA: c9t11C18:2; ARA: C20:4n-6; EPA: C20:5n-3; DHA: C22:6n-3; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6 PUFA: n-6 polyunsaturated fatty acids; n-3 PUFA: n-3 polyunsaturated fatty acids; n-6/n-3: n-6/n-3 PUFA ratio; TMR: total mixed ratio; L: lowland grazing supplemented with second harvest; M1–M5: mountain pastures; D: diet; DIM: days in milk

CONCLUSION

The results suggested that grazing on M1 pastures at 1100-1300 m above the sea level already improved the fatty acid profile of milk compared to lowland grazing supplemented with the second harvest. When ewes were moved to the highest pastures, milk fatty acid profile became even more beneficial probably due to the high ALA content in grazing from mountain pastures. The traditional Alpine grazing management of Bovec sheep could become a useful strategy to manipulate dietetic characteristics of milk and cheese.

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