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Original Contribution

EFFECT OF C3 GENOTYPES ON SERUM LYSOZYME CONCENTRATIONS IN VARIOUS SHEEP BREEDS

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ABSTRACT

The aim of this investigation was to study the effect of C3 complement component genotypes in different sheep breeds on blood serum lysozyme concentrations. The sheep included in this experiment were from 3 productive types: A. Milk type crossings: 118 sheep - Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven breed; B. Merino type: 72 sheep from the Trakia merino breed and C. Meat type: 107 sheep from the Ile-de-France breed and 87 sheep from the Charollais breed. Five C3 genotypes were found: SS, FF, FS, F7S and F5S, formed by two allele genes – $C3^F$and $C3^S$. The SS genotype was characterized by the highest significant lysozyme concentrations ($p<0.01$). The other genotypes exhibited lower values, although lysozyme levels were within the reference range for sheep. The variations in lysozyme levels among the C3 genotypes in the other sheep breeds were not significant.

Key words: lysozyme; C3 genotypes; sheep breeds

1. INTRODUCTION

Lysozyme is a primary humoral factor of innate immunity in both animals and humans. It is known to be effective against some bacteria (1, 2, 3) and viruses (4, 5)

Considerable breed-related differences have been reported in sheep (6), swine (7) and cattle (8, 9). Stoyanchev et al. (10) observed that the major histocompatibility complex (MCH) in chickens influences significantly blood serum lysozyme levels.

All these facts motivated our investigation on the influence of C3 complement component genotypes in different sheep breeds on blood serum lysozyme concentrations.

2. MATERIALS AND METHODS

2.1. Animals

The sheep included in this study were from 3 productive types:

A. Milk type crossings: 118 sheep - Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven breed.

B. Merino type: 72 sheep from the Trakia merino breed.

C. Meat type: 107 sheep from the Ile-de-France breed and 87 sheep from the Charollais breed.

At the beginning of the experiment, the sheep were aged 2-3 years. They were housed in separate premises. Blood for analysis was collected from *v. jugularis* and stored in 10 ml tubes. The blood was allowed to clot for one hour at room temperature (25°C) and then subsequently centrifuged for 10 min at 4000 rpm.

2.2. Methods

Blood serum lysozyme concentrations were determined according to the method of Lie (11). Twenty milliliter of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na_2HPO_4 and NaH_2PO_4, pH = 6.2) was mixed with 20 ml suspension of 24-hour culture of *Micrococcus lysodeicticus* at 67°C. This mixture was poured out in Petri dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm

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diameter). Fifty microliter of undiluted sera was poured out in each well. Eight standard dilutions (from 0,025 to 3,125 μg/ml) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity as well and they served as control. The samples were incubated at 37°C for 20 hours and lytic diameters were measured. The final lysozyme levels were calculated using special computer program developed in the Trakia University.

The polymorphism of C3 complement component was determined according to the method of Teisberg (12) stated below:

1. Buffers

a. Stock solutions for gels – 5.5-diethylbarbituric acid sodium salt (Diemal Na, Loba-Chemie, Austria) 0.0230 M; 5.5-diethylbarbituric acid (Reanal, Hungary) 0.0037 M; Calcium-L(+)- lactate (Fluka AG, Switzerland) 0.0009 M; pH=8.6.

b. Stock solution for tray buffer – 5.5-diethylbarbituric acid sodium salt (Diemal Na, Loba-Chemie, Austria) 0.061 M; 5.5-diethylbarbituric acid (Reanal, Hungary) 0.01 M; Calcium-L(+)- lactate (Fluka AG, Switzerland) 0.0018 M; pH=8.6.

The first buffer was used for preparation of agarose gel (1%) where serum samples were applied. After their absorption, the electrophoresis was carried out at 20 V/cm for about 2.5 h. It was stained for 1 min with Amido Black 10 B and destained overnight.

2. Destaining solution: ethanol, distilled water and glacial acetic acid (5:5:1) were mixed (for example, 1 l destaining solution contains 450 ml ethanol, 450 ml distilled water and 100 ml glacial acetic acid). Then 2 tablespoons of active charcoal powder were added to allow the manifold use of the destaining solution.

3. Staining solution – 1% Amido black 10B was added to 1 l destaining solution (without active charcoal ethanol), shaken well and left overnight. If necessary it could be filtered after a week.

3. RESULTS

The results on milk type sheep are presented on **Table 1**. It shows that the principal genotypes were five: SS, FF, FS, F7S and F5S, formed by two allele genes – $C3^F$and $C3^S$.

In this breed type, the SS genotype was characterized by the highest significant lysozyme concentrations ($p<0.01$). The other genotypes exhibited lower values, although lysozyme levels were within the reference range for sheep. The variations in lysozyme levels among the C3 genotypes in the other sheep breeds were not significant **(Tables 2, 3, 4).**

Table 1. Effect of different C3 genotypes on serum lysozyme levels in milk type sheep (μg/ml)

Genotype	Mean±SE	VC(%)	n
SS	0,25±0,02**	65,04	44
FF	0,19±0,04	63,61	9
FS	0.19±0,02	75,78	39
F7S	0,24±0,04	63,28	18
F5S	0,17±0,02	27,14	8

***p<0,01 vs F5S, FS and FF.*

Table 2. Effect of different C3 genotypes on serum lysozyme levels in sheep of Ile de France breed (μg/ml)

Genotype	Mean±SE	VC(%)	n
SS	0,11±0,007	36,79	38
FF	0,11±0,02	48,32	9
FS	0,11±0,01	46,79	26
F7S	0,16±0,03	113,56	30
F5S	0,15±0,05	62,96	4

Table 3. Effect of different C3 genotypes on serum lysozyme levels in sheep of Charollais breed (μg/ml).

Genotype	Mean±SE	VC(%)	n
SS	0,22±0,02	39,48	19
FF	0,26±0,03	49,46	18
FS	0,23±0,01	23,71	21
F7S	0,23±0,02	29,37	14
F5S	0,24±0,01	21,00	15

Table 4. Effect of different C3 genotypes on serum lysozyme levels in sheep of Trakia merino breed (μg/ml).

Genotype	Mean±SE	VC(%)	n
SS	0,26±0,02	25,93	19
FF	0,27±0,05	61,96	13
FS	0,21±0,03	57,06	15
F7S	0,23±0,01	27,95	25

4. DISCUSSION

At the moment, the structure of the gene coding for lysozyme is known to a considerable extent (13, 14, 15, 16). From a theoretical and especially a practical point of view, it is not sufficient. It is not clear what interrelationships do exist between this gene's polymorphism and the actual synthesis of lysozyme. Finally, the latter determines whether a living organism (or an entire population) should be with a high natural resistance or not. Investigation of this could follow two approaches:

1. Through a direct selection of animals according to high lysozyme concentrations, that would, however, absolutely require the inclusion of this sign in selection programs. At this stage, selectionists are not inclined to do this.

2. Through indirect selection, i.e. looking for genetic markers that highly correlate with lysozyme concentrations. Both approaches have advantages and disadvantages. The former is more time-consuming and more expensive and would be impeded by the same problems, encountered by the selection according to traditional quantitative signs. The latter is quicker, easier and cheaper, but requires the detection of a suitable genetic marker. It is however the only problem when this approach is preferred. Our results suggested that the C3 genotypes of the complement system in sheep from various breeds had an effect on blood serum lysozyme levels. Our previous study (6) allowed us to make more definitive conclusions. It aimed at checking out whether transferrin genotypes would influence blood serum lysozyme concentrations in different ovine breeds. Four alleles were detected (Tf^A, Tf^B, Tf^C and Tf^D), that formed 10 different genotypes. The analysis of results obtained within a particular breed showed that the highest lysozyme level was that of the TfBB genotype or genotypes where the Tf^B allele was participating. The homozygous genotype TfBB was observed only in the Caucasian breed. With respect to the average lysozyme concentration, it was superior to the other genotypes within the same breed, although the differences were not significant. The explanations of this fact rest in the small population of animals with this genotype as well as in the considerable phenotypic variety of the sign. The incidence of the TfBB genotype was exceptionally low – 7.4% of all studied animals of this breed. The high average lysozyme levels in this genotype combined with the high coefficient of variation showed that the predominant number of animals was with a high individual lysozyme concentration and these could serve as a basis in a future selection according to this sign.

In the Trakia merino and Northeast merino breeds, the TfCC genotype exhibited the highest concentrations. The average values in sheep from this genotype were insignificantly higher than those in other genotypes. On the other hand, if the results are interpreted only from the point of view of genotype without considering the breed affiliation, the TfBB genotype showed the highest levels again. In this case, the differences between it and the average values in this genotype were highly significant ($p<0.001$) and that was despite the considerable phenotypic variety (CV=146.88%)! The explanation of this phenomenon could be only one: the prevailing number of animals from this genotype was with exceptionally high lysozyme concentrations.

All those facts suggested that the sheep from the TfBC heterozygous genotype should be expected to demonstrate high average lysozyme levels as well. Such values were observed in the South Corriedale breed: indeed, the sheep from this breed with the TfBC genotype were with higher levels compared to those from other genotypes, although quite insignificant. This genotype was the second after TfBB and showed higher levels versus the other genotypes ($p<0.01$). Other authors also reported a relationship between the genotype affiliation, lysozyme levels and the resistance of animals against various diseases. Stoyanchev et al. (10) stated that broiler-chickens, belonging to the $B^{21}B^{21}$ homozygous genotype were with the highest serum lysozyme and complement levels. On the other hand, Briles et al. (17), Bennejean et al. (18) and Iotova et al. (19) reported that broiler chickens from this genotype were the most resistant to the Marek disease's virus. Therefore, this high resistance of $B^{21}B^{21}$ broiler chickens to Marek's disease should inevitably be attributed to high lysozyme and complement activities.

Other authors produced evidence about various animal genotypes (determined through various polymorphic genetic systems) and resistance or susceptibility with respect to infectious and parasitic diseases. Yotsov et al. (20, 21, 22) observed that the resistance of chickens (broiler and layer types) against Marek's disease was related to the alkaline phosphatase genotype AkpSS, the cholinesterase genotypes $E_{S4}AB$ and $E_{S5}AB$ and the heterozygous hemoglobin genotype Hb^2AB. Other authors (7, 23, 24, 25, 26, 27, 28) stated that there was a relationship between various transferrin genotypes in swine, cattle, sheep and carps and resistance or susceptibility to leptospirosis in cattle, facial eczema in sheep and bronchiomycosis in carps.

In conclusion our results show a relationship between the C3 genotypes and blood serum lysozyme concentrations in sheep from various breeds.

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