The effect of adding herbs to the summer diet on the fatty acid profile of the lipid fraction of sheep milk

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The study was carried out on bulk milk samples collected at two-week intervals during the summer (June-August) and individually from ewes at the end of the experiment. The milk was obtained from ewes of the Koluda prolific dairy breed, housed indoors and fed green alfalfa forage and hay with a mixture of concentrate feeds. Three diet groups were established: group I – control (without the addition of herbs to the concentrate feed) and groups II and III, in which an herbal mixture was added to the concentrate feed in the amount of 10 and 20 g/sheep/day, respectively. The effect of the herb supplement in the sheep diet on the fatty acid profile of the milk fat was analysed. The results showed that the fat composition changed in all groups during the three-month milking period. In the first two months, an improvement was noted in the health-promoting properties of the milk fat (a decrease in SFA and an increase in MUFA and PUFA), but in the final period of the experiment they deteriorated. The unfavourable changes were smaller where the proportion of herbs in the diet was higher. As a consequence, in the final stage of research, lower SFA content was noted in the fat of the group III sheep than in the control group and II, by 6.0% ($P\leq0.01$) and 4.1% ($P\leq0.05$), respectively, lower content of OFA by 9.1% ($P\leq0.01$) and 5.6% ($P\leq0.05$), and higher content of UFA by 17.7% ($P\leq0.01$) and 11.8%. ($P\leq0.05$), including MUFA by 20.1% ($P\leq0.01$) and 11.3% and PUFA by 11.7% (NS) and 15.0% ($P\leq0.05$), as well as CLA, by 26.0% ($P\leq0.01$) and 17.9% (ns) and DFA by 23.8% ($P\leq0.01$) and 13.0% ($P\leq0.05$). This also meant favourable changes in the UFA/SFA, PUFA/SFA and DFA/OFA ratios. The milk fat of group III also had higher content of *n-3* PUFA (by 25.3%) than in group II, and thus a lower *n-6/n-3* ratio.

KEY WORDS: sheep / herbs / fatty acid profile / summer diet

Increasing awareness among consumers about the relationship between food, diet and health has led them to search for products that not only are tasty, fresh and safe for health, but also are natural and have health benefits [21, 32, 36]. At a time when diet-dependent diseases are increasingly common, functional food, i.e. food with health-promoting constituents having a proven beneficial effect on one or more functions of the body beyond nourishment, takes on importance [18, 23]. Consumers have favourable opinions of the improved quality of products of animal origin, resulting in part from proper diet and rearing conditions [19]. One such product is milk, whose fat (consisting of about 500 fatty acids), apart from its unquestionable nutritional value, has numerous health-promoting properties [3]. Scientific research has demonstrated the beneficial effect of butyric acid (C4:0) in the treatment of intestinal inflammation and functional disorders, as well as in anticancer therapy [20, 25]. Oleic acid (C18:1) lowers blood cholesterol levels, while vaccenic acid (C18:1 *n-7*) slows tumour cell growth in the colon [2]. Polyunsaturated fatty acids (PUFAs, including *n-3* and *n-6* PUFAs) are effective in preventing and treating cardiovascular diseases and are essential for the proper development of the body and organ function, especially the brain and retina [28, 29]. A highly valuable constituent of the milk fat of ruminants is conjugated linoleic acid (CLA). Research results indicate a positive effect of this acid in the prevention of obesity (*trans*-10, *cis*-12 isomer) [8], while its *cis*-9, *trans*-11 configuration has immune, antioxidant, anti-sclerotic and anti-cancer effects [5, 6].

Previous studies have demonstrated that the fatty acid profile of milk fat can be modified in a way that is beneficial for health by adding oilseed plants to the diet of ruminants [9, 34]. The fat of milk obtained from pasture-grazed animals has also been shown to have better health-promoting properties than that of conventionally-fed animals [7, 10, 11, 13]. Studies have also found seasonal changes in the composition of milk fat [4, 12, 15, 22, 27]. Gerchev et al. [17] reported significant differences in the composition of the milk fat of sheep grazing on mountain pastures in the period from April to July (lower content of C4:0, C10:1 and C18:1 in July and higher content of C6:0 and C14:1 in June as compared to April). Mel'uchova et al. [30] showed that seasonal changes in the fatty acid profile of sheep milk fat are the result of changes in the fat composition of pasture vegetation. An improvement in the functional properties of cow milk fat through the use of herbs, either as natural feed supplements [24] or sown in the pasture [33], has been demonstrated as well.

Summing up, previously conducted research has shown that the composition of milk can be modified for greater health benefits through the use of grazing or by adding herbs to the feed. Therefore we postulated that introducing a suitably composed mixture of herbs to the diet of milking sheep housed indoors and fed green forage from monoculture field crops would improve the fatty acid profile of their milk. For this purpose, varied amounts of a herb mixture were added to the diet of sheep – 10 or 20 g/head/day.

Material and methods

The research was carried out at the National Research Institute of Animal Production, Experimental Station in Kołuda Wielka. The experimental material consisted of 66 ewes of the Koluda prolific dairy breed (aged 2 to 8 years), milked commercially after their lambs were weaned at the age of 8-9 weeks, during the summer (June-August). The ewes were housed indoors and fed alfalfa greens, hay and a mixture of concentrate feeds. Nutrition levels were established according to INRA-88 standards for milking sheep, based on the requirements of a ewe with a body weight of 70 kg producing on average 0.7 kg of milk.

The ewes were assigned to three feeding groups, which were analogous in terms of date of lambing, ewe body weight, average daily weight gain of lambs, and number of lambs reared. Group I (control) was fed bulky feed and a concentrate mixture without herbs. The experimental groups received the same feed as group I, but with herbs added to the concentrate feed, in the amount of 10 g/head/day for group II and 20 g/head/day for group III.

The herb mixture used in the experiment was composed by the authors of the study from 9 herbs (common nettle *Urtica dioica*, fennel *Foeniculum capillaceum*, caraway *Carum carvi*, coriander *Coriandrum sativum*, fenugreek *Trigonella foenumgracum*, peppermint *Mentha piperita*, English marigold *Calendula officinialis*, chamomile *Matricaria chamomilla*, and milk thistle *Silybum marianum*). The mixture was intended to benefit the animals mainly by improving their digestion and metabolism, by acting as galactogogues, and by exerting bacteriostatic and anti-inflammatory effects.

The fatty acid profile of the milk fat was determined in bulk milk samples collected in each group from morning milking at two-week intervals, as well as in milk samples taken from 20 ewes from each group from the last control morning milking (at the end of the experiment). Fatty acids in milk fat were determined by gas chromatography [26] with modifications used at the Institute of Agricultural and Food Biotechnology in Warsaw. The analyses were performed with a Hewlett-Packard model 6890 gas chromatograph with a flame ionization detector, using an Rtx-2330 column (105 m x 0.25 m x 20 µ). Milk fat was extracted according to standard procedures described by Folch et al. [14].

The results of the experiment were analysed statistically using the STATISTICA 6 PL package, by one-way analysis of variance (ANOVA), where the factor was the addition of herbs in three groups. Statistical differences between groups were verified by Duncan's test.

Results and discussion

Analysis of the fat composition of the bulk milk showed seasonal changes in the fatty acid profile. In all feeding groups, the content of saturated fatty acids (SFAs) showed a downward trend until the 97th day of lactation and then increased, most sharply in the final period of the experiment (Fig. 1). SFA content was found to be lowest in the group III milk fat, as compared to groups I and II, and in the final stage of the experiment it was also lower in the group II milk fat as compared to group I. The content of UFAs and MUFAs increased in all feeding groups until the 97th day of lactation, and then decreased. From the 83rd day of lactation the milk fat of the group III ewes had higher content of UFAs and MUFAs than that of the other groups, and in the final stage of the study there were also marked differences between groups I and II. The PUFA content curves were generally similar to the MUFA curves, but the absolute differences in the content of these acids between groups were smaller (Fig. 2). The *n-3* PUFA curves were similar, but it should be noted that from the 111th day of lactation, the content of these acids in the milk fat was comparable in all groups. The content of CLA in the milk fat of all groups was similar and increased up to the 125th day of lactation. However, in the final stage of the study, a decrease in the content of this acid and marked differences between groups were observed. The lowest content of this health-promoting acid was found in the group I milk fat, and the highest in the group III fat. To sum up, in the final stage of the research the milk fat had a less beneficial fatty acid profile in terms of SFAs, UFAs and MUFAs content than in the initial stage of research. The use of the herb supplement in the amount of 20 g/head/day in the diet of milking sheep mitigated the adverse seasonal changes in the fatty acid profile of the milk fat.

Different results than in our research were reported by Ptasińska-Marcinkiewicz [35] in a study on the milk fat of mountain sheep and crosses of this breed with Friesian sheep (25% Friesian), while the same author obtained similar results to ours for the milk of Olkuska sheep. The author also reported that changes in the fatty acid profile depended on the breed of milking sheep. In the milk of mountain sheep and crosses of this breed with Friesian sheep, the amount of SFAs decreased and that of UFAs increased during the 3rd and 4th month of lactation. In the milk of the Olkuska sheep, on the other hand, the content of SFAs was significantly higher while that of MUFAs and PUFAs was lower in the 1st and 3rd month of lactation than in the 2nd and 4th month. A similar pattern of seasonal changes was observed by Strzałkowska et al. [37] during lactation of goats lasting 200 days, and thus in winter and summer feeding conditions (decrease in SFA content, and increase in MUFA and PUFA content, including CLA). Similar changes in the fatty acid profile to those obtained in our research were reported by Gerchev et al. [17] and by Mihaylova et al. [31] for ewes grazed on mountain pastures – an increase in SFA and a decrease in MUFA, but in contrast with our research, a decrease was observed in these studies for PUFA and CLA. Seasonal fluctuations in the fat composition of the milk of sheep kept in the pasture from April to September were studied by Mel'uchowa et al. [30]. On the basis of the results, the authors suggest that seasonal changes in CLA content in sheep milk fat (a decrease from May to August, and then an increase in September) are primarily associated with the seasonal content of α-linolenic acid in grass lipids (analogous fluctuations to CLA content) .

The analysis of the fatty acid profile of milk collected individually from ewes during the last control milking confirms the beneficial effect, in terms of health, of the use of herbs on the composition of milk fat. In group III, a lower content of SFAs was found in comparison to groups I and II, by 6.0% ($P \leq 0.01$) and 4.1% ($P \leq 0.05$), respectively (Table 2). The highest content of butyric acid (C4:0) was found in the milk fat from group II, and the difference in comparison to group III was 9.4% ($P \leq 0.05$) – Table 1. Lower content of medium-chain fatty acids (MCFAs) was observed in the milk fat of the experimental groups than in the control (the differences between groups II and III and group I were 9.5% and 18.0%, respectively at $P \leq 0.01$). The content of the dominant saturated fatty acid C16:0 was

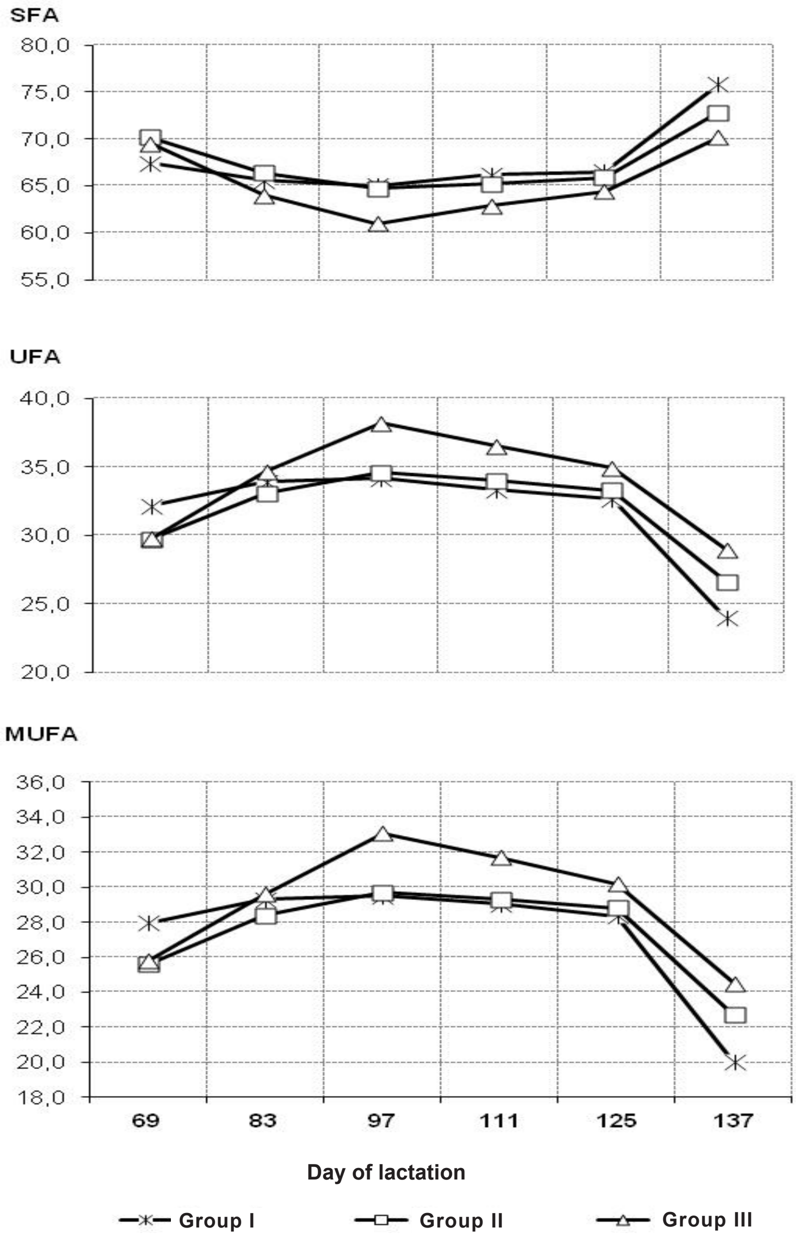

Fig. 1. Content of SFA, UFA and MUFA in the milk fat of bulk milk (g/100 g of fat) during the sheep milking period

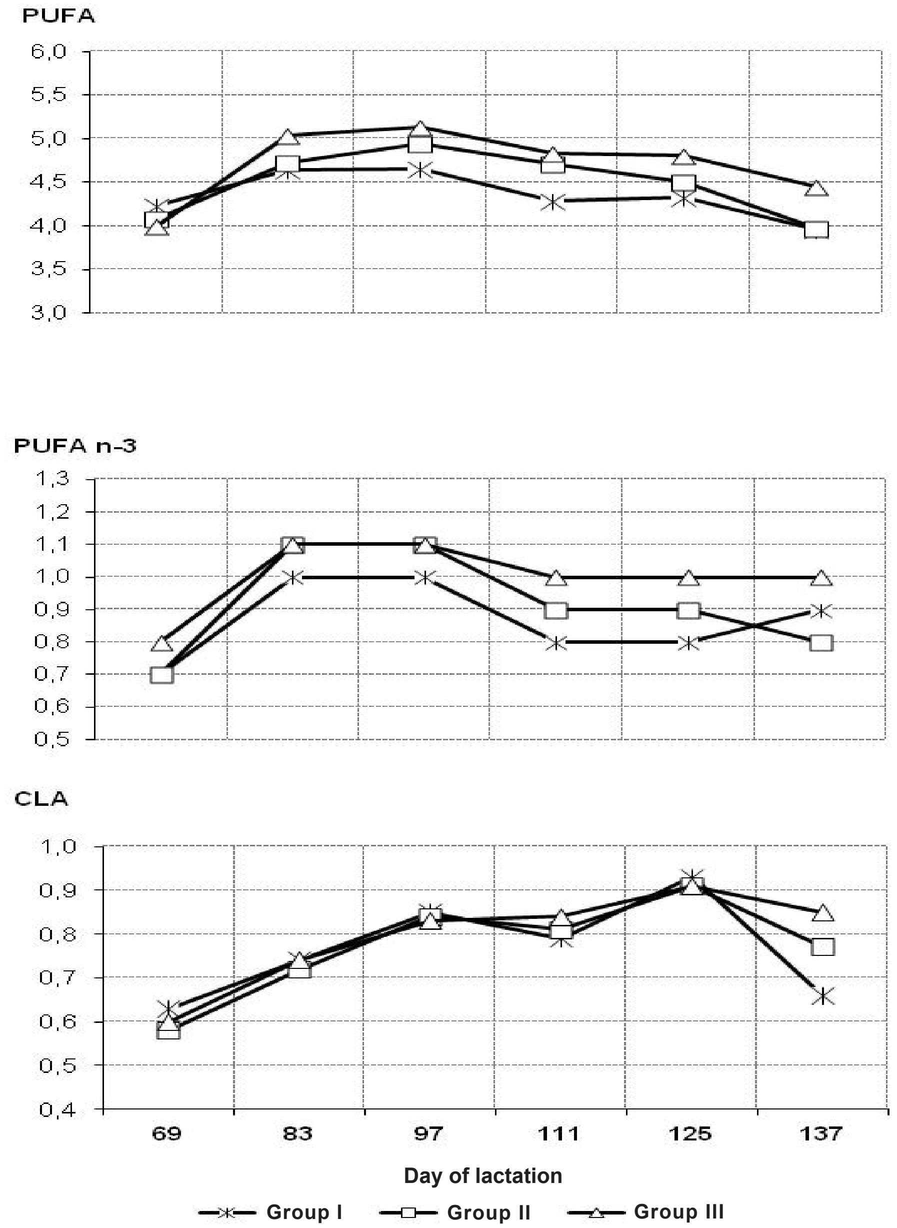

Fig. 2. Content of PUFA, *n-3* PUFA and CLA in the milk fat of bulk milk (g/100 g of fat) during the sheep milking period

similar in all groups. On the other hand, the content of SFAs with the longest carbon chains (C17:0 and C18:0) was higher in the milk fat of the ewes in the experimental groups, and the differences between groups III and I for these acids were statistically significant. The milk fat from group III contained more unsaturated fatty acids (UFA) than that of groups I and II, by 18.7% (P≤0.01) and 11.8% (P≤0.05), respectively. The milk fat of this group also had more monounsaturated acids (MUFAs) in comparison to groups I and II, by 20.1% (P≤0.01) and 11.3% (P≤0.05), respectively. In terms of polyunsaturated acids (PUFAs), there was a tendency towards higher content of PUFAs in the milk fat of group III than for group I (by 11.7%, NS) and there were significant differences between experimental groups – PUFA content was 15.0% higher in group III than in group II (P≤0.05). The differences in the content of *n-3* and *n-6* PUFAs were similar, but the differences between group III and groups I and II for *n-3* PUFAs were much greater: 20.7% (NS) and 25.3% (P≤0.05), respectively. This affected the *n-6*/*n-3* ratio, which is important for the health quality of milk. It was more favourable in the group III milk fat as compared to groups I and II, as it was 13.2% (NS) and 13.8% (P≤0.05) lower, respectively. In the milk fat of ewes receiving the larger herb supplement, CLA content was higher than in the other groups; the difference with respect to the control was statistically significant and amounted to 26.0%

Table 1

Content of fatty acids in milk fat (g/100 g)

Fatty acids	Group			SEM
	I	II	III	
C4:0	2.13	2.32^{a}	2.12^{b}	0.032
C6:0	1.98^{a}	2.10^{A}	1.80^{Bb}	0.032
C8:0	2.06^{B}	2.01^{B}	1.74^{A}	0.038
C10:0	7.86^{B}	7.05^{B}	5.93^{A}	0.174
C12:0	5.73^{A}	4.52^{Ba}	3.78^{Bb}	0.159
C14:0	14.1^{A}	13.0	12.3^{B}	0.212
C14:1	0.79^{a}	0.69^{b}	0.72	0.014
iso-C15:0	0.61	0.56	0.65	0.014
C15:0	1.39	1.27	1.32	0.024
C16:0	34.25	34.47	33.52	0.433
C16:1	2.44	2.08	2.06	0.069
iso-C17:0	0.91^{b}	0.96	1.07^{a}	0.026
C17:0	0.54^{B}	0.59	0.67^{A}	0.016
C18:0	3.48^{B}	4.63	5.53^{A}	0.242
C18:1 *T*	1.60	1.47	1.73	0.056
C18:1 *c*9	12.49^{B}	14.50^{b}	16.59^{Aa}	0.408
C18:1 *c*11	0.40	0.36	0.38	0.008
C18:1 *c* (other)	1.00^{B}	1.18	1.27^{A}	0.035
C18:2	2.04	1.93	2.14	0.052
C18:3	0.60^{b}	0.61^{b}	0.77^{a}	0.024

Least squares means with different letters differ significantly: A, B – P≤0.01; a, b – P≤0.05

Table 2
Fatty acid profile of milk fat (g/100 g)

Fatty acids	Group			SEM
	I	II	III	
SFA	75.43^{A}	73.94^{a}	70.92^{Bb}	0.507
MCFA	35.48^{A}	32.10^{Ba}	29.09^{Bb}	0.568
UFA	23.91^{B}	25.38^{b}	28.38^{Aa}	0.502
including:				
MUFA	19.99^{B}	21.57^{b}	24.01^{Aa}	0.429
PUFA	3.92	3.81^{b}	4.38^{a}	0.097
including:				
PUFA *n-3*	0.82	0.79^{b}	0.99^{a}	0.031
PUFA *n-6*	2.14	2.03	2.24	0.054
CLA	0.73^{B}	0.78	0.92^{A}	0.027
UFA/SFA	0.319^{B}	0.345^{b}	0.405^{Aa}	0.010
PUFA/SFA	0.052^{b}	0.052^{b}	0.063^{a}	0.002
PUFA *n-6*/*n-3*	2.659	2.679^{a}	2.309^{b}	0.064
DFA	27.39^{B}	30.01^{b}	33.92^{Aa}	0.707
OFA	71.95^{A}	69.30^{a}	65.39^{Bb}	0.711
DFA/OFA	0.384	0.439	0.530	0.015

MCFA = C6:0 + C8:0 + C10:0 + C12:0 + C14:0+ C14:1 + iso-C15:0 + C15:0;
SFA = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C13:0 + C14:0 + iso-C15:0 + C15:0 + C16:0 + iso-C17:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0;
UFA = MUFA + PUFA;
MUFA = C10:1 + C12:1 + C14:1 + C15:1 + C16:1 + C17:1 + C18:1 *T* + C18:1 *c*9 + C18:1 *c*11 + C18:1 *c* other + C20:1;
PUFA = C18:2 + CLA + C18:3 + C20:2 + C20:4 + C20:5 + C22:5 + C22:6;
PUFA *n-3* = C18:3 + C20:5 + C22:6;
PUFA *n-6* = C18:2 + C20:2 + C20:4;
DFA = UFA + C18:0;
OFA = SFA – C18:0
Least squares means with different letters differ significantly: A, B – P≤0.01; a, b – P≤0.05

(P≤0.01). Milk fat from both experimental groups had a favourably higher UFA/SFA ratio; it was highest in group III – higher than in groups I and II by 27.0% (P≤0.01) and 17.4% (P≤0.05), respectively. Similar relationships were noted for the PUFA/SFA ratio, which was 21.2% higher in group III than in groups I and II (P≤0.05%). The milk fat from group III, in comparison with groups I and II, had a higher content of hypocholesterolaemic acids (DFA), by 23.8% (P≤0.01) and 13.0% (P≤0.05), respectively, and lower content of hypercholesterolaemic acids (OFA), by 9.1% (P≤0.01) and 5.6% (P≤0.05), respectively. This resulted in a higher DFA/OFA ratio for the milk of ewes from both experimental groups – by 14.3% when 10 g of the herb mixture was used, and by 38.0% with the addition of 20 g/head/day.

The results of our research indicate that the milk fat of sheep fed the larger amount of herbs had more beneficial health-promoting properties as compared to results obtained by Atti et al. [1]. The authors cited investigated the milk fat of sheep kept on a pasture on which perennial ryegrass was predominant. They reported lower (as compared to group III in our study) content of UFAs (26.1%), PUFAs (3.09%) and MUFAs (23.0%), and similar content of CLA (1.03%). The milk fat in that study had more beneficial health-promoting properties in terms of SFAs (68.7%) and DFAs (35.2%). Gerchev et al. [17] reported a higher content of SFAs (72.2%), lower content of PUFAs (4.1%) and similar content of MUFAs (24.2%) in milk fat obtained in the 4th month of milking (July) from sheep of the local Tetevenska breed kept in mountain pastures as compared to the ewes in our study receiving the herb supplement in the amount of 20 g/head/day. Mihaylova et al. [31], in their study on the composition of the milk fat of local breeds of sheep grazing on mountain pastures, showed a similar content of SFAs (70.1%), lower MUFAs (22.2%) and higher PUFAs (7.7%) and CLA (2.5%) in the third month of milking (July). The use of a larger dose of the herb supplement in our study had a similar effect to that observed by Gerchev and Mihaylova [16] in the milk fat of local breeds grazed by traditional methods in mountain pastures. The SFA content in the milk fat of sheep of the Srednostaroplaninska and Tetevenska breeds was 70.8% and 71.0%, respectively, while MUFA content was 25.0% and 25.0% and PUFA content 4.5% and 3.9%.

To sum up, during the three-month period of milking of ewes of the Koluda prolific dairy breed, the composition of the milk fat underwent changes in all feeding groups. The health-promoting properties of the fat improved in the first two months of milking (decrease in SFAs and increase in MUFAs and PUFAs) and deteriorated in the final milking period. The use of the herbal supplement in the amount of 20 g/head/day reduced these changes. As a consequence, at the end of the study, the sheep fed the larger amount of herbs produced milk with a more favourable fatty acid profile in terms of health benefits, i.e. a significantly lower content of SFAs and OFAs, higher content of UFAs (including MUFAs, PUFAs, *n-3* PUFAs and CLA) and DFAs, and more favourable UFA/SFA, PUFA/SFA, *n-6*/*n-3* and DFA/OFA ratios. The analysis suggests that the addition of the tested herb mixture to concentrate feed in the amount of 20 g/head/day for sheep housed indoors and fed green forage from field crops resulted in milk comparable to that of sheep raised in natural pasture conditions in terms of fatty acid composition and health-promoting properties.

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