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# Varieties of sheep and their effects on blood lysozyme and complement activity

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#### Abstract.

Using 432 blood samples from the following sheep breeds, we were able to assess the influence of breed on blood serum lysozyme and complement activities: There are a total of 96 sheep from the following breeds: White Maritsa, Patch-faced Maritsa, Stara Zagora, Pleven Blackhead, Milk crosses, Ile de France, Trakia Merino, and Mouton Charollais. The livestock was raised on farms owned by individuals as well as by the Agricultural Institute in Stara Zagora and Karnobat. Ile de France sheep showed the greatest activity in the alternative route of complement activation (APCA) (p<0.01), whereas milk crosses raised at the Agricultural Institute - Stara Zagora had the lowest activity. In contrast, the amounts of blood lysozyme were found to be lowest in White Maritsa and Ile de France sheep and greatest in milk crosses (p<0.01).

Keywords: lysozyme, complement, sheep breeds

#### Introduction

According to many studies (Arsov et al., 1979; Ado and Mayanski 1983; Kolivanova 1987; Kishko and Ganova 1989; Zyczko and Zyczko, 1998), the specific and non-specific immune responses are dictated by the activities of phagocytosis, complement, beta lysins, interferon, and immunoglobulins. It is possible to utilize all of these metrics to biologically assess the state of the systemic immune system. One of the earliest and most essential systemic defensive systems against invaders carrying alien genetic material is complement. With nine main components numbered C1 through C9, the complement enzyme cascade incorporates almost twenty serum fractions. Its key role in systemic natural defense is supported by the fact that the complement system might function either alone or in conjunction with phagocytosis. In the absence of antibodies or when antibodies are attached to microbial cells, the complement system might be triggered by carbohydrate compounds produced by microbes. A link between the two types of immune responses, innate and acquired, is therefore provided by it. The lysis of erythrocytes, bacterial cells, viruses, virus-infected cells, neoplastic cells, and so on are among complement's protective roles (Lie, 1985; Tanchev, 2006).

members of the animal and human species. The lytic, cationic, and hydrophobic features of lysozyme are responsible for its bactericide activity against Gram-positive (Aliev et al., 1973; Buharin and Vasilev, 1974; Blotskii et al., 1976) and certain Gram-negative (Angelo, 1965; Huang et al., 1999) bacteria and viruses. Cattle, horses, pigs, rabbits, sheep, and goats have blood lysozyme variations that depend on breed, age, and species (Emelyanenko, 1977; Lie, 1980; Lie and Solbu, 1983; Sotirov, 2006; Blotskii et al., 1976; Arsov et al., 1979; Sotirov, 1991; Zyczko and Zyczko, 1998; Γanchev, 2006; Bivolarski and Sotirov, 2001; Sotirov, 2006; and Semerdjiev et al., 2010).

The studies that were referenced include data about the type of animals involved, as well as serum lysozyme concentrations and complement activity. These two criteria varied among breeds, according to the majority of writers. Our research were primarily motivated by the absence of thorough knowledge about the



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association between breed and these elements of humoral innate immunity, as well as by their relevance.

#### Material and methods

Muller, 1983; Emelyanenko, 1985; Kulberg, 1985) found that it has properties comparable to immunoglobulins, including as precipitation, phagocytosis, and C3 complement component binding. For the summer of 2010, it was a blood system. In a study conducted by Kulberg (1985), Andonova and Goundasheva (2007), Pleven Blackhead 2007, and others, the blood complement and lysozyme serum proteins were examined in sheep and rams from Mouton. The complement activates through three pathways: classical, alternative, and Charollais, Ile de France, Trakia Merino breeds, milk crosses, and White lectin pathways. Include Stara Zagora breeds produced by the Stara Agricultural Institute Breed, age, Zagora, the Agricultural Institute-Karnabat, and private farms all have typical complement levels. There were a total of 432 samples taken from cattle, with 48 samples from each breed. The results include information on the season and their physiological states. Blood samples were taken from sheep using disposable needles in simple vacutainers after fixation, as well as from pigs (Emelyanenko, 1977; Sotirov et al., 2007; Canfield, 1963; Canfield and Liu, 1965; Γanchev, 2006; Sotirov, 1991). Goats were kept in cooling bags at 6-7 °C while blood was collected (Bivolarski and Sotirov, 2001; Sotirov et al., 2005). What the alternative and poultry activities are (Semerdjiev et al., 2008; Sotirov et al., 1989). APCA was used to ascertain the route of complement activation.

Table 1. Blood APCA activity in studied breeds

Breed	n	Mean ± SEM	VC

20 ml suspension of 24-hour culture of Micrococcus lysodeicticus at

67°C. The mixture was poured out in 14-cm Petri dish. After solidifying at room temperature, thirty-two 5-mm wells were madewith a special device. Fifty microliters of undiluted sera were pipettedin each well. Eight standard lysozyme dilutions (from 0.025 to 3.125μg/ml) were prepared and pipette in the wells. The samples were incubated for 20 hours at 37°C and lytic zone diameters were measured. The final lysozyme concentrations were calculated by special software developed at the Trakia University.

Data were processed by one-way analysis of variance (ANOVA) with the fixed effect model using Data analysis tool pack, Microsoft Excel 2010, Microsoft Corporation Ltd.

**Table 2.** Serum lysozyme concentrations in studiedsheep breeds

Breed	n	Mean ± SEM	VC
Mouton Charollais	48	0.28 ± 0.02	55.38
Ile de France	48	$0.16 \pm 0.02$	75.15
Trakia Merino	48	$0.20 \pm 0.01$	49.52
Milk crosses	48	0.76 ± 0.11*	97.20
White Maritsa	48	$0.13 \pm 0.01$	31.90
Patch-faced Maritsa	48	$0.29 \pm 0.02$	55.27
Karnobat local	48	$0.17 \pm 0.01$	40.95
Pleven Blackhead	48	$0.46 \pm 0.09$	132.77
Stara Zagora	48	$0.71 \pm 0.43^*$	419.61

<sup>\* -</sup> p<0,05



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there were only minor differences across the groups. Given the role of innate immunity in natural selection, this may suggest that breed had a substantial role in determining this parameter. Surprisingly, the breeds with the lowest blood serum lysozyme concentrations were the ones with the greatest complement activity: Isle de France and White protection. Maritsa. This is because the complement system plays a crucial role in maintaining systemic homeostasis. Concurrently, there is evidence that complement activity varies among breeds; specifically, milk crosses between breeds that have the lowest

The greatest lysozyme levels were observed in rams with medium APCA activity (Sotirov et al., 2006). A functional ram's results could be explained by factors such as the breed (159.258±3.883 CH50), the different levels of 169.085±2.883 CH50, and the lowest activity in Karakachan, which are influenced by external and heritability factors. The highest level of activity was found in Mouton Charollais (203.949±5.544 CH50). The study conducted by Semerdjiev et al. (2008) found that goats' increased complement activity in the winter, in contrast to their high serum lysozyme concentrations, acts as a compensates for summer season. This demonstrated that seasonal factors also influencedSuch a negative correlation did not exist for this measure, notwithstanding these recommendations. There were three breeds where variations in APCA activity were noted. So, it's safe to believe that Wambura et al. (1998) reported on the Zebu breeds. Researchers found that three different Zebu breeds' tick resistance and complement system did not inherit together or were very weak in purebred Zebus due to genetic crosses with Friesian cattle (Irwin and Wilson, 1989; Kulberg, 1985). The with substantially greater complement activity than crosses. Serum lysozyme coefficients of variation ranging from 31.90% to 419.61% highlight the significant complement activity in the Zebu breeds investigated. Crucial for the development of ectoparasite resistance in milk crosses. The level of variety is rather considerable. The strong association between parasite and complement activity, which was postulated to explain the high rates of genetic infection, may have contributed to this. Variation in these sheep was due to the changing seasons. Sotirov (2006) also found variances in purebred horses, which likely arose from the entrance of breeders; in this case, the increased activity was associated with the animals' temperaments and types.variety of genes. Some breeds' very high phenotypic diversity was Table 2 displays the serum lysozyme concentrations in the sheep breeds that were investigated. These concentrations were rather high because of multiple animals. There was a significant increase in blood lysozyme levels in concentrations. Despite the polygenic control of the Stara Zagora breed and this milk cross, the primary gene in these animals was shown to be dominant at 0.71±0.43 µg/ml and 0.76±0.11µg/ml, respectively, with a p-value less than 0.05. In a homozygous condition, the levels were at their lowest. Other studies (Irwin and Wilson, 1989) also found the same thing when it came to White Maritsa and Ile de France sheep (0.13±0.01). They reported that there were

in ruminants, this enzyme is controlled by a minimum of ten genes. The corresponding work by Bivolarski B and Sotirov L in 2001. There seems to be a significant level of non-specific resistance in sheep, according to seasonal examinations on this parameter's values in several of the breeds that have been investigated. Bulgarian level of uniformity, even though there has been no deliberate selection for this characteristic Jurnal of Veterinary Medicine, (suppl. 1), 7-12. has been carried out.I.A. Blotskii, V.K. Shiliakin, NY Tereshchenko, and AI. Kundrukov The theory that serum lysozyme was a crucial component in 1976. Discovered lizard in the flesh during leptospirosis. Veterinariya, 4, 55—innate immunity, which plays a significant role in the accumulation of systemic antibodies 56.

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agalactiae. Also, the reference to Canfield RE, 1963 was inferred. (Shabanov et al., lysozyme. Journal of Biological Chemistry, 238:2698-707. 1973) The amino acid sequence of egg white is one of the explanations for the chronicization of the condition. Someone else discovered that 10% of Canfield RE and Liu AK, 1965 was discovered by Mutovin and Mamatov in 1978. Low levels of lysozyme (muramidase) were found in the disulfide bonds of sheep who did not have egg white mastitis or sheep that had subclinical mastitis. Published in the Journal of Biological Chemistry in 1997 and 2002, the article discusses serum lysozyme.

Blood lysozyme concentrations vary with breed. In 1985, Emelyanenko published articles. Problems of Veterinary Immunology. findings in cattle (Meyer et al., 1981; Seifert, 1983). On pages 40–46 of the Moscow-based

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Lee Huang S, Sun Y, Kung H, Blithe D, and Chen HC, 1999 found that this occurred at a low frequency in populations. extraordinarily lofty personal principles. Then, after a while, Lie and Solbu Research using beta-core (1983) and lysozyme as anti-HIV components found that lysozyme activity in human chorionic gonadotropin preparations in Norwegian Red cattle was significant. It was likely passed down in a straightforward Mendelian fashion and was reported in the Proceedings of the National Academy of Sciences,

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prevalence of the dominant gene responsible with elevated lysozyme levels From 1989 by Irwin DM and Wilson AC. The percentage of multiple cDNA sequences was 6%. It may be the evolution of bovine stomach lysozyme, according to this facts and our findings. The primary gene responsible for elevated blood lysozyme levels was identified in a study conducted by Kishko YG and Ganova LA in 1989, according to the Journal of Biological Chemistry, namely in breeds that exhibit a high degree of phenotypic variability (264, 11387-11393). Controlling the bactericidal effect in a homozygous pattern. Interferon, which has a high blood lysozyme level, might be employed for animal selection and to increase the phagocytic activity of blood in nursing piglets (Sotirov, 2006). Proceedings of the Republic Kiev. Seminar. any relevant study helps to fully understand serum lysozyme fluctuations It was 1987, Kolivanova GE. The phagocytic activity of rabbit leukocytes in experimental fusario- and T-2-toxicosis is influenced by factors such as breed, species, age, gender, and production of pig blood. On the flip side, the procedures type provides insight on the effects of lysozyme on production, particularly on animals' resistance to microbes, and on the increase of systemic resistance in animals (27-31). In 1985, Kulberg AY published... Medical immunology. Moskva, 178–175: diseases.In 1980, Lie O. Cattle serum lysozyme activity varies according to genetics. The citation is from the Acta Veterinaria Scandinavica, volume 21, pages 448– 450.

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### Conclusion

The activity of the alternative route of complement activation (APCA) was found to be greatest in milk crosses (p<0.01), and lowest in Ile de France and White Maritsa sheep. Milk crosses and Stara Zagora sheep had the greatest blood lysozyme contents (p<0.01), whereas White Maritsa sheep had the lowest.

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