TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2021, vol. 6, No 2(11): 95–103

INFLUENCE OF THE IMMUNOMODULATOR "AVIGEN" ON THE NATURAL RESISTANCE OF ROSS HYBRID BROILER

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ABSTRACT

Some basic indicators of nonspecific immunity in ROSS 308 hybrid broilers receiving a polybacterial immunomodulator were studied by enzyme-linked immunosorbent assay and spectrophotometric methods. The experiments were performed in flocks of broiler breeders and broilers under production conditions. In the control herds- under the same breeding conditions and in compliance with the prophylactic programs in the farms, including the accepted antibiotic therapy. The birds from the experimental flocks were raised without the use of antibiotics.

As a result of the performed studies, we found higher concentrations of lysozyme, IFN γ, IL-2; IL-6 in experimental herds receiving the immunomodulator compared to controls. For example, in the experimental broiler herd, we found twice as high levels of serum lysozyme relative to controls: 6.17 ± 0.49 mg / L and 2.99 ± 0.27 mg / L, respectively. The activity of complement (CH50) in the blood serum of broilers treated with AVIGEN - 549.00 ± 19.69, also significantly exceeded the values in the control herd - 377.40 ± 9.58. We received similar results from broiler breeders. Serum IgY concentrations showed a significant difference in adolescent broiler breeders but not in broilers.

We tracked the changes in bursa Fabricii by histological methods. Under the influence of the immunomodulator AVIGEN in the bursa of experimental birds, we observed delayed involution and good functional activity, while in the controls, involution processes were advanced and accompanied by follicle death and connective tissue growth. The morphological and functional activity of bursa Fabricii contributes to the increased natural resistance in the birds treated with the immunomodulator, which enabled their breeding without the use of antibiotics in production conditions.

Key words: lysozyme, complement, IFN γ, IL-2; IL-6, IgY, bursa Fabricii, immunomodulators, biotechnology.

Introduction

In birds, type II interferons (IFN γ) are products of T lymphocytes and natural killer cells. IFN α / β is primarily involved in the formation of antiviral immunity, and IFN γ is a pleiotropic molecule that, to one degree or another, affects all stages of the immune response (Rosenberger et al., 2000; Seo et al., 2002; Wigley and Kaiser, 2003). Bacterial endotoxins are known to be able to induce interferon production in the body (Bailey et al., 2007; Petrunov et al., 2007; Sato and Iwasaki, 2005). Lysozyme is a major factor in humoral nonspecific protection of birds and the natural resistance of the embryo (Gilbert, 1971). Serum lysozyme is secreted by macrophages, and the intensity of its formation depends on various factors (Eshbailat et al., 2004; Bazlamit et al., 2009). Complement proteins are produced by macrophages and heterophiles and make up about 5% of all serum proteins. In the body complement is in an inactive form. After activation its action is cascading and includes a series of proteolytic reactions aimed at enhancing humoral and cellular interactions (Sotirov and

Koinarsky, 2003). IgY is found in the blood serum and egg yolk of birds and is formed by plasma cells in response to antigenic exposure (Tizard, 2002). Its effect by affecting the mucous membranes may be a guideline for the relationship between mucosal and systemic immunity.

In the present experiment, we set out to study the effect of the AVIGEN polybacterial immunomodulator on some key indicators of nonspecific immunity and on the functional activity of the bursa of Fabricius in broilers and growing broiler breeders reared under production conditions.

Materials and methods

Broilers. We made field experiments with broilers hybrid ROSS 308, from two halls, experimental and control, raised under the same conditions. The immunoprophylactic program adopted in the poultry farm was applied. The experimental flock of 15,500 birds received an immunomodulator from day 1 to day 10 and was reared without antibiotics. The control flock (15,520 birds) did not receive an immunomodulator. They used Linco-Spectin (Zoetis Belgium SA) from day 1 to day 7 and lincomycin (Lincocin 40%, Zoetis Belgium SA) from day 19 to day 22.

Broiler breeder. One experimental flock of growing broiler breeders from the ROSS hybrid, and one control flock reared under the same conditions but not treated with the immunomodulator. The birds received the same immunoprophylactic program adopted in the poultry farm. The experimental flock was raised without antibiotics.

All birds from the experimental and control flocks received during the experiments the antistress preparation ASPIVIT AS (Heldiva Ltd, Bulgaria) 5 ml per 10 kg of body weight once a day for 3 days and biostimulating supplement BIOXAN emulsion (Heldiva Ltd, Bulgaria) in a dosage of 5 ml per 10 L of drinking water for three consecutive days.

Method of treatment. We administered immunomodulator AVIGEN in liquid form for a period of 10 consecutive days, containing 3000 doses in 1000 ml. The polybacterial immunomodulator AVIGEN (Heldiva Ltd, Bulgaria) is containing in concentrated form lipopolysaccharide components of the thermostable endotoxin, extracted from Gram-negative bacteria from the family Enterobacteriaceae. In the broilers AVIGEN was applied orally with drinking water from the 1st to the 10th day. For broiler breeders AVIGEN was used, during growing up, with drinking water from the 1st to the 10th day and from the 120th to the 130th day of the bird's life.

Samples. We randomly took 45 blood samples from broilers 26 days after the last intake of the immunomodulator - on the 36th day in broilers and 16 days after the last intake- on the 146th day in broiler breeders. Blood samples were taken from the brachial wing vein, with a vacutainer, in a volume of 2.5 ml. At the same time, we took blood samples from the control herds. Then the samples were stored in room temperature to clot for 30 minutes, centrifuged at 1000 rpm with centrifuge Jouan MR23i (France) for separating the blood serum and stored in a refrigerator for 18-24 hours at a temperature of -18 to -20^{o}C.

Determination of IFN-γ, IL-2, IL-6 and IgY. For this purpose we used Chicken interferon γ, (IFN γ ELISA kit, CUSABIO, China), Chicken Interleukin 2 (IL-2 ELISA kit, CUSABIO, China), Chicken Interleukin 6 (IL-6 ELISA kit, CUSABIO, China) and Chicken Immunoglobulin Y (IgY ELISA kit, CUSABIO, China). We measured the optical density using the ELISA rider Beckman Coulter DTX 880 (USA) at a wavelength of 450 nm. We used a software product (Curve Expert, CUSABIO) to calculate interferon, interleukin and IgY concentrations based on a standard curve.

Control of immunity against Newcastle disease and Infectious bronchitis. Using the enzyme-linked immunosorbent assay, software and ELISA kits of IDEXX Laboratories, we determined the

immune strain in experimental and control broiler flocks in order to exclude possible side effects from the application of the immunomodulator on the planned vaccinations.

Average daily mortality. To monitor this indicator, we used the data from the production diaries of the respective poultry farms.

Statistical analysis of the results. The obtained experimental data were subjected to variation-statistical analysis in order to determine the confidence intervals at the respective values of the indicator (ANOVA, StatSoft Inc.).

Histological examination. In order to track the functional activity of bursa Fabricii we collected samples from the experimental and control groups of birds. The materials were fixed in 10% neutral formalin and processed according to the classical paraffin embedded tissue method. Sections 4-5 μm thick were prepared on Microm HM 335E (Germany) and stained with hematoxylin-eosin (Lillie, 1964). Histographs were taken with a microscope OLYMPUS CX23 (JAPAN), magnification 10x.

Results

In the experimental broiler flock receiving an immunomodulator, lysozyme values ranged from 2.21 mg/L to 8.83 mg/L or, on average, 6.17 ± 0.49 mg/L. In comparison, in control birds these levels were significantly lower. Lysozyme concentrations in the blood serum of birds not receiving an immunomodulator ranged between 1.10 mg/L and 4.41 mg/L or an average of 2.99 ± 0.27 mg/L (Table 1).

In birds receiving the immunomodulator, we also found higher complement activity. The mean value (CH50) in the blood serum of broilers treated with AVIGEN was 549.00 ± 19.69, while in controls this value reached 377.40 ± 9.58 (Table 1).

Table 1: **Lysozyme and complement and IgY in the blood serum of broilers at 36 days of age and of broiler breeders at 146 days of age.**

Researched birds	Lysozyme, mg/mL	Complement activity (APAC),(CH50)	IgY, mg/mL
Broilers			
Experimental group, n=45	6.17*±0.49	549.00*±19.69	4.74±0.62
Control group, n=45	2.99±0.27	377.40±9.58	4.68±0.58
Growing broiler breeders			
Experimental group, n=45	6.42*±0.76	465.00*±15.08	18.10±1.45
Control group, n=45	2.64±0.25	312.00±10.52	12.26±1.28

**statistical significance at p<0.001*

We received similar results from broiler breeders. The mean values of lysozyme (6.42 ± 0.76 mg/mL) and complement (465.00±15.08 CH50) in birds treated with the immunomodulator were significantly higher than in controls. Regarding IgY concentrations, it should be noted that they did not respond to the immunomodulator in broilers, in contrast to that found in growing breeders who received the immunomodulator twice.

Table 2 presents the mean concentrations of interferon γ and interleukins in broilers. After treatment with AVIGEN, in the experimental group we measured the concentration of IFN γ -

548.23 ± 2.18 pg/mL, and in the control group not receiving the immunomodulator – 127.04 ± 1.09 pg/mL. These data show that birds receiving AVIGEN had much higher concentrations of IFN γ than those in the control group. Similar data were found for the studied cytokines IL-2 and IL-6. Their serum concentrations in the experimental flock were higher than those measured in the control group.

Table 2: Mean values of IFN γ, IL-2 and IL-6 concentrations in the blood serum of broilers at 36 days of age and of broiler breeders at 146 days of age.

Researched birds	IFN γ, pg/mL	IL-2, pg/mL	IL-6, pg/mL
Broilers			
Experimental group, n=45	548.23*±2.18	6.28*±0.76	612.55*±2.54
Control group, n=45	127.04±1.09	1.87±0.61	204.18±1.32
Growing broiler breeders			
Experimental group, n=45	635.18*±2.56	6.89*±0.64	541.16*±2.74
Control group, n=45	109.32±2.01	1.45±0.90	165.14±1.65

** statistical significance at p<0.001*

The influence of the immunomodulator AVIGEN on the involution processes of the bursa of Fabricius in broilers are shown in Fig. 1–2. Examination of broilers from the experimental flock on the 36th day of their life revealed that the follicles of the bursa were in an active functional state. The core of the fragments was dense and filled with lymphoblasts (Fig. 1).

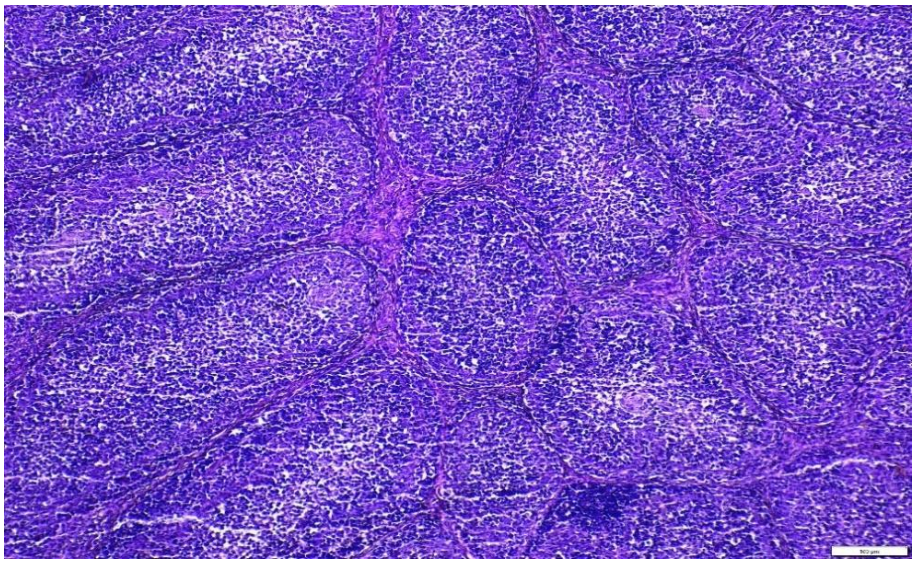

Figure 1: Bursa Fabricii in a 36-day old experimental broiler. Relatively dense and active follicles, X/E, bar = 100 µm.

The research of bursa Fabricii in broilers from the control flock that did not receive the immunomodulator showed at the same age (36 days) accelerated processes of deactivation of the parenchyma and its replacement with connective tissue (Fig. 2).

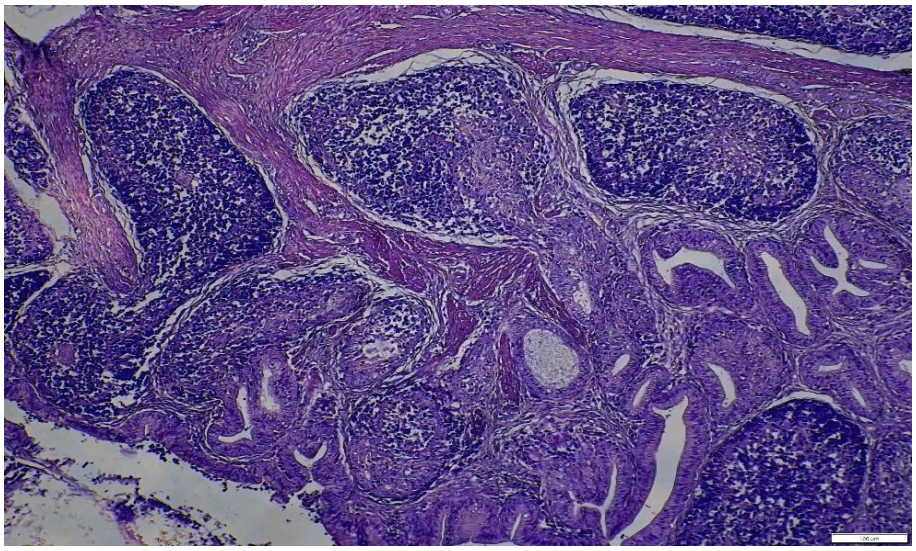

Figure 2: Bursa Fabricii in a 36-day old control broiler.
Process of follicle inactivation and connective tissue growth, X/E, bar = 100 µm.

In growing broiler breeders who took the immunomodulator twice, we observed "the phenomenon of delayed involution"of bursa Fabricii (Fig. 3). After the first sampling on day 136, well-structured, active and dense follicles are seen.

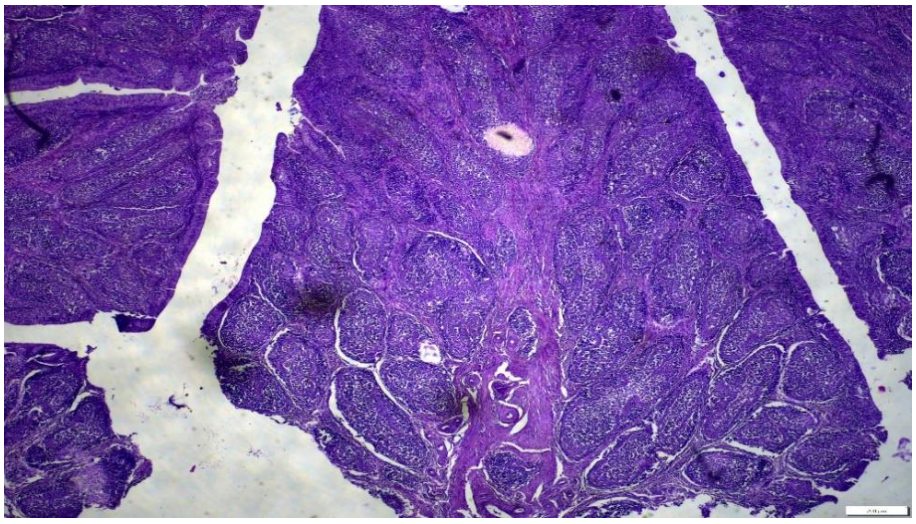

Figure 3: Bursa Fabricii in a breeder from the experimental group of 136 days age.
Active follicles filled with lymphoblastic cells, X/E, bar = 100 µm.

Control breeders of the same age already develop a process of deactivation of the parenchymal tissue - empty and shrunken follicles without lymphoblasts in them (Fig. 4).

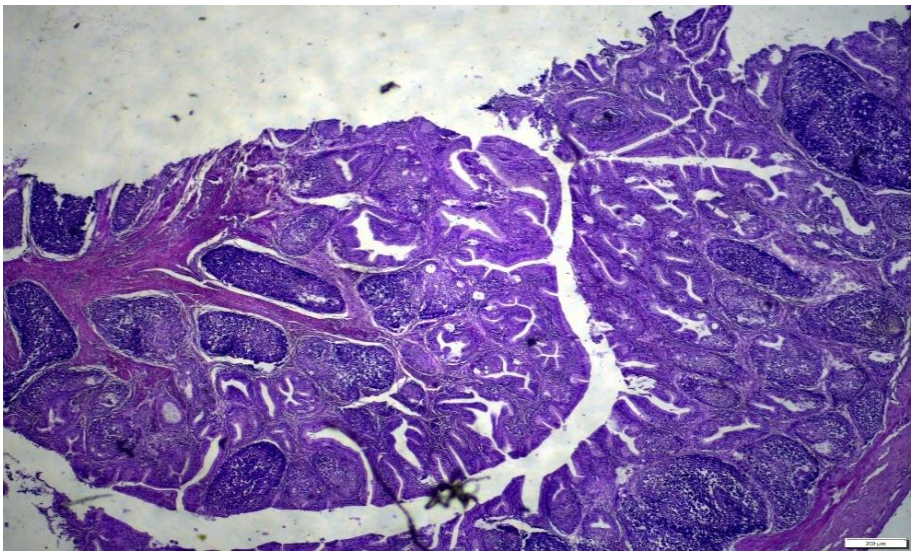

Figure 4: Bursa Fabricii in a 136-day old control breeder.
Many empty follicles and connective tissue growth, X/E, bar = 100 µm.

The second sampling on day 146 confirmed the results. Breeders treated with the immunomodulator had bursa Fabricii in an excellent functional state for the age (Fig. 5), while in the controls we found a deepening of the involution processes (Fig. 6).

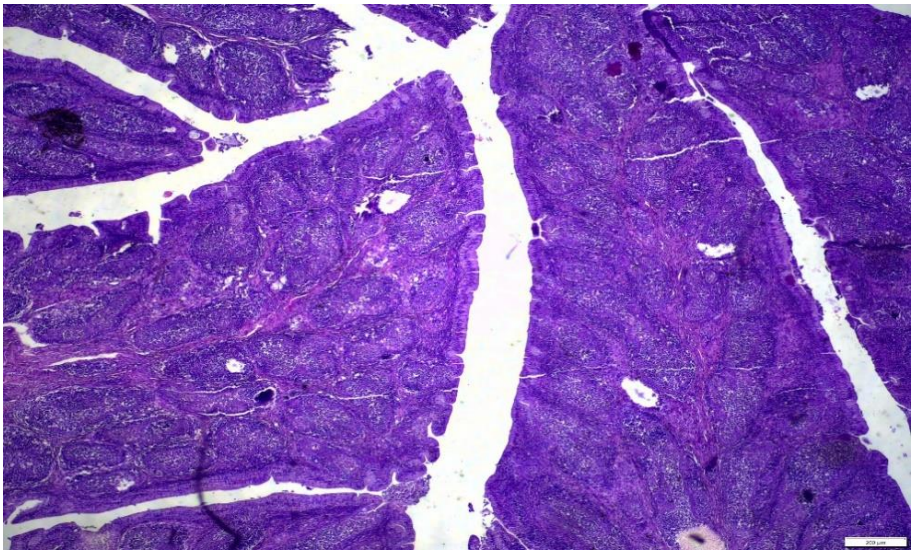

Figure 5: Bursa Fabricii in breeder from the experimental group of 146 days age.
Active and dense follicles filled with lymphoblastic cells, X/E, bar = 100 µm.

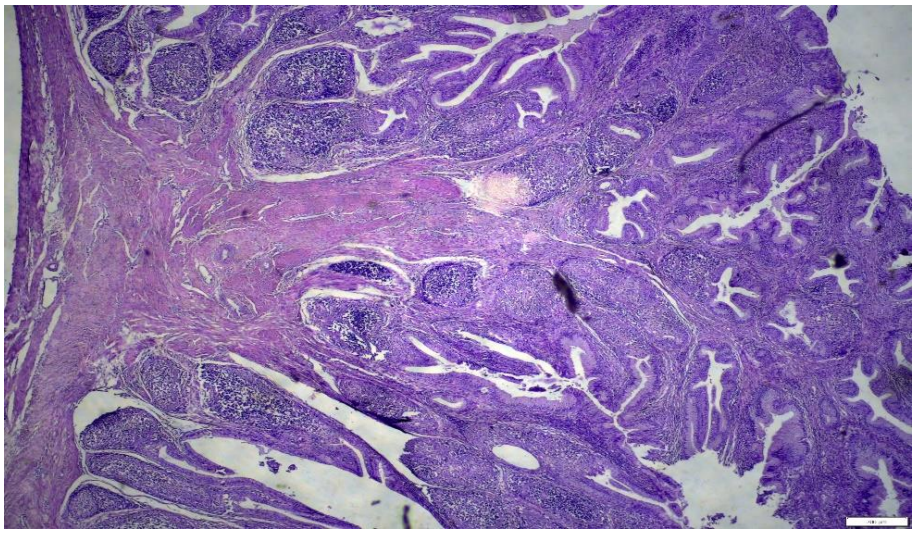

Figure 6: Bursa Fabricii in a 146-day old control breeder.
Newly grown connective tissue displaces the parenchyma, many dying follicles, X / E, bar = 100 µm.

Antibiotics in any form were not administered throughout the rearing period of the broilers treated with the immunomodulator. The immunomodulator-treated breeder flock was also reared without antibiotics not only during growing but also throughout the productive period.

In general, it can be concluded that the average daily mortality in experimental broilers, as well as in the breeders, was lower than that registered in the control flocks.

Discussion

In recent years, great importance has been attached to the factors of natural resistance in birds, in connection with the search for alternatives to the use of antibiotics and overcoming some of the harmful effects of their impact. In modern poultry farms it is often difficult to create such feeding and rearing conditions as they were during the selection of the respective hybrid in the breeding farms. Therefore, even if targeted selection is made by indicators of natural resistance, in production conditions they do not always appear or it is difficult to judge which hybrid has greater natural resistance. As is known, serum lysozyme is a major non-specific protective factor in birds (Eshbailat et al., 2004; Besarabov, 2013).Our results show that stimulation of intestinal mucosal tissue with lipopolysaccharides from enterobacteria contained in the applied immunomodulator leads to increased levels of lysozyme and complement in broilers of the hybrid ROSS 308. According to Besarabov (2013), lysozyme content in blood serum or in egg white, below 3.5 mg/L, shows low natural resistance of birds, or exposure to adverse factors. Interferons and interleukins play a major role in this regard as mediators of immune responses (Seo et al., 2002; Wigley and Kaiser, 2003). In this sense, the application of polybacterial immunomodulators, which can increase the natural resistance in production conditions, is of interest to poultry and biotechnology. Our experiments show that the immunomodulator AVIGEN can be a successful alternative to antibiotics in broiler farming.

Involution of bursa Fabricii is a natural process that takes place in parallel with the sexual maturation of birds. It is accompanied by a certain immune rearrangement. As can be seen from the presented histological findings (Figs. 1 and 2), the lobules of broilers bursa Fabricii at 36 days of

age from the experimental group are dense and active, while in the control flock is observed at the same age, as a rule, inactive functional units.

It is known that in bursa Fabricii not only antigen-dependent process of B-lymphocyte formation takes place, but also antigen-dependent reactions associated with antibody-secreting plasma cells. Immunoreactive B-lymphocytes are able to form protein factors of interleukocyte interaction - interleukins 2 and 6 and turn into effector cells of the humoral immune response, they can also respond to signals from T-helpers. Therefore, the effect of IgY formation in birds receiving an immunomodulator indicates possible links between innate and acquired immunity, which may be activated from the mucosa to bursa Fabricii.

According to Dunon et al. (1999), Ciriaco et al. (2003) bursa Fabricii is a major immunocompetent organ in birds with a crucial role in the formation and regulation of the immune response in young birds, and its physiologically induced involution affects immunoreactivity after puberty. Consistent with these notions, we found that birds receiving the immunomodulator showed a delay in involution of the bursa, accompanied by increased complement activity, higher levels of lysozyme, interferon, and serum interleukins. This "prolongation of the life" of the bursa can be explained by the presence of epithelial tufts in the bursal epithelium, which according to Schaffner et al. (1974) act as Payer's plaques and are directly connected to the intestinal mucosa through the bursal duct.

Conclusion

1. The AVIGEN immunomodulator delays the involution of bursa Fabricii in broilers and broiler breeders and provides better immunoreactivity to birds.
2. The use of the immunomodulator AVIGEN increases the natural resistance in broilers of the hybrid ROSS 308, by increasing the activity of complement, lysozyme, interferon-gamma and interleukins in the blood serum.
3. Based on the increased natural resistance, birds receiving the AVIGEN immunomodulator may be kept in production conditions without the use of antibiotics.

Acknowledgements

The scientific research in this publication has been carried out by the author team under contract № КП-06-Н36/16, funded by The Bulgarian National Science Fund (BNSF), Bulgaria.

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