Lysozyme and complement activities in broiler-chickens with coccidiosis. II. Experiment with *E. acervulina*

V. KOINARSKI1 and L. SOTIROV2*

1 Department of Parasitology ; Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria
2 Department of Genetics ; Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

* Corresponding author : E-mail : sotirov20@yahoo.com

SUMMARY

The aim of this study was to elucidate the potential role of humoral factors of innate immunity in broiler-chickens infected with *E. acervulina* under influence of Copper (Cu) and Zinc (Zn) cations. The experiment was performed on 39 broiler chickens, divided into 4 groups: group I : untreated and non-infected ; group II : treated with a double salt of copper and zinc at a dose of 0.17 g/kg forage ; group III : infected with *E. acervulina* and treated with Cu and Zn ; group IV : infected with *E. acervulina* and untreated. It was found out that lysozyme concentrations in avian intestines depended on the species and the pathogenicity of *Eimeria* and the resulting lesions. Copper and zinc cations decreased blood serum lysozyme levels and *E. acervulina*-infected birds exhibited a higher activity of the alternative pathway of complement activation.

Keywords : broiler-chickens - lysozyme - complement - coccidiosis - Copper - Zinc.

Introduction

It is known that the pathogenicity of the various *Eimeria* species in broiler chickens is varied. It may be related to its impact upon lysozyme and complement activities. KOVANSKIIH [4] observed a decrease of lysozyme and procerdin plasma levels in chickens infected with *E. tenella* and *E. maxima* simultaneously with increased production of IgM and IgG. SUTEU et al. [11] reported that lysozyme concentration in caecal content of chickens infected with *E. tenella*, *E. maxima* and *E. hagani* increased statistically significantly compared to non-infected control chickens. The blood serum lysozyme levels were the lowest in infected chickens and the highest in infected birds treated with Sulfaquinoxaline and Olaquindox.

In a precedent study, we observed decreased serum lysozyme concentrations in chickens infected with *E. tenella* and in the same time increased caecal lysozyme levels that were 78 times higher than those of non-infected birds [10].

The aim of the present study was to elucidate the potential role of humoral factors of innate immunity in broiler-chickens infected with *E. acervulina* under influence of Cu and Zn cations.

Materials and methods

Four groups of ten day-old broiler chickens (*White Plymouth Rock x †White Cornish*) were performed. The broiler-chickens were reared in batteries.

Group I - broiler chickens were non-infected and untreated (n=10).

Group II - broiler chickens received additionally double basic salt of copper and zinc in the food. Mixed copper-zinc crystals (CuO•(ZnO)2•(OH)3Cl) was synthesized by the method of continuous co-precipitation under standard conditions with pH=7. The salt was given 0.170 g per kg food starting 2 days post-inoculation during 10 days (n=10).

Group III - broiler chickens were infected with *E. acervulina* and treated with (CuO•ZnO)2•(OH)3Cl at the same dose (n=10).

Group IV - broiler chickens were infected with *E. acervulina* only (n=9).

Chickens from groups III and IV were individually infected three times on the 10th, 13th and 16th days after hatching with 4.105 sporulated *E. acervulina* oocysts using ingluvial tube [according to the method of WILLIS and BAKER, 12].

During the experiment, the chickens were fed a standard mixed diet without antibiotic or coccidiostatic supplements. They were housed on a slat floor under conditions minimizing the risk of spontaneous infection with *Eimeria*.

A) INFECTION MATERIAL

An *E. acervulina* strain, isolated from naturally infected
birds in Bulgaria (2003), was used. The strain was applied after passages through 2-week old chickens, cultivated according to routine methods in 2.5% potassium bichromate solution. One milliliter of the oocyst culture was used in a sterility test on McConkey’s agar in a Petri dish.

B) METHODS

Blood was sampled from v. ulnaris profunda (after 10 days treatment with Cu and Zn salt), allowed to clot for 1 h and then centrifuged for 10 min at 4000 rpm. Then the chickens were sacrificed via cervical dislocation and samples for duodenal lysozyme content were done by obtaining 10 g duodenum from each bird, washing with an equal amount of saline (2 mL) and suspensions were centrifuged for 5 min at 4000 rpm.

Serum and duodenum lysozyme concentrations were determined by the method of LIE et al. [6]. Briefly, 20 ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na2HPO4 and NaH2PO4, pH = 6.2) were mixed with 20 mL suspension of 24 hour-culture of Micrococcus lysodeicticus at 67°C. This mixture was poured out in Petri’s dishes (14 cm diameter). After solidifying at room temperature, 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured out in each well. Eight standard dilutions (from 0.025 to 3.125 mg/L) of lysozyme (Veterinary Research Institute, Veliko Tarnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured. The alternative pathway of complement activation (APCA) was studied by the method of SOTIROV [9]. In this aim we used veronal-veronal Na buffer (85 g NaCl - High School of Biotechnology, Bulgaria; 3.75 g 5.5-Diethylbarbitur-saure Natrium salz - Loba - Chemie, Austria; 5.75 g 5,5-Diethylbarbituric acid - Reanal, Hungary; 0.01 M EGTA - Sigma, USA; 0.008 M MgCl2 - Polskie Odczynniki Chemiczne, Poland. All of these chemicals were diluted in 2 L distilled water with pH = 7.5. This buffer must be diluted 1:5 before use.) Then 100 µL from each serum sample were mixed with 300 µL buffer. From these mixtures using U bottomed microplates (Flow Laboratories, UK) seven dilutions were made - 70 µL diluted serum (dil. ser.) + 30 µL buffer ; 60 µL dil. ser. + 40 µL buffer ; 50 µL dil. ser. + 50 µL buffer; 40 µL dil. ser. + 60 µL buffer ; 30 µL dil. ser. + 70 µL buffer ; 20 µL dil. ser. + 80 µL buffer ; 10 µL dil. ser. + 90 µL buffer and 50 µL buffer were additionally added to each well. 100 µL of 1% rabbit erythrocyte suspension to each dilution were droped and were incubated at 37°C for 1 hour. Optical density were measured by « Sumal-PE2 » ELISA reader (Karl Zeiss, Germany) at 540 nm. Lysozyme content, APAC activity and statistical analysis were calculated using special computer programs developed in Thracian University.

Results

Data presenting the dynamics of serum lysozyme concentrations are presented in Table I. Serum lysozyme levels were the highest in the non-infected and untreated group.

They were significantly higher (P<0.05-0.01) vice versa (vs) the other three groups. No significant differences in lysozyme concentrations in the duodenal content (Table II) were evidenced. Therefore, it could be assumed that neither the infection nor the treatment with Cu and Zn did seriously damage the epithelium and the deeper layers of the duodenum.

The investigation of the alternative pathway of complement activation (APCA) (Table III) showed that the highest activity was present in the infected and untreated group (group IV). The differences between this group and the other three were significant (P<0.05).

Discussion

Our data about serum lysozyme concentrations suggested that applied treatments exerted a marked immunosuppressive effect. This conclusion is further supported by the significant differences between the untreated and treated groups.
LYSOZYME AND COMPLEMENT ACTIVITIES IN BROILER-CHICKENS WITH \textit{E. ACERVULINA}

(P<0.05-0.01). On the other hand, the differences among groups II, III and IV were not considerable thus showing that Cu, Zn and \textit{E. acervulina} had a comparable negative effect on serum lysozyme levels. A plausible explanation to those facts could be found in metal cations’ nature from one part and in the pathogenic action of \textit{E. acervulina} on the other. KATZ and ROBERSON [3], having studied the interaction among various single-chain globular proteins and various metal cations observed that the latter provoked an increase in protein chain volume. This increase was variable and dependent particularly on metal ion nature and then, on the protein itself. Those studies explain why the impact of various metal cations on lysozyme and complement levels in animals are different. KORNEGAY \textit{et al.} [5] reported that the supplementation of the diet of pigs with copper at doses of 200 and 400 mg/kg body weight decreased serum lysozyme activities. BENKOVA \textit{et al.} [1] showed that industrial heavy metal emissions (Cu, Pb, Cr and Zn) decreased the phagocytic activity, the phagocytic index and complement levels in lambs, experimentally infected by \textit{Fasciola hepatica}. The results of MARKOV \textit{et al.} [7] were different (they gave evidence that the supplementation of calves with 244 mg/kg magnesium acetate resulted in higher lysozyme, bactericidal and phagocytic activities than in control calves or calves, treated with sodium acetate. Therefore, the communications of KORNEGAY \textit{et al.} [5], BENKOVA \textit{et al.} [1] and MARKOV \textit{et al.} [7] supported the assumptions of KATZ and ROBERTSON [3].

In a previous study, we observed decreased serum lysozyme concentrations in chickens infected with \textit{E. tenella}. At the same time, unlike \textit{E. acervulina}, the infection with \textit{E. tenella} in chickens resulted in caecal lysozyme levels that were 78 times higher than those of non-infected birds [10].

COZMA \textit{et al.} [2] showed that lambs infected with coccidiae exhibited low levels of blast transformation of lymphocytes, a low phagocytic index and lysozyme. MONTGOMERY \textit{et al.} [8] found out that a \textit{F. hepatica} extract, dissolved in phosphate buffer, inhibited \textit{in vitro} the classic and the alternative pathway of complement activation in bovine serum. In cases when the bovine serum contained antibodies against the parasite, no changes in haemolytic activity of complement were present.

The changes in serum APCA in chickens infected with \textit{E. acervulina}, were completely different from lysozyme changes. This parameter reached its highest values in chickens, infected with the parasite, but untreated (P<0.05). Chickens that were infected and treated with Cu and Zn showed a lower APCA activity, then followed the group that was treated and non-infected and the lowest APCA values were observed in controls. The highest values in group IV (infected with \textit{E. acervulina}) could be explained with the antigenic challenge of \textit{Eimeria} on immune system (most probably, by bacteria having penetrated through the wall of injured duodenum as well). A similar result was obtained in the third experiment of a previous study [10]. The fact that the APCA activities in the group infected and treated with Cu and Zn (group III) were lower than those in group IV provided evidence that both metal ions inhibited APAC.

Our data allowed us to conclude that:

1. The lysozyme concentration in intestines of broiler chickens depended on the species and pathogenicity of \textit{Eimeria} and on the lesions they caused.

2. Obviously, lysozyme played a primary role in the defense of chickens against coccidiosis although the mechanism of this response is still unknown.

3. The copper and zinc cations decreased blood serum lysozyme levels, but not duodenal lysozyme concentrations.

4. The blood serum APAC activities increased in chickens, infected with \textit{E. acervulina}.

References


