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Original Contribution

BREED AND AGE-RELATED DIFFERENCES IN LYSOZYME CONCENTRATIONS AND COMPLEMENT ACTIVITY IN RAMS

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ABSTRACT

One hundred and forty one rams from the following breeds, Karakachan, Tsigay, Staroplaninska, Ile de France, Suffolk, Mouton Charollais, Trakia Merino and Pleven Blackhead reared at the Institute of Mountain Animal Breeding and Agriculture, Troyan, were studied. It was established that in Karakachan rams, lysozyme concentrations and complement activity decreased with age whereas in Tsigay rams – increased. In rams, as in other animal species, breed-related differences in lysozyme concentration and complement activity were present.

Key words: rams, breeds, lysozyme, complement

INTRODUCTION

The activity of phagocytosis, complement activity, beta lysins, lysozyme concentration, interferon and immunoglobulins determine the levels of both innate and specific systemic immune responses (1 – 5) All of them could be used also as biological tests for the system's immune status.

Lysozyme is one of the principal factors of innate immunity in humans, animals and birds (6 – 8). Its bactericidal effect against Gram-positive and some Gram-negative microorganisms and viruses is attributed to its lytic, cationic and hydrophobic properties (9 – 14). The complement, as a blood serum protein system, performs various protective functions via activation through three different pathways – alternative, classical and lectin pathways (15, 16).

The studies of some investigators evidenced that lysozyme and complement levels are different in the variety of animal species and are influenced by breeds. Significant breed-related differences in lysozyme concentrations and complement activity were reported in swine, turkeys, sheep, horses and cattle (17 – 23). We did not find enough information about serum lysozyme concentration and complement activity in rams and this motivated us to develop this study.

MATERIALS AND METHODS

The experiment was performed in June 2006 with 141 rams from the Karakachan (n=50), Tsigay (n=22), Staroplaninska (n=3), Ile de France (n=17), Suffolk (n=4), Mouton Charollais (n=10), Trakia Merino (n=29) and Pleven Blackhead (n=6) breeds, reared at the Institute of Mountain Animal Breeding and Agriculture, Troyan. Blood was aseptically sampled from the jugular vein. Serum lysozyme concentrations were determined according to the method of Lie (24) after centrifugation for 10 min at 2000 g. Briefly 20 ml of 2 % agarose (ICN, UK, Lot 2050) dissolved in sodium phosphate buffer (pH = 6.2) was mixed with 20 ml suspension of 24 hours culture of *Micrococcus lysodeicticus* at 67°C. This mixture was poured into Petri's dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured in each well. Eight standard

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dilutions (from 0,025 to 3,125 μg/ml) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured. Final lysozyme concentrations were calculated by using special computer program developed in Trakia University and expressed as μg/ml.

The activity of the alternative pathway of complement activation (APCA) was assayed by the method of Sotirov (17). Each serum sample was first diluted by mixing 100μl serum with 350 μl veronal - veronal Na buffer (in final concentrations: 146 mM NaCI, 1,8 mM 5,5-diethylbarbituric acid sodium salt, 3,2 mM 5,5-diethylbarbituric acid, 1 mM EGTA and 0,8 mM $MgCl_2$). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer: 80 μl diluted serum + 20 μl buffer, 70 μl diluted serum + 30 μl buffer, 60 μl diluted serum + 40 μl buffer, 50 μl diluted serum + 50 μl buffer, 40 μl diluted serum + 60 μl buffer, 30 μl diluted serum + 70 μl buffer and 20 μl diluted serum + 80 μl buffer. The final serum dilutions were, respectively, 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then 50 μl buffer and 100 μl of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 μl of each supernatant was removed and placed in flat bottomed plates for measurement of optical density at 540 nm using "Sumal-PE2" ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer programs developed in the Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement-induced haemolysis of applied erythrocytes).

Statistical analysis:

Data were analysed using the fixed effect MANOVA model (Program STATISTICA, StatSoft, Inc., USA).

RESULTS AND DISCUSSION

Serum lysozyme concentrations in the different breeds of rams are shown on **Table 1**. Karakachan rams, aged 1.5 years, presented the highest (3.207 ± 3.205 μg/ml). The levels gradually decreased with age to 0.234 ± 0.123 μg/ml at the age of 4-6 years. The mean value for this sample was 1.309 ± 1.235 μg/ml. The high standard errors (SE) and high coefficients of variation (CV) are due to the significant variability of the studied trait. In Tsigay rams, lower lysozyme concentrations were observed in animals younger than 1.5 years (0.266 ± 0.055 μg/ml), whereas the levels increased in young adults aged 2–4 years – 4.481 ± 3.054 μg/ml. The mean value of the sample was low – 1.416 ± 0.873 μg/ml. In rams of the Staroplaninska breed, lysozyme concentration was the lowest (0.126 ± 0.024 μg/ml). Low lysozyme levels were also observed in Suffolk, Trakia Merino and Pleven Blackhead breeds. Medium concentrations were assayed in Mouton Charollais rams (0.437 ± 0.226 μg/ml). High variability of lysozyme levels in Ile de France rams (5.148 ± 2.032 μg/ml) demonstrated that there are high individual differences, allowing to perform a selection for improving the studied trait. This breed had significantly higher lysozyme levels compared to the other breeds ($p < 0.05$). It should be outlined that the high average lysozyme concentrations, observed in some samples, are due to animals with exceptionally high individual values of the enzyme – ram № 239 from the Ile de France breed, (24.5 μg/ml), ram № 0165293 from the Karakachan breed (23.5 μg/ml) and ram № 0164959 of the Tsigay breed (19 μg/ml). The frequency of animals with high lysozyme concentrations was extremely low. This was shown throughout previous studies of ours in the South Bulgarian Corriedale and Caucasian sheep breeds (18). The studies of Walawski et al. (25) showed that, among cattle, there were animals with high lysozyme activity. Their frequency was also very low – among 10 000 animals, two bulls and several cows were only discovered. Their progeny, regardless of gender, age or other traits, inherit a very high or normal lysozyme activity.

During the last fifteen years a number of investigators showed, to a considerable extent, the genetic structures of genes coding for lysozyme in various animal species and its significance for the protection of the organisms against pathogens. On the basis of reports of these authors and our results, we hypothesise that breeds with a high phenotypic diversity of the trait have the so-called main gene (or allele) encountered in the homozygous state and that it determines the high lysozyme levels. This hypothesis is supported by the studies of Irwin and Wilson (26) and Irwin et al. (27), stating that in ruminants there were at least 10 genes coding for the synthesis of this enzyme. Davies and Maddox (28) discovered the polymorphism of repeated dinucleotide sequences in one lysozyme locus in sheep. Five alleles within the locus have been determined (A, B, C, D and E), each with a different frequency. The most commonly discovered allele is D (0.81).

Table 1. Lysozyme concentration in rams from different sheep breeds (expressed in μg/ml). Results are expressed as mean ± standard error (SE). VC(%) : Coefficients of variations.

Breed	*n*	*X ± SE*	*VC (%)*
Karakachan 1,5 г.	7	3,207 ± 3,205	244,76
2-4 г.	3	0,488 ± 0,378[c]	109,50
4-6 г.	15	0,234 ± 0,123[c]	81,03
Average	25	1,309 ± 1,235	145,09
Tsigai 1,5 г.	8	0,266 ± 0,055[c]	54,19
2-4 г.	3	4,481 ± 3,054	96,38
Average	11	1,416 ± 0,873	75,28
Staroplaninska	3	0,126 ± 0,024[c]	27,08
Ile de France	17	5,148 ± 6,032[c]	102,21
Suffolk	4	0,242 ± 0,164[c]	116,95
Mouton Charollais	10	0,437 ± 0,226	59,57
Trakia merino	29	0,13 ± 0,02[c]	42,13
Pleven Blackhead	6	0,26±0,03[c]	32,07

[c] *Mean values with superscript within a column differ significantly (p < 0,05) vs Ile de France.*

Table 2. APCA activity in rams from different sheep breeds (expressed in CH50 units). Results are expressed as mean ± standard error (SE). VC(%) : Coefficients of variations.

Breed	*n*	*X ± SE*	*VC (%)*
Karakachan 1,5 г.	7	160,671 ± 4,196	6,40
2-4 г.	3	163,319 ± 5,567	4,82
4-6 г.	15	151,898 ± 17,237	16,05
Average	25	159,258 ± 3,883[a]	8,45
Tsigai 1,5 г.	8	161,312 ± 3,662	6,01
2-4 г.	3	175,788 ± 10,267	8,26
Average	11	165,260 ± 3,919[a]	7,50
Staroplaninska	3	179,067 ± 9,814	7,75
Ile de France	17	168,504 ± 3,62[a]	8,89
Suffolk	4	163,104 ± 14,637	15,54
Mouton Charollais	10	164,745 ± 6,827[a]	8,59
Trakia merino	29	131,25±5,62[a]	12,11
Pleven Blackhead	6	198,44 ± 3,54[a]	3,98

[a] *Mean values with superscript within a column differ significantly (p < 0,001) vs Ile de France.*

The complement activities (APCA) according to the breeds are presented on **Table 2.** The age-related features of complement activity in Karakachan and Tsigay rams correspond to trends observed for blood lysozyme. Significant breed-related differences were observed, the highest APCA activities being those in Pleven Blackhead rams ($p < 0.001$). The lowest complement activity was in Trakia Merino rams (131.25 ± 5.62 CH50). In the other breeds, the levels of this important innate immunity trait are intermediate (between 131.25 ± 5.62 and 198.44 ± 3.54 CH50). In our previous studies, APCA activity in sheep has also exhibited breed-related differences (20). The results of Audran

et al. (29) were similar to ours. They have studied five sheep breeds – Préalpe, Ile de France, Limousine, Mérinos d'Arles and Bouchara and found the highest complement activities in Ile de France sheep (171.4 CH50), Préalpe (168.8 CH50) and Mérinos d'Arles (167.7 CH50), but the differences among the mean APCA values in these breeds were not significant. According to them, the breeds Limousine (159.6 CH50) and Bouchara (138.1 CH50) had significant lower APCA activity.

CONCLUSIONS

1. In Karakachan rams, blood lysozyme concentration and complement activity decreased with age, whereas in Tsigay rams it increased.
2. In rams there were breed-related differences in blood lysozyme concentrations and complement activities, as are found in other animal species.

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