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EFFECT OF C3 GENOTYPES UPON THE ACTIVITY OF THE ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION IN DIFFERENT SHEEP BREEDS

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Summary

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The effect of C3 genotypes (FF, FF1, FS, SS) upon the activity of the alternative pathway of complement activation (APCA) was studied in ewes from three different productive types: Milk type – 118 crossings of Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven sheep and 100 Blackhead Pleven sheep; Merino type – 72 Trakia Merino sheep; Meat type – 107 sheep of each of Ile-de-France and Charollais breeds. The obtained results did not suggest that C3 genotypes had a considerable impact on APCA activity in sheep.

Key words: breeds, C3 genotypes, complement, sheep

INTRODUCTION

The complement system is among the most important humoral factors of natural immunity in vertebrates. The alternative pathway of its activation has an ancient phylogenetic origin and protects both animals and humans against various pathogens – bacteria (predominantly Gram-negative), viruses, virus-infected cells, malignant cells. It could be also activated by some organic (inulin, agarose, streptokinase etc.) and inorganic (radiocontrast media) substances (Kulberg, 1985). A number of authors have reported that the total APCA activity is determined by the genetic potential of animals (Lie *et al.*, 1983; Lie, 1985, Sotirov, 1991). It is accepted that complement activity in swine is considerably influenced by swine lymphocyte antigens (SLA) genotype affiliation (Vaiman *et al.*, 1978). In men, there are a lot of studies about the polymorphism of the C3 component of the complement system and the link of some of its alleles to blood serum C3 component concentration and the susceptibility to some diseases (Dissing *et al.*, 1972; Brönnestam, 1973; Brönnestam & Cedergren, 1973, Sörensen & Dissing, 1975; Botto *et al.*, 1990).

In animals, there are several reports about the polymorphic structure of the C3 complement component (Gorman *et al.*, 1981; Kay *et al.*, 1986; Gahne & Amorena, 1987; Bowling & Dileanis, 1990), but the relationship between a definite C3 haplotype and the higher activity of classical and alternative pathways of complement activation is shown only

in Duroc, Belgian Miniature and Tai pigs (Meckchay *et al.*, 2003).

The lack of information about the relationship of C3 genotypes and APCA activity in different sheep breeds motivated the present study.

MATERIALS AND METHODS

Animals

Ewes (n=504) from 3 productive types were included in this study:

A. Milk type – Crossings (n=118) [Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven breed]; Blackhead Pleven breed (n=100); B. Merino type – Trakia Merino breed (n=72); C. Meat type – Ile-de-France breed (n=107) and Charollais breed (n=107);

By the beginning of the experiment, the sheep were at the age of 2–3 years. They were housed in separate premises. Blood for analysis was sampled in 10 mL tubes from *v. jugularis*. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min.

Methods

Determination of the alternative pathway of complement activation (APCA)

APCA was studied by the method of Sotirov (1991). For this aim we prepared veronal-veronal Na buffer. Eighty five g NaCl (High School of Biotechnology, Dimitrovgrad, Bulgaria), 3.75 g 5,5-diethylbarbituric acid sodium salt (Diemal Na, Loba-Chemie, Austria), 5.75 g 5,5-diethylbarbituric acid (Reanal, Hungary), 0.01 M EGTA (Sigma, USA), 0.008 M $MgCl_2$ (Polskie Odczynniki Chemiczne, Poland) were diluted in 2 L distilled water with pH=7.5 (the buffer must be diluted 1:5 to preparae working buffer solution before use). The working buffer solution was used for preparation of initial dilution of each serum sample by mixing 350 μL buffer with 100 μL serum in a 0.5 mL tube. Using U bottomed microplates (Flow Laboratories, UK), seven dilutions were made from these sera – 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; 20 μL diluted serum + 80 μL buffer. Another 50 μL buffer were additionally added to each well. One hundred μL of 1% rabbit erythrocyte suspension to each dilution were dropped and the sera were incubated at 37°C for 1 hour. Then, the reaction mixture was centrifuged at 1000 rpm for 3 min. The supernatants were separated and 150 μL of each supernatant were placed in flat-bottomed plates. The optical density were measured by a "Sumal-PE2" ELISA reader (Karl Zeiss, Germany) at 540 nm. The final APCA activity was calculated using special computer programmes developed in the Trakia University.

Determination of the polymorphism of C3 complement component (Teisberg, 1970)

1. Buffers 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.

2. Buffer for trays: 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.

3. Gels: the first buffer (1) was used for preparation of agarose (Agarose, Fluka AG, 05070) gel (1%) where serum samples were applied.

4. Staining solution: 10 g amido black 10B was added to 1L destaining solution (without active charcoal ethanol), shaked well and left for overnight. If necessary it could be filtered after a week.

5. Destaining solution: ethanol, distilled water and glacial acetic acid (5:5:1) were mixed (for example, 1L 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 in order to allow the manifold use of the destaining solution.

After serum absorption, the electrophoresis was carried out at 20 V/cm for about 2.5 h. The gels were stained for 1 min with Amido Black 10 B and destained for overnight.

Statistical analysis

The statistical analysis of data within each breed and milk productivity type was performed using one-way ANOVA and the following linear additive model with fixed effects:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where:

y_{ij} – the values of the jth observation of the respective level of studied trait;

μ – population mean;

α_i – the differential effect of the C3 genotype;

ε_{ij} - random error.

The coefficients of organized factor's effect (C3 genotypes) were calculated according to Plohiskyi (1970) and Snedekor & Cohran (1961).

RESULTS AND DISCUSSION

The data of the study are presented in Table 1–5. It could be seen that in some instances, the highest APCA values were observed in the SS genotype (milk crosses and Ile-de-France sheep) whereas in others, the FF1 (Charollais), FF (Trakia Merino) or the FS7 (Blackhead Pleven) genotypes were dominating. The differences between dominating and the other genotypes were usually statistically insignificant with the exception of the FF genotype from the Trakia Merino breed and the FS7 genotype from the Blackhead Pleven breed, where the differences between FF, FS7 on one hand and the other genotypes on the other within the respective breed were significant ($P<0.05–0.01$). All these results did not suggest that C3 genotypes could be used as genetic markers for performing an indirect selection in sheep with the purpose of APCA activity increase. Another proof for this are the values of the coefficient of organized fac-

Table 1. APCA activity (CH50) in milk type ewes belonging to various C3 genotypes (n=118)

Genotype	Mean ± SEM	CV (%)	n
SS	137.46 ± 3.68	17.56	44
FF	135.47 ± 6.85	14.31	9
FS	130.58 ± 3.71	17.49	39
FS7	129.19 ± 3.48	11.11	18
FS5	135.81 ± 9.08	17.69	8

Table 2. APCA activity (CH50) in Pleven Blackhead ewes belonging to various C3 genotypes (n=100)

Genotype	Mean ± SEM	CV (%)	n
SS	183.62 ± 3.55	12.24	41
FF	179.25 ± 3.36	3.25	4
FS	177.98 ± 4.39	12.59	27
FS7	193.69 ± 4.37*	10.08	21
FS5	171.22 ± 11.68	16.71	7

* $p<0.05$ vs all genotypes.

Table 3. APCA activity (CH50) in Ile-de-France ewes belonging to various C3 genotypes (n=107)

Genotype	Mean ± SEM	CV (%)	n
SS	156.00 ± 3.28	12.81	38
FF	142.65 ± 7.03	13.94	9
FS	154.38 ± 4.35	14.08	26
FS7	152.36 ± 3.89	13.78	30
FS5	144.85 ± 10.25	12.26	4

tor's effect (i.e. C3 genotypes) – $\acute{\eta}_x^2$ = 0.198 (after Plohinskyi, 1970) and $\acute{\eta}_x^2$= 0.168 (after Snedekor & Cohran, 1961).

Similar results were obtained in horses (Sotirov, 2003) showing that with regard to APCA, the C3(3,3) genotype corresponding to the FF ovine genotype, was dominating. In pigs, the highest complement activity was observed in animals belonging to the homozygous SLA-12/SAL-12 genotype unlike other genotypes (SLA-13-3/SLA-12 and SLA-13-3/SLA-13-3) (Vaiman *et al.*, 1978). Different complement activity was observed in miniature pigs from various SLA genotypes (Mallard *et al.*, 1989). In German Landrace, Belgian Landrace, British Landrace, British Large White, Swedish Large White, Ch and Duroc, the average APCA valeus also varied within a broad range. The highest activity was observed in German Landrace and in most cases it was significantly different vs the other breeds ($P<0.05–0.01$, Sotirov, 1991). The hereditary coefficient (h^2) in purebred German Landrace pigs was 0.093 for male and 0.186 for females and in crosses of German and Belgian Landrace – 0.156 (males) and 0.216 (females), respectively (Sotirov, 1991).

These results show that in crosses, the genetic diversity of the studied trait was higher, as indicated by the h^2 coefficient. Similar data were observed in 130 young bulls sons of 14 elite sires. The calculated h^2=0.69 is indicative for a relatively high genetic variety with statistically significant parental effect ($P<0.01$) (Lie *et al.*, 1983).

All cited reports evidence that the complement system is under genetic control. Additional proofs with this regard are data obtained from the study of the lysozyme, APCA and the classical

Table 4. APCA activity (CH50) in Charollais ewes belonging to various C3 genotypes (n=107)

Genotype	Mean ± SEM	CV (%)	n
SS	127.01 ± 5.77	19.29	19
FF	134.52 ± 3.98	12.19	18
FS	130.97 ± 3.99	13.65	21
FS7	126.53 ± 6.11	17.39	14
FS5	129.30 ± 6.75	19.53	15
FS10	130.29 ± 5.79	14.07	11
FF1	137.94 ± 6.27	12.87	9

Table 5. APCA activity (CH50) in Trakia merino ewes belonging to various C3 genotypes (n=72)

Genotype	Mean ± SEM	CV (%)	n
SS	152.89 ±4.10	11.39	19
FF	157.84 ±5.11**	11.22	13
FS	148.65 ±2.59	6.53	15
FS7	137.42 ±7.17	25.56	25

** P<0.01 vs FS7

pathway of complement activation (CPCA) in the progeny of a wild boar, showing that lysozyme leves was exceptionally high (2.5 ± 0.17 μg/mL) that could be explained by the high serum enzyme levels in the sire (2.4 μg/mL) (Sotirov, 1991). For APCA, there is a breakdown in progeny's levels – only 35.72 ± 7.32 CH50. It is assumed that this is due to the fact that APCA levels of the boar were similarly low (41.70 CH50) (Sotirov, 1991). CPCA concentrations in the progeny were again high (366.09 ± 48.46 CH50) probably because of the high CPCA activity of the sire (258.80 CH50, Sotirov, 1991). Following out the changes of these three signs in the progeny of the boar and comparing them to its own values, it could be definitely stated that these parallel changes were only possible if the effect of sire's heredity was involved.

The role of the hereditary potential should be accounted for as an option for increasing complement concentrations in producing new breeds by hybridization of cultured breeds with biologically similar species that are highly resistant to infectious or parasitic diseases. An example is reported in three zebu breeds (Meru, Mbullu, and Iringa Red) and their crosses with Friesian cattle by respect to their resistance against ticks by Wambura *et al.* (1998). It was observed that purebred zebu were more resistant to parasites compared to crosses and that the activity of complement in purebred zebu was higher than that in crosses. It was assumed that the highest complement activity was important for the higher resistance of the three zebu breeds. This suggestion was supported by the statistically significant negative correlation between the degree of

infection with parasites and the complement levels.

In conclusion, on the basis of obtained results, it could be assumed that unlike horses and swine, C3 genotypes had no significant effect upon APCA activities in sheep.

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