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The changes in the milk composition and its lipid fraction during the rearing of lambs in non-milked sheep

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Abstract: *The changes in the milk composition and its lipid fraction during the rearing of lambs in non-milked sheep*. Studies regarding the effect of lactation stage on milk content and lipid fraction composition are mostly conducted on milked animals. The aim of this study was to analyze the changes in the basic milk composition and content of fatty acids in the fat fraction during the rearing of lambs in non-milked sheep. The study was carried out on 22 ewes of Polish lowland sheep of Żelazneńska strain, which reared lambs sold at low weight classes (up to 22 kg). Milk samples were collected at 10th (period 1), 25th (period 2) and 52nd (period 3) day of lactation. There were no differences in the amount of the basic components of milk in the studied periods of lactation beside the fat ($P \leq 0.05$) content. There were also no difference in the content of fatty acid groups in the fat fraction of ewe's milk examined at 10th, 25th and 52nd day of lactation. Although, in the third period of lactation, the content of saturated fatty acids (SFA) was slightly higher compared to first period. In turn, the content of oleic acid ($P \geq 0.30$) and C18:3 ($P \leq 0.05$) was higher in 10th day of rearing then in 52nd day. A slightly larger share of essential C18 unsaturated fatty acids in ewe's milk in early lactation may suggest the involvement of adipose tissue in the formation of milk fat.

Key words: non-milked sheep, milk composition, stage of lactation

INTRODUCTION

Sheep's milk is one of the most valuable product of animal origin. In addition to basic nutrients it contains many biologically active compounds, which ensure the proper development of lambs, and can affect the quality and health-promoting properties of their meat after slaughter. They have also a positive impact on human health. Content of sheep's milk and the milkfat composition may depend on many factors, i.e. breed, diet, age of ewes, as well as the stage of lactation (Atti et al. 2006, De La Fuente et al. 2009, Rozbicka-Wieczorek et al. 2015).

There are not many research on changes in the composition of sheep's milk during lactation especially in non-milked sheep. During lactation, secretory cells of mammary gland utilize 80% of the blood circulating metabolites for milk synthesis, depending on the speed of infiltration of precursors of milk compounds (i.e. free amino acids, glucose or fatty acids). The reduction of lipogenesis and increase of fatty acid mobilization from adipose tissue at the beginning

of lactation, induces an increase in the activity of enzymes of mammary gland, to provide substrates for milk fat synthesis. The increase in protein catabolism in the serum with the progress of lactation, provides a steady increase substrates for the synthesis of milk protein (Nazifi et al. 2002, Krajnicakova et al. 2003, Darwesh et al. 2013). Changes in these processes during lactation may cause changes in the content of the milk components depending on its stage. Major changes in the composition of milk fat during lactation are recorded in dairy animals producing more milk as cows or goats (Darwesh et al. 2013, Billal et al. 2014). Also in sheep, stage of lactation may differently influence the composition of milk in milked sheep and those, which only rearing lambs.

The aim of this study was to analyze the changes in the basic milk composition and content of fatty acids in the fat fraction during the rearing of lambs in non-milked sheep.

MATERIAL AND METHODS

Animals, treatment and sampling

The study was carried out on ewes of Polish lowland sheep of Żelazneńska strain, which reared lambs sold at low weight classes. Reared lambs required weight reached about 60 days of age. Chosen 22 ewes at the age of 3–4 years, which were fed according to standards for lactating ewes (Osikowski et al. 1998). The diet was based on meadow hay (3.89 MJ/kg of dry matter [DM], 11.8% of total protein [CP]/kg DM, 29.32% crude fiber [CF]/kg SM) and concentrate (7.02 MJ/kg DM, 18% CP/kg DM) consisting of: oat meal (30.5%) wheat meal (23%), rapeseed (30.5%), wheat bran (15%) and compound mineral (1%). Ewes were fed twice a day, morning and evening. Fresh water was available ad libitum. All the ewes lambing in approximately the same day.

Milk samples were collected at 10th (period 1), 25th (period 2) and 52nd (period 3) day of lactation. The lambs were separated from their dams 2 h before milk collection. Then ewes after injection 5 units of oxytocin were hand milked. A representative milk sample (100 ml) was taken from the full udder together with residual milk of each ewe and placed in a sterile bottle with a preservative (Mlekostat CC). Immediately after collection, milk samples were transferred to the Milk Testing Laboratory in order to determine the chemical composition and the content of fatty acids in the fat fraction.

Chemical analysis

The basic chemical composition, i.e. protein, fat, lactose and total solid (TS) amount were determined by infrared spectrophotometry using Milkoscan FT-120 (Foss Electric, Hillerod, Denmark).

Milk fat was extracted according to Röse-Gotlieb method (AOAC 1990). Methylation of the fatty acids was made by transesterification according to EN-ISO 5509:2000. The separation and quantification of fatty acid methyl esters (FAMEs) were carried out by gas chromatography using a Hewlett Packard 5890 with FID detector equipped with capillary column (length – 60 m; internal diameter – 0.25 mm; film thickness 0.25 μm; Agilent Technologies, Wald-

bronn, Germany). Operating conditions were as follows: carrier helium flow 20 cm per 1 s; detector temperature at 240°C, injector temperature at 220°C. The temperature program was as follow: 130°C for 1 min; 130–210°C at 10°C for 1 min; 210°C for 25 min, 210–230°C at 2.5°C for 1 min and 230°C for 18 min. On the basis of retention time relative to the palmitic acid C16:0 selected fatty acids was identified. By the method of the external calibration for both their total fatty positional and geometric isomers of quantitative analysis was carried out using reference Supelco and Sigma.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 23.0 software (2016) using paired t-test for dependent samples.

RESULTS AND DISCUSSION

The content of the basic ingredients of milk in the studied periods of rearing lambs are presented in Table 1. The differences in the content of the most components were not statistically significant, although as expected during the peak of lactation, which in sheep falls to four weeks, the fat content in milk was the lowest ($P \leq 0.05$) in comparison to period 3.

Similar changes in the chemical composition of milk during lactation in Corriedale and Friesian sheep were registered by Niżnikowski et al. (1999). In Wrzosówka sheep, unlike in the present study, milk fat content was lower at the beginning of lactation. The increase in fat content was observed from the fourth week of milk secretion (Nowak and Niżnikowski 1994). In another study on the effect of lactation stage on milk composition in the local goats breeds, also the lowest part in fat amount in its middle stage has been reported (Strzałkowska et al. 2010, Darwesh et al. 2013, Mahmoud et al. 2014). The decrease in the content of this component during the major part of lactation may be related with the effect of dilution due to the increase in milk volume and a decrease in fat mobilization from adipose tissue that decreases the availability of plasma non-esterified fatty acid (NEFA) for mammary lipid synthesis (Chilliard et al. 2003).

No difference in the content of fatty acid groups in the fat fraction of ewe's

TABLE 1. The content of basic ingredients in the milk of examined lactation periods (%)

Item	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
Dry matter	18.86	2.66	18.22	2.46	19.18	1.46
Protein	4.48	0.61	4.56	0.81	4.05	1.40
Fat	8.11	2.16	7.57[a]	1.13	9.31[b]	1.54
Lactose	4.83	0.61	5.08	0.39	4.71	0.60
Ash	0.76	0.13	0.84	0.08	0.79	0.13

Means with different letters in rows (a, b) differ significantly at $P \leq 0.05$.

TABLE 2. The content of fatty acids group in fat milk of examined lactation periods (g/100 g fat)

Item	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
SFA	58.19	2.99	58.78	1.48	59.13	1.09
MUFA	29.12	2.39	29.09	2.28	28.61	0.70
PUFA	3.51	0.24	3.52	0.27	3.45	0.26
UFA	32.63	2.51	32.61	2.48	32.06	0.72
n-6	1.98	0.17	2.01	0.17	1.95	0.19
n-3	1.06	0.07	1.05	0.08	1.00	0.06

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, UFA – unsaturated fatty acids.

milk examined at 10th, 25th and 52nd day of lactation was recorded (Table 2). Although, the content of saturated fatty acids (SFA) was higher ($P \geq 0.40$) in the third period of lactation, especially when compared to the first, while the UFA content was lower ($P \geq 0.50$) in period 3 compared to the first. It can only suggest, a slight decrease in the *de novo* synthesis of fatty acids in early stage of lactation and greater mobilization of lipids, mainly NEFA, from adipose tissue for the synthesis of milk fat in connection with possible negative energy balance in this period. The significantly higher concentration of UFA and lower SFA in fat of cow's milk during early lactation has been reported by many researchers (Auldist et al. 1998, Stoop et al. 2009, Arnould et al. 2010).

In fat milk of studied ewes the largest share in SFA group constituted medium chain fatty acids (MCFA) (C12–C16), and their content at 10th, 25th and 52nd day of the milk secretion was 61.5, 62.4, 62.5% respectively. Of these acids, the special attention is paid to palmitic acid C16:0, whose content can reflect the involvement of the mammary gland in the synthesis of milk fat (Barber et al. 1997). In the first stage of lactation the content of that acid was lower by about 3% when compared to the second and third period, which may indicate a slight reduction in the *de novo* synthesis in the early lactation (Table 3). The second, regarding amount, in SFA group was acid C18:0. Its highest level was recorded in third period considered. The increase in C16:0 and C18:0 in the final stage of lactation in Churra sheep breed also have been obtained by De La Fuente et al. (2009). It should be noted that the above-mentioned sheep are used for milk production, whose lactation is much longer.

In the group of monounsaturated fatty acids the acid C18:1*cis*9 was dominated. In the first studied period, its quantity represented 77% of the total MUFA, in the second and third period 76 and 75%, respectively. This acid which is one of the most important fatty acids in the fat fraction of milk, may come from various sources, including adipose tissue. Its slightly higher content ($P \geq 0.30$) in the first period especially when compared to the third, may suggest that transferred

TABLE 3. The content of fatty acids in fat milk of examined lactation periods (g/100 g fat)

Fatty acids	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
C6:0	2.27	0.25	2.16	0.25	2.25	0.23
C8:0	1.29	0.11	1.25	0.10	1.24	0.09
C10:0	5.73	1.03	5.61	1.11	5.47	0.99
C12:0	3.14	0.25	3.19	0.26	3.07	0.15
C14:0	8.25	1.41	8.50	1.19	8.79	0.82
C15:0	1.16	0.19	1.14	0.20	1.18	0.16
C16:0	23.26	1.17	23.83	0.98	23.90	1.38
C17:0	1.04	0.15	1.06	0.12	1.05	0.15
C18:0	12.05	0.72	12.04	1.16	12.18	0.76
C10:1	0.14	0.03	0.13	0.02	0.14	0.02
C14:1	1.26	0.22	1.22	0.45	1.25	0.31
C15:1	0.22	0.05	0.21	0.03	0.22	0.04
C16:1	2.38	0.36	2.59	0.21	2.51	0.21
C18:1*t*11	1.85	0.35	1.93	0.43	2.08	0.55
C18:1	0.74	0.09	0.73	0.09	0.8 0	0.05
C18:1*c*9	22.41	2.64	22.16	2.42	21.48	0.86
C20:1	0.12	0.03	0.12	0.03	0.14	0.02
C18:2	1.81	0.17	1.83	0.16	1.77	0.18
C18:2*c*9*t*11	0.45[a]	0.07	0.47[a]	0.10	0.51[b]	0.12
C18:3	0.68[a]	0.04	0.66[a]	0.05	0.63[b]	0.03
C20:3	0.08	0.03	0.08	0.01	0.07	0.01
C20:4	0.17	0.02	0.17	0.01	0.18	0.02
C20:5	0.09	0.01	0.09	0.01	0.10	0.01
C22:5	0.15	0.04	0.15	0.03	0.14	0.03
C22:6	0.07	0.01	0.07	0.01	0.06	0.01

Means with different letters in rows (a, b) differ significantly at $P \leq 0.05$.

from a pool of plasma NEFA replaced acids *de novo* synthesized by the mammary gland (Table 3). Negative correlation –0.73 ($P \leq 0.05$) between oleic acid and palmitic acid tested on 10th day of rearing lambs and positive (0.62) correlation with a total fat content seems to confirm this. Due to the deficit of energy in early lactation enzymes activity involved in the synthesis of milk fat may be somewhat reduced even in not milked animals characterized by lower milk production (Chiliard et al. 2003). Similar relationships between the above-

mentioned acids was obtained by De La Fuente et al. (2009) in milk of Churra ewes, by Chilliard et al. (2003) in goats and Billal et al. (2014) in cows.

The differences in the content of polyunsaturated fatty acids in tested periods of lactation, besides linolenic acid (C18:3) and conjugated linoleic acid (C18:2*c*9*t*11) have been not confirmed statistically. The content of C18:3 acid in 10th day of rearing was higher ($P \leq 0.05$) compared to 52nd day of lactation (Table 3). It is known that these fatty acids in milk fat does not come from endogenous synthesis, but only from the feed or adipose tissue after their release by lipoprotein lipase in the blood (Clegg et al. 2001). In the study conducted by De la Fuente et al. (2009) on the influence of the stage of lactation in dairy Churra breed in contrast to the results obtained in this study the content of C18:3 increased with advancing lactation. In the present study together with advancing lactation increased ($P \leq 0.05$) the C18:2*c*9*t*11 acid content of which the presence in the milk is mainly related to endogenous synthesis from vaccenic acid.

CONCLUSION

The period of lactation of studied ewes during the rearing lambs did not affect the content of the basic components of milk.

No clear effect was noted of lactation stage on the contents of groups and individual fatty acids in the fat fraction in milk of studied ewes. An exception was the linolenic acid (C18:3 n-3) which higher contents was registered in the early phase of the rearing lambs.

Both fatty acids content in milk and relationships between some fatty acids only suggest trends for the occurrence of negative energy balance in early lactation in ewes whose milk is used only for rearing lambs. A slightly larger share of essential C18 unsaturated fatty acids in milk in early lactation may suggest the involvement of adipose tissue in the formation of milk fat.

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Streszczenie: *Zmiany w składzie mleka i jego frakcji tłuszczowej w trakcie odchowu jagniąt u owiec nieużytkowanych mlecznie*. Badania dotyczące wpływu laktacji na składniki mleka i skład frakcji lipidowej są w większości prowadzone na zwierzętach użytkowanych mlecznie. Celem tych badań było przeanalizowanie zmian w podstawowym składzie mleka i w zawartości kwasów tłuszczowych w jego frakcji tłuszczowej w trakcie odchowu jagniąt u owiec niedoskonalonych w kierunku użytkowania mlecznego. Badania prowadzono na 22 maciorkach nizinnych odmiany żelazneńskiej, które odchowywały jagnięta sprzedawane w niskich standardach wagowych (do 22 kg). Próby mleka pobierano w 10. (okres 1), 25. (okres 2) i 52. (okres 3) dniu laktacji. Nie znaleziono różnic w zawartości podstawowych składników mleka w badanych okresach laktacji, oprócz różnic w zawartości tłuszczu. Różnice nie występowały także w zawartości grup kwasów tłuszczowych w frakcji tłuszczowej mleka maciorek badanych w 10., 25. i 52. dniu laktacji, chociaż zawartość nasyconych kwasów tłuszczowych (SFA) była nieznacznie większa w trzecim okresie laktacji w porównaniu z okresem pierwszym. Z kolei zawartość kwasu oleinowego ($P \geq 0{,}30$) i C18:3 ($P \leq 0{,}05$) była większa w 10. dniu od-

chowu jagniąt niż w 52. dniu. Niewiele większy udział niezbędnych nienasyconych kwasów tłuszczowych C18 w mleku maciorek we wczesnej laktacji może wskazywać na zaangażowanie tkanki zapasowej w tworzenie tłuszczu mleka.

Słowa kluczowe: owce niedojone, skład mleka, stadium laktacji

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