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EFFECTS OF BREEDING AND HOUSING SYSTEMS OF LAYERS ON EGG QUALITY AND THE ACTIVITY OF CYSTATIN AND LYSOZYME

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Key words: housing system, breed of hens, egg quality, lysozyme, cystatin

The study was carried out on eggs collected from 4 groups. Group I – Green leg (Gl) layers, kept in a free-range system, Group II –Lohmann Brown (LB) layers kept in a battery system, Group III –Lohmann Brown (LB) layers, kept in a free-range system, Group IV – Lohmann Brown (LB) layers, kept on litter containing straw and sawdust.

Standard feeding system with the use of a DJ feed mixture containing 17% of protein and metabolic energy of 2800 kcal was applied. The layers were in the peak of laying, *i.e.* between 26 and 34 weeks of age.

The purpose of the study was to find the effects of the breeding and housing systems on commercial quality of the eggs. The measurements included cystatin and lysozyme activities, as these substances are of great interest as regards their use in biomedical and nutraceutical applications.

The measurements of the egg quality included: whole egg weight, the percentages of the egg-shell, white and yolk, the pH of albumen, Hough's units and sensory analysis of the eggs. The essential goal was to determine lysozyme and cystatin activities, using standard methods. The highest activities were found in the eggs from the Lohmann layers, kept in cages, and in a floor system. The lowest values were obtained with the eggs of Green-leg layers, which could have been affected by the winter time. It is worth noting that the differences observed were significant.

The effects of the genetic breed of the layers, feeding and housing systems and age of the hens on lysozyme and cystatin activities have been measured, but the results did not give us a unanimous answer to the problem under investigation. There is very great interest in obtaining lysozyme and cystatin from egg white and their application as nutraceutical or medical preparations. Industrial applications of the biologically active substances present in eggs are possible, provided that the problem of the differences in their activities in the raw material is solved. It is also important to know how to predict the values of these parameters.

INTRODUCTION

Recently, significant changes have been observed in food safety requirements and consumer approaches to food products and diets. This, in turn, requires new marketing strategies from food businesses. Today, the consumer is interested in buying natural, unprocessed food products, or such products that have been processed to the least possible degree. As for eggs, the consumer is now looking for ecological eggs from layers kept in free-range systems rather than those laid by hens kept in cages or battery systems. Besides, enriched, healthy eggs have been of an increasing interest to a number of consumers. Since eggs are not only used as food ingredients, but also as a raw material for the production of nutraceuticals, it seems justifiable to study the differences between the eggs from layers kept in cages and those kept in free-range systems as well as those laid by the primary chicken breeds (*e.g.* green--leg), genetically modified (most recent chicken lines).

In recent years, various branches of food and pharmaceutical industries have directed their attention to eggs, considering them a source of biomedical preparations and diet supplements [Ball, 2004; Froning, 2004].

The substances present in the egg white are very important for biological regulation of numerous metabolic processes and for the protection of human body from microorganisms and cancer.

The most thoroughly examined biologically active substance of the egg white is lysozyme (a protein, enzyme, 14.2 kDa). Lysozyme exhibits antibacterial activity, esp. against G+ bacteria. Its antiviral and anticancer properties have also been reported. Using modern technologies, it is possible to isolate lysozyme from the egg white. The substance thus obtained can be used as a natural food preservative and for pharmacological purposes [Davis & Reeves, 2002; Kijowski *et al*., 1998; Kopeć 2000; Trziszka, 2000].

Cystatin is a very important proteinase inhibitor in the egg white (concentration of only 0.05%). It is a small molecule (12.7 kDa) of high thermal stability, free of carbohydrates [Trziszka 2000; Davis & Reeves.2002].

This inhibitor is not toxic and its biological function is connected with protein protection by the process of the embryo developing. It was found in numerous *in vitro* experiments that cystatin inhibited enzyme activity of cysteine endopeptidases, the key substances initiating a cascade of en-

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zyme changes in cancer development. The data presented in recent publications show that there is a promising future for the use of egg white preparations in pharmaceutical industry and health care [Kennedy, 1993].

The differences in the activity of cystatin resulting from numerous factors are very important. Earlier studies showed that the activities of biological substances in the egg white were affected by the conditions prevailing in housing systems (free-range/cages) for laying hens. It was found that the hens in a free range system laid eggs with a higher activity of lysozyme and cystatin, as compared to eggs from the layers kept in battery systems with cages [Swierczewska *et al*., 2003, 2005].

Modifications of hen diets by supplementing them with mineral-humine preparations and concentrates containing fish oil only slightly affected the activity of biologically active substances present in fresh eggs. Storage of eggs up to 4 weeks at 15°C decreased the activity of lysozyme, approximately by 10%, while the activity of cystatin was observed at trace levels. [Kopeć *et al*., 2005].

The research on biologically active substances present in eggs is constantly developing and it is expected that in the near future, new properties of eggs will find a wide variety of applications not only in food products manufacturing, but also in pharmaceutical industry.

The purpose of the present study was to find the effects of breeding and housing systems for layers on commercial quality of the eggs and also on cystatin and lysozyme, important in biomedical and nutraceutical applications.

MATERIALS AND METHODS

The study was carried out on eggs collected from 4 groups (30 layers in each group).

Generally, standard feeding systems were applied for all layers, *i.e.* a DJ mixture, with average protein content of 17% and metabolic energy of 2800 kcal. The layers were in the peak of laying, within the ages from 26 to 34 weeks.

Group I – Green-leg (Gl) – kept in a free-range system, in voliers. Daily feed intake averaged 130 g/bird.

Group II –Lohmann Brown (LB) layers kept in a typical battery system, with 5 birds in each cage. (500 cm^2/bird. Daily feed intake averaged 125 g/bird.

Group III –Lohmann Brown (LB) layers kept in a free range system (with no grass) and had free access to drinking water and feed. Daily feed intake averaged 130 g/bird.

Group IV –Lohmann Brown (LB) layers kept in henhouses on straw and sawdust litter. Daily feed intake averaged 130 g/bird.

Eggs were collected every day and eggs weighing from 55 to 65 g were selected for the investigation. The age of eggs for the study was 2–5 days. Egg quality measurements included: whole egg weight, the percentages of egg-shell, egg white and yolk, the pH of the egg white, the Hough units and sensory assessment by a panel of 6 judges from the Sensory Quality Laboratory at the Department of Food Products Technology and Quality Management.

The activity of lysozyme was determined, using a spectrophotometric (turbidimetric) method at a wavelength of 450 nm. The changes in absorbance were recorded in a suspension of *Micrococcus lysodeikticus* bacteria, during a 6 minutes' reaction of the enzyme containing the bacterial cells [Kopeć *et al*., 2005].

The activity of cystatin was determined with regard to its ability to inhibit the enzyme activity of papain, using BANA (N-benzoil-DL arginyl-beta-naphtylamide hydrochloride) as a substrate, after incubation of the samples at 37°C. The reaction was terminated by adding DMBA (p-dimethyloaminobenzaldehyde), and next, a decrease in the absorbance was measured spectrophotometrically at a wavelength of 450 nm [Kopeć *et al*., 2005].

The results were analysed statistically using Statistica 6.0 program and one-way analysis of variance, at p=0.05.

RESULTS AND DISCUSSION

The purpose of the study was to find the effects of housing systems, chicken genotype (primary breed and commercial lines of layers) on the commercial quality of the eggs and biological activities of the lysozyme and cystatin. The reason for conducting these studies was to provide food and pharmaceutical industries with a better knowledge about eggs and their quality, depending on some factors in the food production chain, consequently affecting their suitability for biomedical applications. Special attention was focused on minimizing adverse conditions prevailing in a battery system of housing and highlighting favourable environmental conditions of a free-range system. The data obtained in the studies are shown in Tables 1-3.

As can be seen in Table 1, the egg weight was relatively low, which was due to the young age of the layers, but it is also noteworthy that the weight of the eggs from green-leg layers was lower than that of the other lines.

The highest percentage content of yolk was observed in eggs with the lowest mass*, i.e.* from green-leg and battery-kept Lohmann Brown. The highest percentage content of white was observed in the eggs with the highest mass, *i.e.* from birds kept on straw and sawdust. The highest pH was observed in the whites from green-leg layers (9.02%), which may indicate an advanced ageing process, whereas the value of Hough units was high, which indicated high content of thick white. Lower pH values in the whites from Lohmann Brown layers were not confirmed by higher content of thick white.

TABLE 1. The quality of eggs from various breeding and housing systems for layers.

Chicken breeds/housing systems	Parameters					
	Egg weight (g)	Shell (%)	White (%)	Yolk (%)	pH	Hough units
Green-leg Free-range	39.3a (4.2)*	13.5c (1.1)	56.2a (3.9)	30.3c (1.8)	9.02b (0.3)	86b (4.1)
Lohmann Battery	46.8b (3.1)	12.5b (0.9)	56.8a (3.7)	30.7c (2.3)	8.66a (0.2)	83b (2.8)
Lohmann Free-range	55.3c (4.2)	11.6a (0.7)	59.6b (4.6)	28.8b (2.1)	8.70a (0.1)	75a (6.1)
Lohmann Litter	56.7c (4.8)	12.2b (1.0)	60.6b (4.3)	27.2a (1.9)	8.70a (0.2)	95c (7.8)

* the values in brackets present standard deviation; identical letters mean no statistically significant differences

Statistically relevant deviations depending on the housing system were observed. The best quality features were found in the group of layers kept on straw and sawdust.

Table 2 shows biological activities of the lysozyme and cystatin present in the egg white. Generally, the lowest activity of these substances were found in the eggs from layers kept in free-range systems (layers of Green-leg breed and also Lohmann Braun line). The highest activity of lysozyme (116.120 units) were found in the eggs from the Lohmann Brown layers, kept in a battery system. Especially high cystatin activity (4.237) was found in the eggs from the Lohmann Brown layers, kept in a floor system.

The assessment of the activity of lysozyme and cystatin is very important due to various conditions of egg production and the breed of layers. In the conditions of industrial obtaining of these substances, it is necessary to predict their activity which may depend on numerous factors. Trziszka *et al*. [2006] observed that the activity of biologically active substances was higher in ecological conditions. Other studies [Świerczewska *et al*., 2003] show that the activity of lysozyme and cystatin was highest in the spring-summer period and lowest in winter. Kopeć *et al*. [2005] showed that the use of feed enriching the yolks with PUFA results in a lower activity of cystatin. Moreover, its activity decreases during storage. That is why it is so important to determine what factors influence the biologically active substances contained in egg white.

The results obtained by Trziszka *et al.* [2006] proved that not only the production system but also other factors, like breeding, influenced the level and activity of the egg white substances. Significant differences were observed between Lohman and Tetra layers. The level of lysozyme contents from the Tetra layers was similar in the battery and free-range production. Apart from that, the level of cystatin indicated that eggs from layers kept in batteries and ecological system showed similar and high activity (approx. 21 units/5 mg of protein), while keeping hens on litter resulted in a very low cystatin activity. In another study, Trziszka *et al*. [2004] isolated and characterized cystatin from various layers between 20 and 80 weeks of age, and found that the layers aged 50 weeks showed higher activity of cystatin against papain and also higher yield by extraction.

The impact of external factors on enzyme activity of the lysozyme and inhibiting action of cystatin is still being studied, since it is of great interest due to biomedical applications and nutraceuticals. However, the results of the present investigation and those obtained in earlier studies [Świerczewska *et al.,* 2003, 2005; Kopeć *et al*., 2005; Trziszka *et al.,* 2006] did not give enough replications, therefore, further studies are still required.

Table 3 shows the results of sensory analysis of the eggs on a 1–5 scale. Generally, the score was high in all the groups under investigation, but the highest was noted with the eggs from layers kept in a free-range system. It seems likely that this was connected with higher content of the yolk. Apart from that, the investigated material was fresh, good for consumption. Eggs from free-range system, esp. from green-leg layers, should be offered as special value eggs.

TABLE 2. Activity of lysozyme and cystatin present in the egg white, depending on breeding and housing system.

Substances	Chicken breeds/housing systems			
	Green-leg Free-range	Lohmann battery	Lohmann Free-range	Lohmann litter
Lysozyme per 100 mg of protein	83940a (5280)	116120c (6400)	100590b (4780)	98200b (4800)
Cystatin per1000 mg of protein	1797b (105)	2623c (150)	1483a (85)	4237d (125)

* the values in brackets present standard deviation; identical letters mean no statistically significant differences

CONCLUSIONS

1. Generally, small differences between egg quality features were observed in all variants. The number of Hough units was lower in eggs from free-range system.

2. The highest activity of lysozyme and cystatin was observed in the eggs from the breeds of Lohmann Brown, esp. kept in battery system.

3. The best sensory properties were found in the eggs from free-range system, especially from Green-leg layers.

The results obtained in the present study and those obtained by other authors do not provide us with enough information on the relationships between the genetic lines of layers, feeding and housing systems and age of layers as regards the biological activities of the lysozyme and cystatin.

The two substances under investigation are of great interest for food products manufacturers and pharmaceutical industry. If manufacturing companies decide to use biologically active substances present in the eggs in their products, it is necessary to find what procedures can be applied to reduce the

TABLE 3. Sensory analysis of boiled eggs.

Chicken breeds/ housing systems	Parameters						
	Odour	Taste	Overall impression	Foreign bodies	Texture of egg white	Texture of yolk	Σ
Green-leg Free-range	4.1 (0.3)	4.2 (0.3)	3.9 (0.4)	4.7 (0.1)	4.4 (0.3)	4.1 (0.2)	25.4 (0.3)
Lohmann battery	4.1 (0.2)	3.4 (0.5)	4.0 (0.5)	5.0 (0.1)	3.8 (0.2)	3.7 (0.2)	24.0 (0.3)
Lohmann Free-range	4.3 (0.4)	3.9 (0.4)	3.8 (0.2)	5.0 (0.1)	4.4 (0.4)	3.8 (0.2)	25.2 (0.3)
Lohmann Litter	3.7 (0.3)	3.6 (0.3)	3.9 (0.2)	4.7 (0.1)	4.3 (0.3)	3.7 (0.1)	23.9 (0.2)

* the values in brackets present standard deviation; identical letters mean no statistically significant differences

differences in their content of the raw material and also to find the methods for predicting their yield and activity. The finished biomedical products containing eggs can by no means show significant differences in curing effects they can result in.

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WPŁYW GRUP GENETYCZNYCH I SYSTEMU UTRZYMANIA NIOSEK NA JAKOŚĆ JAJ ORAZ AKTYWNOŚĆ LIZOZYMU I CYSTATYNY

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Badania prowadzono na jajach pochodzących od czterech grup niosek. Grupa I – nioski Zielononóżka kuropatwiana (ZK) utrzymywane w systemie półintensywnym z możliwością korzystania z wybiegów, Grupa II – nioski Lohmann Brown (LB) utrzymywane w typowych klatkach bateryjnych, Grupa III – nioski Lohmann Brown (LB) utrzymywane w systemie półintensywnym (jak grupa I), Grupa IV – nioski Lohmann Brown (LB) utrzymywane w systemie fermowym na ściółce słomiasto-trocinowej [Dobrzański, 2000].

Kury otrzymały standardowe żywienia z udziałem mieszanki paszowej DJ o zawartości białka przeciętnie 17% i energii metabolicznej 2800 kcal. Wiek niosek obejmował przełom szczytu nieśności, tj. 26 – 34 tygodnie życia.

Celem prowadzonych badań było wykazanie wpływu systemu utrzymania i grupy genetycznej niosek na jakość handlową jaj oraz aktywność cystatyny i lizozymu jako ważnych substancji w zastosowaniach biomedycznych i nutraceutycznych.

W badaniach jakościowych jaj uwzględniono; masę jaj i procentowy udział skorupy, białka i żółtka, wartość pH białka, jednostki Hougha oraz przeprowadzono ocenę sensoryczną jaj. Najważniejszym zadaniem było oznaczenie aktywności lizozymu i cystatyny metodami standardowymi. Najwyższą aktywnością lizozymu i cystatyny charakteryzowały się jaja niosek Lohmann utrzymanych w klatkach i w systemie podłogowym, natomiast najniższymi wartościami charakteryzowały się jaja Zielononóżek, co również może mieć związek z zimową porą roku. Na podkreślenie zasługują relatywnie duże zmienności.

Przeprowadzone badania pozostawiają nie wyjaśniony problem zależności pomiędzy grupą genetyczną niosek, systemem żywienia i utrzymania oraz wiekiem kur w aspekcie wpływu tych czynników na aktywność biologiczną lizozymu i cystatyny. Istnieje zainteresowanie wykorzystaniem zarówno lizozymu jak i cystatyny jako nutraceutyków i preparatów biomedycznych. Chcąc wdrażać biologicznie aktywne substancje z jaj do praktycznego wykorzystania w przemyśle należy rozwiązać problem występującej nadmiernej zmienności aktywności tych substancji w surowcu wyjściowym oraz stworzyć możliwość prognozowania wartości tych parametrów.