



## Effect of “Avigen” Immunomodulator on Beta-lysine Activity in Broilers

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**Abstract:** The beta-lysine activity of blood serum and egg white in broilers and broiler breeders from the ROSS 308 hybrid reared in industrial conditions was studied using a photometric method. The birds from the experimental groups were treated with polybacterial immunomodulator "AVIGEN", the anti-stress preparation "ASPIVIT C" and the preparation "BIOXAN", which helps to increase the resorption surface of the intestinal mucosa. We found increased activity of beta-lysine in the blood serum in immunomodulator-treated broilers. No significant differences were found in the activity of beta-lysine in the blood serum of the experimental and control parent herds until the onset of lay. On the onset of lay, we observed an increase in beta-lysine activity in the blood serum, especially pronounced in the experimental herd. We also registered an increase in this indicator in the egg white of the birds from the experimental flock from the 2nd to the 7th day after laying. Elevated serum beta-lysine concentrations during the onset of lay, legitimizes beta-lysine as a stress-induced factor of non-specific immunity. The higher levels of beta-lysine in the blood serum and egg white of the experimental herds are probably due to the stimulation on the mucous membranes with the polybacterial immunomodulator AVIGEN.

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## 1. Introduction

Beta-lysine is a non-specific immune defense factor in birds that has been little studied. "Beta-lysine" means the thermostable bactericidal substances in the serum, active against bacilli, which do not need the presence of complement to perform their function (Weinert, 2013). The beta-lytic activity of the serum provides information about the state of the body in animals and humans in different physiological and pathological processes of different etiology, so it is important in clinical trials in veterinary and human medicine (Sarukhanov et al., 2016). Henriksen et al. (2012), Wu, (2013), Xie et al. (2015), Ouidir et al. (2015) performed experiments on lysine synthesis with the help of other microorganisms – *Saccharomyces cerevisiae*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*. Ayasan and Okan (2010) investigate the effects of containing lysine on the fattening performance of broiler chickens. Beta-lysine is also known to be a precursor to the biosynthesis of antibiotics, neurotransmitters, and several polymers with high molecular weight (Hungetal., 2013). Our previous

studies (Karakolev et al., 2014; Karakolev, 2015; Karakolev and Nikolov, 2015) followed the physiological fluctuations in the beta-lysine activity of the blood serum in broilers and hen embryos from two hybrids of laying hens, and in the egg white of commodity eggs, depending on the age and hybrid affiliation of the laying hens. In a detailed study of the role of *Schizochytrium limacinum* in the diet of broilers on their natural immunity, Sotirov et al. (2021) also found a positive effect of the beta-lysine index in the experimental group. Investigating the possibilities for influencing the humoral immune response in laying hens, Bozakova et al., (2018) also draw attention to the changes in the activity of beta lysine under the influence of the immunomodulator «Immunobeta». According to Zemskov et al. (2018) beta lysine is involved in the mechanisms of immune homeostasis, in induction, regulation, and possibly in the correction of the immune response. The protective effects of other bacteriolysins, such as lysozyme and the complement system, have been studied in sufficient depth. However, literature data on beta lysine are extremely scarce, with no data on the beta-lysine activity of blood serum and egg white in broiler breeders, as well as the factors potentially influencing this activity.

With the present studies, we aimed to determine the beta-lysine activity of the blood serum of broilers and in the blood serum and egg white in broiler breeders treated with the immunomodulator "AVIGEN", as one of the main factors of natural immunity in the studied birds.

## 2. Material and Methods

### 2.1. Polybacterial immunomodulator "AVIGEN"

The product is a concentrated form of lipopolysaccharide components of the thermostable endotoxin extracted from Gram-negative bacteria from the Enterobacteriaceae family.

#### 2.1.1. Method of treatment

The immunomodulator was administered in liquid form, containing 3,000 doses (ten days) in 1000 ml.

For broilers - orally, through drinking water from the 1st to the 10th day. The experimental and control flocks of broilers numbered 12,000 birds each. From the birds treated with the immunomodulator in the experimental flock, as well as from the control one, 45 birds were randomly selected, from which blood was taken for testing.

For broiler breeders - during the growing up period, orally, through drinking water from the 1st to the 10th day and from the 120th to the 130th day of life. The experimental and control flocks of broilers breeders numbered 12,500 birds each. From them, we took 45 samples of blood serum for the tests.

The field experiments were conducted in a poultry farm of Planeta 98 Ltd.

The subject of the study were broiler breeders and broilers from the hybrid ROSS 308, grown on the floor under industrial conditions. The birds from the experimental flocks received with the water and the preparations "ASPIVIT C" and "BIOXAN" in a dosage of 5 ml per 10 l of water. Both preparations have an ancillary effect. The first has an anti-stress effect on birds, and the second improves the permeability of mucous surfaces.

### 2.2. Sampling

At certain intervals (6-, 12- 18-, 24-, 36-week-old), we took blood from the axillary vein. The separated blood serum was stored at 4°C. We collected blood samples from broilers at 10, 18, and 28 days of age. Serum testing was performed no later than 24 hours after sampling. We collected 45 eggs in a day (2, 7, 10, and 14) from control and experimental parent flock in the first two weeks after laying. The egg white test was performed no later than 6 hours after sampling.

### 2.3. Determination of beta-lysine activity

The beta-lysine activity of blood serum and egg white was determined by a spectrophotometric method described by Bucharin et al. (1977), modified by Karakolev and Nikolov (2015). The research was performed in flat-bottomed plates. We used a pre-prepared spore suspension of *Bacillus subtilis* ATCC 6633. We added the controls with an automatic pipette - 80 µl of saline + 80 µl of spore

suspension in each of the first 4 wells. We then instilled the experimental sera with an automatic pipette - 80 µl serum + 80 µl spore suspension in each of the following wells, according to the number of samples tested. We homogenized by a plate shaker. Optical density measurements were performed using a BioTek L80 spectrophotometer at a wavelength of 630 nm, before incubation. We incubated the plate in a plate incubator with a timer (37°C for 2 hours). Immediately after incubation, we again measured the optical densities at the same wavelength. Since the optical densities of the controls did not change for 2 hours in the incubator, we performed the calculations by taking the changes in the optical densities of the samples for each well separately, according to the formula:

$$\% \text{ of lysis} = \text{OD1} - \text{OD2} / \text{OD1} \times 100, \quad (1)$$

where OD1 is the optical density of the sample before incubation and OD2 is the optical density of the sample after incubation.

## 2.4. Statistical analysis

The optical density results were calculated for the control wells and experimental sera expressed as a percentage of the test culture lysis. Data were processed by independent t test with the fixe defect model using Data analysis tool pack, Microsoft Excel 2016, Microsoft Corporation Ltd. at a level of significance  $P < 0.05$ .

## 3. Results

### 3.1. Values for beta-lysine activity in the blood serum of broiler breeders

Measured values for beta-lysine activity in the blood serum of broiler breeders are presented in Table 1. Monitoring of changes in the indicator began at 6 weeks of age and continued until 36 weeks of age. Significant differences were found in beta-lysine activity at 18 weeks of age, coinciding with the laying of birds and the strong physiological stress that accompanies this process. Not coincidentally, at this stage, there is a slight increase in serum beta-lytic activity in the control group. The changes are physiologically conditioned.

Table 1. Beta-lysine activity (%) of blood serum in broiler breeders ROSS hybrid, n=45

Age, in weeks	Experimental flock $\bar{X} \pm \text{SD}$	Control flock $\bar{X} \pm \text{SD}$	P value
6	18.26±0.23	12.34±0.46	P<0.00001
12	21.58±0.42	14.05±0.63	P<0.00001
18	47.50±4.95	32.29±1.55	P<0.00001
24	29.86±1.05	15.27±0.95	P<0.00001
36	20.41±0.61	15.70±0.35	P<0.00001

The increase in beta-lysine activity in the blood serum of laying birds from the experimental flock is significantly more pronounced - 47.50% vs. 32.29% in controls. As can be seen from the table, the values of the studied indicator are higher during the whole observed period in the birds treated with the immunomodulator, while in the controls, the activity of beta-lysine is close to the physiological norm with a small increase during the laying period. The obtained data show an increase in beta-lysine activity in the blood serum of laying birds (18th week), significantly higher in the experimental flock. After the onset of lay, beta-lysine levels again decreased at 24 and 36 weeks, and in birds receiving the immunomodulator, it remained higher compared to controls.

### 3.2. Values for beta-lysine activity of egg white in broiler breeders

In research of egg white, we found higher beta-lysine activity in the experimental parent flock compared to the control. These differences are particularly pronounced on the 2nd and 7th days of laying. The results obtained by us show that beta-lysine is one of the earliest factors of non-specific

protection of the chicken embryo, which is active at the beginning of prenatal development. In the control herd, these values fluctuate physiologically between 28% and 15% activity. In birds treated with a polybacterial immunomodulator, beta-lysine activity in egg white was almost twice as high. This is an indicator directly related to the non-specific, humoral immune defense and the development of the embryo, as well as to the hatchability of the breeding eggs.

Table 2. Beta-lysine activity (%) of egg white in broiler breeders ROSS hybrid, n=45

Days of laying	Experimental flock $\bar{x} \pm SD$	Control flock $\bar{x} \pm SD$	P value
2	42.06±1.27	28.45±0.24	P<0.001
7	40.20±1.44	22.19±0.82	P<0.001
10	28.45±0.56	22.63±0.85	P<0.001
14	20.82±0.55	15.50±0.57	P<0.001

### 3.3. Values for beta-lysine activity in the blood serum of broilers

In research of blood sera from broiler chickens, we found that the activity of beta lysine generally had higher values in immunomodulator-treated birds. Already on the 10<sup>th</sup> day of the life of broilers, the studied indicator has an average value of 24.59, or 37 % more than the controls. On day 28, beta-lysine activity increased again to 37.35%, while in untreated birds, it remained at 16.54 % activity.

Table 3. Beta-lysine activity (%) in the blood serum of broilers ROSS hybrid, n=45.

Age in days	Experimental flock $\bar{x} \pm SD$	Control flock $\bar{x} \pm SD$	P value
10	24.59±0.69	15.53±0.52	P<0.001
18	25.14±0.36	17.44±0.27	P<0.001
28	37.35±0.86	16.54±0.69	P<0.001

## 4. Discussion

In all our blood serum and egg white tests, we found higher beta-lysine activity in birds from the experimental flocks receiving an immunomodulator. Probably, it is positively influenced by stimulation of the mucous membranes with the polybacterial immunomodulator, as well as by the action of the preparations "ASPIVIT C" and "BIOXAN", which help to increase the resorption surface of the intestinal mucosa. In addition, the application of the anti-stress preparation "ASPIVIT C" provides additional equalization in the conditions of the experiments that were conducted under production conditions. This makes it possible to exclude the influence of some technological stressors and to believe with a high degree of confidence that the increase in beta-lysine activity in experimental birds is due to the effect of the immunomodulator "AVIGEN". Our results also show that age is not a determinant of beta-lysine expression. Birds respond well regardless of age and category, and breeders treated with immunomodulator, lay eggs containing more beta-lysine, especially in the first 7 days after the onset of laying. Several studies on germ-free animals demonstrate that symbiotic bacteria and/or bacterial molecules (for instance, lipopolysaccharide,  $\beta$ -glucan, and peptidoglycan) can fully trigger adaptive immunity (Gensollen et al., 2016; Ganalvonarburg et al., 2016, Macpherson et al., 2017). The intestinal mucosa plays an important role in the initial triggering of the immune response and the following regulation of its maturation (Hrnciv et al., 2008).

Polybacterial immunomodulators are a powerful inducer of lysozyme and complement (Karakolev et al., 2014). The induction of beta lysine in egg white and blood serum is studied for the first time under the influence of the preparations we use – "AVIGEN", "ASPIVIT C" and "BIOXAN". The present experiments also establish their influence on the activity of beta-lysine, which is part of the non-specific immune factors in the blood serum. Obviously, beta-lysine is one of the earliest factors of innate immunity, as it is already present in the avian embryo, and its additional activity can be induced by lipopolysaccharides from enterobacteria included in the drinking water of birds, as our experiments

show. We evaluate the effect of the other two preparations only as supportive - anti-stress in "ASPIVIT C" and increasing the resorptive capacity of the mucous membranes in "BIOXAN".

In our previous studies, the physiological values of beta-lysine in the blood serum in broiler breeders and broilers were monitored. Unlike broilers, in parent flocks, there is a strong stress factor associated with the onset of laying and accompanying hormonal and immune rearrangement in the body (Karakolev, 2015; Karakolev and Nikolov, 2015). Nevertheless, the concentration of beta-lysine may be influenced by some factors that have a beneficial effect on the activity of complement, lysozyme, and other indicators of the natural (innate) immune response. A number of authors (Ganalvonarburg et al., 2016; Zemskov et al., 2018; Bozakova et al., 2018) consider the factors that may influence the mechanisms of non-specific, innate resistance and emphasize that polysaccharide substances are one of the most effective for this purpose. Hung et al. (2013) consider that AblAs from methanoarchaea are lysine 2,3-aminomutases that may function as potential biocatalysts for the synthesis of  $\beta$ -lysine *in vivo* and *in vitro*. Okanishi et al.(2013), and Zhang, et al.(2013), Weinert et al.(2013), also developed genetic methods for the biosynthesis of lysine for biotechnological purposes.

From the present experiments, it is clear that lipopolysaccharides from enterobacteria contained in concentrated form in the immunomodulator "AVIGEN" have a positive effect on the activity of beta-lysine fractions in the blood serum of broilers and especially in the parental forms during laying and about two weeks thereafter. The increased activity of beta-lysine is an important part of the non-specific immune defense and the possibilities for its activation in stressful periods of breeding are of particular importance for the health status of birds. Similar data in broilers was reported by Sotirov et al.(2021), which compared the effect of the application of *Schizochytrium limacinum* on some indicators of natural immunity in broilers. The authors found an increase in the activity of beta-lysine in the blood serum in experimental group III, accompanied by a slight decrease in the values of lysozyme and complement. These data also confirm the results of our previous studies on the inverse correlation between lysozyme and complement values on the one hand and beta-lysine activity on the other. Comparison of the results regarding the effect of *Schizochytrium limacinum* in the diet of broiler chickens and the measured activity of beta lysine of 11.49% show that beta-lysine is less affected than as a result of exposure to lipopolysaccharides from enterobacteria. Bozakova et al.(2018) investigated the effect of the immunomodulator "Immunobeta" on innate humoral immunity in laying hens and also found some effect on beta lysine activity. In the conditions of temperature stress, Bozakova et al.(2018) tracks the effect of the immunomodulator "Immunobeta" on innate humoral immunity in turkeys and laying hens, which supports our data on the special role of beta-lysine in stressful situations for the body.

After administration of the immunomodulator "AVIGEN", the activity of beta-lysine in the blood serum of broilers from the ROSS 308 hybrid reached 37.35%, which is significantly above the physiological limit observed in the control flock. The increase in the activity of beta-lysine in the blood serum in broiler breeders who took the immunomodulator in our experiments is especially significant and long-lasting. The discovery of almost twice the activity of beta-lysine in egg white shows the importance of beta-lysine as one of the earliest protective factors of innate immunity in birds and the importance of immunomodulators for their health status.

## Conclusion

Based on the results obtained, it can be concluded that the immunomodulator "AVIGEN", containing lipopolysaccharides from enterobacteria and taken with drinking water in an appropriate dosage, causes an increase in beta-lysine activity in the blood serum of broilers and broiler breeders. The effect of beta-lysine activity does not depend on the age and category of the birds.

For the first time, it is established that breeding eggs laid by broiler breeders receiving the immunomodulator "AVIGEN" have higher levels of beta-lysine, especially in the first weeks after laying.

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