Effect of layer genotype on physical characteristics and nutritive value of organic eggs

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ABSTRACT
The aim of the study was to evaluate the quality of organic eggs from hens of different genotypes and ages. The study investigated the physical characteristics, nutritive value and foaming properties of egg whites from Hy-Line Brown commercial layers, native Greenleg Partridge hens included in the conservation program, and Araucana hens laying blue-shelled eggs. Genotype had an effect on egg weight, albumen height and Haugh units. The vitamin A content of egg yolks was greater for Greenleg Partridge compared to Hy-Line Brown hens (P < 0.05). The n-6/n-3 PUFA ratio in egg yolks from Greenleg Partridge hens was lower, while the EPA, DHA and PUFA n-3 content were higher than in egg yolks from Hy-Line Brown hens (P < 0.05). Egg yolk cholesterol concentration was similar for the studied hen breeds (P > 0.05). Layer age had a significant effect on egg weight, yolk weight and color, and the presence of meat and blood spots (P < 0.05).

KEYWORDS Organic eggs; genotype; vitamins; cholesterol; fatty acids; foaming properties

PALABRAS CLAVE huevos orgánicos; genotipo; vitaminas; colesterol; ácidos grasos; propiedades espumantes

Efecto del genotipo de gallinas ponedoras en las características físicas y el valor nutritivo de huevos ecológicos

RESUMEN El presente estudio se propuso evaluar la calidad de huevos ecológicos producidos por gallinas de diferentes genotipos y de distintas edades. En este sentido, el estudio investigó las características físicas, el valor nutritivo y las propiedades espumantes de las claras de huevos provenientes de ponedoras comerciales Hy-Line Brown, de gallinas arborófilas pativerdes nativas incluidas en un programa de conservación y de gallinas araucanas (o mapuche), cuyos huevos tienen cáscara azul-verdosa. Al respecto se estableció que el genotipo determina el peso del huevo, la altura de la albúmina y las unidades Haugh. En comparación con las gallinas Hy-Line Brown, se constató que el contenido de yemas de los huevos procedentes de la variedad arborófila pativerde fue mayor (P < 0.05), mientras que la ratio PUFA n-6/n-3 de las yemas de huevos de esta última variedad de gallina fue menor y el contenido de EPA, DHA y PUFA n-3 más elevado (P < 0.05). La concentración de colesterol de las yemas fue similar en todas las razas estudiadas (P < 0.05). Por último, se comprobó que la edad de las ponedoras tuvo un efecto significativo en el peso del huevo, el peso de la yema y el color, así como en la presencia de manchas de carne y de sangre (P < 0.05).

Introduction

Eggs are an important food because they are an economically affordable source of high-quality protein and nutrients (Exler, Phillips, Patterson, & Holden, 2013). According to Council Directive 1999/74/EC, in all European countries table eggs can be produced in cage, litter, free-range and organic housing systems. In many European countries, the majority of eggs are produced in enriched cage and litter systems, but recent years have seen a growing interest in alternative systems, including the organic system. According to Commission Regulation (EC) No. 589/2008 of 23 June 2008, eggs from organic hens are identified by the number 0 in the egg code (EC, 2008). It is estimated that organic eggs account for 5–20% of the total egg production depending on the country. The purchase and consumption of organic eggs have become particularly popular among consumers who believe that organically produced eggs are of better quality. Although the concept of quality is not clear, the quality of eggs is assessed based on their physical characteristics, nutritive value, and functional properties of egg albumen.

Native breed hens are most often recommended for organic farming, but commercial hybrids are also used in practice in this production system. The diversity of hens used to produce organic eggs results in eggs with different shell colors. Shell color is unrelated to the nutritive value of eggs, but consumers in different parts of the world have specific preferences regarding eggshell color. In North America, consumers prefer white-shelled eggs, and brown eggs are more popular in Asia and Europe (Hooge, 2007). One of such native breeds commonly found in organic farms located in areas associated with their existence is the green-legged partridge hen. They are known to adapt easily to prevailing local environments and make efficient use of paddocks. Eggs sourced from such hens are commonly available in retail with great consumer interests. Recent years

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have witnessed an interest in eggs with a unique blue and green color. Araucana is a well-known breed of chicken that lays greenish eggs (Peterson et al., 1978).

The results of nutritive value (fatty acid profile, cholesterol and vitamin content) of eggs from different breeds of hens may be important for consumers who purchase organic eggs. Therefore, the aim of the present study was to evaluate the effect of layer genotype on the nutritive value and foaming properties of organically produced eggs.

Material and methods

Birds and management

The experiment used eggs from the native Greenleg Partridge hens, which are included in the list of the world genetic resources that need to be conserved (World Watch List, FAO, Rome, 2000); blue- and green-shelled eggs from Araucana hens; and eggs from organically raised Hy-Line Brown commercial hybrids. The hens were housed and fed, from the 16th week of life, in accordance with regulations pertinent to organic rearing, i.e. EC Directive 1804/1999 and Regulation of European Economic Community (EEC) Council 2092/91. Hens were housed in a deep litter (6 hens/m²) poultry house with growing trees (5 m²/hen). The hens were also given organic poultry feed (16.1% protein, 11.2 MJ).

Sampling

To evaluate the physical characteristics, 30 eggs were collected from layers of each breed at 26, 42 and 56 weeks of age. Eggs were evaluated after 24 h of storage at 12°C and 55% humidity. The egg quality evaluation included egg weight and yolk weight (g); shape index (%), yolk, albumen and shell percentage; shell traits – L*a*b* color, weight (g), thickness (μm), density (mg/cm³), crushing strength (N); physical characteristics of interior egg quality – thick albumen height (mm), Haugh units (HU), yolk color (1–15 on the DSM scale) and the presence of meat and blood spots.

Egg weight

Egg weight was determined by weighing individually with a digital laboratory balance (Ohaus Navigator NOB 110, Sweden) exact to 0.1 g.

Shape index

Shape index of eggs was determined as a ratio of short-to-long axis which was measured using an electronic caliper MITUTOYO Absolute Digmatic Caliper model CD-15DCX (Japan) exact to 0.01 mm.

Relative weight of egg components

Percentage of egg morphological components (albumen, yolk and shell) was calculated based on their weights measured individually for each egg.

Shell color

Shell color was measured by the L*a*b* color system using a Konica Minolta spectrophotometer Model CR-400 (Japan). L* represents the grading between white (100) and black (0). The higher the value for L*, the lighter is the shell color, a* – egg’s color tone as a function of a red-green scale (green a* < 0 and red a* > 0); b* – egg’s color tone as a function of a blue-yellow scale (blue b* < 0 and yellow b* > 0).

Eggshell traits and interior egg traits

Eggshell weight, density and thickness, albumen height, Haugh units, yolk color according to DSM scale were measured using electronic equipment for egg quality measurements (EQM – Egg Quality Measurements, Technical Services and Supplies, UK).

Eggshell strength

Eggshell strength (N) was measured using a multipurpose testing system, model BT1-FR1.OTH.D14 with measuring head 100 N and software testXpert (Zwick/Roell GmbH&Co. KG, Germany).

Biochemical analysis

Nutritive value (cholesterol content, vitamin A and E content, fatty acid profile of egg yolk, foaming properties (foaming capacity, foam drainage) and pH of yolk and albumen were evaluated in eggs laid by the birds at 56 days of age.

Egg yolks were analysed for vitamin A and E content using an accredited procedure based on Polish standards PN-EN ISO 14565 (all-trans-retinol, vitamin A) and PN-EN ISO 6867 (α-tocopherol, vitamin E). Determinations were made by HPLC (Merck-Hitachi) on a LiChroCART™ 250–4 Superspher™ 100 RP–18 column, 4 μ, using UV-VIS (324 nm, vitamin A) and FL detectors (Ex295 nm, Em350 nm, vitamin E). A 1 ml/min eluent flow (methanol:H₂O, 96.5:3.5, v/v) was used. Vitamins were determined using a standard solution of all-trans-retinol and α-tocopherol (Sigma-Aldrich) after saponification of the sample components in ethanol KOH solution, extraction of vitamins with petroleum ether, evaporation of solvent and dissolution of the dry residue in ethanol. Before use, the standards were standardized using tabular extinction values for ethanol solutions (1000 μg/ml) of vitamin A and vitamin E, which were 1835 (λ = 325 nm) and 7.58 (λ = 292 nm), respectively.

A gas chromatographic validated method was used for determination of cholesterol in egg yolks (Gąsior & Pietras, 2013). The method involves KOH (60 g/100 ml) saponification and hexane extraction of the cholesterol. Analysis was performed in isothermal conditions (265°C) on Shimadzu GC 2010Plus gas chromatograph, on a column (30 m, i. D. = 0.25 mm, df = 0.25 μm) with 5% phenyl, 95% dimethylpolysiloxane phase, at helium flow, 1.9 ml/min, and FID detector temperature, 300°C. Cholesterol standard 5-Cholest-3-α-ol, C₂₇H₄₆O, for chromatography (Sigma-Aldrich, USA) and internal standard 5 α-cholestanol, C₂₇H₄₈O, >97% (Sigma-Aldrich, USA) for quantification was used.

Higher fatty acids in egg yolks were quantified as methyl esters by gas chromatography. The samples were prepared
according to Folch, Lee, and Sloan-Stanley (1957) whereby the sample was homogenized in chloroform: methanol in a 2:1 ratio, the solvent was evaporated, and evaporation residue was saponified (0.5 N NaOH in methanol) and esterified (BF₃ in methanol). The fatty acid methyl esters were extracted in hexane and analysed with a VARIAN 3400 gas chromatograph, using a column filled with acid-modified polyethylene glycol (e.g. Zebron ZB-Wax 30 m), an 8200 CX autosampler and data processing software.

The measurement of albumen and yolk pH was performed with a Hanna Instruments pH-meter.

**Foaming properties**

Foaming properties were evaluated by foaming capacity (FC) and foam stability (FS) based on the method described by Ferreira, Benringer, and Jost (1995) with some modification. Foams were processed by whipping 100 ml of albumen for 3 min at 20°C by a mixer operating at 1220 rpm. The volumes of the foam and the drained liquid were assessed just after whipping and during holding up for 30 min at 20°C. For the determination of FC and FS the following formulae are used:

\[
\text{FC\%} = \left( \frac{\text{FV}}{\text{ILV}} \right) \times 100\%
\]

\[
\text{FS\%} = \left( \frac{\text{ILV} - \text{DV}}{\text{ILV}} \right) \times 100\%
\]

where: FV – volume of foam; ILV – volume of the initial liquid phase; DV – volume of drainage.

**Statistical analysis**

The results were statistically analysed by STATISTICA 12 PL. The results for the effect of layer genotype and age on egg quality traits (e.g., egg weight, shape and proportion of morphological components, eggshell traits, interior egg traits) were subjected to a two-way analysis of variance. The main effects (G – effect of genotype, A – effect of age) and the effect of G × A interaction were determined. The results for the effect of layer genotype on egg quality traits (foaming properties and pH of egg albumen and yolk, content of vitamins A and E and cholesterol in egg yolk) were subjected to a one-way analysis of variance. Significant differences between the means in groups were estimated by Duncan’s test.

Differences were considered as significant if \( P < 0.05 \), and highly significant if \( P < 0.01 \). The results for the effect of layer genotype and age on the number of eggs with meat and blood spots were determined by Kruskal-Wallis nonparametric test, and the frequency of meat and blood spots was expressed in per cent.

**Results and discussion**

**Egg weight**

The present study showed that layer genotype and age had an effect on egg weight. Hy-Line Brown commercial layers produced heavier eggs than Araucana and Greenleg Partridge hens (\( P < 0.05 \)) (Table 1). Also, Lukanov, Genchev, and Pavlov (2015, 2016) observed that White Leghorn and Rhode Island Red commercial layers produced heavier eggs than Araucana hens and native Dutch Schijndelaar hens. Pintea, Dulf, Bunea, Matea, and Andrei (2012) found that under organic farming conditions Araucana hens laid significantly smaller eggs compared to Isa Brown hens. In the study by Millet et al. (2006), Araucana hens produced smaller eggs compared to ISA Brown and Lohmann Selected Leghorn hens. The effect of genotype on the weight of organic eggs was also reported by Hammershøj and Steenfeldt (2015), who observed that genetically raised Lohmann Silver hens laid heavier eggs than New Hampshire hens. In our study, layers tended to produce heavier eggs with advancing age (\( P < 0.05 \)). Our results correspond with the findings of other authors, who showed that egg weight depends on the age of the layers (Ferrante, Loi, Vezzoli, & Guidobono Cavalcini, 2009; Samiullah, Omar, Roberts, & Chousalkar, 2017; Simčić, Stibilj, & Holcman, 2009).

**Shape index**

Our study demonstrated the effect of genotype on egg shape index (\( P < 0.05 \)). The shape index of eggs from Hy-Line Brown commercial layers was higher compared to Araucana and Greenleg Partridge hens. The literature provides varying results for the effect of genotype on the egg shape index. Hanusová, Hrnčár, Hanus, and Oravcová (2015) found no significant differences in shape index between the eggs from Rhode Island Red and Oravka hens. In contrast, Krawczyk (2009) and Calik (2011) reported that this trait differed significantly between the native hens and the strains of hens from a domestic pedigree farm. Our study found no effect of layer age on egg shape index (\( P > 0.05 \)), whereas the study by Nikolova and Kocevski (2006) revealed that Rosa Brown hens aged less than 45 weeks produced more rounded eggs, and hens older than 45 weeks laid more elongated eggs.

**Percentage constituent of morphological elements in eggs**

Our study showed that genotype but not the age of laying hens had an effect on shell percentage, albumen and yolk percentage in the egg (Table 1). Eggs obtained from Green-legged Partridge and Araucana hens were characterized with higher yolk content than in eggs from commercial Hy-line Brown hens. Likewise, Biesiada-Orzazga et al. (2014) found the yolk weight and percentage content to be higher in Araucana compared to Greenleg Partridge eggs. Smaller egg weight and greater yolk percentage in eggs from native hens in contrast with commercial breeds were also reported by Simčić et al. (2009) and Sokolowicz, Krawczyk, and Dykiel (2018).

**Shell color**

Shell color is an important external characteristic of an egg. Our study showed that shell color depends on layer genotype. Eggshells from Araucana hens were greenish, eggs from Greenleg Partridge hens were cream colored, and those from Hy-Line Brown hens were brown. According to Samiullah, Roberts, and Chousalkar (2016), shell color is a genetically determined trait, the gene responsible for pigment synthesis has been identified, but the amount of pigment deposited depends, among others, on hen breed and age. The eggshell color intensity (\( L^* \)) in hens of all the
studied breeds decreased with age of the layers (Table 2).
Our results are consistent with the findings of Zita, Tumova, and Stolc (2009). Also, Nedup and Phurba (2014) showed that shell color depends on the breed and its intensity on layer age. The decrease in shell color intensity with bird age can be linked to an increase in egg size without a proportionate increase in pigment deposition on the surface of the shell (Odabasi, Miles, Balaban, & Portier, 2007).

**Eggshell quality**

Our study showed the effect of genotype on shell characteristics related to breaking strength, namely shell weight, thickness, and density, which are important in commercial handling and transport. On all evaluation dates, the shell weight of eggs from Hy-Line Brown hens was higher than that from Greenleg Partridge and Araucana hens \( (P < 0.01) \). Eggshell thickness was greatest in Araucana hens and smallest in Greenleg Partridge hens \( (P < 0.05) \). The shells of eggs from Hy-Line Brown hens were characterized by higher density compared to the birds of the other breeds. Eggshell strength in the hens of all the studied breed was similar on all evaluation dates \( (P > 0.05) \). Other studies concerning the influence of breed on different shell quality traits present varying results. Van Den Brand, Partmentier, and Kemp (2004) and Zita et al. (2009) failed to show the effect of hen breed on shell thickness, whereas Hanusová et al. (2015), who investigated the quality of eggs from Orávka and Rhode Island Red hens, found the eggs to differ in shell thickness. Krawczyk (2009) reported that eggshells from native Greenleg Partridge hens had lower weight, density and thickness compared to the eggs of Messa 45 commercial crosses, but these traits improve with layer age. Also in the study by Suijiwo et al. (2017), eggs from commercial layers were characterized by lower shell thickness compared to eggs from crossbred blue hens. Hammershøj and Steenefeldt (2015) reported the effect of genotype on shell strength parameters of eggs from organically raised Lohmann Silver and New Hampshire hens. There was no effect of layer age on shell weight and thickness \( (P > 0.05) \). The shell density of eggs from hens of the studied breeds was observed to decrease with layer age \( (P < 0.05) \) (Table 2). As the hens aged, shell strength showed a downward trend, but the effect of age on this trait was not significant \( (P > 0.05) \). Wang et al. (2009) observed shell percentage to decrease, and shell thickness to increase with hens age. The effect of age on shell thickness was also reported by Küçüköymüz, Bozkurt, Nur Herken, and Çinar (2012). The tendency for decreased eggshell strength in our study may be associated with increased egg weight, which was not paralleled by a corresponding increase in shell weight, with a reduction in calcium and phosphorus availability, and with slower mineralization of the eggshell in older layers, as reported by Lichovníková and Zeman (2008). A deterioration in eggshell quality parameters with the age of laying hens was also observed by Calik (2011).

**Interior egg traits**

In our study, we found that genotype has an effect on Haugh units and albumen height. On all evaluation dates, Haugh score for the albumen of Araucana eggs was lower than for the egg albumen of the other hen breeds \( (P < 0.01) \). Albumen height was highest for the eggs of Hy-Line Brown hens, and lowest for the eggs of Araucana hens \( (P < 0.01) \) on all evaluation dates. Suijiwo et al. (2017) found no effect of genotype on Haugh units and albumen height in eggs from commercial Hy-Line Brown and crossbred blue hens. Wang et al. (2009) and Dudek and Rabszyn (2011) observed Haugh units and albumen height to decrease with the age of the layers. A similar but non-

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**Table 1. Egg weight, shape and proportion of morphological components.**

| Parameters \(^1\) | Layer age (wks) | Genotype | Effect\(^2\) |
|------------------|----------------|----------|-------------|
|                  |                | Greenleg Partridge | Araucana | Hy-Line Brown | SEM | G  | A | GaA |
| Egg weight (g)   | 26             | 52.45 ± 4.28\(^b\) | 51.87 ± 3.50\(^b\) | 59.99 ± 3.36\(^b\) | 0.049 | * | * | 0.72 |
|                  | 42             | 55.94 ± 3.30\(^b\) | 55.96 ± 7.63\(^b\) | 60.53 ± 6.55\(^b\) | *  | * | 0.05 |
|                  | 56             | 56.70 ± 4.33\(^b\) | 56.23 ± 6.97\(^b\) | 62.04 ± 5.14\(^b\) | *  | * | 0.05 |
| Shape index (%)  | 26             | 74.31 ± 3.14 | 74.69 ± 1.65 | 75.92 ± 2.58 | 0.25 | *  | 0.12 | 0.65 |
|                  | 42             | 74.24 ± 2.40 | 74.34 ± 3.34 | 74.92 ± 2.44 | *  | * | 0.05 |
|                  | 56             | 72.94 ± 4.97 | 72.70 ± 3.04 | 75.38 ± 3.02 | *  | * | 0.05 |
| Percentage in egg: |                |            |            |            |            |
| - shell (%)      | 26             | 10.90 ± 1.76\(^b\) | 11.52 ± 1.64\(^ab\) | 12.38 ± 1.33\(^b\) | 0.13 | *  | 0.52 | 0.93 |
|                  | 42             | 10.88 ± 1.78\(^b\) | 11.03 ± 1.61\(^b\) | 12.06 ± 1.83\(^b\) | *  | * | 0.05 |
|                  | 56             | 10.69 ± 1.66\(^b\) | 10.96 ± 1.00\(^b\) | 11.96 ± 1.20\(^b\) | *  | * | 0.05 |
| - albumen (%)    | 26             | 58.20 ± 4.62 | 58.74 ± 4.66 | 60.77 ± 2.85 | 0.33 | *  | 0.85 | 0.33 |
|                  | 42             | 58.17 ± 3.23 | 58.67 ± 4.85 | 60.29 ± 2.97 | *  | * | 0.05 |
|                  | 56             | 58.04 ± 4.40 | 58.55 ± 4.18 | 59.59 ± 3.02 | *  | * | 0.05 |
| - yolk (%)       | 26             | 30.90 ± 2.19\(^a\) | 29.80 ± 3.46\(^a\) | 26.86 ± 2.33\(^b\) | 0.28 | *  | 0.97 | 0.29 |
|                  | 42             | 30.95 ± 2.19\(^a\) | 30.23 ± 4.09\(^ab\) | 27.75 ± 2.36\(^b\) | *  | * | 0.05 |
|                  | 56             | 31.27 ± 3.70\(^ab\) | 30.49 ± 3.54\(^a\) | 28.35 ± 2.23\(^b\) | *  | * | 0.05 |

\(^1\) n = 30 eggs per group.

\(^2\) Effect: G – genotype, A – layer age, * significant effect \( (P < 0.05) \);

\( a, b \) values in rows with different letters differ significantly \( (P < 0.05) \);

\( A, B \) values in rows with different letters differ significantly \( (P < 0.01) \);

\( X, Y, Z \) values in columns with different letters differ significantly within a trait \( (P < 0.01) \).
Eggs from hens of all the studied breeds showed the highest least intensive yolk color, by Hy-Line Brown hens (56 weeks of age, eggs with the most intensive yolk color to brown-feathered hens. In the study by Pintea et al. 2012), yolk weight from Isa Brown eggs, but egg yolk percentage was significantly higher for Araucana compared to Isa Brown hens. Rizzi and Marangon (2010) found that the effect of layer age on albumen height corresponds with the results of Van Den Brand et al. (2008) and Nonga, Kajuna, Ngowi, and Karimuribo (2010) reported the effect of hen breed on egg quality and found the number of blood and meat spots to be higher in brown – compared to white-shelled eggs. Smith and Musgrave (2008) and Nonga, Kajuna, Ngowi, and Karimuribo (2010) report that blood spots are caused by ovipositional bleeding and their occurrence in the egg is influenced, among others, by genetic factors and layer age. In our study, we showed the effect of layer genotype and age on egg yolk color. At 26, 42 and 56 weeks of age, eggs with the most intensive yolk color were laid by Greenleg Partridge hens, and those with the least intensive yolk color, by Hy-Line Brown hens ($P < 0.05$). Eggs from hens of all the studied breeds showed the highest yolk color intensity at 56 weeks of age and the lowest intensity at 42 weeks of age ($P < 0.01$). Eggs from Greenleg Partridge hens had more intensive yolk color compared to the commercial hens, which could be associated with the fact that these hens used outdoor runs more frequently and spent more time forage eating than the commercial hens. The more intensive yolk color in the initial and final period of egg production in our study can be linked to the outdoor access, where layers were able to consume grass and herbs during the autumn (week 26) and spring (week 56). Hammershøj and Johansen (2016) stated that yolk color is largely influenced by the grasses and herbs consumed on the free-range. The less intensive yolk color during the winter period (week 42) can be attributed to the shorter time spent by the layers on the free-range, adverse weather conditions (snowfall), and less food available on the free-range. **Foaming capacity and pH** One of the important functional properties of egg albumen is the foaming capacity. Based on its excellent foaming ability, egg white is widely used in a variety of food products, including cakes and pies. For these food products, foams provide unique and desirable textures and largely affect the final quality. Thus, foaming properties of egg white are greatly desired for food industry. Foam comprised of millions of bubbles each encapsulated by a protein film and separated by a thin water filled lamella. Egg white foam is created as liquid egg whites are whipped. During this process, air comes into
the solution to form bubbles, white proteins adsorb rapidly at the air–water interface and undergo rapid conformational changes to form cohesive viscoelastic films around bubbles (Xiang et al., 2017). The basic parameter used to assess foam quality is foam stability. Foam stability is related to the properties of interfacial films surrounding air bubbles, in terms of their strength. Our study showed no effect of layer genotype on the functional properties of egg albumen. Egg albumen foaming capacity and stability in hens of the studied breeds and commercial Hy-Line Brown hens were similar (P > 0.05) (Table 4). In our study, we found the effect of hen genotype on albumen pH. pH value was lowest for eggs from commercial Hy-Line Brown layers and highest for Greenleg Partridge eggs (P < 0.05). Also, Hammershøj and Steenfeldt (2015) showed the effect of genotype on pH of egg albumen from organically raised hens. The yolks of eggs from the studied hen breeds were characterized by similar pH (P > 0.05). No significant differences in yolk pH between the eggs from Hy-Line Brown and hybrid blue hens were also reported by Sujiwo et al. (2017).

Table 4. Foaming properties and pH of egg albumen and yolk.

| Parameters | Greenleg Partridge | Araucana | Hy-Line Brown | SEM | G | A | GaX |
|------------|--------------------|----------|---------------|-----|---|---|-----|
| Foaming capacity (%) | 550 ± 81.03 | 497 ± 71.28 | 478 ± 38.34 | 17.45 |
| Foam stability (%) | 98.69 ± 0.95 | 98.59 ± 0.51 | 98.36 ± 0.90 | 0.20 |
| pH of albumen | 8.76 ± 0.34 | 8.68 ± 0.15 | 8.54 ± 0.13 | 0.03 |
| pH of yolk | 6.24 ± 0.22 | 6.27 ± 0.32 | 6.32 ± 0.21 | 0.04 |

Table 3. Interior egg traits.

| Parameters | Greenleg Partridge | Araucana | Hy-Line Brown | SEM | G | A | GaX |
|------------|--------------------|----------|---------------|-----|---|---|-----|
| Albumen height (mm) | 26 | 7.96 ± 1.34 | 5.93 ± 0.54 | 8.37 ± 1.14 | 0.13 | * | 0.39 | 0.97 |
| | 42 | 7.84 ± 1.18 | 5.86 ± 0.88 | 8.05 ± 1.00 | 0.00 |
| | 56 | 7.69 ± 1.16 | 5.74 ± 0.84 | 7.77 ± 1.94 | 0.00 |
| Haugh units (HU) | 26 | 89.06 ± 7.60 | 76.13 ± 3.68 | 89.94 ± 6.30 | 0.77 | * | 0.52 | 0.88 |
| | 42 | 88.88 ± 6.19 | 76.37 ± 8.50 | 89.21 ± 4.85 | 0.00 |
| Yolk weight (g) | 26 | 16.92 ± 0.93 | 15.38 ± 1.30 | 16.08 ± 1.29 | 0.11 | * | * | 0.58 |
| | 42 | 17.28 ± 1.11 | 16.69 ± 1.45 | 17.06 ± 1.23 | 0.00 |
| | 56 | 17.66 ± 1.77 | 16.97 ± 1.48 | 17.47 ± 0.95 | 0.00 |
| Blood spots (%) | 26 | x1.00 ± 0.00 | 0.00 ± 0.00 | 11.76 ± 33.21 | 2.86 | * | * | * |
| | 42 | x1.00 ± 0.00 | 0.00 ± 0.00 | 6.67 ± 25.82 | 0.00 |
| | 56 | x1.00 ± 0.00 | 0.00 ± 0.00 | 23.81 ± 43.64 | 0.00 |
| Meat spots (%) | 26 | 0.00 ± 0.00 | 0.00 ± 0.00 | 17.65 ± 39.30 | 2.60 | * | 0.43 |
| | 42 | 0.00 ± 0.00 | 0.00 ± 0.00 | 20.00 ± 41.00 | 0.00 |
| | 56 | 0.00 ± 0.00 | 0.00 ± 0.00 | 42.86 ± 50.71 | 0.12 | * | * | * |
| Yolk color (DSM) | 26 | x1.00 ± 0.00 | x1.00 ± 0.00 | x7.41 ± 1.77 | 0.12 | * | * | * |
| | 42 | x1.00 ± 0.00 | x1.00 ± 0.00 | x7.27 ± 1.03 | 0.00 |
| | 56 | x1.00 ± 0.00 | x1.00 ± 0.00 | x10.24 ± 0.54 | 0.00 |

Table 3. Rasgos del interior del huevo.

| Parameters | Genotipo | Hy-Line Brown | SEM | G | A | GaX |
|------------|----------|---------------|-----|---|---|-----|
| Albumen altura (mm) | 26 | 7.96 ± 1.34 | 5.93 ± 0.54 | 8.37 ± 1.14 | 0.13 | * | 0.39 | 0.97 |
| | 42 | 7.84 ± 1.18 | 5.86 ± 0.88 | 8.05 ± 1.00 | 0.00 |
| | 56 | 7.69 ± 1.16 | 5.74 ± 0.84 | 7.77 ± 1.94 | 0.00 |
| Altura de la yema (HU) | 26 | 89.06 ± 7.60 | 76.13 ± 3.68 | 89.94 ± 6.30 | 0.77 | * | 0.52 | 0.88 |
| | 42 | 88.88 ± 6.19 | 76.37 ± 8.50 | 89.21 ± 4.85 | 0.00 |
| Peso de la yema (g) | 26 | 16.92 ± 0.93 | 15.38 ± 1.30 | 16.08 ± 1.29 | 0.11 | * | * | 0.58 |
| | 42 | 17.28 ± 1.11 | 16.69 ± 1.45 | 17.06 ± 1.23 | 0.00 |
| | 56 | 17.66 ± 1.77 | 16.97 ± 1.48 | 17.47 ± 0.95 | 0.00 |
| Coeficiente de sangre (%) | 26 | 0.00 ± 0.00 | 0.00 ± 0.00 | 11.76 ± 33.21 | 2.86 | * | * | * |
| | 42 | 0.00 ± 0.00 | 0.00 ± 0.00 | 6.67 ± 25.82 | 0.00 |
| | 56 | 0.00 ± 0.00 | 0.00 ± 0.00 | 23.81 ± 43.64 | 0.00 |
| Coeficiente de carne (%) | 26 | 0.00 ± 0.00 | 0.00 ± 0.00 | 17.65 ± 39.30 | 2.60 | * | 0.43 |
| | 42 | 0.00 ± 0.00 | 0.00 ± 0.00 | 20.00 ± 41.00 | 0.00 |
| | 56 | 0.00 ± 0.00 | 0.00 ± 0.00 | 42.86 ± 50.71 | 0.12 | * | * | * |
| Color de la yema (DSM) | 26 | x1.00 ± 0.00 | x1.00 ± 0.00 | x7.41 ± 1.77 | 0.12 | * | * | * |
| | 42 | x1.00 ± 0.00 | x1.00 ± 0.00 | x7.27 ± 1.03 | 0.00 |
| | 56 | x1.00 ± 0.00 | x1.00 ± 0.00 | x10.24 ± 0.54 | 0.00 |

The content of vitamins, cholesterol and fatty acids in yolk

The content of vitamins, cholesterol and fatty acids in yolk has an effect on the nutritive value of eggs. The study by Bunea et al. (2017) evidenced that the chemical composition of yolk varies widely according to hen breed. Our study demonstrated the effect of genotype on vitamin A content, whereas the effect of genotype on vitamin E content was not significant (Table 5). The vitamin A content of yolk from Hy-Line Brown eggs was lower than in the yolk of Greenleg Partridge eggs (P < 0.05). The higher content of vitamin A in egg yolks from Greenleg Partridge hens may be due to the fact that the hens of this breed were more willing to use free-range and spent more time foraging compared to the commercial hens; therefore, it can be assumed that on the free-range they ingested more carotenoids, which had a favourable effect on the vitamin A content of egg yolk, because in the layer's body part of β-carotene is converted to vitamin A (Hencken, 1992). Vitamin E is one of the most efficient natural antioxidants. According to Bunea et al. (2017), α-tocopherol content is correlated to the total carotenoid content on the free-range. Our study did not show any effect of genotype on cholesterol concentration in egg yolk (Table 5). Also, Pintea et al. (2012) observed that organically raised Araucana hens produced eggs with a similar cholesterol content to Isa Brown hens. Our results fail to
Table 5. Contenido de vitaminas A y E y de colesterol en la yema de huevo.

| Parameters | Greenleg Partridge | Araucana | Hy-Line Brown | SEM |
|------------|-------------------|---------|--------------|-----|
| vitamin A (µg/g) | 6.23 ± 0.40<sup>a</sup> | 7.86 ± 0.55<sup>b</sup> | 5.68 ± 0.55<sup>b</sup> | 0.11 |
| vitamin E (µg/g) | 76.61 ± 10.03 | 78.95 ± 10.41 | 71.36 ± 4.41 | 2.12 |
| cholesterol (mg/g) | 14.21 ± 0.24 | 14.26 ± 0.18 | 14.60 ± 0.31 | 0.09 |

<sup>1</sup>n = 5 muestras por grupo (genotipo); 1 muestra = 3 yemas.

<sup>a,b</sup> Los valores en las filas con distintas letras son significativamente diferentes (<i>P</i> < 0.05).

In their study, the concentration of cholesterol was higher in the yolks of eggs from local breeds of hens compared to the eggs from the commercial hybrids. Wang et al. (2009) reported that Araucana hens lay eggs with a lower cholesterol content compared to commercial layers, while Millet et al. (2006) noted higher cholesterol content in the yolks of Araucana eggs compared to the yolks of eggs commercial hens. Similar cholesterol content in the yolk of eggs from different genotypes support previous findings (Krawczyk, Sokolowicz, & Szymczyk, 2011), which showed lower cholesterol content in the yolks of eggs from the native Greenleg Partridge breed compared to the yolks of eggs from hens of other breeds. Rizzi and Marangoz (2012) also demonstrated that genotype has an effect on the level of egg yolk cholesterol.

Table 6. Perfil de ácidos grasos de la yema de huevo.

| Fatty acids<sup>1</sup> | Greenleg Partridge | Araucana | Hy-Line Brown | SEM |
|-------------------------|--------------------|---------|--------------|-----|
| SFA                     | 0.05 ± 0.07<sup>a</sup> | 0.07 ± 0.04<sup>a</sup> | 0.00 ± 0.00<sup>b</sup> | 0.02 |
| C12                     | 0.07 ± 0.03<sup>a</sup> | 0.04 ± 0.02<sup>a</sup> | 0.13 ± 0.04<sup>b</sup> | 0.01 |
| C14                     | 0.82 ± 0.09<sup>a</sup> | 0.70 ± 0.11<sup>ab</sup> | 0.65 ± 0.06<sup>b</sup> | 0.03 |
| C16                     | 31.41 ± 0.59<sup>a</sup> | 30.52 ± 1.72<sup>a</sup> | 31.06 ± 1.16<sup>a</sup> | 0.31 |
| C18                     | 7.16 ± 0.17<sup>a</sup> | 8.86 ± 0.36<sup>b</sup> | 7.85 ± 0.49<sup>a</sup> | 0.20 |
| C20                     | 0.02 ± 0.00<sup>a</sup> | 0.03 ± 0.00<sup>ab</sup> | 0.00 ± 0.00<sup>b</sup> | 0.003 |
| C22                     | 0.03 ± 0.03<sup>ab</sup> | 0.07 ± 0.02<sup>a</sup> | 0.01 ± 0.04<sup>b</sup> | 0.01 |
| MUFA                    | 5.39 ± 0.98<sup>a</sup> | 3.51 ± 0.93<sup>b</sup> | 5.33 ± 0.48<sup>a</sup> | 0.31 |
| C18:1                   | 40.81 ± 1.17<sup>a</sup> | 41.35 ± 0.58<sup>a</sup> | 42.60 ± 0.26<sup>a</sup> | 0.32 |
| C22:1                   | 0.01 ± 0.00<sup>a</sup> | 0.01 ± 0.000<sup>a</sup> | 0.01 ± 0.000<sup>a</sup> | 0.003 |
| PUFA n-3                | 0.07 ± 0.05<sup>a</sup> | 0.10 ± 0.04<sup>a</sup> | 0.08 ± 0.04<sup>a</sup> | 0.01 |
| C18:1                   | 0.68 ± 0.17<sup>a</sup> | 0.62 ± 0.17<sup>a</sup> | 0.50 ± 0.03<sup>a</sup> | 0.04 |
| C20:4                   | 1.14 ± 0.16<sup>ab</sup> | 0.94 ± 0.10<sup>a</sup> | 0.05 ± 0.01<sup>b</sup> | 0.05 |
| CLAc9-t11               | 0.51 ± 0.31<sup>ab</sup> | 0.61 ± 0.03<sup>a</sup> | 0.05 ± 0.02<sup>b</sup> | 0.08 |
| CLAc9-t11               | 0.06 ± 0.06<sup>ab</sup> | 0.06 ± 0.01<sup>a</sup> | 0.12 ± 0.05<sup>a</sup> | 0.01 |
| Σ                        | 39.54 ± 0.81<sup>a</sup> | 40.29 ± 1.91<sup>a</sup> | 39.70 ± 1.29<sup>a</sup> | 0.37 |
| MUFA                    | 0.06 ± 0.02<sup>a</sup> | 0.07 ± 0.02<sup>a</sup> | 0.10 ± 0.04<sup>a</sup> | 0.01 |
| C20:4                   | 1.16 ± 0.03<sup>a</sup> | 1.32 ± 0.04<sup>a</sup> | 1.16 ± 0.03<sup>a</sup> | 0.47 |
| PUFA n-3                | 1.93 ± 0.21<sup>a</sup> | 1.79 ± 0.33<sup>a</sup> | 1.10 ± 0.24<sup>a</sup> | 0.13 |
| CLA                     | 0.62 ± 0.03<sup>a</sup> | 0.70 ± 0.02<sup>a</sup> | 0.22 ± 0.03<sup>a</sup> | 0.08 |
| DHA                     | 67.61 ± 0.75<sup>a</sup> | 68.57 ± 1.78<sup>a</sup> | 68.15 ± 1.24<sup>a</sup> | 0.33 |
| SFA                     | 32.39 ± 0.58<sup>a</sup> | 31.43 ± 1.76<sup>a</sup> | 31.85 ± 1.24<sup>a</sup> | 0.26 |
| DHA/OFAs                | 1.53 ± 0.05<sup>a</sup> | 1.49 ± 0.12<sup>a</sup> | 1.52 ± 0.08<sup>a</sup> | 0.02 |
| MUFA/SFA                | 0.03 ± 0.09<sup>a</sup> | 0.37 ± 0.09<sup>a</sup> | 0.32 ± 0.03<sup>a</sup> | 0.02 |
| MUFA/SFA                | 0.08 ± 0.08<sup>a</sup> | 7.19 ± 3.03<sup>a</sup> | 10.56 ± 2.23<sup>a</sup> | 0.80 |

<sup>1</sup>n = 5 yemas por grupo (genotipo).

<sup>a,b</sup> Los valores en las filas con distintas letras son significativamente diferentes (<i>P</i> < 0.05).
commercial hens and from hybrid blue hens was reported by Suijwio et al. (2017). Wang et al. (2009) found that blue-shelled eggs from Dongxiang hens had greater yolks and lower cholesterol content. Fatty acid profile of the human diet is essential to the prevention of cardiovascular diseases. Dietary fatty acids, in particular EPA and DHA, are recommended by food agencies around the world (DiMarco et al., 2017; Lamas et al., 2016). In our study, the main fatty acids in the egg yolks of all the hen breeds under study were oleic (C18:1), palmitic (C16:0) and linoleic acids (C18:2). Also, Hidalgo, Rossi, Clerici, and Ratti (2008) showed that the principal yolk fatty acids are oleic, palmitic, linoleic and stearic acids. The yolk fatty acid profile in the analysed eggs was characterized by a relatively low proportion of saturated fatty acids (SFA), which was accompanied by a high proportion of unsaturated fatty acids (UFA). In our study, we showed the effect of genotype on the fatty acid profile of egg yolk (Table 6). The highest concentration of monounsaturated fatty acids (MUFA) was characteristic of yolks from Hy-Line Brown eggs. The egg yolks from Hy-Line Brown hens contained no caprylic acid (C8) and arachidic acid (C20), which were found in the egg yolks from the other breeds. The yolk of eggs from Greenleg Partridge hens had a higher content of docosahexaenoic acid (DHA) compared to the egg yolk of Hy-Line Brown hens (P < 0.05). The presence of EPA acids was found in the egg yolk from Greenleg Partridge and Araucana hens, but not in the egg yolk from Hy-Line Brown hens (P < 0.01). The yolk of eggs from Hy-Line Brown hens contained a lower percentage of n-3 PUFA compared to the Greenleg Partridge and Araucana eggs (P < 0.05). The content of CLA in egg yolks from Hy-Line Brown hens was lower compared to Greenleg Partridge and Araucana egg yolks (P < 0.01). The PUFA n-6/n-3 ratio was more beneficial in the egg yolk of Greenleg Partridge and Araucana hens compared to Hy-Line Brown hens. Pintea et al. (2012) demonstrated that egg yolk lipids in Araucana hens had a lower SFA content, a higher MUFA, EPA and DHA content, and a more favourable n-6/n-3 ratio than the egg yolk lipids of ISA Brown hens. Stanić et al. (2015) did not confirm the effect of breed on the percentage of n-3 fatty acids. Rizzi and Maranong (2012) showed the effect of breed on the fatty acid profile of yolks in organic eggs and found the egg yolks from Hy-Line Brown hens to contain less saturated fatty acids, less monounsaturated fatty acids and more polyunsaturated fatty acids compared to the eggs of the other breeds. In the same study, egg yolks from Hy-Line Brown hens also showed a higher n-6/n-3 ratio compared to the eggs from the other hen breeds. Millet et al. (2006) determined the fatty acid content in Araucana eggs compared to Isa Brown eggs. In the Araucana eggs, they found more SFA and more UFA, as well as less PUFA. However, the Araucana eggs compared with the Isa Brown eggs contained less n-6 PUFA and slightly less n-3 PUFA. The n-6/n-3 ratio was also similar amongst the eggs of the two breeds. In the opinion of Hammershøj and Johansen (2016), the fatty acid profile is largely determined by grasses and herbs consumed on the free-range.

Conclusions
The outcomes of the study are likely to be of practical significance for both consumers and producers of organic eggs. Producers can make use of the knowledge genotype impacts of various quality properties of eggs match the choice of layers to consumers’ expectation in respect of eggs. In response to consumers expectations regarding eggs with enhanced nutritive values (higher proportions of EPA, DHA acids as well as a more benefiting ratio of n-6/n-3 acids), it is recommended that the breeding of Greenleg Partridge or Araucana layers in organic farms should be encouraged. However, if market surveys indicate that customers pay higher attention to egg weight then the commercial Hy-line Brown layers should be preferred. A significant information gained from the study for the needs of consumers and producers is that cholesterol levels in yolks from Greenleg Partridge, Araucana and Hy-Line Brown hens held in organic farms is similar.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
Biesiada-Drzazga, B., Banaszewska, D., Andraszek, K., Bombik, E., Kaluza, H., & Rojek, A. (2014). Comparison of egg quality of free range Araucana and Green-legged partridge chickens. European Poultry Science, 78, 8.
Bunea, A., Copaciu, F. M., Paşca, D., Dulf, F., Rugină, D., Chira, R., & Pintea, A. (2017). Chromatographic analysis of lyophilic compounds in eggs from organically fed hens. The Journal of Applied Poultry Research, 26(4), 498–508.
Calik, J. (2011). Ocena jakości jaj sześciu rodów kur niesących w zależności od ich wieku [Assessing the quality of eggs produced by six breeds of egg-laying hens in relation to their age]. Zywność Nauko Technologia Jakost, 85, 85–93.
DiMarco, D. M., Missimer, A., Murillo, A. G., Lemos, B. S., Malysheva, O. V., Caudill, M. A., ..., Fernandez, M. L. (2017). Intake of up to 3 eggs/day increases HDL cholesterol and plasma choline while plasma trimethylamine-N-oxide is unchanged in a healthy population. Lipids, 52(3), 255–263.
Dudek, M., & Rabsztyn, A. (2011). Egg quality of dual-purpose hens intended for small-scale farming. Acta Scientarium Polonorum, Zootechnica, 10(1), 3–12.
EC. (2008). Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Official Journal of the European Communities, 203, 53–57.
Exler, J., Phillips, K. M., Patterson, K. Y., & Holden, J. M. (2013). Cholesterol and vitamin D content of eggs in the U.S. retail market. Journal of Food Composition and Analysis, 29, 110–116.
Ferrante, V., Lolli, S., Vezzoli, G., & Guidobono Cavaichini, L. (2009). Effects of different two rearing systems (organic and barn) on production performance, animal welfare traits and egg quality characteristics in laying hens. Italian Journal of Animal Science, 8, 165–174.
Ferreira, M., Benninger, R., & Jost, R. (1995). Instrumental method for characterizing protein foams. Journal of Food Science, 60, 90–93.
Flock, D. K., Preisinger, R., & Schmutz, M. (2001, September). Egg quality – A challenge for breeders of laying hens. In R. W. A. W. Mulder & S. F. Bilgili (Eds.), WPSC. Turkish Branch 2001. Proceedings of IX European Symposium on the Quality of Eggs and Egg Products (pp. 51–55). Kusadasi, Turkey: WPSC. Turkish Branch 2001.
Folch, J., Lee, M., & Sloan-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226(1), 497–509.
Gasior, R., & Pietras, M. (2013). Validation of a method for determining cholesterol in egg yolks. Annals of Animal Science, 12(1), 143–153.

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Hammershøj, M., & Johansen, N. F. (2016). Review: The effect of grass and herbs in organic egg production on egg fatty acid composition, egg yolk color and sensory properties. Livestock Science, 194, 37–43. Hammershøj, M., & Steenfeldt, S. (2015). Organic egg production. II. The quality of organic eggs is influenced by hen genotype, diet and forage material analyzed by physical parameters, functional properties and sensory evaluation. Animal Feed Science and Technology, 208, 182–197. Hanusová, E., Hrnčiar, E., Hanus, A., & Oravcová, M. (2015). Effect of breed on some parameters of egg quality in laying hens. Acta Phytotechnica and Zootecnica, 18(1), 20–24.

Hencken, H. (1992). Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. Poultry Science, 71, 711–717. Hidalgo, A., Rossi, M., Clerici, S., & Ratti, S. (2008). A market study on the quality characteristics of egg from different housing systems. Food Chemistry, 106, 1031–1038.

Hooge, D. M. (2007). Bacillus subtilis spores improve brown egg color. World Poultry, 23(3), 14–15.

Krawczyk, J. (2009). Effect of layer age and egg production level on changes in quality traits of eggs from hens of conservation breeds and commercial hybrids. Annals of Animal Science, 9(2), 185–193.

Krawczyk, J., Sokolowicz, 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. Archiv für Geflügelkunde, 75(3), 151–157.

Kučukyilmaz, K., Bozkurt, M., Nur Herken, E., & Çelik, M. (2012). Chemical and physiological behavior of feed carotenoids. CytA - Journal of Food, 14(2), 289–295.

Lichovníková, M., & Zeman, L. (2008). Effect of housing system on the calcium requirement of laying hens and on eggshell quality. Czech Journal of Food Sciences, 4, 162–168.

Lukanov, H., Genchev, A., & Pavlov, A. (2015). Egg quality and shell color characteristics of crosses between Araucana and Schijndelaar with highly productive White Leghorn and Rhode Island red strains. Agricultural Science and Technology, 7(3), 366–371.

Lukanov, H., Petrov, P., Genchev, A., Halil, E., & Ismail, N. (2016). Productive performance of easter egger crosses of Araucana and Schijndelaar roosters with White Leghorn hens. Trakia Journal of Sciences, 17, 72–79.

Millet, S., De Ceulaer, K., Van Paemel, M., Raes, K., De Smet, S., & Janssens, G. P. J. (2006). Lipid profile in egg of Araucana hens compared with Lohmann selected Leghorn and Isa Brown hens given diets with different fat sources. British Poultry Science, 47(3), 294–300.

Ndong, H. E., Kajuna, F. F., Ngowi, H. A., & Karimuribo, E. D. (2010). Physical egg quality characteristic of free-range local chickens in Morogoro municipality, Tanzania. Livestock Research for Rural Development, 22(12), 218–219.

Odabasi, A., Miles, R., Balaban, M., & Portier, K. (2007). Changes in brown eggshell color as the hen ages. Poultry Science, 86, 356–363.

Petersen, D. W., Lilyblade, A., Cliford, C. K., Ernst, R., Cliford, A. J., & Dunn, P. (1978). Composition of and cholesterol in Araucana and commercial eggs. Journal of the American Dietetic Association, 72, 45–47.

Pinte, A., Dulf, F. V., Bunea, A., Matea, C., & Andrei, S. (2012). Comparative analysis of lipophilic compounds in egg of organically raised Isi Brown and Araucana hens. Chemical Papers, 66, 955–963.

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

Samuliš, S., Omar, A. S., Roberts, J., & Chousalkar, K. (2017). Effect of production system and flock age on eggshell and egg internal quality measurements. Poultry Science, 96, 246–258.

Simčič, M., Stibilj, V., & Holcman, A. (2009). The cholesterol content of eggs produced by the Slovenian autochtonous Styrian hen. Food Chemistry, 114, 1–4.

Smith, D. P., & Musgrove, M. T. (2008). Effect of blood spots in table egg albumen on Salmonella growth. Poultry Science, 87, 1659–1661.

Sokolowicz, Z., Krawczyk, J., & Dykel, M. (2018). The effect of the type of alternative housing system, genotype and age of laying hens on egg quality. Annals of Animal Science, 18, 541–555.

Staniš, N., Petricević, V., Škrbić, Z., Lukić, M., Pavlovski, Z., Lillc, S., & Petricević, M. (2015). Effects of age and time of day of sampling on proximate and fatty acid composition of whole eggs from two strains of laying hens. Archives Animal Breeding, 58, 151–158.

Suijwo, J., Kim, D., Yoon, J., Kim, H., Kim, J., Lee, S., & Jang, A. (2017). Physicochemical and functional characterization of blue-shelled eggs in Korea. Korean Journal for Food Science of Animal Resources, 37(2), 181–190.

Van Den Brand, H., Parmentier, H. K., & Kemp, A. B. (2004). Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. British Poultry Science, 45(6), 745–752.

Wang, X. L., Zheng, J. X., Ning, Z. H., Xu, L. J., Xu, G. Y., & Yang, N. (2009). Laying performance and egg quality of blue-shelled layers as affected by different housing systems. Poultry Science, 88, 1485–1492.

World Watch List. (2000). World Watch List for domestic animal diversity. Rome: FAO.

Xiang, D., Li, J., Zhang, Q., Zhao, T., Li, M., Xu, X., & Liu, X. (2017). Effect of a multiple freeze-thaw process on structural and foaming properties of individual egg white proteins. Food Chemistry, 228, 243–248.

Zita, L., Turnova, E., & Stoic, L. (2009). Effects of genotype, age and their interaction on egg quality in brown egg laying hens. Acta Veterinaria Brno, 78, 85–91.