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Assessment of performance and egg quality in laying hens of Spanish indigenous breed Black Castellana as compared with a selected white egg-layer strain

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ABSTRACT Indigenous animal genetic resources should be preserved because of their well adaptation to the environment, their tolerance to low food availability and their sociocultural importance. The characterization of the quality of the products generated by heritage breeds may bring more arguments to encourage the raising of these animals. This study aimed at evaluating the egg performance and quality of Spanish indigenous Black Castellana (**BC**) breed as compared with a selected strain (Lohmann LSL-Classic). Four groups of 30 hens were arranged: 1) Lohmann hens fed a control diet; 2) BC hens fed the control diet; 3) Lohmann hens fed a diet including linseed at 70 g/kg (omega-3 diet); 4) BC hens fed the omega-3 diet. Egg production was higher by 12.3% for Lohmann hens but, since BC eggs were heavier by 15.4%, no effect of genetics was found on daily egg mass. Feed intake was higher by 5.0% for BC hens. Nonetheless, no difference was detected for feed conversion ratio. Eggshell was thicker by 6.78% in Lohmann eggs. Haugh units did not differ among freshly laid and stored eggs in Lohmann hens, whereas Haugh units decreased in stored BC eggs (80.5 vs. 76.7 vs. 72.3 at 0, 14, and 30 d of storage). Yolks of BC eggs contained less fat (57.5 vs. 60.8% DM), more protein (32.8 vs. 31.9% DM) and more cholecalciferol (1.25 vs. 1.22 μg/g DM), and showed lower proportion of saturated fatty acids (29.0 vs. 37.0%) and higher proportions of monounsaturated (45.7 vs. 39.6%) and polyunsaturated (25.2 vs. 23.4%) fatty acids. Feeding the omega-3 diet reduced the yolk proportions of saturated (32.5 vs. 33.5%) and monounsaturated (42.0 vs. 43.3%) fatty acids and increased those of polyunsaturated (25.4 vs. 23.2%) and ω-3 (7.05 vs. 2.42%) fatty acids. No effect due to genetics or diet was found on yolk color score or on yolk content in cholesterol, cobalamin, retinol and γ-tocopherol. This study represents the first exhaustive characterization of eggs from Spanish indigenous Black Castellana breed.

Key words: Black Castellana, indigenous breed, laying hen, egg quality, fatty acid

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INTRODUCTION

Spain displays a wide range of indigenous breeds for many species of domestic animals, in particular as regards poultry. According to the Official Catalogue of Livestock Breeds of Spain (Ministerio de Agricultura, Pesca y Alimentación, 2023), twenty indigenous breeds of chickens are currently recognized. Among these indigenous breeds, the Black Castellana (**BC**) hen, a white-egg layer registered in the Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organization of the United Nations (FAO, 2024), stands out for being one of the oldest Mediterranean chicken breeds. The BC breed is known to have originated other worldwide known chicken breeds like the Minorca breed and the White-Faced Spanish (Dávila et al., 2009), admitted, respectively, in the American Poultry Association as early as in 1888 and 1874 (Ekarius, 2007). As compared with commercial strains of white-egg layers, BC breed remains highly polymorphic as it has proven to possess higher heterozygosity and greater number of alleles per locus (Hillel et al., 2003; Dávila et al., 2009). The higher gene diversity across markers found in this indigenous unselected breed is of utmost importance as this finding entails that BC laying hens harbor a reservoir of alleles that can be useful both for future commercial and research purposes (Blackburn, 2006).

In the treatises on animal science of the nineteenth century (Tratado de aves de corral, 1857), BC layers

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were reported to be the hens with the highest egg-laying performance among the different Spanish chicken breeds (Orozco, 1986). However, since the 1960s this indigenous breed has been largely displaced in commercial farms by the selected hybrids that came along with the industrial development of the poultry sector. Consequently, the BC is currently considered a breed threatened of extinction (Ministerio de Agricultura, Pesca y Alimentación, 2023). Even lacking interest for intensive producers, indigenous unselected breeds should be preserved since they represent part of the heritage of a country (Mendelsohn, 2003; Marsoner et al., 2018) and because of their intrinsic genetic value. Indeed, the conservation of indigenous animal genetic resources is urged by target 2.5 of the Sustainable Development Goals of the 2030 Agenda (United Nations, 2015). Furthermore, consumers are increasingly demanding animal products originating from heritage breeds and obtained in non-industrial systems (Menger and Hamm, 2021; Tampaki et al., 2022). This type of animal production may provide a non-negligible niche market for indigenous poultry breeds like BC (Zander et al., 2013; Martin-Collado et al., 2014), since these rustic and locally well-adapted breeds are very suitable for generating quality chicken products in free-range systems (Lordelo et al., 2017).

In this sense, FAO's report on the State of the World's Animal Genetic Resources for Food and Agriculture (FAO, 2007a) brought out that in many cases the success in the conservation of indigenous threatened breeds has stemmed from the achievement of public recognition of the high organoleptic quality of the animal products originating from livestock of these breeds. Nevertheless, in order to gain this recognition, the performance of these animals and the quality of their products need to be objectively assessed. Indeed, the Global Plan of Action for Animal Genetic Resources of FAO (FAO, 2007b) emphasized that characterizing the performance of indigenous animal resources is essential when aiming at promoting the commercial use of these heritage breeds and in order to ensure their conservation. Despite this, to the authors' knowledge, hitherto no exhaustive characterization of the quality of eggs laid by BC hens has been published. The assessment of the nutritional composition of the eggs produced by the BC breed is all the more pertinent as previous research (Campo, 1995; González Ariza et al., 2021; Ianni et al., 2021) has shown that hen genetics influences several egg quality parameters such as yolk contents in protein, cholesterol and α-tocopherol, as well as yolk fatty acid profile.

There is general consensus on the diverse positive health effects of consuming ω-3 polyunsaturated fatty acids and on the need to increase their intake by the population of western countries (Swanson et al., 2012; Dempsey et al., 2023). The latter could be achieved by enriching with this type of fatty acids the foodstuffs most commonly consumed by people. Research conducted over the last 2 decades has proven that the enrichment of eggs with ω-3 fatty acids can be reached by feeding laying hens with sources of this kind of fat, among which linseed, fish oil or, more recently, microalgae (Fraeye et al., 2012). Nevertheless, all this research has so far been carried out with high-yielding selected strains of layers (Omri et al., 2019; Rey et al., 2021; Antony et al., 2024) and hence, the transfer of ω-3 fatty acids from the diet to the egg yolk has hitherto not been confirmed in heritage breeds of laying hens. The ability of converting linolenic acid into eicosapentaenoic acid and docosahexaenoic acid in the liver has been reported in different species of birds (Petzinger et al., 2014; Alagawany et al., 2019; Irawan et al., 2022) but still some differences due to genetics could exist in the hepatic synthesis of these long-chain fatty acids since the egg yolk fatty acid profile has been observed to vary among laying hens of different breeds fed the same diet (Ianni et al., 2021; Jiang et al., 2023). Furthermore, the hepatic conversion of linolenic acid into long-chain fatty acids has also been found to be influenced by the dietary concentration of linoleic and linolenic acid (Elkin et al., 2015). Thus, an interaction between diet composition and genetic type of hens could happen to occur. In summary, for farmers breeding BC hens, it would be useful to confirm whether their hens have also the ability to produce ω-3 enriched eggs when fed a diet containing linseed.

Therefore, the aim of the present research work was to assess productive performance and egg quality traits (yolk color score, shell thickness and proportion, albumen Haugh units, yolk contents in fat, crude protein, cholesterol and vitamins, and yolk fatty acid profile) in Black Castellana and Lohmann LSL-Classic laying hens reared under free-range conditions and fed 2 commercial diets (either a common diet for laying hens or a diet including linseed).

MATERIALS AND METHODS

Experimental Design

This study lasted 4 wk, starting on April 11th 2023, and was carried out in a commercial farm of free-range laying hens in Viloria (Valladolid, Spain). Sixty Lohmann LSL-Classic hens (average liveweight at the beginning of the study: 1,502 $\pm$ 17.1 g) and sixty BC hens (average liveweight at the beginning of the study: 1,789 $\pm$ 48.5 g) were used in this study. Black Castellana hens were all purebred (Figure 1) and recorded in the official pedigree book of the breed. The strain Lohmann LSL-Classic was chosen as control commercial strain because both Lohmann LSL-Classic and BC hens are light white-egg layers. When this study began, all hens had just started laying eggs 2 wk ago. Because of the different age at which Lohmann LSL-Classic and BC hens reach maturity, Lohmann hens were 22 wk old at the beginning of the study, whereas BC hens were 28 wk old. Nevertheless, all hens were at the same physiological state. The Ethics Committee of the Regional Government of Castilla y León (Spain) declared that no specific ethical approval was needed for this study since hens were kept under conventional conditions in a commercial free-range farm with no samples being taken on the birds and with no bird having been sacrificed during the trial. This research work only implied the collection of eggs on which several quality parameters were measured. Nonetheless, hens were handled at all times in

Figure 1. Laying hens aged 26 weeks of Spanish indigenous breed Black Castellana (Photo Credit: C. Romero).

keeping with the principles for the Care and Use of Animals for Scientific Purposes of the Ministry of Agriculture, Fishery and Food.

Two commercial diets for free-range laying hens (a common diet and a diet enriched in omega-3 fatty acids) were purchased at NANTA (Tudela de Duero, Valladolid, Spain). Table 1 provides the ingredient and nutrient compositions of these diets. For each genetic type of hens, half of the birds were fed the common diet (Control diet), whereas the other half received the diet containing linseed at 70 g/kg (Omega-3 diet). Hence, 4 groups of thirty hens each were considered in the present study: 1) Lohmann LSL-Classic hens fed the Control diet; 2) Black Castellana hens fed the Control diet; 3) Lohmann LSL-Classic hens fed the Omega-3 diet; 4) Black Castellana hens fed the Omega-3 diet. Before the beginning of the study, hens had a 2-wk adaptation period to the diets. Hens had ad libitum access to feed (provided in mash form) and water throughout the whole study. The thirty hens of each group were randomly distributed to 6 outdoor fenced parks of 80 m^2 provided with a shed of 15 m^2 (each park constituted a replicate; six replicates per group; five hens per replicate). Feeders, watering troughs and nests were placed within the sheds. Hens had 24-h free access to the shed (sheds remained open for the whole day).

Hen Performance Assessment

Daily, all laid eggs were collected manually, counted and weighed. Daily egg production was calculated as follows:

$$\textit{Daily egg production } (\%) = (\textit{total number of eggs obtained on a day} / \textit{total number of hens present on that day}) \; x \; 100$$

Feed intake by laying hens from each park was monitored weekly and divided by 5 and 7 to obtain the daily feed intake by hen. Daily egg mass was determined by multiplying daily egg production (%) by average egg weight divided by 100. Feed conversion ratio was then calculated by dividing feed intake by egg mass.

Egg Collection and Egg Quality Assessment

On the first, second and third day of week 4 of the study, twelve freshly laid eggs were collected per replicate (72 eggs collected per group of hens). In 4 out of the 12 eggs originating from each replicate, the following parameters were measured: egg weight, yolk color score, albumen Haugh units, and shell thickness and proportion. Yolk color was determined according to the Roche Yolk Color Fan and data were expressed based on the standard DSM Roche Fan values (from 1 for light yellow to 15 for orange). Albumen height of the 96 eggs was determined with a QCH device (TSS, York, UK). Thereafter, Haugh units were calculated using the following formula:

Haugh units = 100 x log (h − 1.7 x $w^{0.37}$ + 7.57), where h = albumen height (mm) and w = egg weight (g) (Eisen et al., 1962)

Shell weight (including testaceous membranes) was determined after rinsing and drying at room temperature for 24 h. The proportion of eggshell was calculated by dividing the dried shell weight over egg weight. Shell thickness was measured at the equator of eggs with a digital Mitutoyo micrometer (Kawasaki, Japan).

Yolks from these 4 eggs of the same replicate were collected and pooled (six pools per group of hens). The yolk pools were frozen at -20°C, freeze-dried using a Telstar LyoQuest lyophilizer (Terrassa, Spain) and subsequently, used for the determination of yolk content in fat, crude protein, cholesterol, cobalamin, cholecalciferol, retinol, and α-and γ-tocopherol, and for the assessment of yolk fatty acid profile.

In order to assess the effect of egg storage on albumen quality for the different groups of laying hens, the remaining 48 eggs per group (8 eggs per replicate) were

Table 1. Ingredient and nutrient compositions of the commercial diets used (g/kg as fed).

Ingredients	Experimental diets	
	Control	Omega-3
Barley	308	173
Corn	162	242
Soybean meal (47% crude protein)	224	220
Wheat flour (30% starch)	150	150
Soybean oil	49.2	37.2
Linseed	0.0	70.0
Calcium carbonate	90.0	90.0
Monocalcium phosphate	7.0	7.0
Salt	3.5	3.5
DL-Methionine	1.3	1.3
Butylated hydroxytoluene	0.0	1.0
Vitamin-mineral premix[1]	5.0	5.0
Analyzed composition		
Crude protein	174	178
Crude fiber	40.1	40.4
Starch	307	298
Fat	70.0	82.6
Ash	125	126
Calcium	37.0	37.2
Total phosphorus	5.65	5.77
Sodium	1.53	1.55
Lysine	8.94	9.02
Methionine	3.85	3.97
Fatty acid profile (%)		
Palmitic acid (C16:0)	16.4	10.4
Stearic acid (C18:0)	2.99	4.27
Arachidic acid (C20:0)	ND[2]	0.62
Palmitoleic acid (C16:1)	0.55	ND
Oleic acid (C18:1)	23.5	21.7
Gadoleic acid (C20:1)	0.52	0.33
Linoleic acid (C18:2)	51.2	35.8
Linolenic acid (C18:3)	4.77	26.9
Saturated fatty acids	19.4	15.3
Monounsaturated fatty acids	24.6	22.0
Polyunsaturated fatty acids	56.0	62.7
ω-6 fatty acids	51.2	35.8
ω-3 fatty acids	4.77	26.9
Ratio ω-6/ω-3	10.7	1.33
Calculated composition		
AME[3] (MJ/kg)	11.3	11.4

[1]Vitamin-mineral mix supplied the following per kilogram of diet: sulphur, 0.15 g; vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 20.0 IU; vitamin K, 3.00 mg; thiamine, 1.00 mg; riboflavin, 5.00 mg; vitamin B_6, 2.00 mg; vitamin B_{12}, 30.0 μg; niacinamide, 30.0 mg; pantothenic acid, 6.44 mg; folic acid, 1.00 mg; biotin, 100 μg; choline, 150 mg; Fe, as $FeSO_4$, 25.0 mg; Zn, as ZnO, 60.0 mg; Mn, as MnO, 100 mg; Cu, as $CuSO_4$, 4.00 mg; I, as KI, 1.50 mg; Se, as Na_2SeO_3, 0.20 mg: phytase, 900 FTU; β-glucanase, 152 U; and β-xylanase, 1220 U;
[2]ND = not detected;
[3]AME = apparent metabolisable energy.

stored in darkness at a constant temperature of 4°C. After 14 d of storage, 24 eggs (4 eggs per replicate) were taken and broken for determination of albumen Haugh units. The same procedures were followed with the remaining 24 eggs after 30 d of storage.

Chemical Analyses

Chemical analyses were conducted in triplicate. Dry matter (930.15), crude protein (976.05), crude fiber (978.10), starch (996.11), ashes (942.05), cholesterol (994.10), calcium, phosphorus and sodium (985.01), cholecalciferol (2002.05) and lysine and methionine (982.30) were determined in accordance with the methods of the Association of Official Analytical Chemists (AOAC, 2006). Fat was analyzed by Soxhlet analysis (method 4. B) after 3 M HCl acid hydrolysis (Boletín Oficial del Estado, 1995). The determination of the fatty acid profile was done in keeping with method 996.06 of AOAC (2006) and as described by Romero et al. (2022). Fatty acids from samples were methyl esterified and then, fatty acid methyl esters were analyzed with a gas chromatograph (Agilent 7820A) equipped with a flame-ionization detector and an Agilent HP-88 column (60 m x 250 μm x 0.2 μm). The analyses of fatty acids were performed in duplicate. Analyses of cobalamin were performed following the procedure of Tekin et al. (2019) and those of retinol, α-tocopherol and γ-tocopherol were done as described in a previous publication of our team (Herranz et al., 2024).

Statistical Analysis

Data of variables were subjected to a one-way analysis of variance (**ANOVA**) with hen genetics, diet consumed and their interaction as the main sources of variation by using the general linear model procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). When interactions were significant ($P < 0.05$), comparisons among all the treatment means were made using a t-test.

The batch of five hens represented the experimental unit for egg-laying performance results (parameters assessed: egg production, egg weight, egg mass, feed intake and feed conversion ratio). The egg was the experimental unit for shell thickness and proportion, yolk color score and albumen Haugh units. A pool of 4 yolks originating from eggs of the same treatment constituted the experimental unit for the contents in fat, crude protein, cholesterol and vitamins and for the fatty acid profile.

RESULTS

Hen Performance

The results on egg-laying performance are reported in Table 2. Daily egg production was higher for Lohmann LSL-Classic laying hens than for BC (79.6 vs. 70.9%, $P < 0.001$). However, eggs of BC hens were heavier on average than those of Lohmann LSL-Classic hens (60.5 vs. 52.4 g, $P < 0.001$). This led to a lack of significant difference between the 2 genetic types of hens for daily egg mass, which amounted on average to 42.3 g/d. Higher feed intake was observed for BC than for Lohmann LSL-Classic hens (125 vs. 119 g/d, $P < 0.001$). Nevertheless, feed conversion ratio did not differ significantly between the 2 types of hens (2.90, on average). No hen died during the study in any of the groups considered.

No significant effect of diet was detected on feed intake or on any of the variables measured to assess egg-laying performance.

Table 2. Effect of hen genetics and diet consumed on egg-laying performance.

Hen genetics	Black Castellana		Lohmann LSL-Classic			*P*-value		
Diet	Control	Omega-3	Control	Omega-3	SEM[1]	Hen genetics	Diet	Hen genetics x Diet
Daily egg production (%)	70.6	71.2	79.6	79.7	1.27	< 0.001	0.75	0.84
Average egg weight (g)	60.7	60.4	52.5	52.4	0.558	< 0.001	0.77	0.93
Daily egg mass (g/d)	42.8	43.0	41.8	41.8	0.814	0.17	0.90	0.90
Feed intake (g/d)	125	126	120	119	0.406	< 0.001	0.84	0.19
Feed conversion ratio (g feed/g egg mass)	2.93	2.94	2.87	2.85	0.057	0.17	0.91	0.78

[1]n = 6 replicates per treatment (5 hens per replicate).

Table 3. Effect of hen genetics and diet consumed on egg weight, shell thickness and proportion, yolk color score and albumen Haugh units.

Hen genetics	Black Castellana		Lohmann LSL-Classic			*P*-value		
Diet	Control	Omega-3	Control	Omega-3	SEM[1]	Hen genetics	Diet	Hen genetics x Diet
Egg weight (g)	60.9	59.5	52.3	52.6	1.06	< 0.001	0.58	0.43
Shell thickness (μm)	342	336	364	361	5.47	< 0.001	0.44	0.75
Shell (%)	10.9	11.0	12.7	13.2	0.218	< 0.001	0.26	0.87
Yolk color score	11.3	11.2	11.3	10.8	0.297	0.58	0.26	0.58
Haugh units at 0 days of storage[2]	81.2	79.9	86.1	90.3	1.56	< 0.001	0.80	0.17
Haugh units at 14 days of storage	77.2	76.2	89.9	88.1	1.58	< 0.001	0.15	0.43
Haugh units at 30 days of storage	70.6	74.0	88.9	85.9	1.59	< 0.001	0.25	0.23

[1]n = 24 eggs per treatment;
[2]Eggs were stored in darkness at 4°C.

Egg Quality

Table 3 presents the effect of hen genetics and diet on egg weight, shell thickness and proportion, yolk color score and albumen Haugh units. In keeping with the results reported in Table 2, it was observed again that eggs of BC hens were heavier (60.2 vs. 52.4 g, $P < 0.001$). Shell thickness (362 vs. 339 μm, $P < 0.001$) and shell proportion (12.9 vs. 10.9%, $P < 0.001$) were higher in eggs of Lohmann LSL-Classic laying hens than in those of BC hens. No significant effect of hen genetics was found for yolk color score, which was on average 11.1. In freshly laid eggs, albumen Haugh units were higher for Lohmann LSL-Classic laying hens than for BC ones (88.2 vs. 80.5, $P < 0.001$). The same happened in eggs stored refrigerated for 14 (89.0 vs. 76.7, $P < 0.001$) or 30 days (87.4 vs. 72.3, $P < 0.001$). Indeed, the interaction between egg storage time and hen genetics was significant ($P = 0.006$; Figure 2), since no significant differences were found among the 3 different times for albumen Haugh units of eggs laid by Lohmann LSL-Classic hens, while Haugh units decreased ($P < 0.04$) during storage in the albumen of eggs from BC hens.

The effect of diet was not significant on shell thickness or proportion, yolk color score or albumen Haugh units. Furthermore, neither was the interaction between egg storage time and diet significant ($P = 0.81$) for albumen Haugh units.

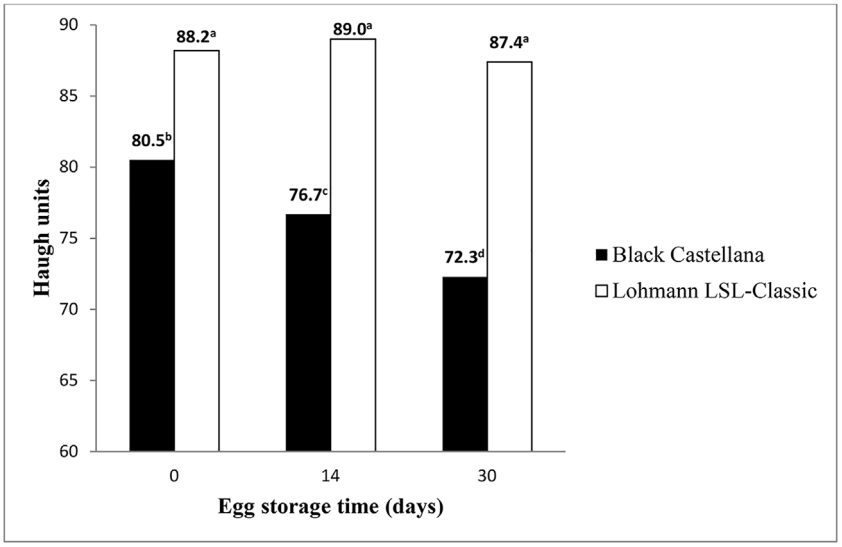

Figure 2. Effect of egg storage time on albumen Haugh units depending on hen genetics (SEM = 0.963; n = 48 eggs at each day; $P_{time} < 0.001$; $P_{hen\ genetics} < 0.001$; $P_{time\ x\ hen\ genetics} = 0.006$). Eggs were stored in darkness at a constant temperature of 4 °C. [a-d]Means with different superscripts differ significantly ($P < 0.05$).

Egg Nutritional Composition

The effect of hen genetics and diet on egg yolk content in fat, crude protein, cholesterol and vitamins is shown in Table 4. Yolks of BC eggs contained less fat (57.5 vs. 60.8% DM, $P < 0.001$), more crude protein (32.8 vs. 31.9% DM, $P = 0.003$), more cholecalciferol (1.25 vs. 1.22 μg/g DM, $P = 0.012$) and less α-tocopherol (104 vs. 163 μg/g DM, $P = 0.032$) than egg yolks of Lohmann LSL-Classic hens. Feeding the omega-3 diet increased the yolk content in fat (60.0 vs. 58.3% DM, $P = 0.006$) and in cholecalciferol (1.27 vs. 1.20 μg/g DM, $P < 0.001$), as compared with the control diet.

Neither hen genetics nor diet had a significant effect of egg yolk content in cholesterol (1,986 mg/100 g DM, on average), cobalamin (5.40 μg/100 g DM, on average), retinol (49.3 μg/g DM, on average) and γ-tocopherol (13.7 μg/g DM, on average).

The fatty acid profile in egg yolks is reported in Table 5. Yolks from eggs of BC hens showed lower proportion of saturated fatty acids (29.0 vs. 37.0%, $P < 0.001$) and higher proportions of monounsaturated (45.7 vs. 39.6%, $P < 0.001$) and polyunsaturated fatty acids (25.2 vs. 23.4%, $P < 0.001$) than yolks in eggs laid by Lohmann LSL-Classic hens. These differences are mainly due to the lower proportions of myristic acid (0.136 vs. 0.278%, $P < 0.001$) and palmitic acid (18.3 vs. 25.5%, $P < 0.001$) and also to the higher proportions of oleic acid (43.0 vs. 36.2%, $P < 0.001$), linoleic acid (18.2 vs. 16.6%, $P < 0.001$) and docosahexaenoic acid (1.61 vs. 1.26%, $P < 0.001$) found in the egg yolks of BC hens, as compared with eggs of Lohmann LSL-Classic hens.

In eggs from hens fed the omega-3 diet, the yolk proportion of saturated (32.5 vs. 33.5%, $P < 0.001$) and monounsaturated fatty acids (42.0 vs. 43.3%, $P < 0.001$)

Table 4. Effect of hen genetics and diet consumed on egg yolk content in fat, crude protein, cholesterol and vitamins.

Hen genetics	Black Castellana		Lohmann LSL-Classic			*P*-value		
Diet	Control	Omega-3	Control	Omega-3	SEM[1]	Hen genetics	Diet	Hen genetics x Diet
Fat (% DM)	56.9	58.1	59.8	61.9	0.494	< 0.001	0.006	0.39
Crude protein (% DM)	32.4	33.3	32.1	31.8	0.243	0.003	0.26	0.12
Cholesterol (mg/100 g DM)	1975	1967	1982	2020	17.2	0.11	0.40	0.22
Cobalamin (μg/100 g DM)	5.18	5.23	5.76	5.44	0.285	0.19	0.64	0.52
Cholecalciferol (μg/g DM)	1.22	1.29	1.18	1.26	0.013	0.012	< 0.001	0.93
Retinol (μg/g DM)	56.3	44.3	47.7	49.1	5.27	0.72	0.33	0.22
α-tocopherol (μg/g DM)	111	98.0	194	132	25.4	0.032	0.16	0.35
γ-tocopherol (μg/g DM)	10.1	14.0	12.0	18.7	3.13	0.29	0.11	0.67

[1]n = 6 pools of 4 yolks/pool per treatment.

Table 5. Effect of hen genetics and diet consumed on the egg yolk fatty acid profile (% of total fatty acids).

Hen genetics	Black Castellana		Lohmann LSL-Classic			*P*-value		
Diet	Control	Omega-3	Control	Omega-3	SEM[1]	Hen genetics	Diet	Hen genetics x Diet
C14:0	0.140[c]	0.132[c]	0.270[b]	0.287[a]	0.005	< 0.001	0.33	0.025
C14:1	ND[2]	ND	0.04	ND	0.007	0.015	0.015	0.015
C15:0	0.057[b]	ND	0.067[a]	0.070[a]	0.002	< 0.001	< 0.001	< 0.001
C16:0	18.7	18.0	25.6	25.4	0.139	< 0.001	0.007	0.098
C16:1	2.03	2.19	3.05	2.99	0.048	< 0.001	0.34	0.064
C17:0	0.292[a]	0.232[b]	0.230[b]	0.217[b]	0.007	< 0.001	< 0.001	0.006
C17:1	0.102[a]	0.092[b]	0.072[c]	0.087[b]	0.003	< 0.001	0.45	0.002
C18:0	9.88[b]	10.1[b]	11.1[a]	10.3[b]	0.154	< 0.001	0.078	0.007
C18:1	42.8[a]	43.2[a]	37.3[b]	35.1[c]	0.260	< 0.001	0.005	< 0.001
C18:2	19.5[a]	16.9[b]	16.5[b]	16.7[b]	0.260	< 0.001	< 0.001	< 0.001
C18:3	0.757[c]	3.95[b]	0.927[c]	5.22[a]	0.080	< 0.001	< 0.001	< 0.001
C20:1	0.287	0.242	0.220	0.170	0.005	< 0.001	< 0.001	0.62
C20:2	0.302[a]	0.212[b]	0.220[b]	ND	0.005	< 0.001	< 0.001	< 0.001
C20:3	0.187[c]	0.252[c]	0.412[a]	0.352[b]	0.010	< 0.001	0.80	< 0.001
C20:4	2.71[a]	1.70[b]	2.58[a]	1.20[c]	0.046	< 0.001	< 0.001	0.002
C20:5	ND	0.130	ND	0.132	0.003	0.70	< 0.001	0.70
C22:5	0.217	0.390	0.132	0.140	0.042	0.002	0.050	0.071
C22:6	1.20[c]	2.02[a]	1.01[d]	1.52[b]	0.035	< 0.001	< 0.001	< 0.001
C24:0	0.377[a]	0.157[b]	0.192[b]	0.200[b]	0.016	0.001	< 0.001	< 0.001
C24:1	0.500[a]	ND	0.202[b]	ND	0.018	< 0.001	< 0.001	< 0.001
Saturated fatty acids (%)	29.5	28.6	37.5	36.5	0.115	< 0.001	< 0.001	0.54
Monounsaturated fatty acids (%)	45.7[a]	45.7[a]	40.9[b]	38.3[c]	0.284	< 0.001	< 0.001	< 0.001
Polyunsaturated fatty acids (%)	24.8[a]	25.7[a]	21.6[b]	25.2[a]	0.318	< 0.001	< 0.001	0.001
ω-6 fatty acids (%)	22.5[a]	18.8[b]	19.3[b]	17.9[c]	0.251	< 0.001	< 0.001	0.001
ω-3 fatty acids (%)	2.36[c]	6.74[b]	2.48[c]	7.37[a]	0.110	0.006	< 0.001	0.039
Ratio ω-6/ω-3	9.51[a]	2.79[c]	7.81[b]	2.43[c]	0.122	< 0.001	< 0.001	< 0.001
Trans-fatty acids	0.25	0.22	0.24	0.22	0.005	0.23	< 0.001	0.80

[a-d]Means within a row with different superscripts differ significantly ($P < 0.05$);
[1]n = 6 pools of 4 yolks/pool per treatment;
[2]ND = Not Detected.

decreased, while that of polyunsaturated fatty acids increased (25.4 vs. 23.2%, $P < 0.001$), as compared with eggs of control hens. Moreover, feeding the omega-3 diet enabled an increase in the yolk proportion of ω-3 fatty acids (7.05 vs. 2.42%, $P < 0.001$) and a reduction of the ratio ω-6/ω-3 (2.61 vs. 8.66, $P < 0.001$). These results were mainly achieved thanks to the increase in the proportions of linolenic acid (4.58 vs. 0.842%, $P < 0.001$) and docosahexaenoic acid (1.77 vs. 1.10%, $P < 0.001$) in the eggs of hens fed the omega-3 diet, as compared with the eggs of hens that received the control diet. Finally, the proportion of trans-fatty acids was lower in eggs from hens fed the omega-3 diet (0.22 vs. 0.24%, $P < 0.001$), but it remained unaffected by hen genetics.

DISCUSSION

The diet consumed by hens did not influence productive results but, as expected, hen genetics had a significant effect on egg-laying performance. Indeed, Lohmann LSL-Classic hens used in this study belong to a strain that has been genetically improved for decades (Habig et al., 2012; Lohmann, 2024), whereas no intensive genetic selection for increased egg production has been performed in indigenous breed BC. Thus, daily egg production of Lohmann LSL-Classic hens was higher by 12.3% in the period considered in this research. Nevertheless, since BC hens reach sexual maturity later than Lohmann LSL-Classic hens and hence, are heavier when starting at laying eggs, the average weight of the eggs laid was greater for BC hens than for Lohmann LSL-Classic birds. It is commonly observed in field conditions that a positive relationship exists in laying hens between the body weight of the hen and the egg weight (Bish et al., 1985). In the case of BC hens, higher average egg weight made up for lower daily egg production and made it possible to achieve a daily egg mass that did not differ significantly from that of Lohmann high-yielding hens. Also because of higher body weight (Harms et al., 1982; Bish et al., 1985), BC hens ate more feed than Lohmann LSL-Classic hens. Nevertheless, no significant differences were eventually detected for feed conversion ratio between the 2 genetic types of hens. Results on productive performance reported here for BC are in keeping with data published by Orozco (1986), while the performance attained in this trial by Lohmann LSL-Classic hens met the targeted results established in the Lohmann LSL-Classic free-range management guide (Lohmann, 2024).

Shell was thinner and shell proportion was lower in eggs of BC hens than in those laid by Lohmann birds. Previous studies (Nordstrom and Ousterhout, 1982; Sooncharenying and Edwards, 1989; Sabah and Şahan, 2018) have shown that shell thickness and shell percentage are negatively correlated with egg weight in hens. Eggshell breaking strength has also been reported to decrease when egg weight increases (Kocevski et al., 2011). Hence, heavier eggs like those of BC hens are more prone to present worse eggshell quality. Moreover, differences in the formation of eggshell due to hen genetics (Buss, 1982) seem also to exist. Indeed, Sooncharenying and Edwards (1989) revealed that hen breed is one of the main sources of variation affecting shell weight and thickness. In this context, Pandey et al. (1986) compared six different strains of White Leghorn hens and found that strain significantly influenced both the percentage and the thickness of shell. Furthermore, in a more recent research (Dunn et al., 2012), it was brought out that a large genetic variability exists in the size of the calcium carbonate crystals formed in hen's uterus. In turn, this variability of genetic origin could account for the differences observed among breeds and strains of laying hens as regards eggshell quality parameters, such as shell thickness.

Hen genetics is also known to affect albumen height (Johnson and Merrit, 1955; Scott and Silversides, 2000; Silversides et al., 2006) and albumen Haugh units (Pandey et al., 1986; Herranz et al., 2024). Taking into account that the heritability of albumen traits ranges from moderate to high (Washburn, 1979) and that one of the quality parameter long time selected in Lohmann LSL-Classic strain has been albumen Haugh units, it becomes clearly understandable why Haugh units are higher in freshly laid eggs of Lohmann LSL-Classic hens than in those of BC hens. Based on previous publications (Jones and Musgrove, 2005; Herranz et al., 2024), it is well known that albumen Haugh units decrease during storage of eggs, showing thereby that albumen loses quality. In the current research work, Haugh units were determined in eggs that had been stored at 4°C for 14 and 30 d. Albumen Haugh units of eggs laid by Lohmann LSL-Classic hens remained unaffected by storage time but in eggs of BC hens Haugh units were 4.7% and 10.2% lower after 14 and 30 d of refrigerated storage, respectively. Nevertheless, it should be highlighted that these decreases are not large, especially if taking into consideration that in BC breed no selection for improved albumen quality has been conducted. Jones and Musgrove (2005) evaluated the effect of egg storage (also at 4°C) on albumen Haugh units in commercial eggs, likely originating from a genetically selected strain of laying hens, and reported decreases of 9.6% and 11.7% in Haugh units after 14 and 30 d of storage, respectively. Indeed, even larger reductions in albumen Haugh units have been reported in stored eggs from high-performing hybrids, since Scott and Silversides (2000) obtained a reduction of 23.3% for the Haugh units measured in eggs from ISA-White and ISA-Brown laying hens after only 10 d of egg storage. Nevertheless, it must be pointed out that in the latter case eggs were stored at room temperature (on average, 20.2°C).

Even if some authors have reported significant effect of hen genetics on yolk color (Pandey et al., 1986; Hassan et al., 2018), yolk color score is usually more affected by the ingredient composition of the diet consumed by hens. In this sense, Kojima et al. (2022) found that the Roche yolk color score varied significantly with the dietary inclusion of different vegetal extracts (e. g. paprika, marigold petal) but no significant effect of hen breed on

this parameter was detected, even if 2 very different hen breeds were compared (Rhode Island Red hens and Chinese Silky Fowl). Concerning the influence of diet on yolk color, dietary xanthophylls, like zeaxanthin, have been observed to be very efficiently deposited in yolk fat (Hammershøj et al., 2010). The 2 diets used in the present study contained corn, which is a source of zeaxanthin, and were both devoid of synthetic pigments. Hence, it is normal that similar color scores were obtained in eggs originating from the 2 diets, with no significant difference being achieved between them.

Yolks from eggs of BC hens contained less fat (fat content lower by 5.50%) and more protein (protein content higher by 2.82%) than those of Lohmann LSL-Classic hens. Concerning fat profile, yolks of eggs laid by BC hens presented lower proportion of saturated fatty acids and higher proportions of monounsaturated and polyunsaturated fatty acids than yolks from eggs of Lohmann hens. In a previous study (González Ariza et al., 2021), another Spanish indigenous breed of white-egg layer (Utrerana breed), with an adult body weight similar to that of BC, was also compared with Lohmann LSL-Classic lineage and it was found that the protein content was 2.96% higher in yolks from eggs of Utrerana hens. This result is in keeping with the finding of the present research work since here eggs from BC breed showed a protein content in yolk 2.82% higher than that found in eggs of Lohmann LSL-Classic hens. Moreover, similarly to what was found in this study with BC hens, egg yolks from Utrerana hens contained higher proportions of oleic acid and polyunsaturated fatty acids than yolks of eggs laid by Lohmann birds. The omega-3 diet increased the yolk fat content because this diet turned out to be richer in fat than the control diet (8.26 vs. 7.0%). The same effect was observed, also with white-egg laying hens, by Omri et al. (2019), who included linseed at 45 g/kg in the diet and obtained higher fat content both in the diet and in the egg yolks, as compared with the control group. Logically, feeding the omega-3 diet, which contained much more linolenic acid than the control diet (26.9 vs. 4.77%), caused an increase in the proportions of polyunsaturated and ω-3 fatty acids in the egg yolks. These increases in the egg yolks when feeding the diet including linseed at 70 g/kg were achieved not only because of a higher yolk percentage of linolenic acid but they were also due to higher yolk percentages of eicosapentaenoic acid and docosahexaenoic acid. It is known that birds have the capacity to convert in the liver linolenic acid into long-chain ω-3 fatty acids by means of desaturation and elongation enzymes (Alagawany et al., 2019; Irawan et al., 2022). Thereafter, these newly synthesized ω-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are transferred to the egg yolk. Fraeye et al. (2012) mentioned that old hens make this conversion more efficiently. However, results of the present study demonstrate that young laying hens also have the hepatic ability to form these long-chain ω-3 fatty acids and then depose them in the egg yolk. The enrichment of egg yolks with ω-3 fatty acids through the inclusion of linseed in hen diet has largely been studied with high-yielding strains of laying hens (Cherian and Quezada, 2016; Omri et al., 2019; Huang et al., 2020) but this work shows for the first time that hens from an indigenous unselected breed are also capable to enrich their egg yolks in linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, which are fatty acids that have proven to possess many health-related benefits. Egg yolks from both BC and Lohmann LSL-Classic hens fed the omega-3 diet reached low values for the ratio ω-6/ω-3 (2.61, on average). The ratios ω-6/ω-3 obtained in this work when feeding the hens with the omega-3 diet can be considered desirable healthy ratios. Ratios ω-6/ω-3 between 2 and 3 have shown to exert positive effects in human beings like reduced proliferation of cancerous cells in the rectum, lower risk of breast cancer, suppressed inflammation in patients with rheumatoid arthritis, to name but a few (Simopoulos, 2002). Finally, irrespective of hen genetics or the diet consumed, it can be concluded that the fatty acids present in egg yolk in the higher percentages were palmitic acid, oleic acid and linoleic acid. The abundance of these same fatty acids has been repeatedly observed in the egg yolk of laying hens by other researchers (Mikulski et al., 2012; Cherian and Quezada, 2016; Lordelo et al., 2017; Rey et al., 2021).

Although some studies support that a genetic basis exists for yolk cholesterol concentration (Bair and Marion, 1978; Han and Lee, 1992), in the present study cholesterol content in egg yolks was not affected by hen genetics. Likewise, Campo (1995) had already reported a lack of significant difference between BC and Leghorn hens for yolk cholesterol level. The yolk cholesterol concentration was neither influenced by the diet consumed by hens in the current research work. As reviewed by Naber (1979), variations in dietary composition have little impact on egg cholesterol content. Accordingly, more recent research works (Küçükersan et al., 2010; Ceylan et al., 2011) found that feeding laying hens with diets including fat sources like fish oil or linseed oil modified the fatty acid profile of the egg yolk (by increasing the proportion of ω-3 polyunsaturated fatty acids) but it did not alter the yolk cholesterol content. Values in laying hens of yolk cholesterol concentration obtained in the current research work fall within the range of values previously obtained by other authors (Han and Lee, 1992; Campo, 1995; Mikulski et al., 2012).

When assessing egg nutritional quality, it is important to determine egg content in cobalamin since eggs represent the main source of this vitamin for non-strict vegetarians, that is, people only excluding meat from their diet but still eating eggs and dairy products (O'Brien et al., 2019; Hess et al., 2023). Even so, there is a dearth of studies quantifying cobalamin concentration in eggs from hens of different breeds or fed different diets. Therefore, authors were unable to find other works also evaluating the effect of hen genetics on yolk cobalamin level. Nonetheless, it can be said that cobalamin concentrations found in the yolks of the present research work are in agreement with the values reported by Squires and Naber (1992).

Cholecalciferol plays a key role in calcium and phosphorus homoeostasis and consequently, is crucial for bone and eggshell mineralization. This vitamin is principally synthesized in the skin after adequate exposure to sunlight. Several factors such as genetics, age, breeding status, egg production or growth as well as the calcium and phosphorus levels in the diet might affect the requirements of birds in cholecalciferol (de Matos, 2008). Our results show that both hen genetics and omega-3 dietary enrichment modified the egg yolk content in cholecalciferol. Specifically, higher yolk cholecalciferol content was observed in eggs laid by BC hens and when hens were fed the omega-3 diet. Taking into consideration that both BC and Lohmann hens received the same diets and sunlight exposure, it can be surmised that the difference in yolk cholecalciferol content between BC and Lohmann hens can be of genetic origin and could be due to different absorption/metabolism of cholecalciferol depending on hen breed. In this sense, a recent study (Liermann et al., 2023) compared the effect of the dietary dose of this vitamin in hens of different strains and also reported different responses to increasing dietary cholecalciferol concentration among genetic lines. Feeding laying hens with diets supplemented in cholecalciferol and sunshine exposure (as it happens in free-range farming conditions) are the main ways to produce vitamin D_3-enriched eggs (Kühn et al., 2014; Barnkob et al., 2020). The higher cholecalciferol deposition observed in yolks of BC hens could suggest that hens of this indigenous hardy breed are genetically capable of producing more cholecalciferol than selected high-yielding hybrids because of having been raised for centuries in open-air conditions. The present study also showed that feeding a diet with higher percentage of ω-3 fatty acids enabled an increase in yolk cholecalciferol content. The omega-3 diet of the current study presented a fat content greater than that of the control diet and led to a higher yolk fat content. It is assumed that lipids increase the bioavailability of fat-soluble vitamins (Borel et al., 2015). Consequently, the increase in the amount of cholecalciferol observed in the eggs of hens fed the omega-3 diet could be attributed to a greater bioavailability of this fat-soluble vitamin. However, this effect was not observed with the other studied fat-soluble vitamins, so it seems that the relationship between consuming the omega-3 diet and the increased yolk cholecalciferol concentration could not be only explained by the higher dietary and yolk fat content. In fact, the connection between the consumption of ω-3 fatty acids enriched diets and the body cholecalciferol content has been recently reviewed in a meta-analysis based on human beings (Alhabeeb et al., 2022). These authors concluded that dietary supplementation with ω-3 fatty acids increased the plasma vitamin D_3 content, with this effect being nonetheless dependent on dosage and on the duration of the supplementation. However, the physiological processes underlying this relationship remain yet to be elucidated.

The main function of α-tocopherol is to protect lipids from oxidative damage and this important role in membrane stabilization and oxidative damage protection justifies increased requirement in α-tocopherol in polyunsaturated fatty acid-enriched foods, since polyunsaturated fatty acids are known to be more susceptible to oxidation than saturated fat (Cherian et al., 1996; Raederstorff et al., 2015). Accordingly, increased dietary intake of polyunsaturated fatty acids has been shown to decrease vitamin E levels in plasma and tissues in both animals and human beings. Nevertheless, in the work of Trebunová et al. (2007) the dietary use of linseed, as source of ω-3 fatty acids, had no effect on egg yolk α-tocopherol level, just like it has also been observed in the present study. On the other hand, in the current research work, no effect of hen genetics on the amount of retinol and γ-tocopherol in egg yolk was noted. However, the amount of α-tocopherol was higher in the eggs laid by the commercial hybrid strain than in those laid by BC hens. In previous studies in which the retinol content was determined in eggs of both native breeds and commercial hybrids, researchers concluded that there was no significant effect of the genotype on this vitamin level (Sokolowicz et al., 2020; Gumułka et al., 2022). Nevertheless, when analyzing the effect of genetics on vitamin E concentration in egg yolk, contradictory results can be found in the literature. While Sokolowicz et al. (2020) reported no significant effect on vitamin E content due to the genotype of hens, Gumułka et al. (2022) detected a higher amount of this vitamin in eggs laid by a native Polish breed. In the aforementioned research work of González Ariza et al. (2021), a higher content of vitamin E was found in eggs from Spanish indigenous breed Utrerana, as compared with eggs of Lohmann LSL-Classic laying hens. Additionally, in a remarkable study in which nine hen breeds were compared (Bunea et al., 2017), authors highlighted the wide range of variation in the content of vitamin E in egg yolk, revealing thereby the influence of genetic factors on the nutritional composition of the egg yolk.

CONCLUSIONS

This study constitutes the first exhaustive characterization of laying performance and egg quality in Spanish indigenous Black Castellana breed. Despite their lower laying rate as compared with a selected high-yielding commercial strain, Black Castellana hens showed that they can compete in egg quality, since their egg yolks contained less fat, more protein and more cholecalciferol, and showed lower proportion of saturated fatty acids and higher proportions of monounsaturated and polyunsaturated fatty acids. Furthermore, it was proven that Black Castellana hens are also capable to enrich the yolks of their eggs with ω-3 fatty acids when fed a diet containing a source of this type of fat. In summary, this work provides breeders of Black Castellana hens with information supporting the market differentiation of the eggs produced by this threatened indigenous breed.

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DISCLOSURES

The authors declare no conflict of interest.

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