Content of protein and lipid fraction components in the milk of Wrzosówka Polish Heath sheep depending on somatic cell count

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The aim of the study was to determine the effect of somatic cell count (SCC) on components of protein and lipid fractions in the milk of Wrzosówka Polish Heath sheep. The experiment was conducted on 30 ewes at the age of 3-4 years. The milk samples were taken during the 4th week of lactation. Basic milk composition, somatic cell count (SCC), whey protein content and fatty acid profile were determined. Based on SCC the samples were divided into four classes: class I – up to 100 x103 SCC/ml; class II – 100-200 x103 SCC/ml; class III – 200-500 x103 SCC/ml; and class IV – over 500 x103 SCC/ml. A high SCC in the milk decreased (P≤0.01) the total protein content and casein fraction. Content of β-lactoglobulin (β-LG) also decreased as SCC increased. The content of lactoferrin (LF), a protein with strong antibacterial properties, increased (P≤0.01) as SCC increased. In the milk samples with the highest SCC, lower content of C20 PUFA was noted in comparison to the other classes. Among selected functional fatty acids, the content of C20:3 and C22:5 (DPA) was higher in class II (100-200 x103) and III (200-500 x103), respectively. The lowest percentage of functional acids was found in the milk with the highest SCC. The somatic cell count had a greater effect on protein fraction components than on fatty acid content.

KEY WORDS: sheep / milk / somatic cell count / protein fraction / fatty acids

The health of the mammary gland has a significant impact on the quantity of sheep milk produced and its quality. Udder infection adversely affects not only the level of milk production, but also its processing value [2, 4, 13], which is important in dairy sheep farming, and lamb rearing.

According to some authors [6, 12], an increase in the somatic cell count (SCC), caused by increased penetration mainly of neutrophils from the blood to the mammary gland, is associated with an increased percentage of protein in sheep milk. Other authors have reported a decrease in total protein content with increased SCC [9] or have indicated no differences in the content of this constituent for low or high counts of somatic cells [14]. According to Leitner [10], Nuddy et al. [12], Pirisi et al. [14] and Rodrigez-Nogales et al. [17], the content of fat, lactose and certain elements, such as calcium, phosphorus, potassium and magnesium, decreases during udder infection. Many studies indicate that an increase in the somatic cell count also results in decreased milk yield in sheep, which can unquestionably have an adverse effect on rearing conditions for lambs and thus on their growth and development [8, 11, 13].

While studies on the quality of sheep's milk have analysed the relationship between SCC and components of the protein fraction of milk [1, 12, 17], the number of studies on the association between mammary gland health and the lipid fraction is limited.

The aim of the study was to determine the effect of somatic cell count on the content of basic constituents of the milk of Polish Heath sheep, as well as the content of selected proteins in the protein fraction and of fatty acids in the fat fraction of the milk. Although sheep of this breed are not used for dairy purposes, due to their high prolificacy both the quantity and quality of their milk are important in the breeding of lambs.

Material and methods

The research was carried out on 30 Polish Heath ewes from the Agricultural Experimental Station in Żelazna. The ewes' diet was in accordance with requirements for lactating ewes and consisted of a concentrate feed and hay supplemented with straw and a mineral and vitamin mixture. The chemical composition and nutritional value of the basic feeds are given in Table 1.

Milk was obtained from ewes at the age of 3-4 years, in the fourth week of lactation. The sheep were milked manually, after separating them from their lambs for 2 hours. From this milk 100 ml samples were collected to determine their chemical composition, somatic cell count and content of casein, whey proteins and fatty acids.

The percentage content of basic milk constituents, i.e. protein, fat, lactose, dry matter and casein, was determined by infrared spectrophotometry in a Foss Milkoscan FT analyser. Somatic cell count (SCC) was determined by flow cytometry with a Bentley Somacount 150.

Whey proteins were determined using an Agilent 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a variable wavelength UV-VIS detector and Supelcosil LC-318 Supelco and Supelguard columns, according to the method described by Puppel et al. [16]. The following eluents were used to determine the most important whey proteins: solution A – mixture of 0.1% TFA (Merck) in

Table 1
Chemical composition and nutritional value of fodder

Specification	Grain meal	Grass hay
Dry matter (%)	89.24	90.82
Crude protein (%)	11.8	8.8
Ether extract (%)	1.72	1.36
Crude fibre (%)	4.89	34.55
UFL/kg DM	1.06	0.6
PDI/kg DM	69	68

UFL – feed unit for milk production; PDI – protein digestible in the small intestine

acetonitrile with water (5:95); solution B – mixture of acetonitrile with water (95:5). Phase flow: 1.0 ml/min, UV detection at 220 nm.

Milk fat was extracted by the Röse-Gotlieb method according to AOAC [3], in order to determine the fatty acid profile. Separation and quantitative analysis of fatty acid methyl esters were performed by gas-liquid chromatography using a Hewlett Packard 5890 gas chromatograph with a flame ionization detector and a DD 23 column (60 m length, 0.25 mm inner diameter and 0.25 µm film thickness). The following conditions were used for separation of fatty acids: carrier gas (helium) 20 cm/s; detector temperature 240ºC; injector temperature 220ºC; split 1:40.

The separation was conducted at a programmed temperature: initial temperature of 130ºC/min; temperature increase from 130 to 210ºC at 10ºC/min; 210°C for 25 min; temperature increase from 210 to 230ºC at 2.5ºC/min; 230°C for 18 min. Identification and quantitative analysis of fatty acids were carried out using Sigma and Supelco standards.

The effect of somatic cell count on milk constituents was estimated by one-way analysis of variance. The material was divided into four classes according to somatic cell count: class I – less than 100 $x10^3$ SCC/ml, class II – 100-200 $x10^3$ SCC/ml, class III – 200-500 $x10^3$ SCC/ml; and class IV – over 500 $x10^3$ SCC/ml. A statistical evaluation of the differences between the classes of somatic cell counts was performed using Duncan's test.

Results and discussion

The somatic cell count in the milk of the Polish Heath ewes did not exceed an average of 260 $x10^3$/ml milk (log SCC 2.09). The proportion of ewes whose milk had a somatic cell count above 1,000 x10^3 per ml of milk was 7%, while for 53% of ewes the SCC in the milk did not exceed 100 x10^3/ml. Świderek [18], in a study on mammary gland health in this breed of sheep, found that over 70% of tested milk samples had an SCC of no more than 100 x10^3/ml. Despite numerous studies, the physiological limit for somatic cell count in sheep milk has not been conclusively determined. According to the author cited above [18], the SCC value considered to be the normal physiological level is highly varied and can range from 100 x10^3 to 500 x10^3 in 1 ml of milk, or even exceed that value.

Table 2 presents the results of the analysis of the effect of somatic cell count on the content of basic constituents and individual protein fractions in the milk of the ewes. A high SCC in the milk resulted in a decrease ($P \leq 0.01$) in the level of protein and its main fraction – casein, especially as compared to samples with counts below 100 x10^3. As SCC increased, β-lactoglobulin (β-LG) content decreased ($P \leq 0.05$, $P \leq 0.01$). On the other hand, α-lactalbumin (α-LA), the second most important whey protein, was higher in milk with higher SCC, although the differences between the classes were not statistically significant (Table 2).

In contrast to the results obtained in our study, Bianchi et al. [6], in a study on Sardinian ewes, and Olechnowicz et al. [13], in maternal line 05, noted an increase in protein

Table 2
The content of milk components depending on SCC

Milk components	SCC per ml of milk							
	I <100 x10³ n=16		II 100-200 x10³ n=4		III 200-500 x10³ n=6		IV >500 x10³ n=4	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Fat (%)	8.66	0.32	10.27	0.65	8.76	0.53	8.98	0.65
Protein (%)	5.15^{A}	0.07	4.84	0.15	4.8	0.12	4.48^{A}	0.15
Casein (%)	4.61^{aBC}	0.03	4.39^{a}	0.07	4.21^{B}	0.05	4.24^{C}	0.07
Lactose (%)	5.17	0.04	5.16	0.08	5.29	0.06	5.3	0.08
Total solids (%)	19.85^{a}	0.26	21.68^{ab}	0.53	19.72^{b}	0.43	19.77	0.53
Lactoferrin (g/l)	0.17^{ABC}	0.01	0.25^{AD}	0.02	0.28^{BE}	0.01	0.73^{CDE}	0.02
α-lactoalbumin (g/l)	2.30	0.14	2.72	0.27	2.38	0.22	2.54	0.27
β-lactoglobulin (g/l)	7.86^{aA}	0.28	8.26^{bC}	0.56	6.11^{ab}	0.46	5.18^{AC}	0.56

Values with the same letters in the same row differ significantly: A, B...E at $P \leq 0.01$; a, b at $P \leq 0.05$

and casein content in milk with a higher somatic cell count. On the other hand, a decrease in casein content as SCC increased was reported by Rodriguez-Nogales et al. [17] in the milk of the Spanish sheep breeds Assaf, Churra and Castellana, as in the Polish Heath ewes in the present study. The decrease in the amount of casein and total protein can be attributed to the increase in proteolytic processes in milk with a high somatic cell count. This increase in proteolytic activity is associated with greater plasmin activity in udders with deteriorating health [1].

Nudda et al. [12], investigating the effect of somatic cell count in the milk of Sarda sheep on the composition of the protein fraction, found that as SCC increased, the proportion of casein decreased and that of whey proteins increased. While in the present study an increase in SCC significantly ($P \leq 0.05$, $P \leq 0.01$) reduced the β-LG content and slightly increased that of α-LA (Table 2), in studies by Pirisi et al. [14] and Nuddy et al. [12], it increased the content of both β-LG and α-LA. It should be noted that in the studies cited a marked increase in these proteins was observed in milk with SCC milk greater than $1{,}000 \times 10^3$, which was not observed in our study. This was attributed mainly to their greater concentration due to the reduction in milk yield, and not to increased synthesis. A decrease in the content of β-lactoglobulin and α-lactalbumin with increasing SCC was found by Rodriguez-Nogales et al. [17] in sheep milk with the highest SCC ($> 2{,}500 \times 10^3$).

As expected, the content of lactoferrin (LF), a protein with strong antimicrobial properties, belonging to non-specific cellular immunity, increased in the milk of the sheep in our study ($P \leq 0.01$) with increasing SCC (Table 2). An increase in LF in milk with more somatic cells was also reported by Duranti and Casoli [7] and by Nudda et al. [12]. An increase in lactoferrin levels in sheep milk may be evidence of deteriorating udder health and may be an important indicator in assessment of the health of the mammary gland in sheep.

Table 3

Content of fatty acid groups depending on SCC in milk

Acid groups	SCC per ml of milk							
	I $<100 \times 10^3$ n=16		II $100\text{-}200 \times 10^3$ n=4		III $200\text{-}500 \times 10^3$ n=6		IV $>500 \times 10^3$ n=4	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
SFA	57.25	2.38	56.67	3.02	51.67	3.37	53.34	4.77
MUFA	36.62	1.69	38.21	2.14	33.89	2.39	34.99	3.39
PUFA	4.36	0.37	5.02	0.47	5.38	0.52	3.82	0.74
PUFA *n-3*	0.95	0.07	1.16	0.09	1.08	0.10	0.76	0.14
PUFA *n-6*	2.90	0.31	3.32	0.39	3.82	0.43	2.67	0.61
C20 PUFA	0.66	0.06	0.86^{a}	0.08	0.88^{b}	0.09	0.51^{ab}	0.13

SFA – saturated fatty acid, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids
Values with the same letters in the same row differ significantly: a, b at $P \leq 0.05$

The results of the analysis of the effect of somatic cell count on the fatty acid content of the fat fraction of the milk of the ewes are presented in Tables 3 and 4.

No statistically significant differences were noted in the level of fatty acid groups depending on SCC, apart from 20-carbon polyunsaturated fatty acids (PUFA), the content of which was lower ($P \leq 0.05$) in milk samples with the highest SCC as compared to the other classes (Table 3).

The content of other groups of acids, especially PUFAs, was lower in the milk samples assigned to class IV, mainly in comparison with milk with a somatic cell count below 100 x 10^3 (Table 3). The general rule that increased mastitis infection is accompanied by an increase in the transport of blood components and thus a possible increase in the content of acids released from very low density lipoproteins (VLDL), i.e. PUFA, was not confirmed in our study. No differences in PUFA content depending on SCC were observed by Radzik-Rant et al. [16] in their study on the milk of Pomeranian sheep. Bernatowicz et al.

Table 4
Fatty acid content depending on SCC in milk

Fatty acids	SCC per ml of milk							
	I <100 x10^3 n=16		II 100-200 x10^3 n=4		III 200-500 x10^3 n=6		IV >500 x10^3 n=4	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
C4:0	1.59	0.25	1.10	0.32	2.10	0.36	1.93	0.51
C6:0	1.20	0.09	1.10	0.11	1.28	0.12	0.99	0.17
C8:0	1.38	0.09	1.34	0.11	1.43	0.13	1.10	0.18
C10:0	4.35	0.27	4.29	0.35	4.48	0.39	3.38	0.55
C10:1	0.11	0.01	0.10	0.01	0.12	0.01	0.10	0.02
C12:0	2.67	0.15	2.77	0.19	2.67	0.22	2.17	0.30
C12:1	0.04	0.03	0.04	0.04	0.05	0.04	0.03	0.06
C14:0	7.08	0.34	7.22	0.44	6.26	0.49	5.92	0.69
C14:1	0.46	0.03	0.44	0.04	0.48	0.04	0.39	0.06
C15:0	0.96	0.05	1.04	0.07	0.94	0.08	0.94	0.11
C15:1	0.26	0.01	0.28	0.02	0.24	0.02	0.23	0.03
C16:0	24.69	1.03	24.84	1.31	22.21	1.46	23.09	2.07
C16:1	0.76	0.12	0.75	0.15	0.56	0.16	0.37	0.23
C17:0	1,44	0.06	1.48	0.08	1.28	0.09	1.29	0.13
C17:1	0.74	0.04	0.76	0.04	0.73	0.05	0.63	0.07
C18:0	11.88[a]	0.59	11.51[b]	0.75	9.03[abc]	0.84	12.54[c]	1.19
C18:1 *trans*11	2.24	0.12	2.22	0.16	2.17	0.18	1.81	0.25
C18:1 *cis*9	30.25	1.43	31.78	1.81	27.88	2.03	29.50	2.87

Values with the same letters in the same row differ significantly: a, b, c at $P \leq 0.05$

[5], on the other hand, observed an increase in the proportion of functional PUFA in cow milk with increasing SCC.

Among saturated fatty acids, there was an increase (P≤0.05) in the content of C18:0 in the milk of SCC class IV as compared to class III. The level of C18:1*cis*9 oleic acid, which was dominant in the MUFA group, decreased, albeit insignificantly, in milk with higher content of somatic cells (Table 4).

This does not confirm the results previously obtained for the milk of Pomeranian sheep, in which the level of this acid markedly increased with higher SCC. In the same study, in the SFA group, there was an increase in the content of palmitic acid with increasing somatic cell count, and not in stearic acid, as in our analysis [16].

Among the selected functional acids, the level of C20:3 was higher (P≤0.05) in milk samples with a somatic cell count of 100-200 x 10^3, while the content of docosapentaenoic acid C22:5 (DPA) was higher (P≤0.01) in milk with a somatic cell count of 200-500 x 10^3 (Table 5). The content of functional acids, including eicosapentaenoic C20:3 and docosapentaenoic C22:5, was the lowest in the milk with the highest SCC, although the differences between classes were not statistically significant (Table 5). Pirisi et al. [14] also found lower content of fatty acids in cheese produced from milk with the highest somatic cell count (1,000 <SCC <2,000), although not in the milk itself.

In conclusion, the SCC in the milk of ewes of the Polish Heath breed influenced the constituents of the protein fraction. An increase in SCC decreased the proportion of total protein and casein and the content of β-lactoglobulin. The content of lactoferrin, a whey protein with antibacterial activity, increased with increasing SCC.

Table 5

Content of functional fatty acids depending on SCC in milk

Functional fatty acids	SCC per ml of milk							
	I <100 x10^3 n=16		II 100-200 x10^3 n=4		III 200-500 x10^3 n=6		IV >500 x10^3 n=4	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
C18:2	2.42	0.42	2.80	0.54	3.30	0.60	2.29	0.85
C18:2 *cis*9, *trans*11 (CLA)	0.51	0.04	0.54	0.05	0.48	0.05	0.39	0.07
C18:3 *n-3*	0.56	0.05	0.61	0.07	0.54	0.08	0.45	0.11
C20:3 *n-3*	0.16^{a}	0.04	0.30^{abC}	0.05	0.09^{C}	0.06	0.13^{b}	0.08
C20:4 *n-6*	0.27	0.04	0.31	0.05	0.33	0.06	0.19	0.08
C20:5	0.03	0.00	0.03	0.00	0.02	0.00	0.03	0.00
C22:5	0.16^{A}	0.06	0.18^{B}	0.08	0.38^{ABC}	0.09	0.13^{C}	0.12
C22:6	0.05	0.01	0.05	0.01	0.05	0.01	0.04	0.02

Values with the same letters in the same row differ significantly: A, B, C at P≤0.01; a, b at P≤0.05

The number of somatic cells in the milk did not affect the content of functional fatty acids, apart from C20:3 and C22:5 (DPA). At the same time, analysis of the relationship between SCC and the fatty acid profile indicated a decrease in the content of C20 PUFA in the milk with the highest somatic cell count.

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