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Relationships between eggshell quality parameters and albumen lysozyme activity of eggs in local amateur chickens*

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The aims of the study were (1) to compare eggshell quality parameters and enzymatic activity of lysozyme in the albumen of eggs obtained from local amateur chickens; (2) to evaluate correlations between eggshell quality parameters and lysozyme activity and content. The study was carried out on eggs collected from two age groups of three local amateur chicken populations: Polish Liliputy Bantams (PLB), native Polish Crested Chickens (PCr, CP-22 strain) and Gold Laced Polish Chickens (GLP). A total of 135 PLB, 75 PCr and 75 GLP chickens were kept on litter in an experimental station. Using appropriate research equipment, the physical quality parameters of the eggshell and the content and activity of lysozyme were determined. Differences were observed among these populations in some physical eggshell parameters and overall elastic deformation of eggs. On the other hand, shell strength was similar among the three populations. In addition, differences were found in lysozyme content and activity in the albumen, with higher values recorded for white eggs than for cream-colored eggs. Positive monotonic relationships were estimated between eggshell

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strength and shell weight, proportion of shell in the egg, and shell thickness. The variability of eggshell color parameters and age-related values of Spearman's correlation coefficients between these parameters and shell strength indicate that non-invasive measurement of shell color can be used to predict egg resistance to damage only for the GLP population.

KEY WORDS: chicken / eggshell quality / lysozyme / correlation

Eggshell quality is important in poultry reproduction as well as in table eggs production. The physical quality of an eggshell is assessed mainly by its color, strength, thickness, and ability to deform elastically [Kett and Tůmová 2016, Knaga *et al.* 2019]. Eggshell color and strength are considered some the most important factors that influence consumer choice; moreover, these qualities indirectly ensure safety and are therefore of fundamental importance to egg producers [Kett and Tůmová 2016]. Studies performed on commercially used chickens have shown differences in eggshell strength between white and brown eggs [Ledvinka *et al*. 2000] and within brown strains [Tůmová *et al.* 2011, Ketta *et al*. 2020].

Using a reflectometer Yang *et al*. [2009] observed a correlation between eggshell color and shell strength in native Chinese chickens. Moreover, Sirri *et al.* [2018] showed a relationship between eggshell lightness (L*) and shell strength in commercially used laying chickens by measuring eggshell color using the CIElab (L*, a*,b*) scale methods. More recently, Drabik *et al.* [2021] showed differences in eggshell strength between seledine-egg-laying Araucana and white-egg-laying Leghorn chickens. It is noteworthy that in the aforementioned studies, the L*, a*, b* color parameters correlated with the proportion of some mineral content of eggshells, and eggshell color was correlated with pigment content. The CIElab method is currently recommended as an effective tool for assessing pigment deposition in the breeding of egg-laying chickens [Zeng *et al*. 2022].

An eggshell is a natural protective coating, the formation of which is based on the phenomenon of biomineralization. Over 900 identified proteins are involved in this process. One of them is lysozyme, a globular protein with high enzymatic activity and strong antioxidant and anti-inflammatory properties [Nimalarante and Wu, 2015; Seweryn *et al*. 2018]. This enzyme helps stabilize amorphous calcium carbonate and affects the morphology of calcite crystals [Gautron *et al*. 2021]. Another important property of lysozyme that protects the developing embryo is its ability to form a complex with ovomucin, which is responsible for the gel structure of the albumen, thus preventing migration of microorganisms into the egg content [Trziszk*a et al.* 2013]. Interestingly, Bilková *et al*. [2018] and Gvoždíková Javůrková *et al.* [2019] have shown that in native and non-commercial chicken breeds there is a relationship between eggshell pigmentation content and the concentration of lysozyme and ovotransferrins in egg albumen.

Andreson *et al.* [2004] compared historical and current strains of commercial Single Comb White Leghorn chickens and showed that eggshell thickness and strength has changed over time. Furthermore, as compared to commercial hybrids, studies have demonstrated differences in the physical parameters of eggshells between native chicken breeds [Radwan 2015, Fathi *et al.* 2019, Franco *et al*. 2020, Lordelo *et al.* 2020, Gumułka *et al.* 2022] and non-commercial breeds [Ketta *et al*. 2020, Lewko *et al.* 2024], thus highlighting the impact of selective genetic pressure on eggshell traits. Furthermore, a study by Rizzi [2023] and Rizzi *et al*. [2023] showed high variability in eggshell color in local Italian chicken breeds.

Therefore, the characterization of eggshell quality parameters, their variability, and the relationship between them in amateur (traditional) chicken breeds in Poland is interesting and important for the future development of optimal methods of storing and transporting eggs. The term "traditional chickens" refers to breeds that have not been subjected to the intense selection pressures characteristic of modern commercial farming. These breeds were developed using traditional breeding methods that focus on practical traits such as hardiness and adaptability to traditional farming conditions, rather than on specialized parameters that increase egg production efficiency. To date, research conducted on the internal and external parameters of eggs from traditional chickens indicates that they can be recommended for niche egg production for consumers who demand an original product [Gumułka *et al.* 2022, Lewko *et al.* 2024]. Moreover, it is worth exploring the relationship between eggshell color and lysozyme enzymatic activity in these specific chicken genotypes.

The aims of the study were (1) to compare eggshell quality parameters and the enzymatic activity of lysozyme in the albumen of eggs obtained from amateur chickens; (2) to evaluate correlations between eggshell parameters and color as well as lysozyme content and activity.

Material and methods

Experimental birds and management

The study was carried out on eggs collected from three amateur chicken populations: Polish Liliputy Bantams (PLB), native Polish Crested Chickens (PCr, CP-22 strain) and Gold Laced Polish Chickens (GLP). The exterior of these breeds, which are traditional in Poland, is presented in Figures1A-C. Polish Liliputy Bantams are miniature chickens of native origin, popular in backyard farming due to their low maintenance requirements, decorative value, and strong brooding instinct. Gold Laced Polish Chickens are maintained in Poland in traditional farming as ornamental birds and egg layers, although there is no historical evidence linking these chickens with poultry breeding in Poland. The purpose of using GLP in this research was to compare the parameters of this breed's eggs with those of native Polish Crested Chickens, and thus to detect possible differences between these two breeds. A total of 135 PLB, 75 PCr and 75 GLP chickens were kept on litter in on extensive farming production system in an experimental building at the Research and Education Centre of the Faculty of Animal Sciences of the University of Agriculture in Krakow, Poland. There were 3 pens (2.0 × 2.5 m) connected with gravel runs (2.0 × 4.5 m) for each chicken genotype

Fig. 1 A-C. The exterior of amateur chicken populations studied: A–Polish Liliputy Bantams (PLB), B–native Polish Crested Chickens (PCr, CP-22 strain), C–Gold Laced Polish Chickens (GLP).

(3 replications). The runs allowed the birds to express their natural behaviors. Chickens were kept at a stocking density of 9 birds/m^2 for PLB and 5 birds/m^2 for PCr and GLP. A different stocking density was used for PLB chickens due to their smaller body size. Each pen was equipped with 6 individual nests, a round feeder (circumference: 133 cm) and an automatic bell drinker (circumference: 125 cm). A natural and artificial lighting schedule was used: 16h L, 8h 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 65-70%. The chickens had free access to feed and water. A commercial granulated layer-breeder mixture was fed to the chickens. The chemical composition of the mixture was 15.0 % CP, 5.1% crude fat, 4.6% crude fiber, 90.5% DM, and 18.6% crude ash (11.3 MJ ME_N, 0.35% P and 3.80% Ca according to data provided by the producer).

The experiment did not require the permission of the II Local Ethical Committee for Animal Experiments in Krakow at the Institute of Pharmacology in Krakow, Poland (PL).

Eggs for analysis (n = 66 per genotype) were collected from each chicken population (11 eggs per pen) at 33 and 55 weeks of age (two age groups). After 24 h of storage in a refrigerator at 4°C and 55% humidity, the shell quality and lysozyme parameters of the albumen were evaluated.

Parameters of eggshells and lysozyme

The following parameters of eggshell quality were assessed: weight, color, number of pores, elastic deformation and strength. The weight of the eggshell (g) was determined using Egg Quality Measurements Electronic Equipment (Technical Services and Supplies Ltd., Dunnington, York, UK). Shell color (L*, a*, b* color space) was measured using a Minolta C580 Chroma Meter reflectance spectrophotometer (aperture 8 mm) (Konica Minolta Sensing Business Unit, Osaka, Japan), equipped with a 50-mm reading head and 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 measurement was performed at 3 egg measurement points (sharp end, blunt end, equator). The shell thickness (µm) was measured after removal of shell membranes using a Mitutoyo Digital Micrometer 293-766-30 (Mitutoyo America Corporation, Aurora, IL USA). The membranes were removed with tweezers after the shell had been cleaned and washed with distilled water. The test was performed at 3 egg measurement points (sharp end, blunt end, equator). Eggshell porosity (pores per cm^2) was assessed using Tyler's [1953] method. The inside of the eggshells were dyed, and pore density was measured using a stereomicroscope (4× magnification) over 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 measurement (accurate to 1 µm) was performed at 3 egg measurement points (on the equatorial circumference of the egg and on the sharp and blunt ends 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 (U/ml) in the albumen were determined following the method of Leśnierowski *et al.* [2021]. 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 unit in 1 min, measured at a wavelength of 450 nm and a temperature of 25°C. The decrease in the absorbance of the solution was calculated from the following 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 for normal distribution using the Kolmogorov-Smirnov test, and the Levene test was used to assess the homogeneity of the variance. The values of the analyzed parameters in the three populations of chickens at 33 and 55 weeks of age were compared using two-way analysis of variance and Tukey's post-hoc test. P values of less or equal to 0.05 were considered to be statistically significant. The values in the tables were expressed as mean, coefficient of variation (CV), and range. Analysis of the relationship between the eggshell quality parameters and between some of these parameters and the activity and content of lysozyme were performed by estimating the Spearman rank-order correlation coefficients. The statistical analysis was processed using Statistica version 13.3 (TIBCO Software Inc., Palo Alto, CA).

Results and discussion

Eggshell parameters

The eggshell quality parameters are presented in Table 1. Both PCr age groups produced eggshells of the simile weight, and these were heavier than those of eggs from PLB and GLP. The proportion of shell in PCr and PLB eggs was higher than in GLP eggs at 33 and 55 weeks of age, respectively. Moreover, the values of this parameter decreased with age for PCr chickens. Shell thickness of PCr was greater than that of the other populations for eggs obtained at 33 weeks of age, and to PLB at only 55 weeks. For all populations, the values of this parameter were lower for eggs obtained at 55 weeks of age than at 33 weeks. PLB shells had the lowest porosity compared to GLP and PCr at 33 weeks of age, but porosity was similar across the populations for eggs obtained at 55 weeks. For PCr and GLP, a decrease in shell porosity with the age of chickens was noted. Shell strength was similar across the three populations but lower for eggs obtained at 55 weeks of age compared to 33 weeks. The highest variability for most eggshell quality parameters was noted for PCr chickens. The variability in both age groups together was CV 10-30% for shell weight, proportion of shell in egg, shell thickness and strength parameters.

The eggshell color parameters are presented in Table 2. The color of the shells ranged from white for GLP and PLB to cream for PCr, and it did not depend on the age of the chickens. The variability of shell color parameter L* was low (CV<5%) and similar for the three populations. For the color parameter a*, higher CV (CV>40%) was noted for PLB and GLP, while higher CV (CV>80%) was found for PCr for color parameter b*.

The results of the analyses of the elastic deformation of eggshells are presented in Table 3 and 4. For deformation measured at the sharp and blunt end under 0.5 and 1.0 kg loads at 33 weeks of age and for the blunt end in both loads at 55 weeks, the lowest values were recorded for GLP eggs. Also, for elastic deformation calculated for the sharp, blunt and equator egg regions, the lowest values were recorded for

Table 1. Quality parameters of the eggshells (n = 66 eggs /population) in amateur chickens at 33 and 55 weeks of age

Trait	Age		Population			Effect (*P* value)		
			PLB	PCr	GLP	P	A	P × A
Shell weight (g)	33	mean	3.4^{c*}	5.6^{a*}	4.9^{b*}	<0.0001	0.4040	0.0142
		CV %	8.9	10.1	8.7			
		range	1.1	2.2	2.0			
	55	mean	3.6^{c*}	5.3^{a*}	4.8^{b*}			
		CV %	9.2	15.9	10.3			
		range	1.3	4.5	2.3			
	total	mean	3.5^{c}	5.4^{a}	4.9^{b}			
		CV %	9.0	13.0	9.0			
		range	2.3	4.5	2.3			
Proportion of shell in egg (%)	33	mean	10.4^{ab*}	10.6^{a*}	9.9^{b*}	<0.0001	<0.0001	0.0682
		CV %	6.9	8.3	6.9			
		range	3.5	3.9	2,5			
	55	mean	10.2^{a*}	9.8^{ab**}	9.5^{b*}			
		CV %	6.5	9.6	6.6			
		range	2.6	4.5	2.1			
	total	mean	10.3^{a}	10.2^{a}	9.7^{b}			
		CV %	7.0	10.0	7.7			
		range	4.1	4.5	2,9			
Shell thickness (μm)	33	mean	331.2^{c*}	375.1^{a*}	351.4^{b*}	<0.0001	0.0005	0.1138
		CV %	8.4	9.6	6.7			
		range	115.0	145.0	95.0			
	55	mean	327.7^{b**}	350.1^{a**}	335.2^{ab**}			
		CV %	7.9	9.2	7.7			
		range	101.0	147.0	102.0			
	total	mean	329.6^{c}	363.2^{a}	343.6^{b}			
		CV %	8.0	10.0	7.0			
		range	118.0	166.0	113.0			
Shell porosity (pore number/cm^2)	33	mean	27.4^{c*}	33.2^{a*}	30.8^{b*}	<0.0001	<0.0001	<0.0001
		CV %	10.0	5.8	8.3			
		range	10.0	8.0	10.0			
	55	mean	28.4^{a*}	28.1^{a**}	28.0^{a**}			
		CV %	13.4	11.4	10.2			
		range	12.0	13.0	10.0			
	total	mean	27.8^{c}	30.8^{a}	29.5^{b}			
		CV %	12.0	12.0	10.0			
		range	12.0	18.0	12.0			
Shell strength (N)	33	mean	37.6^{a*}	40.4^{a*}	38.9^{a*}	0.2160	<0.0001	0.9199
		CV %	16.6	26.2	16.2			
		range	23.4	44.7	24.8			
	55	mean	31.7^{a**}	33.5^{a**}	32.9^{a**}			
		CV %	24.6	20.0	15.5			
		range	25.6	28.4	23.1			
	total	mean	34.8^{a}	37.1^{a}	36.1^{a}			
		CV %	22.0	26.0	18.0			
		range	25.2	45.6	26.2			

PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; a,b,c – values in the same column (population) with different superscripts differ significantly P≤0.05;*,**– values in the same column (age) with different superscripts differ significantly P≤0.05.

Table 2. The eggshells color parameters (n = 66 eggs /population) in amateur chickens at 33 and 55 weeks of age

Trait	Age		Population PLB	PCr	GLP	Effect (*P* value) P	A	P × A
Shell color L*	33	mean	89.7[a*]	84.1[b*]	90.2[a*]	<0.0001	0.4402	0.1644
		CV %	2.4	3.8	1.8			
		range	9.7	12.1	6.7			
	55	mean	89.4[a*]	85.3[b*]	90.1[a*]			
		CV %	2.1	3.9	2.0			
		range	9.0	12.5	6.6			
	total	mean	89.5[a]	84.7[b]	90.1[a]			
		CV %	2.0	4.0	2.0			
		range	9.7	13.8	7.5			
Shell color a*	33	mean	5.6[b*]	14.7[a*]	6.4[b*]	<0.0001	0.1202	0.0521
		CV %	43.2	21.8	39.0			
		range	11.8	12.7	9.2			
	55	mean	6.1[b*]	12.6[a*]	6.0[b*]			
		CV %	54.2	29.9	49.7			
		range	10.9	13.6	10.6			
	total	mean	5.8[b]	13.7[a]	6.2[b]			
		CV %	49.0	26.0	44.0			
		range	12.2	14.4	10.8			
Shell color b*	33	mean	-1.1[b*]	2.4[a*]	-0.9[b*]	<0.0001	0.2365	0.1054
		CV %	-47.2	68.5	-31.7			
		range	2.0	5.7	1.1			
	55	mean	-0.8[b*]	1.8[a*]	-1.1[b*]			
		CV %	-50.6	94.9	-37.4			
		range	1.6	6.3	1.6			
	total	mean	-0.9[b]	2.1[a]	-0.9[b]			
		CV %	-50.0	80.0	-38.0			
		range	2.0	6.3	1.8			

PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; a,b,c – values in the same column (population) with different superscripts differ significantly P≤.05;*,** – values in the same column (age) with different superscripts differ significantly P≤0.05.

GLP eggs. An increase in elastic deformation was noted with the age of the chickens, with the greatest increase recorded for GLP. The variability in total eggshell elastic deformation was similar across the three populations (CV<10%).

Lysozyme content and activity

The results of the estimation of lysozyme parameters in the eggs' albumen are presented in Table 5. The GLP eggs were distinguished by the highest content of lysozyme, and thus the highest enzymatic activity. In turn, the opposite was noted for PCr eggs, which were characterized by the lowest content of lysozyme and the lowest enzymatic activity. For all three populations, lysozyme content and activity were higher for eggs obtained at 55 weeks of age compared to 33 weeks, with the greatest increase with age noted for PLB.

Table 3. Elastic deformation (μm) of eggshells (n = 66 eggs /population) measured at 3 points (sharp end, blunt end, equator) under load of 0.5 kg in amateur chickens at 33 and 55 weeks of age

Trait	Age		Population			Effect (*P* value)		
			PLB	PCr	GLP	P	A	P × A
Deformation – sharp end, load of 0.5 kg	33	mean	33.5^{a*}	31.6^{a*}	29.0^{b*}	<0.0001	0.0614	0.2734
		CV %	10.2	10.4	6.7			
		range	17.0	15.0	8.0			
	55	mean	34.8^{a*}	32.1^{b*}	31.3^{b*}			
		CV %	16.1	10.4	9.0			
		range	25.0	15.0	12.0			
	total	mean	34.1^{a}	31.9^{b}	30.1^{c}			
		CV %	13.0	10.0	9.0			
		range	25.0	15.0	14.0			
Deformation – blunt end, load of 0.5 kg	33	mean	35.9^{a*}	35.2^{a*}	29.0^{b*}	<0.0001	<0.0001	<0.1112
		CV %	12.7	13.9	6.7			
		range	23.0	22.0	8.0			
	55	mean	40.5^{a**}	37.7^{a*}	36.3^{b**}			
		CV %	31.0	15.2	10.0			
		range	71.0	25.0	16.0			
	total	mean	38.2^{a}	36.4^{a}	32.5^{b}			
		CV %	25.0	15.0	14.0			
		range	71.0	26.0	22.0			
Deformation – equator, load of 0.5 kg	33	mean	41.7^{a*}	38.2^{b*}	40.4^{ab*}	<0.0085	0.3855	0.4468
		CV %	15.1	10.5	8.9			
		range	34.0	18.0	13.0			
	55	mean	41.2^{a*}	39.8^{a*}	41.0^{a*}			
		CV %	12.1	7.9	10.1			
		range	18.0	13.0	16.0			
	total	mean	41.5^{a}	38.9^{b}	40.7^{ab}			
		CV %	14.0	9.0	9.0			
		range	34.0	19.0	17.0			

PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; a,b,c – values in the same column (population) with different superscripts differ significantly at $P \le 0.05$;*,**– values in the same column (age) with different superscripts differ significantly at $P \le 0.05$.

Phenotypic correlation coefficients

Tables 6-8 present the results of the correlation analysis between eggshell quality parameters and lysozyme content and activity. For PLB (Tab. 6), a positive monotonic relationship was found between eggshell strength and the a* color parameter for eggs obtained at 33 weeks of age, and for the b* color parameter at 55 weeks. Moreover, the b* color parameter at 55 weeks of age was negatively related to elastic shell deformation calculated for all three egg regions. Albumen lysozyme content and activity were positively related to proportion of shell in eggs at the age of 55 weeks. For PCr (Tab. 7), a positive monotonic association was found between eggshell strength and the a* color parameter for eggs obtained at 55 weeks of age. On the other hand, a negative correlation coefficient was found between albumen lysozyme content and eggshell porosity at the age of 33 weeks. For GLP (Tab. 8), shell strength was positively related to the b* eggshell color parameter at 33 weeks of age and to the L* and a* parameters at

Table 4. Elastic deformation (µm) of eggshells (n = 66 eggs /population) measured at 3 points (sharp end, blunt end, equator) under load of 1.0 kg and overall elastic deformation in amateur chickens at 33 and 55 weeks of age

Trait	Age		Population			Effect (P value)		
			PLB	PCr	GLP	P	A	P × A
Deformation – sharp end, load of 1.0 kg	33	mean	65.1^{a*}	60.8^{a*}	56.1^{b*}	<0.0001	0.0095	0.4702
		CV %	8.8	9.3	6.8			
		range	25.0	22.0	14.0			
	55	mean	66.9^{a*}	62.3^{b**}	60.0^{b**}			
		CV %	14.3	9.9	8.1			
		range	48.0	26.0	22.0			
	total	mean	65.9^{a}	61.5^{b}	57.9^{c}			
		CV %	12.0	10.0	8.0			
		range	48.0	27.0	26.0			
Deformation – blunt end, load of 1.0 kg	33	mean	69.6^{a*}	69.4^{a*}	56.1^{b*}	<0.0001	<0.0001	0.0002
		CV %	9.8	14.9	6.8			
		range	29.0	43.0	14.0			
	55	mean	73.9^{a*}	73.9^{a*}	71.8^{b**}			
		CV %	14.4	14.6	9.5			
		range	41.0	40.0	32.0			
	total	mean	71.8^{a}	71.4^{a}	63.6^{b}			
		CV %	13.0	15.0	15.0			
		range	41.0	45.0	40.0			
Deformation – equator, load of 1.0 kg	33	mean	80.9^{a*}	76.3^{b*}	81.9^{a*}	0.0102	0.1440	0.4465
		CV %	9.6	10.7	9.4			
		range	31.0	35.0	27.0			
	55	mean	81.3^{a*}	80.1^{a*}	82.9^{a*}			
		CV %	11.1	8.5	9.6			
		range	34.0	27.0	28.0			
	total	mean	81.1^{ab}	78.1^{b}	82.4^{a}			
		CV %	10.0	10.0	9.0			
		range	36.0	35.0	30.0			
Elastic deformation (µm)	33	mean	54.1^{a*}	51.9^{a*}	48.7^{b*}	<0.0001	<0.0001	0.0395
		CV %	7.3	8.7	6.5			
		range	15.7	19.1	12.8			
	55	mean	55.8^{a*}	53.9^{b*}	53.8^{b**}			
		CV %	8.2	7.6	6.2			
		range	17.6	18.0	14.7			
	total	mean	54.9^{a}	52.9^{b}	51.1^{c}			
		CV %	8.0	8.0	8.0			
		range	21.0	21.5	21.0			

PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens; a,b,c – values in the same column (population) with different superscripts differ significantly $P \leq 0.05$;*,** – values in the same column (age) with different superscripts differ significantly $P \leq 0.05$.

55 weeks of age. Moreover, a negative monotonic relationship were estimated between albumen lysozyme content and activity and the b* color parameter at 55 weeks of age.

Eggshell damage during preparation of eggs for transport and delivery to the customer can be a serious problem causing economic losses. In addition, damage to the structure of the eggshell increases the likelihood of bacteriological contamination of egg content, thus reducing the safety of this food product. Handling eggs in a way that

Table 5. Lysozyme content and enzymatic activity in the albumen of eggs (n = 66/ population) from amateur chickens at 33 and 55 weeks of age

Trait	Age		Population			Effect (*P* value)		
			PLB	PCr	GLP	P	A	P × A
Lysozyme content in albumen (%)	33	mean	0.20^{b*}	0.19^{b*}	0.28^{a*}	<0.0001	<0.0001	<0.0001
		CV %	6.4	7.9	5.9			
		range	0.05	0.07	0.07			
	55	mean	0.30^{a**}	0.21^{c**}	0.28^{b*}			
		CV %	5.9	8.3	6.5			
		range	0.07	0.07	0.08			
	total	mean	0.25^{b}	0.20^{c}	0.28^{a}			
		CV %	22.5	9.5	6.3			
		range	0.15	0.08	0.09			
Lysozyme activity in albumen (U/ml)	33	mean	42140^{b*}	41039^{b*}	59416^{a*}	<0.0001	<0.0001	<0.0001
		CV %	6.5	7.8	5.7			
		range	10780	14476	14476			
	55	mean	64677^{a**}	45302^{c**}	60674^{b*}			
		CV %	6.0	8.0	6.5			
		range	14876	13814	17002			
	total	mean	52872^{b}	43069^{c}	60015^{a}			
		CV %	22.0	9.0	6.0			
		range	33002	16509	17464			

PLB – Polish Liliputy Bantams; PCr – Polish Crested Chickens; GLP – Gold Laced Polish Chickens;
a,b,c – values in the same column (population) with different superscripts differ significantly $P \leq 0.05$;
*,** – values in the same column (age) with different superscripts differ significantly $P \leq 0.05$.

protects the eggshell structure and preserves the high biological value of the bacterial defense of albumen is especially important for eggs from small unique populations of chickens that provide products intended for consumers with special needs.

The present study found the eggshell quality parameters of amateur chickens to be largely consistent with those reported in previous reports for non-commercial used chicken breeds [Franco *et al*. 2020, Lordelo *et al*. 2020, Ketta *et al*. 2020, Lewko *et al*. 2024] and confirmed the variability in eggshell quality characteristics across genotypes and age that were shown in previous studies. Variation in eggshell color may be attractive for consumers and have a positive impact on their choice, but it makes difficult to introduce egg handling standards within commercial practices. Sirri *et al.* [2018] reported CV values ranging from 5 to 10% for the L* and b* color parameters and >10% for the a* parameter. These values are lower than those calculated for amateur chickens, but Sirri *et al.*'s research was carried out on commercial hybrids kept in a cage system. Lewko *et al*. [2021] and Krawczyk *et al*. [2024] noted variability in eggshell lightness from L*32.6 (Yellow-legged Partridge) to L*92.8 (Leghorn) for eggs obtained from breeds/varieties of chickens covered by the genetic resources protection program in Poland. Rizzi *et al*. [2023] assessed the color of eggshells from eight autochthonous chicken breeds from Italy and showed that the darkest eggshells were found from Robusta Maculata (L* 67.6) and the lightest were from Padovana Dorata (L* 92.7). In the present study, despite variations in eggshell thickness among amateur chicken populations, no significant differences in strength in both age groups

Table 6. Spearman rank–order correlation coefficients for eggshells characteristics and lysozyme content and activity in albumen of eggs from Polish Liliputy Bantams (PLB) at 33 (above the diagonal) and 55 weeks of age (below the diagonal)

Item	Shell W	Shell %	Shell T	Shell L*	Shell a*	Shell b*	Shell P	Shell S	ED	Lys. C	Lys. A
Shell W		0.641***	0.597***	-0.133	0.243	0.166	0.164	0.505**	-0.223	-0.192	-0.207
Shell %	0.661***		0.651***	-0.179	0.331	0.199	-0.102	0.537**	-0.181	-0.243	-0.310
Shell T	0.621***	0.700***		-0.140	0.165	0.181	-0.186	0.433**	-0.148	-0.214	-0.284
Shell L*	-0.223	-0.134	-0.134		-0.076	-0.617***	0.037	0.179	0.289	0.047	0.100
Shell a*	-0.176	-0.466**	-0.180	-0.279		0.056	-0.118	0.381*	-0.128	0.111	0.143
Shell b*	0.440**	0.309	0.429*	-0.502**	-0.038		0.105	0.031	-0.220	-0.193	-0.245
Shell P	-0.185	-0.054	-0.234	0.015	0.054	-0.371*		0.051	-0.025	-0.234	-0.213
Shell S	0.557***	0.701***	0.502**	-0.312	-0.304	0.425*	-0.139		-0.231	-0.089	-0.071
ED	-0.394*	-0.229	-0.237	0.228	-0.110	-0.515**	-0.180	-0.282		-0.026	0.049
Lys. C	0.233	0.389*	0.091	-0.047	-0.275	0.055	0.166	0.165	-0.120		0.975***
Lys. A	0.214	0.381*	0.045	-0.044	-0.277	0.028	0.215	0.148	-0.113	0.989***	

Shell W – shell weight; Shell % –Shell % in egg; Shell T – Shell thickness; Shell color L* – Shell L*; Shell color a*– Shell a*; Shell color b* – Shell b*; Shell porosity – Shell P; Shell strength – Shell S; Elastic deformation – ED; Lysozyme content – Lys C; Lysozyme activity – Lys A; Correlation is significant at the $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$ level.

Table 7. Spearman rank–order correlation coefficients for eggshells characteristics and lysozyme content and activity in albumen of eggs from native Polish Crested Chickens (PCr, CP–22 strain) at 33 (above the diagonal) and 55 weeks of age (below the diagonal)

Item	Shell W	Shell %	Shell T	Shell L*	Shell a*	Shell b*	Shell P	Shell S	ED	Lys. C	Lys. A
Shell W		0.839***	0.711***	-0.081	0.205	0.230	-0.159	0.744***	-0.654***	0.057	0.024
Shell %	0.818***		0,742***	-0.222	0.249	0.354*	-0.154	0.665***	-0.729***	-0.057	-0.088
Shell T	0.835***	0.696***		-0.076	0.172	0.172	-0.017	0.648***	-0.688***	-0.162	-0.153
Shell L*	0.163	0.201	0.077		-0.850***	-0.943***	0.089	-0.046	0.103	-0.044	0.002
Shell a*	0.185	0.013	0.258	-0.735***		0.780***	-0.005	0.137	-0.212	-0.032	-0.058
Shell b*	0.082	-0.086	0.179	-0.820***	0.882***		-0.162	0.139	-0.198	0.088	0.033
Shell P	0.188	0.318	0.316	0.115	-0.089	-0.112		0.105	0.139	-0.369*	-0.335
Shell S	0.609***	0.500**	0.513**	-0.247	0.430**	0.361	0.173		-0.752***	-0.049	-0.058
ED	-0.361	-0.500**	-0.228	0.022	-0.013	-0.029	-0.010	-0.379*		0.077	0.075
Lys. C	-0.124	0.002	0.044	0.055	-0.218	-0.161	0.285	-0.155	-0.032		0.971***
Lys. A	-0.048	0.056	0.106	0.105	-0.209	-0.187	0.298	-0.211	-0.010	0.953***	

Shell W – shell weight; Shell % –Shell % in egg; Shell T – Shell thickness; Shell color L* – Shell L*; Shell color a*– Shell a*; Shell color b* – Shell b*; Shell porosity – Shell P; Shell strength – Shell S; Elastic deformation – ED; Lysozyme content – Lys C; Lysozyme activity – Lys A; Correlation is significant at the $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$ level.

and overall elastic deformation of eggs obtained in the second half of production were observed. However, a decrease in shell strength and an increase in elastic deformation with age of chickens were noted. Lewko *et al.* [2021] showed differences in eggshell thickness, porosity, strength and elastic deformation between different genotypes of non-commercially used chicken breeds. Krawczyk *et al*. [2024] showed differences in eggshell thickness and porosity but comparable shell damage resistance. Therefore, future research on local amateur chickens should be extended to include analyses of eggshell ultrastructure.

As expected, amateur chickens' eggshell strength in both age groups was estimated to be moderately to highly positively correlated with shell weight, proportion of shell in the egg, and shell thickness. Furthermore, for PCr and GLP, a negative monotonic relationship was observed between some of these eggshell characteristics and elastic deformation, with moderate to high correlation coefficients. Similarly, in previous research on native Egyptian chicken breeds, Fathi *et al*. [2019] noted a positive correlation between eggshell strength and shell toughness and thickness. For these breeds, it was further suggested that eggshell strength is the most effective factor for predicting eggshell breaking force, followed by thickness. In the present study, the values of the correlation coefficient between the basic eggshell characteristics varied among the chicken populations, thus indicating different strengths of the monotonic relationship, particularly in the case of PLB. These differences may result from variation in the ultrastructural features of the shell related to both the palisade [Radwan, 2015] and mamillary layers [Krystianiak *et al*. 2005, Fathi *et al*. 2019]. Krystianiak *et al.* [2005] showed that in ring-necked pheasants, blue eggshells are characterized by low hatchability, a thinner mamillary layer and other structural abnormalities, as compared to dark-brown, light-brown and olive eggs. Radwan [2015] noted a positive correlation between palisade length and eggshell breaking strength and thickness, but a negative

Table 8. Spearman rank–order correlation coefficients for eggshells characteristics and lysozyme content and activity in albumen of eggs from Gold Laced Polish Chickens (GLP) at 33 (above the diagonal) and 55 weeks of age (below the diagonal)

Item	Shell W	Shell %	Shell T	Shell L*	Shell a*	Shell b*	Shell P	Shell S	ED	Lys. C	Lys. A
Shell W		0.684***	0.782***	-0.217	0.317	0.456**	-0.114	0.576***	-0.661***	0.061	0.032
Shell %	0.541**		0.844***	-0.338	0.254	0.546***	-0.132	0.847***	-0.725***	0.024	0.019
Shell T	0.704***	0.776***		-0.479**	0.404*	0.599***	-0.089	0.631***	-0.653***	-0.038	-0.103
Shell L*	0.092	0.269	0.209		-0.444**	-0.807***	0.002	-0.114	0.094	0.203	0.274
Shell a*	0.374*	-0.028	0.115	0.131		0.615***	0.187	0.219	-0.098	-0.095	-0.177
Shell b*	0.094	-0.141	0.014	-0.797***	-0.125		-0.025	0.434*	-0.237	-0.246	-0.287
Shell P	0.130	-0.147	0,063	-0.104	0.344	0.1621		-0.008	0.0721	-0.103	-0.106
Shell S	0.522	0.282	0.327	0.484**	0.404*	-0.204	-0.030		-0.717***	-0.013	0.006
ED	-0.556**	-0,517**	-0.525**	-0.147	-0.016	-0.133	0.195	-0.5411**		-0.091	-0.074
Lys. C	0.001	0.003	-0.182	0.236	0.118	-0.404**	-0.190	0.162	0.071		0.954***
Lys. A	0.019	0.015	-0.197	0.213	0.064	-0.366*	-0.205	0.155	0.039	0.988***	

Shell W – shell weight; Shell % –Shell % in egg; Shell T – Shell thickness; Shell color L* – Shell L*; Shell color a*– Shell a*; Shell color b* –Shell b*; Shell porosity – Shell P; Shell strength – Shell S; Elastic deformation – ED; Lysozyme content – Lys C; Lysozyme activity – Lys A; Correlation is significant at the *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ level.

correlation was found for elastic deformation, both for native breeds (Fayoumi and Gimieizah) and commercially used chickens (Hy-Line Brown). Moreover, Fathi *et al.* [2019] observed low shell mammillary alignment and extra crystallization material between caps in eggs of the native Fayoumi breed, which is characterized by high breaking force.

When handling eggs from amateur chickens, it is important to use non-invasive procedures that correlate with these eggs' resistance to damage. In this regard, the color of the eggshell is of practical interest. The results of this study indicate that eggshell strength in both amateur chicken age groups is in a moderate monotonic correlation with some shell color parameters, but mainly for PLB and GLP. Moreover, the significance of Spearman's rank-order correlation coefficients for eggshell strength and some different shell color parameters varies depending on the age of chickens. It is worth noting that for GLP, increased color lightness also means increased eggshell strength, but only for eggs obtained from chickens in the second half of the egg production period. Overall, elastic deformation is negatively moderately correlated with the b* color parameter, but only for PLB eggs and also from older chickens. Considering also the high variability of the a* and b* eggshell color parameters, it is not practical to use measurement of eggshell color to infer the tested PCr and PLB shell resistance to damage across the whole egg production period. For GLP, the L*, a*, b* scale parameter designations for eggs obtained mainly in the second half of production can be used for this purpose. In ring-necked pheasants, a moderate negative correlation was found between lightness in eggshell color, its thickness and egg shape [Nowaczewski *at al*. 2013]. Nowaczewski *et al*. [2013] concluded that the lightness of pheasant eggshell pigment rather than its color is related to eggshell thickness. Yang *at al*. [2009] showed that in brown-egg-laying Yangzhou chickens, eggshell strength and thickness reduced as the shell color became paler, and the number of pores increased. Thus authors concluded that this eggshell quality trait could be assessed through the shell color. However, Sirri *et al.* [2018] showed a weak correlation between eggshell strength and color parameters L* and a* in commercial brown-egg-laying hybrids assessed throughout the production period, thus they do not recommend the practical use of these parameters. Drabik *et al.* [2021] found that the highest breaking strength was characteristic of eggs from Marans chickens with the darkest shells compared to Araucana and Leghorn breeds, with the lowest values for this trait recorded for white-shelled eggs.

In this research, differences in the analyzed lysozyme parameters were noted for eggs obtained from amateur chickens from both age groups. Similarly, Yu *et al.* [2022] noted variation in eggs' lysozyme content among indigenous Chinese breeds. Also Hejdysz *et al.* [2024] showed differences in lysozyme content among several pure breeds and lines of chickens, with some breeds showing double the lysozyme content in eggs compared to others. Eggshell color may determine some biological egg characteristics, including lysozyme activity related to bacteriostatic properties of albumen. It has been shown that the highest content and activity of lysozyme is characteristic of GLP eggs with a white shell color, and the lowest parameters are recorded for PCr eggs with a cream color. The highest increase in lysozyme content and activity with the age of the chickens was recorded for PLB, as compared to the other populations. Moreover, this study found that an increase in eggshell porosity in PCr chickens in the first half of production was associated with a decrease in lysozyme content, although the correlation coefficient was low. Interestingly, for older GLP chickens, an increase in the b* color parameter was associated with a decrease in lysozyme content and activity. In ring-necked pheasants, higher lysozyme content and activity were found in lighter-colored eggs, i.e., blue and light-brown color, compared to dark-brown and olive shell color [Kożuszek *at al*. 2009]. The authors reported that high lysozyme content in eggs with thinner and more porous blue shells may indicate a stronger natural protective barrier in these eggs. Gvoždíková Javůrková *et al.* [2019] documented a positive correlation between concentration of lysozyme in egg albumen and eggshell cuticle protoporphyrin in tinted and dark brown eggs, but not brown, white and blue eggshells. The authors suggest that this relationship results from the combination of both genetic and hormonally regulated extrinsic factors that significantly influence the content of lysozyme in egg albumen.

Conclusions

In summary, the research results show differences in some physical eggshell parameters between populations of amateur chickens traditionally bred in Poland. On the other hand, breaking strength was similar for the three genetic groups, but it decreased with the age of the chickens. In addition, differences were found in the content and activity of lysozyme in the albumen, with higher values recorded in white eggs than in cream eggs and in the second half of egg production. Eggshell strength was in a positive monotonic correlation with shell weight, shell proportion in the egg, and thickness. The variability of the eggshell a* and b* color parameters and the differences in the Spearman's rank-order correlation coefficients between shell strength that depend on the age of the chickens indicate that non-invasive measurement of shell color cannot be used to predict egg resistance to damage for PCr and PLB. It can be suggested that in the case of GLP, the L*, a*, b* scale parameters for eggs obtained mainly in the second half of production can be used for this purpose.

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