Scientific Annals of Polish Society of Animal Production - Vol. 15 (2019), No 4, 59-72

DOI: 10.5604/01.3001.0013.6480

The effect of herbs added to the winter diet 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 rennet cheese produced from it. The milk was obtained from ewes of the coloured variety of Polish Merino, from February to April. The sheep were housed indoors and fed preserved roughage 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 indicated that the addition of herbs to the winter diet of sheep in the amount of 10 and 20 g/sheep/day significantly increased the content of the acid C4:0 in the raw milk used for cheese making, by 11.5% and 20.0%, respectively (P≤0.01), relative to the control group. C4:0 content in the raw milk was also increased in group III by 7.6% compared to group II (P≤0.05). The herbs had no statistically confirmed effect on other parameters of the health-promoting quality of the milk fat and the rennet cheese. In the cheese fat from group I produced from sheep milk obtained in the period from 69 to 137 days of lactation, there was an increase in the content of SFA and a decrease in UFA during the course of the experiment. The addition of herbs to the concentrate feed of the sheep improved the fatty acid profile of the fat of bundz cheese made from milk obtained from the 97th day of lactation. The experimental factor also reduced the cholesterol content in the raw milk in group II by 26.8% and in group III by 21.2% (P≤0.01) compared to group I. It did not affect the content of this lipid in rennet cheese.

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

Functional food, i.e. food with health-promoting components having a proven beneficial effect on one or more body functions beyond nutrition, is very popular among consumers, who want products that are not only tasty and safe, but also natural and beneficial

for their health [18, 22, 26, 34, 39]. According to surveyed consumers, food quality can be improved by returning to extensive livestock farming, natural feeding, and traditional food production methods, without added ingredients [2]. The results of recent studies, however, indicate acceptance of measures taken to reduce the content of components negatively affecting health, e.g. cholesterol and fat [38, 42]. Milk fat is composed of about 500 fatty acids [3], many of which have health-promoting effects. Butyric acid (C4:0) relieves inflammation and intestinal dysfunction and exerts anti-cancer effects [20, 28]; oleic acid (C18:1) lowers blood cholesterol; monounsaturated fatty acids (MUFA) prevent atherosclerosis; vaccenic acid (C18:1 *n*-7) slows the growth of colon cancer cells [1]; and polyunsaturated fatty acids *n*-3 PUFA and *n*-6 PUFA prevent and treat cardiovascular disease and are essential for the proper development of the body and organ function, especially of the brain and retina [31, 32]. Particularly important for our health is CLA, which prevents obesity and has immunological, antioxidant, anti-atherosclerotic and anticancer properties [4, 5, 7]. In response to the expectations of contemporary consumers, numerous studies have been undertaken with the aim of modifying the functional properties of milk fat. The fatty acid profile has been improved by using supplements in animal diets, including linseed [23], *Camelina sativa* cake [11, 37], dried distillers grains with solubles (DDGS) [41] and various herbs [27, 36]. Pasture grazing has also been shown to have a beneficial effect in this regard in comparison to conventional feeding [6, 10, 13].

The results of the cited studies indicate that milk fat composition can be modified to benefit human health by means of a suitable animal diet, including the addition of herbs to dairy cow feed. In view of the above, we hypothesized that the inclusion of a suitably composed herb mixture in the diet of milking sheep (fed with preserved bulky feed from monoculture field crops) could improve the lipid fraction of their milk, and thus the cheese produced from it. For this purpose, varied levels of a herb mixture supplement were used, in the amount of 10 or 20 g/sheep/day.

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 raw sheep milk obtained from Merino ewes of the coloured variety (aged 2 to 8 years) and bundz rennet cheese produced from it. After rearing their lambs (at the age of 8-9 weeks), the sheep were used for milking, which lasted for 3 months (from February to April). The ewes were housed indoors and fed preserved bulky feed (haylage, sugar beet pulp silage, and hay) and compound concentrate feed. The feeding level was established 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 0.5 kg of milk. Three feeding groups were formed for

the experiment, with 25 ewes assigned to each group in such a way that the groups were similar in age, body weight, lambing date and litter size. The experimental factor was a herb mixture composed of 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*. It was intended to affect the animals mainly by improving their metabolism and digestion, exerting galactogogue, bacteriostatic and anti-inflammatory effects, and improving the lipid profile of the raw milk used for cheese making. All groups were fed the above-mentioned bulky feed and concentrate feed: group I (control) without herbs, and groups II and II with the addition of herbs in the amount of 10 or 20 g/sheep/day, respectively.

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 by the vat method using 10 kg of milk from each group, at the milk processing plant associated with the farm at the Kołuda Wielka Experimental Station of the National Research Institute of Animal Production. 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 the raw milk and cheese (6 batches x 3 groups = 18 samples of raw milk and cheese). Fat was extracted according to standard procedures given by Folch et al. [14], and fatty acids in the fat were analysed by gas chromatography [29], with modifications used at the Institute of Agricultural and Food Biotechnology in Warsaw, 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), Cholesterol content was also determined by gas chromatography, 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

There were no statistically confirmed differences between groups in the total content of saturated fatty acids (SFA) in the bulk milk fat (Table 1), except for the content of C4:0, which was significantly higher in the milk fat from the experimental groups compared to the control group (by 11.5% and 20.0% in groups II and III, respectively; $P \leq 0.01$); additionally, the content in group III was higher than in group II (by 7.6%; $P \leq 0.05$). Among SFA, fairly characteristic tendencies were observed towards higher content of C6:0 and C8:0

(on average by 6.0% and 4.0%, respectively) and of C17:0 and C18:0 (by 6.6% and 6.4%) in the experimental groups than in the control, and lower content of C12:0 and C14:0 (by 8.6% and 5.3%). There were also no statistically significant differences between groups in UFA content in the fat of the bulk milk, including the total content of MUFA, PUFA, *n*-3 and *n*-6 PUFA, and CLA, and the *n*-6/*n*-3 PUFA ratio. However, total MUFA content was slightly higher in the milk fat of ewes from the experimental groups (by 3.3% on average), mainly due to the higher content of C18:1 *c*9 and C18:1 *c*11 for these groups (by 5.6% and 7.0%, respectively). The fat of the raw milk from the experimental groups also showed a tendency towards higher UFA/SFA (on average by 4.6%) and DFA/OFA (by 4.0%) ratios, which is more favourable in terms of health quality. This was due to the higher content of DFA and lower content of OFA in the experimental groups than in the control group. Bulk milk obtained from ewes in groups II and III contained significantly less cholesterol; the differences in relation to group I were 26.7% and 21.3%, respectively ($P \leq 0.01$; Table 1).

In a study by Pakulski and Pakulska [35], the fat of milk of coloured Merinos used to make bundz during the winter feeding period had a more beneficial fatty acid composition than in the present study, in terms of the content of PUFA, including CLA, and of SFA and MUFA in the second year of the experiment. However, in the first year of the experiment, the milk fat had similar content of SFA and lower content of MUFA in comparison to the experimental groups in the present study. Few studies in the available literature deal with the composition of sheep milk fat during winter feeding. Most studies on this subject have been carried out under grazing conditions. Gerchev et al. [17], 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 similar content of SFA (72.2%) and PUFA (4.1%) to that found in our research. The content of MUFA (24.2%) in the cited study was slightly higher. Mihaylova et al. [33], in a study of the composition of milk fat from sheep of local breeds grazing in mountain pastures, showed a slightly lower content of SFA (70.1%), higher PUFA (7.7%) and CLA (2.5%) and similar MUFA content (22.2%) to the control group in our research, while MUFA content was lower than in the experimental groups.

In a study by Gerchev and Mihaylova [16], sheep grazing on mountain pastures produced milk with a similar fat composition to that obtained in the experimental groups in our research in the case of SFA and PUFA content, but higher content of MUFA. The differences in milk fat composition were probably due to breed and dietary factors. This is confirmed by Borys et al. [9], who showed a clearly more favourable fatty acid profile, in terms of human health, in milk obtained from sheep during the summer than in the winter, as well as by numerous studies conducted on dairy cattle [6, 15, 25, 30]. In general, however, the fatty acid profile of the sheep milk in the present study, despite the use of winter feed, was only slightly worse than in the case of pasture feeding in the studies cited above (except for the content of CLA).

The fat of bundz rennet cheese did not differ in fatty acid composition from the fat of the milk from which it was produced (Table 2). As in the case of the raw milk, there were

Table 1

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

Item	Group			SEM
	I	II	III	
C4:0	2.00^{C}	2.23^{Bb}	2.40^{Aa}	0.047
C6:0	2.08	2.18	2.23	0.033
C8:0	2.47	2.57	2.57	0.038
C10:0	9.17	8.87	9.05	0.126
C12:0	6.33	5.75	5.82	0.174
C14:0	12.98	12.23	12.35	0.209
C14:1	0.65	0.63	0.60	0.014
iso-C15:0	0.92	0.93	0.92	0.017
C15:0	1.38	1.42	1.33	0.029
C16:0	25.40	25.05	24.55	0.214
C16:1	1.45	1.42	1.28	0.065
iso-C17:0	1.45	1.42	1.43	0.021
C17:0	0.68	0.73	0.72	0.018
C18:0	7.22	7.47	7.90	0.280
C18:1 *T*	1.45	1.50	1.43	0.053
C18:1 *c*9	16.15	16.90	17.22	0.386
C18:1 *c*11	0.50	0.55	0.52	0.010
C18:1 c other	0.92	0.90	0.88	0.027
C18:2	2.37	2.38	2.30	0.059
C18:3	0.60	0.63	0.58	0.030
SFA	72.72	71.72	71.76	0.404
UFA	26.27	27.25	27.02	0.405
including				
MUFA	22.22	23.14	23.06	0.318
PUFA	4.04	4.12	3.96	0.103
including				
PUFA *n-3*	0.85	0.88	0.82	0.040
PUFA *n-6*	2.58	2.60	2.52	0.065
CLA	0.51	0.52	0.54	0.012
UFA/SFA	0.362	0.380	0.377	0.008
PUFA/SFA	0.055	0.058	0.055	0.002
PUFA *n-6/n-3*	3.131	3.044	3.113	0.110
DFA	33.48	34.72	34.92	0.627
OFA	65.50	64.25	63.87	0.625
DFA/OFA	0.515	0.542	0.548	0.015
Cholesterol (mg/100 g of milk)	26.9^{A}	19.7^{C}	21,2^{B}	0.932

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, C – P≤0.01; a, b – P≤0.05

no statistically confirmed differences in the profile of the lipid fraction of bundz between feeding groups. The fat of cheese obtained from the milk of ewes from the experimental groups had a slightly lower content of SFA (by 1.8% on average) compared to the control group, with a tendency towards a higher (by 5.9%) content of UFA, including 6.2% more MUFA (7.5% more of the dominant C18:1 *c*9) and 4.4% more PUFA (mainly C18:2 and C18:3, on average by 8.1% and 5.8%, respectively). Among SFA, more pronounced differences between the experimental and control groups were observed for C12:0 and C14:0 (on average less in bundz from the experimental groups, by 9.0% and 5.3%, respectively) and for C18:0, higher content of which was found in bundz fat from groups II and III than from group I, by 5.4% and 11.3%, respectively. Characteristic, though statistically unconfirmed, differences were found in the content of *n*-3 and *n*-6 PUFA and CLA, which are important in terms of health quality. More favourable results in this respect were found in group II bundz fat compared to group I (by 10.8%, 4.0% and 10.0%, respectively). On the other hand, bundz made from the milk of ewes from group III contained fat with a similar amount of *n*-3 PUFAs as bundz from group I, and higher *n*-6 PUFA and CLA, by 4.4% and 4.0%, respectively. Generally, bundz made from the milk of group II sheep had a 6.8% lower *n*-6/*n*-3 PUFA ratio than bundz from group I, and bundz from group III had a higher ratio than cheese from groups I and II, by 4.5% and 12.1%. The UFA/SFA and PUFA/SFA ratios were similar in the cheese obtained from the milk of ewes from both experimental groups, higher than for group I by 7.6% and 6.5% on average. The DFA/OFA ratio was also higher in groups II and III compared to group I (by 9.2%), due to the higher content of DFA and lower OFA in the bundz fat.

The fat of bundz produced in the present study differed in fatty acid profile from that of bundz produced in a similar period from sheep of the same breed by Pakulski and Pakulska [35]. The results for groups II and III in our study were less favourable than in the cited study in terms of SFA, UFA and DFA content, and more favourable in terms of MUFA and *n*-3 PUFA content. Bonczar et al. [8], in their analysis of the fat of bundz made from the milk of pasture-grazed mountain sheep, showed higher content of saturated fatty acids C4:0-C14:0 (39.5%) and lower content of C18:1 (13.8%), C18:2 (1.4%) and C18:3 (0.5%). Bundz fat in that study contained a similar amount of C16:0 (25.5%) as in the control group in the present study, but more than in the experimental groups. It also contained more CLA (1.1%) than the bundz fat obtained in any of the groups in our study.

The profile of the lipid fraction of bundz underwent adverse changes in terms of health quality during the research (Figs. 1 and 2). At the beginning of the study, the curves for SFA content were nearly the same for all groups, with a clear upward trend. From the 97th day of lactation (3rd batch of cheese), SFA content continued to increase, although less rapidly, in the cheese from group I. In the group II fat it decreased slightly and then remained constant, while in the group III fat it decreased until the 125th day lactation (5th batch) and then increased. The increase in the content of SFA acids during the research was

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

Item	Group			SEM
	I	II	III	
C4:0	2.22	2.28	2.67	0.035
C6:0	2.23	2.25	2.28	0.027
C8:0	2.57	2.57	2.60	0.030
C10:0	9.37	8.95	9.00	0.146
C12:0	6.42	5.88	5.80	0.184
C14:0	13.07	12.38	12.37	0.215
C14:1	0.63	0.63	0.63	0.011
iso-C15:0	0.92	0.95	0.92	0.016
C15:0	1.38	1.43	1.38	0.018
C16:0	25.32	25.00	24.68	0.203
C16:1	1.40	1.40	1.28	0.065
iso-C17:0	1.43	1.45	1.40	0.021
C17:0	0.68	0.70	0.72	0.016
C18:0	7.07	7.45	7.87	0.292
C18:1 *T*	1.42	1.54	1.44	0.056
C18:1 *c*9	15.90	16.90	17.28	0.403
C18:1 *c*11	0.53	0.53	0.53	0.012
C18:1 *c* other	0.90	0.97	0.95	0.029
C18:2	2.23	2.40	2.42	0.062
C18:3	0.60	0.65	0.62	0.034
SFA	73.28	71.98	71.90	0.417
UFA	25.87	27.40	27.40	0.437
including				
MUFA	21.91	23.22	23.31	0.346
PUFA	3.96	4.18	4.09	0.108
including				
PUFA *n-3*	0.83	0.92	0.83	0.043
PUFA *n-6*	2.52	2.62	2.63	0.069
CLA	0.50	0.55	0.52	0.013
UFA/SFA	0.354	0.381	0.381	0.008
PUFA/SFA	0.054	0.058	0.057	0.002
PUFA/MUFA	0.180	0.180	0.175	0.003
PUFA *n-6/n-3*	3.11	2.90	3.25	0.118
DFA	32.94	34.85	35.26	0.670
OFA	66.22	64.53	64.03	0.652
DFA/OFA	0.501	0.542	0.552	0.016
Cholesterol (mg/100 g of cheese)	32.73	32.98	33.40	0.856

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

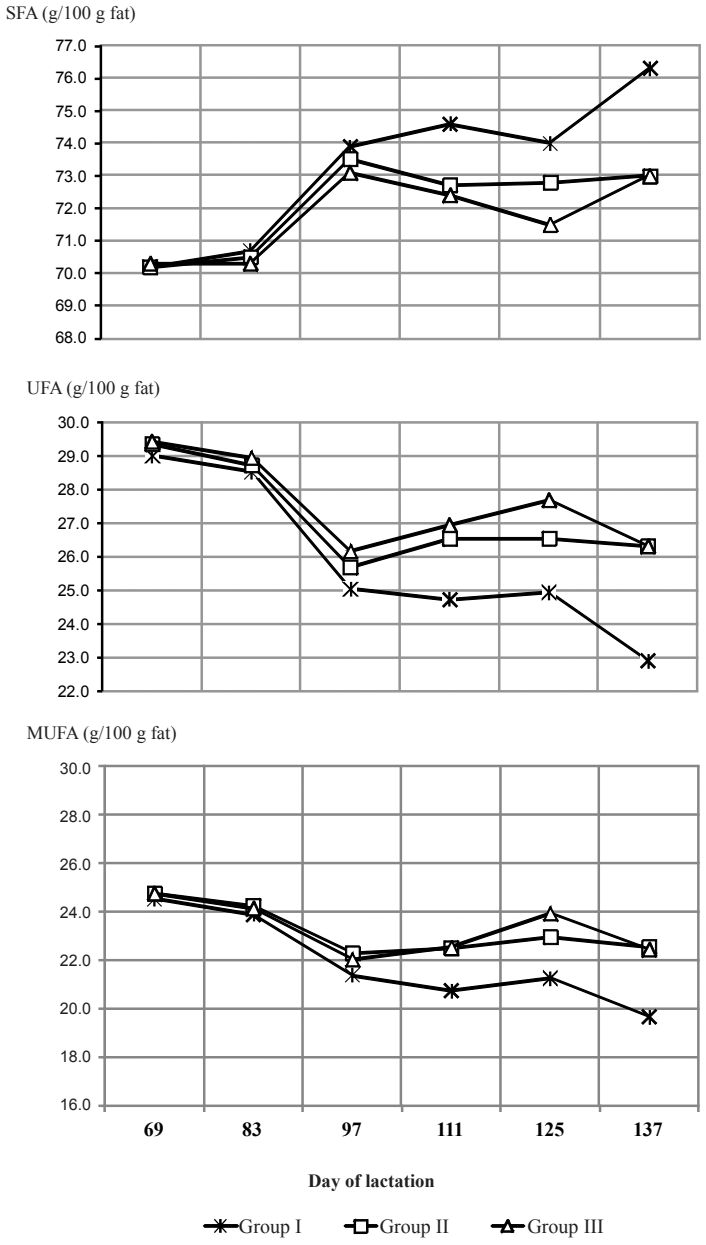

Fig. 1. Changes in the content of SFA, UFA and MUFA in the rennet cheese fat produced from sheep milk obtained in the period from 69 to 137 days of lactation

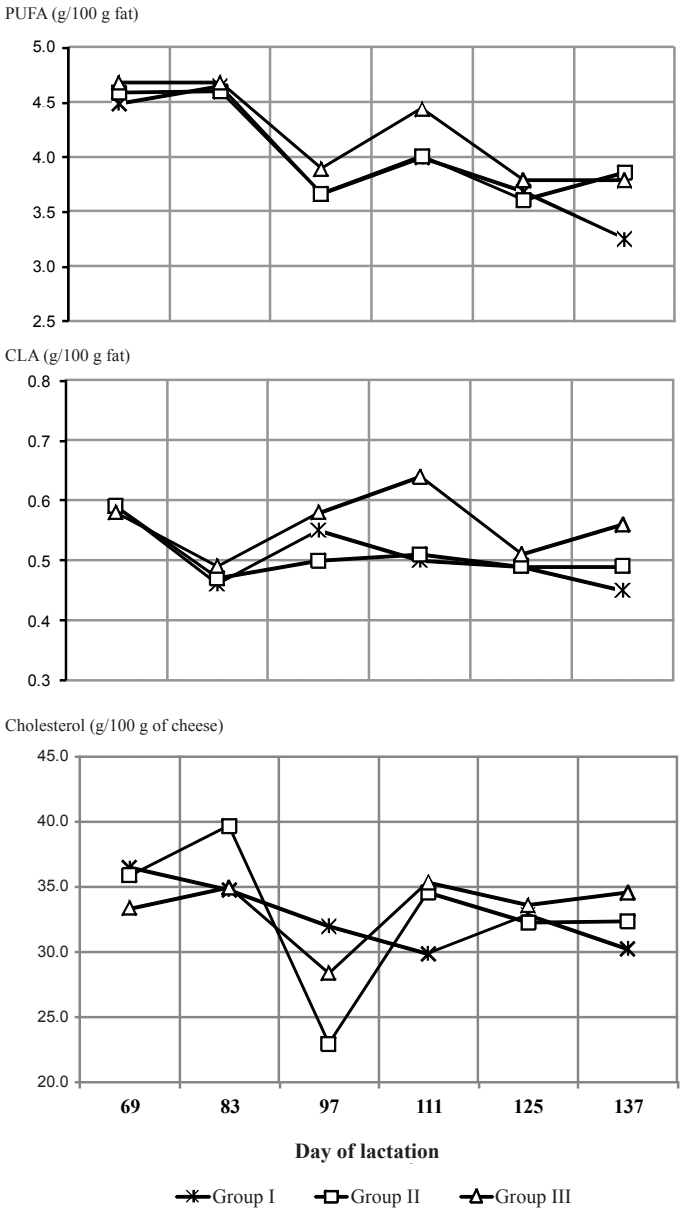

Fig. 2. Changes in the content of PUFA, CLA and cholesterol in the lipid fraction of cheese produced from sheep milk obtained in the period from 69 to 137 days of lactation

8.5% in group I and only 4.3% in the experimental groups, while the curves plotted for UFA content in bundz fat ran in the opposite direction to those for SFA. Until the 97th day of lactation (3rd batch), the UFA content markedly decreased in all groups, followed by inhibition of this process in the experimental groups, and even an increase, which was particularly pronounced in group III. In the group I bundz fat, however, there was a further decrease in UFA content. It should be noted that in the final stage of the study, compared to the initial stage, UFA content decreased by 10.2% in the experimental groups and by as much as 20.7% in the control group. The changes in the MUFA content were similar to the changes in UFA content. During the experiment, the health-promoting properties of bundz fat deteriorated in the experimental groups by 8.2% and in the control group by as much as 22.9%. The curves plotted for the content of acids from the PUFA group also show a downward trend during the period of research. The curves for groups I and II practically coincide, except for the fat of cheese made from milk obtained on the 137th day of lactation, in which the content of these acids increased in group II, and further decreased in group I. The curve plotted for group III indicates higher content of PUFA in the fat of cheese obtained throughout the research period. Changes in the content of CLA in bundz obtained in successive stages of the study were of a similar nature, although, as in the case of PUFA, they were less characteristic than for SFA and MUFA content.

Changes in the fatty acid profile of the bundz lipid fraction probably resulted from changes that occurred in the milk fat during the research, which the authors have demonstrated in an earlier publication [21]. Technological processes have not been shown to affect the fat composition of sheep and goat cheese [12, 35], which indicates that the quality of the final product can be modified at the stage of raw milk production.

Cheese made from the milk of sheep fed winter feeds did not differ significantly in cholesterol content between groups (Table 2). Changes in its content in the bundz cheese during the study were generally not highly characteristic, either in the case of trends in changes in cheese made from milk from different feeding groups or in the shape of the curves at each stage of the study (Fig. 2). There was a decrease in cholesterol content in the bundz made from milk obtained from sheep on the 97th day of lactation (3rd batch), which was much more pronounced in the experimental groups than in the control group. It should be emphasized that the cholesterol content was much higher in the bundz than in the raw milk it was made from. This was due to the higher fat content in cheese than in milk. Kovacs et al. [24] found a positive correlation of $r = 0.98$ between fat and cholesterol content in milk, while according to Talpura et al. [40] the correlation was 0.63. The fluctuations in the cholesterol content in cheese in our research could have been due to production under experimental conditions in which the pasteurization process was not fully standardized (vat method). Grega et al. [19], in a study of milk with the same fat content, showed higher cholesterol content in pasteurized and sterilized milk than in raw milk.

To sum up, the addition of herbs in the amount of 10 or 20 g/sheep/day to feed for milking sheep significantly increased the content of C4:0 in the raw milk used to make cheese, by 11.5% and 20.0%, respectively (P≤0.01), relative to the control group, and the addition of 20 g/sheep/day resulted in a further 7.6% increase (P≤0.05) compared to the amount of 10 g/sheep/day. The herbs had no statistically confirmed effect on other parameters of the health-promoting quality of the fat of the milk or the rennet cheese produced from it. This could be due to an insufficient number of observations, as well as to adverse changes in the lipid fraction profile of the bundz (increased SFA and decreased MUFA and PUFA) occurring during the experiment. It should be noted that from the 97th day of lactation there was a tendency towards improved health-promoting properties of the bundz fat in the experimental groups. The addition of herbs to the diet of sheep in the amount of 10 or 20 g/sheep/day reduced the cholesterol content in the raw milk by 26.8% and 21.2%, respectively (P≤0.01), compared to the control group, without affecting the content of this lipid in the rennet cheese.

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