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RESEARCH ARTICLE

Effect of Immunomodulator "Immunobeta" on Humoral Innate and Acquired Immune Response in Layer Hens

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ABSTRACT

The effect of immunomodulator "Immunobeta" was investigated on 6750 layers hybrid Loman Brown divided in two equal groups – control and experimental. The layers were housed in enriched cages constructed in large battery for 3375 hens – one for each group. The experiment started when hens were at 18 weeks of age. The control hens received the usual diet for their age, whereas the experimental birds received a diet supplemented with "Immunobeta" at a dosage of 4 kg/tone for 2 months. The first analysis was conducted prior to treatment (Test I), while the second study was done at the end of treatment, i.e. 2 months later (Test II). The evaluation of residual effect of the immunomodulatory substance was checked one month after the end of treatment (Test III). Twelve hens from each group were analysed in each test. The results indicated that "Immunobeta" increased lysozyme concentration in blood sera and hens' egg white. It was found that "Immunobeta" elevated the activity of alternative pathway of complement activation and increase the concentration of IgM as well.

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INTRODUCTION

The present stage of social development, characterized with excessive use of antimicrobial drugs in poultry farming and rapidly increasing antibiotic resistance, requires research for new alternatives for treatment and prevention of diseases. Nowadays the goal is the reduction of antibiotics used and improvement in poultry immunity, production and disease resistance. Since 2006, the European Union has banned the use of nutritional antibiotics as growth promoters in livestock husbandry. An EU-wide ban on the use of antibiotics as growth promoters in animal feed enters into effect on January 1, 2006. The last 4 antibiotics which have been permitted as feed additives to help fatten livestock will no longer be allowed to be marketed or used from this date (file:///C:/Users/Work%20PC%201/Downloads/IP-05-1687_EN.pdf).

A possible alternative for restriction of the excessive use of antibiotics and to promote antibiotic-free animal production is the utilization of yeasts and active products obtained from yeasts – beta glucans, mannan-oligosaccharides, and nucleotides. Their use in poultry farming is reported to result in improved immunity and welfare through positive effect on non-specific humoral immunity, growth development and production under both normal conditions and stress. Data for the beneficial effect of yeast products on gastrointestinal physiology, morphology and microbiology in broiler chickens are reported by Al-Mansour *et al.* (2011), Fathi *et al*. (2012), and Bozakova *et al.* (2016). The effects of the dietary supplementation of poultry with yeasts on enhancing humoral immunity, disease resistance and reduction of death rates was investigated by Fathi *et al.* (2012). The beneficial effects from the application of the probiotic Lacto-Sacc on serum lysozyme activity and alternative pathway of complement activation in broiler chickens was observed in previous projects by our research group (Sotirov *et al.*, 2000). In an experiment with 180 turkeys treated with the same preparations, Sotirov *et al.* (2001) obtained comparable results. In sows and piglets treated with Sel-plex Sotirov *et al.* (2007) have shown stimulating effects on serum lysozyme and complement.

Karakolev *et al.* (2013a&b) and Gospodinova *et al*. (2013) reported statistically significant stimulating effect

of the immunostimulant "Helpankar" on blood serum lysozyme, alternative pathway of complement activation and gamma interferon in layer hens.

The above mentioned authors and our data motivated us to investigate the possibilities for improvement of humoral immunity in layer hens via administration of the immunomodulator "Immunobeta".

MATERIALS AND METHODS

The experiment was performed with 6750 layers (hybrid Loman Brown) divided in two equal groups (n=3375) – one control and one experimental. The birds were owned by Divex-2-Georgi Georgiev Co. and the poultry farm was located in the land of Malka Polyana settlement, Aytos municipality. The layers were housed in enriched cages constructed in large battery for 3375 hens each. The experiment started when the birds were 18 weeks of age. Those housed in the control facility received the usual diet for their age, whereas the ratio of layers from the experimental facility was supplemented with "Immunobeta" (CHEMIFARMA S.p.A., Animal nutrition products, Italy) at a dosage 4 kg/tone in continuation of 2 months. The "Immunobeta" contains three important active ingredients: ß-glucans-30%, mannan oligosaccharides-25%, nucleotides-5%, vitamins B1, B2, B6, Niacin, Pantothenic acid, Folic acid, Holin, Iron, Zinc, Manganese and Cooper. The first investigations were done prior to the treatment with "Immunobeta" (I Test), the second – at the end of treatment, i.e. after 2 months (II Test) and the third – one month after the end of treatment to evaluate the residual effect of the immunomodulator (III Test). Twelve hens from each group were analysed.

Serum lysozyme concentrations were determined by the method of Lie *et al.* (1985), alternative pathway of complement activation (APCA) by the method of Sotirov (1986), beta-lysine concentrations by method of Buharin *et al.* (1977) modified by Karakolev and Nikolov (2015). Concentrations of IgG and IgM were measured using ELISA kits (Chicken IgM and IgG ELISA kits, Life Diagnostics, Inc., USA).

RESULTS

As is seen from Table 1, serum lysozyme concentrations at the beginning of the experiment were equal for the both groups, i.e. a good background for proper experimentation. At the time of the second sampling (end of treatment), serum lysozyme concentrations in experimental group were significantly higher compared to the beginning of experiment (P < 0,001). One month later lysozyme concentrations in the same group were also significantly higher compared to the beginning of experiment (P<0.001). In controls, lysozyme concentrations were also increased but with lower values than the experimental group. In the same test periods APCA activity was significantly higher in the control group (P<0.001). This fact provides a proof that "Immunobeta" has activated APCA and thus, has markedly improved the innate resistance of birds. The results allow us to affirm that the preparation "Immunobeta" has a positive effect on humoral factors of innate immunity in layer hens. This conclusion was also supported by the results from the third sampling (one month after the end of the treatment), when the obtained results were almost identical to those from the second treatment. Additionally, Table 1 presents the data from the analysis of serum beta-lysine. It was demonstrated that beta-lysine concentrations in layers are increased under influence of two factors: age and egg production. At the same time, in experimental birds, this parameter was insignificantly higher which could be attributed to the antigen challenge from beta-glucans and mannan oligosaccharides-important ingredients of the preparation "Immunobeta". The findings evidenced that the immunomodulator had a marked influence on the humoral innate immunity of layers. The same table presents the blood serum concentrations of IgG and IgM in layers. It is evident that both parameters are significantly influenced by the age of birds. In the experimental group, increased IgM concentration was due not only to age, but also to the supplementation with "Immunobeta" because prior to the treatment IgM concentration was as low as 35.01 ng/ml, whereas at the end of the experiment (two months later) it became significantly higher than controls (P<0.01), which is exclusively due to the immunogenic response to "Immunobeta". Since IgM is an early humoral factor of the primary specific immunity, our findings show that "Immunobeta" had a positive impact not only on the humoral innate immunity, but also on specific immunity, i.e. it boosts the effect of vaccinations in layers.

Following the end of treatment, we studied the lysozyme concentrations in egg white. Table 2 presents that lysozyme levels were considerably higher in eggs produced by treated hens, compared with the controls. A second investigation was performed two months after the first one to verify if the immune booster had any residual effect. Acceding to the results, residual effect was not observed but a highly significant effect of age was noticed.

Table 1: Effect of immunomodulator "Immunobeta" on serum lysozyme concentrations, APCA activity, Beta-lysins IgG and IgM in layer hens

Time of treatment	Lysozyme concentrations (mg/L)		APCA activity (CH50)		Beta-lysins (%)		IgG (ng/ml)		IgM (ng/ml)	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
I test	0.22±0.02	0.22±0.03	450.44±17.67	589.11±18.44	34.7± 2.9	47.8±5.5	54.6±5.2	45.2±11.5	42.7±4.6	35.01±3.08
II test	1.10±0.11	1.38±0.17***	618.46±18.07***	530.58±12.76[a]	47.6± 5.01	57.9± 6.8	113.8±2.6	114.8±2.7	45.7±2.6	45.8±2.9
III test	0.94 ±0.12[a]	1.23±0.22***	714.65±16.44[a]	696.88±24.27	81.3±32.4	59.7±1.8	121.1±0.5	120.9±0.6	43.1±4.5	45.8±2.9**

* differences between each test into the groups; ** P<0.01; *** P<0.001; [a]differences between control and experimental groups in each test; [a]P<0.001.

Table 2: Effect of immunomodulator "Immunobeta" on egg white lysozyme concentrations in layer hens (mg/L)

Stage	Lysozyme	
	Control group	Experimental group
To the end of treatment (after 2 months treatment)	778.38±51.5	1289.76±250.86
One month after the treatment	1839.06±157.34***	1636.77±180.23

*differences between each test into the groups; *** P<0.001.

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DISCUSSION

The above presented results exhibit that "Immunobeta" has positive influence on natural and specific humoral immunity of layer hens. As described above, at the end of treatment serum lysozyme concentrations in experimental group were significantly higher compared to the beginning of experiment (P<0.001). One month later lysozyme concentrations in the same group were also significantly higher compared to the beginning (P<0.001). Czech *et al.*, (2014) also observed that the addition of *Yarrowia lipolytica* yeast in a dose of 6% increase plasma lysozyme in turkey hens. Tawab *et al*., (2015) revealed that broilers fed on prebiotic increase the lysozyme concentrations more than broilers fed on probiotic. In our experiment APCA activity was significantly higher in the control group (P<0.001). This fact provides proof that "Immunobeta" has activated APCA by its consumption and by this way „wake up" the innate immunity of birds. The results from the third sampling (one month after the end of the treatment), were almost identical to those from the second treatment. These results allow to affirm that the preparation "Immunobeta" has a positive effect on humoral factors of innate immunity in layer hens. Similar data reported Sotirov *et al*. (2000, 2001, 2007) in chicken broilers and pigs treated with different immunomodulators.

We have found that beta-lysins are increased under influence of two factors - age and egg production. In experimental birds, this parameter was insignificantly higher which could be attributed to the antigen challenge from beta-glucans and mannan oligosaccharides-important ingredients of the medicament "Immunobeta". These findings evidence that the immunomodulator had a marked influence on the humoral innate immunity of layers. Karakolev *et al*. (2013a&b) and Gospodinova *et al*. (2013) reported significant stimulating effect of the immunostimulant "Helpankar" on blood serum lysozyme, gamma interferon and APCA in layer hens. Our results are confirmed by the experiments of Karakolev and Nikolov (2015), which have also found that beta-lysine concentrations increased proportionally to egg production and hence, that beta-lysins had not only a protective function but were also related to certain extent to the productive performance of layers.

The blood serum concentrations of IgG and IgM as main humoral factors of adapted immunity in layers, were also tested. It is easily noticed that to the end of experiment IgG concentrations were the same in control and experimental groups. Meanwhile the concentrations of IgM were significantly higher in experimental group (P<0.01). It is known that IgM is humoral factor of early specific immunological response after antigen challenge. Similar results reported Ullah *et al.* (2014) and Li *et al*. (2016). Veterinary practice requires very high antibody titers after vaccination of hens against infectious agents. Positive effect of mananoligosacchrids and betaglucans were reported by Rajapakse *et al.* (2010), Khatoon *et al.* (2017) and Ghasemiana and Jahanian (2016). These findings show that "Immunobeta" had a positive impact not only on the humoral innate immunity, but also on specific immunity, which means that it boosted the effect of vaccinations in layers.

It is well known that manan-oligosacchrids and beta-glucans have additional positive effects on the immune system. Zhang *et al*., (2012) demonstrated that yeast cell walls supplementation has beneficial effects on the immunosuppressive effects of cyclosporine A challenge in broiler chickens. Sadeghı *et al*., (2013) showed that dietary inclusion of prebiotic-based mannan-oligosaccharide and β-glucan displays an efficacy on chicks infected with pathogens and can improve the immune responses and health of infected chicks. Similar results for positive effect of mannan-oligosaccharide and β-glucan on chicken's immune system reported by other authors (Shao *et al*., 2013; Shanmugasundaram *et al*., 2013; Huff *et al.*, 2013). Muzaffar *et al*., (2016) let us know for antitoxic and immunostimulating effects of dietary supplementation with β-glucan and mannan-oligosaccharides isolated from Saccharomyces cerevisiae (S. cerevisiae) in broiler chickens challenged with naturally feed-borne *A. fumigatus*. The results from immunological analysis found that when the feed was supplemented with β-glucan and mannan-oligosaccharides and broiler chickens were challenged with *A. fumigatus*, spleen and thymus indices were improved, the cytokine concentrations in serum were increased, and the activities of heterophils and lymphocytes were up-regulated. Similar results reported Valtchev *et al*., (2015) in ducks.

Our data displays lysozyme concentrations in egg white many times higher than these in blood sera (Table 2). We suggest that the higher concentrations of lysozyme and beta-lysine in egg white, the better health and productive traits of new hatched chickens can be expected. This is due to the important role of lysozyme for the durability of eggs and the better protection of chicken's embryos during incubation. This means more hatched chickens with higher viability.

On the base of our results described above we can conclude that the immune booster "Immunobeta" improves the natural and acquired humoral immune response in layer hens.

Authors contribution: NB-JV and MP conceived and designed the study. LS and TK–DL, MC and IL executed the experiment and analyzed the sera. DG-IJ analyzed the data.

REFERENCES

Al-Mansour S, Al-Khalf A, Al-Homidan I *et al.*, 2011. Feed efficiency and blood hematology of broiler chicks given a diet supplemented with yeast culture. Int J Poult Sci 10:603-7.

Bozakova N, Dimitrov D, Sotirov L, *et al*., 2016. Effect of immunomodulatory Immunobeta on histological features of intestinal villi and crypts in broiler chickens. Cienc Tec Vitivinic, 4:141-9.

Buharin OV, Luda AP and Bigeev RI, 1977. Fotonefelometricheskij metod opredelenia beta-lizinov v sivarotke krovi. In: Sistema beta-lizina I ee rol v klinicheskoj I eksperimentalnoj medicine (Karpov SP, ed): Izdatelstvo Tomskogo Universiteta, Tomsk, USSR pp:52-3.

CHEMIFARMA S.p.A., Catalogue Products for domestic animals, TZAR-05 Ltd. I-st Nutrative supplementations Line I, 4. http://veterinarna-apteka.com.

Czech A, Merska M and Ognik K, 2014. Blood immunological and biochemical indicators in turkey hens fed diets with a different content of the yeast Yarrowia lipolytica. Ann Anim Sci 14:935-46.

Fathi MM, Al-Mansour S, Al-Homidan A, *et al*., 2012. Effect of yeast culture supplementation on carcass yield and humoral immune response of broiler chicks. Vet World 11:651-7.

Uncorrected Proof

Ghasemiana M and Jahanian R, 2016. Dietary mannan-oligosaccharides supplementation couldaffect performance, immunocompetence, serum lipidmetabolites, intestinal bacterial populations, and ilealnutrient digestibility in aged laying hens. Anim Feed Sci Technol 213:81-9.

Gospodinova K, Rumen K, Sotirov L, *et al*., 2013. Quantitative assessment of Interferon in blood serum of layer hens following treatment with polybacterial immunomodulator. 8th Balkan Congress of Microbiology "Microbiologia Balkanika' 2013", October 2-5, 2013, Veliko Tarnovo, VM13 p:89.

Huff GR, Huff WE, Jalukar S, *et al.,* 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/Escherichia coli challenge. Poult Sci 92:655-62.

Karakolev R, Sotirov L, Bonovska M, *et al*., 2013(a). Quantitative assessment of lysozyme and complement in blood serum of layer hens treated with polybacterial immunomodulator. 8th Balkan Congress of Microbiology "Microbiologia Balkanika' 2013", October 2-5, 2013, Veliko Tarnovo, Bulgaria, VM14, p. 89.

Karakolev R, Sotirov L, Bonovska M, *et al*., 2013(b). Influence of age, technologies of growing and polybacterial immunomodulator on serum lysozyme concentrations and complement activity in laying hens. 8th Balkan Congress of Microbiology "Microbiologia Balkanika' 2013", October 2-5, 2013, Veliko Tarnovo, Bulgaria, VM15 p:90.

Karakolev R and Nikolov D, 2015. Fluctuations in serum beta-lysin activity in laying hens depending on age, hybrid type, and rearing method. Ptitsevadstvo 3:18-9.

Khatoon A, Khan MZ, Zain ul Abidin *et al.,* 2017. Mitigation potential of distillery sludge (DS) against ochratoxin A induced immunological alterations in broiler chicks. World Mycotox J 10:255-62.

Li XH, Chen YP, Cheng YF *et al*., 2016. Effect of yeast cell wall powder with different particle sizeson the growth performance, serum metabolites, immunityand oxidative status of broilers. Anim Feed Sci Technol 212:81-9.

Lie O, Solbu H and Sued M, 1985. Improved agar plate assays of bovine lysozyme and haemolytic complement activity. In: Markers for resistance to infection in dairy cattle. Dissert., National Veterinary Institute, Oslo, Norway, Chapter V 1-12.

Muzaffar H, Khaliq T, Ali Khan J, *et al*., 2016. Effect of Protein, Probiotics and Vitamins Supplementation on Semen Quality and Immunohistochemistry of Pituitary Gland in Molted Male Layer Breeders. Pak Vet J 36:149-52.

Rajapakse JRPV, Buddhik MDP, Nagataki M, *et al*., 2010. Effect of Sophy β-Glucan on Immunity and Growth Performance in Broiler Chicken. J Vet Med Sci 72:1629-32.

Sadeghı AA, Mohammaı A, Shawrang P *et al*., 2013. Immune responses to dietary inclusion of prebiotic-based mannan-oligosaccharide and β-glucan in broiler chicks challenged with Salmonella enteritidis. Turk J Vet Anim Sci 37:206-13.

Shao Y, Guo Y and Wang Z, 2013. β-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with Salmonella enterica serovar Typhimurium. Poult Sci 92:1764-73.

Shanmugasundaram R, Sifri M and Selvaraj RK, 2013. Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection. Poult Sci 92:358-363.

Sotirov LK, 1986. Method for determination of the alternative pathway of complement activation in some animals and man. In: Forth Scientific Conference of Agriculture, Stara Zagora, Bulgaria 1-10.

Sotirov LK, Denev SA and Georgieva VK, 2000. Effect of different growth promoters on lysozyme and complement activity of broiler chicks. Bul J Agric Sci 6:75-82.

Sotirov L, Denev S, Tsachev I, *et al*., 2001. Effect of different growth promoters on lysozyme and complement activity. II. Studing in turkeys. Rev Med Vet 152:67-70.

Sotirov L, Goundasheva D and Andonova M, 2007. Sel-plex and sodium selenite dietary supplements with resulting serum lysozyme and complement activities in sows and progeny during post partum periods. TJS 5:20-7.

Tawab AAA, Amar AM, Elshorbagy MA *et al*., 2015. Impact of probiotic and prebiotic on the lysozyme response of broilers against clostridial infection. BVMJ-28:208-13.

Ullah MI, Akhtar M, Iqbal Z *et al*., 2014. Immunotherapeutic activities of mushroom derived polysaccharides in chicken. Int J Agric Biol 16:269-76.

Valtchev I, Koynarski T, Sotirov L, *et al*., 2015. Effect of aflatoxin B1 on Moulard duck's natural immunity. Pak Vet J 35:67-70.

Zhang S, Liao B, Li X, *et al*., 2012. Effects of yeast cell walls on performance and immune responses of cyclosporine A-treated, immunosuppressed broiler chickens. Brit J Nutr 10:858-66.

Uncorrected Proof