ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 06, pp. 4713-4715, June, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

EGG WHITE LYSOZYME CONCENTRATIONS IN WHITE PLYMOUTH ROCK HENS TREATED WITH THE IMMUNOMODULATOR HELPANKAR

[1]Rumen Karakolev, [2*]Lilyan Sotirov, [3]Angel Angelov, [2]Tsvetoslav Koynarski and [2]Nadya Bozakova

[1]National Diagnostic and Research Veterinary Medicine Institute "Prof. Dr. G. Pavlov "- Sofia
[2]Department Animal Genetics, Faculty of Veterinary Medicine, Trakia University, Student campus, 6000 Stara Zagora, Bulgaria
[3]Hipro Bulgaria Ltd.

ARTICLE INFO

Article History:
Received 19th March, 2015
Received in revised form
23rd April, 2015
Accepted 05th May, 2015
Published online 28th June, 2015

Key words:
Lysozyme,
Immunomodulator,
Hens,
Egg white.

ABSTRACT

Egg white lysozyme concentrations were assayed in White Plymouth Rock hens, treated twice with the polybacterialimmunomodulator HELPANKAR. The experimental flock exhibited higher levels of egg white lysozyme compared to untreated birds throughout the observation period from 24 to 64 weeks of age. Maximumvalueswereattainedby42–44 weeks of age: 10325.64±158.43 mg/L and 10475.67±133.55 mg/L respectively. The lysozyme values in control flock were comparable, with highest values from 3448.15±212.82 mg/L to 3842.25±185.28 mg/L corresponding to 33.4% to 36.7% of those in the experimental flock. The twofold treatment of experimental breeder flock with the immunomodulator Helpankar resulted in 2-3 times higher lysozyme concentrations in breeder eggs.

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 (Burrell, 1994).Lysozyme is a primary factor of avian humoral defense and innate resistance of the embryo (Gilbert, 1971; Irwin, 2014). Egglysozyme is produced by specialised cells in the oviduct mucosae.

Corresponding author: Lilyan Sotirov
Department Animal Genetics, Faculty of Veterinary Medicine, Trakia University, Student campus,6000 Stara Zagora, Bulgaria

The intensity of its production depends on a variety of factors (Eshbailat *et al*., 2004; Bazlamit *et al*., 2009). We hypothesized that the treatment of the White Plymouth rock hens with immunomodulator Helpankar would elevate lysozyme concentration in egg white.

MATERIALS AND METHODS

Birds. White Plymouth Rock hens at the same age were reared under equal conditions, on a permanent litter. The experiments were conducted with two flocks of 100 000 hens each – one experimental and one control. Eggs for lysozyme testing were collected when hens achieved 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64 weeks of age. Thirtyeggs were used for every test. Blood samples for analysis were collected from 30 birds from each flock at 24 weeks of age (38 days after the second treatment). *Polybacterialimmunomodulator* (Helpankar, reg. No. 002366) - a natural product containing in concentrated form the lipopolysaccharide components of the thermally stable endotoxin from Gram-negative bacteria of the

Enterobacteriaceae family. *Administration method* - during the growing period, orally, in drinking water, for a period of 10 days, twice: from the 1st to 10th day and from 120 to 130ty day of the birds' life. In the first 10 days one dose of Helpankar (0.4 ml) was dissolved in 700 ml water (this water is for 10 days for each bird in the group), while in the second period (120-130 days) one dose (0.4 ml) was dissolved in 2250 ml water (this water is for 10 days for each bird in the group). *Lysozyme assay.*Egg white lysozyme concentrations were assayed by the method of Lie *et al*. (1985). As indicator, 24-hour *Micrococcus lysodeicticus* culture dispersed in 1% agarose gel was used (Immuno Agarose, MP Biomedicals, USA). Petridisheswereincubatedfor 20 hoursat37°C and diameters of lytic zones were measured. Lysozyme concentrations were calculated against a standard curve built from 8 standards containing between 3.125 and 0.024 mg/L lysozyme (Sigma-Aldrich, USA, Lot SLBF1341V).

RESULTS

The results from the experiments are presented in Table 1.Theyrefertothetimeafterthesecondsupplementation of birds with the immunomodulator.

Table 1.Lysozyme concentrations in egg white at White Plymouth Rock hens (mg/L). The lysozyme concentrations were expressed as mean ± SE (X ± SE), n = 30eggs

Ages (weeks)	Control hens	Experimental hens
24	3466.12±186.24	5212.48±165.20
26	3487.22±165.19	5529.19±150.17
28	3448.15±212.82	5844.32±148.64
30	3562.54±188.34	6345.28±174.32
32	3568.17±154.65	6871.25±145.40
34	3684.30±182.92	7434.12±188.05
36	3657.22±166.40	8062.14±183.25
38	3754.61±184.63	8690.37±166.54
40	3780.55±164.72	9418.82±150.16
42	3842.25±185.28*	10325.64±158.43
44	3652.67±156.35	10475.67±133.55***[a]
46	3641.19±173.22	9456.45±215.65
48	3668.73±126.20	8764.30±185.05
50	3676.45±159.24	8440.26±145.12
52	3670.50±152.62	7832.35±164.11
54	3557.21±168.14	7355.50±224.18
56	3581.18±133.45	6490.56±172.40
58	3562.20±147.38	6432.64±155.30
60	3514.45±173.44	6359.45±176.82
62	3440.20±192.48	6265.34±165.15
64	3352.47±155.23	6054.21±144.55

* - P < 0,05; *** - P < 0,001 (differences between groups in both flocks).

At 24 weeks of age, the average lysozyme concentrations in control hens was 3466.12±186.24 mg/L. At 30 weeks of age, a certain increase in lysozyme level was detected (3562.54±188.34 mg/L), which further increased parallelly to egg production up to 3842.25±185.28 mg/L by the 42nd week (P<0.05). After that, lysozyme concentrations gradually declined to 3352.47±155.23 mg/L at 64 weeks of age. In hens supplemented with the immunomodulator, egg white lysozyme concentrations were substantially higher. At 24 weeks of age average lysozyme level was 5212.48±165.20 mg/L and tended to increase throughput the subsequent weeks. At 30 weeks of age, lysozyme was already 6345.28±174.32 mg/L, to attain maximum values by 42–44 weeks of age – 10325.64±158.43 mg/L and 10475.67±133.55 mg/L respectively (P<0.001). Until the end of the observation period and the weeks that followed, egg white lysozyme levels in the experimental flock declined up to 6054.21±144.55 mg/L. These values were 2 to 3 times higher than respective control measurements.

DISCUSSION

Recently, the innate resistance factors in birds have raised considerable interest as alternatives to antibiotics and their harmful effects are extensively sought. Often, in modern intensive poultry farms, it is difficult to reproduce feeding and rearing conditions similar to those in breeder farms. That is why, even if a purposeful selection for innate resistance parameters had been carried out, the desired qualities could not always appear in industrial production systems or sometimes, it is difficult to evaluate which breed is more resistant. As generally acknowledged, egg white lysozyme is the primary factor of non-specific defense of the embryo, transmitted by hens to their progeny at highest concentrations at the time of peak egg production (Eshbailat *et al*., 2004; Besarabov, 2013). Our results showed that the stimulation of intestinal mucous tissue with enterobacterial lipopolysaccharides resulted in increased egg white lysozyme levels in broiler chicken breeders, thus enhancing the natural resistance of embryos.

According to Besarabov (2013), egg white lysozyme concentrations lower than 3.5 mg/L, indicate a low natural resistance of chickens. Therefore, the utilization of breeder eggs with higher egg white lysozyme content is of real interest to poultry industry. The data obtained from our preliminary experiments showed that the Helpankarpoly bacterial immunomodulator had a stimulating effect on the serum lysozyme concentration and the activity of alternative pathway of complement activation in layer hens and increased blood serum IFN-α and IFN-γ concentrations in broiler chickens (unpublished data).On the basis of our results it could be concluded that the immunomodulator Helpankar increases egg white lysozyme concentrations and thus, enhanced the natural resistance of chicken embryos.

REFERENCES

Besarabov, B.F. 2013. Natural resistance and productivity in birds. Ptitsevodstvo, 5, 16-17.

Bazlamit, Z. 2009. The distribution of lysozyme in certain fluids and tissues of the chick and goose with special reference to polymorphism.M.Sc. Thesis, University of Jordan, Jordan.

Burrell, R. 1994. Human responses to bacterial endotoxin.Circulatory shock**,** 43, 137-153.

Eshbailat, S. and Ibrahimi, I. 2004.Turnover of lysozyme during the development of chicken embryo. Dirasat, medical and biological sciences, 31, 103-115.

Gilbert, A.B. 1971a. The egg: It's physical and chemical aspects. In: Bell, D.J. and Freeman, B.M. (Eds), Physiology and Biochemistry of the Domestic fowl. Academic Press, London/New York, 3, 1379-1399.

Irwin, D.M. 2014. Evolution of the vertebrate goose-type lysozyme gene family.*BMC Evolutionary Biology*, 14: 188. http://dx.doi.org/10.1186/s12862-014-0188-x.

Lie, O., Solbu, H., 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, 1-12.

Sunwoo, H., Nakano, T., Dixon, W., Sim, J. 1996.Immune responses in chickens against lipopolisaharide of *E.coli* and *Salmonella typhimurium*. *Poultry Science,* 75, 342-345.

Webel, D.M., Finck, B.N., Baker, D.H., Jonson, R.W. 1997. Time course of increased plasma cytokines, cortisone and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. *Journal of Animal Science*, 75, 1514-1520.

****