Effect of different growth promoters on lysozyme and complement activity. II. Studing in turkeys

*L. SOTIROV, **ST. DENEV, ***I. TSACHEV, ****M. LALEV, *****M. OBLAKOVA and *****Z. PORFIROVA

** Department of Animal Genetics, Veterinary Faculty, Thracian University, 6000 Stara Zagora, Bulgaria
*** Department of Microbiology and Biotechnology, Agrarian Faculty, Thracian University, 6000 Stara Zagora, Bulgaria
**** Department of Epizootology and Infectious diseases, Veterinary Faculty, Thracian University, 6000 Stara Zagora, Bulgaria
***** Institute of Poultry Science, 6000 Stara Zagora, Bulgaria

SUMMARY
An experiment with 180 turkeys (White Imperial breed) was carried out to study the effect on the lysozyme, complement activity, phagocyte number and phagocyte index of Lacto-Sacc probiotic (Alltech, USA) at a dose of 1 g/kg feed, Pharmastim nutritive premix (Biovit, Bulgaria) at a dose of 0.3 g/kg feed and administered for 16 weeks. Both preparations, in particular Lacto-Sacc, stimulated immunity according to their effect on the examined indices.

KEY-WORDS: lysozyme - complement - turkeys.

RÉSUMÉ
Influence de quelques suppléments fourragers sur l’activité de la lysozyme et du complément. II. Étude chez le dindon. Par L. SOTIROV, ST. DENEV, M. LALEV, M. OBLAKOVA et Z. PORFIROVA.
L’influence de l’additif nutritif "Pharmastim" (Biovet, Bulgarie, en dose 0,3 g/kg de fourrage) et de la probiotique "Lacto-Sacc" (Alltech, USA, en dose 1 g/kg de fourrage) sur l’activité de la lysozyme, sur le complément, le nombre et l’indice phagocytaires chez des 180 dindons (la Blanche Impériale), traités pendant 16 semaines a été étudié. On a constaté que les deux produits possèdent une activité de stimulation immunitaire sur les indices étudiés, laquelle est, toutefois plus marquée chez la probiotique “Lacto-Sacc”.

MOTS-CLÉS : lysozyme - complément - turkeys.

Introduction
There are a number of researchers working hardly on the possibilities to improve resistance of farm animals to infectious diseases. Some are trying to achieve that goal by the methods of selection (direct or indirect) in resistance to a particular infectious agent [3, 4, 5, 6], while others are trying the effect of various immuno-modulators [1, 8, 9, 11, 12, 13, 15].

We have already made an experiment carried out to determine effects of Lacto-Sacc probiotic (Alltech, Inc., USA), nutritive premixes Pharmastim (Biovet, Bulgaria), Avilamycin (Elanco, USA) and Lactose (Merk, Germany) on the lysozyme and complement activity in broiler chicken. Results obtained encouraged our next trial with turkeys.

Material and methods
Three groups of 60 turkeys each (White Imperial breed) were used and 10 birds from each group were examined. Group I was used as a control one, group II was treated with Pharmastim (Biovet, Bulgaria) at a dose of 0.3 g/kg feed and group III received Lacto-Sacc (Alltech, Inc., USA) at a dose of 1 g/kg feed. Turkeys were treated for 16 weeks, then blood samples from the wing vein were collected and tested. Blood was left to clot for an hour at room temperature and centrifuged (2000 rpm/min); sera were examined for lysozyme activity [7]. Briefly 20 ml of 2 % agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0,07 M Na₂HPO₄ and NaH₂PO₄, pH = 6,2) was mixed with 20 ml suspension of 24
hours culture of Micrococcus lysodeicticus at 67°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 were poured out in each well. Eight standard dilutions (from 0.025 to 3.125 (µg/ml) 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 method of Sotirov [14]. For 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 dropped 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 noma caused by chemical compounds.

Results and discussion

Results of Pharmastim and Lacto-Sacc effects on the lysozyme level are given in fig. 1. Values from the Lacto-Sacc group are the highest, followed by that from the Pharmastim and the control groups. All inter-group differences are insignificant. However, the above trend cannot be occasional. Similar results (at significant differences between the average group values) have already been obtained with broiler chicken treated with the same immunity modulators (not published data). It has been found that Lacto-Sacc shows an evident immuno-stimulating effect on the lysozyme level even under the effect of a non-specific antigen (bovine serum albumin). Both studies have shown that Lacto-Sacc can be used as an immunity stimulator, especially when applied prior vaccinations. Our suggestion is supported by the results of Perdigon et al. [10] in mice who have shown that mice treated with Lactobacillus casei and yoghourt develop higher resistance to an infection with Salmonella typhimurium, increased blood number of lymphocytes, also increased IgM and IgA concentration, inhibition of small-intestine carcinoma caused by chemical compounds.

Changes in APCA under the effect of Pharmastim and Lacto-Sacc are summarized in fig. 2. Here again the values found in the Lacto-Sacc treated group are highest, followed by those of the Pharmastim treated group and the control group. Differences in those cases were significant (p < 0.05-0.001). Results cannot be accidental because both indices are changed in the same direction. A similar trend was determined in broiler chicken in which the APCA values were highest in the Lacto-Sacc treated group as well.

The results for the phagocyte index and the phagocyte count are similar to the above described data (figures 3 and 4). Both figures show that the Lacto-Sacc treated group developed significantly higher values compared to the other two groups (p < 0.05-0.001) and also there are minor differences between the Pharmastim treated and the control groups. These results have shown, beyond a doubt, that only Lacto-Sacc probiotic has a definite immuno-stimulating effect and that Pharmastim nutritive premix has not. Similarity of the changes in all of the four traits is not an occasional one. It is

![Figure 1. — Lysozyme levels in turkeys treated with different growth promoters (µg/ml).](image-url)
well known that the major components of activated complement system start to expel small sub-components, such as C3a and C5a. These possess strong anaphilla-toxic and haemmo-toxic properties [2]. They cause inflammation where the infectious agent penetrates the body thus attracting and enhancing the phagocyte effect of all cells that possess phagocyte properties.

Stimulating effect of various substances on the relevant factors of the immune system has been described by other authors as well. Sokolov [13] has determined that low doses of ampicyline and tetracycline (100 and 200 mg/kg w.) increase lysozyme level and that their high doses (500 mg/kg w.) reduce lysozyme level. Riedel-Caspari et al. [12] have determined that vitamin E administered at a dose of 150 mg/kg w. increases lysozyme level and number of granulocytes in swine; vitamin C given at a dose of 100 mg/kg w. to hens restores lysozyme level faster when it has been reduced after hyper-thermal stress [1]. Selenium, magnesium acetate and levamisol all have a favourable effect on lysozyme, phagocytosis and the bactericide activity of serum in cows, calves, and hens [8, 9, 11]. Data of other authors cited, without any doubt, show that it is possible to increase resistance to the infectious diseases by feeding different substances at proper doses.

Finally, we would like to express our belief that the successful control of the infectious diseases in turkeys depends
on the efforts to increase lysozyme levels, complement values, phagocyte index and phagocyte number by selection of turkeys in these particular indices, on the one side, and on the application of different immunity stimulators fed to turkeys with feed or water, on the other side.

Conclusion

Lacto-Sacc (probiotic) and Pharmastim (nutritive compound) stimulate the lysozyme level, complement activity, phagocyte index and phagocyte count in turkeys.

References