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DOROTA BANASZEWSKA

BARBARA BIESIADA-DRZAZGA

DOMINIK OSTROWSKI

KAMIL DRABIK

JUSTYNA BATKOWSKA

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The impact of breeder age on egg quality and lysozyme activity

Dorota BANASZEWSKA[1,*], Barbara BIESIADA-DRZAZGA[1], Dominik OSTROWSKI[1], Kamil DRABIK[2], Justyna BATKOWSKA[2]

[1]Department of Breeding Methods and Poultry Breeding, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland
[2]Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, Lublin, Poland

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Abstract: The aim of this study was to analyze the biological components of eggs as well as lysozyme activity in relation to the age of hens. The research material consisted of eggs from parent stock of Ross 308 meat chickens. The eggs were evaluated at peak laying, i.e. eggs from 30-week-old hens, and after peak laying, i.e. from hens aged 60 weeks. Ninety eggs were evaluated in each period (180 eggs in total). Egg quality traits were divided into destructive and nondestructive traits. In addition, lysozyme activity in the albumen of the eggs was assessed. The quantitative characteristics of the eggs were found to increase with the age of the hens, with a smaller proportion of albumen and a larger of yolk. Egg weight, egg shape index, albumen and yolk weight, and air cell depth were increased with the hen's age. The eggshell qualitative traits were changed with the hen's age. Analyses showed that the shell weight and color of shells increased with the decrease of the density and strength of this element. In eggs from older hens marbled shells and pimples were found significantly more frequently. The lysozyme activity in the egg albumen also changed with the age of the hens. The activity of this enzyme was higher in the eggs of older hens.

Key words: Hen's age, broiler breeders, laying period, eggs, lysozyme

1. Introduction

Chicken eggs are a source not only of proteins with various physicochemical, biological, or technological properties, but of other nutrients as well. The antimicrobial activity of lysozyme, for instance, can be widely exploited in the preservation of meat or meat products [1]; in cheese-making, beer-brewing [2], and wine-making; and also in human and veterinary medicine.

Of all the bioactive components of the hen's egg, only lysozyme is on the list of permitted food additives. It is toxicologically safe because it is also present in the human body; it has properties that are desirable in substances used for preserving food and is considered harmless. Numerous studies have shown that lysozyme isolated as a pure enzyme can prolong the shelf life of many food products. To extend its activity to gram-negative bacteria, lysozyme must be modified in various ways and combined with other substances [3,4]. Owing to its alkalinity, lysozyme forms complexes with other proteins such as ovomucin or biopolymers. The physicochemical state of such a complex of lysozyme with ovomucin is an indicator of egg freshness, because it is responsible for the gel structure of the albumen [5].

The properties of chicken eggs change under the influence of many factors, such as diet, production system, the genetic origin of the layers, veterinary prophylaxis, or housing conditions [6–10]. The age of laying hens may also be considered as an important factor in the case of modification of eggs' quality traits. With the age of hens, changes in egg mass and the quality parameters of yolk and albumen, as well as their stability during storage, are registered [11,12]. The quality of egg contents and shells has also been shown to deteriorate as the laying rate increases [13,14]. Studies also indicated changes in lysozyme activity depending on the hen's age [15].

The aim of this study was to analyze the quality of eggs as well as lysozyme activity in relation to the age of hens.

2. Materials and methods

The research material consisted of eggs bought from parent stock of Ross 308 meat chickens. The birds were kept on deep litter in enclosed housing (Borzychy, Masovian Voivodeship, Poland) in accordance with the requirements given in "Instructions for Raising a Ross 308 Flock" [16]. The microclimate conditions of the chicken houses were strictly controlled in accordance with accepted standards.

* Correspondence: dorota.banaszewska@uph.edu.pl

The chickens came from the same breeder and were fed a complete compound feed adjusted for their age (Table 1). The birds were exposed to 14 h of light per day.

The eggs were evaluated twice, at peak laying, i.e. eggs from 30-week-old hens, and after peak laying, i.e. from hens aged 60 weeks. Ninety eggs were evaluated in each period (180 in total).

An Instron Mini 55 apparatus tensile tester was used to measure the force necessary to crush the shell (N). An EQM (egg quality measurements) set from TSS was used for further analysis. The following characteristics of individual egg components were assessed:

1. Whole egg traits:

· the mass and specific gravity of eggs – according to the Archimedes principle,

· egg shape index – calculated as a ratio of egg axis measurement (long and equator),

· air cell depth - with an Ovolux candling lamp and a millimeter scale.

2. Shell evaluation:

· nondestructive shell thickness measurement – using an ultrasonic eggshell thickness gauge (ORKA, ESTG-1, ORKA Technology Ltd., Israel), the measurement was made at the midpoint between the blunt end and the equator [17],

• color – using a reflectometer, expressed as a percentage of reflected light (in integers from 0 to 100),

• weight – using an electronic scale with accuracy of 0.01 g,

• thickness – using a micrometer, at midheight (at the equator),

• density – expressed in g/cm^3, calculated from established formulas [18].

3. Albumen evaluation:

• height – after breaking an egg against a table, through contact between the EQM sensor and the surface of the thick albumen; based on the thick albumen height and weight of the egg, Haugh units were calculated according to an established formula [19],

• concentration of hydrogen ions (pH) using a CP-251 pH meter.

4. Yolk evaluation:

• weight – measured with an Ohaus electronic scale with accuracy of 0.01 g,

• color – with a colorimeter, according to a yolk fan (DSM) 16-point scale,

• concentration of hydrogen ions (pH) using a CP-251 pH meter.

The data obtained were used to calculate the percentage share of each morphological component of the egg in the weight of the entire egg.

In addition, lysozyme activity in the albumen of the eggs was assessed according to the spectrophotometric method of Kijowski and Leśnierowski [20]. The first stage of measurement was applied to determine the standard curve based on differences in absorbance level (λ = 450 nm using a GENESIS 10S VIS spectrophotometer) in a suspension of *Micrococcus lysodeikticus* bacteria at the beginning and 1 min after adding the lysozyme standard in various concentrations. Egg albumen samples (homogenized fresh albumen) were diluted 100 times and then lysozyme activity was measured in the same way. Final results were calculated using the standard curve.

Table 1. Nutritional value of feeds used in the hens' diet during the laying period.

Compound feed constituents	Type of feed–period of use (weeks)	
	Laying period	
	24–45	Over 45
Crude protein (%)	14.5–15.5	14.0–14.5
ME (MJ)	11.7	11.7
Fat (%)	4.0–5.0	4.0–5.0
Amino acids (%):		
lysine	0.56	0.55
methionine + cysteine	0.53	0.52
methionine	0.29	0.28
Minerals (%):		
Ca	3.0	3.3
P (available)	0.34	0.33

2.1. Statistical analysis

Statistical analysis of the data was performed with the age of hens as the experimental factor. Statistical differences between samples were tested using ANOVA (STATISTICA version 10.0, StatSoft Inc., Poland). Nonparametric data (shell defects) were analyzed using chi-square tests. The exact values of significance probability are given in tables.

3. Results

Table 2 presents data on selected morphological characteristics of hens' eggs depending on the age of the hens when they laid the eggs. These data indicate that age affects most of the physicochemical characteristics of eggs. The Ross 308 hens at their peak laid eggs with a significantly lower weight (by more than 10 g), but the weight of the eggs was more even, as evidenced by smaller standard deviation. The shape index of the eggs of young hens was also slightly smaller, which indicates a more spherical shape of the eggs of hens at their laying peak. Due to the lower weight of eggs from hens at the age of

30 weeks as compared to older hens (60 weeks), the shell, albumen, and yolk weights of these eggs were lower, as well, with a higher proportion of albumen and a smaller share of yolk as compared to the eggs of older hens. These differences were confirmed statistically. In addition, in the eggs of younger hens, the air cell depth was nearly 0.08 mm lower ($P < 0.05$).

The eggshell characteristics depending on the layer's age are presented in Table 3. There are no statistically significant differences in the thickness of shells regardless of the evaluation method. However, a significant difference was observed in the case of such parameters as shell mass, color, and strength. The eggs from older hens were characterized by heavier and brighter shells. However, the shells of the eggs of younger hens were stronger by nearly 6 N than those of older hens.

We also analyzed the frequency of egg defects depending on the age of laying hens (Table 4). It was observed that although the eggs of young hens had thicker and stronger shells, in some cases there was a higher percentage of shell defects in the eggs of hens at the age of 30 weeks as compared to 60-week-old hens. There was a high frequency, exceeding 30%, of severe stripe marks on the shells (in the case of eggs of both young and old

Table 2. Selected egg characteristics according to the age of the hens.

Trait	Age of hens		Probability
	30 weeks	60 weeks	
	Mean ± SD	Mean ± SD	
Egg weight (g)	55. 35 ± 4.73	65.67 ± 5.78	0.000
Egg density (g/cm^3)	1.09 ± 0.01	1.08 ± 0.01	0.006
Egg shape index	1.28 ± 0.03	1.33 ± 0.06	0.000
Shell weight (g)	7.10 ± 0.75	7.98 ± 0.74	0.000
Albumen weight (g)	32.99 ± 5.09	38.61 ± 7.61	0.011
Yolk weight (g)	16.71 ± 1.32	19.23 ± 6.49	0.000
Air cell height (mm)	1.26 ± 0.82	2.04 ± 0.52	0.000
Percentage in egg weight of (%):			
shell	12.63 ± 1.41	12.15 ± 1.21	0.121
albumen	58.25 ± 5.90	55.34 ± 3.29	0.011
yolk	29.82 ± 3.10	32.51 ± 2.94	0.000

SD: Standard deviation.

Table 3. Selected eggshell characteristics according to the age of the hens.

Trait	Age of hens		Probability
	30 weeks	60 weeks	
	Mean ± SD	Mean ± SD	
Shell weight (g)	7.10 ± 0.75	7.98 ± 0.74	0.000
Shell color (%)	56.53 ± 4.90	59.98 ± 5.06	0.004
Shell thickness (mm)	0.315 ± 0.03	0.313 ± 0.02	0.783
Shell density (g/cm^3)	3.26 ± 0.27	3.33 ± 0.33	0.366
Shell strength (N)	45.70 ± 11.08	39.81 ± 9.41	0.014
Shell thickness measured by ultrasound (mm)	0.438 ± 0.04	0.427 ± 0.04	0.314

SD: Standard deviation.

Table 4. Eggshell defects according to the age of the hens (%).

Shell defects	Age of hens		χ^2 (probability)
	30 weeks	60 weeks	
Intact	6.8	7.9	0.822
External crack	1.4	0.0	0.354
Hairline crack	6.8	3.2	0.358
Star crack	4.1	1.6	0.399
Severe stripe marks	30.1	38.1	0.492
Wrinkled	1.4	9.5	0.042
Marbled	35.6	12.7	0.016
Displaced air cell	4.1	9.5	0.237
Weak ends	6.8	4.8	0.626
Pimples	0.0	1.6	0.284
Broken chalaza (%)	2.7	11.1	0.067

hens) and of marbled shells ($P = 0.016$). In addition, internal cracks (hairline or star cracks) and weak ends were fairly common. It is worth noting that in older hens, the frequency of internal cracks, marbled shells, and shells with weak ends decreased while the percentages of shells with stripe marks, wrinkled shells, displaced air cells, and shells with pimples increased.

Table 5 presents selected features of the egg albumen and yolk depending on the age of the hens. The data indicate that both albumen and yolk characteristics change with the age of the hens. In the eggs of older hens, aged 60 weeks, the albumen weight was nearly 5 g greater than in the eggs of young hens aged 30 weeks ($P < 0.05$). At the same time, the albumen height in the eggs of younger hens was greater than in the eggs of older hens ($P < 0.05$). Lysozyme activity also changed with the age of the hens. The activity of this enzyme was higher by 1680 U/mL in the egg albumen of older hens than that of younger ones. These differences were confirmed statistically. The egg yolks of chickens at the age of 60 weeks were also considerably heavier (by about 2.5 g) than in the eggs of younger hens. The color of the yolk also changed with the age of the hens. The yolks of older hens were darker according to a yolk fan (DSM). These differences were confirmed statistically ($P < 0.05$). No statistically significant differences were observed in the case of the pH of the albumen and yolk of eggs laid by hens at different ages. However, a significantly higher frequency of eggs with broken yolks was observed in older hens, by about 8%, which may indicate chalaza damage.

4. Discussion

The level of egg consumption is currently quite high, and consumers value not only their availability and low price but also their taste and nutritional value. In the last few decades, the use of modern poultry breeding and feeding methods has led to marked progress in the productivity of hens [21]. At the same time, increased laying rates have been associated with a deterioration in the quality of egg contents and shells [13,14]. According to Nys [6], the weight of eggs depends on genetic and environmental factors, particularly on the birds' diet and the temperature conditions where they are kept. Studies by Silversides and Budgell [8] and Czaja and Gornowicz [22] clearly indicated the influence of the genotype of hens on the physicochemical characteristics of eggs (including the weight of the egg, shell, and yolk). According to Akyurek and Okur [11], the egg-laying rate of hens is a factor influencing egg weight. Lower laying rates cause birds to store more of the material necessary to build and increase the weight of the egg. Genetically determined differences in the intensity of eggshell color have been demonstrated as well, with a tendency towards lighter shells in the eggs of older hens [23]. Roberts [14] emphasized that the trait that is most correlated with the genotype of hens is eggshell color, and its intensity depends on age, which was also confirmed by the studies by other authors [7,12,24]. The present study confirmed the lower intensity of the shell color of eggs laid by older hens. Dark shell color is caused by a brown pigment derived from hemin. The intensity of the eggshell color is inversely proportional to the laying rate [9]. On the other hand, a tendency towards increased intensity of yolk color may result from decreased production by hens or from a larger amount of carotenoids in their feed, since, as demonstrated by Nys [6], yolk color is particularly influenced by the diet and laying rate of the hen. Our study found statistically significant differences in yolk color depending on the age of the layers. Hens at the age of 60 weeks laid eggs with a more intense yolk color compared to younger hens. Many authors pointed out the influence of age on egg characteristics [25]. Research confirms that egg and shell quality characteristics change with hens' ages. As hens grow older, the weight of the egg, yolk, and shell increases, while albumen quality and shell strength deteriorate [25,26], which was confirmed by our research. Hunton [9] emphasized that the mechanical properties of eggshells are mainly influenced by the concentration of proteins in the matrix and the structure of the shell, while mechanical changes in the properties of the shell, progressing with the age of the hen, are integrally linked to the reduction in phosphorus and calcium absorption from feed by the hens and the slowdown of the mineralization process. In our research, the eggs of older hens had a slightly thinner shell with lower strength than the eggs laid by hens at their laying peak. According to some authors, eggshell quality decreases as hens age because the increased weight of the

Table 5. Selected characteristics of the albumen and yolk of eggs according to the age of the hens

Trait	Age of hens		Probability
	30 weeks	60 weeks	
	Mean ± SD	Mean ± SD	
Albumen weight (g)	32.99 ± 5.09	38.61 ± 7.61	0.000
Albumen height (mm)	6.81 ± 1.29	6.02 ± 1.58	0.022
Haugh units	81.99 ± 7.59	73.23 ± 10.99	0.000
Albumen pH	8.92 ± 0.15	8.94 ± 0.15	0.564
Lysozyme activity (U/mL)	33,120 ± 1,800	34,800 ± 2,640	0.011
Yolk weight (g)	16.71 ± 1.32	19.23 ± 6.49	0.011
Yolk color (yolk fan (DSM) 16-point scale)	3.12 ± 0.96	4.59 ± 1.31	0.000
Yolk pH	6.30 ± 0.58	6.31 ± 0.08	0.585

SD: Standard deviation.

egg contents is not accompanied by a sufficient increase in shell weight. As a result, the strength of the eggshell decreases with age [9,14]. The present study confirmed a significant difference in the strength of the shell. The shells of eggs laid by hens at the age of 30 weeks were nearly 6 N stronger than those of hens aged 60 weeks. Calik [26] also showed a decrease in shell quality parameters (thickness, density, and strength) with the age of layers. A study by Premavalli and Viswanagthan [27] indicated a positive relationship between the thickness and strength of shells and their density. The authors showed that the age of layers affects the frequency of internal cracks in the shell. In the present study, a higher frequency of internal and external cracks was found in hens aged 30 weeks than in older ones. Pantheleux et al. [28], on the other hand, showed that a thicker eggshell only partially contributes to greater strength.

Eggs play a significant role in human nutrition. They are an excellent and affordable source of protein of the highest quality, and they have a relatively low energy level. In addition, eggs have very high concentrations of nutrients [29], and due to the chemical composition and functional properties, they increasingly play a nutraceutical role. Eggs contain proteins; nearly all vitamins (except vitamin C), including the valuable vitamin B_9 (folic acid); carotenoids; choline; niacin, which regulates the level of sugar and cholesterol in the blood; and lecithin, necessary for heart and brain function. Another asset of eggs is their high content of zinc, calcium, phosphorus, and iron. Eggs are one of the few food products that contain natural selenium and iodine [29]. Egg albumen has high biological value and is treated as a standard for assessing the value of other proteins, as it contains large amounts of essential amino acids. Egg albumen contains lysozyme, an enzyme with bactericidal and antiviral properties [4]. Lysozyme (N-acetyl-muramyl hydrolase, E.C. 3.2.1.17) is also called muramidase. This low-molecular-weight enzyme is present in virtually all tissues and secretions of humans and animals. It has also been isolated from some plants, bacteria, and bacteriophages [30]. Many researchers are interested in determining the activity of this enzyme in egg albumen. Our study showed that the activity of lysozyme in 1 mL of egg albumen increased with the age of the hens and with albumen weight. However, the number of Haugh units estimated in this study, which is indicative of albumen quality [31], was slightly higher in the eggs of younger hens than in the eggs of hens past their laying peak. According to some authors, in the eggs of older hens there is a decrease in the proportion of albumen in the weight of the egg [13,32] and in the height of the thick albumen [33], which is associated with the deterioration of egg albumen characteristics. It seems likely that deterioration of the quality of the albumen as a whole with the age of hens may result in the deterioration of its individual components, including lysozyme activity, which is confirmed by our research. Some studies indicated a relationship between egg weight, which is known to increase with the age of laying hens, and the hydrolytic activity of lysozyme [34]. The eggs with the lowest weight were shown to have the highest percentage content of lysozyme in the thin and thick albumen and the highest hydrolytic activity of this enzyme. The hydrolytic activity of the enzyme has also been shown to depend on the pH it is in [35]. The pH of albumen increases with storage time [36], and at higher pH values (5.3–6.6) the enzyme is inactivated [35]. This indicates the indirect role of lysozyme in preserving egg quality. In our study, many more eggs with broken yolk came from older hens, which may indicate poorer albumen quality in the eggs of hens after peak egg production. Some authors linked the activity of this enzyme to the influence of genetic factors [37] and environmental factors. A study by Krawczyk and Gornowicz [38] found that eggs from hens with access to a chicken run had a greater share of lysozyme in the weight of the albumen than eggs from hens kept in a litter system. In addition, the proportion of lysozyme in the albumen of eggs from younger hens (aged 32–36 weeks) was higher than that in the eggs of older hens (aged 52–56 weeks), irrespective of the housing system. A study by Trziszka et al. [39] indicated high variation in the biological activity of lysozyme in the eggs of laying hens of different genetic groups raised in different systems. That study found the highest lysozyme activity in the eggs of caged Lohmann hens. In the same genetic group, lysozyme activity decreased with the hen's age [15]; however, in our study, a reverse relation was found, which could result from the fact that in the cited study the authors analyzed laying-type hybrids while our study focused on meat-type breeders. The data therefore indicate the impact of not only age but also the type of bird on lysozyme activity. According to some authors, feed supplementation with appropriate vitamins and herbs has a positive effect on the hydrolytic activity of lysozyme [40,41] and causes changes in the quality and biological characteristics of eggs [42,43].

In conclusion, the quantitative characteristics of eggs (the weight of the whole egg and its morphological components) were found to increase with the age of the hens, with a smaller proportion of albumen and a larger proportion of yolk. In addition, hens past their peak laying period, at the age of 60 weeks, laid eggs with a more elongated shape, and the shells of these eggs were thinner and less resistant to crushing. The lysozyme activity in the egg albumen also changed with the age of the hens. The activity of this enzyme was higher in eggs of older hens; however, this may also have resulted from the hens' type.

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