PHYSIOLOGICAL THRESHOLD OF SOMATIC CELL COUNT IN MILK OF POLISH HEATH SHEEP AND POLISH LOWLAND SHEEP*

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Abstract

The study was conducted on 320 sheep of two breeds: Polish Heath Sheep (PHS) and Polish Lowland Sheep (PLS). Udder health status was assessed based on somatic cell count (SCC) and bacteriological examination of milk. Cells of the immune system (leukocyte populations and lymphocyte subpopulations) were identified in the blood and milk of sheep by flow cytometry, using a panel of specific monoclonal antibodies and fluorescent dyes. The study showed that the highest proportion of neutrophils and a lower percentage of lymphocytes (CD4$^+$, CD8$^+$, CD19$^+$, WC1-N2$^+$) in milk and blood occurred in sheep of both breeds in which milk SCC ranged between 201 and 300×10^3 cells/ml. In light of existing research, these results suggest that fluctuations in somatic cell count of ewe milk are physiologically determined up to 200×10^3 cells/ml and result from udder health disturbances above this level.

Key words: sheep, mastitis, bacteria, SCC, WBC

Mammary gland health status is one of the main factors that determines the efficiency of sheep production. Inflammation of the mammary gland (mastitis) may adversely affect milk production and quality, cause rearing losses (lower weight gains of lambs, mortality) and result in the need to remove sick ewes from the flock. Microorganisms, in particular pathogenic bacteria, are the main cause of mastitis (Ber-

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gonier and Barthelot, 2003; Mørk et al., 2007; Albenzio et al., 2012). *Staphylococcus aureus*, which is among the most dangerous pathogens as it can spread very rapidly within the herd and is not easily amenable to conventional treatment, was isolated from the milk of sheep with acute mastitis much more frequently than other bacteria (Watkins et al., 1991; Lafi et al., 1998). In the case of subclinical mastitis, which may affect several dozen percent of the ewe herd, coagulase-negative staphylococci were isolated most often (Gonzalez-Rodriguez et al., 1995; Leitener et al., 2001). Also coagulase-positive staphylococci are responsible for a considerable proportion of subclinical mastitis cases (Stefanakis et al., 1995; Suarez et al., 2002). Many authors indicate a role of streptococci in the etiology of mastitis. Research shows that they may be responsible for 10–20% of udder diseases in sheep (Gonzalez-Rodriguez et al., 1995; Stefanakis et al., 1995; Lafi et al., 1998).

The mammary gland becomes infected most often in the first period of lactation, as well as just after weaning (Gonzalo et al., 1994; Watkins and Jones, 2004). Udder inflammations vary in pathogenesis, clinical course and symptoms, and have different effects on udder function. It is widely believed that in addition to clinical changes, the main indicators of udder health are somatic cell count (SCC) and the presence of pathogenic bacteria in milk. Estimated genetic correlations between infection state of the udder and SCC, and also the estimated genetic parameters for these traits, suggest that selection for SCC can lead to a reduction in the incidence of mastitis (Riggio et al., 2010; Tolone et al., 2012). During mastitis, milk somatic cell count increases mainly as a result of increased migration of leukocytes from blood to mammary tissue (Leitner et al., 2003; Le Roux et al., 2003). Pillai et al. (2001) found a two-fold higher percentage of granulocytes in cow's milk samples with over 250×10^3/ml somatic cells compared to the samples with lower SCC. Similar results were obtained by Schwarz et al. (2011). Meanwhile, Persson-Waller and Colditz (1999 a), who infected the udders of dry sheep with *S. aureus* and *E. coli*, found the proportion of neutrophils to increase, and the proportions of lymphocytes and monocytes to decrease in blood within 8 to 24 h of infection, but in dry udder secretion neutrophil percentage increased significantly much earlier, i.e. 4 hours after infection. Similar results were obtained by the same authors (Persson-Waller and Colditz, 1999 b) in sheep infused intramammarily with the immunostimulants β-1,3-glucan and recombinant ovine interleukin-2 (rOvIL-2). It was also found that the proportion of eosinophils and monocytes decreases during the course of mastitis in both blood and dry udder secretion (Piccinini et al., 2005; Persson-Waller and Colditz, 1999 a, b).

Variation in lymphocyte subpopulations depending on udder health status was analysed by many authors. Chaffer et al. (1999) and Benić et al. (2012) demonstrated that milk collected from infected udder quarters contained a significantly lower percentage of T lymphocytes (CD4^{+} and CD8^{+}) compared to milk drawn from healthy quarters. In the milk of sheep whose udders were infected with *S. epidermidis*, Winter and Colditz (2002) observed a much lower percentage of CD4^{+} and CD8^{+} lymphocytes 24 and 48 h after infection compared to milk of the control animals. In the case of Tγδ lymphocytes (WC1-N2^{+}), Soltys and Quinn (1999) observed a higher percentage of these cells in the blood and milk of cows with signs of mastitis com-

pared to healthy cows. Different results were obtained by Persson-Waller and Colditz (1999 a) and Winter and Colditz (2002), who found a lower percentage of WC1-N2+ (Tγδ) in dry udder secretion and in milk of sheep experimentally infected with mastitis pathogens (*S. aureus*, *S. epidermidis*, *E. coli*) compared to healthy sheep. A lower percentage of WC1-N2+ lymphocytes in the milk of susceptible compared to mastitis-resistant cows was also reported by Park et al. (2004).

B lymphocytes (CD19+) are involved in a complex process of humoral response, namely another stage after inflammation in which neutrophils play an essential role. The increased proportion of neutrophils in the early stage of the body's response to the infectious agent changes the proportions of cells involved in subsequent humoral response. This conclusion results from many studies in which animals were experimentally infected. Winter and Colditz (2002), who infected sheep udders with *S. epidermidis*, observed that the milk of these animals had a considerably lower proportion of B lymphocytes compared to control sheep. A similar result was obtained after sheep and goat udders were stimulated with various immune modulators (Persson-Waller and Colditz, 1999 b) and after cows were inoculated with *S. aureus* (Lee et al., 2005).

Despite many years of research, the physiological threshold of somatic cell count in ewe milk has not been conclusively determined. It ranges from 100×10^3 cells/ml (Lascelles, 1979) to 645×10^3 cells/ml (Riggio et al., 2013).

The present study attempts to determine the physiological threshold of somatic cell count in milk of Polish Heath Sheep and Polish Lowland Sheep, based on different proportions of leukocytes in sheep blood and milk depending on udder health status.

Material and methods

The present study was carried out on 160 Polish Heath Sheep (PHS) and 160 Polish Lowland Sheep (PLS) from the Experimental Station in Żelazna that belongs to Warsaw University of Life Sciences. Polish Heath Sheep (Wrzosówka) is the only surviving primitive sheep breed in Poland. It is unique among breeds, since it is extremely adaptable to difficult environmental conditions, disease-resistant and prolific. Polish Lowland Sheep (the Żelaźnieńska variety) was created by crossing Łowicka ewes, with the Merino and Lester rams. This breed is characterized by high prolificacy, good meat and wool production. Both breeds are subject to genetic resources conservation programme. Lactation in these breeds only lasts for a period of rearing lambs.

The study was conducted over 4 years. In each year of the study, all sheep from both flocks were examined for udder health in their 4th week of lactation, based on somatic cell count per ml milk. These data were used only to select animals for experiment. Two groups were formed in each flock (2×20 head). The first group included 20 ewes with the highest milk SCC, and the second included 20 ewes with the lowest SCC. In both groups, milk samples for analysis were collected from each

udder half of the ewes in their 6th week of lactation at the same time of day (8:00 a.m., 2 h after separating lambs). Concurrently, blood from the external jugular vein was drawn from the same ewes into K2EDTA tubes.

Somatic cell count per ml milk was determined by flow cytometry (Somacount 150, Bentley Instruments, Inc. Chaska, Minnesota, USA). Microorganisms were identified and differentiated using standard procedures and commercial test kits (BioMerieux). To characterize staphylococci, coagulase test was used with rabbit plasma, whereas, for *Streptococcus agalactiae* presence, CAMP test was performed.

Total leukocyte count (WBC) in peripheral blood of the sheep studied was determined using a Danam HC 510 hematology analyser. T and B lymphocyte subpopulations in blood and milk (CD2 – T, CD4 – Th, CD8 – Ts/c, CD19 – B, WC1_N2 – Tγδ) were determined flow cytometrically (FACStrak, Becton-Dickinson) using monoclonal antibodies (Pullman and Serotec) and the fluorochromes fluorescein and phycoerythrin (Medac) (Winnicka et al., 1999). For statistical analysis purposes, milk somatic cell count was divided into five classes:

1) $\leq 100 \times 10^3$,
2) $101-200 \times 10^3$,
3) $201-300 \times 10^3$,
4) $301-500 \times 10^3$,
5) $>500 \times 10^3$ cells/ml.

Data (SCC, leukocyte and lymphocyte populations) were transformed to a logarithm scale in order to balance the distribution. The effect of various factors on the number of somatic cells in milk were evaluated by analysis of variance using the model:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + e_{ijklm}$$

where:

Y_{ijklm} – lnSCC;
μ – population mean;
A_i – effect of year of study ($i = 1,.., 4$);
B_j – effect of bacteria (1 – *Streptococci*, 2 – *Staphylococci (-)*, 3 – other bacteria, 4 – bacteria negative);
C_k – effect of breed (1 – PHS, 2 – PLS);
D_l – effect of lactation ($i = 1,.., 5$);
e_{ijklm} – random error.

The relationship between SCC and percentage of immune system cells (leukocyte and lymphocyte populations) in sheep blood and milk was determined by analysis of variance using the model (for every breed):

$$Y_{ijk} = \mu + A_i + B_j + e_{ijk,}$$

where:

Y_{ijk} – leukocyte and lymphocyte populations (%);
μ – population mean;

A_i – effect of year of study ($i = 1,.., 4$);
B_j – effect of milk SCC ($j = 1,...5$);
e_{ijk} – random error.
This model did not account for the age (lactation) of sheep because this factor was not significant.

In order to determine the critical point of the SCC as a physiological norm of healthy udder, the method of ROC curves was used (Fawcett, 2006). Because the main cause of mastitis are bacteria, the result of bacteriological test (presence or absence of bacteria in milk) was used as the reference point for SCC.

Results

The results of cytological and bacteriological examination of milk of PHS and PLS sheep are presented in Table 1. In the milk of PLS sheep, various types of bacteria were almost twice as frequent (29.3%) as in the milk of PHS sheep (16.2%). Of all the bacteria isolated, the most numerous in both sheep breeds were *Staphylococci* (12.8–21.3%, incl. *Staph. aureus:* 2.2–1.9%, respectively) and *Streptococci* (2.8–6.4%). The incidence of other bacteria was low (0.6–1.6%). In both sheep breeds, the presence of bacteria increased (PLS 8.1–49.4%, PHS 10.2–50%) with the increase in SCC (classes I – V). The only exception was class IV milk in PHS ewes, in which the lowest proportion of samples with bacteria was found (6.3%).

Table 1. Percentage of milk samples with isolated bacteria in different milk classes

Breed of sheep	Milk class	SCC ×10³	No. of milk samples	Bacteria in milk			
				Streptococci[a]	*Staphylococci*[b]	other[c]	total
				%	%	%	%
	I	≤100	155		10.2		10.2
	II	101–200	51		12.8		12.8
PHS	III	201–300	24		16.7		16.7
	IV	301–500	44		6.3		6.3
	V	>500	46	19.4	25.0	5.6	50.0
Total PHS			320	2.8	12.8	0.6	16.2
	I	≤100	122		7.1	1.0	8.1
	II	101–200	28		13.0	8.7	21.7
PLS	III	201–300	24	10.0	30.0		40.0
	IV	301–500	33		40.7		40.7
	V	>500	107	16.1	32.2	1.2	49.4
Total PLS			314	6.4	21.3	1.6	29.3

PHS – Polish Heath Sheep, PLS – Polish Lowland Sheep.
[a]*Streptococcus sp. alpha hem.*, [b]*Staphylococcus sp. coag. (–) Staphylococcus aureus, coag. (+)*, [c]*Escherichia coli, Corynebacterium sp., Micrococcus sp.*

The analysis of variance showed a significant (P≤0.01) influence of breeds of sheep, bacteria and year of research on the variability of somatic cell count in the milk and thus on the state of health of the mammary gland. There was no significant

effect of lactation. Detailed results of the analysis of variance relating to the bacteria are presented in Table 2. Significantly higher increase in the number of somatic cells in the milk of sheep was observed in the infections of the mammary gland caused by *Streptococci* (lnSCC = 7.17) than with infections caused by *Staphylococci* (5.79). Lowest average of lnSCC (4.81) was obtained for samples of milk without bacteria.
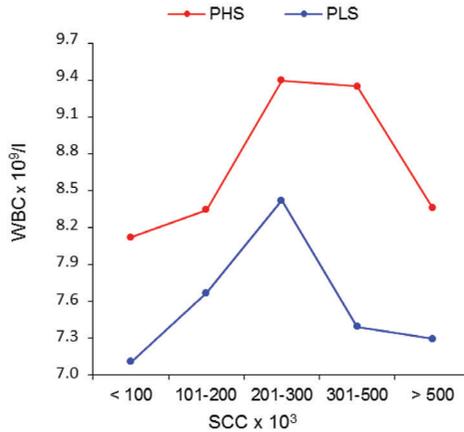
Figure 1. Relationship between peripheral blood leukocyte count (WBC) and milk somatic cell count (SCC) of PHS and PLS sheep

Figure 1 presents the relationship between SCC and leukocyte count (WBC) in peripheral blood of the sheep breeds studied. Leukocyte count was found to increase as milk SCC increased to 300×10^3/ml. Above this level, blood leukocyte count was observed to decrease.
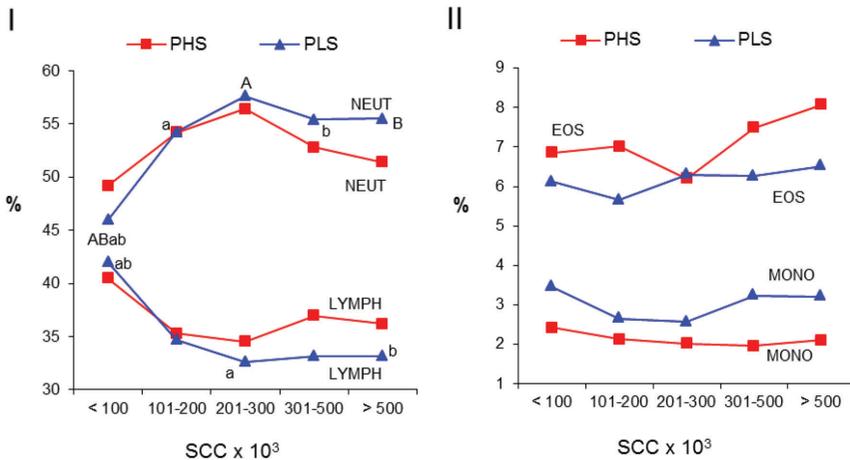
Figure 2. Relationship between percentage of peripheral blood leukocyte populations and milk somatic cell count of PHS and PLS sheep (AB – P≤0.01, ab – P≤0.05). NEUT – neutrophils, LYMPH – lymphocytes, EOS – eosinophils, MONO – monocytes

Changes in percentage composition of leukocytes and their relationship with milk SCC are shown in Figure 2. The lowest percentage of neutrophils in peripheral blood (Figure 2 I) was found in sheep in which milk SCC did not exceed 100×10^3/ml. An increase in milk SCC was paralleled by a rapid rise in the proportion of blood neutrophils (significant in PLS), which peaked at SCC of $201–300 \times 10^3$/ml. Above this level, percentage of blood neutrophils showed a downward tendency (greater in PHS) in both sheep breeds. An inverse relationship was observed for lymphocytes. The highest proportion of these cells was found in sheep with the lowest milk SCC ($<100 \times 10^3$/ml), and the lowest proportion was observed when milk SCC ranged between 201 and 300×10^3/ml. A similar trend was noted for monocytes (Figure 2 II). In both sheep breeds, the proportion of these cells in blood was found to decrease as milk SCC increased to 300×10^3/ml. Above 300×10^3, PLS sheep showed a slight increase in blood monocyte percentage, and in PHS sheep the proportion of these cells remained unchanged. Eosinophil variation showed a slightly different pattern. The lowest proportion of these cells was found in ewes whose milk contained from 101,000 to 200,000 somatic cells (PLS sheep) and from 201,000 to 300,000 somatic cells (PHS sheep). As milk SCC exceeded 300×10^3/ml, a slight increase in the proportion of blood eosinophils (greater in PHS) was found in both sheep breeds.

The relationship between leukocyte percentage and milk SCC (Figure 2) was also reflected in differences in the lymphocyte subpopulations (Figure 3). In both sheep breeds, the increase in milk SCC to 300×10^3/ml was paralleled by a considerable percentage decrease in CD2$^+$, CD4$^+$ and CD8$^+$ lymphocytes in both blood and milk. The proportion of these lymphocytes reached the lowest values in sheep the milk samples of which contained between 200,000 and 300,000 somatic cells. It is necessary to stress, however, that the dynamics of changes in lymphocyte percentage was much higher for milk (a significant percentage decrease in CD2$^+$ and CD8$^+$ in PLS sheep) than for blood. What is more, the proportion of lymphocyte subpopulations in blood and milk increased in both sheep breeds as milk SCC exceeded 300,000/ml. Special consideration should be also given to the relationship between CD4$^+$ and CD8$^+$ lymphocytes in milk. CD8$^+$ percentage was greater than CD4$^+$ percentage in PHS sheep, and an opposite trend was observed in PLS sheep, as reflected in the CD4$^+$/CD8$^+$ ratio (0.72–0.78 in PHS; 1.18–1.96 in PLS).

Greater between-breed differences than for T lymphocytes were observed for the percentage composition of B lymphocytes (CD19$^+$) and T$\gamma\delta$ cells (WC1-N2$^+$) (Figure 4). In the milk of PHS sheep, the increase in SCC was paralleled by a decrease in the proportion of all analysed cells to the lowest values when SCC was $201–300 \times 10^3$/ml, and by an increase in their proportion when SCC exceeded 300×10^3/ml. In the milk of PLS sheep, a similar relationship only applied to CD19$^+$ lymphocytes. In the case of WC1-N2$^+$ lymphocytes, their proportion was the lowest in sheep in which milk SCC ranged between 101,000 and 200,000/ml. Differences in the proportions of immune system cells analysed in blood were distinctly smaller but showed a similar tendency for changes (depending on SCC) as in milk.

The results of ROC analysis for SCC are shown in Figure 5. The cut-off was estimated at 205 (74% – sensitivity, 66% – specificity), AUC = 0.752 (SE = 0.028, P<0.00001).
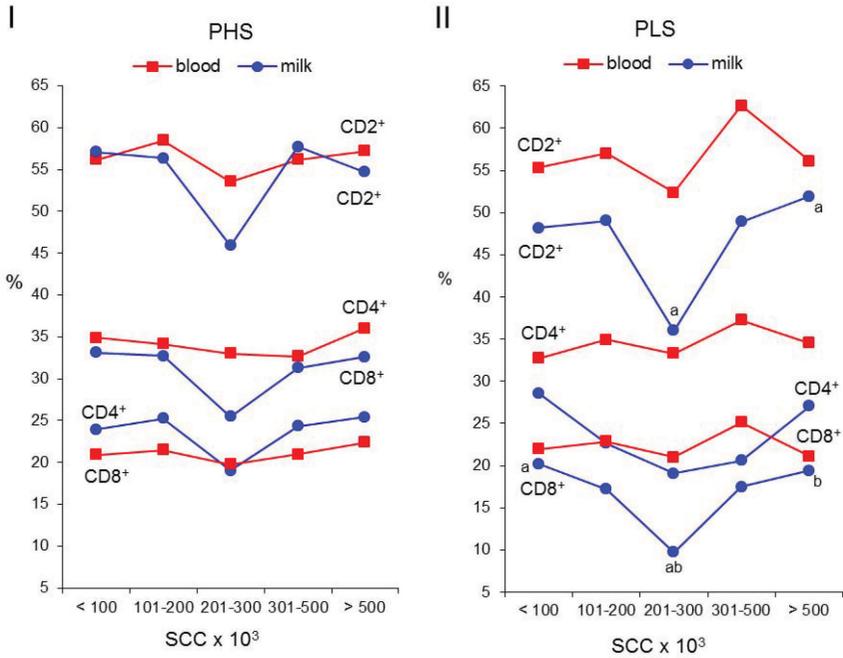
Figure 3. Relationship between percentage of T lymphocyte subpopulations in blood and milk, and milk somatic cell count of PHS and PLS sheep (ab – P≤0.05)
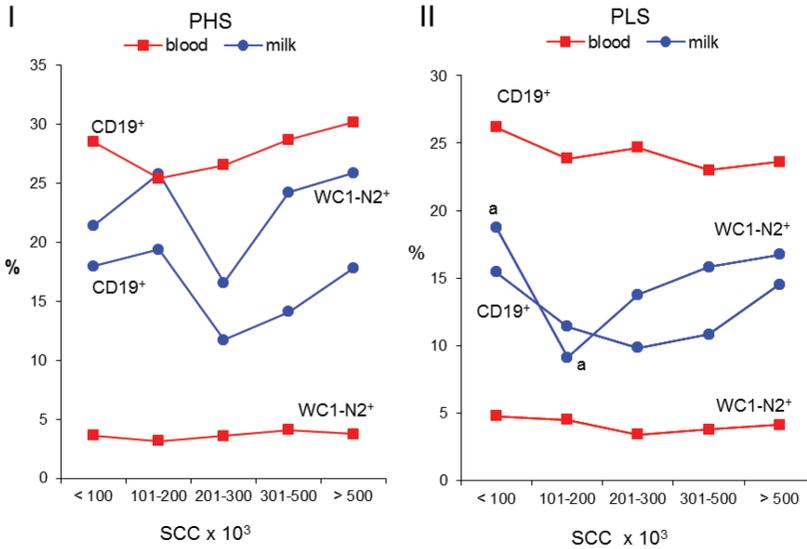
Figure 4. Relationship between percentage of CD19^{+} and WC1-N2^{+} lymphocytes in blood and milk, and milk somatic cell count of PHS and PLS sheep (a – P≤0.05)
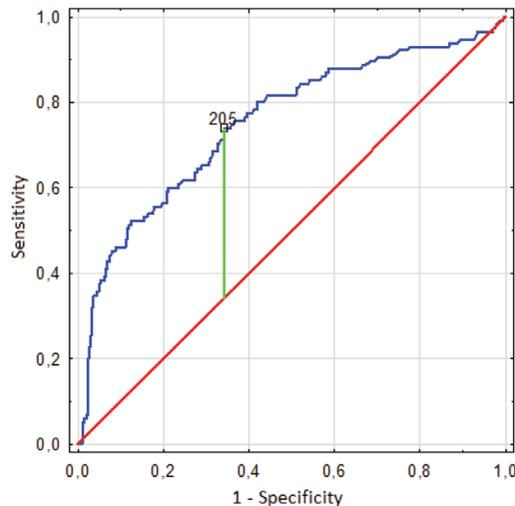
Figure 5. The ROC curve for the somatic cell count (10^3/ml) in milk of PHS and PLS sheep

Discussion

The results obtained for the presence of pathogenic bacteria in milk (Table 1) are consistent with the findings of other authors (Watkins et al., 1991; Gonzalez-Rodriguez et al., 1995; Stefanakis et al., 1995; Lafi et al., 1998; Leitener et al., 2001; Suarez et al., 2002).

Table 2. The results of analysis of variance for lnSCC

Bacteria	n	Mean lnSCC	SE
Streptococci	29	7.17 AB	0.27
Staphylococci	108	5.79 AC	0.13
Other bacteria	7	6.52 d	0.54
Bacteria negative	490	4.81 BCd	0.06

lnSCC – natural logarithm SCC, SE – standard error.
A, B, C – P≤0.01, d – P≤0.05.

The relationship observed between the presence of bacteria in milk and somatic cell count is supported by previous studies with different sheep breeds. Fthenakis et al. (1991) found that 85% of positive bacteriological samples contained more than one million somatic cells per ml of milk. Similar findings were reported by Pengov (2001) for sheep infected with coagulase-negative staphylococci. Jones (1991) found the presence of pathogenic bacteria in 90% of milk samples collected from sheep with subclinical mastitis.

Somatic cell count in milk and the presence of pathogenic bacteria are regarded as indicators of udder health status in ruminants. By comparing these indicators to

the flow cytometric assay of immune system cells, it is possible to follow changes in their composition in both body fluids and udder tissue. Many studies have demonstrated that during bacterial infections of the mammary gland there is an increase in the number of blood leukocytes, which enter the milk in large numbers because of increased permeability of glandular epithelium of the udder, thus increasing the number of somatic cells (Le Roux et al., 2003). In our study, the highest increase in blood leukocyte count of sheep with milk SCC above 200×10^3/ml is probably the result of the body's response to bacterial infections of the mammary gland (Figure 1). The differences observed in blood leukocyte counts between PHS and PLS sheep may be due to both breed differences and different body responses to various pathogens that were identified in the milk of these animals (Table 1). This is corroborated by Soltys and Quinn (1999), who reported that in cows with subclinical mastitis blood leukocyte count varied according to the type of pathogen (staphylococci 147.5 $\times 10^5$/ml, streptococci 150.2 $\times 10^5$/ml WBC). In healthy cows, leukocyte count was 142.6 $\times 10^5$/ml. Different responses to the infectious agent (different WBC levels) were also observed after sheep and goat udders were stimulated with specific (*S. aureus*, *E. coli*) and non-specific (β-1,3 glucan, rOvIL-2, Baypamun) mastitis-causing organisms (Persson-Waller and Colditz, 1999 a, b; Winnicka et al., 2000). Leukocyte count in the blood and milk of ruminants increases most often within 4 and 24 h of udder infection, and consistently decreases over the next few days to reach near physiological levels (Winter and Colditz, 2002). In view of the above, the decrease in blood leukocyte count, observed in sheep (Figure 1) in which milk somatic cell count exceeded 300×10^3/ml, should be regarded as a result of changes in percentage composition of leukocytes. This is because leukocyte count is largely associated with the percentage of granulocytes, which may form over 90% of all leukocytes during the early stage of inflammation. The decreasing percentage of granulocytes in successive stages of the immune response contributes to a change in leukocyte count, while the increase in milk SCC is mainly caused by an increasing proportion of sloughed follicular epithelial cells and secretory ducts of the mammary gland (Sordillo et al., 1987; Sordillo et al., 1989).

The observed differences in peripheral blood leukocyte populations in the analysed sheep (Figure 2) depending on udder health status (somatic cell count) are confirmed by many studies. Boulanger et al. (2003) reported 13% of lymphocytes and 8% of neutrophils in the milk of healthy cows, a significantly lower proportion of lymphocytes (8%) and a significant increase in the proportion of neutrophils (90%) in cows with acute mastitis, and 12% of lymphocytes and 82% of neutrophils in cows with chronic mastitis. In light of the studies cited above (Persson-Waller and Colditz, 1999 a, b; Pillai et al., 2001; Piccinini et al., 2005; Schwarz et al., 2011), the significantly higher proportion of blood neutrophils in sheep with milk SCC of 200,000–300,000, shown in our study (Figure 2 I) when compared to sheep with lower SCC, may be indicative of ongoing udder inflammation, when the increase in neutrophils is greatest.

Variation in lymphocyte subpopulations depending on udder health status was analysed by many authors. Persson-Waller and Colditz (1999 a) found a significantly lower percentage of CD8^{+} lymphocytes in dry udder secretion of sheep as early as

4 hours after infection with *S. aureus* and *E. coli* and a significantly lower percentage of CD4^{+} lymphocytes in blood after 8 hours compared to the control group. A percentage decrease in CD4^{+} and CD8^{+} blood lymphocytes of cows whose udders were infected with *S. aureus* was also reported by Lee et al. (2005). We obtained similar results (Figure 3) but only for sheep in which milk SCC ranged between 200,000 and 300,000 per ml. The lowest percentage of CD2^{+}, CD4^{+} and CD8^{+} lymphocytes in both milk and blood of these sheep is probably the result of the body's early immune response to the infectious agent, characterized by a considerable decrease in the proportion of lymphocytes. Meanwhile, the increase in the percentage of all lymphocytes analysed in sheep with milk SCC above 300×10^3/ml, may be indicative of the next stage of immune response, in which lymphocytes play a major role in recognizing and eliminating the infectious agent. This interpretation is substantiated by the results of some studies. During the seven consecutive days of the experiment, Taylor et al. (1994) observed the proportion of CD4^{+} lymphocytes to rise consistently (from 34% to 46%) in the milk of cows with signs of mastitis, and to remain at a similar level (from 32% to 33%) in healthy cows. An increased proportion of CD4^{+} in the blood and milk of cows on day 8 after experimental challenge with *S. aureus* was also found by Rivas et al. (2000). The predominance of CD4^{+} in the milk of sheep and cows during bacterial infections of the mammary gland was also reported by Taylor et al. (1994), Riollet et al. (2000) and Park et al. (2004). Meanwhile, Leitner et al. (2000) found a greater percentage of both CD4^{+} and CD8^{+} lymphocytes in cow's milk collected from infected quarters compared to milk drawn from uninfected quarters.

The differences in the results of studies discussed above and our own results (Figure 3) concerning the proportion of CD4^{+} and CD8^{+} in blood and milk may spread not only from varying study conditions (breed, physiological status, lactation period, time after infection), but also from the type of microorganisms that cause udder infections (Albenzio et al., 2012). This is confirmed by Soltys and Quinn (1999), who showed that T lymphocytes (CD4^{+}, CD8^{+}) are selectively recruited in udder infections depending on the type of bacteria found in the mammary gland. The same authors demonstrated that CD4^{+} lymphocytes (CD4^{+}/CD8^{+} = 1.39) were dominant cells in the milk of cows with signs of mastitis caused by staphylococci, while CD8^{+} lymphocytes (CD4^{+}/CD8^{+} = 0.65) predominated in infections caused by streptococci, just like in milk obtained from healthy cows (CD4^{+}/CD8^{+} = 0.68). Completely different results were obtained by Park et al. (2004), who found that CD8^{+} lymphocytes predominated in both blood and milk (CD4^{+}/CD8^{+}: blood 0.15, milk 0.42) of cows susceptible to mastitis (SCC>200×10^3/ml) caused by staphylococci (*S. aureus*) and CD4^{+} lymphocytes predominated (CD4^{+}/CD8^{+}: blood 2.5, milk 3.2) in resistant cows (SCC<200×10^3/ml). The discrepancies noted between the results cited above hamper proper interpretation of the CD4^{+}/CD8^{+} values obtained in our study (Figure 3). Special consideration should be given to PLS sheep, the milk of which contained more CD4^{+} than CD8^{+} lymphocytes for all milk SCC ranges. This was reflected in the CD4^{+}/CD8^{+} ratio, which was similar in both the milk (1.18–1.96) and blood (1.48–1.64) of these sheep. The results obtained are supported by few studies (Hurley et al., 1990; Concha et al., 1995) and differ considerably from the results of

many experiments that confirm predominance of CD8^{+} cells in the milk of different ruminants (Persson-Waller and Colditz, 1998; Asai et al., 2000; Banos et al., 2013; Bonelli et al., 2013). In general, the CD4^{+}/CD8^{+} ratios in the blood of both sheep breeds and in the milk of PHS sheep (Figure 3) did not differ from the results of studies cited previously.

Our findings (Figure 4), especially those concerning the changes in the proportion of B lymphocytes (CD19^{+}) in sheep milk containing from 200 to 300 × 10^{3}/ml somatic cells are in agreement with the results of studies cited above (Persson-Waller and Colditz, 1999 b; Winter and Colditz, 2002; Lee et al., 2005). Slightly different results were obtained by Park et al. (2004), who noted a lower percentage of B lymphocytes in milk and a higher percentage of B lymphocytes in blood of cows susceptible to mastitis compared to healthy cows.

In our study (Figure 4), the lowest T$\gamma\delta$ (WC1-N2[+]) percentage was found in the milk of PLS sheep (100 to 200 × 10^{3}/ml SCC) and in the milk of PHS sheep SCC ranged from 200 to 300 × 10^{3}/ml. A considerable proportion of T$\gamma\delta$ lymphocytes in ruminant milk and their concentration in the vicinity of mammary tissue epithelium show that these lymphocytes play an important role in udder defence mechanisms. Some authors suggest that T$\gamma\delta$ lymphocytes play a role in modulating local cell movement by stimulating the flow of lymphocytes and monocytes and by taking an active part in regulating response to mastitis (Park et al., 1994; Burton and Kehrli, 1996; Mukasa et al., 1998; Saunders et al., 1998).

Research published to date shows considerable differences between authors in threshold value of milk SCC in healthy sheep. Filev (1972) and Lascelles (1979) recognized that 100 × 10^{3} SCC in 1 ml of milk is the physiological threshold. Romeo et al. (1998) proposed two threshold values: 140 × 10^{3}/cells/ml for healthy sheep and 340 × 10^{3} cells/ml for sheep with subclinical mastitis. Meanwhile, Gonzalez-Rodriguez et al. (1995) suggested the need to determine the physiological threshold of milk SCC for each sheep breed separately (Churra – 200 × 10^{3} cells/ml, Castellana, Assaf – 400 × 10^{3} cells/ml). Similar threshold limit values, from 200 to 374 × 10^{3} cells/ml somatic cells for healthy sheep, were also proposed by other researchers (Green, 1984; Fruganti et al., 1985; De la Cruz et al., 1994; Mavrogenis et al., 1995; Pengov, 2001; Ozenc et al., 2011). Some authors considered 500 × 10^{3} cells/ml as the physiological threshold of SCC (Travniček et al., 1976; Bergonier and Berthelot, 2003) and even much higher (645×10^{3} cells/ml; Riggio et al., 2013).

Conclusion

In light of the studies cited above, the significant increase in the proportion of neutrophils and the considerable decrease in lymphocyte percentage in both blood and milk of sheep whose SCC ranged from 200,000 to 300,000 per ml (Figures 1–4) can be interpreted as an early stage of the body's response to the infectious agent, which is characterized by a rapid increase in neutrophil percentage and a decrease in lymphocyte population percentage. The results obtained lead to the suggestion that fluctuations in milk SCC of sheep are physiologically determined up to 200,000/ml and result from udder health disturbances above this level. The proposed limit of the physiological SCC in the milk of sheep (PHS, PLS) is confirmed by the results of

ROC analysis (Figure 5) – cutoff point 205×10^3 cells/ml. As can be seen from the presented data, the estimated threshold of SCC 200,000 cells/ml can be a useful detection criterion for the subclinical mastitis in sheep (PHS, PLS).

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