Possibilities for improvement of humoral innate immunity in turkeys and hens in conditions of thermal stress by the immunomodulator Immunobeta

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Summary

The experiments for evaluation of the effect of Immunobeta were performed with turkeys and organically reared layer hens. Turkeys received Immunobeta at a dose of 4 g/kg feed during the beginning of lay (cold period), thermoneutral period and hot summer period. In hens, two dietary levels of Immunobeta were tested: 2 g/kg and 4 g/kg. Blood serum and egg lysozyme levels, alternative pathway of complement activation (APCA) and beta-lysins concentrations were determined. It was found out that Immunobeta had a beneficial effect on serum lysozyme, APCA activity and beta-lysins in turkeys and hens. The lysozyme concentration in egg white was statistically significantly higher in eggs produced by birds treated with both doses of the immunomodulator.

Key words: Immunobeta, lysozyme, complement, beta-lysins, turkeys, hens.

Introduction

In modern society, the welfare aspects of birds' prophylaxis and treatment become especially important. During the last two decade, there are a number of potential non-therapeutic alternatives of antibiotic growth promoters (AGPs) in animal nutrition and production of safety (antibiotic-free) products, including probiotics, prebiotics, symbiotics, immunostimulants, direct feed microbials and etc. (Denev, 1996, 2006, 2006a, 2008; Denev *et al.*, 2000, 2006b, 2009; Staykov *et al.*, 2007; Huyghebaert *et al.*, 2011). A lot of them demonstrated positive results, equivalent to the AGPs, but without the stigma of increasing antimicrobial-resistant bacteria. They stimulated probiotic intestinal flora, ecological balance, inhibition of pathogens, humoral immunity, gut health, animal welfare, growth performance, quality and safety of animal products (Denev, 2008; Zhang and Kim, 2014; Chen *et al.*, 2017).

There are no reports about the effects of the immunomodulator Immunobeta on the innate immunity of turkeys and layer hens at the time of beginning of lay as well as in birds under heat stress. The purpose of these experiments were to investigate the effects of the immunomodulator Immunobeta on serum lysozyme concentrations, alternative pathway of complement activation and beta-lysins in turkeys and organically reared layers in conditions of environmental thermal stress.

Material and Methods

Experimental design

The effects of Immunobeta were evaluated on turkeys and hens in a free-range system during 2015-2016. The birds were treated with Immunobeta as per recommendations of the manufacturer Chemifarma, Italy. The immunostimulatory preparation was produced by enzymatic autolysis of selected yeast strains (*Saccharomyces cerevisiae*) followed by natural extraction of components of yeast cells. The immunomodulator contains three important components: beta-glucans (30%), mannanoligosaccharides (25%) and nucleotides (5%). The experiments for evaluation of immunomodulator's effects in turkey breeders and organic hens were performed during different periods of the year: beginning of lay (cold period), thermoneutral period and hot summer period.

1. The study on the effect of the immunomodulator on turkey breeders took place in the turkey farm of the Agricultural Institute, Stara Zagora with 30 turkey breeders (15 experimental and 15 control birds). The influence of the preparation was tested from February

to August 2015. Tested birds received the immunomodulator Immunobeta at a dose of 4.0 g/kg feed during the beginning of egg lay (February-March 2015, cold period), the thermoneutral period (April-May 2015) and the hot summer period (June-July 2015). During these periods, the blood serum and egg white lysozyme, alternative pathway of complement activation (APCA) and beta-lysins were determined. The duration of the experiment was 7 months.

2. The study on the effect of Immunobeta on humoral immunity of hens reared in a free-range system was performed in the Experimental farm of the Agrarian University – Plovdiv from November 2015 (19 weeks of age) to July 2016 (54 weeks of age). The tests were carried out on 75 dual-purpose rural hens Tetra Super Harco (originating in Babolna Tetra Kft, Hungary), housed in sleeping houses and walking yards. The effects of two dietary Immunobeta levels were followed out: 2.0 g/kg and 4.0 g/kg. The birds were divided in three groups: Group I (control), Group II (supplemented with 2.0 g/kg) and Group III (supplemented with 4.0 g/kg). The effect of the immunomodulator was monitored during three subperiods: beginning of egg lay (January-March 2016, cold period), thermoneutral period (April-May 2016) and hot summer period (June-July 2016). During these periods, the blood serum and egg white lysozyme, alternative pathway of complement activation (APCA) and beta-lysins were determined.

Assay methods

Serum lysozyme concentrations were determined by method of Lie (1985), alternative pathway of complement activation (APCA) by method of Sotirov (1986) and beta lysins by method of Buharin *et al.* (1977).

Statistical analysis

Data were statistically processed by on-way analysis of variance (ANOVA) using statistical software GraphPad InStat 3.06 at a level of significance $P < 0.05$.

Results

The results about the effect of Immunobeta supplementation on serum lysozyme, APCA activity and beta-lysins in turkey breeders are presented in Table 1. Under the influence of Immunobeta lysozyme activity tended to increase during all three subperiods. This factor of innate immunity was increased statistically significantly during the thermoneutral period ($P < 0,001$). A similar tendency occurred in the time course of APCA after supplementation with

the immunomodulator. Although not statistically significant, the differences between control and experimental birds marked a tendency for higher serum APCA in supplemented birds at the time of beginning of lay. The beta-lysins percentage also tended to be higher during the beginning of lay and during the thermoneutral period despite the insignificant differences.

As egg white lysozyme was concerned, there was no positive effect of Immunobeta on this parameter in experimental turkeys (Table 2).

The results summarizing the effects of Immunobeta on serum lysozyme, APCA and beta-lysins in organic layer hens are shown in Table 3. Lysozyme concentrations decreased parallelly to increase in Immunobeta dose and during the hot period, the reduction is statistically significant ($P<0.05$). This dose-dependent change confirmed the effects of Immunobeta on the innate humoral immunity of hens.

A relevant proof for the improved resistance of free-range hens was the change in APCA activity in blood. There was a statistically significant increase in APCA activity during the three subperiods after supplementation with 4.0 g/kg immunomodulator ($P<0.05$). A similar trend for higher APCA activity was also observed in hens treated with 2.0 g/kg Immunobeta as a dietary supplement although the differences vs controls were not significant.

Table 4 presents the results for egg white lysozyme concentrations in organic layer hens under the influence of Immunobeta. They were statistically significantly higher in eggs produced by hens treated with 4.0 g/kg Immunobeta ($P<0.05$). A similar change was noted in eggs produced by hens treated at 2.0 g/kg immunomodulator – the differences vs controls was statistically significant during the hot summer period ($P<0.05$).

Discussion

The results about the effect of Immunobeta on parameters of natural humoral immunity in studied birds affirmed categorically the positive effect of the immunomodulator *Immunobeta*[®]. Improvements of gastrointestinal morphology, physiology, microbiology and growth performance are reported by Denev (2006b, 2008), Yin *et al.* (2008), Al-Mansour *et al.* (2011), Silva *et al.* (2009), Fathi *et al.* (2012) and Bozakova *et al.* (2016) in broiler chickens, and by Huff *et al.* (2013) in turkeys. Other studies have evidence enhanced humoral immunity, resistance to diseases and lower death rates in birds after dietary supplementation of yeasts (Gao *et al.*, 2008; Yin *et al.*, 2008; Fathi *et al.*, 2012). Bozakova *et al.* (2012) demonstrated that the welfare of chicken breeders during the hot summer period has been substantially improved through supplementation of zinc (100 mg/kg Zinteral 35, Lohmann

Animal Health, Germany), containing 35 mg zinc/kg as zinc oxide) and zinc + 250 mg/kg vitamin C (100 mg/kg Zinteral 35 together with 250 mg/kg vitamin C). Bozakova *et al.* (2013a) confirmed the beneficial effects of these dietary supplements on the welfare of New Hampshire breeders reared during the cold winter period. Similar positive influence was reported after supplementation of 1% L-arginine, Zinteral 35, 250 mg/kg vitamin C and 1% Arginine and 250 mg/kg vitamin C to the feed of turkeys and chicken breeders both during the hot and cold periods of the year (Bozakova *et al.*, 2009; Bozakova *et al.*, 2013b; Bozakova *et al.*, 2014). Sotirov *et al.* (2000) and Denev (2008) observed a positive effects of the probiotic Lacto-Sacc[®] and at a lesser extent of lactose (as prebiotic) on the serum lysozyme concentrations and the alternative pathway of complement activation in broiler chickens. In turkeys treated with the above feed supplements, Sotirov *et al.* (2001) obtained similar results for the same factors of non-specific resistance. Sotirov *et al.* (2007) established also the stimulating effect of organic selenium (Sel-Plex[®]) in sows and their progeny, on blood serum lysozyme concentrations and complement levels. Karakolev *et al.* (2013a,b) and Gospodinova *et al.* (2013) provided proofs for significant stimulating effect of the preparation Helpankar on serum lysozyme, gamma interferon and alternative pathway of complement activation in layer hens. Zhang *et al.* (2012) established that a dietary supplement containing yeast cellular wall products boosted the immune system of broiler chickens treated with the immunosuppressing agent cyclosporine A. Furthermore, Sadeghi *et al.* (2013) reported about a positive effect of a dietary prebiotic based on mannan-oligosaccharides and beta-glucans on the immune response of infected chickens. Comparable data about favourable influence of mannan-oligosaccharides and beta-glucans on immune performance of chickens are also reported by Shao *et al.* (2013), Shanmugasundaram *et al.* (2013), Huff *et al.* (2013). Similar data have been published by Valchev *et al.* (2015) in ducks. According to Czech *et al.* (2014) the dietary supplementation with 6% *Yarrowia lipolytica* yeast increased lysozyme levels in turkey breeders. Results about increased egg white lysozyme concentrations are reported in Lohmann Brown hens, treated with 0.4% (4.0 g/kg feed) Immunobeta in previous studies of ours (Bozakova *et al.*, 2017). Bozakova *et al.* (2016) reported that immunomodulator Immunobeta, applied as dietary supplement at doses of 3.0 and 4.0 g/kg feed, stimulated the intestinal villi height and outer diameter of glandular crypts in the small intestine of broiler chickens. The application of the preparation at doses of 2.0 and 4.0 g/kg had beneficial effects on the growth of epithelium lining the glandular crypts and adjacent intestinal villi.

Conclusions

Immunobeta, offered at a dose of 4.0 g/kg feed had a beneficial effect on natural humoral immunity factors – it increased significantly lysozyme levels in turkeys, enhanced APCA activity and beta-lysins in hens. Applied at a dose of 4.0 g/kg, the tested immunomodulator increased significantly egg white lysozyme concentrations in hens, presuming a higher shelf life of eggs and possibly, better protection of chick embryos.

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Table 1. Effect of Immunobeta on serum lysozyme concentrations, APCA and beta lysines in

Periods	Lysozyme (mg/L)		APCA (CH50)		Beta lysines (%)	
	Controls	Immunobeta 4g/kg	Controls	Immunobeta 4g/kg	Controls	Immunobeta 4g/kg
Cold	0,96±0,29	1, 13±0,27	692,78±24,54	695,90±9,84	34,7± 2,9	47,8 ± 5,5
Termoneutral	0,32±0,05	0,72±0,11**	619,45±29,34	632,54±24,83	47,6± 5,01	57,9± 6,8
Hot	0,31±0,03	0,35±0,04	468,55±11,06	488,66±9,36	56,9±1,6	56,5±1,9

turkey breeders.

** - P<0.01

Table 2. Effect of Immunobeta on egg white lysozyme concentrations in turkey breeders

Periods	Controls	Immunobeta 4g/kg	(mg/L).
Cold	499,33±37,19	511, 04±17,98	
Termoneutral	532,67±59,55	475,09 ± 10,01	
Hot	587,83±51,83	306,51±21,59**	

** - P<0.01

Table 3. Effect of Immunobeta on serum lysozyme concentrations, APCA and beta lysines in hens.

Groups	Lysozyme (mg/L)	APCA (CH50)	Beta lysines (%)
Cold period			
Controls	1,71±0,22	543,77±28,15	40,56±1,29
0,2% Immunobeta	1,50±0,25	567,12±25,96	48,99±5,62
0,4% Immunobeta	1,53±0,15	613,74±26,39*	53,17±3,23**
Termoneutral period			
Controls	1,88±0,35	493,77±32,67	40,56±2,22
0,2% Immunobeta	1,50±0,41	533,79±24,84	52,32±5,82*
0,4% Immunobeta	1,53±0,49	563,74±14,48*	50,34±3,90*
Hot period			
Controls	2,05±0,50	443,77±18,99	38,90±4,35
0,2% Immunobeta	1,33±0,39	464,45±31,19	30,65±3,51
0,4% Immunobeta	0,87±0,29*	530,41±10,13*#	33,67±4,01

*- P<0.05; **- P<0.01; #- statistical difference between group treated with 0,4% *Immunobeta* and control group in the hot period.

Table 4. Effect of Immunobeta on egg white lysozyme concentrations in hens.

Groups	Lysozyme (mg/L)		
	Cold period	Termoneutral period	Hot period
Controls	482,66±22,60	494,73±30,82	521,16±30,12
0,2% Immunobeta	527,71±19,01	525,09±22,36	539,84±17,05
0,4% Immunobeta	561,04±27,76*	575,09±25,82*	589,84±11,13*#

*- $P < 0,05$; #- statistical difference between group treated with 0,4% *Immunobeta*[®] and control group in the hot period.