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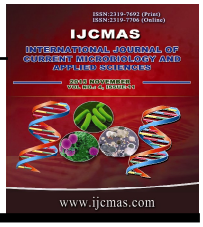


**Original Research Article**

# Growth Performance and Natural Humoral Immune Status in Broiler Chickens Treated with the Immunomodulator Natstim®

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**K e y w o r d s**

Natstim®, Chicken-broiler, Growth performance, Lysozyme, Complement activity

**A B S T R A C T**

The effect of the immunomodulator Natstim® (lyophilised killed bacterial cells from Escherichia coli 29, Escherichia coli J5, Staphylococus aureus 27/58 at amounts corresponding to $10^{10}$ cells of each species, stabilized with Dextrane 40) on growth performance and two factors of natural humoral immunity – serum lysozyme concentrations and the activity of the alternative pathway of complement activation (APCA) was investigated. The results for the experimental group showed a tendency towards higher live weight at 35 and 49 days of age. Experimental broilers had a higher productivity index than control broilers. The relative ratios of slaughter traits to grill weight showed better breast and thigh proportions in the treated group – 34.5%, 30.1% vs. 31.9%, 29.9% in the untreated group. Natstim® had a significant effect on serum lysozyme concentrations (decreased almost 200%) but the activity of the alternative pathway of complement activation was not affected. These results indicate that the immune system was already activated and could effectively control infectious penetrating in the chicken's body.

## Introduction

The improvement of innate resistance to infectious disease is one of mechanisms for achievement of better health status and higher productivity in poultry. The immune system could be activated not only through infectious agents, but also via injection of lipopolysaccharides isolated by bacteria (Webel *et al.,* 1997). The immune response after challenge with natural products containing lipopolysaccharides from *E. coli and Salmonella typhimurium* is manifested initially in the blood serum and after that in the eggs (Sunwoo *et al.,* 1996). The treatment with purified lipopolysaccharide preparations imitates the acute response to Gram-negative infections (Brunel, 1994). The components of the immune system are not isolated from each other (Adinolfi *et al.,* 1966; Glynn, 1969; Heddle *et al.,* 1975). The classical pathway of the complement system, which is activated in the presence of antibodies, kills bacteria over a longer period of time, but the lytic reaction is enhanced by the presence of lysozyme.

Sotirov *et al.* (2000) established a positive effect of the probiotic Lacto-Sacc on serum lysozyme concentration and on the alternative pathway of complement activation in broiler chickens. The growth promoters Pharmastim and Avilamycin did not have any effects on those parameters. However, an experiment with the same preparations in 180 turkeys (Sotirov *et al.*, 2001) showed comparable results to those in broilers. Karakolev *et al.* (2013a,b) and Gospodinova *et al.* (2013) reported a statistically significant stimulating effect of the immune booster Helpankar on serum lysozyme concentration, gamma interferon and the activity of the alternative pathway of complement activation in layer hens. Treatment of layer hens with the immune stimulator Natstim® increased egg production by 1.72%, average egg weight by 0.92 g, and the number of hatched chickens by 1.31% (Chotinski *et al.*, 2011).

In the available literature, there are no data for application of the polybacterial immune stimulator Natstim® on productive performance and natural humoral immunity in broiler chickens. The aim of presented experiment is to determine the effect of Natstim® on growth performance and two factors of natural humoral immunity – serum lysozyme concentrations and the activity of the alternative pathway of complement activation (APCA).

## Material and Methods

### Design

The experiment was conducted in 2014 in the Experimental Poultry Base at the Institute of Agriculture, Stara Zagora. One hundred male Ross 500 broiler chickens were divided into two groups of 50 birds: experimental and control. The birds were reared on deep, permanent wooden shavings. The feeding was done with compound feeds with and without Natstim®. Feeding was performed according to a schedule providing all necessary nutrients for the respective fattening stage – starter (days 1–10), grower (days 11–35) and finisher (days 36–49). All birds were weighed at the end of the respective periods, i.e. on days 10, 21, 35 and 49.

### Treatment

The experimental chickens were treated with Natstim® from day 2 to day 11 at a dose of 300 mg/kg feed daily. The used preparation is a polybacterial immunostimulant containing lyophilised killed bacterial cells from *Escherichia coli 29*, *Escherichia coli J5*, *Staphylococcus aureus* 27/58 at amounts corresponding to $10^{10}$ cells of each species, stabilized with Dextrane 40. The preparation was dissolved in 50 ml water and pulverized in 3 kg feed. The first blood samples were collected on 20 days of age from 25 chickens and the dose was increased to 400 mg/L in drinking water over 10 days to achieve a better immune response. The second blood sampling was on 40 days of age and serum lysozyme concentrations and complement activity (determined as alternative pathway of complement activation) were investigated. The amount of Natstim® for the period between 2 and 11 days of age was 106.77 mg per chick and 818.43 mg per chick during the second period (days 30–40).

### Methods

Serum lysozyme concentrations were assayed by the method of Lie *et al.* (1985) and APCA by the method of Sotirov (1986).

Feed consumption was measured on a daily basis during the different periods and the total calculated for the entire period of the

trial. The death rate and the health of chickens were also assessed on a daily basis. At 49 days of age after 12-hour fasting, slaughter analysis was performed on 3 broiler chickens from each group and feed conversion ratio was calculated. Slaughter yield and percentage ratios between the different body parts and productivity index (PI) were calculated.

PI = Live weight (kg) x survival rate (%) x 100/fattening period (days) x feed expenditure per 1 kg weight gain)

**Statistical analysis**

Data were submitted to statistical analysis by means of ANOVA/MANOVA and LSD post hoc with the software package Statistica 8 (StatSoft, 2009).

**Results and Discussion**

Table 1 shows the live weight of broiler chickens from the two groups during the different age periods. On the 10th day of age, the chickens' weights were 265.62 g and 262.82 g respectively, and the difference was non-significant. On the 21st day, the experimental group was heavier by 71.32 g or 6.9%. The tendency towards more intensive growth in broilers supplemented with Natstim® was preserved and by the 35th day, they were 203.34 g (9.3%) heavier. The difference was statistically significant at $P<0.001$. By 49 days of age, the supplemented and control groups attained mean slaughter weights of 3250 g and 3066.66 g respectively. During the grower period, the growth performance of experimental broiler chickens was better, which coincided with the repeated dietary supplementation of Natstim® for another 10 days after the first blood sampling. We believe that this fact was responsible for the high survival rate of 100% and this suggestion was supported by blood serum lysozyme and complement levels.

Presented as feed intake per 1 kg weight gain (Table 2) the experimental group has consumed 1.988 kg feed vs. 2.055 kg for controls or by 3.2% lower. For a more objective evaluation of broiler performance, the productivity index was calculated as a measure of economic efficiency. The analysis of data for this index (Table 3) showed that the experimental group exhibited higher values - 333.7% versus 298%. Experimental broiler chickens had a higher productivity index by 35.7 points (10.7%) than control birds.

Table 4 presents the absolute values of studied slaughter traits of both broiler chickens groups. The differences between breast and thigh weights (785 g and 684 g vs 699.33 g and 656.66 g) were statistically significant ($P<0.01$). The other studied slaughter traits did not exhibit any statistical differences between the groups. The slaughter yield of experimental chickens was 74.9% and that of controls – 76.5%. The grill to bratfertig ratio was identical in both groups. The calculated ratio of studied slaughter traits to grill in both groups showed a superiority of breast and thigh weight by 34.5% and 30.1% in the treated groups vs. 31.9% and 29.9% in the control group. The breast weight in the experimental group was 4.4% higher while thigh weights were 2% higher. Despite the statistically significant differences in slaughter weight between groups (Table 4), there were also difference between bratfertig/grill, boneless breast/grill and thigh/grill ratios. The other studied carcass parts ratios in both groups of broilers were similar.

As could be seen from table 5 serum lysozyme concentration and complement (APCA) activity were almost identical in

both groups of chickens at 20 days of age. This could be attributed to the very early age of chickens and the immature immune system. Furthermore, the sampling was done rather early after the treatment and the immune system of chicks probably had not yet reacted to the treatment. This required a second series of application but this time Natstim® was given with drinking water at a dose of 400 mg/L water over 10 days. The results from the table 6 showed that the preparation had a serious effect on serum lysozyme concentrations which was almost 50% lower in treated chickens compared to that of controls. This interesting fact could be explained by the circumstance that lysozyme activity was mainly directed to gram-positive bacteria, such as *Staphylococcus aureus*. The result showed that the already activated immune system of birds was capable to cope with bacterial cells and lysates, which penetrated the body. It should be emphasized that mucosal immunity is activated prior to systemic immunity and this had an effect on both the time for response from the part of the latter and on the type of immune reaction. The results about APCA levels, in our opinion, were rather strange, as they were almost identical in both groups. This could be attributed to the specifics of used E. coli strains, which are not specific for gallinaceous birds and thus, did not elicit an APCA response. The APCA activity was affected only by the age of the chickens ($P<0.001$).

**Table.1** Time course of live weight in broiler chickens (g)

| Groups | Day 10 | Day 21 | Day 35 | Day 49 |
|---|---|---|---|---|
| Control | 262.82±11.82 | 963.02±47.37 | 1976.66±36.66 | 3066.66±33.33 |
| Experimental | 265.62±9.74 | 1034.34±19.98 | 2180±20*** | 3250±50*** |

*** P<0.001

**Table.2** Feed conversion (kg/kg)

| Groups | Days 1-10 | Days 11-21 | Days 22-35 | Days 36-49 |
|---|---|---|---|---|
| Control | 1.387 | 1.583 | 1.782 | 2.055 |
| Experimental | 1.340 | 1.561 | 1.765 | 1.988 |

**Table.3** Productivity index (PI)

| Groups | Live weight at 49 days of age, kg | Survival rate, % | Feed conversion ratio (kg/kg) | PI | |
|---|---|---|---|---|---|
| | | | | absolute | relative |
| Control | 3.066 | 98 | 2.055 | 298 | 89.3 |
| Experimental | 3.25 | 100 | 1.988 | 333.7 | 100 |

**Table.4** Slaughter analysis at 49 days of age (g)

| Parameters (g) | Control group | Experimental group | Carcass parts ratios (%) | |
|---|---|---|---|---|
| | | | Control group | Experimental group |
| Live weight | 3066.66±33.33 | 3250±50.00*** | | |
| Bratfertig | 2346±28.57 | 2431.50±37.5 | 76.5 | 74.9 |
| Grill | 2197±38.73 | 2278±26.00 | 93.7 | 93.7 |
| boneless breast | 699.33±10.98 | 785±29.00** | 31.9 | 34.5 |
| Thighs | 656.66±9.93 | 684±9.00** | 29.9 | 30.1 |
| Wings | 243±4.58 | 228±9.00 | 11.1 | 10 |
| Gizzard | 35.33±3.92 | 29±4.00 | | |
| Liver | 68.66±8.5 | 70±10.00 | | |
| Heart | 15.33±2.60 | 13±2.18 | | |
| Neck | 49±3.05 | 49±2.64 | | |
| edible offal | 115±7.00 | 120.66±12.38 | 5.3 | |
| rib cage | 577.5±13.5 | 582±3.00 | 26.3 | |
| abdominal fat | 22.5±12.5 | 20.66±2.18 | 1.1 | |

** P<0.01; *** P<0.001

**Table.5** Serum lysozyme concentration and activity of alternative pathway of complement activation (APCA) in chickens treated with Natstim®

| Parameters / Groups | lysozyme | APCA |
|---|---|---|
| Controls, 20 days of age | 0.48±0.04 | 365.79±14.91 |
| Treated, 20 days of age | 0.49±0.049 | 378.82±8.74 |
| Controls, 40 days of age | 31.11±7.34*** | 526.33±21.69 |
| Treated, 40 days of age | 14.12±2.52 | 526.42±13.66*** |

*** P<0.001

Analysing our results and combining them with the results of the other authors, we could see that both the period of time and the dose of administrated immunomodulator influenced the humoral innate immune response and growth performance of the chicken broilers. We established that higher doses and longer period of challenge have significant effect on productive traits and serum lysozyme concentrations. Similar results reported Awaad *et al.* (2013). These authors reported that treatment of broiler chickens with a combination of soluble β1.3, D-Glucan and *Propionibacterium granulosum* (Betamune®) improved chicken growth performance and chalenged their immune response and enhanced their vaccination effectiveness. The non-specific defence factor lysozyme is mainly found in body fluids and secrets, being active mainly against Gram-positive bacteria, but assisting also the bactericidal activity of complement against Gram-negative pathogens (Adinolfi *et al.*, 1966; Glynn, 1969; Heddle *et al.*,

1975). Phagocytizing cells are a source of lysozyme and when they are activated, lysozyme concentrations are increased. The activity of the humoral specific immunity presented by the different immunoglobulin classes is manifested considerably later, only after invasion of the microbial pathogen in the host and the subsequent challenge of its immune system. This takes at least 2-3 weeks and that is why the natural immunity status is so important, being the incentive behind the numerous investigations for detection and production of new, more efficient immunomodulators.

Due to the short life of broilers, their immune system is not able to response adequately to antigenic challenge, but the fact that serum lysozyme was altered is an evidence that the preparation Natstim® had an effect on the general susceptibility of chickens, making them more resistant to bacterial infections. Sotirov *et al.* (2000) established a positive effect of the probiotic Lacto-Sacc on serum lysozyme concentration and on the alternative pathway of complement activation in broiler chickens. Similar experiment was performed by Sotirov *et al.* (2001) in turkeys and the results were comparable to those in broilers. Karakolev *et al.* (2013a,b) and Gospodinova *et al.* (2013) reported for stimulating effect of the immunomodulator Helpankar on serum lysozyme concentration, gamma interferon and the activity of the alternative pathway of complement activation in layer hens. Chuammitri (2010) suggests that the genetic background underlying the innate cellular immunological functions of heterophils could be enhanced by feeding dietary immunomodulators, leading to partial reduction in *Salmonella enterica* shedding and colonization in chickens. According to the same author combined approach of genetic selection and dietary immunomodulation could be used to assist the poultry industry in controlling and reducing risk of public health risk associated with *Salmonella enterica* contamination of chicken products. Chotinski *et al.* (2011) let us know that treatment of layer hens with Natstim® increased egg production by 1.72%, average egg weight by 0.92 g, and the number of hatched chickens by 1.31%

The results from the present study showed a tendency towards higher live body weight, higher productivity index, better breast and thigh proportions and substantial effect on serum lysozyme in chickens broilers treated with the immunomodulator Natstim®.

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