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The effect of herbs added to the summer diet of sheep on the lipid fraction profile of raw milk for cheese making and rennet cheese produced from it

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A study was conducted on samples of raw sheep milk and bundz rennet cheese produced from it. The milk was obtained from ewes of the Koluda prolific dairy breed, from June to August. The sheep were housed indoors and fed alfalfa green forage, hay, and a mixture of concentrate feeds. Three feeding groups were established: group I – control, fed without the addition of herbs to the concentrate feed, and groups II and III, in which a herb mixture was added to the concentrate feed in the amount of 10 and 20 g/sheep/day, respectively. Six experimental batches of bundz rennet cheese were made from the milk, and the effect of the addition of herbs to the diet on the lipid profile of the milk and cheese was analysed. The results indicate that the experimental factor had no statistically confirmed effect on the lipid profile of the raw milk used to make cheese. However, the milk fat from sheep fed a diet with herbs at 20 g/sheep/day showed a tendency towards reduced content of SFA (by 3.6%) and increased content of UFA (by 6.8%) and DFA (by 7.2%) relative to the control group. The experimental factor had essentially no statistically confirmed effect on the fatty acid profile of the cheese, apart from an 11.3% increase in the content of *n*-6 PUFA ($p \leq 0.05$) in the fat of bundz produced from the milk of sheep fed a diet with herbs at 20 g/sheep/day with respect to the control group. The cheese fat also showed a tendency towards lower content of SFA (by 3.4%) and OFA (by 4.8%) and higher content of UFA (by 7.4%), MUFA (by 7.1%), PUFA (by 9.4%), *n*-3 PUFA (by 11.5%) and DFA (by 7.6%) compared to the control group. The experimental factor had no statistically confirmed effect on cholesterol content in the raw milk or the bundz cheese produced from it.

KEY WORDS: sheep feeding, herbs, sheep cheese, lipid profile

In times of increased incidence of diet-related illnesses, consumers are interested in products that are not only tasty and safe for their health, but also natural and beneficial for their health [20, 31, 36]. This has led to an increase in the importance of functional food, i.e. food that contains health-promoting components with proven benefits for one or more body functions, beyond nutrition [16, 24]. Surveyed consumers expect food from animals raised in a traditional manner, produced using traditional methods, without added ingredients [2, 35]. On the other hand, consumers accept measures taken to reduce the content of components that negatively affect health, such as cholesterol and fat [39]. Studies of milk fat have shown that it is composed of about 500 fatty acids, many of which have health-promoting properties [3]. Butyric acid (C4:0) relieves intestinal dysfunction and has anti-cancer effects [18, 26]; oleic acid (C18:1) lowers blood cholesterol; monounsaturated fatty acids (MUFA) prevent atherosclerosis; vaccenic acid (C18:1 *n*-7) slows the growth of cancer cells in the colon [1]; and polyunsaturated fatty acids *n*-3 PUFA and *n*-6 PUFA prevent and treat cardiovascular diseases and are essential for the proper development of the body and organ function, especially the of brain and retina [28, 29]. Particularly important for our health is CLA, which has antioxidant, immunological, anti-atherosclerotic and anticancer properties and prevents obesity [4, 5, 7]. Therefore, many studies have been undertaken with the aim of modifying the functional properties of milk fat. The intended effect has been obtained by using supplements in animal diets, including linseed [21], *Camelina sativa* cake [10, 34], dried distillers grains with solubles (DDGS) [38] and herbs [25, 33]. The literature also indicates that pasture grazing is preferable to conventional feeding in this regard [6, 9, 12].

Summing up, it has been demonstrated that milk fat composition can be modified to enhance benefits to human health by means of a suitable animal diet, including the addition of herbs, primarily in dairy cattle. In view of the above, we hypothesized that the inclusion of a suitably composed herb mixture in the diet of milking sheep raised indoors would make it possible to improve the lipid fraction of milk, and thus the cheese produced from it. For this purpose, varied levels of a herb mixture (10 or 20 g/sheep/day) were added to concentrate feed for sheep, which were fed mainly with green alfalfa forage and hay from monoculture field crops.

Material and methods

The research was carried out at the Koluda Wielka Experimental Station of the National Research Institute of Animal Production. The subject of the study was sheep milk obtained from 66 ewes of the Koluda prolific dairy breed (aged 2 to 8 years) and bundz rennet cheese produced from it. After rearing their lambs to the age of 8-9 weeks, the sheep were used for milking, which lasted from June to August (3 months). They were housed indoors and fed according to INRA-88 standards for milked sheep (based on the requirements of a ewe with a body weight of 70 kg, producing on average 0.6 kg of milk). They received green alfalfa, hay, and compound concentrate feed. Three feeding groups were formed for the experiment, with 22 ewes assigned to each group in such a way that the groups were similar in age, body weight, lambing date and litter size. The sheep in group I (control) were fed bulky feed and concentrate feed without herbs. Sheep in groups II and III were fed as in group I, but a mixture of herbs was added to the concentrate feed in the amount of 10 or 20 g/sheep/day, respectively. The herb mixture consisted of 9 herbs: common nettle *Urtica dioica*, fennel *Foeniculum capillaceum*, caraway *Carum carvi*, coriander *Coriandrum sativum*, fenugreek *Trigonella foenumgracum*, peppermint *Mentla piperita*, English marigold *Calendula officinialis*, chamomile *Matricaria chamomilla*, and milk thistle *Silybum marianum*. The herbs were intended to benefit the animals mainly by improving their metabolism and digestion and by exerting galactogogue, bacteriostatic and anti-inflammatory effects. They were also meant to improve the lipid profile of the raw milk used for cheese making.

Six experimental batches of bundz cheese were made from the sheep milk (at two-week intervals; the first batch was made in the second week of the experimental diet). The cheese was produced at the milk processing plant associated with the farm, at the Kołuda Wielka Experimental Station of the National Research Institute of Animal Production, by the vat method, using 10 kg of milk from each group. The milk was pasteurized at 75°C for 30 min and then cooled to 34°C, after which calf rennet was added (0.15 ml/L milk). The lipid profile and cholesterol content were determined in samples taken from bulk raw milk and cheese (6 batches x 3 groups = 18 samples of raw milk and cheese). Lipids were extracted according to standard procedures given by Folch et al. [13], and fatty acid analysis was performed by gas chromatography [27], on a Hewlett Packard 6890 gas chromatograph with a flame ionization detector, using an Rtx-2330 column (105 m x 0.25 mm x 20 μ), with modifications used at the Institute of Agricultural and Food Biotechnology in Warsaw. Cholesterol content was determined by the same method, with a Hewlett Packard 5890 sII gas chromatograph with a flame ionization detector, on an HP-1 column, 25 m long, 0.20 mm in diameter and 0.11 μm thick.

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

Results and discussion

The tests carried out on bulk raw milk samples showed no statistically confirmed differences between groups in the lipid profile of the raw milk used to make cheese (Table 1). The group III milk fat contained less SFA (by 3.6%) than group I, and more UFA (by 6.8%), including more MUFA and PUFA, by 6.5% and 8.3%, respectively. Among saturated fatty acids, this fat contained more beneficial stearic acid C18:0 (by 8.9%) than the control group. Content of C18:1 was higher in the milk fat from group II than from group I (by 7.8%). The highest content of MUFA in the group III milk fat was due to the higher content of C18:1 *c*9 (dominant in this group of acids) compared to group I (by 9.3%). Milk fat from sheep whose feed was supplemented with herbs had higher content of PUFA. It should be noted that the differences relative to group I were greater in group III than in group II, by 14.9% and 5.7%, respectively, for *n*-3 PUFA and by 7.4% and 2.9% for *n*-6 PUFA.

The most beneficial *n*-6/*n*-3 PUFA ratio was found in the milk of group III ewes, on average 7.1% lower than in groups I and II, which were similar in this respect. The group III milk also had the most favourable UFA/SFA and PUFA/SFA ratios, by 10.6% and 12.3%, respectively, relative to group I and by 9.9% and 9.0% relative to group II. In the group III milk fat, a favourable tendency in terms of health quality was found for the content of hypocholesterolaemic (DFA) and hypercholesterolaemic (OFA) acids and in the DFA/OFA ratio. Group III milk fat compared to groups I and II contained more DFA (on average by 6.2%) and less OFA (by 4.5%), and thus had an 11.1% higher DFA/OFA ratio. There were no significant differences between groups in the cholesterol content of the raw milk or in the CLA content of its fat. There was only a tendency towards lower (by 5.4%) cholesterol content in the milk of the experimental groups as compared to the control.

Bonczar et al. [8] found that the fat of the milk of pasture-grazed mountain sheep had a less favourable composition in terms of single fatty acids, apart from the content of CLA (1.4%), as compared to our results. Gerchev et al. [15], in the fat of milk obtained in the 4th month of milking (July) from sheep of the local Teteven breed kept on mountain pastures, showed a higher content of SFA (72.2%) and lower content of MUFA (24.2%) and PUFA (4.1%) than in all feeding groups in the present study. Mihaylova et al. [30], in their analysis of the fat composition of milk of local sheep grazing on mountain pastures, showed generally higher content of SFA (70.1%), PUFA (7.7%) and CLA (2.5%), and lower content of MUFA (22.2%) in the third month of milking (July). A study by Gerchev and Mihaylová [14] found that the milk fat of Srednostaroplaninska and Teteven sheep grazed in mountain pastures according to a traditional method contained more SFA (70.8% and 71.0%, respectively) and less MUFA (25.0% and 25.0%) compared to all feeding groups in our research, and less PUFA (4.5% and 3.9%) relative to group III. In general, it can be concluded that in the present study the composition of the fat of the raw milk for cheese making, especially in group III, was comparable in terms of health benefits to that found in the cited studies on grazed sheep.

Table 1
Lipid profile of raw milk for cheese making (g/100 g)

Item	Group			SEM
	I	II	III	
Number of batches	6	6	6	
C4:0	2.18	2.05	2.15	0.043
C6:0	1.87	1.85	1.70	0.050
C8:0	1.80	1.77	1.60	0.062
C10:0	5.98	5.83	5.07	0.252
C12:0	3.82	3.60	3.22	0.173
C14:0	10.90	10.48	10.02	0.303
iso-C15:0	0.67	0.67	0.67	0.018
C15:0	1.23	1.25	1.23	0.014
C16:0	29.38	29.45	29.03	0.514
iso-C17:0	1.25	1.27	1.28	0.037
C17:0	0.82	0.83	0.87	0.030
C18:0	7.28	7.85	7.93	0.372
C14:1	0.63	0.60	0.58	0.019
C16:1	1.83	1.68	1.80	0.063
C18:1 *T*	1.83	1.83	1.81	0.031
C18:1 *c*9	20.12	20.33	22.00	0.778
C18:1 *c*11	0.48	0.50	0.48	0.011
C18:1 *c* other	1.23	1.25	1.25	0.026
C18:2	2.35	2.40	2.52	0.045
C18:3	0.58	0.58	0.63	0.028
SFA	67.75	67.53	65.33	0.854
UFA	31.71	31.89	33.86	0.825
including				
MUFA	27.36	27.40	29.15	0.751
PUFA	4.35	4.49	4.71	0.088
including				
PUFA *n-3*	0.87	0.92	1.00	0.032
PUFA *n-6*	2.72	2.80	2.92	0.055
CLA	0.77	0.77	0.79	0.025
UFA/SFA	0.472	0.475	0.522	0.018
PUFA/SFA	0.065	0.067	0.073	0.002
PUFA *n-6/n-3*	3.187	3.116	2.928	0.099
DFA	38.99	39.74	41.79	1.176
OFA	60.47	59.68	57.40	1.204
DFA/OFA	0.657	0.673	0.739	0.031
Cholesterol (mg/100 g of milk)	20.2	19.1	19.1	0.398

SFA: Σ 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: Σ 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: Σ C18:2, CLA, C18:3, C20:2, C20:4, C20:5, C22:5, C22:6
PUFA *n-3*: Σ C18:3, C20:5, C22:5, C22:6
PUFA *n-6*: Σ C18:2, C20:2, C20:4
DFA = UFA + C18:0
OFA = SFA – C18:0

Table 2
Lipid profile of bundz rennet cheese (g/100 g)

Item	Group			SEM
	I	II	III	
Number of batches	6	6	6	
C4:0	2.22	2.05	2.05	0.045
C6:0	1.95	1.88	1.75	0.052
C8:0	1.87	1.82	1.67	0.061
C10:0	6.13	5.90	5.28	0.247
C12:0	3.90	3.67	3.35	0.169
C14:0	11.15	10.68	10.27	0.266
iso-C15:0	0.65	0.67	0.67	0.020
C15:0	1.20	1.25	1.23	0.018
C16:0	29.70	29.62	29.45	0.332
iso-C17:0	1.23	1.27	1.30	0.036
C17:0	0.82	0.82	0.83	0.029
C18:0	7.05	7.58	7.65	0.298
C14:1	0.62	0.62	0.60	0.016
C16:1	1.78	1.73	1.77	0.048
C18:1 *T*	1.81	1.81	1.80	0.028
C18:1 *c*9	19.68	19.88	21.52	0.627
C18:1 *c*11	0.48	0.50	0.50	0.015
C18:1 *c* other	1.22	1.25	1.28	0.027
C18:2	2.30	2.40	2.53	0.046
C18:3	0.57	0.62	0.67	0.032
SFA	68.43	67.77	66.08	0.688
UFA	31.05	31.45	33.35	0.698
including				
MUFA	26.79	26.99	28.69	0.622
PUFA	4.26	4.46	4.66	0.096
including				
PUFA *n-3*	0.87	0.92	0.97	0.035
PUFA *n-6*	2.65[b]	2.78	2.95[a]	0.051
CLA	0.75	0.76	0.78	0.025
UFA/SFA	0.456	0.466	0.507	0.015
PUFA/SFA	0.063	0.066	0.071	0.008
PUFA *n-6/n-3*	3.130	3.077	3.069	0.098
DFA	38.10	39.04	41.00	0.973
OFA	61.38	60.18	58.43	0.965
DFA/OFA	0.629	0.653	0.708	0.025
Cholesterol (mg/100 mg of cheese)	49.4	43.0	44.2	1.801

SFA: Σ 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: Σ 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: Σ C18:2, CLA, C18:3, C20:2, C20:4, C20:5, C22:5, C22:6
PUFA *n-3*: Σ C18:3, C20:5, C22:5, C22:6
PUFA *n-6*: Σ C18:2, C20:2, C20:4
DFA = UFA + C18:0
OFA = SFA – C18:0
a, b – $P \leq 0.05$

The fat of the rennet cheese generally did not differ in composition from the fat of the raw milk from which it was made. No statistically confirmed differences in the fatty acid profile of the bundz were found between groups, except for 11.3% higher content of *n*-6 PUFAs in the fat of group III bundz relative to group I ($P \leq 0.05$; Table 2). However, there was a tendency towards lower content of SFA (by 3.4%) and higher content of UFA (by 7.4%), MUFA (by 7.1%), PUFA (by 9.4%) and *n*-3 PUFA (by 11.5%) in the fat of cheese made from the milk of sheep whose feed was supplemented with herbs in the amount of 20 g/sheep/day, relative to the control group. Bundz fat did not differ statistically significantly in CLA content between groups. Cheese fat from groups II and III, compared to group I, contained more DFA (by 2.5% and 7.6%, respectively) and less OFA (by 2.0% and 4.8%), which translated to a higher DFA/OFA ratio, by 3.8% and 12.6%, respectively. In the case of bundz obtained from the milk of ewes of both experimental groups, there was a tendency towards improvement, in terms of health quality, in the UFA/SFA and PUFA/SFA ratios, but while there was only a slight increase in these two parameters in group II (by 2.2% and 4.8%, respectively, relative to group I), the corresponding increases in the case of group III was 11.2% and 12.7%.

The bundz fat obtained in our research contained less SFA, OFA, *n*-6 PUFA and CLA, and more DFA and UFA, including MUFA, as compared to results reported by Pakulski and Pakulska [32], who produced the same type of cheese in winter. On the other hand, Bonczar et al. [8], in a study of the fat of bundz made from the milk of pasture-grazed mountain sheep, showed a less favourable fatty acid composition than in our research. They found higher content of saturated C4:0-C14: 0 (39.5%) and lower content of C18:1 (13.8%), C18:2 (1.4%) and C18:3 (0.51 %). The bundz fat in that study contained less C16:0 (25.5%) and more CLA (1.13%) than the cheese fat in the present study.

The composition of the lipid fraction of rennet cheese produced from milk obtained from sheep in the period from 69 to 137 days of lactation underwent changes (Figs. 1 and 2). The curves for the content of individual acid groups were fairly characteristic, similar for all feeding groups. The content of SFA in bundz fat dropped between 69 and 83 days of lactation, remained at a similar level until 125 days of lactation, and then increased most in group I (control). The curves for the content of all analysed categories of unsaturated fatty acids (UFA, MUFA and PUFA) were similar and ran opposite to the curves for SFA (Figs. 1 and 2). The content of these unsaturated fatty acids increased in the fat of cheese made from milk obtained between 69 and 83 days of lactation, remained at an elevated level up to 125 days of lactation, and then declined. CLA content (Fig. 2) increased in the cheese fat of all groups until the 125th day of lactation (by about 50% from the initial value), after which it decreased most in group I. It should be noted that the curves plotted for the profile of the main fatty acid groups in the bundz fat indicate a more favourable composition in terms of health benefits in group III than in groups I and II.

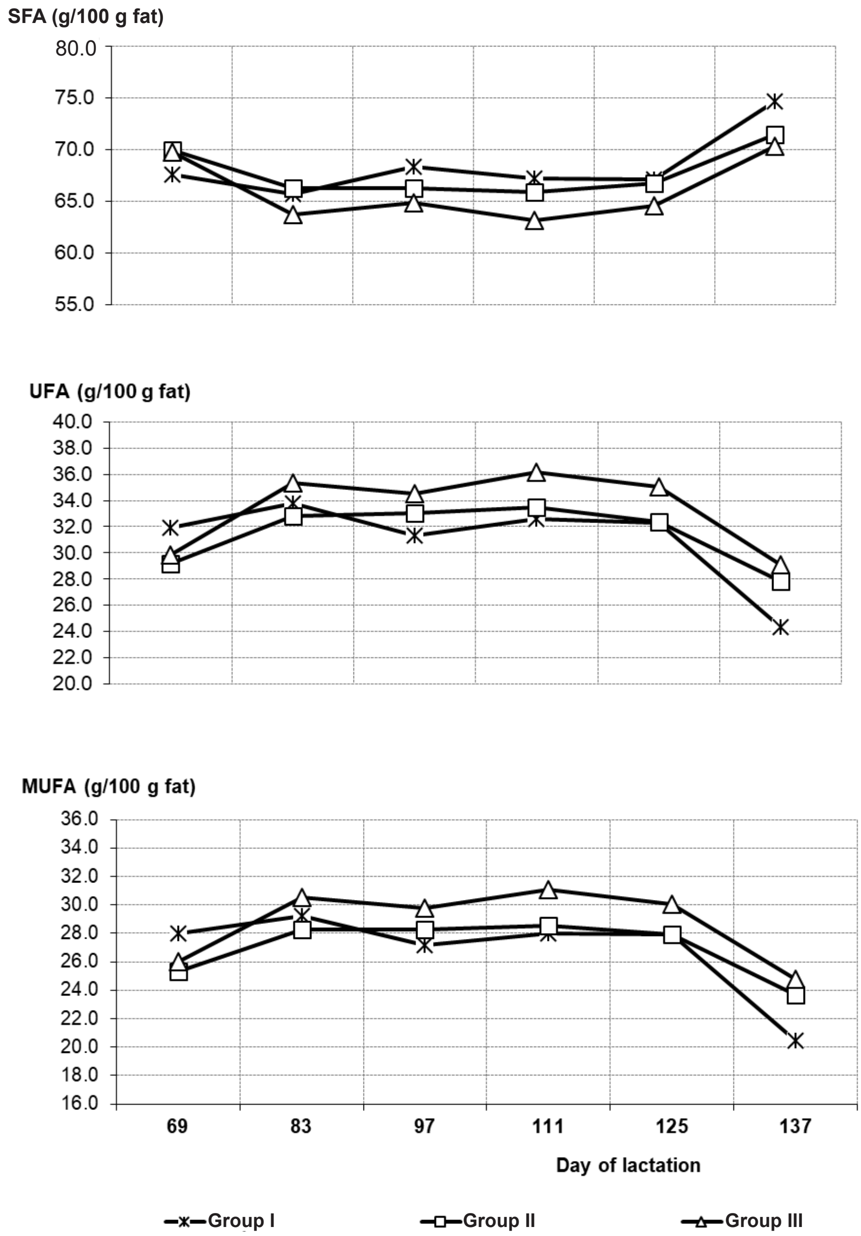

Fig. 1. Changes in the content of SFA, UFA and MUFA in the rennet cheese fat produced from sheep milk obtained in the period from 69 to 137 days of lactation

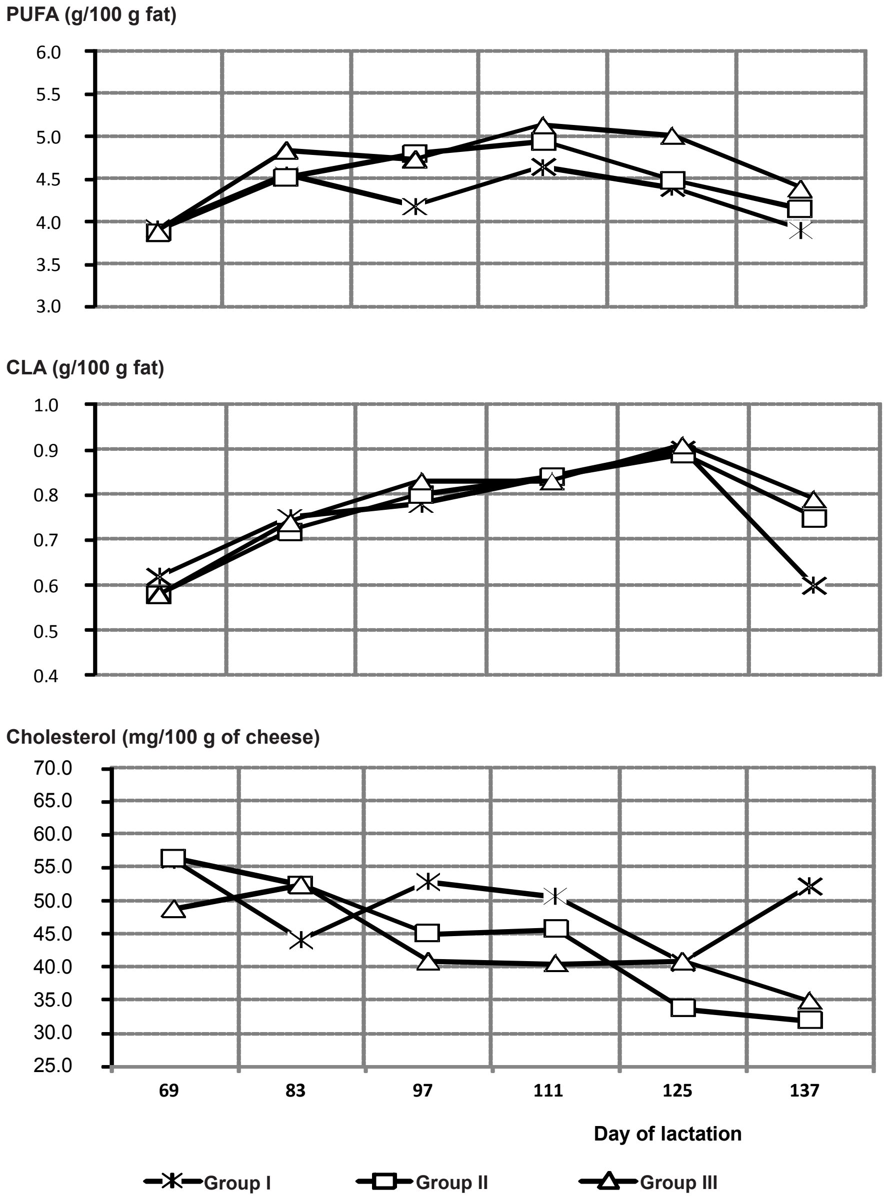

Fig. 2. Changes in the content of PUFA, CLA and cholesterol in the lipid fraction of cheese produced from sheep milk obtained in the period from 69 to 137 days of lactation

Similar changes in fatty acid profile have been found by Kawęcka [22] in the fat of oscypek cheese made from the milk of Polish Mountain sheep and Cakiel Podhalański sheep. The fat of this cheese produced in August contained significantly more SFA and less UFA than in May. Changes in the fatty acid profile of the lipid fraction of bundz observed in our study were probably due to changes taking place in the milk fat during the experiment, which the authors have demonstrated in a previous publication [19]. Pakulska and Pakulski [32] revealed no effect of technological processes on the fat composition of cheese. This is confirmed by studies by Bonczar et al. [8] and Čermák et al. [11] conducted on goat milk and cheese. This indicates the possibility of effective modification of the quality of the final product at the stage of raw milk production.

Bundz made from the milk of ewes in groups II and III contained less cholesterol compared to group I, by 13.0% and 10.5%, respectively, but these differences were not confirmed statistically (Table 2). In the cheese made from sheep milk from the experimental groups, a downward trend in cholesterol was observed during the study (Fig. 2). On the other hand, the content of this lipid in bundz obtained from the milk of the control group ewes showed substantial fluctuations in successive stages of the study, without a clear tendency. It should be noted that the cholesterol content was much higher in the bundz than in the raw milk it was produced from. This is due to the higher fat content in cheese than in milk. A study by Kovacs et al. [23] found a positive correlation ($r = 0.98$) between fat and cholesterol content, while Talpur et al. [37] reported a correlation of 0.63. The fluctuations in the cholesterol content of cheese in our research could also be due to production under experimental conditions, where the pasteurization process was not fully standardized (vat method). Grega et al. [17] showed higher cholesterol content in pasteurized and sterilized milk than in raw milk, while the fat content was the same.

To sum up, there was a clear tendency towards improved health-promoting parameters in the fat of milk from ewes whose diet was supplemented with herbs in the amount of 20 g/sheep/day, as there was a reduction in SFA content (by 3.6%) and an increase in UFA (by 6.8%), including MUFA (by 6.5%), PUFA (by 8.3%), *n*-3 PUFA (by 14.9%), *n*-6 PUFA (by 7.4%) and DFA (by 7.2%), compared to the control group. However, the differences were not statistically confirmed. This may have been due to an insufficient number of observations, as well as to changes in the lipid fraction of milk occurring during the experiment. At the start of the experiment, bundz fat showed a decrease in SFA content and an increase in MUFA and PUFA content (greater in the group III cheese), which is beneficial for human health. This lasted for two months, but decreased in the final stage of the study. The CLA content in the bundz fat from all feeding groups increased up to the 125th day of lactation (by about 50%), and then decreased significantly. In general, the experimental factor had no statistically confirmed effect on the fatty acid profile of bundz, except for an increase in *n*-6 PUFA content (by 11.3%; $P \leq 0.05$) in the fat of bundz produced from the milk of sheep whose diet was supplemented with herbs in the amount of 20 g/sheep/day, relative to the control group. In the fat of cheese made from this milk, there was also a tendency towards lower content of SFA (by 3.4%) and OFA (by 4.8%) and higher content of UFA (by 7.4%), MUFA (by 7.1%), PUFA (by 9.4%), *n*-3 PUFA (by 11.5%) and DFA (by 7.6%) compared to the control group. The experimental factor had no statistically confirmed effect on the cholesterol content in the raw milk or in the bundz rennet cheese type produced from it.

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