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Relationship between transferrin and globulin antigen polymorphism and sheep resistance to mastitis

Krystyna M. CHARON[1], Czesława LIPECKA[2], Tomasz SIUDEK[1], Wiesław P. ŚWIDEREK[1], Elżbieta SKIBA[3]

[1] Department of Genetics and Animal Breeding, Warsaw Agricultural University, Warszawa
[2] Department of Sheep Breeding, Agricultural University, Lublin
[3] Department of Immunology, Institute of Animal Production, Kraków

Abstract. The effect of transferrin genotypes and genes and globulin antigens on sheep resistance/susceptibility to mastitis was analysed. The udder health condition was diagnosed on the basis of the somatic cell count in 1 ml of milk and results of bacteriological tests. It was found that sheep with the transferrin genotype AC, AD and CC were characterised by a better health of the mammary gland than the remaining ewes. Among 9 transferrin alleles observed in the examined sheep, only the presence of transferrin I allele in the ewes genotype was connected with the somatic cell count below the mean value of this trait. The presence of transferrin A allele in the sheep's genotype was connected with a small infection of milk with mastitis pathogens. The serum globulin antigens analysed did not significantly affect the somatic cell count in the milk of the examined ewes. However, the antigens A2, NS1 (antigens of β-globulin) and GB2 (antigen of class IgG immunoglobulins) as well as the globulin antigen A6 and GA1 antigen of class IgG immunoglobulins showed to have a significant effect on the level of sheep milk infection.

Key words: globulin antigen, mastitis, resistance, sheep, transferrin.

Introduction

Health condition of animals is one of the factors influencing production economy. In sheep breeding a significant reason for culling ewes arises from inflammations of the mammary gland – mastitis (MAISI et al. 1987). The lack of effective pharmacological and veterinary agents induces to seek for other

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Correspondence: K.M. CHARON, Department of Genetics and Animal Breeding, Warsaw Agricultural University, ul. Przejazd 4, 05-840 Brwinów, Poland.

means limiting the occurrence of this disease. Variability in the susceptibility of ewes to mammary gland infections indicates a possibility of introducing breeding methods.

Among factors affecting resistance of organism to bacteria infections are transferrins and globulin antigens.

The investigations conducted aimed at an analysis of a possible effect of transferrin and globulin antigen polymorphism on the health condition of the mammary gland in sheep. As both the transferrin genotype and the presence of a specific globulin antigen may be determined in males and females there is a possibility of using these markers in sheep breeding for resistance to mastitis.

Material and methods

The investigations were conducted during the years 1992-1994 on the Warsaw Agricultural University Experimental Farm in Żelazna, on lowland sheep of the Żelazna type.

Health condition of the mammary gland of 375 experimental ewes (in the 1st and 2nd lactation only) was examined three times throughout 100 days of lactation, i.e., at the end of the first, second and third month. Milk samples were taken separately from each half of the udder. The somatic cell count (SCC) was determined in milk with the use of the Fossomatic electronic counter 15610, while the presence of pathogenic bacteria was checked by routine microbiological tests.

Polymorphism of transferrins was determined once by horizontal electrophoresis on starch gel for 354 ewes. The presence of globulin antigens in the blood serum was determined for 277 ewes using the method of double immunodiffusion (SKIBA, WĘGRZYN 1993).

The frequency of milk samples with a specified somatic cell count and those infected with pathogenic bacteria was analysed.

The frequency of transferrin genotypes and genes as well as globulin antigens in the blood serum of the examined sheep was estimated. Moreover, investigations were conducted on the effect of transferrin genotypes and genes as well as globulin antigens on the health condition of the mammary glands of the examined ewes expressed in the somatic cell count.

The influence of biological (i.e., the number of reared lambs, age of ewes) and environmental (management system) effects on the udder health condition of the examined sheep was analysed and the results are presented in an article by ŚWIDEREK (1996).

For statistical analysis the number of somatic cells per 1 ml of milk was transformed to the logarithmic scale (LSCC) to normalise this trait distribution (ALI, SHOOK 1980).

Data were analysed by the least squares method (one classification ANOVA). Only fixed models were used to handle different studied traits. For simplicity, envirnomental and polygenic effects were excluded. The effect of each transferrin genotype on the somatic cell count in milk was evaluated. Also the effect of individual transferrin alleles (two classes : allele present in the genotype vs allele absent in the genotype) on SCC in milk was evaluated. The effect of globulin antigens and of the interaction between them on the somatic cell count in milk was evaluated. Since the number of globulin antigens loci is still unknown, the relationship one antigen – one gene was assumed (SKIBA, WĘGRZYN 1993).

Differences in LSCC means were evaluated using F statistics. The significance of differences in the number of milk samples infected with mastitis pathogens or free from pathogenic bacteria between ewes with a given transferrin allele or globulin antigen was evaluated using chi-square statistics.

Results and discussion

The data referring to the health condition of mammary glands of 375 examined ewes of the lowland Żelazna sheep are presented in Table 1. They show that milk infected with pathogenic bacteria was characterised by a considerably higher somatic cell count. The highest level of somatic cells was observed in the milk containing *Staphylococci.*

Milk samples with less than 300 thousand cells per 1 ml constituted 72.2% of all the samples tested. Among the samples of this group only 6.5% was infected with pathogenic bacteria. 4.8% of all the milk samples contained 300 to 500 thousand somatic cells per 1 ml. The presence of pathogenic bacteria in these samples was observed more frequently (21.9% of samples). A small percentage of samples (5.3%) contained between 500 thousand and 1 mln cells per 1 ml, and in the majority of them (85.2% in relation to all the samples in this group) bacteria were observed. A considerable proportion of milk samples (17.6%) obtained from Żelazna ewes contained over 1 mln somatic cells per 1 ml, and over half of them contained mastitis pathogens.

Mammary gland inflammation is principally caused by bacteria – *Streptococci, Staphylococci* and rod-bacteria (WATSON 1988). The degree of infection

Table 1. Somatic cell count (SCC) and the presence of bacteria in the ewes' milk

Milk samples	Month of lactation	Milk samples with defined SCC (ths/1 ml of milk)								Total milk samples	
		< 300		(300; 500 >		(500; 1000 >		> 1000			
		n	%	n	%	n	%	n	%	n	%
A	I	541	73.81	32	4.37	38	5.18	122	16.64	733	100.0
B		28	5.18	8	25.00	21	55.26	69	56.56	126	17.19
A	II	572	76.57	31	4.15	41	5.49	103	13.79	747	100.0
B		35	6.12	5	6.13	19	46.34	64	62.14	123	16.47
A	III	452	65.89	42	6.12	36	5.25	156	22.74	686	100.0
B		39	8.63	10	23.81	35	97.22	80	51.28	164	23.91
A	I-	1565	72.2	105	4.85	115	5.31	381	17.59	2166	100.0
B	III	102	6.52	23	21.90	98	85.22	213	55.91	436	20.13

A – milk samples with defined SCC per 1 ml, B – milk samples infected with mastitis pathogens in the range of SCC, n – number of milk samples.

by individual types of bacteria in the milk samples from the examined ewes is presented in Fig. 1.

Pathogenic bacteria were found in 19.3% of milk samples. *Staphylococci* were the most frequently isolated bacteria (12.2% of samples), and principally *Staphylococcus epidermidis*. *Staphylococcus aureus* was present in 2% of samples.

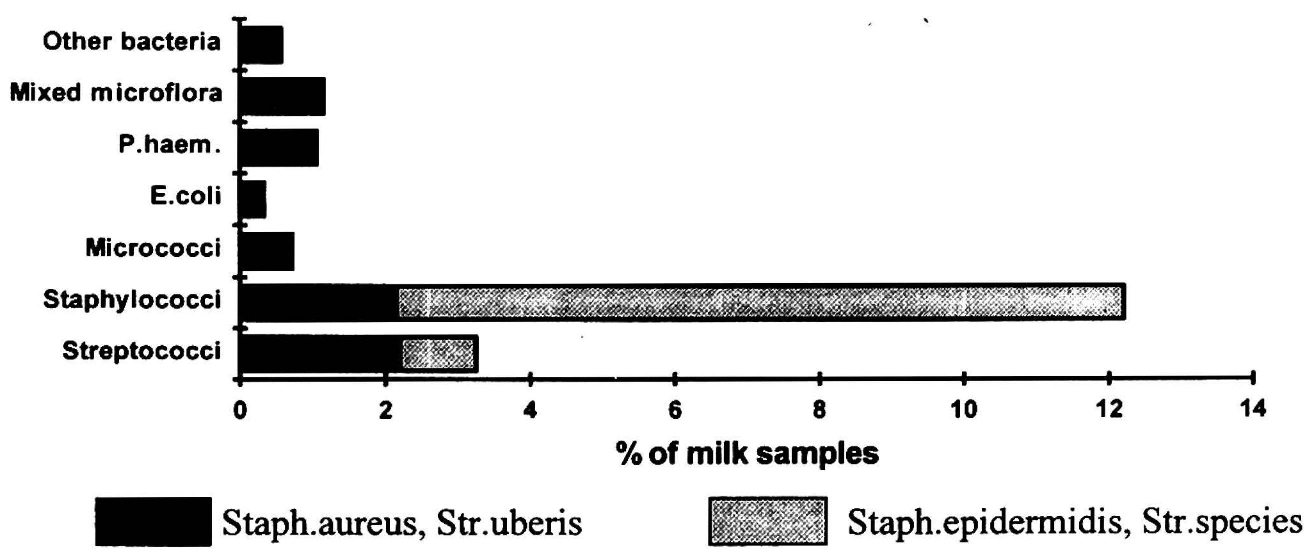

Fig. 1. The percentage of sheep milk samples infected with bacteria

The presence of *Streptococci* was observed in 3% of milk samples, *Streptococcus uberis* being dominant. *Pasteurella haemolytica* was observed to occur in about 1% of milk samples, while *Escherichia coli* – in 0.3%. Also

Table 2. The frequency of transferrin genotypes and alleles in the studied population of ewes

Transferrin genotype	Number of ewes	Frequency of genotype	Transferrin allele	Frequency of allele
AA	14	0.039		
AB	24	0.068		
AC	90	0.254	A	0.267
AD	43	0.121		
AM	2	0.006		
BB	4	0.011	B	0.113
BC	28	0.079		
BD	16	0.045		
BE	1	0.003	E	0.001
BM	2	0.006		
CC	52	0.147		
CD	39	0.110	D	0.162
CM	18	0.050		
CP	1	0.003	P	0.001
DD	8	0.023		
GC	2	0.006	G	0.004
GD	1	0.003		
IA	2	0.006	I	0.010
IB	1	0.003		
IC	3	0.008	C	0.403
IM	1	0.003		
MM	2	0.006	M	0.038
Total	354	1.000		1.000

other bacteria were isolated, i.e., *Micococcus sp.*, *Bacillus sp.*, *Pseudomonas aeruginosa* and gram negative bacillus.

Health condition of the mammary gland of dams affects lamb rearing. A clear, negative effect of mastitis on production results (quantity and quality of milk produced, results of rearing lambs, ewe deaths) was observed in all sheep breeds, irrespective of utilisation type (MACKIE, RODGERS 1986, FTHENAKIS, JONES 1990).

Milk of many animal species contains iron binding proteins – lactoferrin and transferrin. Lactoferrin is synthesised in the mammary gland while transferrin is transferred from the blood serum (SANCHEZ et al. 1992). Transferrin (syderofilin) is a β-globulin, demonstrating considerable polymorphism, revealed by the occurrence of various genotypes in one species (WELCH 1990).

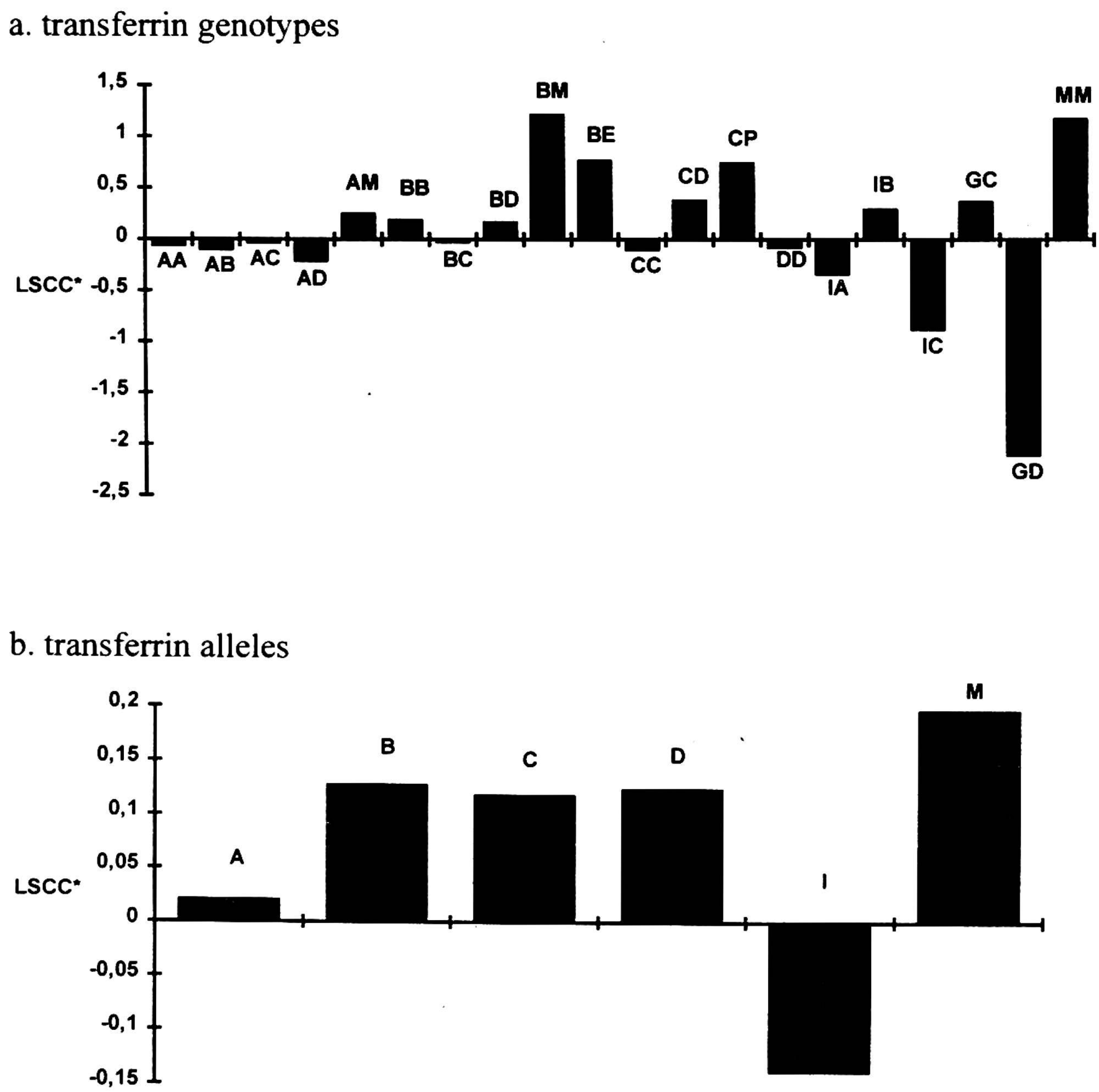

Fig. 2. The effect of transferrin genotypes (a) and alleles (b) on somatic cell count in milk of the investigated ewes

* Deviation in somatic cell count in logarithmic scale (LSCC) from the mean LSCC (4.48) accepted as 0

Its bacteriostatic activity protects the organism from bacterial infections. This kind of resistance is based on the transferrin and pathogenic bacteria competing for iron (HUEBERS, FINCH 1987).

The group of the examined Żelazna ewes was found to have 9 transferrin alleles controlling 22 genotypes (Table 2). The most frequent were A and C alleles, while B and D alleles were slightly less frequent. The frequency of the I and M alleles oscillated around 1-4%. As E, G and P alleles occurred in less than 1% of the animals, ewes containing these alleles were ignored in our further investigations.

Among transferrin genotypes the highest frequency (0.254) was observed for the genotype AC, which is connected with the frequency of A and C alleles in the population. The analysis of variance on the number of somatic cells in the milk of the examined sheep showed a significant effect of transferrin genotype on this trait ($P \leq 0.05$). Milk obtained from ewes with transferrin genotypes characterised by the highest frequency of AC, AD and CC (Table 2) had slightly less somatic cells in comparison to the mean value of this trait in the population (Fig. 2). The diagram presents a deviation in the logarithmic value of the somatic cell count from the mean LSCC (4.48 ± 1.89) obtained for animals tested in the experiment (n = 354), and accepted as 0 in the diagram. The CD genotype, occurring in 39 ewes (frequency 0.11), increased the number of somatic cells in the milk. The remaining genotypes showed different effects, but due to their very low frequency it was impossible to draw conclusions.

An analysis of associations between a specified transferrin allele and the mammary gland health condition showed that, among the 6 alleles discussed, the presence of I allele in the ewes' genotype was connected with a lower somatic cell count, as compared to the mean obtained for the examined animals. The remaining alleles (A, B, C, D, M) occurred in the ewes with the mean SCC value higher than the general mean (Fig. 2). However, an analysis of variance of the somatic cell count in the milk showed that only sheep with the M allele in the genotype differed significantly ($P \leq 0.05$) from sheep without this transferrin allele.

There is no information in the literature on associations between transferrin genotype and resistance of sheep to the mammary gland infections. However, such investigations conducted on cows (MALIK et al. 1970) revealed significant differences in their resistance to mastitis depending on the transferrin genotype in the blood serum. Cows with the EE transferrin genotype were resistant, while those with the genotype D_1D_1 were susceptible to infections of the mammary gland.

Transferrin takes part in the protection of organism from bacterial infections (HUEBERS, FINCH 1987, DEJONG et al. 1990, SANCHEZ et al. 1992). Thus the cognizance of the connection between the transferrin alleles and the presence of pathogenic bacteria in the milk is particulary important. An analysis of this association showed that the lowest proportion of infected milk samples was found in sheep with A allele in the genotype (Table 3; $P \leq 0.01$). The frequency of this allele was quite high, when compared to other alleles, and occurred in the genotype of almost 50% of the ewes (Table 2). Thus, it may suggest that it could be taken into consideration when selecting yearling ewes for resistance to mammary gland inflammations.

Investigations conducted by LINGAAS et al. (1992) on cows revealed no significant differences in the frequency of transferrin alleles between animals susceptible and resistant to mastitis. However, results reported by SANCHEZ et al. (1988) point to a considerable role of transferrin in protecting organism of both cows and sheep from mastitis infection. According to SANCHEZ et al. (1992) the biological function of transferrin, i.e., binding of iron, is significant for the health of both lactating dam and newly born lamb.

Table 3. The relationship between the presence of pathogens in milk and transferrin alleles in the studied population of ewes

Allele		Number of milk samples	Milk samples		χ^2
type	presence.		non-infected	infected	
A	0	889	694	195	
	1	956	798	158	8.71**
B	0	402	332	70	
	1	1443	1160	283	0.98
C	0	1223	1004	219	
	1	622	488	134	3.52
D	0	563	467	96	
	1	1282	1025	257	2.27
I	0	35	30	5	
	1	1810	1462	348	1.69
M	0	138	112	26	
	1	1707	1380	327	0.01

** differences significant at $P \leq 0.01$
0 – absence of transferrin allele, 1 – presence of transferrin allele

Globulins play an important role in resistance of organism to bacterial infections. Antigen determinants which differentiate individuals of the same species are localised in these proteins. The role of protein-carriers of these antigens has not yet been sufficiently explained. However, it is known that immunoglobulins take part in immunologic response of organism (de BENEDICTIS 1979).

The frequency of globulin antigens determined in the 277 Żelazna ewes examined is presented in Table 4. Each sheep had simultaneously two or more antigens, so the total of antigen frequencies did not exceed 1. In the group of examined animals a high frequency was observed for β-globulin antigens – NS1 and A2, both immunoglobulin antigens class IgG-GA1 and GB2 and immunoglobulin antigen class IgM-MA2. However, neither α-globulin antigen A3 nor β-globulin antigen A7 were found. The frequency of the remaining antigens ranged between 14% and 20%.

Table 4. The frequency of globulin antigens in the studied population of ewes

Antigen of globulin	Number of ewes with antigen	Frequency of globulin antigen
α-globulin antigen :		
A3	0	0.00
β-globulin antigen :		
A2	164	0.59
A4	40	0.14
A5	67	0.24
A6	56	0.20
A7	0	0.00
NS1	184	0.66
immunoglobulin IgG antigen :		
GA1	227	0.82
GB2	151	0.55
immunoglobulin IgM antigen :		
MA2	147	0.53
Total number of investigated ewes	277	–

The analysis of variance conducted for the somatic cell count (in logarithmic values) in sheep milk did not indicate globulin antigens to have a significant effect on this trait. It was, however, observed that in ewes with the antigen A5 the number of somatic cells per 1 ml of milk was lower than the logarithmic mean (4.65 ± 0.63) for the examined animals. In the case of all the remaining antigens a reverse tendency was observed (Fig. 3).

The effect of interaction between certain antigens and the somatic cell count proved to be significant, but only the interaction between antigens A2 and MA2 lowered the number of somatic cells in milk. The remaining interactions (A5 × GB2, A5 × MA2, A6 × MA2 and GA1 × GB2) significantly increased the quantity of somatic cells in milk.

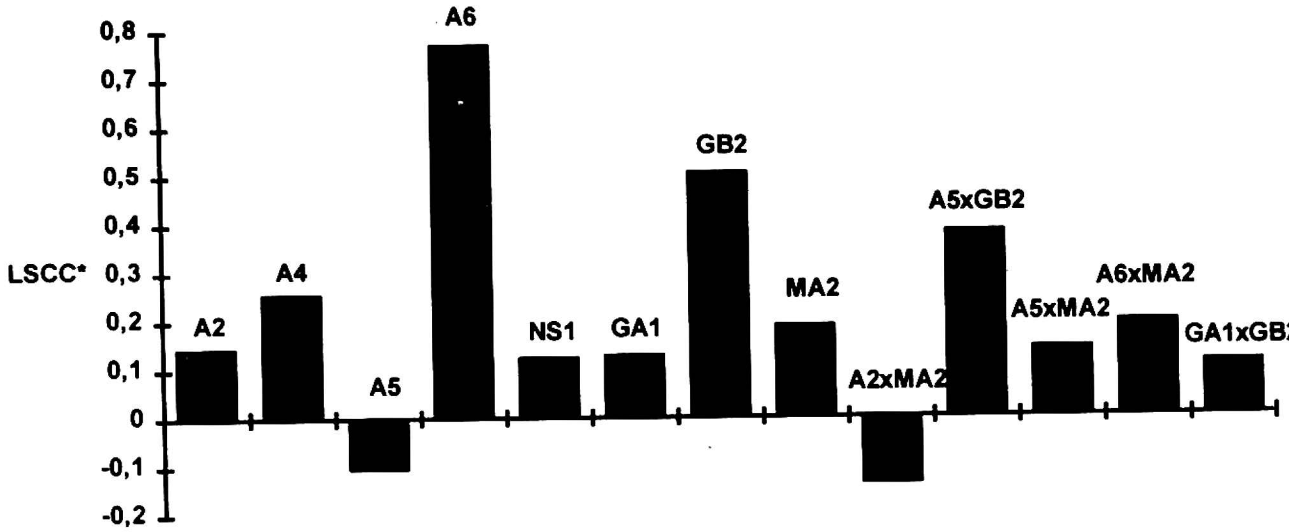

Fig. 3. The effect of globulin antigens on somatic cell count in milk of the investigated ewes
*Deviation in somatic cell count in logarithmic scale (LSCC) from the mean LSCC (4.65) accepted as 0

Table 5. The relationship between the presence of mastitis pathogens in milk and globulin antigens in the studied population of ewes

Antigen type	Antigen presence	Number of milk samples	Milk samples non-infected	Milk samples infected	χ^2
A2	0	591	446	145	
	1	865	720	145	13.30**
A4	0	1231	983	248	
	1	225	183	42	0.26
A5	0	1101	876	225	
	1	355	290	65	0.76
A6	0	1153	911	242	
	1	303	255	48	4.01*
NS1	0	483	355	128	
	1	973	811	162	19.64**
GA1	0	265	197	68	
	1	119	961	230	5.39*
GB1	0	639	487	152	
	1	817	679	138	10.66**
MA2	0	667	520	147	
	1	789	646	143	3.45

** differences significant at $P \leq 0.01$
* differences significant at $P \leq 0.05$
0 – absence of antigen, 1 – presence of antigen

Moreover, it was observed that, depending on the allotype, the studied ewes exhibited different susceptibility to mastitis infections (Table 5). Sheep with the antigens A2, NS1, GA1 and GB2 were observed to have a significantly higher ($P \leq 0.01$) percentage of milk samples not infected with pathogenic bacteria as compared with the milk of sheep without these antigens, whereas sheep with the antigen A6 had a significantly lower percentage of uninfected milk samples.

The allotypes determined in sheep are localised on α-, β- and γ-globulins (de BENEDICTIS 1979, IANNELLI 1979, SKIBA, WĘGRZYN 1993, SKIBA et al. 1994). They are determined by autosomal dominant genes and are inherited according to Mendel's law. These traits may, therefore, be used as genetic markers in analysing synthesis and catabolism of these proteins in studying passive and active resistance.

The relation between certain antigen determinants and the degree of milk infection creates possibilities for effective selection aimed at improving udder health condition in sheep.

Conclusions

Results of the conducted investigations indicate that mastitis in the lowland Żelazna sheep was caused principally by *Staphylococci*, and that the milk infected by *Streptococci* had the highest somatic cell count.

The transferrin genotype in the blood serum may affect health condition of ewes' mammary glands. Sheep with the AC, AD and CC transferrin genotypes (the highest frequency) produced milk with a slightly lower somatic cell count than the mean of all the sheep examined. Inflammation of the udder was most often diagnosed in ewes with the CD genotype (frequency 0.11) and BD genotype (frequency 0.045). The remaining transferrin genotypes occurred only in individual ewes, and for that reason it was impossible to draw conclusions.

The study on the effect of transferrin alleles on the somatic cell count in the milk showed that the presence of I allele in the genotype resulted in lower number of somatic cells in the milk. On the other hand, the proportion of infected milk samples was much lower in sheep with A allele. The same effect was found for β-globulin antigens A2 and NS1 as well as for GB2 antigen of class IgG immunoglobulins. These relationships suggest that the mentioned above globulin antigens as well as transferrin A allele can be useful as selection criteria for resistance to mastitis in sheep.

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