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SHORT REPORT

A note on the effect of immunostimulation of laying hens on the lysozyme activity in egg white

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Four immunostimulating preparations (IPs) – Levamisol, Lidium KLP, Echinacea and Baymix Se+E – were administered to Hy-Line hens aged 8 months (group I, II, III and IV, respectively, 10 birds in each). The egg white lysozyme activity (LA) was determined before administering the IP, and next 17, 24, 31, 45 and 60 days after. All IPs led to an increase in the egg white LA which was maintained over a period of 45 (group II, III and IV) and even 60 days (group I) after administration. Levamisol was shown to be most effective IP, while Baymix Se+E the least.

KEY WORDS: chicken / egg / immunostimulation / lysozyme

Egg white lysozyme is described as an "endogenous antibiotic" and is widely used in the therapy of bacterial and viral infections, as also a "natural preserving agent" for the protection of food against bacterial deterioration [Cunningham *et al*. 1991]. Its use for food preservation eliminates the necessity of applying nitrites or hydrogen peroxide. The lysozyme content of the egg white may be determined by analysing its activity [Trziszka and Clostermann 1993]. The higher the activity the more lysozyme may be obtained from the egg white, what is important e.g. for the production of Lidium KLP used for treating viral and bacterial infections, as also for the production of preparations used as food preservatives. The activity of egg white lysozyme is affected by

numerous factors and among them by hen's maintenance and nutrition [Świerczewska *et al*. 1998a], as well the immunostimulatory effect of certain preparations, such as Levamisol.

This report presents the effects of selected immunostimulators on the lysozyme activity of hen's egg white.

Material and methods

Birds

The examinations were conducted on eggs obtained from eight-month old Hy-line hens with a live body weight of about 2 kg, maintained in individual cages and fed a commercial DJ concentrate. The tests aimed at determining the effect of four immunostimulating preparations (IPs) – Levamisol, Lidium KLP, Echinacea and Baymix Se+E – offered to four groups of laying hens (each of 10) on the lysozyme activity (LA) of their egg white. The LA was determined in the egg white of each hen before administering the IP and next 17, 24, 31, 45 and 60 days after. Each time determined was the LA in 10 eggs obtained from each group, what led to a total of 240 eggs analysed (Tab. 1).

Table 1. Experiment scheme

Group	Number of hens	Preparation	Application
I	10	Levamisol	2 mg/kg live body weight/day over 3 consecutive days (*per os*)
II	10	Lidium KLP	0.025 mg/kg live body weight. Single injection into breast muscle
III	10	Echinacea	10 ml solution (2.5 ml preparation + 7.5 ml water)/hen/day over 3 consecutive days (*per os*)
IV	10	Baymix Se + E	10 ml solution (2.5 ml preparation + 7.5 ml water)/hen/day over 3 consecutive days (*per os*)

Immunostimulating preparations (IPs)

Used were four common preparations widely used for immunizing animals.

Levamisol (POLFA, Poland) is a 10% tiasol compound inducing the synthesis of interferone (cytokinases are released from stimulated lymphocytes). The preparation leads to an increased immunity of cells to viral infections and has an anti-carcinogenic effect. It is a synthetic preparation. At small concentrations it stimulates, and at higher inhibits certain immune responses. Levamisol is used also in the treatment of nematode

infestations in the digestive tract of chicken and turkeys.

The active substance in **Lidium KLP** (STREULI) consists of a lysozyme dimmer, isolated from egg white. It is a preparation widely used in the treatment of viral and bacterial diseases of cattle, pigs, horses and dogs. It helps cure diseases of a complicated etiology, appearing in homeostatic disturbances and a lowered organism immunity. It is also used as a preparation stimulating mechanisms regulating the cellular and hormonal defense of the organism.

Echinacea (RATIOPHARM) is composed of an extraction of *Echinacea angustifoliae*, 70% sorbital, 96% ethanol and distilled water. The preparation is comparatively easy to use as it may be offered as a drink. Echinacea contains derivatives of coffeic acid, as well as polysaccharides, glycoproteins and alanine together with arabinose, glucose, glucosamine, and traces of pyrolysidinic alkaloids. It is used externally on wounds difficult to heal and internally (as a prophylaxis or treatment agent) in cases of chronic inflammations of the respiratory tract, influenza, arthritis, prostate hypertrophy and cancer. Echinacea stimulates the activity of the immune system and thus decreases the susceptibility to bacterial and viral infections. Moreover, it helps remove toxins from the organism.

Baymix Se+E (BAYER) is a liquid preparation for poultry, containing selenium and vitamin E. Baymix Se+E prevents the occurrence of selenium of vitamin E deficiency, especially when animals are subjected to stress, show low body weight gain, a low egg production and an insufficient absorption of vitamins from the digestive tract.

Analytical

The LA was determined at the Warsaw Agricultural University Laboratory, 12 hours after the egg was laid, using the spectrophotometric method (Epol 20), at a weave length of 450 nm [Trziszka and Clostermann 1993]. The determination consisted of the steps given below.

1. Manual separation of white from yolk and next homogenization (rubbing three times through a strainer).
2. Preparation of a standard – 1 mg of lysozyme (SIGMA, 21252 U/mg) is dissolved in fresh, ice-cold distilled water and transferred into a bulb so as to obtain 250 ml solution.
3. Preparation of a buffer consisting of KH_2PO_4, Na_2HPO_4, NaCl, NaN_3 and dissolving it in 500 ml of sterile water with 0.1 n HCl.
4. Preparation of a *Micrococcus luteus* suspension by mixing the bacteria with the buffer prepared (12-14 mg bacteria plus 40 ml of buffer).
5. Diluting the suspension as to obtain an extinction at 450 nm amounting to 0.750 in relation to air.
6. Measuring the LA against the standard prepared and registering the extinction by a spectrophometer at 450 nm.

Results and discussion

Prior to the administration of the IPs the LA of the egg white was found similar in all groups and ranged from 11,306 to 12,358 U (Tab. 2), the inter-group differences being not significant. This corroborates the results obtained by Świerczewska *et al*. [1998b] and confirms that between hens of similar origin and age, and maintained in the same environment, no significant differences in the LA of egg white occur. However, later results reported by Świerczewska *et al*. [2002] showed the trait to be significantly affected by bird's maintenance. In the egg white of hens maintained extensively (unlimited access to runs) the LA was significantly higher than in those maintained in closed compartments on litter, or in cages. Moreover, at low (about 6°C) or high (25°C) ambient temperatures the LA decreased considerably.

On day 17 after the administration of Lidium KLP, Echinacea ratiopharm or Baymix Se+E (group II, III and IV, respectively), the egg white LA increased slightly

Table 2. Least squares means (LSM) for lysozyme activity found in consecutive tests, as affected by different immunostimulating preparations

Test number	Lysozyme activity (U)			
	Group I (Levamisol)	Group II (Lidium KLP)	Group III (Echinacea ratiopharm)	Group IV (Baymix Se + E)
1. LSM	12,358	11,528	11,377	11,306
SE	235	235	235	235
different from the result of test no. *	*3, 4, 5, 6*	*3, 4, 5, 6*	*3, 4, 5*	*3, 4, 5, 6*
2. LSM	11,896	11,571	11,776	11,835
SE	204	204	204	204
different from the result of test no. *	*3, 4, 5, 6*	*3, 4, 5, 6*	*3, 4, 5*	*3, 4, 5, 6*
3. LSM	16,627	17,109	17,028	16,823
SE	1,312	1,245	1,245	1,245
different from the result of test no. *	*1, 2, 4, 5, 6*	*1, 2, 5, 6*	*1, 2, 6*	*1, 2, 4, 5, 6*
4. LSM	14,838	17,322	17,049	15,382
SE	963	963	963	963
different from the result of test no. *	*1, 2, 3, 5, 6*	*1, 2, 5, 6*	*1, 2, 6*	*1, 2, 3, 6*
5. LSM	18,283^{A}	15,955^{AB}	17,727	14,340^{B}
SE	987	987	987	987
different from the result of test no. *	*1, 2, 3, 4, 6*	*1, 2, 3, 4, 6*	*1, 2, 6*	*1, 2, 6*
6. LSM	16,650^{A}	13,281^{B}	12,997^{B}	12,875^{B}
SE	722	722	685	685
different from the result of test no. *	*1, 2, 4, 5*	*1, 2, 3, 4, 5*	*3, 4, 5*	*1, 2, 3*

^{AB}Within rows means bearing different superscripts differ significantly at $P \leq 0.01$.
*Within columns, in italics, shown are test numbers, mean LAs for which differ significantly ($P \leq 0.01$) from the mean LA found in the given test.

as compared to the initial level and was similar in all three groups (11,571, 11,776 and 11,835 U, respectively), the inter-group differences being not significant (Tab. 2). At this stage Levamisol (group I) caused a not significant decrease of the LA – from 12,358 to 11,896 U. A clear effect of all IPs was observed on day 24 after administration. An increased LA was maintained over 45 days in II, III and IV group of hens, while in group I (Levamisol) even over 60 days. This shows that the administration of the preparations selected stimulated the immune system and had a stimulating effect on the LA activity.

At present natural immunostimulators are considered to be of considerable importance in the organism's protection against infection. Siwicki *et al* [1998] in studies conducted on trauts demonstrated that an injection with preparation KLP-602 (Lidium) stimulated the cellular and humoral defence of the organism and thus protected it against furunculosis. After a single injection the mortality amounted to 45%, after three injections – to 25%, while in the control group as much as 85% of fish died. However, another immunostimulator – Echinacea – did not show such a positive effect on antibody formation in studies conducted on rats [South and Exon 2001].

Bessei *et al* [1995] reported that after protective vaccinations commonly applied to chicken an increased level of lysozyme of the blood plasma and egg white was observed, while Kato *et al*. [1994] showed that the addition of immunostimulators to the feed is one of the methods for obtaining eggs with an increased lysozyme concentration. The highest largest number of investigations refers to the possibility of using Levamisol as an immunostimulator for animals [Dębowy and Obmińska-Domaradzka 1987]. It has also been demonstrated that certain antibiotics, such as zincbacitracine, significantly lower the concentration and activity of lysozyme, simultaneously lowering the egg pH [Bessei 1994].

The results presented here show that it is feasible to increase the level of lysozyme in the egg white by adding immunostimulating preparations to the feed of hens.

Administration of the immunostimulating preparations tested led to a significant increase in the lysozyme activity of the egg white.The significant increase in lysozyme activity was maintained over a period of 45 days in eggs obtained from hens stimulated with Lidium KLP, Echinacea and Baymix Se+E and over a period of 60 days in those obtained from hens stimulated with Levamisol.

Levamisol proved to be the most effective immunostimulator, while Baymix the least.

REFERENCES

1. BESSEI W., 1994 – Effect of Zincbacitracin on the performance and egg quality of laying hens given different levels and sources of protein. ***Archiv für Geflügelkunde*** 58, 219-223.
2. BESSEI W., GRASHORN M., 1995 – Einfluss von Fütterung und Impfung der Legehennen auf Eiqualität verangerungen von Kleintierzucht. Thesis. Hohenheim University.
3. CUNNINGHAM F.E., PROCTOR V.A., GOETSCH S.J., 1991 – Egg white lysozyme as food preservative: an overview. ***World's Poultry Science Journal*** 47, 142.

4. DĘBOWY J., OBMIŃSKA-DOMARADZKA B., 1987 – Wykorzystanie immunostymulujących właściwości Levamisolu w praktyce weterynaryjnej (Use of immunostimulant properties of levamisole in veterinary practice). In Polish with English summary. ***Medycyna Weterynaryjna*** 43, 357-359.
5. KATO A., IBRACHIM H.R., NAKAMURA S., KOBAYASHI K., 1994 – New methods for improving the functionality of egg white proteins. In: Egg Uses and Processing Technologies (J.S. Sim, S. Nakai, Eds). CAB International 250-268.
6. SIWICKI A., KLEIN P., MORAND M., KICZKA W., STUDICKA M., 1998 – Immunostimulatory effects of dimerized lysozyme (KLP-602) on the nonspecific defense mechanisms and protection against furunculosis in salmonids. ***Veterinary Immunology and Immunopathology*** 27 (61), 369-378.
7. SOUTH E., EXON J., 2001 – Multiple immune functions in rats fed Echinacea extracts. ***Immunopharmacology and Immunotoxicology*** 23, 411-421.
8. ŚWIERCZEWSKA E., NIEMIEC J., STĘPINSKA M., RIEDEL J., SIENNICKA A., GRZYBOWSKA A., KAKOWSKA R., 1998a – Lysozyme activity in egg white as influenced by egg storage time and temperature and by management system of laying hens. Proceedings of the VII European Symposium on the Quality of Eggs and Egg Products. Poznań, Poland, p. 162.
9. ŚWIERCZEWSKA E., NIEMIEC J., STĘPIŃSKA M., RIEDEL J., GRZYBOWSKA A., KAKOWSKA R., SIENNICKA A., ŁUCZYŃSKI T., 1998b – Aktywność lizozymu jaja kurzego zależnie od pochodzenia, utrzymania i wieku niosek, miejsca jaja w serii oraz warunków przechowywania jaj (Lysozyme activity in chicken egg white as affected by origin, management and age of layers or clutch position and egg storage) In Polish with English summary. ***Prace i Materiały Zootechniczne*** 53, 83-89.
10. ŚWIERCZEWSKA E., KOPEĆ W., NOWORYTA-GŁOWACKA J., 2002 – Wpływ warunków utrzymania kur na aktywność lizozymu, owomukoidu oraz owoinhibitora w białku jaja (Effect of housing system on lysozyme, ovomukoid and ovoinhibitor activity in egg white). In Polish with English summary. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 60, 291-298.
11. TRZISZKA T., CLOSTERMANN G., 1993 – Measuring the lysozyme activity as method for estimating the egg quality. ***Archiv für Geflügelkunde*** 57, 22-26.

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Wpływ immunostymulacji kur na aktywność lizozymu białka ich jaj

Streszczenie

Badania przeprowadzono na ośmiomiesięcznych kurach Hy-Line utrzymywanych indywidualnie w klatkach. Ptaki podzielono na cztery grupy (I, II, III i IV), w których podawano odpowiednio Levamisol, Lidium KLP, Echinacea i Baymix Se+E. W każdej grupie aktywność lizozymu (LA) białka jaja określano przed zastosowaniem preparatu, a następnie w 17, 24, 31, 45 i 60 dniu od jego podania. Wszystkie preparaty przyczyniły się do zwiększenia aktywności lizozymu, które utrzymywało się do 45 (grupa II, III i IV) i do 60 dnia (grupa I) od podania. Najskuteczniejszy okazał się Levamisol, a najmniej skuteczny Baymix Se+E.