EFFECT OF LAYING HENS AGE AND HOUSING SYSTEM ON PHYSICOCHEMICAL CHARACTERISTICS OF EGGS*

Sebastian Nowaczewski¹*, Lidia Lewko², Marta Kucharczyk¹,
Kinga Stuper-Szablewska⁴, Magdalena Rudzińska⁵, Renata Cegielska-Radziejewska³, Agata Biadala³,
Karolina Szulc¹, Łukasz Tomczyk³, Sebastian Kaczmarek⁶, Marcin Hejdysz¹, Tomasz Szablewski³,

¹Department of Animal Breeding and Product Quality Assessment, Poznan University of Life Sciences, 60-637 Poznań, Poland

²National Research Institute of Animal Production, Experimental Station Koluda Wielka, Waterfowl Genetic Resources Station in Dworzyska, 62-035 Kórnik, Poland

³Department of Food Quality and Safety Management, Poznan University of Life Sciences, 60-637 Poznań, Poland

⁴Department of Chemistry, Poznan University of Life Sciences, 60-637 Poznań, Poland

⁵Department of Food Technology of Plant Origin, Poznan University of Life Sciences, 60-637 Poznań, Poland

⁶Department of Animal Nutrition, Poznan University of Life Sciences, 60-637 Poznań, Poland

*Corresponding author: sebastian.nowaczewski@up.poznan.pl

Abstract

In this study, the objective was to evaluate the effect of the age of Hy-line Brown hens – 44 and 60 weeks of age (26 and 42 weeks of laying) and housing system (enriched cages and litter) on the quality of eggs. According to our results, eggs with a better quality of albumen were obtained from younger hens than from older hens. Moreover, older hens produced eggs with inferior quality related to shell parameters, such as greater elastic deformation, poorer breaking strength, and thinner shells. However, hens kept in a litter housing system laid eggs with superior shell quality characteristics of less flexible and greater breaking strength, even though the thickness, weight, and percentage were similar in both housing systems. In addition, a higher amount and activity of lysozyme were obtained from eggs laid by hens kept in the litter housing system than those obtained from hens kept in cages. The maintenance system and age of birds also had a significant ($P \leq 0.05$) effect on the basic chemical composition of eggs, as well as on the proportion of individual elements and fatty acid profile. Taking these results into account, we can conclude that the more valuable characteristics from the morphological and nutritional point of view were observed in eggs obtained from younger Hy-line hens kept in the litter housing system.

Key words: laying hen, housing system, age, egg quality, biochemical traits

The qualitative traits of eggs, which are characterized by high variability, are conditioned by many factors including the genotype, physiological and physical con-

*Source of research financing: work financed from statutory activity, project no. 506.569.04.00.

dition of birds, nutritional status, storage condition and age of the bird in addition to the environment and the housing system they are kept in (Ahn *et al.*, 1999; Suk and Park, 2001; Tauson, 2005; Basmacioğlu and Ergül, 2005; Czaja and Gornowicz, 2006; Bozkurt and Tekerli, 2009).

The various housing systems of laying hens still raise disputes among producers and consumers. Previous studies have focused on determination of the effect of various housing systems on the birds' health and welfare, as well as on the quality of the obtained products (eggs and meat). These studies show that poultry rearing and maintenance systems affect behavior, performance, and health of the birds, as well as affect quality of eggs directly and indirectly (Van den Brand *et al.*, 2004; Hidalgo *et al.*, 2008; Biesiada-Drzazga and Janocha, 2009; Holt *et al.*, 2011).

Currently, the production of table eggs in the European Union is chiefly based on the enriched cage housing system. It has been shown that it is the most economical form of production of eggs. According to Biesiada-Drzazga and Janocha (2009), the cage housing system has many advantages over the litter housing system, because it allows increased numbers of birds per unit area (up to 50 birds/m², which is dependent on number of cage rows vs. approximately 9 laying hens per m² in alternative systems), and increased profit per egg. In addition, good hygienic conditions of the cage housing system, for example via collection and removal of manure from this housing system, cleanliness of the egg transporting system and of the drinking water and feeding equipment are very important in obtaining good quality eggs. The caged housing system also protects birds from predators and ensures controlled environmental conditions. However, it should be remembered that any caged housing system in table egg production is very strongly criticized. Public opinion is putting more and more pressure on egg producers to limit or cease use of this hen housing system. This is even reflected in the gradual withdrawal of cage eggs in many stores. Therefore, it seems that the litter housing system together with free range or ecological production will be soon the only possible solution.

It is well known that the quality of eggs becomes poorer with increasing age of hens. Deterioration of the eggshell quality may affect the appearance of eggs, which might generate real losses in production due to increased damage. For example, Yilmaz and Bozkurt (2009) observed a lower shell thickness, shell strength and density of eggs obtained from laying hens aged 80 weeks than for those obtained from the hens at the peak of the laying period (28th week of age).

As mentioned earlier, consumers are increasingly interested in the quality of eggs and housing systems which generate greater welfare. Therefore, in times of high competition, the aim is to create a high-quality and safe product (e.g. eggs with low levels of LDL in their yolk). This can be achieved by creating optimal environmental conditions and ensuring proper veterinary prophylaxis, as well as providing feeds that ensure the supply of nutrients favoring high production, and guaranteeing good quality eggs.

Considering the aforementioned reasons and the fact that there is continuous genetic improvement of birds, as well as the simultaneous effect of many environmental factors on the quality of the eggs in repeatedly different and often forced conditions (e.g., the obligatory introduction of new, enriched cages in the EU from

2012), it seems that research in this area should be conducted periodically, and often experiments should be repeated on various farms and at different latitudes.

Therefore, the aim of this study was to compare egg quality of 44 and 60 week old hens, kept in enriched cages and a litter housing system.

Material and methods

The experimental material consisted of eggs obtained from Hy-line Brown laying hens at two stages of the first year of egg production, i.e. 44th and 60th week of age (26th and 42nd week of laying). Birds were kept in two different housing systems (cages and litter) on the same commercial farm located in Wielkopolska, Poland.

Housing systems

Twenty thousand laying hens in 3-level cage batteries were kept in one henhouse. Birds were kept in cages of a new type (enriched), which in accordance with the Directive 1999/74/EC (19 July 1999) took effect for cage housing of hens in Poland from 1 January 2012. There were 48 birds per cage, each with an area of 3.6 m². Stocking density was 750 cm² per hen. Each cage was equipped with a nest, perches, bedding, nipple drinkers, and material to wipe claws. A chain feeding system was installed throughout the henhouse.

Table 1. Composition of diet for laying hens (in % unless specified)

Components per kg	18–31 weeks of age	32–60 weeks of age
Metabolizable energy (kcal/kg)	2 760	2 720
Crude protein	16.5	16
Crude fibre	4	4.5
Crude ash	12.2	12.6
Crude fat	4.4	4.5
Methionine	0.44	0.4
Lysine	0.88	0.79
Ca	3.6	3.8
P available	0.55	0.5
Na	1.6	1.5
Vitamin A (IU/kg)	11 000	11 000
Vitamin D ₃ (IU/kg)	2 500	2 500
Vitamin E (mg/kg)	15	15

Approximately seven thousand laying hens were kept in one henhouse in the floor housing system. A rye straw was used as a litter. Stocking density in the litter housing system was 8 birds per m². The number of hens per nest was 5.

Birds in both housing systems were kept in the same environmental conditions. We used artificial light with a lighting program of 16 h light and 8 h darkness. The temperature in the henhouse fluctuated around 20°C. Feeding of birds was also identical in both housing systems and was based on a complete diet. Table 1 shows the compositions of the mixtures.

Egg quality parameters

A total of 360 eggs (90 eggs in each housing system per flock age) were evaluated. In the cage system, eggs were randomly selected from one row of cages (in the middle in a vertical orientation), both at the beginning, in the middle and at the end of the henhouse (horizontal orientation). In case of the litter, eggs were randomly chosen from entire stock from nests located at the beginning, in the middle and at the end of the henhouse. Before testing, eggs were stored for 1 day under standard microclimatic conditions (about 17°C and 70–75% relative humidity). At first, all eggs were used to assess the morphological and physical characteristics, then their yolks and albumen were assigned to evaluate the biochemical parameters.

Before assessing the parameters of egg quality, each egg was weighed. The following egg quality traits were evaluated:

1. Weight of egg (g), albumen, yolk, and shell (0.01 g accuracy) with the assistance of a WPS 360C type balance;
2. Percentage quantity of the albumen, yolk, and eggshell in the egg mass;
3. Egg shape index (%) by measuring egg's length and width (slide calipers, with 0.01 mm accuracy);
4. Albumen quality parameters:
 - albumen index (%)
 - Haugh units (points) were calculated according to the formula (Haugh, 1937):
$$JH = 100 \log (H + 7.7 - 1.7 W^{0.37}),$$
where:
 H – thick white height, evaluated using a three-legged micrometer screw;
 W – egg weight;
5. Yolk quality parameters – index (%) according to the method described by Funk (1948);
6. Yolk color (pcs.) according to La Roche scale. In order to eliminate the subjectivity of assessment of yolk color, this test was always performed by the same person in the same artificial lighting conditions;
7. Eggshell quality parameters:
 - Thickness (μm) with the shell membranes at the blunt and sharp ends, as well as at the equator part of the egg, with an accuracy of 1 μm was determined by using a screw micrometer (Mitutoyo);
 - The color of the eggshell (% of white) was measured using a nephelometer [Unigalvo (Corning-ELL)] scaled from 0 to 100%;
 - Examination of the elastic deformation and the breaking strength of the eggshell was measured using a TA.XT Plus Texture Analyzer (Stable Micro Systems) with a set of suitable starters;

- Elastic deformation (μm) was measured with an accuracy of $0.1 \mu\text{m}$, in three-point measurements of eggs after the application of three different loads: 0.50 kg, 1.00 kg, and 1.50 kg. This study allowed for the determination of the degree of elastic deformation of the eggshell (μm) under the influence of the applied pressure;
 - To analyze the breaking strength of the eggshell, the pressure was applied gradually until the shell cracked. This measurement allowed for the definition of the force (N) needed to break, crush, or puncture the shell;
8. Chemical composition of the eggs such as water content, crude protein, fat, and ash content was analyzed. Different methods were used to analyze each of the aforementioned chemical components: water was measured using the drier method at 105°C (PN-A-86509: 1994); protein was measured using the Kjeldahl method ($\text{N} \times 6.25$) (PN-A-04018: 1975/Az3: 2002); fat content was measured using the Soxhlet method (PN-A-86509: 1994); and ash content was determined by burning the sample in the muffle furnace at 600°C (BS-A-86509: 1994);
 9. Fatty acids profile in yolk was also analyzed. Lipids were extracted from homogeneous yolk samples using a mixture of methylene chloride and methanol. The fatty acid composition was determined by gas chromatography after performing methylation of the fatty acids (ISO/FDIS 17059). Fatty acid methyl esters (FAMES) were prepared according to the AOCS Method Ce 1k-07;
 10. The amount of selected trace elements in eggs was analyzed by performing flame atomic absorption spectrometry (FAAS) [Agilent Technologies AA Duo-AA280FS/AA280Z spectrometer (Agilent Technologies, Mulgrave, Victoria, Australia)] equipped with a Varian hollow-cathode lamp. Calibration curves were drawn prior to the analysis with four replicates per each concentration of the trace element;
 11. The quantity of lysozyme in egg albumen ($\mu\text{g}/\text{ml}$) was determined by performing electrophoresis (Leśnierowski and Kijowski, 1995), whereas the hydrolytic activity of this enzyme (U/mL liquid protein) was analyzed by performing spectrophotometry.

Statistical analysis

For the statistical evaluation, SAS® v. 9.4 (SAS, 2011) statistical package was used. Two-way ANOVA was performed according to the following formula:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij},$$

where Y_{ij} is the measured dependent variable, μ is the overall mean, α_i is the effect of the housing system (cage or litter), β_j is the effect of hens' age (44th or 60th week), $(\alpha\beta)_{ij}$ is the interaction between housing system and hens' age, and ε_{ij} is the random error. Means were compared pairwise using Duncan's multiple range test. In case of the absence of significance in interactions, main effects are discussed. All data are presented as means with a pooled standard error of the mean (SEM).

Results

Egg physical parameters and albumen lysozyme content

Effect of housing system (HS) and age of hens (A) on physical traits of eggs is presented in Table 2. There was no relationship between HS and egg weight. Interaction between housing system (HS) and age of hens (A) in relation to this trait was also not significant. On the other hand, eggs of older hens were heavier ($P < 0.0001$), by about 5.3 g. Eggs obtained from cages were characterized by a more spherical shape than those obtained from litter. In comparison to the cage housing system, eggs obtained from the litter housing system were characterized by lower yolk percentage (by about 1.8 p.p.) and higher albumen percentage (by about 2.3 p.p.) but only in the 60th week. An inverse relationship was found at the 44th week of hen age (HS $\times$ A interaction was significant). The eggs obtained from litter housing system were characterized by greater (44th week) and lower (60th week) yolk weight than those obtained from cages, resulting in a significant interaction between HS and A ($P < 0.0001$). In comparison to the cage housing system, eggs obtained from the litter housing system were characterized by lower eggshell percentage and weight. Older hens laid eggs with greater shell weight but lower eggshell percentage.

Table 2. Physical characteristics of eggs depending on housing system and hen's age

Housing system (HS)	Age (A)	Egg weight (g)	Egg shape (%)	Morphological components					
				Yolk		Albumen		Shell	
				(%)	(g)	(%)	(g)	(%)	(g)
Cage	44	64.9	79.0	23.1 b	14.9 c	66.4 a	43.1 b	10.6	6.9 ab
	60	69.4	79.6	25.5 a	17.6 a	64.2 b	44.6 ab	10.3	7.1 a
Litter	44	63.0	78.4	25.3 a	15.9 b	64.4 b	40.6 c	10.4	6.5 c
	60	69.0	77.7	23.7 b	16.3 b	66.5 a	45.9 a	9.8	6.8 bc
SEM		0.67	0.47	0.38	0.23	0.42	0.64	0.15	0.27
Main effects									
Cage		67.1	79.3 a	24.3	16.3	65.3	43.9	10.4 a	7.0 a
Litter		66.0	78.0 b	24.5	16.1	65.4	43.3	10.1 b	6.7 b
SEM		0.63	0.39	0.31	0.21	0.33	0.56	0.11	0.18
	44	63.9 a	78.7	24.2	15.4	65.4	41.8	10.5 a	6.7 a
	60	69.2 b	78.6	24.6	17.0	65.4	45.3	9.5 b	7.0 b
	SEM	0.54	0.42	0.30	0.19	0.34	0.50	0.10	0.21
P-value									
HS		0.128	0.009	0.626	<.0001	0.734	0.402	0.023	0.001
A		<.0001	0.893	0.285	0.364	0.963	<.0001	0.004	0.013
HS $\times$ A		0.346	0.189	<.0001	<.0001	<.0001	0.009	0.401	0.886

a-d – mean values in column with no common letters differ significantly ($P \leq 0.05$).

SEM – Standard error of the means.

Table 3 shows the quality of egg albumen and yolk. The cage housing system resulted in an increase in the value of albumen index, which was independent of the age of hen. Eggs obtained from the older birds were distinguished by higher ($P = 0.003$) quality of the albumen expressed in Haugh units. Neither housing system

nor the age of hens had any significant effect on the value of the yolk index. Yolk in eggs obtained from the litter housing system was found to be darker than that of yolk obtained from the cage housing system (by about 1.0 points). Older birds and hens kept on litter laid eggs with a greater quantity of lysozyme and greater activity than those kept in cages.

Table 3. Physical and enzymatic characteristics of egg albumen and yolk depending on housing system and hen's age

Housing system (HS)	Age (A)	Albumen index (%)	Haugh units (points)	Yolk index (%)	Yolk color (points)	Lysozyme	
						quantity (µg/ml)	activity (U/mL)
Cage	44	7.0	76.2	44.1	11.6	2.4	50 493
	60	6.4	68.6	42.7	11.5	3.1	65 768
Litter	44	6.3	71.2	42.5	12.4	3.0	63 950
	60	5.9	68.1	42.7	12.8	3.4	73 065
SEM		0.31	1.77	0.47	0.18	0.14	1 230
Main effects							
Cage		6.7 a	72.4	43.4	11.6 a	2.7 a	58 130 a
Litter		6.1 b	69.7	42.6	12.6 b	3.2 b	68 507 b
SEM		0.21	1.35	0.32	0.11	0.27	1 456
	44	6.7	73.7 a	43.3	12.0	2.7 a	57 221 a
	60	6.2	68.3 b	42.7	12.2	3.3 b	69 416 b
	SEM	0.35	1.28	0.35	0.13	0.15	1 367
P-value							
HS		0.033	0.120	0.067	<.0001	<.0001	<.0001
A		0.113	0.003	0.181	0.256	<.0001	<.0001
HS × A		0.668	0.208	0.082	0.081	0.075	0.062

a-d – mean values in column with no common letters differ significantly ($P \leq 0.05$).

SEM – Standard error of the means.

Eggshell characteristics

There was a significant amount of interaction (Table 4) between the housing system and age of hens on eggshell thickness (blunt end and overall) and shell elastic deformation. Eggs obtained from the cage housing system were characterized by greater elastic deformation (by about 6.6 µm) than those obtained from the litter housing system but only in the 60th week. Eggs from older hens had greater elastic deformation (overall and after the application of three different loads: 0.50 kg, 1.00 kg, and 1.50 kg) than those obtained from younger hens. However, increase in elastic deformation with the age of hen was more pronounced in the cage housing system than in the litter housing system, which resulted in a significant interaction between HS and A. Younger birds and hens kept in litter housing system laid eggs with a greater breaking strength of the eggshell than those kept in cages. Eggs obtained in the 60th week were characterized by poorer strength (by about 9.4 N). The eggshell color was decreased with increased bird age.

Table 4. Eggshell parameters depending on housing system and hen's age

Housing system (HS)	Age (A)	Eggshell thickness (µm)				Elastic deformation (µm)				Breaking strength (N)	Eggshell color (points)
		blunt end	sharp end	equatorial part	overall	0.5 kg	1.0 kg	1.5 kg	overall		
Cage	44	407 a	405	432	414 a	31.5 bc	60.7 c	90.0 bc	60.7 bc	45.7	30.6
	60	369 b	367	400	379 c	35.7 a	69.8 a	104.0 a	69.8 a	38.1	34.2
Litter	44	389 ab	401	408	399 ab	30.7 c	59.1 c	88.0 c	59.2 c	57.5	29.8
	60	388 ab	398	394	393 bc	32.8 b	63.2 b	93.5 b	63.2 b	46.3	33.8
SEM		0.009	0.008	0.006	0.005	0.53	0.98	1.60	1.07	0.20	0.732
Main effects											
Cage		388	386	416 a	397	33.6	65.2	97.2	65.3	41.9 a	32.4
Litter		387	399	401 b	396	31.8	61.2	90.6	61.3	51.8 b	31.8
SEM		0.01	0.007	0.003	0.01	0.59	1.45	1.90	0.78	0.45	1.15
	44	398	403 a	420 a	407	31.1	59.9	88.9	59.9	51.6 a	30.2 a
	60	379	382 b	397 b	386	34.3	66.4	98.9	66.6	42.2 b	33.9 b
SEM		0.008	0.006	0.009	0.01	0.62	1.22	2.40	1.28	0.38	1.24
P-value											
HS		0.963	0.185	<.0001	0.967	.0002	<.0001	<.0001	<.0001	<.0001	0.442
A		0.018	0.039	0.026	.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
HS × A		0.021	0.076	0.081	0.012	0.034	0.006	0.002	0.004	0.398	0.786

a-c – mean values in column with no common letters differ significantly ($P \leq 0.05$);
SEM – Standard error of the means.

Egg chemical composition

Younger laying hens and birds kept in litter housing system were characterized by the significantly greatest amount of crude protein ($\bar{X} = 13.7\%$, Table 5) and lower content of water ($\bar{X} = 75.2\%$) in eggs compared to older birds and those kept in cages. Independent of the housing system, the hens at the 44th week had a higher amount of fat and ash in egg mass.

Table 5. Egg chemical composition depending on housing system and hen's age

Housing system (HS)	Age (A)	Chemical components (%)			
		Water	Crude protein	Crude fat	Crude ash
Cage	44	76.1	13.2	10.6	0.94
	60	77.7	12.1	10.3	0.86
Litter	44	75.5	14.4	10.1	0.94
	60	77.4	13.0	9.7	0.87
SEM		0.14	0.19	0.24	0.03
Main effects					
Cage		76.0 a	12.7 a	10.4	0.90
Litter		75.5 b	13.7 b	9.9	0.91
SEM		0.28	0.24	0.17	0.02
	44	74.9 a	13.8 a	10.4 a	0.94 a
	60	76.6 b	12.5 b	10.0 b	0.86 b
	SEM	0.12	0.20	0.11	0.02
P-value					
HS		0.0037	<.0001	0.112	0.748
A		<.0001	<.0001	0.027	0.009
HS $\times$ A		0.119	0.401	0.790	0.796

a-c – mean values in column with no common letters differ significantly ($P \leq 0.05$);

SEM – Standard error of the means.

Macro- and microelements content

Eggs obtained from the litter housing system were found to have increased content of macroelements (sodium, potassium, calcium, and magnesium) in their egg yolk and albumen but only in the 60th week, resulting in a significant amount of interaction between HS and A (Table 6). We also obtained significant interaction between housing system and age of hens on the content of heavy metals (nickel and lead) and microelements (Table 6). Eggs collected at the 60th week of age were characterized by a lower content of iron and zinc than those obtained at 44th week. However, decrease in the content of iron and zinc with increased age of hen was more pronounced in the litter housing system than in a cage housing system, which resulted in a significant interaction between HS and A. Independent of the housing system, the age of hens (60th week) resulted in an increase in the content of cadmium in egg. Younger hens and birds kept in cages were characterized by the larger ($P < 0.0001$) amount of selenium and a lower amount of cobalt than that of older birds and those kept in the litter housing system.

Table 6. Content (mg·kg⁻¹) of macro-, microelements and heavy metals in egg (yolk and albumen) depending on housing system and hen's age

Housing system (HS)	Age (A)	Na	K	Ca	Mg	Ni	Pb	Cd	Fe	Zn	Cu	Mn	Se	Co
Cage	44	1374 c	1455 b	340 b	141 c	4.3 b	0.07 b	0.021	63.4 a	10.5 a	0.4 b	0.3 c	0.32	0.034
	60	1564 b	1240 c	353 b	134 c	4.4 b	0.06 c	0.023	62.0 a	7.9 c	0.2 c	0.4 b	0.18	0.038
Litter	44	1245 c	1386 bc	359 b	246 a	4.6 a	0.06 c	0.022	65.3 a	13.0 b	0.1 d	0.3 c	0.24	0.039
	60	1946 a	1655 a	411 a	164 b	4.3 b	0.09 a	0.024	44.2 b	8.0 c	0.6 a	0.5 a	0.11	0.046
SEM		44.40	49.12	8.16	5.25	0.04	0.003	.0006	1.64	0.51	0.01	0.02	0.01	0.001
Main effects														
Cage		1469	1347	347	138	4.3	0.06	0.022	62.7	9.2	0.3	0.3	0.25 a	0.036 a
Litter		1596	1521	385	205	4.4	0.07	0.023	54.8	10.5	0.4	0.4	0.17 b	0.042 b
SEM		68.81	54.37	7.81	9.12	0.03	0.01	0.004	2.81	0.77	0.05	0.03	0.02	0.10
	44	1309	1421	350	194	4.4	0.07	0.021 a	64.4	11.8	0.3	0.3	0.28 a	0.037 a
	60	1755	1447	382	149	4.3	0.08	0.024 b	53.1	8.0	0.4	0.4	0.15 b	0.042 b
	SEM	55.70	53.63	8.35	11.46	0.04	0.03	0.002	2.21	0.43	0.05	0.02	0.01	0.13
P-value														
HS		0.013	0.003	.0003	<.0001	0.019	.0008	0.147	<.0001	0.007	0.001	0.039	<.0001	.0001
A		<.0001	0.603	0.001	<.0001	0.023	.0002	.0003	<.0001	<.0001	<.0001	<.0001	<.0001	.0002
HS × A		<.0001	.0002	0.030	<.0001	.0004	<.0001	0.868	<.0001	0.009	<.0001	0.022	0.976	0.229

a-c – mean values in column with no common letters differ significantly (P≤0.05);
SEM – Standard error of the means.

Table 7. Amount of fatty acids (%) in egg yolks depending on housing system and hen's age

Housing system (HS)	Age (A)	C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:1 n-7	C20:0	SFAs	MUFAs	C18:2	C18:3	C20:4	C20:5	PUFAs
Cage	44	0.3 c	22.6 c	3.0 c	8.2 b	38.2	1.8	0.3 a	31.4 b	43.0 d	0.5 b	0.5 a	2.2 b	0.3 b	3.5 b
	60	0.3 c	25.2 a	3.0 c	8.7 a	42.6	2.3	0.2 b	34.4 a	47.9 c	0.4 c	0.4 b	2.5 a	0.6 a	3.8 a
Litter	44	0.4 b	23.5 b	4.6 b	4.7 c	44.3	2.4	0.1 c	28.7 c	51.3 b	0.5 ab	0.5 a	0.6 c	0.3 b	1.9 c
	60	0.5 a	23.2 bc	5.4 a	4.8 c	49.5	2.9	0.2 b	28.6 c	57.8 a	0.6 a	0.5 a	0.4 d	0.2 c	1.6 d
SEM		0.007	0.18	0.03	0.04	0.26	0.02	0.007	0.18	0.26	0.01	0.01	0.03	0.008	0.03
Main effects															
Cage		0.3	23.9	3.0	8.5	40.4 a	2.1 a	0.3	32.9	45.4	0.4	0.4	2.4	0.4	3.7
Litter		0.4	23.3	5.0	4.8	46.9 b	2.6 b	0.2	28.6	54.5	0.5	0.5	0.4	0.3	1.8
SEM		0.01	0.27	0.08	0.05	0.82	0.12	0.008	0.24	0.32	0.02	0.04	0.05	0.03	0.12
	44	0.3	23.0	3.8	6.5	41.2 a	2.1 a	0.2	30.0	47.2	0.5	0.5	1.4	0.3	2.7
	60	0.4	24.2	4.2	6.7	46.0 b	2.6 b	0.2	31.5	52.8	0.4	0.4	1.4	0.4	2.7
SEM		0.02	0.24	0.35	0.61	1.07	0.09	0.007	0.20	0.18	0.01	0.02	0.35	0.03	0.07
P-value															
HS		<.0001	0.004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
A		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.237	<.0001	<.0001	.0005	.0005	0.139	<.0001	0.741
HS × A		<.0001	<.0001	<.0001	<.0001	0.128	0.754	<.0001	<.0001	0.007	<.0001	<.0001	<.0001	<.0001	<.0001

a-d – mean values in column with no common letters differ significantly ($P \leq 0.05$); SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids.

SEM – Standard error of the means.

Fatty acids content

Eggs obtained from the cage housing system showed increased content of saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) and showed decreased content of monounsaturated fatty acids (MUFAs) in egg yolk, resulting in a significant interaction ($P < 0.0001$) between HS and A (Table 7).

Discussion

Egg physical parameters and albumen lysozyme content

Voslářová *et al.* (2006) compared the quality of eggs of ISA Brown laying hens kept in two housing systems: litter and conventional cages. They found that hens kept in cages laid heavier eggs than those kept in the litter housing system (53 vs. 40 g). Gerzilov *et al.* (2012) also found that hens kept in cages (conventional and enriched) laid heavier eggs ($\bar{x} = 57.30$ g) than those kept in floor/litter systems (55.50 g). It seems that these results may be caused by lower energy requirement of birds from the caged housing system, where their movement is definitely more limited. However, literature also contains contradictory results. For example, Singh *et al.* (2009) and Ojedapo (2013) observed that floor housing system resulted in the production of heavier eggs than cage housing of hens. In our study, interaction between housing system (HS) and hen age (A) was insignificant. On the other hand, Singh *et al.* (2009) observed that interaction in relation to egg weight ($P < 0.01$).

In this study, eggs obtained from the younger birds kept in cages were characterized by more favorable parameters of albumen quality (index, number of Haugh units) than those obtained from older hens. However, we did not find an interaction between housing system and bird age. In contrast to our results, Yilmaz Dikmen *et al.* (2017) obtained such a significant interaction in case of all the analyzed traits that describe the quality of the albumen. Samiullah and Chousalkar (2014) also noted significant interaction between production system and age of laying hens. A more rapid decline in the value of Haugh units as laying progressed was found in free-range systems than in the cage housing system. Singh *et al.* (2009) found less beneficial qualities of albumen (lower height) in eggs obtained from the litter system. In contrast to these authors, Trziszka *et al.* (2007) noted that the eggs from the litter system were characterized by more favorable albumen parameter (Haugh units of 95.0) than those obtained from conventional battery cages (83.0). Minelli *et al.* (2007) showed that the oldest hens (70–73 weeks of age) laid eggs with higher albumen and yolk weight (43.80 g and 17.1 g, respectively) compared to younger hens, which was also partially (albumen) confirmed in our study. Roll *et al.* (2009) also confirmed this tendency in relation to yolk weight.

Research by Biesiada-Drzazga and Janocha (2009) conducted on eggs of Tetra SL and Hy-line Brown hens kept for comparison in cages and on litter did not confirm any significant impact of the housing system on eggshell weight or its percent-age content in egg mass. Similarly, Van den Brand *et al.* (2004), as well as Lewko and Gornowicz (2011), did not find an effect of housing system on egg weight or

percentage of the morphological elements of eggs in experiments on selected hybrids of laying hens. Similar results were obtained by Lichovníková and Zeman (2008). In contrast, in this study we found a significant effect of the housing system and the age of hens on the weight of eggshell and its percentage content in egg. However, the interaction between housing system and bird age with reference to above mentioned traits was not confirmed statistically in our research. On the other hand, a greater decrease in the value of this trait with progressing laying period was found in free-range systems in comparison with cages (Vlčková et al., 2018). Similar to our results, Angelovičová et al. (2014) found significant differences in the eggshell weight in case of eggs laid by 30- and 40-week-old ISA Brown hens. Zita et al. (2009) noted that in the initial laying period (20–26 weeks of age) in hens, the percentage of eggshell was greatest ($\bar{x}$ = 12.5%), which decreased as laying progressed. Similar results were obtained in our study. This is a logical relationship assuming that the increase in egg weight with the age of hens is a result of an increase in the weight of albumen (Kontecka et al., 2012).

In this study, the amount of lysozyme and its hydrolytic activity in egg protein were found to be greater in eggs obtained from older hens. Świerczewska et al. (2005) obtained similar results. They found an increase in the activity of lysozyme over the course of the production period. The difference in activity between the first (35th week of age) and the last (50th week) date of the investigation was on average 55.0 U/mL. One of the hypotheses of this phenomenon may be an increased mobilization to produce eggs with greater protection against pathogens in the later period of reproduction. During this time, the thickness of the eggshell usually decreases and creates greater possibility of harmful microorganisms penetrating into the eggs. The negative effect of laying hens' age on eggshell thickness was also confirmed in our research. However, Krawczyk and Gornowicz (2010) did not observe any significant differences in the values of lysozyme between younger (32–36 weeks of age) and older hens (52–56 weeks of age). Trziszka et al. (2007) observed higher activity of this enzyme in eggs laid by hens kept in cages (116,120 U/100 mg of protein) than in those from the litter housing system (98,200 U/100 mg of protein). On the other hand, Krawczyk and Gornowicz (2010) demonstrated a greater amount of lysozyme in the eggs obtained from free-range systems than those obtained from the litter housing system.

Eggshell characteristics

Zita et al. (2009) showed that in comparison with younger hens, eggs obtained from laying hens within 54–60 weeks of age were characterized by the lowest elastic deformation of the shell (average was approximately 0.34 mm) and breaking strength. This was partially confirmed in our study. Laying hens in their 60th week of age laid eggs with poorer eggshell breaking strength compared with birds in their 44th week of age. Unlike the study published by Zita et al. (2009), in this study we observed a significantly greater amount of flexibility of eggshell in eggs obtained from older hens than those obtained from younger hens. Sokołowicz et al. (2012) and Calik (2014) showed no effect of age of hens on the eggshell strength. In our study, we confirmed the effect of housing system on the eggshell strength. However, the

interaction between housing system and hen age with reference to eggshell strength was not observed. Vlčková *et al.* (2018) found the aforementioned interaction. The authors noted that the value of this trait decreased with the progression of the laying period but only in the cage housing system. On the other hand, Svobodová *et al.* (2014) reported no differences in the elastic deformation of the eggshell of Lohmann hens, which were kept for comparison in conventional cages and litter. Samiullah and Chousalkar (2014) and our results showed the interaction between hens' age and housing system in case of elastic deformation of the eggshell. Pištěková *et al.* (2006), Wang *et al.* (2009), Krawczyk and Gornowicz (2010), and Ketta and Tümová (2014) found no effect of housing system on the eggshell breaking strength. Lolli *et al.* (2013) showed better eggshell strength when the birds were kept in cages than on litter (37.8 N vs. 35.1 N). Similar results were obtained by Lichovníková and Zeman (2008).

Calik (2014) observed a decrease in the thickness of the eggshell only after the 83rd week of laying hens' age. Conversely, Van den Brand *et al.* (2004) and Zita *et al.* (2009) found no effect of age of laying hens on the value of this feature. In our study, the effect of hens' age on eggshell thickness was found only in the cage housing system. Poorer results were obtained for older birds. However, Vlčková *et al.* (2018) did not confirm the effect of hens' age on the thickness of eggshell either in cages or in the free-range system (no interactions between housing system and birds' age). Analyzing eggshell thickness during the laying period of Hy-line Brown hens, Jiang *et al.* (2014) noticed that, as birds aged, the thickness increased from 0.388 mm to 0.398 mm at 27–28 and 87–89 weeks of age, respectively.

Biesiada-Drzazga and Janocha (2009) showed that eggshells of laying hens kept on litter were slightly thinner (no statistically significant difference) than those of hens kept in cages. In our experiment, the eggshells of older hens (60th week of age) kept in the cage housing system were less thick than those of the litter housing system. Lolli *et al.* (2013) and Lichovníková and Zeman (2008) also obtained the lowest eggshell thickness from litter housing systems compared to other housing systems. In contrast, Englmaierová *et al.* (2014) conducted research on laying hen hybrids (Hisex Brown) and did not find the effect of housing system (enriched cages and litter) on the mean values of eggshell thickness, which were 0.379 mm and 0.376 mm, respectively.

In this study, we did not find any difference between the analyzed housing systems in relation to the color of the eggshell. However, Sekeroglu *et al.* (2010) reported a significantly greater brightness of the eggshell in laying hens (ATAK) kept on litter than those kept in the cage housing system. As the age of hen increases, the eggshell becomes lighter, which was also confirmed in this study and by Odabaşı *et al.* (2007) and Zita *et al.* (2009). On the other hand, Chang-Ho *et al.* (2014) performed experiments with Hy-line Brown hens and observed no statistically significant differences in the color of eggshells between the 20th and 70th week of age. Ledvinka *et al.* (2012) found that eggshell colour was affected by housing system ($P \leq 0.001$) with statistically significant interaction of age and housing system. In our study, that interaction was not confirmed ($P = 0.786$).

Egg chemical composition

In this study, we found that the eggs obtained from the litter housing system were characterized by a greater level of protein content compared to eggs obtained from the hens kept in cages. In contrast, a higher proportion of fat and water content was found in eggs laid by the hens kept in cages than those kept in the litter housing system. These results agree with those obtained by Matt et al. (2009). Minelli et al. (2007) showed that the eggs obtained from the youngest birds (28–32 weeks) had lower contents of protein ($\bar{X} = 6.50\%$) and ash ($\bar{X} = 1.56\%$) compared to eggs obtained from the older hens. However, their eggs demonstrated higher fat content ($\bar{X} = 30.60\%$) compared to that of older hens. In contrast, in this study we obtained greater content of protein and ash in eggs from younger layers than in those obtained from older hens. The above mentioned large variation and inconsistency of the literature results do not allow for a wider interpretation of the own results.

Similar to the results obtained by Minelli et al. (2007), in this study we found that the fat content was lower in eggs obtained from older hens than in those obtained from younger hens (60th week of age). Radu-Rusu et al. (2014) keeping laying hens in three different housing systems, showed that the eggs from cages had higher content of dry matter ($\bar{X} = 44.3\%$), ash ($\bar{X} = 1.29\%$), proteins ($\bar{X} = 13.7\%$), and lipids ($\bar{X} = 28.4\%$) compared to those obtained from the free-range systems. However, the water content in their study was lower than that in this study, which ranged from 55.7% (battery of cages) to 57.3% (free-range systems).

Macro- and microelements content

In this study, eggs obtained from older hens kept in the litter housing system showed the highest content of sodium, potassium, and calcium compared to those kept in cages. However, eggs obtained from younger hens (44th weeks of age) kept in the litter housing systems showed the highest level of magnesium, iron, and zinc compared to those of older hens kept in the litter housing system. Drabik et al. (2018) obtained lower content of toxic metals, such as cadmium ($\bar{X} = 0.018$ ppm) and lead ($\bar{X} = 1.181$ ppm) in eggs obtained from cages than those obtained from organic production. Higher values of heavy metals recorded for birds kept in the ecological system may be the result of access to free range and green forage, as an integral part of the diet of birds in this housing system. Metals present in soil and plants could therefore accumulate in the body of birds and then pass to egg content. In this study, the level of lead in eggs obtained from cages was lower than in those obtained from the litter housing system. Küçükyılmaz et al. (2012) obtained lower content of zinc in eggs obtained from the organic production than in those obtained from the conventional cage system. In this study, the level of zinc was found to be lower in eggs obtained from cages than those obtained from the litter housing system.

Fatty acids content

Egg yolk is designed to supply the required amount of PUFAs for the development of the embryo; egg yolks contain 15–26% of MUFAs and 3.3–4.6% as PUFAs (Lordelo et al., 2017). Depending on the age of birds and the housing system, we obtained statistically significant differences ($P \leq 0.05$) relating to the fatty acid profile.

Contradictory results were obtained by Scheideler et al. (1998) and Sokołowicz et al. (2012). They stated that, in ecological farming, regardless of the age of laying hens, they found a low $n\text{-}6:n\text{-}3$ ratio in yolks. This result is similar to those obtained by Krawczyk et al. (2011).

Thus, based on our results, we conclude that both the age of Hy-line Brown hens and housing system have an effect on the quality of eggs. Eggs with a better quality of albumen were obtained from younger hens when compared to eggs obtained from older hens. Moreover, older hens produced eggs with inferior shell quality, such as greater elastic deformation, poorer breaking strength, and lower thickness. However, hens kept on litter laid eggs with superior quality of the shell (less flexible and more breaking strength), even though the thickness, weight, and percentage were similar in both housing systems. In addition, the highest amount and activity of lysozyme were obtained for eggs obtained from hens kept in the litter housing system. The maintenance system and the age of birds also had a significant ($P \leq 0.05$) effect on the basic chemical composition of eggs, as well as on the share of individual elements and fatty acid profile.

Conclusions

Taking these results into account, we can conclude that the more valuable morphological and nutritional characteristics were shown by eggs from younger Hy-line hens kept in the litter housing system.

References

- Ahn D.U., Sell J.L., Jo C., Chamruspollert M., Jeffrey M. (1999). Effect of dietary conjugated linoleic acid on the quality characteristic of chicken eggs during refrigerated storage. *Poultry Sci.*, 78: 922–928.
- Angelovičová M., Ševčíková V., Angelovič M., Bučko O. (2014). Comparison of two different breeding systems of laying hens in relation to egg shell quality. *Sci. Pap. Anim. Sci. Biotechnol.*, 47: 166–171.
- AOCS Official Method Ch 6–91. (1997). Determination of the composition of the sterol fraction of animal and vegetable oils and fats by TLC and capillary GLC. *Methods and Recommended Practices of the AOCS*, Sixth edition. Firestone D. (ed.). AOCS Press, Champaign, IL, USA, pp. 1–5.
- Basmacioğlu H., Ergül M. (2005). Research on the factors affecting cholesterol content and some other characteristics of eggs in laying hens. The effects of genotype and rearing system. *Turk. J. Vet. Anim. Sci.*, 29: 157–164.
- Biesiada-Drzazga B., Janocha A. (2009). Impact of hen breed and rearing system on the quality of eggs for consumption (in Polish). *Zywn-Nauk. Technol. Ja.*, 3: 67–74.
- Bozkurt Z., Tekerli M. (2009). The effects of hen age, genotype, period and temperature of storage on egg quality. *Kafkas Univ. Vet. Fak.*, 15: 517–524.
- Calik J. (2014). Effect of length of productive life of Rhode Island Red (R-11) hens on their performance and egg quality. *Acta Sci. Pol. Zootech.*, 13: 39–50.
- Chang-Ho K., Jong-Ho S., Jae-Cheong L., Kyung-Woo L. (2014). Age-related changes in egg quality of Hy-line brown hens. *Int. J. Poult. Sci.*, 13: 510–514.
- Czaja L., Gornowicz E. (2006). Effect of genome and hen's age on table egg quality (in Polish). *Rocz. Nauk. Zoot.*, 33: 59–70.
- Drabik K., Chabroszewska P., Vasiukov K., Adamczuk A., Blicharska E., Batkowska J. (2018). The influence of the breeding system on the content of heavy metals in eggs (in

- Polish). In: *Nauki Przyrodnicze i Medyczne: Świat żywy a technologie w otoczeniu ludzi i zwierząt*, pp. 93–102.
- Englmaierová M., Tůmová E., Charvátová V., Skřivan M. (2014). Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination. *Czech J. Anim. Sci.*, 59: 345–352.
- Funk E. (1948). The relation of the yolk index determined in natural position to the yolk index as determined after separating the yolk from the albumen. *Poultry Sci.*, 27: 367–367.
- Gerzilov V., Datkova V., Mihaylova S., Bozakova N. (2012). Effect of poultry housing systems on egg production. *Bulg. J. Agric. Sci.*, 18: 953–957.
- Haugh R.R. (1937). The Haugh unit for measuring egg quality. *The US Egg Poultry Magazine*, 43: 522–573.
- Hidalgo A., Rossi M., Clerici F., Ratti S. (2008). A market study on the quality characteristics of eggs from different housing systems. *Food Chem.*, 106: 1031–1038.
- Holt P.S., Davies R.H., Dewulf J., Gast R.K., Huwe J.K., Jones D.R., Waltman D., Willian K.R. (2011). The impact of different housing systems on egg safety and quality. *Poultry Sci.*, 90: 251–262.
- ISO/FDIS 17059. Oilseeds — Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography (Rapid method).
- Jiang S., Cui L.Y., Hou J.F., Shi C., Ke X., Yang L.C., Ma X.P. (2014). Effects of age and dietary soybean oil level on eggshell quality, bone strength and blood biochemistry in laying hens. *Br. Poult. Sci.*, 55: 653–661.
- Ketta M., Tůmová E. (2014). Differences in the eggshell quality and tibia strength in Lohmann White and Czech Hen housed in cages and on litter. *Acta Fytotechn. Zootechn.*, 17: 75–78.
- Kontecka H., Nowaczewski S., Sierszuła M., Witkiewicz K. (2012). Analysis of changes in egg quality of broiler breeders during the first reproduction period. *Ann. Anim. Sci.*, 12: 609–620.
- Krawczyk J., Gornowicz E. (2010). Quality of eggs from hens kept in two different free-range systems in comparison with a barn system. *Arch. Geflügelk.*, 74: 151–157.
- Krawczyk J., Sokołowicz Z., Szymczyk B. (2011). Effect of housing system on cholesterol, vitamin and fatty acid content of yolk and physical characteristics of eggs from Polish native hens. *Arch. Geflügelk.*, 75: 151–157.
- Küçükyılmaz K., Bozkurt M., Yamaner C., Çınar M., Çatlı A.U., Konak R. (2012). Effect of an organic and conventional rearing system on the mineral content of hen eggs. *Food Chem.*, 132: 989–992.
- Ledvinka Z., Tůmová E., Englmaierová M., Podsedníček M. (2012). Egg quality of three laying hen genotypes kept in conventional cages and on litter. *Arch. Geflügelk.*, 76: 38–43.
- Leśniewski G., Kijowski J. (1995). Separation, polymer formation and antibacterial activity of lysozyme. *Pol. J. Food Nut. Sci.*, 49: 3–16.
- Lewko L., Gornowicz E. (2011). Effect of housing system on egg quality in laying hens. *Ann. Anim. Sci.*, 11: 607–616.
- Lichovnicková M., Zeman L. (2008). Effect of housing system on the calcium requirement of laying hens and on eggshell quality. *Czech J. Anim. Sci.*, 53: 162–168.
- Lolli S., Hidalgo A., Alamprese C., Ferrante V., Rossi M. (2013). Layer performances, eggshell characteristics and bone strength in three different housing systems. *Biotechnol. Anim. Husb.*, 29: 591–606.
- Lordelo M., Fernandes E., Bessa R.J.B., Alves S.P. (2017). Quality of eggs from different laying hen production systems, from indigenous breeds and specialty eggs. *Poultry Sci.*, 96: 1485–1491.
- Matt D., Veromann E., Luik A. (2009). Effect of housing systems on biochemical composition of chicken eggs. *Agron. Res.*, 7: 662–667.
- Minelli G., Sirri F., Folegatti E., Meluzzi A., Franchini A. (2007). Egg quality traits of laying hens reared in organic and conventional systems. *Ital. J. Anim. Sci.*, 6: 728–730.
- Odabaşı Z., Miles R.D., Balaban M.O., Portier K.M. (2007). Changes in brown eggshell color as the hen ages. *Poultry Sci.*, 86: 356–363.

- Ojedapo L.O. (2013). Effect of two housing systems (cages vs deep litters) on external and internal egg characteristics of a commercial laying birds reared in derived savanna zone of Nigeria. *Transnat. J. Sci. Techn.*, 3: 25–34.
- Pišťáková V., Hovorka M., Večerek V., Straková E., Suchý P. (2006). The quality comparison of eggs laid by laying hens kept in battery cages and in a deep litter system. *Czech J. Anim. Sci.*, 51: 318–325.
- PN-A-04018:1975/Az3 (2002). Agricultural and food products. Determination of nitrogen by the Kjeldahl method and conversion to protein (Az3 change).
- PN-A-86509 (1994). Egg products – Physicochemical tests
- Radu-Rusu R.M., Usturoi M.G., Leahu A., Amariei S., Radu-Rusu C.G., Vacaru-Opriş I. (2014). Chemical features, cholesterol and energy content of table hen eggs from conventional and alternative farming systems. *S. Afr. J. Anim. Sci.*, 44: 33–42.
- Roll V.F.B., Briz R.C., Levirino G.A.M. (2009). Floor versus cage rearing: effects on production, egg quality and physical condition of laying hens housed in furnished cages. *Ciênc. Rural.*, 39: 1527–1532.
- Samiullah R.J.R., Chousalkar K.K. (2014). Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. *J. Appl. Poult. Res.*, 23: 59–70.
- SAS (2011). User's Guide. Statistical Analysis System Institute, Inc. Cary/North California.
- Scheideler S.E., Jaroni D., Froning G. (1998). Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. *Poultry Sci.*, 77: 192–196.
- Sekeroglu A., Sarica M., Demir E., Ulutas Z., Tilki M., Saatci M., Omed H. (2010). Effects of different housing systems on some performance traits and egg qualities of laying hens. *Anim. Vet. Adv.*, 9: 1739–1744.
- Singh R., Cheng K.M., Silversides F.G. (2009). Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poultry Sci.*, 88: 256–264.
- Sokołowicz Z., Krawczyk J., Herbut E. (2012). Quality of eggs from organically reared laying hens during their first and second year of production (in Polish). *Zywn.-Nauk. Technol. Ja.*, 4: 185–194.
- Suk Y.O., Park C. (2001). Effect of breed and age of hens on the yolk to albumen ratio in two different genetic stocks. *Poultry Sci.*, 80: 855–858.
- Svobodová J., Tůmová E., Englmaierová M. (2014). The effect of housing system on egg quality of Lohmann White and Czech hen. *Acta Fytotechn. Zootechn.*, 17: 44–46.
- Świerczewska E., Skiba T., Sokołowska A., Noworyta-Głowacka J., Kopeć W., Korzeniowska M., Bobak L. (2005). Egg white biologically active proteins activity in relation to laying hen's age. *Proc. of XI European Symposium on the quality of eggs and egg products*. Doorwerth, the Netherlands, WPSA, 23–26 May 2005.
- Tauson R. (2005). Management and housing systems for layers – effects on welfare and production. *World Poultry Sci. J.*, 61: 477–490.
- 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.
- Van den Brand H., Parmentier H., Kemp B. (2004). Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. *Br. Poult. Sci.*, 45: 745–752.
- Vlčková J., Tůmová E., Ketta M., Englmaierová M., Chodová D. (2018). Effect of housing system and age of laying hens on eggshell quality, microbial contamination, and penetration of microorganisms into eggs. *Czech J. Anim. Sci.*, 63: 51–60.
- Voslářová E., Hanzalek Z., Večerek V., Straková E., Suchý P. (2006). Comparison between laying hen performance in the cage system and the deep litter system on a diet free from animal protein. *Acta Vet. Brno*, 75: 219–225.
- Wang X.L., Zheng J.X., Ning Z.H., Qu L.J., Xu G.Y., Yang N. (2009). Laying performance and egg quality of blue-shelled layers as affected by different housing systems. *Poultry Sci.*, 88: 1485–1492.
- Yilmaz A.A., Bozkurt Z. (2009). Effects of hen age, storage period and stretch film packaging on internal and external quality traits of table eggs. *Sci. Pap. Anim. Sci. Biotech.*, 42: 462–469.
- Yilmaz Dikmen B., Ipek A., Şahan Ü., Sözcü A., Baycan S.C. (2017). Impact of differ-

ent housing systems and age of layers on egg quality characteristics. Turk. J. Vet. Anim. Sci., 41: 77–84.

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

Received: 8 I 2020

Accepted: 30 VI 2020