

The effect of adding herbs to the winter 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 winter (February–April) and individually from ewes at the end of the experiment. The milk was obtained from ewes of the coloured variety of Polish Merino, housed indoors and fed conserved bulky feeds and 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 in the period from 69 to 137 days of lactation the content of SFA increased in the milk fat of sheep fed without the addition of herbs, while the content of UFA decreased. The addition of the herbal supplement to the concentrate feed in the amount of 10 or 20 g/sheep/day improved the fatty acid profile from the 97th day of lactation. As a result, at the end of the experiment, the milk fat of sheep in groups II and III contained more MUFA acids than that of group I (6.7%; $P \leq 0.05$ and 10.6%; $P \leq 0.01$, respectively), more PUFA (by 11.1% and 12.5%; $P \leq 0.01$), and more DFA (by 6.3%; $P \leq 0.05$ and 11.8%; $P \leq 0.01$). The addition of herbs in the amount of 10 or 20 g/sheep/day (groups II and III) increased the content of *n*-3 PUFAs in the milk fat (by 17.5%; $P \leq 0.01$ and 7.9%; $P \leq 0.05$, respectively), *n*-6 PUFA (by 9.7% and 11.4%; $P \leq 0.01$) and CLA (by 11.9% and 28.6%; $P \leq 0.01$), and decreased the content SFA (by 1.75% and 2.8%, respectively; $P \leq 0.01$) and OFA (by 2.9%; $P \leq 0.05$ and 5.2%; $P \leq 0.01$). In both experimental groups, beneficial changes were also noted in the health quality indicators of the milk, calculated on the basis of the UFA/SFA, DFA/OFA and PUFA/SFA ratios.

KEY WORDS: sheep / herbs / fatty acid profile / haylage

Milk fat is the most complex of natural fats, as it contains about 500 fatty acids. At the same time, it is the most digestible fat of animal origin [2]. A unique component of milk

fat is short- and medium-chain fatty acids (SCFAs and MCFAs). They are hydrolysed faster than long-chain fatty acids and without the involvement of pancreatic lipase, owing to which they are a direct source of energy and do not cause obesity. Apart from its unquestionable nutritional value, milk fat has health-promoting properties. Butyric acid (C4:0) has been shown to have a beneficial effect in the treatment of inflammation and functional disorders of the intestines and to exert an antitumour effect [16, 19]. Oleic acid (C18:1) lowers blood cholesterol levels, while vaccenic acid (C18:1 *n*-7) slows tumour cell growth in the colon [1]. Polyunsaturated fatty acids (PUFAs, including *n*-3 and *n*-6 PUFAs) are a safe and effective therapy in the prevention and treatment of cardiovascular diseases and are essential for the proper development of the body and the functioning of its organs, especially the brain and retina [22, 23]. A highly valuable constituent of ruminant milk fat is conjugated linoleic acid (CLA). The available literature indicates a positive effect of this acid in preventing obesity (the *trans*-10, *cis*-12 isomer) [7], while CLA with the *cis*-9, *trans*-11 configuration has immune, antioxidant, anti-sclerotic and anti-cancer effects [4, 5].

Previous research has shown 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 feed ration [8, 27]. Furthermore, milk fat obtained from pasture-grazed animals has been shown to have better health-promoting properties than milk from animals fed conventionally [6, 10, 12]. Research has also shown seasonal changes in the composition of milk fat. Čermák et al. [11] in a study on pasture-grazed goats, reported positive changes in the composition of milk fat in the months when there was more clover and herbs in the vegetation. Kowal [17] demonstrated that the milk of cows kept in free-stall barns and fed throughout the year in a TMR system was richer in UFAs, including PUFAs, in spring and summer than in autumn and winter. This has been confirmed by other research results [3, 14]. Gerchev et al. [15] found significant differences in the composition of the milk fat of sheep grazing on mountain pastures in the period from April to July. The milk fat obtained in July had lower content of C4:0, C10:1 and C18:1 than in April and higher content of C6:0 and C14:1 in June than in April. Mel'uchova et al. [24] showed that seasonal changes in the fatty acid profile of sheep milk fat are the result of changes in the fat composition of the pasture vegetation. An improvement in the functional properties of cow milk fat through the use of herbs, as natural feed supplements [18] or sown in the pasture [26], has been demonstrated as well.

To sum up, research conducted mainly on dairy cattle has shown that the composition of milk can be modified for greater health benefits through the use of grazing or the addition of herbs to feed. Therefore we hypothesized that introducing a suitably composed mixture of herbs to the diet of milking sheep could improve the fatty acid profile of their milk. For this purpose, varied amounts of a herb mixture supplement were used for ewes housed indoors and fed preserved bulky feed from monoculture field crops.

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 75 ewes of the coloured variety of Polish Merino (aged 2 to 8 years), milked commercially after the lambs were weaned at the age of 8-9 weeks, during the winter (February-April). The ewes were housed indoors and fed preserved bulky feed (haylage, sugar beet pulp silage and 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 0.5 kg of milk.

The ewes were assigned to three feeding groups which were analogous in terms of lambing date, 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 in the concentrate feed wheat bran was replaced with a herb supplement – 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 officinalis*, chamomile *Matricaria chamomilla*, and milk thistle *Silybum marianum*). It 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 samples from bulk morning milk collected in each group at two-week intervals and 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 the milk fat were determined at the Institute of Agricultural and Food Biotechnology in Warsaw. Milk fat was extracted according to standard procedures described by Folch et al. [13]. Fatty acids were determined by gas chromatography [20] 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 mm x 20 µm).

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

The results of the bulk milk tests were used to assess changes in the fatty acid profile of the milk during the three-month milking period. The curves for the content of major

fatty acid groups indicate a general trend of increasing SFA content and decreasing UFA content in the milk fat during the experiment (Fig. 1). In the initial milking period, the curves for SFAs and UFAs (including MUFAs and PUFAs) were similar for all groups of ewes; SFA content increased and that of UFAs (including MUFAs and PUFAs) decreased. In the second half of the experiment (from the 97th day of lactation), there was an increase in the variation in the SFA and UFA content (including MUFAs) in the milk fat of ewes from different groups. During this period, favourable changes in the profile of the milk fat were observed in the experimental groups; SFA content decreased while that of UFAs, including MUFAs, increased. In contrast, in the control group there was a marked increase in SFA content and a decrease in that of MUFAs, and consequently a decrease in the total UFA content. The herb supplement had no clear effect on changes in PUFA, *n-3* PUFA or CLA content in the fat of the bulk milk during the experiment (Fig. 2).

Similar changes in the fatty acid profile of sheep milk fat were demonstrated by Gerchev et al. [15], i.e. an increase in SFA content and decrease in MUFA content, and by Mihaylova et al. [25], who in addition to the above also found a decrease in PUFA and CLA during lactation of ewes grazing on mountain pastures. Different results were reported by Ptasińska-Marcinkiewicz [28], in a study on the milk fat of mountain sheep and crosses of this breed with Friesian sheep (25% Friesian) and the milk of Olkuska sheep. The author found that changes in the fatty acid profile depended on the breed of sheep. In the milk of mountain sheep and crosses of this breed with Friesian sheep, the amount of SFAs decreased (with an increase in short-chain fatty acids) while 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, including SCFAs, was significantly higher and that of MUFAs and PUFAs was lower in the 1st and 3rd months of lactation than in the 2nd and 4th months. In a study by Strzałkowska et al. [29], during 200-day lactation of goats, i.e. in winter and summer feeding conditions, the content of SFAs in the milk fat decreased while that of MUFAs and PUFAs, including CLA, increased.

The beneficial effect of the herb mixture on the fatty acid profile was confirmed by the tests of milk collected individually from the ewes at the end of the experiment. They showed that the milk fat of sheep receiving the herb mixture in the amount of 10 or 20 g/head/day (groups II and III) had higher content of butyric acid C4:0, which is a short-chain fatty acid (SCFA), than the milk fat of sheep from the control group, by 9.5% ($P \leq 0.01$) and 6.5% ($P \leq 0.05$), respectively (Table 1). The milk fat from the experimental groups had a lower total content of medium-chain fatty acids (MCFAs) than the milk from the control group (on average by 6.1%, NS, Tab. 2), with higher content of C6:0 in both experimental groups and lower content of C10:0, C12:0 and C14:0; most of the differences with respect to the control were statistically significant (Table 1). The milk fat of sheep fed the herb mixture had lower content of SFAs and higher content

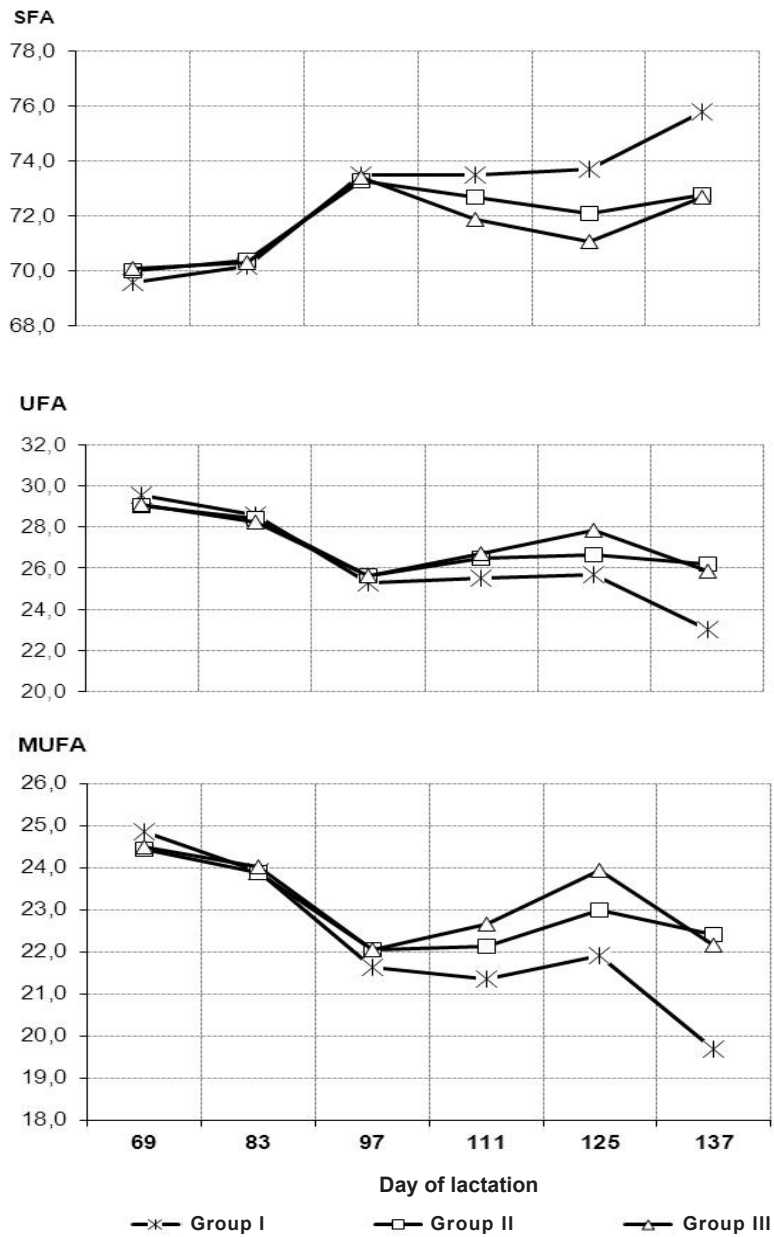


Fig. 1. Content of SFA, UFA and MUFA in the milk fat (g/100 g of fat) during the sheep milking period

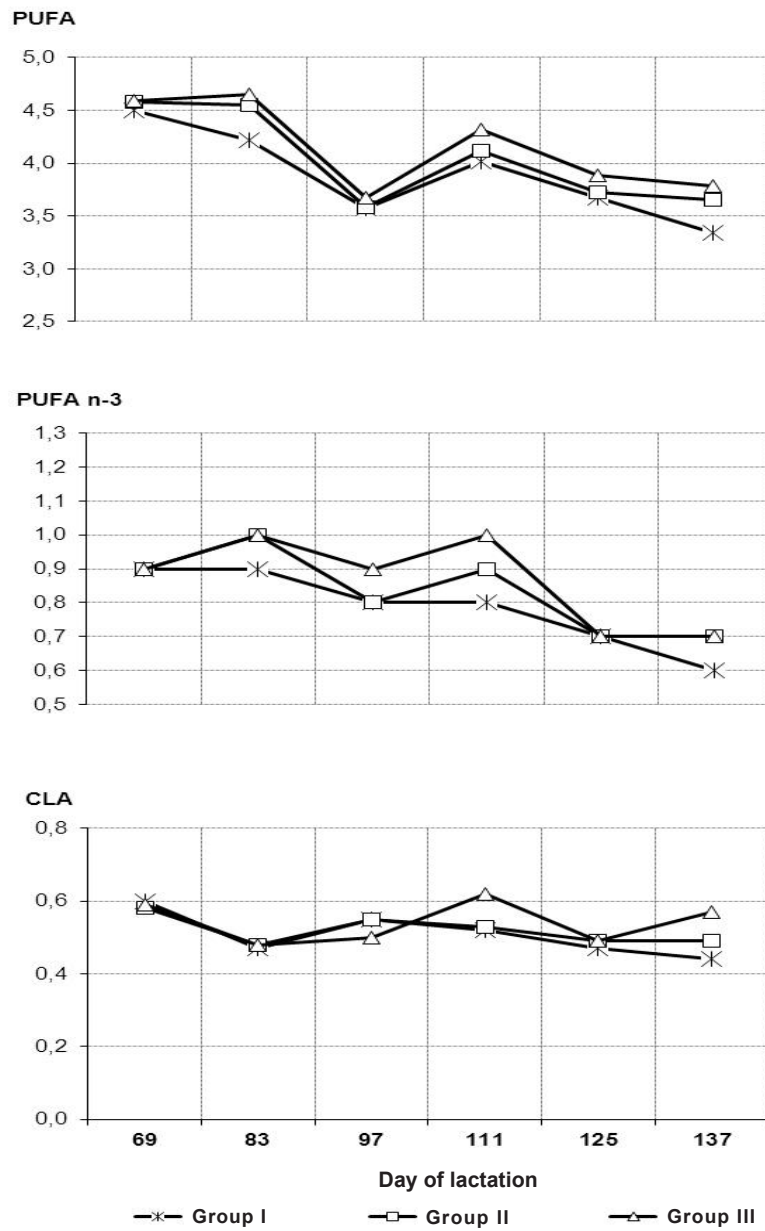


Fig. 2. Content of PUFA, *n*-3 PUFA and CLA in the milk fat (g/100 g of fat) during the sheep milk-ing period

of UFAs than the control, and the differences were greater when the addition of herbs to the feed was greater (Table 2). Milk fat from group II contained 1.8% less SFAs and 7.4% more UFAs than that of group I (control), while the corresponding differences between group III and I were 2.8% and 10.9% (all differences significant at $P \leq 0.01$). The lower total SFA content in the milk fat of the experimental groups was influenced by the lower content of MCFAs in these groups and the lower C16:0 content (by 5.5%, $P \leq 0.05$), despite the higher C18:0 content (by 15.6%, $P \leq 0.05$) in group III as compared to group I. In the case of unsaturated acids, the milk fat of ewes from both experimental groups had higher content of both monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs). The differences between groups II and I were 6.7% for MUFAs and 11.1% for PUFAs, while the corresponding differences between groups III and I were 10.6% and 12.5%, respectively (all statistically significant). The higher total MUFA content in the experimental groups resulted mainly from higher content of acids with 18 carbon atoms, while the higher PUFA content was the result of significantly higher content of C18:2 and C18:3. Due to the higher content of these PUFAs in the milk of the sheep from the experimental groups, the total content of *n*-3 PUFAs (dominant C18:3) and *n*-6 PUFAs (dominant C18:2) was higher in the experimental groups. On the other hand, the *n*-6/*n*-3 PUFA ratio, which is important for the health quality of milk, was most favourable in the milk of the ewes from group II; it was lower than in groups I and III by 6.5% (NS) and 9.2% ($P \leq 0.05$), respectively. The milk fat of ewes from both experimental groups had more favourable UFA/SFA and PUFA/SFA ratios than in the control (Table 2). The increase in the UFA/SFA ratio was 9.8% ($P \leq 0.05$) in the group receiving the herb mixture in the amount of 10 g/head/day (group II), and greater in the group receiving 20 g/head/day (group III), amounting to 14.6% ($P \leq 0.01$). The PUFA/SFA ratio in the two experimental groups was very similar, and was 16.7% higher ($P \leq 0.01$) than in the control. The content of CLA, one of the components determining the health quality of milk, was higher in both experimental groups than in the control – by 11.9% (NS) in group II and by 28.6% ($P \leq 0.01$) in group III. The milk fat of ewes fed the larger amount of herbs (20 g/head/day) contained 14.9% ($P \leq 0.01$) more CLA than the milk of ewes receiving 10 g/head/day. Higher content of hypocholesterolaemic acids (DFA) was found in the milk fat of the group II and III sheep than in the control, by 6.3% ($P \leq 0.05$) and 11.8% ($P \leq 0.01$), respectively, and lower content of hypercholesterolaemic acids (OFA), by 2.9% ($P \leq 0.05$) and 5.2% ($P \leq 0.01$), respectively.

The available literature contains few studies on the composition of sheep milk fat during winter feeding. The vast majority of research in this field has been conducted under grazing conditions. Gerchev et al. [15], in milk fat obtained in the fourth month of milking (July) from the local Tetevenska breed of sheep kept in mountain pastures, showed a similar content of SFAs (72.2%) to that found in our research for the sheep receiving the herb supplements in the amount of 20 g/head/day. The content of MUFAs (24.2%) and PUFAs

Table 1
Content of fatty acids in milk fat (g/100 g)

Fatty acids	Group			SEM
	I	II	III	
n	20	20	20	
C4:0	2.01 ^{Ba}	2.20 ^A	2.14 ^b	0.027
C6:0	2.04 ^{Bb}	2.15 ^a	2.18 ^A	0.021
C8:0	2.46	2.44	2.59	0.030
C10:0	9.83 ^a	9.26 ^b	9.56	0.111
C12:0	7.28 ^A	6.31 ^B	6.37 ^B	0.117
C14:0	14.56 ^A	13.61 ^B	13.30 ^B	0.159
C14:1	0.69 ^B	0.71 ^B	0.62 ^A	0.010
iso-C15:0	0.99	0.96	0.97	0.014
C15:0	1.44	1.37	1.40	0.023
C16:0	26.36 ^a	26.64 ^A	24.92 ^{Bb}	0.259
C16:1	1.65	1.74 ^A	1.50 ^B	0.035
iso-C17:0	1.53	1.51	1.52	0.018
C17:0	0.69	0.66	0.70	0.012
C18:0	5.96 ^b	6.09 ^b	6.89 ^a	0.145
C18:1 <i>T</i>	1.39	1.27	1.55	0.057
C18:1 <i>c</i> ₉	14.15 ^{Bb}	15.36 ^a	16.15 ^A	0.223
C18:1 <i>c</i> ₁₁	0.52	0.56	0.61	0.009
C18:1 <i>c</i> (other)	0.69 ^{Bb}	0.81 ^a	0.88 ^A	0.022
C18:2	2.07 ^A	2.29 ^B	2.30 ^B	0.034
C18:3	0.43 ^{Bb}	0.53 ^A	0.48 ^a	0.011

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

(4.1%), however, was slightly higher than in our study. Mihaylova et al. [25], examining the composition of the milk fat of local breeds of sheep grazed on mountain pastures, observed a lower total content of SFAs (70.1%), higher content of PUFAs (7.7%) and CLA (2.5%), and slightly lower content of MUFAs (22.2%) in the third month of milking (July) than in our study in the case of the larger herb supplement. Research by Borys et al. [9] confirms that feeding sheep a diet with green forage has a more beneficial effect on the fatty acid profile of their milk, in terms of health benefits, than feeding with preserved bulky feeds. A similar tendency was demonstrated by Kowal [17] in cow milk fat obtained in autumn and winter as compared to spring and summer. The beneficial effect of organic feeding of cows (grazing in summer) as compared to conventional feeding (silage year round) on the health-promoting properties of milk fat has been confirmed in studies by other authors [6, 14, 21].

To sum up, in conditions of winter feeding with bulky feed during the period from 69 to 137 days of lactation, indicators of the health-promoting quality of milk fat deteriorated in the group of sheep fed without herbs – SFA content increased and that of UFAs decreased.

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

Fatty acids	Group			SEM
	I	II	III	
SFA	75.11 ^A	73.79 ^B	72.98 ^B	0.309
MCFA	40.18	37.75	37.71	0.351
UFA	23.78 ^A	25.53 ^B	26.38 ^B	0.298
including:				
MUFA	20.36 ^{Bb}	21.72 ^a	22.52 ^A	0.255
PUFA	3.43 ^A	3.81 ^B	3.86 ^B	0.051
including:				
PUFA <i>n-3</i>	0.63 ^{Bb}	0.74 ^{Ab}	0.68 ^a	0.012
PUFA <i>n-6</i>	2.27 ^A	2.49 ^B	2.53 ^B	0.036
CLA	0.42 ^B	0.47 ^B	0.54 ^A	0.012
UFA/SFA	0.316 ^{Bb}	0.347 ^a	0.362 ^A	0.005
PUFA/SFA	0.045 ^A	0.052 ^B	0.053 ^B	0.001
PUFA <i>n-6/n-3</i>	3.618	3.383 ^b	3.725 ^a	0.057
DFA	29.75 ^{Bb}	31.63 ^a	33.27 ^A	0.405
OFA	69.75 ^{Aa}	67.70 ^b	66.09 ^B	0.415
DFA/OFA	0.430 ^{Bb}	0.469 ^a	0.505 ^A	0.009

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 + C22:5 + 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 – $P \leq 0.01$; a, b – $P \leq 0.05$

The inclusion of an herb supplement in the bulky feed of the sheep in the amount of 10 or 20 g/head/day (groups II and III) improved the fatty acid profile of their milk fat from the 97th day of lactation. As a result, at the end of the experiment, the milk fat of the sheep in groups II and III, receiving the herb supplement, contained more MUFAs, PUFAs (including *n-3* PUFAs, *n-6* PUFAs and CLA) and DFAs than that of group I (control). The herb mixture added to the concentrate feed in the amount of 10 or 20 g/head/day reduced the content of SFAs and OFAs, which are unfavourable in terms of health. As a consequence, the health quality indicators of the milk, calculated based on the UFA/SFA, DFA/OFA and PUFA/SFA ratios, also underwent beneficial changes. The analysis suggests that the addition of the herb mixture to the concentrate feed of sheep housed indoors improved the health-promoting properties of milk fat. The larger herb supplement (20 g/head/day) resulted in milk that was comparable in terms of fatty acid composition and health benefits to the milk of sheep raised in natural pasture conditions.

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