



STUDY OF THE EFFECTS OF BREED ON SOME INNATE IMMUNITY PARAMETERS IN RAMS

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ABSTRACT

Investigations were carried out on 26 rams from the breeds Karakachan and Copper-Red Shoumen. The non-specific immune parameters, phagocytic activity of leukocytes, bactericidal activity of phagocytes systems (oxygen-dependent and oxygen independent) and total plasma protein level were evaluated. Phagocytic response was evaluated against *S. aureus* 209-P with a certain percentage of active phagocytes (phagocytic index) and the number of absorbed particles per one phagocytic cells (phagocyte number). Phagocytosis completion index was defined as the percentage of the microbial cells that have been destroyed by phagocytes after incubation. State of the oxygen-dependent bactericidal systems of phagocytes was assessed in vitro using the NBT test, which reflects the ability of superoxide restore NBT in diphormazane. NBT test was evaluated by the degree of reduction in spontaneous and stimulated reactions, taking into account the intracellular deposits diphormazane. Our studies and results shows that the rams from the two local Bulgarian breeds have a high activity of innate immune parameters and that's may be useful and important in the breeding programs as an indicator of resistance and highly tolerance to oxidative stress.

Key words: sheep, indigenous breeds, phagocytic activity, NBT test, total plasma protein

INTRODUCTION

The problem of providing the population with high-grade and organic food is recognized as the second largest problem among the ten closest global problems of mankind, formulated by leading experts in the WHO.

The efficiency of livestock development depends on many factors. Fundamental of these is genetically based level of productivity.

It is known now that the immune system is the most sensitive indicator system that responds to changes in environmental factors. The external effects of impact is on the performance of innate immune resistance and specific immune system (6). In this connection, when acclimatizing highly productive animals, it becomes very important to study the state's natural resistance factors.

The problem of increasing non-specific resistance of farm animals is still relevant today. Breeding for improvement of natural resistance plays a primary role in the problem of disease control, as well as in the creation of animals that are suitable to the conditions of industrial technology (7, 13).

The present study was undertaken to investigate the effects of breed on PMN phagocytic activity, metabolic activity of neutrophils and on the total plasma protein level.

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Competing Interests: The authors have declared that no competing interests exist.

MATERIALS AND METHODS

Animals

The study was conducted on 18 rams from the breed Karakachan and 8 rams from the breed Copper Red Shoumen.

Blood collection

Venous blood was collected From each normal animal with Vacutainer tubes containing heparin as an anticoagulant.

Bacteria

In these experiments we used an alive culture of the *Staphylococcus aureus* strain 209P. Bacterial cultures were grown in standard media at 37 °C during 18h and then taken for our investigations.

Absorptive and metabolic activity of peripheral blood neutrophils

A modified method of Gordienko et al., (5) was used to study the absorptive phagocytosis phase with bacterial cells and oxygen-dependend metabolism of peripheral blood neutrophiles in NBT-reactions. Briefly, a18h microbial suspension of *Staphylococcus aureus* strain 209P with a density of $2 \times 10^9/cm^3$ was used. Phagocytosis was measured by the phagocytosis index (PI) - (% phagocytosis) and phagocyte number (PN). Oxidative metabolism was measured by the nitroblue tetrazolium (NBT) reduction test. In conical tubes 0.1 ml whole

heparinized blood, 0.1 ml medium 199, and 0,1 ml 1% NBT solution were mixed. The samples were incubated at 37°C for 40 minutes, blood smears were performed and Giemsa stained. We counted, at the optical microscope, the proportion of positive NBT polymorphonuclear leukocytes which had included and reduced NBT dye to nitroformazan - a dark-blue precipitate (3). Stimulated NBT test - leukocytes were stimulated with 0,1 ml suspension *St. aureus*.

Total serum proteins

The level of total serum protein was examined with the semi-automatic biochemical analyzer Screen master LIHD-113 (Hospital Diagnostic, Germany).

Statistics

The significance of differences for all parameters was estimated by Student’s T-test.

RESULTS

Phagocytic activity of the blood neutrophils in rams from two breeds is shown on Figure 1. The animals from Karakachan breed had lower phagocytic index and phagocytic number compared to the rams from Copper-Red Shoumen breed ($p < 0,01$).

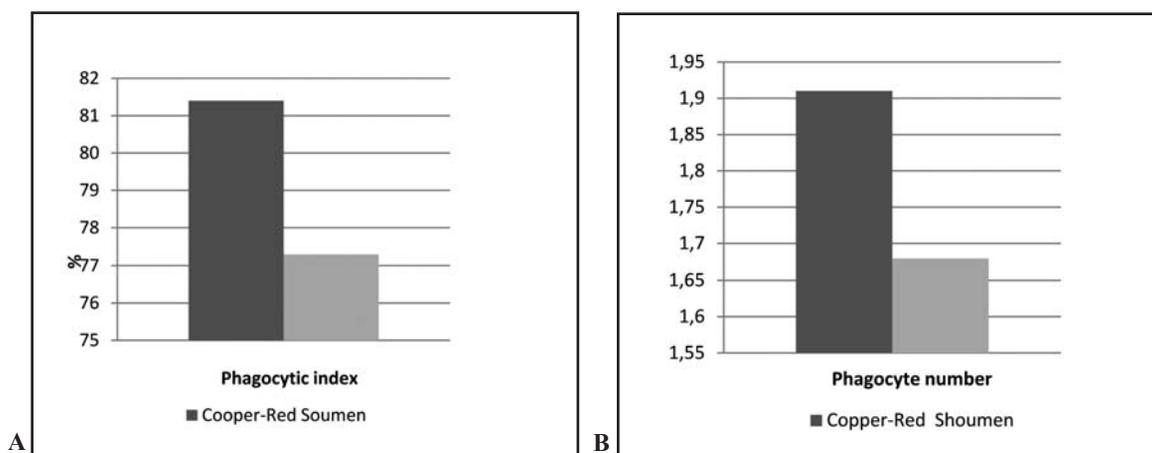


Figure 1 A, B. Phagocytic activity of neutrophils of rams

The capacity of the non-stimulated neutrophils of Cooper-Red Shumen rams to reduce NBT was found to be not significantly increased in comparison to rams from the other breed.

The stimulation of polymorphonuclear and mononuclear cells was accompanied by the significantly higher capacity of leukocytes to reduce NBT in rams from the Karakachan breed (Fig. 2)

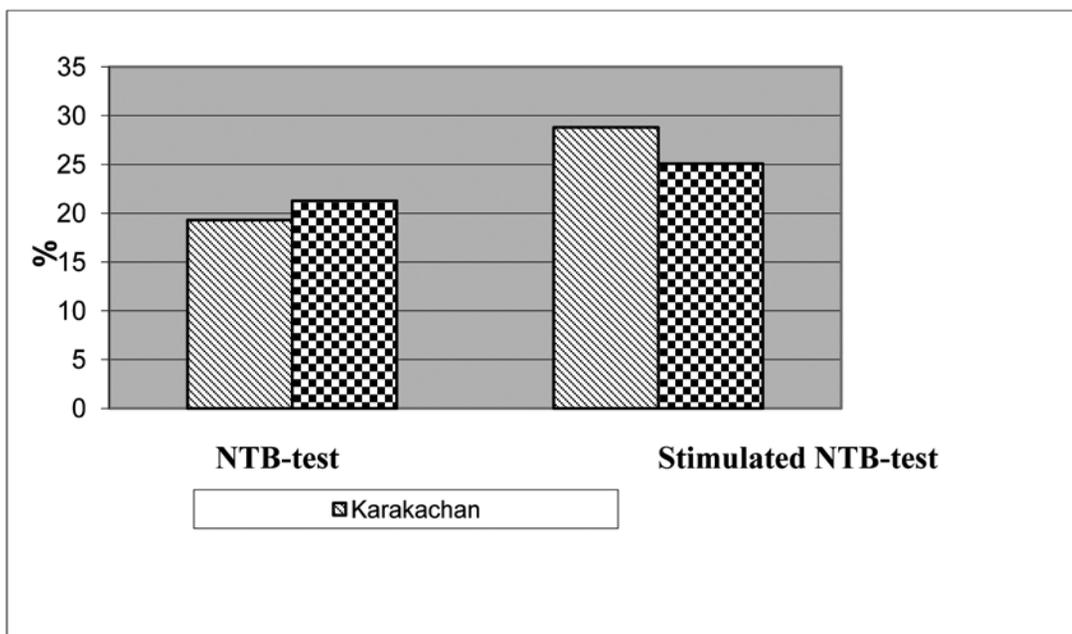


Figure 2. Spontaneous and stimulated NBT-test of neutrophils from peripheral blood of rams

Serum values of total proteins in rams from two breeds are presented in Table 1.

the normal parameters, but they were about 12% higher in the Karakachan rams, when comparing the rams .

Serum levels of the total proteins were within

Table 1. Serum values of proteins in Karakachan and Copper-Red Shoumen rams

Parameters	Normal values	Rams	
		Karakachan	Copper-Red Shoumen
Total protein g/dL	6.8-8.2	8.05±1.31	7.15±1.9

Values are mean±standard error of mean (p<0.05)

DISCUSSION

The natural resistance of the body is essential for animal life. It is caused by humoral factors and by the ability of specific cellular components - phagocytosis. These nonspecific reactions are labile and they depend on various factors, like stress and on the breed. In this regard, the study of non-specific immunity, related with the breed is of great

scientific and practical importance, if we consider the contradictory scientific results (1, 8, 12).

Phagocytosis is an important mechanism of nonspecific immunity. In the blood, polymorphonuclear and mononuclear leukocytes act as “professional” phagocytes. In our study, the pattern of phagocytic activity of the leukocytes was determined by the phagocytic number and index. As a test system we use *St.aureus* 209 P, while other

authors used different particles and crystals (2). The phagocytic activity in rams was determined by Semerjiev (11), who observed tendency for changes of PI and PN depending on the season. Variations of leukocyte phagocytic activity due to the season and breed were found out in goat kids, goats and bucks (9,10). As shown by presented results, the rams from the Cooper–Red Shumen breed had higher phagocytic activity than the animals from the Karakachan breed. In contrast Semerjiev et al. (8) observed lower values of PI and PN in rams from both breeds.

NBT test is used for studies of ability of the innate immune system to kill the bacteria. Statement of the NBT test in two versions helped us to calculate the functional reserve of cells.

The values of the stimulated NBT test characterized the activity of phagocytic cells in the presence of antigenic stimulus. They are considered as criteria for the complete phagocytosis. Spontaneous NBT-test is used to evaluate the degree of activation of intracellular phagocytic systems. Our results indicate a good phagocytic activity, which is in correlation with the results of Deptula et al. (4).

Blood proteins reflect on the state of the metabolic processes in the rams. At the level of blood total protein affect the nature of nutrition and metabolic disorders. Changes in the metabolic rate are reflection of the adaptation processes. The data obtained can serve as a rationale for identifying the productive and breed potential of the rams, as well as of the work aimed to regulate these processes.

CONCLUSION

It was concluded that there are breed-related difference in the innate immune parameters in rams. The phagocytic activity of Cooper–Red Shumen rams was higher than in the animals from Karakachan breed.

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