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Rearing system, utility type and age of hens as factors modifying the hydrolytic activity of lysozyme

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SUMMARY

The aim of study was to analyse the impact of the utility type, age and rearing system of hens on egg quality traits and the enzymatic activity of egg albumen lysozyme (LA). The research material consisted of eggs from two genotypes of hens: Hy-Line Brown laying hens and Ross 308 parental stock (meat-type). Laying-type hens were reared in two systems (60 eggs/system): intensive (litter, balanced feed) and extensive (open runs, on-farm fodder). The second variable was the age of hens from the parent stock (29, 33, 41, 53 and 70 wks; 60 eggs/age). Selected quality traits of eggs (egg weight, albumen weight and its proportion in the egg, albumen height, and Haugh unit) were measured, as well as albumen lysozyme activity.
The rearing system significantly modified egg weight, albumen weight, and thick albumen height, but did not affect LA. LA also did not depend on the utility type of birds; however, it decreased after 33 weeks of age. The enzymatic activity of lysozyme was highly negatively correlated with the age of hens, and positively with albumen height and Haugh unit.

KEY WORDS: meat-type layers; extensive system; muramidase; egg quality; albumen

INTRODUCTION

Lysozyme (muramidase), a protein with enzymatic activity, was discovered by Alexander Fleming in 1922 and since that time has aroused the interest of scientists. It is widely distributed in nature, found in both plant and animal cells, in body fluids and secretion fluids, e.g. tears and saliva, as well as in birds' eggs (Kvasnička, 2003). The basic form of lysozyme is the monomer, but its structure can be modified by changes in the temperature or pH of the environment or by high pressure. Its activity is exploited to inhibit the development of Gram-positive bacteria of the genera *Bacillus* (Abdou et al., 2007; Sung et al., 2011), *Lactobacillus* (Tribst et al., 2008), *Listeria* (Datta et al., 2008), *Staphylococcus* (Wang et al., 2005) and *Streptococcus* (Domenech et al., 2011).

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Moreover, the addition of chelating agents that break down the lipopolysaccharide structure of the outer membrane of the egg, in conjunction with the enzyme, allow for effective action against Gram-negative bacteria as well (Pellegrini et al., 1992). This is also the case for the integrated action of lysozyme with nisin, lactoferrin, glycine and ethylenediaminetetraacetic acid (Yuste et al., 2000). The antibacterial activity of lysozyme against Gram-negative bacteria also increases under the influence of high temperature and pressure (Nakimbugwe et al., 2006). Possibilities of modifying its activity have broadened its spectrum of action against Gram-negative bacteria of the genera *Brucella* (Salton, 1957, Vedmina et al., 1979), *Eschericha* (Chen et al., 2005), *Klebsiella* (Sheffield et al., 2013) and *Salmonella* (Datta et al., 2008), as well as fungi (Wang et al., 2005, Samaranayake et al., 2009) and the HIV-1 virus (Cole and Cole, 2008).

Lysozyme is the only biologically active ingredient of hen's eggs permitted as a safe food additive (FAO/WHO, 1993). This enzyme has been widely used as a preservative of poultry meat and its products (Hughey et al., 1989; Kijowskiet al., 2013), in controlling the growth of microorganisms in beer production (Silvetti et al., 2010), and in the production of 'smart packaging' (Cargi et al., 2004; Kandemir et al., 2005; Malhotra et al. 2015). Three main types of lysozyme are distinguished in the animal kingdom: chicken-type lysozyme, referred to in the literature as c-type, produced by most vertebrates and used a model for studies on the enzyme's activity; goose-type (g-type); and i-type, which has been found in invertebrates (Callewaert and Michiels, 2010). A particularly valuable source of lysozyme, used on an industrial scale, is chicken eggs, which contain about 3.5% of this enzyme in their dry mass (Cegielska-Radziejewska et al., 2008).

The content of lysozyme, and above all its activity, depends on many factors. These include the species of poultry (Jollés, 1967), as well as the birds' genotype (Lewko et al., 2007), the rearing system (Krawczyk and Gornowicz, 2010), diet modifications (Kopeć et al., 2005), storage conditions (Wilcox Jr and Cole, 1954), albumen processing methods (Kvasnička, 2003), the pH of the lysozyme environment (Chang et al., 2000), and methods of determination (Helal and Melzig, 2008). The utility type of birds may also affect the hydrolytic activity of lysozyme. Most studies focus on eggs from laying birds, while information is lacking on the activity of lysozyme in the eggs of meat-type breeder hens. Eggs that are too small or damaged are not classified as hatching eggs, so they can be a valuable source of this enzyme.

The aim of the work was to analyse the influence of the utility type, age, and rearing system of hens on the enzymatic activity of egg albumen lysozyme.

MATERIALS AND METHODS

The research material consisted of 420 eggs from hens of two utility types: laying hens of the Hy-Line Brown line, divided into two rearing systems (intensive and extensive, 60 eggs per group) according to Batkowska and Brodacki (2017), and meat-type breeders (Ross 308 parental stock). The birds came from the same breeder and were fed a balanced diet adjusted for their age and phase of laying. Eggs for analysis were collected from hens at different ages (29, 33, 41, 53 and 70 weeks; 60 eggs/age). Utility types were compared for eggs obtained from birds at the same age (33 wks).

The electronic EQM kit (Egg Quality Measurements, TSS®) was used for the analysis. Egg weight (EW) and egg albumen weight (AW) were determined, and the percentage of albumen in the weight of the whole egg was calculated. The albumen height (AH) was measured, and on this basis the Haugh unit value (HU) was calculated (Wiliams, 1992).

Ten albumen samples were taken from each group, placed individually in sealed containers, and stored for 6 months at -18°C. Then the samples were thawed and the enzymatic activity of lysozyme (LA) was evaluated according to Kijowski and Leśnierowski (1999). Data were analysed using SPSS 20.0 PL statistics software (IBM Corp., 2011). The normality of distribution was assessed using the Kolmogorov-Smirnov test. The statistical significance of differences was verified using Student's t-test (2 categories) and one-way analysis of variance (3 and more categories) by the Tukey post hoc test. Pearson correlation coefficients and the linear regression between egg traits were estimated.

RESULTS

The quality features of egg albumen and the enzymatic activity of lysozyme are presented in Table 1. The tests revealed that the rearing system significantly ($P < 0,05$) affects such qualitative characteristics of chicken eggs as egg weight, albumen weight, and thick albumen height, but does not significantly influence the enzymatic activity of egg albumen lysozyme. In the case of the meat-type breeders, the egg weight and egg albumen weight increased significantly with the age of the birds. However, the thick albumen height decreased by 3,5 mm, the Haugh unit by 39, and lysozyme activity by 10,440 U/ml, with the highest activity observed in eggs collected at 33 weeks of age (45,741 U/ml; $P < 0,05$). Comparison of the quality traits of eggs depending on the utility type of hens revealed that meat-type breeders laid considerably larger eggs than laying-type birds. Eggs from laying hens had higher structural albumen (6,6 mm on average) and higher Haugh unit values (81,5 HU on average). Albumen weight and its proportion of the egg weight were significantly higher in laying-type hens. Lysosyme activity was slightly higher in eggs obtained from meat-type breeders, but the difference (1,580 U/ml) was not confirmed statistically.

Table 2 presents the Pearson correlation coefficients and linear regression between the features analysed in the experiment. Egg weight, albumen height, Haugh unit and albumen weight were significantly dependent on the age of the layers. Albumen weight (AW) was largely dependent ($R = 0,752$; $P < 0,01$) on the egg weight, and the amount of albumen was correlated with the Haugh unit value, which increased by 8,5 with each mm of thick albumen height. The relationships between the percentage of albumen in the egg, Haugh unit, and albumen weight were also significant. With each week of age, the weight of the eggs increased by 0,222 g, accompanied by a decrease in albumen height (0,104 mm) and lysozyme activity (-324,7 U/ml). Significant correlations between lysozyme activity and hens' age ($R = 0,574$; $P < 0,01$), albumen height ($R = 0,423$; $P < 0,01$) and Haugh unit value ($R = 0,459$; $P < 0,01$) were noted as well.

Table 1

Albumen quality traits and enzymatic activity of lysozyme in eggs

Trait	Rearing system of laying hens		Meat-type breeders' age (wks)					UT (at 33 wks)	
	I	E	29	33	41	53	70	Laying hens	Meat-type breeders
EW	51,88*	60,61*	52,33^{a}	60,67^{b}	59,00^{b}	62,17^{b}	63,17^{b}	56,25*	60,67*
AH	5,85*	7,42*	4,50^{b}	6,17^{c}	3,33^{ab}	4,00^{ab}	2,67^{a}	6,64	6,17
HU	77,58*	85,40*	65,17^{cd}	77,67^{d}	48,17^{ab}	57,00^{bc}	38,67^{a}	81,49	77,67
AW	31,09*	39,26*	28,67^{a}	34,00^{ab}	34,17^{ab}	36,67^{b}	36,17^{b}	35,18*	34,00*
AP	59,74*	64,25*	54,33	56,33	58,17	59,00	57,00	62,00*	56,33*
LA	42,574*	45,756*	43,160^{b}	45,741^{b}	45,160^{b}	34,400^{a}	34,740^{a}	44,165	45,741

EW – egg weight (g), AH – albumen height (mm), HU – Haugh unit, AW – albumen weight (g), AP – proportion of albumen in egg weight (%), LA – enzymatic activity of egg albumen lysozyme (U/ml); I – intensive system, E – extensive system, UT – utility type of birds (laying hens vs. meat-type breeders); $^{a,\,b}$Mean values (in row) differ significantly at $P \leq 0,05$; *mean values (in row) differ significantly at $P \leq 0,05$

Table 2

Pearson correlation (R) and linear regression (B) coefficients between analysed features

Trait	Age of birds		EW		AH		HU		AW		AP	
	R	B	R	B	R	B	R	B	R	B	R	B
EW	0,395**	0,222**										
AH	-0,602**	-0,104**	0,050	0,015								
HU	-0,718**	-1,105**	-0,134	-0,367	0,960**	8,529**						
AW	0,122	0,064	0,752**	0,706**	0,318**	0,969**	0,152	0,052				
AP	-0,182	-0,104	0,190	0,194	0,416**	1,379**	0,341**	0,127**	0,785**	0,856**		
LA	-0,574**	-324,7**	-0,190	-191,4	0,423**	1382,3**	0,459**	168,7**	-0,045	-48,00	0,110	108,8

EW – egg weight (g), AH – albumen height (mm), HU – Haugh unit, AW – albumen weight (g), AP – proportion of albumen in egg weight (%), LA – enzymatic activity of egg albumen lysozyme (U/ml); *coefficient significant at $P \leq 0,05$; **coefficient significant at $P \leq 0,01$

DISCUSSION

The quality of eggs may depend on many factors, such as the genotype and the age of the birds, as well as on the environmental and feeding conditions that make up the rearing system (Batkowska and Brodacki, 2017). Diversified technologies for rearing of laying-type hens significantly modify egg characteristics such as their weight, the proportions of individual elements (especially yolks and shells), and albumen height. After conversion to HU, the most favourable albumen features have been noted for free-range eggs, and the least favourable for eggs from the cage system (Minelli et al., 2007; Küçükyılmaz et al., 2012; Batkowska et al., 2016). These relationships are confirmed by our research, as well as by Krawczyk and Gornowicz (2010), who additionally noted improvement in such albumen characteristics as height, Haugh units and lysozyme content in comparison to the litter system. The proportion of lysozyme in the albumen weight was 28% higher in eggs from the free-range system than in eggs from the more intensive system. In contrast, a study by Popova et al. (2020) analysing the effect of rearing systems on egg quality found that eggs from free-range hens had lower weight, a thinner shell, and greater variation in yolk colour and fatty acid profile.

The elements of the rearing system include diet, in which feed additives may be a factor modifying egg quality, as well as lysozyme content and activity. Graszkiewicz et al. (2007) report that enrichment of the standard feed with vitamins with antioxidant properties (A and E) positively influenced the quality of egg albumen, significantly increasing the hydrolytic activity of lysozyme. Another study (Lewko and Gornowicz, 2015) showed that the addition of a properly balanced composition of high-quality dried herbs (sage, mint, and marjoram) in the amount of 5% can significantly improve selected albumen quality parameters (weight, height, and HU) and increase the content and activity of lysozyme in eggs. Feeding birds green fodder had a similar effect.

Another important factor affecting the quality of egg albumen is the genetic origin of birds. According to Lewko and Gornowicz (2009), who analysed the quality of eggs from seven strains of Polish laying hens, i.e. S-55, K-44, K-66, A-88, A-22, M-55 and V-44, egg albumen from A-22 laying hens had the highest concentration (5,14 μg/ml) and hydrolytic activity (108,746 U/ml) of lysozyme. High muramidase parameters justify the use of the A-22 strain to create commercial hybrids of laying hens, whose eggs should contain more lysozyme, which may be of particular importance for their further use in the food industry, pharmacology, and human or veterinary medicine.

The age of laying hens is another factor that strongly influences egg quality. In older birds, there is a deterioration of albumen quality expressed as a reduction in its proportion in the egg weight and in the amount of structural albumen (Silversides and Scott, 2001). There seems to be little literature data on the content and enzymatic activity of lysozyme in eggs of laying hens at different ages. However, since the quality of albumen in eggs deteriorates with the age of birds, it can be inferred that the parameters characterizing the lysozyme in these eggs may deteriorate as well. These tendencies are not confirmed by Banaszewska et al. (2019). The lysozyme activity in the eggs from younger meat-type breeder hens was higher than in eggs from older hens.

The results of our research do not confirm the observations of Fletcher et al. (1983), who found that larger eggs have a higher percentage of albumen and greater albumen height (Nestor and Jaap, 1963), which is indirect evidence of albumen quality. A study conducted by Lewko and Gornowicz (2016) analysing the proportion of lysozyme in individual egg albumen fractions showed that the lightest eggs contained the largest amount of lysozyme, with the highest activity in both the thin and thick albumen (0,61 vs 0,38; 131,105 vs 80,705).

Factors affecting the hydrolytic activity of muramidase include the pH of its environment. In a study by Smolelis and Hartsell (1951), the enzyme was inactivated at higher pH values and lost its properties in an alkaline environment. Lysozyme retained nearly 100% activity after heat treatment at 100°C for 3 min at pH 4,5, while an increase in pH decreased its activity. Phosphate buffer was found to be a factor that stimulates lysozyme activity at higher pH values (Chang et al., 2000). Therefore, apart from the factors considered in this study, the change in the hydrolytic activity of muramidase in stored eggs, in which the pH of the albumen increases over time, may be interesting (Batkowska et al., 2014). The alkaline nature of lysozyme facilitates the formation of complexes with other proteins, including ovomucin. This complex is responsible for the gel structure of the albumen, and its physicochemical state is an indicator of egg freshness. At pH 9,3-9,6, the complex is dissociated and the albumen is thinned. A lack of thick albumen increases yolk motility, which reflects the advancement of ageing processes and deteriorating egg quality.

CONCLUSIONS

The rearing system of laying hens significantly influenced the quality parameters of eggs, i.e. egg weight and albumen quality (weight and height), but did not affect the enzymatic activity of egg albumen lysozyme.

Egg weight and albumen weight increased with the age of hens. However, a deterioration of the quality of the egg albumen (decrease in thick albumen height and Haugh units) was noted, as well as a decrease in the enzymatic activity of lysozyme after the age of 33 weeks.

Among eggs from birds of the same age (33 wks), those from laying hens had a higher egg weight, albumen weight, and proportion of albumen in the egg weight. Other traits (albumen height and Haugh units) were similar. The utility type also had no effect on lysozyme activity.

Among the variables studied, the enzymatic activity of lysozyme was highly negatively correlated with the age of hens, and positively with albumen height and Haugh unit.

An important factor taken into account in analysing the potential use of lysozyme in the food industry is the price of the raw material and improvements in methods of isolating the enzyme. Owing to continual research on lysozyme and methods of its effective modification, its importance in medicine or veterinary medicine may increase.

Conflicts of interest: The authors declare no conflict of interest.

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