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

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ABSTRACT The aim of the study was to determine shell quality of eggs laid by some strains of native breed hens of different ages, with special consideration of their effect on lysozyme concentration and enzymatic activity. Evaluation was made of the eggshells from 6 breeds/strains of laying hens covered by the gene pool protection program in Poland: Greenleg Partridge (Z-11), Yellowleg Partridge (Ż-33), Rhode Island Red (R-11), Rhode Island White (A-33), Sussex (S-66), and Leghorn (H-22). Significant ($P \leq 0.01$) differences were established for all the shell quality characteristics between hen strains. As the birds aged, shell weight and porosity increased, and shell compression strength decreased in all the experimental groups. Lysozyme content was lowest in white-shelled eggs (H-22) and highest in cream-colored and light brown eggs (Z-11, Ż-33, and R-11). Furthermore, age of hens had a greater effect on lysozyme concentration and activity in the eggs than on shell quality traits. Regardless of the layer genotype, eggs from older hens showed higher lysozyme concentration and enzymatic activity.

Key words: eggshell quality, lysozyme, native breed of hen

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INTRODUCTION

In view of the fact that the egg serves to provide nutrients and plays a reproductive role, its content must be protected from mechanical damage and microbial penetration. This role is served by the shell. Shell quality, which is reflected in many parameters (such as shell thickness, density, number of pores, deformation, compression strength), determines the economic performance of farms with hens in lay (Abdallah et al., 1993; Czaja et al., 2005). In addition, shell quality is determined by the impact of many other factors, including bird age, housing system, and genotype (Kuźniacka et al., 2004; Krawczyk and Gornowicz, 2010; Calik, 2011; Lewko and Gornowicz, 2011; Mohiti-Asli et al., 2012). Among the tests to determine mechanical properties of the egg, analysis of shell compression strength and deformation plays a key role in the objective assessment of its quality as easily measurable traits. It is also noteworthy that determination of shell strength through the measurement of its deformation entails no damage to the shell, which is very important in the case of hatching eggs (Hamilton, 1982; Abdallah et al., 1993; Połtowicz, 2001).

Lysozyme (muramidase), a well-studied component of poultry eggs, is a small-molecule hydrolytic enzyme with antifungal, antiviral, and antibacterial properties, especially against gram-positive bacteria (Trziszka et al., 2007; Tomczyk et al., 2016). In the hen's egg albumen, which constitutes from 58 to 64% of egg weight, lysozyme content forms around 3.5% of its albumen (Juneja, 2000; Trziszka, 2000; Mann, 2007; Mann and Mann, 2011; Trziszka et al., 2013). This valuable substance is used in the pharmaceutical industry as an ingredient in medicines and in the food industry for food storage and preservation (Cegielska-Radziejewska et al., 2008; Gajda and Bugla-Płoskońska, 2014; Kijowski et al., 2013 Salejda and Krasnowska, 2014). The concentration and enzymatic activity of lysozyme depend on many factors such as genotype, diet, housing system, layer age, egg storage conditions, as well as

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stress, disease history, and pharmaceuticals used (Trziszka et al., 2007; Lewko and Gornowicz, 2009, 2015a,b; Rizzi and Marangon, 2012; Vlčková et al., 2019).

Wellman-Labadie et al. (2009) used microsequencing to study the location of lysozyme in shell membranes and in the shell during the egg formation process. They observed that lysozyme had a large effect on shell protective function during embryogenesis and on calcium carbonate deposition in the eggshell, which contributes to the egg's compression strength. Bilkova et al. (2018) and Gvoždíková Javůrková et al. (2019) reported that in the native and fancy breeds of hens, shell pigmentation is related to lysozyme and ovotransferrin concentration in eggs. Poland has 11 strains of hens covered by the gene pool protection program; they are kept in small populations (800–1,100 birds) subjected to no selection for production characteristics or egg quality. The main goal of the program is to preserve the genotype and to protect hens from increased inbreeding. Krawczyk and Calik (2018), who evaluated egg quality in Polish native breeds, found shell color to vary widely. Krawczyk (2009) also reported that the percentage of albumen in the eggs of native hens is significantly lower than in eggs from commercial hybrids used for intensive egg production. In turn, Calik (2016) investigated chemical composition of albumen weight of eggs from some strains of hens and found egg albumen to increase with the age of the layers. It is therefore interesting to study the concentration of this valuable enzyme in the albumen of eggs from these populations, as related to some shell quality traits and age of hens. It was assumed that in the large population of hens covered by the gene pool protection program in Poland, which has not been selected for many years, there may be a strong relationship between shell quality traits and the concentration of muramidase. This study will enable choosing strains of hens whose eggs will show higher lysozyme content and enzymatic activity.

The aim of the study was to evaluate shell quality of eggs laid by some strains of native breed hens of different ages and to determine their effect on the concentration and enzymatic activity of lysozyme.

MATERIALS AND METHODS

The study used 6 breeds/strains of laying hens covered by the gene pool protection program in Poland: Greenleg Partridge (Z-11), Yellowleg Partridge (Ż-33), Rhode Island Red (R-11), Rhode Island White (A-33), Sussex (S-66), and Leghorn (H-22). Strains Z-11 and Ż-33 are old, native populations, whereas R-11, S-66, H-22, and A-33 hens are locally adapted breeds, which were imported by pedigree farms after the Second World War and withdrawn from these farms in the 1970s and 1980s. The intensification of poultry production has contributed to the erosion or extinction of many valuable breeds of hens. In the 1970s, this led to the initiative to create conservation flocks, and these breeds were later included in conservation programs. Chickens (120 birds per strain, each strain in 4 replications of 30 birds) were kept on an experimental farm. A total of 720 hens were studied. Hens were kept on litter in a controlled-environment confinement building at a stocking density of 5 birds/m^2. Birds were fed ad libitum with a layer diet containing 89.11% of DM, 11.28% of crude ash, 16.93% of CP, 2.15% of crude fat, 2.5% of crude fiber, 3.55% of calcium, and 0.5% of phosphorus. The standard diet contained no special additives. Thirty hens in each compartment were provided with 6 nests, a round feeder (circumference: 126 cm), and a round drinker (circumference: 110 cm).

At 33 and 35 wk of age, 30 eggs were collected from each experimental group for analysis. Shell quality was tested using a texture analyzer TA.XT PLUS (Stable Micro Systems Ltd, Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, UK) fitted with appropriate attachments. Shell deformation was measured accurate to 1 μm at 3 measurement sites (on the equatorial circumference of the egg and on both egg poles—on the sharp and blunt ends along the long axis of the egg—using a 2.5-mm-diameter cylindrical probe [test speed: 0.50 mm/s, distance: 0.100 mm]) under 3 different loads: 0.50 kg, 1.00 kg, and 1.50 kg. This test determined the degree of elastic deformation of the shell under the applied load. To analyze mechanical strength of the eggshell, the applied load (N) was gradually increased until the shell broke (a 7.5-cm-diameter plate probe, test speed: 10.00 mm/s, distance: 1.000 mm). This measurement determined the load at which the analyzed eggshell was broken, crushed, or perforated. Shell thickness (μm) without shell membranes was measured using a Mitutoyo micrometer (Mitutoyo 293-766-30 Digimatic Digital Micrometer 0-1″; Borsigstraße 8-10 D-41469 Neuss), accurate to 1 μm. After drying the shells at room temperature, shell membranes were removed using pincers. The test was performed at three egg measuring points (blunt and sharp ends of the egg and its side), and the mean from 3 measurements as the result. The pores (number of pores per cm^2) were marked and calculated using the method of Tyler (1953). Porosity was determined using a stereomicroscope (magnification of 4×) in a shell area of 0.25 cm^2. The instrumental measurement of shell color was performed using a Minolta C580 Chroma Meter (Konica Minolta, INC. Made in Japan) (D65 lighting, 10° observer, 8-mm aperture size, calibrated with a white plate: L* – 99.18) at 3 egg measurement points (sharp end, blunt end, side), and the result was averaged.

To make a detailed analysis of the active ingredient (lysozyme) in egg albumen fractions (hydrolytic activity, percentage), the previously collected and weighed eggs from different experimental groups of the birds were broken and separated into albumen and yolk. Next, the albumen was separated into thick and thin fractions (1 mL of different albumen fractions was collected using an automatic pipette with an appropriate tip) 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. The content and

Table 1. Shell color, weight, thickness, porosity, and strength of eggs from native strains of hens.

Item	Age (wk)		Strains of hens: H-22 L*	H-22 a*	H-22 b*	Ż-33 L*	Ż-33 a*	Ż-33 b*	Z-11 L*	Z-11 a*	Z-11 b*	R-11 L*	R-11 a*	R-11 b*	S-66 L*	S-66 a*	S-66 b*	A-33 L*	A-33 a*	A-33 b*	Mean	Effect of (P value): Strain (S)	Age (A)	S × A
Shell color (%)	33	x	85.1^{E}	18.1^{A}	21.2	57.8^{C}	13.9^{B}	21.2	65.4^{D}	13.4^{B}	20.9	40.0^{B}	12.2^{B}	20.3	40.4^{B}	12.8^{B}	21.7	32.6^{A}	17.2^{A}	19.8	53.5			
		SD	1.85	0.51	0.49	4.72	0.46	0.42	6.01	0.50	0.52	4.83	0.46	0.41	4.38	0.44	0.53	3.21	0.50	0.46				
	53	x	84.9^{E}	17.3^{A}	21.0	57.7^{C}	13.4^{B}	20.9	64.5^{D}	13.0^{B}	19.8	39.7^{B}	11.8^{C}	20.0	40.6^{B}	12.0^{C}	20.0	30.7^{A}	16.6^{A}	19.6	53.0			
		SD	2.67	0.47	0.45	1.50	0.41	0.39	2.29	0.44	0.44	1.35	0.40	0.38	0.93	0.40	0.48	1.42	0.43	0.39				
	Mean		85.0	17.7	21.1	57.8	13.6	21.0	65.0	13.2	20.3	39.8	12.0	20.1	40.5	12.4	20.8	31.6	16.9	19.7	53.2	≤0.01	0.127	0.561
	SD		2.28	0.49	0.47	3.46	0.43	0.40	4.53	0.47	0.48	3.52	0.43	0.39	3.13	0.42	0.50	2.64	0.46	0.42				
Shell weight (g)	33	x	6.88^{C}			$6.59^{B,C}$			$5.95^{A,b}$			$6.69^{B,C}$			$6.30^{A,b,a}$			7.18^{C}			6.60			
		SD	0.57			0.51			0.41			0.50			0.50			0.44						
	53	x	8.48^{E}			$7.13^{AB,b}$			$7.23^{B,C,b}$			$7.54^{C,c}$			$6.72^{A,a}$			8.03^{D}			7.52			
		SD	0.74			0.51			0.83			0.57			0.64			0.72						
	Mean		7.68			6.86			6.59			7.11			6.51			7.60			7.06	≤0.01	≤0.01	≤0.01
	SD		1.03			0.57			0.92			0.68			0.61			0.73						
Shell thickness (μm)	33	x	$341.0^{B,C}$			335.0^{AB}			321.0^{A}			$348.0^{B,C}$			$345.0^{B,C}$			357.0^{C}			341.0			
		SD	30.16			25.68			23.67			28.47			29.48			21.73						
	53	x	$365.0^{C,c}$			$329.0^{A,B}$			$330.0^{A,b,a}$			$351.0^{C,b}$			319.0^{A}			$348.0^{BC,b}$			340.0			
		SD	23.23			27.36			29.74			22.73			24.68			33.39						
	Mean		353.0			332.0			325.0			350.0			332.0			352.0			341.0	≤0.01	0.716	≤0.01
	SD		29.4			26.5			27.0			25.6			30.1			28.4						
Shell porosity (pore number /cm^2)	33	x	47.0^{E}			$35.1^{B,C,b}$			39.0^{D}			45.1^{E}			30.1^{A}			$32.0^{A,ba}$			38.0			
		SD	3.89			4.39			4.86			4.53			4.69			3.97						
	53	x	$54.0^{D,d}$			40.0^{B}			45.0^{C}			$51.0^{D,c}$			$35.0^{A,a}$			$38.0^{AB,b}$			43.8			
		SD	7.20			4.84			4.95			5.36			4.95			4.81						
	Mean		50.5			37.5			42.0			48.0			32.5			35.0			40.9	≤0.01	≤0.01	0.857
	SD		6.73			5.20			5.73			5.74			5.38			5.32						
Shell strength (N)	33	x	37.7			$41.1^{B,C,b}$			$35.2^{A,c,a}$			43.5^{B}			34.6^{A}			37.6			38.3			
		SD	8.63			6.95			6.94			6.50			8.53			5.93						
	53	x	37.8^{B}			37.8^{B}			$31.3^{A,b}$			37.0^{B}			$26.3^{A,a}$			27.8^{A}			33.2			
		SD	8.77			8.77			9.35			11.59			9.51			12.51						
	Mean		37.8			39.4			33.3			40.3			30.5			33.4			35.7	≤0.01	≤0.01	0.033
	SD		8.62			8.01			8.39			9.87			10.25			10.34						

A, B …: values in rows with different letters differ significantly $P \leq 0.01$; a, b ...: for $P \leq 0.05$.
Strains of hens: Leghorn (H-22), Yellowleg Partridge (Ż-33), Greenleg Partridge (Z-11), Rhode Island Red (R-11), Sussex (S-66), Rhode Island White (A-33).
Abbreviations: x, mean value; S × A, interaction of strain × age.

activity of lysozyme in the fresh albumen fractions was determined by spectrophotometry (Leśnierowski and Kijowski, 1995), consisting in the use of lysis of cell walls of *Micrococcus lysodeikticus* bacteria. Hydrolytic activity of lysozyme was expressed in lysozyme activity units (U/mL). One such unit is defined as the amount of lysozyme that in 1 min decreases the absorbance of a *M. lysodeikticus* bacterial suspension by 0.001, measured at a wavelength of 450 nm and a temperature of 25°C. The reaction occurred in a mixture of a volume of 2.6 ml (2.5 mL of bacterial suspension + 0.1 mL of lysozyme solution) and pH 6.24, in a cuvette with a length of optical path being 1 cm. After calculating the value of absorbance decrease (ΔA) for the working solution of lysozyme, a correlation curve between ΔA and lysozyme concentration was graphed. Next, based on the standard curve, hydrolytic activity of lysozyme was determined in the studied preparations. The value of the decrease in the solution absorbance (ΔA) was calculated from the following equation:

$$\Delta A = At0 - At \ (U / min.)$$

where At0 is the absorbance value for the bacterial suspension at time t0 and At is the absorbance value for the bacterial suspension after time t.

The results were statistically analyzed, and significant differences were determined using two-way ANOVA (genotype × age of hens) via Duncan's test using the Statistica 12 PL software package (StatSoft Polska Sp. z o.o. ul. Kraszewskiego 36, 30-110 Kraków). Pearson's coefficients of correlation were also calculated between the analyzed traits (shell color only for L*).

RESULTS

Highly significant ($P \leq 0.01$) differences were observed in shell quality traits between strains and between ages of hens (Table 1). Shell color ranged from the white strain H-22 (L*, 85.0; a*, 17.7; b*, 21.1) to creamy strains Ż-33 and Z-11 (L*, 57.8; a*, 13.6; b*, 21.0 and L*, 65.0; a*, 13.2; b*, 20.3, respectively), light brown strains R-11 and S-66 (L* –39.8, a*, 12.0; b*, 21.0 and L*, 40.5%; a*, 12.4; b*, 20.8), and the dark brown strain A-33 (L*, 31.6; a*, 16.9; b*, 19.7) ($P \leq 0.01$). Eggs with the highest average shell weight and shell thickness were obtained from H-22 hens (7.68 g and 353.0 μm), and those with the lowest values were obtained from strains S-66 and Z-11 (6.51 g and 353.0 μm; 6.59 g and 325.0 μm, respectively). At the same time, eggs from S-66 hens showed the lowest number of pores (32.5 pores/cm^2) and the lowest shell compression strength (30.5 N). In turn, the highest number of pores per shell was found for eggs from strains R-11 and H-22 (48.0 and 50.5 pores/cm^2, respectively). The highest compression strength was noted in the eggs of strains Ż-33 and R-11 (39.4 and 40.3 N, respectively). As birds from all the strains aged, there were significant ($P \leq 0.01$) increases in shell weight (by 0.92 percentage points on average) and shell porosity (by 5.80 percentage points on average) and decreases in shell compression strength by an average of 5.10 percentage points, and in addition, significant ($P \leq 0.01$) differences were observed for R-11, S-66, and A-33 hens. For the eggs of H-22 hens, compression strength was at a similar level (37.8 N on average). The age of hens had no significant effect on changing eggshell color. The weight, thickness, and compression strength were concomitantly influenced by both genotype and age of the hens.

The results for shell deformation are presented in Table 2. In general, a significant ($P \leq 0.01$) effect of age was noted for loads of 0.5, 1.0, and 1.5 kg measured at the sharp and blunt end, and the effect of genotype was noted on the circumference. Regardless of the load and range of measurement, there was a significant ($P \leq 0.01$) interaction between genotype and age. For the parameters measured, lowest deformation values were found in the strain H-22, ranging from an average of 31.12 (sharp end/load of 0.5 kg) to 121.1 μm (circumference/load of 1.5 kg). The highest values of this parameter were observed in strains R-11, Ż-33, A-33, and S-66 (from 34.50 N, R-11/sharp end/load of 0.5 kg, to 134.7 N, Ż-33/circumference/load of 1.5 kg).

As is evident from Table 3, both genotype and age had a highly significant ($P \leq 0.01$) effect on lysozyme content and hydrolytic activity. Albumen from the eggs of Ż-33, Z-11, and R-11 birds showed the highest average percentage of lysozyme (range from 0.269 to 0.276%) and thus the highest enzymatic activity of lysozyme (range from 57,749 to 59,347 U/mL). In turn, the lowest enzyme percentage was noted in the albumen of eggs from H-22 hens, wherein the lysozyme content averaged only 0.233% and lysozyme activity was 49,966 U/mL. Lysozyme content and activity in the egg albumen increased significantly ($P \leq 0.01$) with the age of the hens.

Table 4 shows the results of the analysis of the correlations between some shell quality traits and lysozyme parameters. A significant ($P \leq 0.01$) and negative correlation was observed only between shell color and lysozyme content (−0.378) and lysozyme activity (−0.372). For the other traits, the coefficients of correlation were very low and not significant, ranging from −0.058 (shell thickness/lysozyme activity) to 0.060 (shell weight/lysozyme content).

DISCUSSION

A study on eggs from 41 fancy and traditional chicken breeds (Gvoždíková Javůrková et al., 2019) demonstrated a positive correlation between eggshell pigmentation and the concentration of lysozyme and ovotransferrin in tinted and light brown eggs, but not in blue, white, and dark brown eggs. The same study reported that in traditional chicken breeds, the concentration of lysozyme and ovotransferrin in egg albumen was influenced to a large extent by environmental factors (temperature, humidity), genotype, diet, and the year of egg collection. Many studies suggested that eggshell color is a genetically determined trait that is difficult to modify with environmental

Table 2. Mean values of shell deformation (μm) measured at 3 points of the egg (circumference, sharp end, and blunt end) under 3 different loads (0.50 kg, 1.00 kg, and 1.50) of eggshells in the analyzed strains of hens.

Item	Age (wk)		Strains of hens						Mean	Effect of (P value):		
			H-22	Ż-33	Z-11	R-11	S-66	A-33		Strain (S)	Age (A)	S × A
Elastic deformation—sharp end, load of 0.5 kg	33	x	31.23^{a}	30.03^{A,a}	30.77^{a}	34.93^{B,b}	31.43^{a}	32.03	31.74			
		SD	3.38	2.13	6.32	10.27	4.26	7.26				
	53	x	31.00^{a}	34.56^{b}	35.00^{b}	34.07	35.00^{b}	34.81^{b}	34.07			
		SD	4.78	5.85	7.37	5.17	5.10	5.31				
	Mean		31.12	32.30	32.89	34.50	33.22	33.42	32.91	0.056	≤0.01	≤0.047
	SD		4.11	4.93	7.13	8.07	4.96	6.50				
Elastic deformation—sharp end, load of 1.0 kg	33	x	60.10	57.30^{A}	60.90	64.33^{B}	61.23	61.20	60.84			
		SD	4.84	4.03	5.90	10.93	9.08	11.01				
	53	x	59.47^{A,a}	66.20^{b}	65.87^{b}	66.70^{B}	69.65^{B}	68.00^{B}	65.98			
		SD	6.69	9.35	10.54	10.52	10.86	10.62				
	Mean		59.79	61.75	63.39	65.52	65.44	64.60	63.41	≤0.01	≤0.01	≤0.03
	SD		5.80	8.43	8.39	10.71	10.72	11.26				
Elastic deformation—sharp end, load of 1.5 kg	33	x	91.37	85.13^{a}	90.47	93.57^{b}	90.90	89.73	90.20			
		SD	13.23	7.48	8.43	12.15	12.83	11.59				
	53	x	88.07^{A,a}	98.67^{B}	96.47^{b}	98.80^{B}	106.31^{B,c}	102.37^{B,c}	98.44			
		SD	9.18	15.71	14.03	15.99	17.90	16.61				
	Mean		89.72	91.90	93.47	96.19	98.61	96.05	94.32	≤0.01	≤0.01	≤0.01
	SD		11.41	13.97	11.87	14.32	17.10	15.43				
Elastic deformation—blunt end, load of 0.5 kg	33	x	35.93	34.73	34.60	35.97	34.27	35.30	35.13			
		SD	6.07	4.16	3.58	5.27	4.11	3.98				
	53	x	34.17^{A,a}	40.40^{B}	36.40	37.10	38.08^{b}	39.00^{B}	37.52			
		SD	6.57	9.64	5.24	7.63	4.87	5.38				
	Mean		35.05	37.56	35.50	36.54	36.18	37.15	36.33	0.153	≤0.01	≤0.01
	SD		6.33	7.90	4.53	6.53	4.84	5.01				
Elastic deformation—blunt end, load of 1.0 kg	33	x	69.67	67.20	68.13	68.27	66.83	68.00	68.02			
		SD	10.10	7.45	7.16	8.02	8.67	6.99				
	53	x	65.57^{A}	76.90^{B}	70.50	69.93	74.92^{B}	76.30^{B}	72.35			
		SD	9.70	13.97	8.06	10.87	8.27	10.07				
	Mean		67.62	72.05	69.32	69.10	70.88	72.15	70.19	0.051	≤0.01	≤0.01
	SD		10.03	12.13	7.64	9.51	9.35	9.48				
Elastic deformation—blunt end, load of 1.5 kg	33	x	105.33	99.70	101.30	100.70	100.27	97.53	100.81			
		SD	17.20	11.45	10.11	12.65	14.33	19.30				
	53	x	97.30^{A,a}	114.17^{B,C}	107.43^{b}	103.37^{A,B,b}	113.77^{B,C,c}	116.52^{C}	108.76			
		SD	12.87	19.15	12.16	15.56	13.36	18.14				
	Mean		101.32	106.94	104.37	102.04	107.02	107.03	104.79	0.111	≤0.01	≤0.01
	SD		15.60	17.26	11.51	14.12	15.34	20.91				
Elastic deformation—circumference, load of 0.5 kg	33	x	41.40^{a}	45.10^{B,b}	45.53^{B,b}	43.50^{b}	42.93	40.20^{A,a}	43.11			
		SD	5.40	5.16	5.95	5.16	3.98	2.99				
	53	x	38.63^{A,a}	45.37^{B,c}	42.13^{b}	41.97^{b}	45.77^{B,c}	40.33^{a,b}	42.37			
		SD	3.63	7.12	5.91	4.77	4.76	5.53				
	Mean		40.02	45.23	43.83	42.74	44.35	40.27	42.74	≤0.01	0.293	≤0.038
	SD		4.77	6.16	5.99	4.99	4.55	4.34				
Elastic deformation—circumference, load of 1.0 kg	33	x	82.80^{A}	90.40^{B}	88.03^{A,B,b}	86.03^{b}	84.70	80.03^{A,a}	85.33			
		SD	11.58	10.39	11.77	10.50	8.51	6.81				
	53	x	77.50^{A,a}	88.57^{B,C}	84.53^{A,B,b}	82.47^{A,B}	92.46^{C}	81.33^{A}	84.48			
		SD	7.65	9.49	11.87	9.39	10.00	9.70				
	Mean		80.15	89.49	86.28	84.25	88.58	80.68	84.90	≤0.01	0.419	≤0.01
	SD		10.09	9.91	11.85	10.04	9.95	8.25				
Elastic deformation—circumference, load of 1.5 kg	33	x	125.50^{a,c}	135.50^{B,b}	130.60^{B,c}	127.90^{b,c}	126.70^{b,c}	120.13^{A,a}	127.72			
		SD	13.32	14.75	16.50	14.07	13.45	11.70				
	53	x	116.73^{A,a}	134.00^{B,C}	129.80^{B,b,c}	127.03^{B,b}	137.08^{B,C,c}	124.44^{A,B,b}	128.18			
		SD	10.86	11.24	13.16	12.60	11.73	13.24				
	Mean		121.12	134.75	130.20	127.47	131.89	122.28	127.95	≤0.01	0.743	≤0.01
	SD		12.83	13.02	14.80	13.25	13.61	12.53				

A, B …: values in rows with different letters differ significantly $P \leq 0.01$; a, b …: for $P \leq 0.05$.

Strains of hens: Leghorn (H-22), Yellowleg Partridge (Ż-33), Greenleg Partridge (Z-11), Rhode Island Red (R-11), Sussex (S-66), Rhode Island White (A-33).

Abbreviations: x, mean value; S × A, interaction of strain × age.

intervention (Roberts, 2004; Krawczyk, 2009; Krawczyk and Calik, 2018; Sokołowicz et al., 2018). In studies concerning the quality of eggs from native breed hens (Krawczyk and Calik, 2018) and commercial laying hen hybrids (Samiullah et al., 2017), shell color lightness was found to increase with the age of layers, but as in our study, these changes were not always significant.

Table 3. Lysozyme content and enzymatic activity in the albumen of the eggs from native strains of hens.

Item	Age (wk)		Strains of hens						Mean	Effect of (P value):		
			H-22	Ż-33	Z-11	R-11	S-66	A-33		Strain (S)	Age (A)	S × A
Lysozyme content (%)	33	x	0.225^{A}	$0.262^{B,C,b}$	0.269^{C}	0.267^{C}	$0.248^{B,a}$	$0.261^{B,C,b}$	0.255			
		SD	0.02	0.02	0.02	0.02	0.02	0.02				
	53	x	0.242^{A}	0.276^{B}	0.281^{B}	0.286^{B}	0.277^{B}	0.279^{B}	0.273			
		SD	0.02	0.02	0.03	0.01	0.01	0.01				
	Mean		0.233	0.269	0.275	0.276	0.262	0.270	0.264	≤0.01	≤0.01	0.250
	SD		0.02	0.02	0.03	0.02	0.02	0.02				
Lysozyme activity (U/ml)	33	x	$48{,}341^{A}$	$56{,}435^{C}$	$57{,}999^{C}$	$57{,}979^{C}$	$53{,}264^{B,a}$	$55{,}723^{B,C,b}$	54,957			
		SD	3,747.2	3,982.3	5,020.0	5,164.1	4,758.4	5,154.5				
	53	x	$51{,}590^{A}$	$59{,}064^{B}$	$60{,}222^{B}$	$60{,}715^{B}$	$59{,}124^{B}$	$59{,}382^{B}$	58,350			
		SD	3,693.1	3,402.6	7,238.1	3,501.3	2,428.9	3,265.9				
	Mean		49,966	57,749	59,111	59,347	56,194	57,553	56,653	≤0.01	≤0.01	0.260
	SD		4,035.9	3,904.3	6,276.4	4,586.6	4,770.7	4,659.1				

A, B ...: values in rows with different letters differ significantly $P \leq 0.01$; a, b ...: for $P \leq 0.05$.
Strains of hens: Leghorn (H-22), Yellowleg Partridge (Ż-33), Greenleg Partridge (Z-11), Rhode Island Red (R-11), Sussex (S-66), Rhode Island White (A-33).
Abbreviations: S × A, interaction of strain × age; x, mean value.

The scientific substantiation of the relationship between egg lysozyme concentration and eggshell color would allow for a rapid selection of eggs with low or high concentration of this valuable component. It is evident from our study that lysozyme content is lowest in white-shelled eggs (H-22) and highest in creamy-colored and light brown eggs (Z-11, Ż-33, and R-11). Greater differences were found in egg lysozyme activity, which was highest in the old native strains Z-11, Ż-33, and R-11, which were the first to be conserved and had previously not been selected for production traits. The obtained results provide the basis for further studies. Myint et al. (2012) and Kinoshita et al. (2016) pointed to the differences in antimicrobial potential of the commercial lines of birds, which resulted from genetic work in breeds subjected to artificial selection on pedigree farms, considering the biological value of hatching eggs.

Eggshell pores allow exchange of gases between the egg and its environment, which is of crucial importance to embryo development during incubation, and their number per shell depends on poultry species. Hen eggs have more pores per square centimeter than goose eggs, but fewer pores than duck eggs (Trziszka, 2000). Higher eggshell porosity increases water vapor conductance, which, according to Krystianiak et al. (2005), may adversely affect chick hatchability. Many studies (Malec, 2005; Ketta and Tůmová, 2016) have confirmed the correlation between shell porosity and shell strength, and this relationship was also observed in our study. In all the strains, there was an increase in the number of pores per shell in older hens and a decrease in shell compression strength. It could be assumed that greater exchange of gases in the eggs with higher porosity may increase lysozyme activity in terms of its defensive function, but the coefficient of correlation for these traits is very low and nonsignificant. Hincke et al. (2000) suggested that considerable amounts of lysozyme are found in eggshell membranes, which protects the egg content in the event of shell cracks.

The decreasing shell compression strength in the analyzed eggs laid by older native hens was already noticed in our earlier studies (Krawczyk, 2009; Krawczyk and Calik, 2018) and may result from the failure to improve egg quality traits in genetic resource flocks or from lower availability of calcium to older layers (Lichovníková and Zeman, 2008). Recent studies suggest that in eggs from high-producing commercial layers fed with diets appropriate for their age, the decrease in eggshell strength in older layers is small and nonsignificant (Cambell et al., 2017; Petričević et al., 2017).

Elastic deformation of the shell reflects its brittleness and breaking strength. In this way, it is possible to determine how much the shell can deform without damage when it is subjected to a specific load, which is crucial for hatching eggs (Scholtyssek,1993). Gornowicz et al. (2013) measured elastic deformation of the shell in eggs obtained from 4 breeds/strains of laying hens covered by the gene pool protection program, namely, Greenleg Partridge, Yellowleg Partridge, Rhode Island Red, and Sussex, as well as a commercial hybrid designed for

Table 4. Correlations between some shell characteristics and lysozyme content and activity in the albumen of the eggs from native strains of hens.

Item	Shell color	Shell weight	Shell thickness	Shell porosity	Shell strength
Lysozyme content	−0.378[1]	0.060	−0.048	−0.055	−0.040
Lysozyme activity	−0.372[1]	0.032	−0.058	−0.056	−0.027

[1]Statistically significant.

semi-intensive production of eggs (Black D-109). The authors showed that the commercial layer hybrids produced eggs with the highest average elastic deformation of the shell (25.08 µm). For the conserved breeds, this parameter ranged from 20.65 (Sussex) to 21.53 µm (Greenleg Partridge). Similar values of this parameter were reported by Lewko and Gornowicz (2015a,b) for multipurpose layer hybrids—27.19 µm (group I), 25.17 µm (group II), and 26.81 µm (group III). An earlier study (Połtowicz et al., 2003), which examined the same eggshell trait in Hy-Line White layers, reported much higher values of this parameter, ranging from an average of 46.7 (load of 1 kg, group 2) to 170.2 µm (maximum load, group 1). In our study, this parameter averaged between 32.91 (sharp end/load of 0.5 kg) and 127.95 µm (circumference/load of 1.5 kg).

The age of hens had a greater effect on the concentration and activity of lysozyme in the eggs than on shell quality traits, and our results are consistent with the findings of Banaszewska et al. (2019). Regardless of the layer genotype, eggs obtained from older hens were characterized by higher lysozyme concentration and activity.

CONCLUSIONS

It was found from this study that 1) there were significant differences in all the eggshell quality traits between hen strains, 2) with advancing age of the hens from all strains, shell weight and porosity increased while shell compression strength decreased, 3) lysozyme concentration was lowest in eggs from H-22 hens and highest from Z-11, Ż-33, and R-11 hens, and 4) the age of hens had a greater effect on lysozyme concentration and activity in the eggs than on shell quality traits. Regardless of the layer genotype, eggs from older hens showed higher lysozyme content and enzymatic activity.

DISCLOSURES

The authors declare that there is no conflict of interest.

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