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DYNAMIC STUDIES IN BROILER CHICKEN NATURAL IMMUNE FACTORS

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Abstract

Natural immune factors are the first defense mechanism against variety of pathogenic agents. Both the blood serum lysozyme and alternative pathway of complement activation play significant role in stock animals′ welfare. In this study we decided to investigate the dynamics of the aforementioned humoral factors among two of the most popular broiler chicken hybrids – Ross and Cobb. Both hybrids show relatively high concentrations of blood serum lysozyme during the first week of life (Ross-7,79 and Cobb-11,06 mg/L). Which could be explained by the small amounts of yolk sack, left from the egg embryo. During the second week the levels of blood serum lysozyme lowers dramatically. Through the next weeks the concentration of blood lysozyme increases gradually among both hybrids. The comparative analysis show faster and higher levels in favor of the Ross hybrid. The dynamics of the alternative pathway of complement activation (APCA) were relatively similar. Both hybrids have moderate levels of APCA activity through the first week (Ross-325,32 CH50 and Cobb – 346,94 CH50), which lowers in the second week of live and grows gradually trough the life of the bird. Compared with the Cobb, the Ross hybrid exhibited higher levels of APCA, which gives these birds better protection against pathogens. Similar results for both factors of interest, were obtained among the hybrids′ parent flocks. The results of our experiment unambiguously show better humoral immunity protection in favor of the Ross hybrid.

Key words: *Chicken hybrids, Complement system, Lysozyme.*

INTRODUCTION

Nowadays stock animals are bred for fast growth and maximal production. The huge populations reared at relatively small spaces increase the risk of spontaneous infections. Despite the use of different vaccine programs, the mortality rates in chicken farms are relatively high. Innate immune factors such as phagocytosis, complement, beta lysins, lysozyme, interferon etc. play significant role in animal′s protection against variety of pathogens. Blood serum lysozyme is an important factor of the humoral immune response. The serum lysozyme protects the host organism against variety of Gram negative bacteria and some big viruses, such as the *Avipoxvirus* (Nguyen-Huu, 1979; Zyczko and Zyczko, 1998).

Evolutionary the complement system is one of the oldest defense mechanisms against pathogenic bacteria. The alternative pathway of complement activation (APCA) does not require the complex antigen-antibody, so it plays significant role in the first minutes after infection. It is quite efficient against Gram negative bacteria, viruses, neoplastic cells etc. hence it plays significant role in animals′ protection mechanisms (Mueller-Ortiz, et al., 2004; Paape and Capuco, 1997; Zhu et al., 2005).

It has been discovered that both serum lysozyme concentrations and APCA activity play significant role in chicken defense mechanism against *Eimeria tenella* infections. This data combined with the protective effect against bacterial and viral agents defines the two factors of innate immune response as critical for livestock animals (Koinarski and Sotirov, 2005).

Breed, species, age and sex have been evaluated as factors with huge impacts on the levels of blood serum lysozyme and APCA activity (Koynarski and Sotirov, 2012; Sotirov et al., 2011; Sotirov et al., 2007).

The importance of these two innate immune factors motivated us to investigate their variations and dynamics among two of the most common broiler chicken hybrids – Ross and Cobb. Additionally to the broiler flocks, we decided to investigate both factors of humoral immunity among hybrids′ parent flocks.

MATERIALS AND METHODS

Blood serum complement and lysozyme concentrations were analyzed in broiler chicken from the popular Ross and Cobb hybrids, reared in private farms. Samples were collected during the six week growing period of the broiler chicken, where 25 samples were collected for every week of life of the birds. Additionally we analyzed the target immune factors among both hybrids parent flocks between the 45th and 55th week of age. Twenty five samples from each parent flock were collected every five weeks from the aforementioned period of time. The total number of investigated samples was 450. Blood for analysis was collected aseptically from *v. ulnaris* with disposable needles in plain vacuum tubes after fixation. Blood was transported in cool bags at 6^{o}C.

The activity of the alternative pathway of complement activation (APCA) was assayed by the method of Sotirov (1991). Each serum sample was first diluted by mixing 100µl serum with 350 µl veronal-veronal Na buffer (in final concentrations: 146 mM NaCI, 1,8 mM 5,5-diethylbarbituric acid sodium salt; 3,2 mM 5,5-diethylbarbituric acid; 1 mM EGTA and 0,8 mM MgCl2). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer: 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 and 20 µl diluted serum + 80 µl buffer. The final serum dilutions were, respectively, 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then 50 µl buffer and 100 µl of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 µl of each supernatant was removed and placed in flat bottomed plates for measurement of optical density at 540 nm using 'Sumal-PE2' ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer programs developed in the Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement induced haemolysis of applied erythrocytes).

Blood serum lysozyme concentrations were assayed by the method of Lie (1985). Twenty ml of 2% agarose dissolved in phosphate buffer (0.07 M $NaHPO_4$ and NaH_2PO_4) was mixed with 20 mililiters 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 made with a special device. Fifty microliters of undiluted sera were pipetted in each well. Eight standard lysozyme dilutions (from 0.025 to 3.125 µg/ml) were prepared and pipetted in weight 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.

RESULTS AND DISCUSSIONS

The analyzed data for the blood serum lysozyme concentrations among the broiler hybrids is presented on table 1. As seen from the table, both hybrids show extremely high lysozyme concentrations through the first day after hatching. These results unambiguously exhibit the effect of the remaining small amounts of yolk in the first few days of life. The concentration of serum lysozyme was much higher among the Cobb hybrids ($P < 0.01$), compared with the Ross flock. Through the next two weeks of life, both hybrids show decrease of the lysozyme concentrations (about 50%), compared with the first week ($P < 0.05$). Significant differences between the hybrids at this stage were not detected. From the forth to sixth week both hybrids exhibit growth in the lysozyme levels, which indicates activation of animals′ genetic resources. In the fourth week of life (day 22) the Ross hybrid shows significant increase of the serum lysozyme levels (15.72 mg/L). The Cobb flock also exhibited higher concentrations (10.03 mg/L), but levels were in favor of the Ross hybrid ($P < 0.01$). For the last two weeks of life the Ross

flocks show almost constant levels of lysozyme concentrations, while the Cobb hybrid exhibited significant reduction in the fifth week of life, where the lysozyme levels were also in favor of the Ross hybrids ($P < 0.01$).

Table 1. Dynamic analysis of blood serum lysozyme concentrations (mg/L) in broiler chicken hybrids for six week growing period

Hybrid	Ross	Cobb
Age	X ± Sx	X ± Sx
1 day	7.79±0.24^{abc1}	11.06±0.12^{jmq1}
8 days	4.27±0.21^{def}	4.54±0.18^{jkl}
15 days	6.24±0.08^{ghi}	6.04±0.11^{mno}
22 days	15.72±0.14^{cdg2}	10.03±0.22^{knp2}
29 days	10.33±0.13^{aeh3}	6.66±0.06^{pqr3}
36 days	12.37±0.07^{bfi}	12.89±0.10^{lor}

$^{a-r}$ – $P < 0.05$ – significant differences between different ages among each hybrid population.
$^{1-3}$ – $P < 0.01$ – significant differences between hybrids for the same age.

The results from our study exhibit higher and faster increase of serum lysozyme concentrations for the Ross hybrids, which gives these birds better protection against pathogens through the growth period. Although not investigated we suggest that the better lysozyme concentrations will result in better mortality and growth rates in favor of the Ross hybrid.

The obtained results for the APCA activity trough growth period and its variations over time and hybrid type are shown on table 2. Both hybrids show gradual increase of complement activity over time.

Soon after hatching both hybrids show moderate levels of APCA activity, but during the next weeks the values get close to the ones seen in adult birds.

Comparing the data from the first two weeks of life with the last two weeks (week 5 and 6) we determined significantly different results among both hybrid populations ($P < 0.01$). Both broiler types exhibit increase of the APCA activity between week 5 and week 6 ($P < 0.05$), which indicates the enhancement of birds′ immune system. Although not significant, the outcome indicates slightly better APCA activity for the Ross hybrid.

Table 2. Dynamic analysis of blood serum APCA activity (CH50) in broiler chicken hybrids for six week growing period

Hybrid	Ross	Cobb
Age	X ± Sx	X ± Sx
1 day	325.32±4.15^{ab}	346.94±5.12^{h}
8 days	339.27±8.20^{cd}	312.88±6.51^{ij}
15 days	321.34±4.12^{ef}	320.79±5.02^{kl}
22 days	357.36±11.22^{g}	336.79±9.50^{m}
29 days	415.02±4.30^{ace}	398.32±6.21^{ikn}
36 days	522.14±9.25^{bdfg}	518.93±6.61^{hjlmn}

^{a,b,i,j,k,l} – $P < 0.01$; ^{c,d,e,f,g,h,m,n} – $P < .,05$ – significant differences between different ages among each hybrid population.

The results for the dynamics of APCA activity and blood serum lysozyme among the parent flocks are shown on table 3. Similarly to the broiler flocks, the Ross hybrid parent stock exhibited significantly higher results for the blood serum lysozyme concentration ($P < 0.01$), compared with the Cobb hybrids. The established results for the APCA activity among parent stock were again not significant, but with a tendency for higher levels among the Ross hybrids.

Table 3. Blood serum lysozyme (mg/L) and APCA activity (CH50) among broiler chicken parent flocks

Hybrid Age	Lysozyme concentration		APCA	
	Ross	Cobb	Ross	Cobb
	X ± Sx	X ± Sx	X ± Sx	X ± Sx
45 weeks	6.46±0.21^{1}	3.4±0.14^{1}	431.04±7.20	424.65±9.12
50 weeks	8.25±0.48^{2}	3.90±0.30^{2}	438.94±9.02	411.36±6.22
55 weeks	9.19±0.61^{3}	4.39±0.27^{3}	440.19±6.30	428.15±8.32

$^{1-3}$ – $P < 0.01$ – significant differences between hybrids for the same age.

The overall results for both parameters indicate significant advantage for the Ross hybrid. The data concerning the serum lysozyme levels among the stock animals, backed up by the results obtained from the parent flocks, unambiguously show the better genetic potential of the Ross hybrid. According to Lie (1985) the serum lysozyme is under polygenic control.

Irwin (2004) suggests that some individuals carry out some major genes coding higher serum lysozyme levels. We could suggest that somehow the Ross hybrid breeding was able to

detect and maintain some of the lysozyme major genes, which give these birds huge advantage compared with the other hybrid. Koinarski and Sotirov (2005) demonstrate the crucial role of both serum lysozyme concentrations and APCA activity in chickens' protection against *Eimeria* infections. Authors detect less damage in intestinal tract and overall lower mortality rates among chicken with high concentrations of serum lysozyme, compared with those exhibiting low levels. Authors suggest that potential selection of animals towards high lysozyme concentrations will lead to substantial reduction of losses caused by Coccidiosis. Breed related differences were detected among other species too. In sheep Sotirov et al., (2011) established significantly higher lysozyme concentrations in Milk cross sheep, compared with breeds like Mouton Charolaise and Ile de France. Similar differences in lysozyme concentrations were observed in cattle by Semerdjiev et al., (2006).

The results for the other important factor of the innate immune response were not that considerable. Although not significant the higher APCA activity for both parent and stock animals was shown from the Ross hybrid. We should point out the relatively low variation for this indicator among both hybrids. This phenomenon could be explained by the extremely important role of the complement system in the fight against pathogenic agents.

Breed related differences in APCA activity was observed by Sutherland et al., (2005) in pigs. Authors evidenced higher complement activity in pigs from the Duroc breed, compared with the Landrace and Yorkshire breeds.

The absolute superiority for the serum lysozyme concentrations and high levels of complement activity shows that the Ross hybrid has great genetic potential for the intensive ways of rearing in livestock farming.

CONCLUSIONS

The higher concentrations of serum lysozyme and APCA activity suggests better protection for the Ross hybrid against pathogenic bacteria and some viral infectious agents. The present study exhibit better natural immunity of the Ross hybrid, which probably will have effect on the performance traits of the birds.

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