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SERUM LYSOZYME CONCENTRATIONS IN DIFFERENT SHEEP BREEDS

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Summary

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Four sheep breeds from 3 productive types were included in the study: milk type crossings: Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Black-headed Pleven breed (118 female and 14 male); merino type sheep from the Trakia merino breed (80 female and 9 male); meat type: Ile-de-France breed (107 female) and Charollais breed (107 female). Milk-type lambs were also included (10 males and 19 females). The highest lysozyme concentrations were observed in milk type rams, the differences being significant vs the other breeds ($P<0.01$). Then, lysozyme levels were ranked as followed in descending order: Charollais, Trakia merino sheep, milk type crossings, Trakia merino rams and Ile-de-France sheep. Significantly lower levels ($P<0.01$) were determined in milk-type male and female lambs compared to adult animals from the same type. The data were indicative about a considerable breed- and age-dependent variations in blood lysozyme concentrations. Moreover, apart the inter-breed variations, intra-breed ones were significant, evidenced by the coefficients of variations in Trakia merino sheep (3.34%) and milk type rams (up to 91.49%).

Key words: breed-dependent variations, lysozyme, sheep

INTRODUCTION

Lysozyme is a primary humoral factor of innate immunity in both animals and humans. It is known to be effective against Gram-positive bacteria (Aliev, 1973; Buharin & Vasilev, 1974; Blotskyi, 1976) and some viruses (Angelo, 1965; Lee-Huang *et al.*, 1999). Considerable breed-related differences in lysozyme activity were reported in swine, turkeys (Sotirov, 1991; Sotirov *et al.*, 1998) and cattle (Kadimov *et al.*, 1983; Lie, 1980).

The significance of this important humoral factor of innate immunity, the breed-related differences in some animal species and the incomplete information with this regard motivated us to investigate its blood serum concentration in some sheep breeds, reared in Bulgaria.

MATERIALS AND METHODS

The experiment was performed in the sheep breeding farm of the Scientific and Research Institute of Cattle and Sheep Breeding, Stara Zagora. Four sheep breeds from 3 different productive types were used:

- Milk type crossings: 118 sheep and 14 rams - Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Black-headed Pleven crossings;
- Merino type: 80 sheep and 9 rams

from the Trakia Merino breed (TM);

- Meat type: 107 sheep from the Ile-de-France breed and 107 sheep and 6 rams from the Charollais breed;
- Milk type lambs: 10 male and 19 female.

By the beginning of the experiment, the sheep and the rams were at the age of 2–3 years. They were housed in separate premises. Blood for analysis was sampled from *v. jugularis* in 10 mL tubes. The blood was allowed to clot for one hour at room temperature (25 ^{o}C) and the samples were centrifuged at 4000 rpm for 10 min.

Blood serum lysozyme concentrations were determined according to the method of Lie (1985). Twenty mL of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na_2HPO_4 and NaH_2PO_4, pH = 6.2) were mixed with 20 mL suspension of 24 h culture of *Micrococcus lysodeicticus* at 67 ^{o}C. This mixture was poured out in Petri's dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera per 1 well were poured out in 24 wells. Eight standard dilutions (from 0.025 to 3.125 μg/mL) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were also used in the same amount per well in 8 wells. The samples were incubated for 20 h at 37 ^{o}C and lytic diameters were measured. The final lysozyme concentrations were calculated by a special software, developed in the Trakia University.

Data were analysed using the fixed effect MANOVA model (STATISTICA, Statsoft Inc. USA). Difference between means were tested using the t-test.

RESULTS AND DISCUSSION

The results of the experiments are presented in Table 1. The highest lysozyme concentrations were observed in milk type rams and they were statistically significantly higher vs the concentrations in the other breeds ($P<0.01$). Then followed, in descending order the Charollais, TM sheep, milk type sheep, TM rams and Ile-de-France breeds. Significantly lower lysozyme levels ($P<0.01$) were found out in milk type lambs (both genders) vs adult milk type sheep. The data were indicative about important breed- and age-related variations in blood lysozyme values. The variations were also significant within breeds as well, manifested by the coefficient of variations that ranged between 33.34% in TM sheep to 91.49% in milk type rams. These variations in lysozyme concentrations should be interpreted as a fact of importance. First, it evidenced a various degree of equalization within each of studied breeds. The milk type sheep was the most variable and that could be easily explained by the fact that the animals were crossings of forementioned breeds. Among purebred sheep, the highest varibility was that of the Ile-de-France breed. Perhaps, this is related to the use of imported rams that has increased the genotypic and phenotypic diversity in the population. The other breeds were relatively equal with regard to this parameter, probably due to the performed purposeful selection for economically important traits. On the second place, the considerable phenotypic diversity is an opportunity for an intentional selection for lysozyme concentrations that would result in increase in the natural resistance of sheep against infectious and parasitic diseases.

The data in Table 1 could be compared to similar results reported in a previous study of ours (Sotirov *et al.,* 1997) on nine sheep breeds: Ile-de-France, TM, Northeastern Bulgarian merino (NEBM), Tsigay, Caucasian, South Corriedale,

Table 1. Serum lysozyme concentrations in different sheep breeds (μg/mL)

Breeds	Mean ± SEM	CV%	n
Milk type sheep	0.22 ± 0.013	67.56	118
Milk type rams	1.36 ± 0.35**	91.49	14
Trakia merino sheep	0.23 ± 0.01	42.73	80
Trakia merino rams	0.17 ± 0.02	33.34	9
Ile de France sheep	0.13 ± 0.01	84.44	107
Charollais sheep	0.24 ± 0.01	42.24	107
Charollais rams	0.35 ± 0.1**	65.32	6
Male milk type weaned lambs	0.10 ± 0.01	40.25	10
Female milk type weaned lambs	0.13 ± 0.02	64.58	19

** P<0.01 vs all other breeds.

Karakachan and Romanov. Each breed included various categories of animals: rams, sheep, male and female lambs except for the Romanov breed, where only sheep were studied. The comparison shows similar results for the Ile-de-France and TM breeds. For instance, the lysozyme levels in Ile-de-France sheep during the first experiment was 0.14 ± 0.01, and during the second – 0.13 ± 0.01 μg/mL. The same was valid for for the TM breed: 0.22 ± 0.01 μg/mL and 0.23 ± 0.01 μg/mL for the 1st and the 2nd experiment respectively. The data obtained in rams were also similar – 0.13 ± 0.02 μg/mL (experiment I) and 0.17 ± 0.02 μg/mL (experiment II). The lack of statistically significant difference in the average lysozyme concentrations in the two trials, differing in both time (years 1996 and 2003) and place showed that a high homogeny of this trait has been achieved despite that an intentional selection was not done. The average concentration of lysozyme in those two breeds was however low and this shows that the lack of control upon this important factor of natural immunity results in elimination of individuals possessing a high congenital potential for its synthesis.

The comparison of all breeds studied in the course of both experiments demonstrates that the highest lysozyme concentrations were those in ewes and gimmers from the South Corriedale breed, being significantly higher than all other breeds and types of sheep (P<0.001) followed by North Caucasian sheep. The differences among the other breeds were also considerable (P< 0.05 – 0.001). The data of breeds subject to the present study did not show a significant age- or gender-related variations unlike the deviations observed in South Corriedale and Caucassian lambs, that exhibited a high lysozyme activity (Sotirov *et al.*, 1997). This is an evidence that this valuable trait is stably transmitted from parents to the progeny. At the same time, male lambs possessed a higher lysozyme activity than females although the differences were not statistically significant. Significant gender-related differences were reported in Tsigay sheep and rams but not in other studied breeds (Sotirov *et al.*, 1997).

The phenotypic diversity in the present study was lower than that observed in the breeds South Corriedale (0.049 – 99.92 μg/mL in ewes and 0.069 – 7.43 μg/mL in gimmers), North Caucasian (0.049–14.83

µg/mL) and Caucasian (0.049 – 3.122 µg/mL) (Sotirov *et al.*, 1997). This fact is very important because this high phenotypic diversity is achieved in a new breed (South Corriedale) and the other two are involved in a different extent in its creation. Therefore, the most appropriate moment of selection for this trait is when the breed is newly produced and the individuals with high serum lysozyme concentrations are not yet eliminated. This information is important because, according to unpublished data of ours, there is a low or no correlation between serum lysozyme activy and some productive traits as milk productivity, live weight, fleece weight and fertility (r from 0 to –0.17). This permits an individual selection for serum lysozyme concentrations, thus achieving a high resistance of animals against Gram-positive bacteria and some viruses (Buharin & Vasilev, 1974). This approach is supported by the studies of Mamatov (1971) and Burgele *et al.* (1973), Aliev *et al.* (1973) showing that following infection in sheep from 4 breeds – Karabakh, Soviet merino, Red Samukh and Azerbaijan merino with a virulent *Brucella melitensis* strain , lysozyme levels decreased by 23.3% in Karabakh and Soviet merino sheep whereas in the other two remained unchanged. In this case, lysozyme serves as an indicator of resistance in the Red Samukh and Azerbaijan merino breeds.

The hypothesis for lysozyme as a humoral factor of natural resistance participating actively in the formation of systemic defense against bacterial pathogens is confirmed in other studies as well. It is reported that lysozyme concentrations increased during infection of sheep with *Mycoplasma agalactiae.* It is presumed that this is probably one of the causes for the chronic course of the infection (Shabanov *et al.*, 1973). Mutovin & Mamatov (1978) found out that in 10% of sheep, free from mastitis, lysozyme levels were low and 70% of sheep with subclinical mastitis were also with low lysozyme activities.

Breed-related differences in lysozyme levels were also observed in cattle (Meyer *et al.*, 1981; Seifert, 1983; Kadimov *et al.*, 1983).

On the basis of a rich experimental material (294 bulls from 19 lines), Lie (1980) supposed that serum lysozyme activity was influenced by at least two genetic levels: a. according to the observed heretability coefficient ($h^2 = 0.27$) he assumed that lysozyme activity was regulated polygenically and b. an existence of a gene with a relatively low frequency within the population, responsible for the exceptionally high levels in some individuals is also supposed. Later, Lie & Solbu (1983) concluded that the activity of lysozyme in Red Norwegian Cattle was probably inherited as a simple Mendelian sign and that the frequency of the dominant gene determining the high enzymatic concentration was 6%. In this breed Lie (1985) observed a high correlation ($r = 0.63$; $P<0.01$) between serum and colostrum lysozyme concentrations. According to him, this genetical bond was highly influenced by the presence of the dominant gene, described earlier (Lie & Solbu, 1983).

On the basis of cited data and our own results (considered as preliminary), we assume that at least in breeds with a high phenotypic diversity of the sign, a so-called primary gene determining high lysozyme levels was encountered in a homozygous state.This hypothesis does not rule out the polygenic pattern of lysozyme activity inheritance. The supposition is also supported by the studies of Irwin & Wilson (1989) and Irwin *et al.* (1989),

reporting the existence of at least 10 genes coding the synthesis of the enzyme in ruminants.

Using RFLP, Sigurdardottir *et al.* (1990) found out three loci coding lysozyme in cattle. They did not, however, observed any relationship between those loci and the productive traits of bulls with regard to the resistance to diseases and milk productivity.

Data evidencing that serum lysozyme levels were under a genetic control are also found out in studies, reporting about alleles existing within one microsatellite locus and controlling the lysozyme synthesis in macrophages of two half-sib families of Polish Black and White Lowland Cattle and about the correlation of lysozyme activity with alleles inherited from parents. The microsatellite is related to a locus of high lysozyme activity estimated to be 70-95% of the phenotypic diversity of the lysozyme activity in both groups (Pareek *et al.*, 1998).

It is shown that among cattle, there are animals with a high lysozyme activity. Their incidence is howevere exceptionally low – 2 bulls and several cows among 10000 animals. Their progeny, regardless of gender, age or other factors, inherited a very high or normal lysozyme activity. Those data were used by authors for development of an experimental breeding programme that aimed to provide information about the existance of a link between lysozyme polymorphism and the natural resistance in cattle (Walawski *et al.*, 1999).

In swine, heretability coefficients (h^2=0.095 and 0.305) were observed in purebred and crossbred pigs respectively (Zyczko & Zyczko, 1998). It is stated that swine possess a gene, coding lysozyme synthesis that is expressed in the stomach as well as in all other tissues. The size of this gene is similar to the size of genes coding lysozyme in the other mammalian species. All those genes (both inside and outside the stomach) use the same promotor (Mey Yu & Irwin, 1996). In cloven-hoofed ruminants, 4 out of the about 10 genes coding lysozyme are expressed in the stomach. It is accepted that most of lysozyme gene duplicates appeared some 40–50 millions of years ago, prior to the divergence of cows and sheep (Wen & Irwin, 1999).

On the basis of the similarity between structures of lysozyme and histones, Steinrauf *et al.* (1999) supposed that lysozyme, conjugated to nucleic acids, could play other biological functions as well. According to authors, perhaps this fact could explain why Lee Huang *et al.* (1999) call lysozyme a protein killer in AIDS virus control. This could open a road to new studies in the treatment of this disease.

The opinion about the existence of a genetic control on lysozyme synthesis is further confirmed in 4 synthetic lines of chickens (CSML, WSML, CSFL и NNL), selected on the basis of high serum lysozyme levels, that after crossbreeding yielded broilers with a high heterosis effect by respect to lysozyme and thus, a higher innate resistance in chickens against pathogens was achieved (Nath *et al.*, 2002).

Taking into account all that information, it could be stated that in mammals, the genetic organization and structure of genes coding lysozyme are similar. The similarities among them, in general, are that they are relatively small and structured in 4 exons and 3 introns (Irwin *et al.*, 1996). For instance, human lysozyme gene is built of 5856 kb, divided into 4 exons and 3 introns and is homologous to chicken lysozyme gene and the gene, co-

ding human lactalbumin. Human and chicken lysozyme genes differ mainly by the size of their introns and the 3′ non-coding region. Four repeating elements are found out in human lysozyme gene, one in each intron and one in the 4th exon. The human lysozyme gene is located on chromosome 12 (Peters *et al.*, 1989). In geese, the gene is not homologous to that in chickens and exhibits a completely different exon-intron organization. Its tissue expression matches only partially (Nakano & Graft, 1991).

CONCLUSIONS

Our studies showed that there were statistically significant inter-breed differences in sheep with regard to blood serum lysozyme concentrations. This is accompanied by a considerable phenotypic variations of the studied sign within the different breeds. The age also influenced blood serum lysozyme activity in sheep.

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