Proteinases activity inhibitors in the egg white depending on various housing systems of egg layers

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Egg white is rich in biologically active substances playing an important role in the system of growing embryo protection. Inhibitors of proteinases, especially cysteine (cystatin) and serine (ovomucoid) are one of the most important ones. Cystatin and ovomucoid activities depend on various factors, such as genetic origin, feeding/housing system, etc. The aim of the research was to investigate the effect of three different keeping systems, i.e. battery (Lohmann and Tetra), on litter (Lohmann) and free range, semi ecological (Tetra) on the activity of proteinases inhibitors in egg white.Ovomucoid activity, as an ability to inhibit trypsin was determined with the synthetic substrate BapNA using colorimetric method. The inhibitory activity of cystatin against papain was analysed using the method based on colorimetrical measurements of the amount of products released from the substrate BANA.The results indicated that eggs obtained from layers kept in battery and ecological system showed similar activity of cystatin (ca. 21 units/5 mg protein), while keeping hens on litter led to very low activity. The highest antitrypsin activity of egg white was analysed in eggs collected from hens kept in cages (esp. Tetra), whereas eggs derived from hens housed in more ecological systems (free range and on litter) were characterised by slightly lower activity of ovomucoid.

Keywords: layers, housing system, egg white, proteinase inhibitors, cystatin activity, lysozyme.

Introduction

An egg is the largest cell which, apart from a complex of genetic information, contains a range of organic and mineral substances which are necessary to create a new life. Nature does not know a food material which is better than an egg consisting of proteins, fats, vitamins, mineral compounds and biologically active substances necessary for the proper development of an embryo and its protection against microorganisms (Trziszka 2000).

Lysozyme, ovomucoid, ovoinhibitor and cystatin are biologically active proteins of egg white protecting the embryo during the incubation period. Their activity also prolongs the shelf life of table eggs. Some of the biologically active constituents of egg white, especially lysozyme, have wide novel applications in medicine and/or as a preservative substance in food production. Lysozyme protects the embryo up to the time of producing of its own immunoglobulins and it is also active against viruses and some toxins (Kopec and Trziszka, 1997, Trziszka 2000). Another kind of protection is achieved by cysteine proteinases inhibitors, i.e. cystatin and by inhibitors of serine proteinases, i.e. ovomucoid and ovoinhibitor, which inhibits the activity of bacterial enzymes.

Earlier studies showed that the activities of biological substances in egg white are affected by conditions and keeping system (intensive, extensive) of laying hens. It was found that the hens managed in an extensive type of housing system laid eggs with a higher activity of lysozyme, cystatin, ovoinhibitor and ovomucoid, as compared to eggs originating from the layers kept in an intensive housing system (Swierczewska *et al*., 2003, 2005). Low lysozyme activity in egg whites was observed when the temperature in the premises for the laying hens decreased during winter or increased during summer time beyond the standard range established for the layers (Swierczewska *et al*., 2002).

The aim of the research was to investigate the effect of three different keeping systems, i.e. battery (two groups), on litter (one group) and one group of free range (semi ecological) on the activity of lysozyme, cystatin and ovomucoid.

Material and methods

The studies were conducted on 360 hen eggs (4x90 eggs) derived from four groups of different keeping systems, i.e. battery I (Lohmann), battery II (Tetra), litter (Lohmann), semi ecological, free range (Tetra). The hens' age varied from 40 to 50 weeks. Hens feed mixture contained approximately 18% of total protein and 11.5 MJ metabolic energy per kg. Fresh eggs were manually broken and the egg yolk was separated from white, which was the raw material for the study.

The activity of lysozyme was determined by a spectrophotometric (turbidimetric) method at a wavelength of 450 nm. The change in absorbancies in a suspension of *Micrococcus lysodeikticus* bacteria, was registered during a 6 minutes reaction of the enzyme with the bacterial cells.

The activity of cystatin was determined as the ability to inhibit enzymatic activity of papain using BANA (N-benzoil-DL arginyl- □-naphtylamide hydrochloride) as a substrate after incubation of the samples at 37ºC.The reaction was stopped by the addition of DMBA (p-imethyloaminobenzaldehyde) and the decrease of the absorbance was measured spectrophotometrically at a wavelength of 450 nm.

Anti-trypsin activity of ovomucoid was measured spectrophotometrically at 412 nm after a 10 min incubation of trypsin solution with egg white at 37ºC in 0.1 M tris-HCl buffer (pH 8) with the addition of 20 mM CaCl2, using nitroanilide N-benzoil-DL-Arginine (BapNA) as a substrate (Erlanger *et al*., 1961). The results obtained in the study were calculated and expressed as units of anti-trypsin activity per 0.1 mg of egg white protein.

Results and discussion

The effect of egg production systems on the biologically active substances is presented in Table 1.

Table 1. Biologically active substances in egg white in the aspect of various egg production systems

	Bioactive substances		
System of egg production	**Lysozyme in 2.5 mg protein**	**Cystatin in 5 mg protein**	**Ovomucoid in 0.1 mg protein**
Battery I	1235 a	21.1 a	13.3 b
Battery II	1074 b	21.7 a	16.1 a
Litter	1113 ab	1.1 b	12.1 b
Semi-ecological system	1073 b	21.4 a	12.0 b

The same letter (a, b) of means shows no significant differences at p= 0.05

The results proved that not only production system but also other factors, like e.g. breeding, can affect the level and activity of egg white substances. The level of lysozyme content was confirmed in the area from 1073 to 1235 (in 2.5 mg protein), but it depended more on the breed of layers than the production system. Statistically significant differences were observed between Lohman and Tetra layers. The level of lysozyme contents from Tetra layers was similar in the battery and ecological production.

Apart from that, the level of cystatin indicated that eggs obtained from layers kept in battery and ecological system showed similar activity (ca. 21 units/5 mg protein), while keeping hens on litter led to very low activity. The highest antitrypsin activity of egg white was observed in eggs collected from hens kept in cages (Battery II; Tetra), whereas eggs derived from hens housed in more ecological systems (free range and on litter) were characterised by slightly lower activity of ovomucoid.

The study of Swierczewska et al. (2003) showed that the hens managed in an extensive type of housing system laid eggs with a higher activity of lysozyme and cystatin, ovoinhibitor and ovomucoid as compared to eggs originating from the layers kept in an intensive housing system. Another study, Trziszka et al. (2004), aimed to isolate and characterize the cystatin from various layers at different ages between 20 – 80 weeks, indicated that the layer hens at the age of 50 weeks have a higher activity of cystatin against papain and also higher yield by extraction. Swierczewska et al. (2005) showed that cystatin had the lowest activity in egg white collected from 35 weeks old hens, whereas eggs collected in the 50th week of hen's age expressed the highest activity of cystatin. The authors concluded that the activity of biologically active proteins in egg white increases with increasing age of the hens to 45 weeks, in case of trypsin and chemotrypsin inhibitors, and up to 50 weeks in case of cystatin and lysozyme. Cystatin has lower stability of activity in comparison to other investigated substances. Generally, it is very important to continue the study on biologically active substances from egg white because they will play a very significant role in biomedical and nutraceutical uses.

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