*Ann. Anim. Sci., Vol. 24, No. 3 (2024) 965–977* *DOI: 10.2478/aoas-2024-0032*

sciendo

# PHYSICAL QUALITY PARAMETERS AND ACTIVITY OF ALBUMEN LYSOZYME OF EGGS FROM TRADITIONAL HENS BRED IN POLAND COMPARED WITH COMMERCIAL HYBRIDS*

Lidia Lewko[1], Małgorzata Gumułka[2♦], Krzysztof Andres[2], Józefa Krawczyk[1]

[1]Department of Poultry Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[2]Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
♦Corresponding author: malgorzata.gumulka@urk.edu.pl

**Abstract**

**The variation in egg quality parameters of traditional/native hen breeds with good freshness and antibacterial properties of egg albumen may determine the attractiveness of these eggs for modern consumers looking for high quality products. The objective of this study was to evaluate external and internal physical quality parameters and enzymatic activity of lysozyme of eggs from three traditional hens bred in Poland and to compare these egg quality parameters to those obtained from commercial hybrids kept under the same management conditions in extensive farming production system. The study was carried out on eggs collected from 4 genetic groups of hens, i.e. Polish Liliputy Bantams (PLB), native Polish Crested Chickens (PCr, CP-22 strain), Gold Laced Polish Chickens (GLP) and from Hy–Line Brown hybrids (HLB). In total 135 PLB, 75 PCr, 75 GLP and 75 HLB hens were kept on litter (3 pens) and fed commercial feed. The eggs (n = 33/genotype/age) were collected at the 33 and 55 weeks of age. The study concluded that with the age of the hens, there was an increase (P<0.05) in the weight of the egg and the proportion of its main fractions, as well as a decrease (P<0.05) in the quality parameters of the albumen and the eggshell. However the eggs from traditional breeds retain good parameters of albumen quality even at the end of production period. The eggs of traditional hens were characterized by higher (P<0.05) yolk proportion and its color and albumen lysozyme parameters, and a lower (P<0.05) eggshell and albumen height and Haugh unit score in relation to commercial hybrids. The highest (P<0.05) content of lysozyme and enzymatic activity of lysozyme were found in eggs from Gold Laced Polish Chickens. Thus, eggs obtained from traditional hens may meet requirements of modern consumers because of specific physical characteristics, good albumen lysozyme activity and freshness parameters. Particularly noteworthy are Polish Liliputy Bantams eggs, characterized by the low weight and a high proportion of more intensely colored yolk and good Haugh unit score at the end of production period.**

**Key words: genotype, egg, physicochemical parameters, lysozyme**

In recent years, there has been an increasing interest in the quality of products from local breeds and varieties of hens (Franco et al., 2020; Lordelo et al., 2020; Marelli et al., 2020; González Ariza et al., 2022), especially those kept in non-intensive production systems like free-range and organic systems (Sirri et al., 2018; Kumar et al., 2022; Rizzi et al., 2022). The variation in egg quality characteristics from these hens may be attractive to consumers. In addition, an element of product acceptability by consumers is food safety, which is fundamentally influenced by the antibacterial properties of egg albumen. Lysozyme, also called muramidase, is one of the main proteins in hen egg albumen, with well documented bacteriostatic activity (Leśnierowski and Stangierski, 2018). The native form of this enzyme is a monomer that hydrolyzes the β-1,4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine of cell wall peptidoglycan in Gram-positive bacteria (Leśnierowski and Yang, 2021). Hens' eggs are the primary commercial source of biologically active lysozyme preparations (Shahmohammadi, 2018). Their antibacterial properties are used in the food industries as well as in medicine, pharmacology, and veterinary medicine (Leśnierowski and Yang, 2021; Nawaz et al., 2022). Of particular importance is the use in humans as an alternative antibiotic (Ferraboschi et al., 2021). The concentration and hydrolytic activity of lysozyme (Krawczyk et al., 2021; Lewko et al., 2021) as well as the concentration of ovotransferrin, another antimicrobial albumen protein (Gvoždíková Javůrková et al., 2019), vary depend on the genotype of hens farmed under non-commercial conditions. Moreover, hens of native breeds produce eggs with different physical (Krawczyk, 2009) and nutritional characteristics from those of commercial hybrids (Franco et al., 2020; Lordelo et al.,

*This study was financially supported by the Ministry of Education and Science for the University of Agriculture in Krakow (subvention number: 020013-D015) and for the National Research Institute of Animal Production, Poland (subvention number: 501-120-811).

2020; Marelli et al., 2020; Ianni et al., 2021). In Poland, Krawczyk et al. (2021) and Lewko et al. (2021) reported the difference in external and internal quality parameters of eggs obtained from hens kept in accordance with the rules of a biodiversity conservation program.

Thus, eggs obtained from traditional/native hen breeds can open up new market opportunities for the niche products with specific characteristic. The University of Agriculture in Krakow (Poland) carries out breeding work aimed at preserving traditional hen breeds intended for organic, free-range, backyard, and extensive farming (Andres et al., 2008; Gumułka et al., 2020; Węglarz et al., 2020). Thus, some genetic groups of hens such as Polish Liliputy Bantams (PLB), native Polish Crested Chickens (PCr) and Gold Laced Polish Chickens (GLP) are kept at the University's experimental farm. The PCr are maintained as a conservation flock (National Research Institute of Animal Production, 2023) and PLB and GLP as a part of the educational collection. All these hens have specific physical features that enhance their visual appeal, which is also important for small-scale farmers. The PLB hens are an ancient Polish dwarf variety and are characterized by small body weight (about 0.5 kg for a female) but they also show a seasonality in egg production (Antos et al., 2013). The CP-22 strain of PCr hens are light type birds (body weight for female – about 1.7 kg) with a medium-sized feather crest and rose comb. The main color of their plumage is golden and silver wheaten (Andres, 2022). The breeding program for these unique hen resources does not provide for selection for egg production but only for the protection of these flocks against increased inbreeding (National Research Institute of Animal Production, 2023). The GLP is an old breed of European origin of the light type hen (body weight for female – about 1.8 kg), characterized by a relatively large crest of feathers and having muffs and beard (Roberts, 2008; Yoshimura et al., 2012).

All these groups of hens represent the historical traditions of poultry farming in Poland. However, there is no information on the internal and external quality parameters of the eggs of these hens. To date, only a preliminary approach towards the nutritional value of eggs stored under various conditions from strain CP-11of PCr at peak of production has been reported (Gumułka et al., 2022). Importantly, the eggs of this breed match the standards of a commercial egg product, as was shown by a comparison with Hy-Line Brown hybrids.

It is known that the age of hens affects external physical parameters of egg quality in both commercial hybrids (Vlčková et al., 2019) and native breeds of hens (Krawczyk, 2009; Krawczyk et al., 2021). Additionally, Lewko et al. (2021) showed an age-related decrease in eggshell compression strength from native hens kept under a conservation program. Due to the changes in some ultra- and microstructure characteristics of the eggshell with the increasing age of the hens (Benavides-Reyes et al., 2021), it is particularly important to determine the activity of the antimicrobial defence system of the albumen. Vlčková et al. (2019) noted the effect of the age of the hens on the content of ovotransferrin and ovalbumin in the albumen. On the other hand, Krawczyk et al. (2021), Kowalska et al. (2021) and Lewko et al. (2021) also reported an increase in the concentration and hydrolytic activity of lysozyme with the age of the hens. Thus, it seems reasonable to undertake an investigation of the parameters of egg freshness, eggshell resistance, and lysozyme activity in different ages of traditional hens, as it is particularly important for flocks whose duration of egg production is shorter compared to commercial hybrids.

We hypothesizes that eggs from traditional hens bred in Poland match the quality of eggs produced by commercial hybrids in terms of their physical characteristics and lysozyme activity, irrespective of age of birds. Thus, the eggs produced by traditional hens kept in extensive farming production system may be a potential source of new products that are suited to the expectations of modern consumers.

The objective of this study was (1) to evaluate the external and internal physical quality parameters and enzymatic activity of lysozyme of eggs from three traditional hens bred in Poland and; (2) to compare these egg quality parameters to those obtained from commercial hybrids kept under the same management conditions.

## Material and methods

### Experimental birds and management

The study was carried out on eggs collected from 4 genetic group of hens, i.e. Polish Liliputy Bantams (PLB), native Polish Crested Chickens (PCr, CP-22 strain), Gold Laced Polish Chickens (GLP) and from Hy–Line Brown hybrids (HLB). A total of 135 PLB, 75 PCr, 75 GLP and 75 HLB hens were kept on litter (chopped wheat straw) in an experimental building at the Research and Education Centre of the Faculty of Animal Sciences of the University of Agriculture in Krakow, Poland. The birds were hatched and raised on the Research and Education Centre premises. Hens were kept in extensive farming production system. Housing, lighting and feeding conditions were in accordance with welfare standards for laying hens (European Union, 1999). There were 3 pens (2.0 × 2.5 m) connected with runs (2.0 × 4.5 m) for each hen genotype (3 replications). Hens were kept at a stocking density of 9 birds/m$^2$ for PLB and 5 birds/m$^2$ for PCr, GLP and HLB. For PLB different stocking was adopted due to the smaller body size of the birds. Each pen was equipped with 6 individual latching nests, a round feeder (circumference: 133 cm) and an automatic bell drinker (circumference: 125 cm). A natural and artificial lighting schedule was used: 16 h L, 8 h D (lights on from 05:00 to 21:00 h; 10–15 lx).The temperature in the building was around 20°C with relative humidity of 65–70%. Birds had free access to feed and water. A commercial, granulated, layer-breeder mixture, was fed to the hens. The composition of the mixture is given in Table 1. The

chemical composition of the mixture was 15.0% crude protein, 5.1% crude fat, 4.6% crude fiber, 90.5% DM, 18.6% crude ash. Analyses to determine the chemical composition of the mixture were carried out at the Central Laboratory of the National Research Institute of Animal Production in accordance with the standards applicable in the laboratory. There were no special additives in the diet.

Table 1. Composition of the commercial mixture used to feed hens

| Ingredients | g/kg |
|---|---|
| Corn | 350.0 |
| Wheat | 330.0 |
| Soybean meal | 130.0 |
| Sunflower meal | 40.0 |
| Wheat bran | 37.3 |
| Limestone | 91.0 |
| Monocalcium phosphate | 12.0 |
| NaCl | 3.0 |
| DL-Methionine | 1.1 |
| L-Lysine hydrochloride | 0.6 |
| Vitamin–mineral premix[1] | 5.0 |
| Metabolizable energy (MJ/kg) | 11.35 |
| **Nutrient composition (g/kg DM)** | |
| Crude protein | 150.0 |
| Lys | 7.2 |
| Met | 3.4 |
| Ca | 36.0 |
| Available P | 3.75 |
| Na | 1.5 |
| Cl | 2.2 |

[1]Premix is provided with the following content per kilogram: 10,000 IU of vitamin A, 2000 IU of vitamin $D_3$, 20 IU of vitamin E, 1.5 mg of vitamin $K_3$, 1.0 mg of vitamin $B_1$, 4.0 mg of vitamin $B_2$, 1.5 mg of vitamin $B_6$, 0.01 mg of vitamin $B_{12}$, 8.7 mg of calcium pantothenate, 20.0 mg of niacin, 0.8 mg of folic acid, 200.0 mg of choline chloride, 85.0 mg of manganese, 60.0 mg of zinc, 45.0 mg of iron, 8.0 mg of copper, 1.0 mg of iodine, and 0.25 mg of selenium.

**Eggs and physical quality parameters**

Data on the number of eggs laid were recorded in the period from the 30th to the 56th week of the hens age. The eggs for analysis (n = 33/genotype/age) were randomly collected from each hen genotype group (11 eggs/pen) at 33 and 55 weeks of age. After 24 h of storage in a refrigerator at 4°C and 55% humidity, the physical quality and lysozyme parameters of the eggs were evaluated. The average of hen day egg production in the study period was approximately 51%, 60%, 48% and 85% for PLB, PCr, GLP and HLB, respectively. Egg shape index (%) was evaluated with a Shape-Meter (B.V. Apparatenfabriek Van Doorn, De Bilt, The Netherlands), scaled between 65 and 85%. The weight of the egg (g) and internal egg parameters, i.e. the weight of yolk and eggshell (g), albumen height (mm), and Haugh unit score were determined using Egg Quality Measurements Electronic Equipment (Technical Services and Supplies Ltd., Dunnington, York, UK). On the basis of the measurements taken, the following were calculated: proportion of albumen, yolk and shell in egg. They were calculated as a percentage of the weight of the egg part in the weight of the egg.

The color of the yolk was measured using the reflectance spectrophotometer Minolta C580 Chroma Meter (aperture 8 mm) (Konica Minolta Sensing Business Unit, Osaka, Japan) equipped with a 50-mm reading head and with Spectra Magic NX software. The instrument was calibrated with a white reference plate (Konica Minolta, Sensing, Inc. Japan), with setting values (L* = 97.10, a* = -4.88, b* = 7.04) before the measurement. Results were recorded as L*, a*, b* color space. The L* value represents lightness (0 = black, 100 = white), a* indicates redness (−100 = green, 100 = red) and b* gives a value for yellowness (−100 = blue, 100 = yellow). The pH value of the albumen (on whole egg albumen) and yolk was evaluated using a Seven2Go (Mettler-Toledo, Warszawa, Poland) pH meter. The pH meter was calibrated using 3 calibration buffers (pH 4.0, pH 7.0 and pH 10.0).

**Quality parameters of eggshells**

The color of the shell (L*, a*, b* color space) was performed at 3 egg measurement points (sharp end, blunt end, equator) using the Minolta C580 Chroma Meter reflectance spectrophotometer with instrument settings as described in the previous section. The shell thickness (μm) was measured after removal of shell membranes by using a Mitutoyo Digital Micrometer 293-766-30 (Mitutoyo America Corporation, Aurora, IL USA). The test was performed at 3 egg measurement points (sharp end, blunt end, equator). The number of pores per $cm^2$ of shell was determined according to Tyler (1953). The eggshells were covered with dye on the inside and porosity was determined using a stereomicroscope (magnification of 4×) in a shell area of 0.25 $cm^2$.

Shell elastic deformation (μm) and shell strength (N) were tested with a texture analyzer TA.XT PLUS (Stable Micro Systems, Godalming, UK) fitted with appropriate attachments. Shell deformation (accurate to 1 μm) was performed at 3 egg measurement points (on the equatorial circumference of the egg and on egg sharp and blunt end along the long axis of the egg) using a 2.5 mm diameter cylindrical probe (test speed: 0.5 mm/s, distance: 0.1 mm) under 2 different loads: 0.5 kg, 1.0 kg. This test determined the degree of elastic deformation of the shell under the applied loads. In the evaluation of the mechanical strength of the eggshell, a 7.5 cm diameter plate probe was used and the applied load (N) (test speed 10.0 mm/s, distance: 1.0 mm) was gradually increased until the shell broke or was crushed.

**Lysozyme content and enzymatic activity in albumen**

Preparation of the albumen fraction and analysis of the lysozyme content (%) and enzymatic activity (U/ml) were carried out as previously described by Lewko et al. (2021) and Krawczyk et al. (2021). In brief, the albumen was separated into the thin and thick fraction (1 mL of different albumen fractions was collected using an automatic pipette) and placed in disposable sterile containers. The so-prepared albumen samples were used to make preparations to determine the concentration and hydrolytic activity of lysozyme in the albumen fractions. In further procedures, the analytical method developed by Leśnierowski and Kijowski (1995) was used. Analysis was performed using a spectrophotometer (Metertech SP-830 plus; NanGang, Taipei, Taiwan). One enzyme unit is defined as the amount that decreases the absorbance of a *Micrococcus lysodeikticus* bacterial suspension as the substrate by 0.001 in 1 min, measured at a wavelength of 450 nm and a temperature of 25°C. The reaction occurred in a mixture of total volume of 2.6 ml (2.5 ml of bacterial suspension + 0.1 ml lysozyme solution) and pH 6.2, in a cuvette with a 1 cm optical path length. After calculating the value of absorbance decrease (ΔA) for the working solution of lysozyme, a curve of correlations between ΔA and lysozyme concentration was graphed. Next, based on the standard curve, the hydrolytic activity of lysozyme was determined in the preparations. The decrease in the absorbance of the solution was calculated from the equation: $\Delta A = A_{t0} - A_t$ (U/min) ($A_{t0}$, absorbance of bacterial suspension at $t_0$ time; $A_t$, absorbance of bacterial suspension after time *t*).

**Statistical analysis**

The data were examined using the Kolmogorov-Smirnov test for normal distribution and using the Brown-Forsythe test for homogeneity of the variances. A two-way analysis of variance with interaction was applied to all the parameters analyzed. The following factors were included in the analysis: genotype of hens (4 genetic groups) and age of hens (33 and 55 wk). The model of ANOVA was: $Yijk = \mu + Gi + Aj + (G\times A)ij + \varepsilon ijk$, where Yijk = values of variable; μ = overall mean; Gi = effect of genotype; Aj = effect of age; G×A = interaction of genotype × age; εijk = residual random error. Fisher's test was used for multiple comparisons. The values in the tables were expressed as mean and standard error of the mean (SEM). Differences were considered significant at the level of $P<0.05$. The statistical analysis was processed using Statistica version 6.0 (TIBCO Software Inc., Palo Alto, CA).

## Results

Table 2 presents the results of the selected external and internal physical quality parameters of eggs from traditional hens bred in Poland in relation to age and compared with commercial hybrid. The egg shape index was influenced by genotype. The PLB eggs had a similar shape to HLB, while GPL eggs showed the most ($P<0.05$) elongated shape compared to eggs of other genetic groups. Variation in egg weight was observed, which was influenced by genotype and age. Eggs with the lowest weight ($P<0.05$) at both 33 and 55 weeks of age were noted for PLB (32.3 and 34.8 g, respectively) and with the highest weight ($P<0.05$) for HLB (62.7 and 65.3 g, respectively). With the age of the hens, an increase in egg weight ($P<0.05$) was observed, on average, by about 1.3 g. Albumen weight was affected by genotype. It ranged from 18.4 (PLB) to 39.4 g (HLB) for eggs obtained at 33 weeks of age and from 18.4 (PLB) to 41.3 g (HLB) for eggs at 55 weeks of age. The proportion of albumen in the egg was influenced by genotype, age and the interaction of these two factors. The lowest ($P<0.05$) proportion of albumen was found in PLB eggs (52.7%), and the highest ($P<0.05$) in HLB eggs (63.1%) at 55 weeks of age. The yolk weight and its proportion in the egg were influenced by the genotype and age as well as the interaction of these two factors. The weight of the yolk and its proportion in the egg increased ($P<0.05$) with the age of the hens by an average of 1.9 g and 2.8 percentage points respectively. The highest ($P<0.05$) yolk weight was recorded for PCr, GLP and HLB eggs at 55 weeks of age compared to PLB eggs. The highest egg yolk proportion ($P<0.05$) (37.0%) was found in PLB eggs, also obtained at 55 weeks of age.

Table 3 presents the results of the selected internal physical quality parameters of eggs from traditional hens bred in Poland in relation to age and compared with commercial hybrids. The yolk color was influenced by genotype, age (L*, a*, b*) and the interaction of these two factors (L*, a*). It was shown that with the age of the hens there was an increase ($P<0.05$) in color lightness (average L* 1.1), and intensity of red (average a* 9.8) and yellow (b* 2.8). The most intense lightness of yolks color ($P<0.05$) was found in the eggs of the GLP and HLB (L* 56.6 and L* 56.3*, respectively) in comparison to the other genetic group. The pH of the yolk was influenced by genotype, age and the interaction of these two factors. The pH of albumen was dependent on the genotype and the interaction of genotype and age. The pH value of the yolk decreased with the age of the hens ($P<0.05$) (on average by 0.1) with average values from 6.0 (HLB) to 6.3 (PLB). Albumen acidity in both assessed periods of hen age in the total assessment was at the same level – 7.8. The highest albumen pH ($P<0.05$) was found in the GLP egg compared to other genetic groups. Albumen quality parameters depended on genotype, age, and the interaction of these two factors. Irrespective of the age of the hens, the most favorable ($P<0.05$) albumen quality features, i.e. the highest albumen height (9.2 mm/33 week of age and 7.2 mm/55 week of age) and the value of Haugh units (94.9/33 week of age and 83.5/55 week of age) were found in HLB eggs compared to other genetic groups. Moreover, average value of albumen height was the lowest in PLB eggs (4.8 mm). For the value of Haugh units similar low value was noted for PLB and GLP as in other groups.

Table 2. Quality parameters of eggs (n = 66/genetic group) from traditional hens bred in Poland in relation to age and compared with commercial hybrids

| Characteristic | Age (wk) | Genotype of hens | | | | Mean | SEM | Effect (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PLB | PCr | GLP | HLB | | | G | A | G × A |
| Egg shape index (%) | 33 | 77.8 a* | 74.4 b* | 70.4 c* | 77.6 a* | 75.1* | 0.4 | | | |
| | 55 | 76.9 a* | 75.0 b* | 72.0 c** | 77.9 a* | 75.5* | 0.4 | | | |
| | Mean | 77.4 a | 74.7 b | 71.2 c | 77.8 a | 75.3 | 0.3 | <0.0001 | 0.2710 | 0.1135 |
| Weight of egg (g) | 33 | 32.3 d* | 53.1 b* | 49.3 c* | 62.7 a* | 49.3* | 1.0 | | | |
| | 55 | 34.8 d** | 53.2 b* | 50.8 c* | 65.3 a** | 51.0** | 1.0 | | | |
| | Mean | 33.6 d | 53.2 b | 50.1 c | 64.0 a | 50.2 | 0.7 | <0.0001 | 0.0002 | 0.1696 |
| Weight of albumen (g) | 33 | 18.4 d* | 31.4 b* | 28.2 c* | 39.4 a* | 29.4* | 0.7 | | | |
| | 55 | 18.4 d* | 30.8 b* | 28.4 c* | 41.3 a** | 29.7* | 0.8 | | | |
| | Mean | 18.4 d | 31.2 b | 28.3 c | 40.3 a | 29.6 | 0.5 | <0.0001 | 0.3503 | 0.1411 |
| Proportion of albumen in egg (%) | 33 | 56.9 c* | 59.3 b* | 57.2 bc* | 62.9 a* | 59.1* | 0.3 | | | |
| | 55 | 52.7 d** | 57.8 b** | 55.8 c* | 63.1 a** | 57.4** | 0.4 | | | |
| | Mean | 54.9 a | 58.6 b | 56.5 c | 63.0 d | 58.2 | 0.3 | <0.0001 | <0.0001 | 0.0002 |
| Weight of yolk (g) | 33 | 10.5 c* | 16.1 a* | 16.2 a* | 14.6 b* | 14.3* | 0.2 | | | |
| | 55 | 12.9 b** | 17.2 a** | 17.6 a** | 17.2 a** | 16.2** | 0.3 | | | |
| | Mean | 11.7 c | 16.6 a | 16.9 a | 15.9 b | 15.3 | 0.2 | <0.0001 | <0.0001 | 0.0056 |
| Proportion of yolk in egg (%) | 33 | 32.7 a* | 30.2 b* | 32.9 a* | 23.3 c* | 29.8* | 0.4 | | | |
| | 55 | 37.0 a** | 32.3 c** | 34.7 b** | 26.4 d** | 32.6** | 0.5 | | | |
| | Mean | 34.9 a | 31.2 c | 33.8 b | 24.9 d | 31.2 | 0.3 | <0.0001 | <0.0001 | 0.0125 |

a, b, c, d – values in the same row with different letters differ significantly, P<0.05.
*,** – values in the same column with different superscripts differ significantly, P<0.05.
Abbreviations: PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; HLB – Hy-Line Brown; G – genotype; A – age; G × A – interaction genotype × age.

Table 3. Internal physical quality parameters of eggs (n = 66/genetic group) from traditional hens bred in Poland in relation to age and compared with commercial hybrids

| Characteristic | Age (wk) | Genotype of hens | | | | Mean | SEM | Effect (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PLB | PCr | GLP | HLB | | | G | A | G × A |
| Yolk color L* | 33 | 52.0 b* | 53.4 b* | 56.0 a* | 57.1 a* | 54.6* | 0.4 | | | |
| | 55 | 55.1 b** | 54.9 b* | 57.2 a* | 55.6 ab* | 55.7** | 0.3 | | | |
| | Mean | 53.5 b | 54.2 b | 56.6 a | 56.3 a | 55.2 | 0.2 | <0.0001 | 0.0150 | 0.0067 |
| a* | 33 | 32.3 b* | 36.4 a* | 38.1 a* | 38.4 a* | 36.3* | 0.8 | | | |
| | 55 | 47.8 ac** | 42.6 b** | 49.9 a** | 43.9 bc** | 46.1** | 0.8 | | | |
| | Mean | 40.1 b | 39.4 b | 44.0 a | 41.1 b | 41.1 | 0.6 | 0.0126 | <0.0001 | 0.0024 |
| b* | 33 | 7.7 ab* | 7.1 bc* | 7.8 a* | 6.8 c* | 7.4* | 0.1 | | | |
| | 55 | 10.1 b** | 9.8 b** | 11.2 a** | 9.4 b** | 10.2** | 0.2 | | | |
| | Mean | 8.9 b | 8.5 b | 9.5 a | 8.1 c | 8.8 | 0.2 | <0.0001 | <0.0001 | 0.2034 |
| pH of yolk | 33 | 6.3 a* | 6.1 b* | 6.3 a* | 5.9 c* | 6.2* | 0.0 | | | |
| | 55 | 6.3 a* | 6.1 b* | 6.1 b** | 6.1 b** | 6.1** | 0.0 | | | |
| | Mean | 6.3 a | 6.1 b | 6.2 a | 6.0 c | 6.2 | 0.0 | <0.0001 | 0.0054 | <0.0001 |
| pH of albumen | 33 | 7.7 b* | 7.7 b* | 8.0 a* | 7.6 c* | 7.8* | 0.0 | | | |
| | 55 | 7.8 b** | 7.9 a** | 7.8 b** | 7.5 c* | 7.8* | 0.0 | | | |
| | Mean | 7.8 b | 7.8 b | 7.9 a | 7.6 c | 7.8 | 0.0 | <0.0001 | 0.7132 | <0.0001 |
| Albumen height (mm) | 33 | 4.8 d* | 6.3 b* | 5.2 c* | 9.2 a* | 6.4* | 0.2 | | | |
| | 55 | 4.8 c* | 5.0 c** | 6.1 b** | 7.2 a** | 5.6** | 0.1 | | | |
| | Mean | 4.8 c | 5.7 b | 5.7 b | 8.2 a | 6.1 | 0.1 | <0.0001 | <0.0001 | <0.0001 |
| Haugh unit score | 33 | 79.2 b* | 81.1 b* | 74.3 c* | 94.9 a* | 82.4* | 0.8 | | | |
| | 55 | 78.3 c* | 72.2 d** | 80.8 b** | 83.5 a** | 78.7** | 0.5 | | | |
| | Mean | 78.8 b | 76.7 c | 77.6 bc | 89.2 a | 80.6 | 0.5 | <0.0001 | <0.0001 | <0.0001 |

a, b, c, d – values in the same row with different letters differ significantly, P<0.05.
*,** – values in the same column with different superscripts differ significantly, P<0.05.
Abbreviations: PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; HLB – Hy – Line Brown; G – genotype; A – age; G × A – interaction genotype × age.

Table 4. Shell color features of the eggshells (n = 66/genetic group) from traditional hens bred in Poland in relation to age and compared with commercial hybrids

| Characteristic | | Age (wk) | Genotype of hens | | | | Mean | SEM | Effect (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PLB | PCr | GLP | HLB | | | G | A | G × A |
| Shell color | L* | 33 | 89.6 a* | 84.1 b* | 90.0 a* | 60.0 c* | 81.0* | 1.1 | | | |
| | | 55 | 89.3 a* | 85.3 b* | 90.0 a* | 62.9 c** | 81.9** | 1.0 | | | |
| | | Mean | 89.5 a | 84.7 b | 90.1 a | 61.5 c | 81.5 | 0.7 | <0.0001 | 0.0060 | 0.0027 |
| | a* | 33 | 5.6 c* | 14.7 b* | 6.4 c* | 24.9 a* | 12.9* | 0.7 | | | |
| | | 55 | 6.1 c** | 12.9 b** | 6.0 c** | 25.5 a* | 12.6* | 0.7 | | | |
| | | Mean | 5.9 c | 13.8 b | 6 .2 c | 25.2 a | 12.8 | 0.5 | <0.0001 | 0.2563 | 0.0259 |
| | b* | 33 | -1.0 c* | 2.4 b* | -0.9 c* | 16.0 a* | 4.1* | 0.6 | | | |
| | | 55 | -0.8 c* | 1.8 b* | -1.1 c* | 14.4 a** | 3.6** | 0.6 | | | |
| | | Mean | -0.9 c | 2.1 b | -1.0 c | 15.2 a | 3.9 | 0.4 | <0.0001 | 0.0007 | 0.0007 |

a, b, c – values in the same row with different letters differ significantly, P<0.05.
*,** – values in the same column with different superscripts differ significantly, P<0.05.
Abbreviations: PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; HLB – Hy-Line Brown; G – genotype; A – age; G × A – interaction genotype × age.

Table 5. Quality features of the eggshells of eggs (n = 66/genetic group) from traditional hens bred in Poland in relation to age and compared with commercial hybrids

| Characteristic | Age (wk) | Genotype of hens | | | | Mean | SEM | Effect (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PLB | PCr | GLP | HLB | | | G | A | G × A |
| Shell weight (g) | 33 | 3.4 d* | 5.6 b* | 4.9 c* | 8.6 a* | 5.6* | 0.2 | | | |
| | 55 | 3.6 d* | 5.3 b** | 4.8 c* | 6.8 a** | 5.1** | 0.1 | | | |
| | Mean | 3.5 d | 5.5 b | 4.9 c | 7.7 a | 5.4 | 0.1 | <0.0001 | <0.0001 | <0.0001 |
| Proportion of shell in egg (%) | 33 | 10.4 b* | 10.6 b* | 9.9 c* | 13.8 a* | 11.2* | 0.1 | | | |
| | 55 | 10.2 ab* | 9.9 bc* | 9.5 c* | 10.5 a** | 10.0** | 0.1 | | | |
| | Mean | 10.3 b | 10.2 b | 9.7 c | 12.2 a | 10.6 | 0.1 | <0.0001 | <0.0001 | <0.0001 |
| Shell thickness (µm) | 33 | 331 d* | 375 b* | 351 c* | 407 a* | 366* | 3.6 | | | |
| | 55 | 327 c** | 350 b** | 335 bc** | 386 a** | 350** | 3.3 | | | |
| | Mean | 329 d | 363 b | 343 c | 397 a | 358 | 2.5 | <0.0001 | <0.0001 | 0.1938 |
| Shell porosity (pore number/cm$^2$) | 33 | 27.4 d* | 33.2 b* | 30.8 c* | 36.4 a* | 31.9* | 0.4 | | | |
| | 55 | 28.4 b* | 28.1 b** | 28.0 b** | 37.1 a* | 30.4** | 0.5 | | | |
| | Mean | 27.9 d | 30.7 b | 29.5 c | 36.8 a | 31.2 | 0.3 | <0.0001 | <0.0001 | <0.0001 |
| Shell strength (N) | 33 | 37.6 b* | 40.1 b* | 38.9 b* | 48.1 a* | 41.2* | 0.8 | | | |
| | 55 | 31.7 b** | 33.6 b** | 32.9 b** | 44.6 a* | 35.7** | 0.8 | | | |
| | Mean | 34.8 b | 36.9 b | 36.0 b | 46.4 a | 38.3 | 0.6 | <0.0001 | <0.0001 | 0.7813 |
| Elastic deformation (µm) | 33 | 51.0 ab* | 52.0 a* | 48.7 bc* | 48.0 c* | 49.9* | 0.5 | | | |
| | 55 | 54.4 a** | 53.1 a* | 53.4 a** | 47.7 b* | 52.6** | 0.6 | | | |
| | Mean | 52.6 a | 52.5 a | 51.0 a | 47.9 b | 51.2 | 0.4 | <0.0001 | 0.0027 | 0.0819 |

a, b, c, d – values in the same row with different letters differ significantly, P<0.05.
*,** – values in the same column with different superscripts differ significantly, P<0.05.
Abbreviations: PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; HLB – Hy-Line Brown; G – genotype; A – age; G × A – interaction genotype × age.

Table 6. Lysozyme content and enzymatic activity in the albumen of eggs (n = 66/genetic group) from traditional hens bred in Poland in relation to age and compared with commercial hybrids

| Characteristic | Age (wk) | Genotype of hens | | | | Mean | SEM | Effect (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PLB | PCr | GLP | HLB | | | G | A | G × A |
| Lysozyme content in thin albumen (%) | 33 | 0.23 c* | 0.23 c* | 0.38 b* | 0.40 a* | 0.31* | 0.01 | | | |
| | 55 | 0.44 a** | 0.26 c** | 0.39 b* | 0.27 c** | 0.34** | 0.01 | | | |
| | Mean | 0.34 b | 0.25 c | 0.39 a | 0.34 b | 0.33 | 0.01 | <0.0001 | <0.0001 | <0.0001 |
| Lysozyme content in thick albumen (%) | 33 | 0.16 a* | 0.15 b* | 0.17 a* | 0.14 b* | 0.16* | 0.01 | | | |
| | 55 | 0.16 b* | 0.16 b** | 0.18 a* | 0.19 a** | 0.17** | 0.01 | | | |
| | Mean | 0.16 b | 0.16 b | 0.18 a | 0.16 b | 0.16 | 0.01 | <0.0001 | <0.0001 | <0.0001 |
| Lysozyme activity in thin albumen (U/ml) | 33 | 49168 c* | 49989 c* | 81816 b* | 85288 a* | 66565* | 1569 | | | |
| | 55 | 94358 a** | 55467 c** | 82741 b* | 56317 c** | 72221** | 1723 | | | |
| | Mean | 71463 b | 52728 d | 82279 a | 70802 c | 69393 | 1182 | <0.0001 | <0.0001 | <0.0001 |
| Lysozyme activity in thick albumen (U/ml) | 33 | 35112 a* | 32088 b* | 37016 a* | 29866 b* | 33520* | 543 | | | |
| | 55 | 34994 b* | 35136 b** | 38607 a* | 39434 a** | 37043** | 441 | | | |
| | Mean | 35053 b | 33612 b | 37811 a | 34650 b | 35282 | 373 | <0.0001 | <0.0001 | <0.0001 |

a, b, c, d – values in the same row with different letters differ significantly, P<0.05.
*, ** – values in the same column with different superscripts differ significantly, P<0.05.
Abbreviations: PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; HLB – Hy-Line Brown; G – genotype; A – age; G × A – interaction genotype × age.

Table 4 presents the results of the eggshell color evaluation from four genetic groups of hens. The shell color was influenced by genotype, age (L*, b*) and the interaction of these two factors (L*, a*, b*). The lightest (P<0.05) shell color was found in GLP eggs (GLP vs. PCr and GLP vs. HLB) obtained from both 33 and 55 weeks of age (L* 90.0 and 90.0, respectively). On the other hand, the darkest (P<0.05) coloration of the shell at both ages was observed in HLB eggs (L* 60.0 and 62.9, respectively). In addition, the eggshells from these hens were characterized by the highest (P<0.05) intensity of red and yellow, respectively, at the level of a* 25.2 and b* 15.2. The intensity of the red color (a*) ranged on average from 5.9 (PLB) to 25.2 (HLB) and the yellow (b*) from -0.9 (GLP) to 15.2 (HLB). Statistically significant differences were confirmed (P<0.05).

Table 5 presents the quality features of the eggshells from traditional hens bred in Poland in relation to age and compared with commercial hybrids. The age, genotype, and the interaction of these two factors affected the shell weight, shell proportion in egg, shell porosity and elastic deformation of the shell. Other traits were influenced by genotype and age. Irrespective of the age, HLB eggshells were characterized (P<0.05) by the highest weight (7.7 g), proportion in the egg (12.2%), thickness (397 μm), number of pores (36.8 pores/cm$^2$), strength (46.4 N) with the lowest elastic deformation (47.9 μm) compared to other genetic groups. In turn, the mean results for PLB eggshells showed that they were the lightest (P<0.05) (3.5 g) with the lowest shell thickness (329 μm) and porosity (27.9 pores/cm$^2$) compared to other genetic groups. These shells were also the most elastic (52.6 μm) with the lowest strength (34.8 N). Significant differences (P<0.05) for the latter features were confirmed only for HLB eggshells. On average, the lowest (P<0.05) proportion of shell in eggs was found in GLP eggs (9.7%) compared to other genetic groups.

Table 6 shows the results of lysozyme content and enzymatic activity in the albumen from four genetic groups of hens. The content and hydrolytic activity of lysozyme were influenced by genotype, age, and the interaction of these two factors. On average, the highest (P<0.05) concentration of lysozyme was found in eggs obtained at 55 weeks of age in both albumen fractions tested (0.34% – thin albumen and 0.17% – thick albumen, respectively). At the age of 33 weeks, HLB egg thin albumen was characterized by the highest (P<0.05) enzyme content (0.40%). In contrast, at 55 weeks of age, the highest (P<0.05) content of lysozyme was found in PLB egg albumen (0.44%). In the thick albumen fraction, the content of lysozyme ranged from 0.14 (HLB) to 0.17% (GLP) at 33 weeks of age and from 0.16 (PLB, PCr) to 0.19% (HLB) at 55 weeks of age. In the case of lysozyme hydrolytic activity, higher results were obtained in eggs from 55 weeks of age (on average by 5656 U/ml for the thin fraction and by 3523 U/ml for the thick fraction) compared to 33 weeks of age. Moreover, the highest (P<0.05) enzyme activity was found in GLP

eggs, in both the thin albumen (82279 U/ml) and thick albumen (37811 U/ml).

## Discussion

To our knowledge, this is the first report presenting the external and internal parameters of egg quality, including the concentration and activity of lysozyme in the egg albumen of three traditional hens bred in Poland, i.e. PLB, PCr and GLP.

In the present study, the age of the hens had no influence on the egg shape index. However, differences in shape were found depending on the genotype, with similar values obtained for PLB and HLB eggs. Also, a previous study showed the influence of genotype and age, and the interaction of these two factors (Tůmová et al., 2007; Ledvinka et al., 2011; Lordelo et al., 2020; Kumar et al., 2022) on the egg shape index. Rizzi et al. (2022) showed that for eggs of Italian hen breeds, the values of this parameter ranged from 73.2 (Ermellinata di Rovigo) to 77.9% (Padovana Camosciata, chamois plumage). Thus, the shape index values of eggs from traditional hens bred in Poland are within the range recorded for eggs of other genetic groups, with the exception of GLP eggs, which were characterized by a more elongated shape.

The present study showed an increase in the pH value of yolk, egg and yolk weight, and proportion of albumen and yolk in eggs with the age of the traditional hens, as well as in commercial hybrids. This dependence is also indicated by studies conducted by Zita et al. (2009), Krawczyk et al. (2021), and Kraus et al. (2021). In addition, differences in the values of these parameters were noted between traditional hens and commercial hybrids. Differences in egg weight may result from differences in body weight of females of genetic groups. Lacin et al. (2008) found that egg weight was lower in the group of low-weight Lohmann hens than in the groups of medium and heavy hens. In turn, Rizzi and Marangon (2012), Marelli et al. (2020), Ianni et al. (2021) and Gumułka et al. (2022) indicate a higher proportion of yolk in eggs from non-commercial hen breeds, which is beneficial for consumers. Marelli et al. (2020) indicate differences in egg quality parameters in autochthonous Italian breeds, and the egg weight of these genetic groups was lower compared to commercial hybrids.

An important distinguishing feature in the egg trade is the color of the yolk. Consumers are more likely to purchase eggs with a darker yolk, and this choice is guided by the conviction of its higher tastiness (Tomczyk et al., 2016). This study showed, however, that the lightness of the yolk color increases with the age of the hens. Meanwhile, an inverse relationship was demonstrated by Krawczyk (2009), Krawczyk et al. (2021) and Nowaczewski et al. (2021). In turn, our research has shown that the yolks from PLB and PCr were darker in color compared to the yolks of other genetic groups. Rizzi and Marangon (2012) noted differences in lightness, redness, and yellowness of the yolks between eggs from Italian breeds and commercial hybrids. Changes in yolk color as hens age and between genotype may result from differences in the efficiency in pigment absorption, transport, and rate of deposition (Nys, 2000).

The freshness and quality of eggs depends on the time that has passed since the egg was laid. Albumen quality is an indicator of egg freshness. It is better with a higher value of albumen height and Haugh units, and these features are influenced by, inter alia the age of the hens. This study showed a correlation in the deterioration of some albumen quality characteristics with the age of the hens, which has also been confirmed by other authors (Krawczyk and Calik, 2018; Vlčková et al., 2019, Krawczyk et al., 2021). The most favorable albumen quality features were found for HLB compared to other genetic groups. Importantly, in eggs from traditional hens, the albumen height in older birds did not exceed 6.1 mm and Haugh units 80.8, thus qualifying them as fresh eggs of good quality. Differences between genetic groups in albumen traits are consistent with the results of research by Krawczyk and Calik (2018), Krawczyk et al. (2021) and Nowaczewski et al. (2021).

Due to the fact that the egg has a nutritional and reproductive function, its content must be effectively protected against mechanical damage and the penetration of microorganisms. Generally, this role is fulfilled by the shell. The color of the eggshell is one of the criteria that affect the consumer evaluation of eggs. The study showed that with the age of the hens, the lightness of the eggshell color increased and the intensity of the yellow color decreased. This may indicate that with the age of hens there are changes in shell pigment synthesis and transport connected with changes in the abundance of the expression of pigment regulatory genes in the shell gland. In this study, the eggshells from traditional hens were distinguished by a lighter color, with a lower intensity of red and yellow compared to HLB. In addition, PLB and GLP eggshells were characterized by a higher blue saturation. Based on the color of the shell of the eggs they lay hens can be classified according to specific color of shell – white (PLB and GLP), cream (PCr) and brown (HLB). Also, Rizzi et al. (2022) showed differences in lightness, redness, and yellowness indexes of eggshell in indigenous Italian hen breeds. Traditional hens may differ from HLB in the intensity of eggshell pigmentation based on protoporphyrin-IX and/or biliverdin. According to Wang et al. (2023), shell color L* and b* were negatively and positively correlated with protoporphyrin-IX content and total pigment content, respectively, whereas a* was positively correlated with protoporphyrin-IX, but negatively correlated with the biliverdin content of eggshells.

It was shown that the quality parameters of the eggshell decreased with the age of the hens. A previous study by Ledvinka et al. (2011) indicates the influence of the age of hens representing different genotypes on the qualitative characteristics such as proportion of eggshell,

shell thickness and strength. Research by Lewko et al. (2021) on eggs from 6 breeds/strains of native breeds of hens showed that the weight and porosity of the shells increased, with the age of the hens, and their resistance to damage decreased. According to Benavides-Reyes et al. (2021) a decrease in the breaking strength of eggshell in LSL-hens at the end of production is due to changes in the ultra- and microstructure characteristics of the eggshell (i.e., decreased mammillary density; increased size of crystal units).

An important factor influencing the quality parameters of the shell is also the genotype, which is demonstrated by studies on laying hens (Tůmová et al., 2007; Ledvinka et al., 2011). In the present research, eggs from traditional hens were characterized by worse eggshell quality parameters, i.e. lower weight, proportion of shell in egg, thickness, strength and lower porosity compared to commercial hybrids. This is consistent with the results of other studies, e.g. Sirri et al. (2018), Franco et al. (2020) and Rizzi et al. (2022) conducted on non-commercial hens. Moreover, Zita et al. (2009) assessed the quality of eggshells of hens of different genotypes (ISA Brown, Hisex Brown, Moravia BSL) and showed that ISA Brown eggshells were characterized by the highest weight, thickness and proportion in egg. It can be assumed that traditional hens retain their genetic distinctiveness, manifested by poorer shell quality parameters, due to being kept in isolation from other genotypes, and no selection aimed at improving performance characteristics.

Currently, consumers pay special attention to the origin of the product, with a preference for locally sourced eggs. This study has shown that eggs obtained from traditional hens may meet these requirements. Particularly noteworthy are PLB eggs. With special conditions during transport due to the low parameters of the shell quality, they can be offered as a niche product.

Hen eggs are a valuable source of bioactive ingredients, including lysozyme. This enzyme belongs to a group of biologically active proteins providing natural primary protection of the embryo up to the point when the first antibodies are formed. Previous studies indicate that the content and activity of lysozyme is conditioned by many factors, among other things female age, nutrition, rearing system, origin and health of the flock (Graszkiewicz et al., 2007; Trziszka et al., 2007). The present study has shown that the content and activity of this enzyme in the thin and thick albumen increases with the age of the hens. This is consistent with research by Nowaczewski et al. (2021) and Kowalska et al. (2021) conducted on eggs of commercial hybrids. However, Vlčkova et al. (2019) showed no effect of the age of hens on lysozyme content in egg albumen of commercial hybrids. In turn, Lewko et al. (2021) and Krawczyk et al. (2021), by examining the egg albumen of 6 breeds/strains of laying hens kept in accordance with the rules of the biodiversity protection program, showed differences in the content and activity of lysozyme depending on both age and genotype. This research also showed the differences in the level and activity of lysozyme in eggs from traditional hens bred in Poland. This is confirmed by previous studies on hens of conservative breeds and commercial hybrids (Krawczyk, 2009) and conservative breeds (Lewko and Gornowicz, 2009; Krawczyk and Calik, 2018). Interestingly, this study showed greater variability between hens in terms of enzyme content and activity in thin albumen compared to thick albumen. It is also worth noting that the GLP egg albumen was characterized by both a higher content and activity of lysozyme compared to commercial hybrids. It can be suggested that these hens can be used as a model for research on the physiological mechanisms of lysozyme synthesis. Many studies have been conducted to determine the relationship between the hens genome and egg quality, and many genes have been found to be associated with egg quality (Wolc, 2014; Hejdysz et al., 2024). Assessment of eggs of different breeds of hens may facilitate understanding how their genetic potential may modify the chemical composition of eggs, including lysozyme parameters.

## Conclusions

Studies have shown that:

- the genetic origin and age of experimental hens as well as interaction of these two factors influence on physical parameters of the eggs and the content and activity of the lysozyme,
- older hens laid heavier eggs with a higher proportion of major fractions and a more rounded shape, but worse albumen (height and Haugh units) and shell quality (thickness, strength, elastic deformation),
- the eggs of traditional hens bred in Poland were characterized by a more favorable yolk proportion in the egg and its color and albumen lysozyme parameters (best in Gold Laced Polish Chickens), and a poorer eggshell and albumen features in relation to commercial hybrids,
- regardless of age the highest content and activity of lysozyme in thin albumen was found in eggs from PLB hens, and in thick albumen in eggs obtained from HLB hens,
- with the age of the hens the content and hydrolytic activity of lysozyme in egg albumen fractions increased.

## Disclosures

The authors declare that there is no conflict of interest in this research.

## References

Andres K. (2022). Polish crested chicken, a traditional native breed of laying hens (in Polish). In: Cultural heritage from the perspective of various disciplines, Król K., Hernik J., Prus B. (eds). The Publishing House of University of Agriculture in Krakow, pp. 169–176.

Andres K., Kapkowska E., Wójtowicz M. (2008). Characteristics of

local chicken varieties with regard to some production traits (in Polish). Rocz. Nauk. Zoot., 35: 119–129.
Antos P., Andres K., Kapkowska E. (2013). Preliminary studies on genetic diversity of selected Polish local chicken varieties. *J.* Cent. Eur. Agric., 4: 11–22.
Benavides-Reyes C., Folegatti E., Dominguez-Gasca N., Litta G., Sanchez-Rodriguez E., Alejandro Rodriguez-Navarro A.B., Umar Faruk M. (2021). Research note: Changes in eggshell quality and microstructure related to hen age during a production cycle. Poultry Sci., 100: 101287.
Directive of Director of National Research Institute of Animal Production No. 12/23 of February 13, 2023 regarding the implementation of the Program for the Protection of the Laying Hen Population Resources. http://www.bioroznorodnosc.izoo.krakow.pl/drob/dokumenty.
European Union (1999). Council Directive 1999/74/EC laying down minimum standards for the protection of laying hens in the EU. Off. J. Europ. Comm. L, 203: 53–57.
Ferraboschi P., Ciceri S., Grisenti P. (2021). Applications of lysozyme, an innate immune defence factor, as an alternative antibiotic. Antibiotics (Basel), 10: 1534.
Franco D., Rois D., Arias A., Justo J.R., Marti-Quijal F.J., Khubber S., Barba F.J., López-Pedrouso M., Lorenzo J.M. (2020). Effect of breed and diet type on the freshness and quality of the eggs: a comparison between Mos (indigenous Galician breed) and Isa Brown hens. Foods, 9: 1–12.
González Ariza A., Arando Arbulu A., Navas González F.J., León Jurado J.M., Delgado Bermejo J.V., Camacho Vallejo M.E. (2022). Data mining-based discriminant analysis as a tool for the study of egg quality in native hen breeds. Sci. Rep., 12: 15873.
Graszkiewicz A., Kaźmierska M., Niedbalska J. (2007). The effect with mineral-humine preparations and antioxidants addition to feed on the activity of lysozyme and cystatin in egg white (in Polish). Żywn. Nauk. Technol. Ja., 5: 360–366.
Gumułka M., Hrabia A., Avital-Cohen N., Andres K., Rozenboim I. (2020). The effect of parachlorophenylalanine treatment on the activity of gonadal and lactotrophic axes in native Polish crested chickens stimulated to broodiness. Poultry Sci., 99: 2708–2717.
Gumułka M., Andres K., Krawczyk, J., Calik J. (2022). Research note: The nutritional value of eggs from native Polish Crested chickens and commercial hybrids that have been stored in various conditions. Poultry Sci., 101: 101579.
Gvoždíková Javůrková V., Pokorná M., Mikšík I., Tůmová E. (2019). Concentration of egg white antimicrobial and immunomodulatory proteins is related to eggshell pigmentation across traditional chicken breeds. Poultry Sci., 98: 6931–6941.
Hejdysz M., Nowaczewski S., Perz K., Szablewski T., Stuper-Szablewska K., Cegielska-Radziejewska R., Tomczyk Ł., Przybylska-Balcerek A., Buśko M., Kaczmarek S.A., Ślósarz P. (2024). Influence of the genotype of the hen (*Gallus gallus domesticus*) on main parameters of egg quality, chemical composition of the eggs under uniform environmental conditions. Poultry Sci., 103: 103165.
Ianni A., Bartolini D., Bennato F., Martino G. (2021). Egg quality from Nera Atriana, a local poultry breed of the Abruzzo region (Italy), and ISA Brown hens reared under free range conditions. Animals, 11: 257.
Kowalska E., Kucharska-Gaca J., Kuźniacka J., Lewko L., Gornowicz E., Biesek J., Adamski M. (2021). Egg quality depending on the diet with different sources of protein and age of the hens. Sci. Rep., 11: 2638.
Kraus A., Zita L., Krunt O., Härtlová H., Chmelíková E. (2021). Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age. Poultry Sci., 100: 1142–1153.
Krawczyk J. (2009). Effect of layer age and egg production level on changes in quality traits of eggs from hens of conservation breeds and commercial hybrids. Ann. Anim. Sci., 9: 185–193.
Krawczyk J., Calik J. (2018). Quality assessment of eggs laid by hens included in programmes for the conservation of animal genetic resources (in Polish). Food Sci. Technol. Quality, 25: 140–150.
Krawczyk J., Lewko L., Calik J. (2021). Effect of laying hen genotype, age and some interior egg quality traits on lysozyme content. Ann. Anim. Sci., 21: 1119–1132.
Kumar M., Dahiya S.P., Ratwan P., Sheoran N., Kumar S., Kumar N. (2022). Assessment of egg quality and biochemical parameters of Aseel and Kadaknath indigenous chicken breeds of India under backyard poultry farming. Poultry Sci., 101: 101589.
Lacin E., Yildiz A., Esenbuga N., Macit M. (2008). Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. Czech J. Anim. Sci., 53: 466–471.
Ledvinka Z., Zita L., Hubený M., Tůmová E., Tyller M., Dobrovolný P., Hruška M. (2011). Effect of genotype, age of hens and K/k allele on eggshell quality. Czech J. Anim. Sci., 56: 242–249.
Leśnierowski G., Kijowski J. (1995). Methods of investigating enzymatic activity and quantitative determination of lysozyme from hen egg white (in Polish). Przem. Spoż., 49: 476–479.
Leśnierowski G., Stangierski J. (2018). What's new in chicken egg research and technology for human health promotion? – a review. Trends. Food. Sci. Technol., 1: 46–51.
Leśnierowski G., Yang T. (2021). Lysozyme and its modified forms: A critical appraisal of selected properties and potential. Trends. Food. Sci. Technol., 107: 333–342.
Lewko L., Gornowicz E. (2009). Egg albumen quality as affected by bird origin. J. Cent. Eur. Agric., 10: 455–463.
Lewko L., Krawczyk J., Calik J. (2021). Effect of genotype and some shell quality traits on lysozyme content and activity in the albumen of eggs from hens under the biodiversity conservation program. Poultry Sci., 100: 100863.
Lordelo M., Cid J., Cordovil C.M.D.S., Alves S.P., Bessa R.J.B., Carolino I. (2020). A comparison between the quality of eggs from indigenous chicken breeds and that from commercial layers. Poultry Sci., 99: 1768–1776.
Marelli A.P., Zaniboni L., Madeddu M., Sayed A.A., Strillacci M.G., Mangiagalli M.G., Cerolini S. (2020). Physical parameters and fatty acids profiles in Milanino, Mericanel Della Brianza, Valdarnese Bianca and commercial hybrids (*Gallus gallus domesticus*) table eggs. Animals, 10: 1533.
Nawaz N., Wen S., Wang F., Nawaz S., Raza J., Iftikhar M., Usman M. (2022). Lysozyme and its application as antibacterial agent in food industry. Molecules, 27: 6305.
Nowaczewski S., Lewko L., Kucharczyk M., Stuper-Szablewska K., Rudzińska M., Cegielska-Radziejewska R., Biadała A., Szulc K., Tomczyk Ł., Kaczmarek S., Hejdysz M., Szablewski T. (2021). Effect of laying hens age and housing system on physicochemical characteristics of eggs. Ann. Anim. Sci., 21: 291–309.
Nys Y. (2000). Dietary carotenoids and egg yolk coloration – a review. Arch. Geflugelkd., 64: 45–54.
Rizzi C., Marangon A. (2012). Quality of organic eggs of hybrid and Italian breed hens. Poultry Sci., 91: 2330–2340.
Rizzi C., Cendron F., Penasa M.,. Cassandro M. (2022). Egg quality of Italian local chicken breeds: I. Yield performance and physical characteristics. Animals, 13: 148.
Roberts V. (2008). British poultry standards: complete specifications and judging points of all standardized breeds and varieties of poultry as compiled by the specialist breed clubs and recognised by the Poultry Club of Great Britain. Oxford, Great Britain, Blackwell Pub. 6th ed., 467 pp.
Shahmohammadi A. (2018). Lysozyme separation from chicken egg white: a review. Eur. Food. Res. Technol., 244: 577–593.
Sirri F., Zampiga M., Soglia F., Meluzzi A., Cavani C., Petracci M. (2018). Quality characterization of eggs from Romagnola hens, an Italian local breed. Poultry Sci., 97: 4131–4136.
Tomczyk L., Szablewski T., Cegielska-Radziejewska R. (2016). Nutritional value of table eggs originating from laying hens kept under different housing systems (in Polish). Food Sci. Technol. Quality, 23: 20–27.
Trziszka T., Dobrzański Z., Skiba T., Kopeć W. (2007). Effect of breeding and housing systems of layers on egg quality and the activity of cystatin and lysozyme. Polish J. Food Nutr. Sci., 57: 583–586.
Tůmová E., Zita L., Hubený M., Skřivan M., Ledvinka Z. (2007). The effect of oviposition time and genotype on egg quality characteristics in egg type hens. Czech J. Anim. Sci., 52: 26–30.

Tyler C. (1953). Studies on egg shells. II. A method for marking and counting pores. J. Sci. Food Agric., 4: 266–272.

Vlčková J., Tůmová E., Míková K., Englmaierová M., Okrouhlá M., Chodová D. (2019). Changes in the quality of eggs during storage depending on the housing system and the age of hens. Poultry Sci., 98: 6187–6193.

Wang H., Ge Y., Zhang L., Wei Y., Li Q., Zhang X., Pan Y. (2023). The pigments in eggshell with different colour and the pigment regulatory gene expression in corresponding chicken's shell gland. Animal, 17: 100776.

Węglarz A., Andres K., Wojtysiak D. (2020). Slaughter value and meat quality in two strains of Polish Crested cockerels. Ital. J. Anim. Sci., 19: 813–821.

Wolc A., Arango J., Jankowski T., Dunn I., Settar P., Fulton J.E., O'Sullivan N.P., Preisinger R., Frenando R.L., Garrick D.J., Dekkers J.C.M. (2014). Genome-wide association study for egg production and quality in layer chickens. J. Anim. Breed. Genet., 131: 173–182.

Yoshimura K., Kinoshita K., Mizutani M., Matsuda Y., Saito N. (2012). Inheritance and developmental pattern of cerebral hernia in the crested Polish chicken. J. Exp. Zool. B Mol. Dev. Evol., 318: 613–620.

Zita L., Tůmová E., Štolc L. (2009). Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. Acta Vet. Brno, 78: 85–91.

Received: 4 IX 2023
Accepted: 2 II 2024