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EFFECT OF FEEDING SELECTED STRAINS OF CONSERVED BREED HENS WITH DIETS CONTAINING LEGUMES ON EGG QUALITY AND CONTENT AND ACTIVITY OF LYSOZYME*

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Abstract

The aim of the study was to evaluate the effect of replacing soybean meals with legume grains in diets on laying performance of hens and egg quality, with particular emphasis on egg lysozyme content. The study included 4 breeds/strains of laying hens included in the conservation programme in Poland, i.e.: Green-legged Partridge (Z-11), Yellow-legged Partridge (Ż-33), Rhode Island Red (R-11), and Leghorn (H-22). At 41–56 weeks of age, the hens of each strain were divided into two groups: I (control) − fed *ad libitum* with a standard diet based soybean meal, and II (experimental) − fed *ad libitum* with a diet that contained beans, peas and lupins instead of soybean meal. At 56 weeks of age, 32 eggs were taken at random from each group of hens and each strain, which were subjected to quality assessment. The results of the study confirm the possibility of feeding the above-mentioned hens with diets in which soybeans were replaced with 28% of faba bean, pea, and yellow lupin grains without a significant effect on laying performance, but with a change in some egg quality characteristics. In groups of hens fed a diet without soybean, a decrease in egg and albumen weight, albumen and yolk pH values, and an increase in yolk yellowness were recorded, as well as an increase in Haugh units and air cell height and shell porosity. There was a significant effect of genotype and diet and simultaneously of both factors on the content and activity of lysozyme in the thin and thick albumen. In the experimental groups, the average content and activity of lysozyme in the thin albumen decreased and in the thick albumen increased. Regardless of diet, statistically significant variation in egg internal quality and egg shell quality between hen strains was shown, as well as a correlation between the content and activity of lysozyme in eggs and shell colour.

Key words: feeding of hens, legumes, egg quality, lysozyme, native breeds, laying hens

The European Union's ban on the use of animal meals in animal nutrition has led to an increase in demand for high-protein feed materials of plant origin. In this situation, the main ingredient in compound feeds is genetically modified soybean. Although so far the negative impact of GM soybean on animal organisms has not been confirmed (Świątkiewicz and Arczewska-Włosek, 2011; Świątkiewicz et al., 2014), a law was passed in Poland in 2006 to ban the marketing of feed derived from genetically modified (GM) plants on the territory of the country, the implementation of which is postponed for the time being. At the same time, research is underway into the possibility of using legumes of varieties grown in Poland as components to replace soybean in diets for poultry (Danek-Majewska et al., 2023; Krawczyk et al., 2015; Kubiś et al., 2018; Rutkowski et al., 2015, 2017; Szczurek and Świątkiewicz, 2022; Zduńczyk et al., 2014).

Legumes, compared to soybean meal, contain less protein and fewer essential amino acids (methionine and lysine), and more crude fibre and anti-nutrients. The level of crude protein is 30–40% in lupin and 20–30% in peas and beans (Rutkowski et al., 2015). In view of this situation, work is being carried out to produce legume varieties with favourable composition for use in feed mixtures, especially for poultry. Andrzejewska et al. (2016) point out the favourable fatty acid profile of modern legume varieties, which can improve the quality of poultry products. Dänner (2003) draws attention to modern varieties of faba bean, which can be used up to 30% in feed for laying hens without negatively affecting egg production. A study by Osek et al. (2013) conducted on broiler chickens shows that partial replacement of soybean with legume seeds in diets, allowed for significantly better weight gains and slaughter performance of birds with similar muscling and fatness of carcasses and better sensory characteristics to be obtained compared to chickens fed diet in which soybean meal was the only protein component. The results obtained in studies on the use of

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yellow lupin in the feeding of laying hens are not conclusive, and a study by Ciurescu and Panâ (2017) shows that the addition of appropriate enzymes to the feed is of great importance for the efficiency of replacing soybean with pea seeds. Most studies in this field suggest that there is a real possibility of replacing GM soybean in poultry diets without adversely affecting productivity, but the problem is the ability of European countries to produce sufficient quantities of legumes under temperate climate conditions (Brzóska, 2012).

The subject of research in this area has been high-producing commercial hybrids of laying hens. To date, the results of research on reducing or replacing soybean meal with legumes in diets for native breed laying hens with low laying performance, predisposed to free-range systems of rearing due to better adaptation to harsh environmental conditions, are not known. These hens are recommended for small-scale backyard farming, and the eggs obtained from them show good physicochemical and sensory characteristics (Krawczyk, 2009). Recent studies by Krawczyk et al. (2021) and Lewko et al. (2021) indicate that eggs from native breeds of hens also provide valuable lysozyme, and its level and activity in the albumen depends on the colour of the shells and the age of the layers. It is therefore interesting to determine the effect on lysozyme levels of feeding hens with a diet containing legumes. Feeding these hens a GM soybean-free diet, composed on the basis of domestic feed raw materials, may be an opportunity to promote the resulting eggs and, indirectly, the breeding of native breeds/strains of hens.

The aim of the study was to evaluate the effect of replacing genetically modified soybean meal in diet with legumes on hens' laying performance and egg quality with a special focus on egg lysozyme content.

Material and methods

The study material consisted of 4 breeds/strains of laying hens covered by the conservation programme in Poland: Green-legged Partridge (Z-11), Yellow-legged Partridge (Ż-33), Rhode Island Red (R-11) and Leghorn (H-22). The Z-11 and Z-33 strains are old, native populations of hens, while R-11 and H-22 are locally adapted breeds that were brought to Poland for breeding farms after the Second World War, but were withdrawn from intensive production in the 1970s. Populations of these hens are included in the world's genetic resources under conservation (World Watch List, FAO 2007), and their detailed characteristics and the desirability of conservation are described in detail on the website www.bioroznorodnosc.izoo.krakow.pl/drob/kury (in Polish). The birds were kept on an experimental farm, each strain in 4 replicates of 30 hens each, meaning that a total of 480 hens were included in the study. At 41−56 weeks of age, the hens of each strain were divided into two groups: group I (control) fed *ad libitum* with a standard diet based on soybean meal, and group II (experimental) fed *ad libitum* with a diet that contained beans, peas and lupins instead of soybean meal. The detailed ingredient composition of the experimental diets is included in Table 1, and the results of their chemical analysis performed at the Central Laboratory of the National Research Institute of Animal Production are included in Table 2. Both diets were balanced according to the estimated nutritional value of the ingredients, and in the diet fed to the experimental groups, soybeans were replaced with faba beans, peas, and yellow lupins, accounting for 28% of the ingredient composition (Table 1). As can be seen from Table 2 these were not isoproteic diets; in the diet fed to the birds of the experimental groups without soybeans, the protein level was 1% lower and the crude fibre level was higher compared to the diet containing soybeans.

Table 1. Composition of control and experimental diets (%)

Components, chemical composition	Control diet	Experimental diet
Ground maize	58.7	46.17
Extracted soybean meal	15.0	–
Rapeseed expeller	3.00	4.00
Maize DDGS	7.00	7.00
Faba beans	–	8.00
Peas	–	10.00
Yellow lupin	–	10.00
Lucerne meal	2.00	1.50
Wheat bran	4.00	3.00
Ground limestone	8.00	8.00
Monocalcium phosphate	1.00	1.00
NaCl	0.25	0.25
DL-methionine	0.05	0.08
DJ premix	1.00	1.00
Per 1 kg of feed:		
Crude protein (g)	160	
Metabolizable energy (MJ/kg)	11.2	
Lys (g)	7.00	
Met (g)	3.10	
Ca (g)	34.8	
P available (g)	3.20	

Table 2. Results of chemical analysis of diets (%)

Diet	Dry matter	Crude ash	Crude fat	Crude protein	Crude fibre
Control	92.31	11.63	3.09	15.33	3.15
Experimental	92.16	11.94	3.08	14.33	4.14

The hens were raised on a farm with a size-limited population. They were kept in the microclimate conditions recommended for laying hens, in a litter floor system, at a stocking rate of 5 birds/m². The hens were kept under optimum environmental conditions at 16−20°C and relative humidity of 60−80%. The lighting programme in

the henhouse was the same for all groups and included 16 h of light and 8 h of darkness (16L:8D).

At 56 weeks, 32 eggs (128 eggs in total) were randomly taken from each group of hens of each strain and evaluated for quality. The number of pores per cm^2 of shell (shell porosity) was determined according to Tyler (1953). The shell area on which the pores were counted was 0.25 cm^2. Shell crushing strength (N) was tested with a TA.XT PLUS texture analyser (Stable Micro Systems, Godalming, UK, 2010) fitted with appropriate attachments. 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 colour of the yolk and shell (L*, a*, b* colour space) was determined using a Konica Minolta CM-700d spectrophotometer (Osaka, Japan, 2018). The instrument (Konica Minolta Sensing, Inc. Japan) was calibrated with a white reference plate (L* = 97.10, a* = –4.88, b* = 7.04) before the measurement. Results were recorded as L*, a*, b* colour 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). Air cell height was measured using an ovolux (handheld egg candling lamp) and a millimetre-scale ruler. The EQM system (Technical Services and Supplies Limited, England) was used to measure Haugh units (HU).

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 thin and thick fractions and placed in disposable sterile containers. The albumen samples prepared in this way were used to make preparations to determine the concentration and hydrolytic activity of lysozyme in the albumen fractions. The content and activity of lysozyme in the fresh albumen fractions was determined with spectrophotometry using a spectrophotometer (Metertech SP-830 plus; Nangang, Taipei, Taiwan, 2011), consisting in the use of the 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 one minute decreases the absorbance of a *Micrococcus 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 volume of 2.6 ml (2.5 ml of bacterial suspension + 0.1 ml lysozyme solution) at pH 6.24, in a cuvette with the length of the optical path being 1 cm. 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 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 − absorbance value for bacterial suspension at time t0,

At − absorbance value for bacterial suspension after time t.

Data were processed using Statistica 13.0 software. Mean values for all analysed parameters were used. A two-way model of ANOVA was used to analyse variability (variable 1: diet; variable 2: breeds). The significance of differences was verified using the Tukey test. Interactions between experimental variables were assessed. The significance of differences was adopted at $P \leq 0.05$. Pearson's coefficients of correlation were also calculated between the analysed traits.

Results

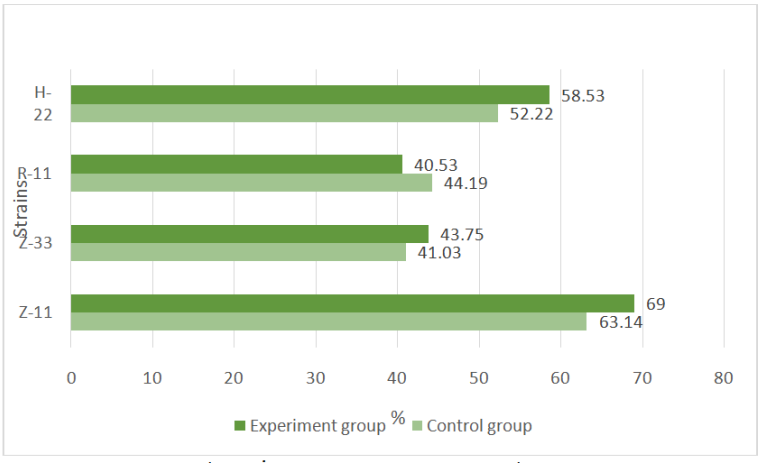
The laying rate of hens of the control groups during the period of the experiment (41−56 weeks) ranged from 41.03 to 63.14%, and in the experimental groups from 40.5 to 69.0% (Figure 1). Regardless of diet, better laying performance was noted in Z-11 and H-22 than in Ż-33 and R-11 hens. Among Z-11 and Ż-33 hens, more eggs were laid in groups fed a diet without soybean meal and with legumes, while the opposite was true among R-11 and H-22 hens. There was a slight variation in the intake of the feed (Figure 2). Yellow-legged Partridge hens consumed 3 g/bird/day more feed mixture compared to the control group, while among Rhode Island Red hens the opposite was true, with 2 g/bird/day higher feed intake found in the control group. Among Green-legged Partridge hens in the experimental group, feed intake was 1 g/bird/day lower than in the control group, while among H-22 hens no differences were found. The average level of daily feed intake per bird in the control and experimental groups was the same, at 145 g.

As can be seen from Table 3, statistically high differences were recorded between hen strains in all interior egg quality traits. The eggs of H-22 hens were characterised by the highest egg weight, yolk weight, and albumen weight as well as by the highest intensity of yolk yellowness (b*) compared to the eggs of the remaining hen breeds ($P<0.01$). On the other hand, eggs of the Green-legged Partridge hens (Z-11) were distinguished by the lowest egg and yolk weight when compared to the eggs of the other breeds and the lowest intensity of yolk redness (a*), but the highest pH levels of yolk and albumen ($P<0.01$). In eggs of the hens of the Yellow-legged Partridge breed (Ż-33), albumen height and the resulting Haugh units were at a significantly lower level than in the eggs of hens of other breeds, with an air cell height that exceeded 3 mm.

Evaluating the average results of all hens, the groups of hens fed the experimental diet compared to the control groups showed a decrease in egg and albumen weight,

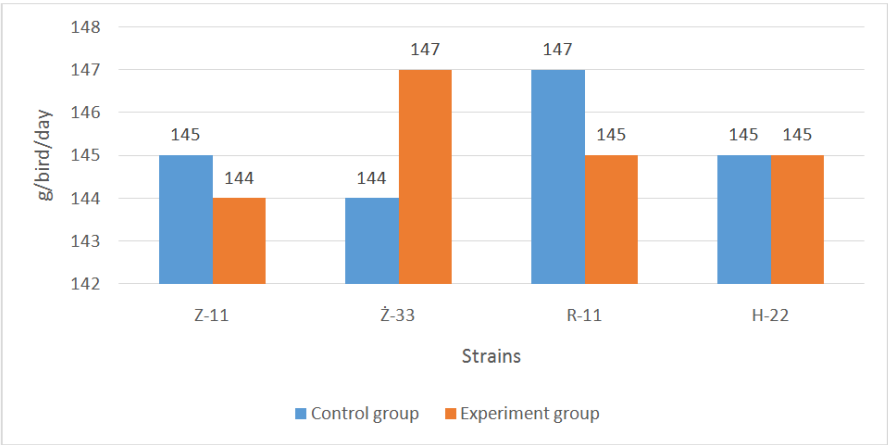
albumen and yolk pH values, and an increase in yolk yellowness (b*), Haugh unit value, and air cell height. The effect of diet on egg quality varied among breeds/strains of hens. In the H-22 strain, feeding hens with a soybean-free diet contributed to a decrease in yolk redness, a decrease in albumen height and Haugh units, and an increase in albumen pH. In the Ż-33 strain, the statistically significant effect of feeding hens a soybean-free diet on the decrease in egg, yolk and albumen weight, and the increase in the intensity of yolk yellowness (b*), and Haugh units and albumen height was confirmed. Among R-11 hens in the experimental group, there was an increase in albumen height and a decrease in albumen pH. In contrast, in the Z-11 strain, there was an increase in both albumen height and Haugh units, and a decrease in pH in both egg albumen and yolk. There was a statistically significant effect of both experimental factors (genotype and diet) simultaneously on such traits as egg, albumen and yolk weight, albumen height and Haugh units, and albumen and yolk pH.

The study found a significant effect of hen genotype on all shell quality traits (Table 4). Eggs of the hens of the Leghorn breed (H-22) were distinguished by the greatest weight and shell thickness as well as the greatest porosity, regardless of diet (P<0.01). The shell colour of these eggs was white and all different in terms of all values (L*, a*, b*) in comparison to the other eggs. The shell crushing strength of eggs from hens of the different breeds varied from 32.7 to 40.3 N, but these differences were not confirmed statistically. In contrast, the effect of diet on shell quality was lower, and was statistically confirmed only in terms of the increase in the experimental group of shell porosity and colour changes in this group consisting of a decrease towards red (a*) and yellow (b*) and an increase towards lightness (L*). The effect of hen strain and diet on shell weight and thickness was noted simultaneously.



Z-11 − Green-legged Partridge; Ż-33 − Yellow-legged Partridge; R-11 − Rhode Island Red; H-22 − Leghorn.

Figure 1. Laying performance of hens at 41−56 weeks (%)



Z-11 − Green-legged Partridge; Ż-33 − Yellow-legged Partridge; R-11 − Rhode Island Red; H-22 − Leghorn.

Figure 2. Feed intake at 41−56 weeks (g/bird/day)

Table 3. Effect of genotype and diet on selected egg quality traits

Item	Group	H-22	Ż-33	R-11	Z-11	Mean	SEM	Effect (P value) of:		
								strain (R)	group (G)	R × G
Egg shape index (%)	Control	74.9	75.4	73.3	73.8	74.35				
	Experimental	75.7 A	75.9 A	72.8 B	73.5 B	74.47	0.191	<0.01	0.945	0.114
Egg weight (g)	Control	65.4 A	60.5 xxB	61.4 Bc	58.2 Bd	61.7				
	Experimental	65.2 AD	55.1 xxBC	61.4 BDe	58.2 BDf	59.9	0.358	<0.01	0.022	<0.01
Yolk weight (g)	Control	19.6 A	20.0 xxC	19.3	17.8 BD	19.2				
	Experimental	19.8 A	18.8 xxB	19.2 C	17.9 BD	18.9	0.107	<0.01	0.160	0.039
Yolk colour (pts)	Control L*	50.6	49.3	49.1	50.1	49.8				
	Experimental L*	51.6 A	49.3 B	48.6 B	50.1	49.9	0.188	<0.01	0.730	0.518
	Control a*	17.4 x	17.2	16.6	15.6	16.7				
	Experimental a*	16.9 xB	19.0 A	17.7	15.9 B	17.4	0.212	<0.01	0.090	0.234
	Control b*	39.7 ADF	29.6 xxBCF	32.3 BDE	30.4 BDF	33.0				
	Experimental b*	40.4 ADF	39.7 xxBCF	35.1 BDE	33.1 BDF	37.1	0.686	<0.01	<0.01	0.050
Albumen height (mm)	Control	7.42 xxA	4.95 xxB	6.84 xxBCE	4.99 xxBD	6.05				
	Experimental	6.19 xxAF	5.45 xxBCF	7.16 xxBDE	6.18 xxDE	6.24	0.076	<0.01	0.057	<0.01
Haugh units (HU)	Control	84.6 xxA	67.9 xxBC	82.0 DE	68.9 xxBF	75.8				
	Experimental	76.0 xxAF	74.0 xxCF	83.1 BDE	78.4 xxDF	77.9	0.539	<0.01	<0.01	<0.01
Albumen pH	Control	7.71 xxAa	8.05 BC	7.79 xxDE	8.10 xxBF	7.91				
	Experimental	8.00 xxA	8.05 CF	7.54 xxBDE	7.90 xxBDF	7.87	0.015	<0.01	0.030	<0.01
Albumen weight (g)	Control	39.7 ADF	35.0 xxBC	37.5 BDE	35.2 BF	36.8				
	Experimental	39.4 A	31.4 xxBC	36.8 BD	35.0 BD	36.6	0.310	0.253	0.021	0.028
Yolk pH	Control	6.00 AFH	6.07 BCF	6.17 BDE	6.36 xxBDF	6.15				
	Experimental	5.95 ADFH	6.04 BCFH	6.14 BDEH	6.28 xxBDFG	6.10	0.011	<0.01	<0.01	0.599
Air cell depth (mm)	Control	3.07 ADFH	3.32 BCFH	2.93 BDEH	2.93 BDFG	3.06				
	Experimental	3.35 ADFH	3.56 BCFH	2.77 BDEH	3.01 BDFG	3.17	0.018	<0.01	<0.01	<0.01

Z-11 − Green-legged Partridge; Ż-33 − Yellow-legged Partridge; R-11 − Rhode Island Red; H-22 − Leghorn.
Values in columns within each strain with different letters differ significantly: xx for P<0.01; x for P<0.05.
Values in rows within each strain with different letters differ significantly: A, B, C, D, E, F, G, H for P<0.01, c, d, e, f for P<0.05.

Table 4. Effect of genotype and diet on selected eggshell quality traits

Item	Group	H-22	Ż-33	R-11	Z-11	Mean	SEM	Effect (P value) of:		
								strain (R)	group (G)	R × G
Shell weight (g)	Control	6.05 A	5.42 xxB	5.53 BC	5.12 BD	5.53				
	Experimental	5.96 ADFH	4.89 xxBCFH	5.38 BDE	5.35 BDG	5.40	0.042	<0.01	0.061	<0.01
Shell thickness (μm)	Control	358 ADF	331 BC	340 BE	325 xxBF	338				
	Experimental	352 ADF	321 BCH	330 BEH	346 xxDFG	337	1.916	<0.01	0.783	<0.01
Shell strength (N)	Control	38.3	34.9	39.8	32.7	36.4				
	Experimental	40.3	36.4	38.9	35.9	37.9	0.613	<0.01	0.226	0.679
Shell colour (pts)	Control L*	92.8 ADFH	83.4 BCFH	75.1 xx BDEH	86.9 BDFG	84.5				
	Experimental L*	93.1 ADFH	84.6 BCFH	78.0 xxBDEH	87.9 BDFG	85.9	0.417	<0.01	<0.01	0.027
	Control a*	–1.05 ADFH	3.31 BCFH	8.24 BDEH	0.63 BDFG	2.78				
	Experimental a*	–1.09 ADFH	2.49 BCFH	7.01 BDEH	0.07 BDFG	2.12	0.232	<0.01	<0.01	0.124
	Control b*	3.58 ADFH	15.7 BCFH	19.7 BDEH	13.3 BDFG	13.1				
	Experimental b*	3.12 ADFH	14.5 BCFH	19.3 BDEH	11.9 BDFG	12.2	0.416	<0.01	<0.01	0.584
Shell porosity (no. of pores/cm^2)	Control	45.7 ADH	36.4 BCFH	45.1 DEH	41.0 BDFG	42.0				
	Experimental	49.7 AB	37.2 BCFH	48.7 DEG	41.7 BDFH	44.3	0.409	<0.01	<0.01	0.060

Z-11 − Green-legged Partridge; Ż-33 − Yellow-legged Partridge; R-11 − Rhode Island Red; H-22 − Leghorn.
Values in columns within each strain with different letters differ significantly: xx for P<0.01; x for P<0.05.
Values in rows within each strain with different letters differ significantly: A, B, C, D, E, F, G, H for P<0.01.

Table 5. Effect of genotype and diet on the content and activity of lysozyme in egg white

Item	Group	H-22	Ż-33	R-11	Z-11	Mean	SEM strain (R)	Effect of:		
								group (G)	R × G	(P value)
Lysozyme content in thin albumen (%)	K	0.305 XXAH	0.313 xCH	0.307 xxEH	0.227 xxBDFG	0.288				
	D	0.218 XXBDFG	0.332 xBDEH	0.249 xxBCFH	0.313 xxADF	0.278	0.003	<0.01	<0.01	<0.01
	mean	0.261	0.322	0.278	0.270	0.253				
Lysozyme content in thick albumen (%)	K	0.166 EH	0.172 xxADE	0.156 xxFBCH	0.171 xxDEG	0.166				
	D	0.173 ADF	0.224 xxBCF	0.191 xxBDEH	0.237 xxBFG	0.206	0.002	<0.01	<0.01	<0.01
	mean	0.169	0.198	0.173	0.204	0.186				
Lysozyme activity in thin albumen (U/ml)	K	64818 xxAD	65314 xD	66589 xxD	48242 xxBC	61241				
	D	46329 xxADFH	52988 xBDFH	70485 xxBCFG	66518 xxEDH	59080	692.0	<0.01	<0.01	<0.01
	mean	55573	59151	68537	57380	60160				
Lysozyme activity in thick albumen (U/ml)	K	35420	36553 xa	33223 xxbD	36482 xxC	35420				
	D	36766 ADFH	47746 xBCF	40520 xxBDEH	50367 xxBFG	43849	499.3	<0.01	<0.01	<0.01
	mean	36093	42149	36871	43424	39634				

Z-11 − Green-legged Partridge; Ż-33 – Yellow-legged Partridge; R-11 − Rhode Island Red; H-22 − Leghorn.
Values in columns within each strain with different letters differ significantly: xx for P<0.01; x for P<0.05.
Values in rows within each strain with different letters differ significantly: A, B, C, D, E, F, G, H for P<0.01.

Table 6. Correlations between selected egg quality traits and lysozyme content in eggs of 56-week-old hens of 4 conservation strains

Item	Lysozyme content (%)	
	thin albumen	thick albumen
Egg weight (g)	–0.153*	–0.305*
Albumen height (mm)	0.011	–0.096
Albumen pH	0.221	0.114
Yolk pH	–0.071	0.104
Albumen weight (g)	–0.172*	–0.263*
Yolk colour: L*	–0.110	–0.068
a*	0.054	0.057
b*	0.180*	0.014
Shell colour: L*	–0.155*	0.082
a*	0.125	–0.144*
b*	0.179*	0.034
Shell weight (g)	–0.116	0.232*
Shell thickness (μm)	–0.050	–0.078
Shell porosity (no. of pores/cm^2)	–0.407*	–0.209*
Air cell depth (mm)	0.210*	0.175*

*Significant correlation.

Significant effects of genotype and diet and simultaneously of both factors on the content and activity of lysozyme in the thin and thick albumen of eggs were recorded (Table 5). The greatest content of lysozyme in the thin albumen of eggs was noticed in the eggs of the hens of the Ż-33 breed, and in the thick albumen of the eggs of the hens of the Z-11 and Ż-33 breeds. Additionally, the highest activity of lysozyme in the thin and thick albumen was noted in the eggs of the hens of the Ż-33 and Z-11 breeds. Among the eggs of the experimental group, the greatest activity of lysozyme was noted in the thin albumen of the eggs of the R-11 hens. In all four experimental groups, the average content and activity of lysozyme in the thin albumen decreased and in the thick albumen increased. Analysing the formation of this trait in individual strains, it was found that in the thin albumen of eggs of H-22 and R-11 hens there was a decrease and in strains Ż-33 and Z-11, while there was an increase in the content of lysozyme in groups fed a diet without soybean. On the other hand, increased lysozyme activity in the experimental groups (soybean-free diet) in the thin albumen was found in the R-11 and Z-11 strains. The lowest content and activity of lysozyme was found in the white-shelled eggs of the H-22 strain.

A significant negative correlation was found between lysozyme levels and egg weight and albumen weight and shell porosity in the thin albumen of the eggs studied (Table 6). A higher content of lysozyme in the thin albumen was observed in eggs with higher redness (b*) of shell and yolk colour and higher air cell height. The lighter the colour of the shell, the less lysozyme in the thin albumen, but this relationship was not confirmed for the thick albumen. The lower the egg weight and albumen weight and the higher the shell weight and porosity, the more lysozyme was found in the thick albumen (Table 6).

Discussion

In our research, faba beans, peas, and yellow lupins together accounted for 28% of the diet to hens in the experimental groups. This ingredient composition made it possible to obtain isoproteic and isocaloric diets in both groups. On the other hand, chemical analysis showed a 1% lower level of crude protein in the diet fed to hens in the experimental groups compared to the control groups, and the results in this regard were similar to the work of Rutkowski et al. (2015). The authors of this study found it possible to completely replace soybeans with yellow lupin seeds, without harming production performance and egg quality, but only when its level in the diet does not exceed 20%. A later study by Rutkowski et al. (2017) found that as the lupin content of hens' diets increases, egg quality deteriorates. Drażbo et al. (2014) found that blue lupin seeds added to laying hens' feed at 20% had a positive effect on improving yolk colour and fatty acid profile, without reducing laying performance. In contrast, Dänner (2003) indicates that up to 30% of faba bean can be added in feed for laying hens without compromising productivity.

The presented results of studies on hen strains with low laying performance demonstrated no clear effect of diet on laying performance of particular breeds/strains. Similar results were obtained by Krawczyk et al. (2015), who showed that the addition of up to 300 g/kg of yellow lupin to the feed mixture did not impair laying performance, physicochemical and sensory properties of eggs. As in the study of Drażbo et al. (2014), lower or the same feed intake was found among hens (except for Ż-33) fed soybean-free diet, suggesting the possibility of fully replacing soybean meal with proposed legume composition.

The study noted the effect of legume-containing diets on lowering egg and albumen weight and increasing shell porosity, as well as a tendency to reduce their crushing strength, and these results are consistent with those obtained by Rutkowski et al. (2017) and Kubiś et al. (2018) in a study on the use of lupin seeds. According to Rutkowski et al. (2015), increased yellow lupin seed content in diets fed to hens decreased egg shell quality (decrease in shell thickness, strength, and proportion in egg weight), among other factors. On the other hand, this study reported a positive effect of lupin on the increase of albumen height, Haugh units, albumen pH, and improvement of yolk colour. In the presented research results, similar relationships were observed for the same traits with the exception of albumen pH, as the value of this trait in eggs of the R-11 and Z-11 strains in the experimental groups decreased. Kubiś et al. (2018) noted that with an increase in the proportion of white lupin meal in the feed, intestinal viscosity increases and the digestibility of the

nutrients deteriorates, which worsens the production performance of laying hens. Krawczyk et al. (2015) suggest that decreased egg production and reduced egg weight in groups fed diets in which soybean meal is replaced with legume seeds may be correlated with the amount of indigestible compounds. The adverse effects of feeding hens with diets in which soybean is replaced with legumes can be effectively reduced by appropriately selected feed enzymes. According to a study by Ciurescu and Panâ (2017), the addition of NSP-hydrolizing enzymes to the diet improved production performance and yolk colour, without compromising other egg quality traits.

The content and enzymatic activity of lysozyme in both thin and thick albumen in the results of our study were comparable to the findings of Krawczyk et al. (2021), but at a much lower level than those found by Kowalska et al. (2020) in the eggs of Hy-Line Brown commercial hens, regardless of their diet. According to a study by Banaszewska et al. (2019), the level of lysozyme activity in the eggs of Ross 308 broilers is even lower compared to the results we obtained for eggs of native breed hens with low laying performance. On the other hand, it is interesting that in the presented research results the level of lysozyme in egg albumen was significantly influenced by both hen genotype and diet, and by both experimental factors simultaneously. A study by Kowalska et al. (2020) shows that feeding laying hens a diet in which soybean meal was completely replaced by legumes contributed to a significant increase in the content and activity of lysozyme in the thin albumen, while in our study a similar relationship was found in the eggs of the old, native breed of Green-legged Partridge hens. In the other strains of hens studied, an inverse relationship was observed in this regard and a different level of influence of diet on the content and activity of lysozyme in egg albumen. Gvoždíková Javůrková et al. (2019) and Lewko et al. (2021), on the basis of their studies, point out that there is also a high correlation between the colour of eggshells and the content of lysozyme in the shells, which was also confirmed by the results of our experiment, in which the least amount of lysozyme was found in white-shelled eggs of strain H-22, and more in those with cream and light brown shells (Z-11, Ż-33 and R-11). In studies by Myint et al. (2012) and Kinoshita et al. (2016) in birds of commercial lines, different antimicrobial potentials (including, among others, lysozyme levels) were observed in birds of commercial lines as a result of genetic work and selection carried out on nucleus farms to improve the biological value of hatching eggs. This seems to be the main reason for the low levels of lysozyme in the eggs studied, since in small populations of native breeds of hens, work on improving egg productivity and quality has not been carried out for many years. A study by Gvoždíková Javůrková et al. (2019) performed on the eggs of 41 amateur and conserved breeds of hens shows that in hens of native free-ranging breeds, the levels of lysozyme and ovotransferrin in egg whites are strongly influenced by climatic conditions (temperature, humidity), genotype, diet, and the year of egg collection. Myint et al. (2012) and Kinoshita et al. (2016) suggest that in native populations and among free-living birds, the levels of various valuable components of eggs show a high dependence on their quality traits, which was also confirmed by the results of our current and previous studies (Krawczyk et al., 2021).

Alkalization of the environment has a major impact on the structure of the albumen, and the maintenance of its gel-like structure is determined by the existence of an interaction between lysozyme and ovomucin. There is no consensus in the literature on the relationship between the pH value of the albumen and the activity of lysozyme. According to Trziszka et al. (2007), this relationship is most stable at a pH of about 8.0, but Salejda and Krasnowska (2014) showed that the enzymatic stability of lysozyme activity is maintained at a pH of 5.3−6.0. In our study, the average albumen pH value was 7.71−8.10 and was similar to the results of similar studies of eggs from different breeds of hens (Trziszka et al., 2007; Rizzi and Marangon, 2012), but the enzymatic activity in both thin and thick albumen was at a much lower level. A similar relationship was found in our earlier study of eggs obtained from native hens of different breeds at different ages (Krawczyk et al., 2021).

Conclusions

The results of the study confirm the possibility of feeding hens of breeds/strains under the conservation programme with a compound feed in which soybean meal was replaced by 28% of beans, peas, and yellow lupins, without significantly affecting productivity, but with a change in some egg quality traits.

In the groups of hens fed the experimental diet (without soybean meal), there was a decrease in egg and albumen weight, albumen and yolk pH values, and an increase in yolk yellowness, an increase in Haugh units and air cell height and shell porosity.

A significant effect of genotype and diet and simultaneously of both factors on the content and activity of lysozyme in the thin and thick albumen of eggs was observed, with the average content and activity of lysozyme in the thin albumen decreasing and in the thick albumen increasing in the experimental groups.

Regardless of diet, statistically significant variation in the interior egg and shell quality of eggs between hen breeds was demonstrated while finding a correlation between the content and activity of lysozyme in eggs and shell colour.

References

Andrzejewska J., Ignaczak S., Barzyk P. (2016). Oil content and fatty acid profile in seeds of Polish breeding lines and cultivars of legumes. Acta Sci. Polon. Agricult., 15: 3–13

Banaszewska D., Biesiada-Drzazga B., Ostrowski D., Drabik K., Batkowska J. (2019). The impact of breeder age on egg quality and lysozyme activity. Turkish J. Anim. Sci., 43: 583–589.

Brzóska F. (2012). Zapotrzebowanie na białko paszowe a zakaz stosowania białka GMO w żywieniu zwierząt w Polsce (in Polish). Pol. Drob., 12: 7–13

Ciurescu G., Panâ C.O. (2017). Effect of dietary untreated field pea (*Pisum sativum* L.) as substitute for soybean meal and enzymes supplementation on egg production and quality of laying hens. Roman. Biotechnol. Lett., 22: 12204–12213.

Danek-Majewska A., Kwiecień M., Samolińska W., Winiarska-Mieczan A., Kiczorowska, B. (2023). Effect of soybean meal substitution with raw chickpea (*Cicer arietinum* L.) seeds on growth performance, selected carcass traits, blood parameters, and bone quality in male broilers. Ann. Anim. Sci., 23: 141–154.

Dänner E.E. (2003). Use of low-vicin/convicin faba beans (*Vicia faba*) in laying hens. Arch. Geflugelkd., 67: 249–252.

Drażbo A., Mikulski D., Zduńczyk Z., Szmatowicz B., Rutkowski A., Jankowski J. (2014). Fatty acid composition, physicochemical and sensory properties of eggs from laying hens fed diets containing blue lupine seeds. Europ. Poult. Sci., 78.

FAO (2007). The state of the world's animal genetic resources for food and agriculture. Roma (Italy) (http:www.fao.org/docrep/010/a1250e/a1250e00.htm).

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.

Kinoshita K. ,Myint S. L., Shimogiri T., Ibrahim H. R., Kawabe K., Okamoto S., Lee Y. P., Matsuda Y., Marda Y. (2016). Chicken ovotransferrin variants OTFB and OTFC harboring substitution of GAT (Asp) to AAT (Asn) in the codon 500 and their antimicrobial activity. J. Poultry Sci., 53: 257–263.

Kowalska E., Kucharska-Gaca J., Kuźniacka J., Lewko L., Gornowicz E., Biesek J. Adamski M. (2020). Quality of eggs, concentration of lyzosyme in albumen and fatty acids in yolk in relation to blue lupin-rich diet and production cycle. Animals, 10: 735.

Krawczyk J. (2009). Optymalizacja warunków utrzymania kur i jej wpływ na produkcyjność, jakość jaj oraz efektywność ekonomiczną chowu niosek (in Polish). Rocz. Nauk. Zoot. Monogr. Rozpr., 100 pp.

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.

Krawczyk M., Przywitowski M., Mikulski D. (2015). Effect of yellow lupine (*L. luteus*) on the egg yolk fatty acid profile, the physicochemical and sensory properties of eggs, and laying hen performance. Poultry Sci., 94: 1360–1367.

Kubiś M., Kaczmarek S. Nowaczewski S., Adamski M., Hejdysz M, Rutkowski A. (2018). Influence of graded inclusion of white lupin (*Lupinus albus*) meal on performance, nutrient digestibility and ileal viscosity of laying hens. British Poultry Sci., 59: 477–484

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 biodiversity conservation programme. Poultry Sci., 100: 100863.

Myint S. L., Kinoshita K., Shimogiri T., Ibrahim H.R., Tsusaki T., Tanoue T., Kawabe K., Maeda Y., Okamoto S. (2012). Effect of polymorphism in egg white lysozyme on muramidase and antibacterial activities as well as hatchability in the Japanese quail (*Coturnix japonica*). J. Anim. Sci., 90: 1747–1755.

Osek M., Milczarek A., Klocek B., Turyk Z., Jakubowska K. (2013). Effectiveness of mixtures with the fabaceae seeds in broiler chicken feeding. Annales Univ. M.Curie-Skłodowska, Lublin, V.XXXI (4): 77–86.

Rizzi C., Marangon A. (2012). Quality of organic eggs of hybrid and Italian breed hens. Poultry Sci., 91: 2330–2340.

Rutkowski A., Kaczmarek S., Hejdysz M., Nowaczewski S., Jamroz D. (2015). Concentrates made from legume seeds (*Lupinus angustifolius*, *Lupinus luteus* and *Pisum sativum*) and rapeseed meal as protein sources in laying hen diets. Ann. Anim. Sci., 15: 129–142.

Rutkowski A., Hejdysz M., Kaczmarek S., Adamski M., Nowaczewski S., Jamroz D. (2017). The effect of addition of yellow lupin seeds (*Lupinus luteus* L.) to laying hen diets on performance and egg quality parameters. J. Anim. Feed Sci., 26: 247–256.

Salejda A.M., Krasnowska G. (2014). Bioaktywne składniki jaja kurzego – możliwości aplikacyjne w biokonserwacji mięsa oraz jego przetworów (in Polish). Bromat. Chem. Toksykol. XLVII: 72–81.

Szczurek W., Świątkiewicz S. (2022). Effects of broiler chicken age and dietary protease on the standardised ileal digestibility of amino acids in seeds from two lupin species. Ann. Anim. Sci., 22: 1351–1362.

Świątkiewicz S., Arczewska-Włosek A. (2011). Prospects for the use of genetically modified crops with improved nutritional properties as feed materials in poultry nutrition. World. Poultry Sci. J., 67: 631–642.

Swiatkiewicz S., Swiatkiewicz M., Arczewska-Wlosek A., Jozefiak, D. (2014). Genetically modified feeds and their effect on the metabolic parameters of food-producing animals: A review of recent studies. Anim. Feed Sci. Technol., 198: 1–19.

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. Pol. J. Food Nutr. Sci., 57: 583–586.

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

www.bioroznorodnosc.izoo.krakow.pl/drob/kury

Zduńczyk Z., Jankowski J., Rutkowski A., Sosnowska E., Drażbo A., Zduńczyk P., Juskiewicz J. (2014). The composition and enzymatic activity of gut microbiota in laying hens fed diets supplemented with blue lupine seeds. Anim Feed Sci. Technol., 191: 57–66.

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