Quality characterization of eggs from Romagnola hens, an Italian local breed

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ABSTRACT In the past years, consumers' concerns about sustainability and animal welfare have increased, strengthening the demand for eggs and meat produced through alternative and extensive farming methods. In addition, producers have also become increasingly interested in the recovery and exploitation of local breeds due to their adaptability to local environmental conditions, to valorize the biodiversity and to provide added value to typical products. Among the Italian local breeds, Romagnola has almost risked extinction and currently is reared in small-scale farms for eggs and meat production. The aim of this study was to characterize the egg quality traits of Romagnola chicken breed (RMG) compared to those obtained by a commercial hybrid (CONV). Ten laying hens of both Romagnola breed and Hy-Line Brown at 40 wk of age were housed in the same outdoor pen and fed the same commercial feed (ME 2,830 kcal/kg, CP 17.2%) for 10 wk. At 5 and 10 wk after housing, all the eggs laid in 4 consecutive days were collected and used for the determination of egg and eggshell characteristics as well as proximate composition and fatty acid profile of egg yolk. As expected, some important productive traits such as egg weight and production resulted higher in CONV chickens. However, eggs from RMG hens presented a higher yolk/egg ratio (31.1 vs. 24.9%; $P < 0.01$) as well as carotenoids (36.8 vs. 20.2 ppm; $P < 0.01$) and cholesterol content (12.8 vs. 11.7 mg/g of yolk; $P < 0.01$) than those laid by the conventional genotype. Moreover, yolks from RMG eggs were characterized by lower polyunsaturated fatty acid (PUFA) n-6 content (22.6 vs. 28.4%; $P < 0.01$) and PUFA n-6/n-3 ratio (11.3 vs. 13.5; $P < 0.01$) showing a healthier fatty acids profile than conventional eggs. These results highlighted several valuable egg quality traits of Romagnola chicken breed that might be exploited for the conservation and the development of this underutilized Italian pure breed.

Key words: chicken, local breed, Romagnola, egg, quality

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INTRODUCTION

In the poultry industry, almost the totality of meat and eggs are provided by commercial chicken hybrids characterized by high productive performance and feed efficiency (Mottet and Tempio, 2017; Tallentire et al., 2018). However, the high specialization of livestock species obtained through selective breeding programs aimed at improving productive traits determined negative effects on the genetic variability and the development of a sustainable agriculture (Hoffmann, 2011). In the last decade, the need to protect animal biodiversity has strongly emerged since it represents an important component of the sustainability of all the natural systems and farms (Hoffmann, 2011; Varst et al., 2015; Henchion et al., 2017). The protection of the biodiversity must not be only considered as the conservation of the animal genetic resources, but also the economical sustainability of many marginal areas, playing an important role in social, ecological, cultural, and traditional profile of rural areas and communities (Hoffmann, 2011; Varst et al., 2015; Castellini and Dal Bosco, 2017).

Recently, consumers' concerns about sustainability and animal welfare have increased (Verbeke, 2009; Leinonen, 2016), strengthening the demand for eggs and meat produced through alternative and extensive farming methods (Gangnat et al., 2018). In addition, producers have also become increasingly interested in the discovery and exploitation of local breeds mainly due to their adaptability to local environmental conditions as well as to diets with poor digestibility and nutrient profile (Castellini and Dal Bosco, 2017). The valorisation of typical products may represent a strategy for the conservation of local breeds as far as their products do not lose the linkage with the production area as usually happens for industrial products (Varst et al., 2015).

In Italy, a registry of local poultry breeds has been created in 2014 and it includes 20 local chicken breeds (MIPAAF, 2014). Among them, the Romagnola chicken

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breed has almost risked extinction and it has been subjected to a recovery program supported by the University of Parma.

Romagnola chicken breed is a rustic animal, extremely variable for plumage, tarsus, and skin color. It is characterized by fine skeletal and thin bones, and a red medium-size comb which is straight for the cock and folded in the hen. The body live-weight ranges from 2.0 to 2.5 kg and from 1.9 to 2.0 kg in males and females, respectively (Zanon et al., 2006). Romagnola chickens are usually extensively reared in small-scale farms where females are generally farmed for producing eggs, whereas males are kept for meat production and commonly sold as whole carcass.

The aim of this study was to characterize the quality traits of eggs from Romagnola hens, an Italian chicken breed, in comparison to those obtained by a worldwide used laying hen hybrid extensively selected for egg production traits.

MATERIALS AND METHODS

Animals and Diet

Ten laying hens of both Romagnola breed (**RMG**) and Hy-Line Brown (**CONV**) of 40 wk of age were housed in the same outdoor pen (of about 200 m^2, corresponding to 10 m^2/hen) at the Department of Agricultural and Food Sciences (Bologna, Italy) for 10 wk. All the birds were reared according to the principles stated in the legislation in force for the protection of animals used for experimental and other scientific purposes. The hens of both groups received the same commercial feed (ME 2,830 kcal/kg, CP 17.2%, Ca 3.94%, Av. P 0.38%) for the whole experimental period (Table 1). Feed and water were available ad libitum. The outdoor pasture was mainly composed by Lolium perenne, Cynodon Dactylon, and Trifolium pratense.

Table 1. Composition of the basal diet given to both conventional and Romagnola hens.

Ingredients	g/kg
Corn	510
Soybean meal	175
Wheat	100
Sunflower meal	69
Vegetable oil	27
Calcium carbonate	105
Dicalcium phosphate	3.4
Sodium bicarbonate	2.1
L-lysine	1.6
MHA	1.8
Choline chloride	1.0
Phytase	0.6
Antioxidant (BHT)	0.2
Vitamin and mineral premix[1]	3.3
Calculated analysis	
Dry matter (%)	88.86
Crude protein (%)	17.19
Total fat (%)	4.44
Crude fiber (%)	2.31
Ash (%)	12.25
Calcium (%)	3.94
Available phosphorus (%)	0.38
Natural xanthophylls (mg/kg)	10.6
ME (kcal/kg)	2830

[1]Provided the following per kg of diet: vitamin A (retinyl acetate), 11,000 IU; cholecalciferol, 3,000 IU; DL-α-tocopheryl acetate, 40 IU; menadione sodium bisulfite, 3.3 mg; riboflavin, 6.0 mg; pantothenic acid, 11.0 mg; niacin, 30 mg; pyridoxine, 4 mg; folic acid, 1 mg; biotin, 0.05 mg; thiamine, 2.5 mg; vitamin B_{12} 20 μg; Mn, 15 mg; Zn, 50 mg; Fe, 30 mg; Cu, 6 mg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

Egg and Eggshell Characteristics

At 5 and 10 wk after housing (corresponding to 45 and 50 wk of hens age), all the eggs laid in 4 consecutive days were collected and used for the determination of their quality traits. Eggshell color was used to identify laid eggs according to the hen genotype (RMG: white; CONV: brown eggshell). Eggs were weighed and length and diameter were measured using a digital caliper. Egg shape index was then calculated as ratio between egg diameter and length (Sauveur, 1988).

Egg shell color was determined using a reflectance colorimeter (Minolta CR-300, Minolta Italia S.p.a., Milano, Italy) and the results were expressed using the CIE $L^*a^*b^*$ system color profile (CIE, 1976).

Eggshell breaking strength was measured by quasi-static compression through an Instron texting machine equipped with a 2 kN load cell. Breaking strength was determined as the minimum force required to fracture the eggshell at a compression speed of 5 cm/min (Sirri et al., 2018).

After that, eggs were broken with care on a glass surface and yolk diameter, as well as Haugh index, were determined (Sauveur, 1988). Yolk and albumen were carefully separated, and yolk weight was obtained. Eggshell was dried overnight at 80°C and subsequently weighed. Albumen weight was calculated as difference between egg weight, and yolk and eggshell weight. These data were used to calculate yolk/egg, albumen/egg and eggshell/egg ratio, and the results were expressed as percentage. Total egg surface was determined with the formula proposed by Sauveur (1988) ($S = 4.68 \times P^{2/3}$, where P = egg weight). As for yolk color, the CIE $L^*a^*b^*$ system color profile was obtained using a reflectance colorimeter using illuminant source C (Minolta CR-300, Minolta Italia S.p.a., Milano, Italy).

Proximate Analysis

A total of 6 pools of 5 yolks for each genotype and sampling session (5 and 10 wk of experiment) were obtained and subjected to proximate analysis. Moisture and ash were determined in duplicate according to the Association of Official Analytical Chemists procedure (AOAC, 1990). Crude protein was determined using the standard Kjeldahl copper catalyst method (AOAC, 1990), whereas total lipid content was assessed through the chloroform: methanol extraction procedure described by Folch et al. (1957).

Determination of Fatty Acids Composition

Fatty acids (**FAs**) were converted to their methyl esters following the method described by Christopherson and Glass (1969). In brief, 250 μg of lipid and 500 μL of the methylating solution (KOH/methanol 2 N) were put in a vial containing 5 mL of hexane and 1 g of anhydrous sodium sulphate. The vial was mixed for 30 s and placed in a water bath at 40°C for 15 min. The sample was then stirred and cooled on ice. The upper phase, containing FA methyl esters, was collected and used for the separation of FAs by using a Shimadzu GC17A gas chromatograph (Shimadzu Corporation, Tokyo, Japan) with a WP-4 Shimadzu integration system, equipped with a Varian CPSIL88 capillary column (100 m length, 0.25 mm i.d., 0.20 mm film thickness) (Varian, Walnut Creek, CA) and a flame ionization detector (Shimadzu Corporation, Tokyo, Japan). The operating conditions of the gas chromatograph were as follows: the oven temperature was kept at 170°C for 15 min, increased to 190°C at a rate of 1°C/min, then increased to 220°C at a rate of 5°C/min, and kept at this temperature for 17 min. The temperature of the injector and detector is 270°C and 300°C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. The identification of individual FA was carried out by using polyunsaturated fatty acid (**PUFA**)-2 FA methyl ester standards (Matreya, Pleasant Gap, PA), and FA quantification by using methyl non-adecanoate 98% (C19:0) (Sigma, Saint Louis, MO) as internal standard added prior lipid extraction.

Cholesterol analysis was carried out on total lipid extract according to Bortolomeazzi et al. (1990) by using a Shimadzu GC17A gascromatograph equipped with a Restek XTI-5 capillary column (30 m length; 0.25 mm i.d.; 0.50 μm film thickness) and a flame ionization detector. The oven temperature was held at 300°C for the whole duration of the analysis whereas the injector and detector were held at 330°C. Helium was used as the carrier gas at the constant flow of 1.9 mL/min. The quantification of total cholesterol content was obtained by using β-sitosterol as internal standard.

β-carotene equivalents of yolk were evaluated according to the method described by Steinberg et al. (2000).

Statistical Analysis

Data were examined using a descriptive statistical analysis (mean, standard deviation, coefficient of variation, variation range) and analyzed by 2-way ANOVA to verify the effect of the genetic strain (CONV vs. RMG) and the sampling time (45 vs. 50 wk of hen age) on the quality traits of eggs. As the effect of the sampling time resulted not significant, it has been excluded from the statistical model and only the effect of the genetic strain was assessed. When the analysis of variance revealed a significant effect ($P < 0.05$), means were separated using the Student Newman-Keuls test (SAS Institute, 1988).

Table 2. Egg physical and eggshell quality traits in conventional (CONV) and Romagnola (RMG) hens.

Trait	Genotype		SEM
	CONV	RMG	
Number	35	31	
Egg weight (g)	64.5^{A}	52.9^{B}	0.77
Yolk weight (g)	16.0	16.5	0.26
Albumen weight (g)	42.3^{A}	31.7^{B}	0.69
Eggshell weight (g)	6.24^{A}	4.67^{B}	0.08
Yolk/egg (%)	24.9^{B}	31.1^{A}	0.45
Albumen/egg (%)	65.3^{A}	60.1^{B}	0.48
Eggshell/egg (%)	9.71^{A}	8.83^{B}	0.11
Shape index	0.79^{A}	0.74^{B}	0.01
Egg surface (cm^2)	75.2^{A}	65.9^{B}	0.61
Eggshell breaking strength (kg)	4.56^{A}	3.26^{B}	0.09
Haugh index	81.8	76.4	2.24
Yolk index	0.40	0.40	0.01

Means within a row not sharing a common superscript are significantly different (A, B: $P < 0.01$).

RESULTS AND DISCUSSION

The quality traits of eggs laid by CONV and RMG hens are reported in Table 2. The eggs from RMG hens had lower egg (52.9 vs. 64.5 g, $P < 0.01$), albumen (31.7 vs. 42.3 g; $P < 0.01$), and eggshell (4.67 vs. 6.24 g; $P < 0.01$) weight compared to CONV ones. Also, Zanon et al. (2006) reported that eggs from Romagnola hens were lighter than commercial brown eggs at the same bird age. Previously, Rizzi and Marangon (2012) observed less egg, albumen, and eggshell weight in other 2 Italian chicken breeds (Ermellinata di Rovigo and Robusta maculata) compared to Hy-Line Brown.

RMG eggs showed lower shape index (0.74 vs. 0.79; $P < 0.01$) and surface (65.9 vs. 75.2 cm^2; $P < 0.01$) than CONV eggs. The value of egg shape index observed in this study is similar to that previously reported for eggs of Romagnola chicken by Zanon et al. (2006). However, contrary to our findings, they observed higher shape index in Romagnola eggs than in commercial brown ones. According to the formula proposed by Sauveur (1988), in which egg surface is proportional to its weight, it resulted that RMG eggs were characterized by lower value of egg surface that the CONV ones (65.9 vs. 75.2 cm^2; $P < 0.01$).

Albeit no significant difference emerged for yolk weight, RMG eggs showed higher yolk/egg (31.1 vs. 24.9%; $P < 0.01$) and lower albumen/egg (60.1 vs. 65.3%; $P < 0.01$) ratio. Similar results regarding yolk and albumen percentage were previously observed for Romagnola (Zanon et al., 2006), Ermellinata di Rovigo, and Robusta maculata (Rizzi and Marangon, 2012).

Eggshell percentage was lower in RMG eggs compared to CONV ones (8.83 vs. 9.71%; $P < 0.01$). On the contrary, Zanon et al. (2006) noticed higher eggshell

Table 3. Proximate composition of the yolk of conventional (CONV) and Romagnola (RMG) hens.

Parameters	Genotype		SEM
	CONV	RMG	
Pool of eggs	35	31	
Moisture (%)	49.8	49.7	0.37
Total fat (%)	32.8	32.7	0.25
Crude protein (%)	17.2	16.9	0.21
Ash (%)	1.79^{a}	1.62^{b}	0.05
Cholesterol (mg/g of yolk)	11.7^{B}	12.8^{A}	0.15

Means within a row not sharing a common superscript are significantly different (A, B: $P < 0.01$; a, b: $P < 0.05$).

Table 4. Yolk color profile of eggs laid by conventional (CONV) and Romagnola (RMG) hens.

Parameters	Genotype		SEM
	CONV	RMG	
n.	35	31	
Lightness (L^*)	65.0	63.4	0.61
Redness (a^*)	-6.53^{B}	-4.32^{A}	0.33
Yellowness (b^*)	42.2^{B}	48.9^{A}	1.16
β-carotene equivalents (ppm)	20.2^{B}	36.8^{A}	0.89

Means within a row not sharing a common superscript are significantly different (A, B: $P < 0.01$).

percentage in eggs from Romagnola than in conventional brown eggs, whereas Ermellinata di Rovigo and Robusta maculata respectively showed lower and higher eggshell percentage compared to Hy-Line Brown (Rizzi and Marangon, 2012).

Eggshell breaking strength was lower in RMG than in CONV eggs (3.26 vs. 4.56 kg; $P < 0.01$) and this result could be related to the lower eggshell percentage observed in RMG eggs. Moreover, CONV eggs presented a more rounded profile, as confirmed by the higher shape index, that could have enhanced the resistance of the eggs to the compression force (Bain, 1991). In general, the eggshell breaking strength of eggs of similar size is far lower in white than in brown ones, as emerged from the comparison of data reported in the performance objectives guides provided by the breeding companies (Hy-Line brown commercial layers, 2016; Hy-Line W36 commercial layers, 2016). However, considering that there are no scientific studies supporting this observation, research is encouraged to confirm it.

No significant difference between the 2 genotypes was observed for Haugh and yolk indexes. Also Rizzi and Marangon (2012) did not find significant differences in Haugh index between eggs of Robusta maculata and Hy-Line, whereas Ermellinata di Rovigo's eggs showed the lowest Haugh index values.

Chemical composition of CONV and RMG yolk is given in Table 3. No significant difference was observed in terms of moisture, total fat, and crude protein between RMG and CONV yolks. A previous study reported that the yolk of Romagnola was characterized by a higher crude protein content and a lower ether extract than those of brown hybrids eggs, whereas no significant difference was detected for moisture content (Zanon et al., 2006). Rizzi and Marangon (2012) observed similar dry matter, protein, and fat content in yolks of Robusta maculata and Hy-Line Brown eggs. However, yolk of Ermellinata di Rovigo exhibited higher fat content when compared to that of brown hybrid hens.

Furthermore, RMG yolk showed lower ash content than the CONV one (1.62 vs. 1.79%; $P < 0.01$), confirming the data previously reported by Zanon et al. (2006). On the other hand, Rizzi and Marangon (2012) reported either similar or higher ash content in the yolk of other Italian local breeds respect to Hy-Line hens.

Even though a similar fat content was observed between the 2 groups, yolk of RMG eggs exhibited higher concentration of cholesterol (12.8 vs. 11.7 mg/g of yolk, respectively, for RMG and CONV; $P < 0.01$). Similarly, Rizzi and Chiericato (2010) and Rizzi and Marangon (2012) observed higher cholesterol content in yolks of other Italian chicken purebreds (Ermellinata di Rovigo and Robusta maculata) compared to Hy-Line hens. Commercial hybrid hens are the result of a strong selective breeding process, which has tremendously increased their productive performance in comparison to unselected lines such as local breeds. It is widely recognized that an inverse relationship exists between yolk cholesterol content and egg deposition rate (Elkin, 2006). Therefore, the higher cholesterol content found in yolk of Romagnola eggs could be explained by the far lower deposition rate of these unselected hens compared to the commercial hybrids ones.

The results of the yolk color evaluation are reported in Table 4. No significant difference between the 2 genotypes was observed in terms of lightness (L^*), whereas the yolk of RMG eggs showed higher values of redness (a^*, –4.32 vs.—6.53; $P < 0.01$) and yellowness (b^*, 48.9 vs. 42.2; $P < 0.01$). Rizzi and Marangon (2012) noticed higher L^*, a^*, and b^* value in yolks of Robusta maculata compared to Hy-Line Brown, whereas for Ermellinata di Rovigo only yellowness was significantly higher than that of hybrid hens.

The amount of β-carotene equivalents was significantly higher in RMG yolk compared to the CONV one (Table 4) (36.8 vs. 20.2 ppm; $P < 0.01$), showing a better pigmentation capacity for the eggs of Romagnola. Since both the hen genotypes received the same basal diet, other factors may have determined the difference observed in terms of cholesterol content in yolks of Romagnola and Hy-Line brown. Sirri et al. (2007) reported a significant effect of the chicken genotype in the efficiency of utilization of the dietary pigments. Other potential factors lying behind this difference could be the lower deposition rate of RMG hens, and consequently the reduced dilution effect of the pigments in the yolk, or also a better exploitation of the pasture, which represents a source of carotenoids. However, without other scientific insights, it is not clear which of the abovementioned factors would have contributed to the chromatic difference observed in the yolks of the 2 groups.

Table 5. Yolk fatty acids composition (expressed in %) in conventional (CONV) and Romagnola (RMG) eggs.

Fatty acids	Group		SEM
	CONV	RMG	
Pool of eggs	12	12	
14:0 Myristic	0.22	0.27	0.02
16:0 Palmitic	22.0^{B}	23.8^{A}	0.22
17:0 Margaric	0.19	0.15	0.03
18:0 Stearic	8.71^{B}	9.78^{A}	0.13
24:0 Lignoceric	0.48	0.48	0.04
Σ SFA	31.6^{B}	34.5^{A}	0.29
16:1 n-7 Palmitoleic	1.73^{B}	2.39^{A}	0.12
18:1 n-9 Oleic	35.2^{B}	37.8^{A}	0.44
20:1 Eicosaenoic	0.26^{B}	0.33^{A}	0.01
Σ MUFA	37.3^{B}	40.6^{A}	0.47
18:2 n-6 Linoleic	25.5^{A}	19.8^{B}	0.51
18:3 n-6 γ-Linolenic	0.18^{A}	0.08^{B}	0.02
20:2 n-6 Eicosadienoic	0.31	0.29	0.01
20:4 n-6 Arachidonic	2.37	2.46	0.04
Σ n-6 PUFA	28.4^{A}	22.6^{B}	0.51
18:3 n-3 α-Linolenic	0.81	0.74	0.04
20:5 n-3 Eicosapentaenoic	0.21	0.24	0.02
22:5 n-3 Docosapentaenoic	0.11	0.10	0.03
22:6 n-3 Docosaesaenoic	0.99	0.95	0.03
Σ n-3 PUFA	2.13	2.03	0.08
Σ PUFA	30.5^{A}	24.6^{B}	0.56
n-6/n-3	13.5^{A}	11.3^{B}	0.40
Others	0.60	0.30	0.11

Means within a row not sharing a common superscript are significantly different (A, B: $P < 0.01$).

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Regarding FAs profile (Table 5), RMG yolks had a higher proportion of saturated (34.5 vs. 31.6%; $P < 0.01$) and monounsaturated FAs (40.6 vs. 37.3%; $P < 0.01$), coupled with a lower proportion of PUFAs (24.6% vs. 30.5%; $P < 0.01$). Similarly, the yolk of other Italian chicken breeds showed higher proportion of saturated FAs when compared to that of Hy-Line Brown (Rizzi and Marangon, 2012). However, monounsaturated FA and PUFA proportions observed in yolk of Romagnola are in disagreement with the findings of Rizzi and Marangon (2012) on Ermellinata di Rovigo and Robusta maculata, suggesting possible differences in lipid metabolism among Italian chicken breeds. No significant difference emerged in terms of PUFA n-3 proportion between RMG and CONV yolk (2.03 vs. 2.13%, respectively). Rizzi and Marangon (2012) observed similar PUFA n-3 proportion in Ermellinata di Rovigo and Hy-Line Brown yolk, whereas Robusta maculata's yolk showed a higher proportion of PUFA n-3 when compared to that of the tested commercial hybrid.

PUFA n-6 content and PUFA n-6/n-3 ratio resulted significantly lower in RMG yolk (22.6 vs. 28.4% and 11.3 vs. 13.5%, respectively, for RMG and CONV; $P < 0.01$). A lower PUFA n-6/n-3 ratio is recommended for a healthy profile of egg lipids being associated to the reduction of the risk of several chronic diseases afflicting the western society (Simopoulos, 2002). Rizzi and Marangon (2012) found that eggs from other Italian chicken purebreds, when compared to Hy-Line Brown, showed either higher or similar PUFA n-6 yolk proportion as well as no significant difference in PUFA n-6/n-3 ratio. The differences in the FA composition between yolks from Romagnola and Hy-Line might be attributable to several factors, including the eating behavior (in terms of different ratio in feed/pasture intake) of the laying hens or to a possible difference in lipid metabolism as observed by Dal Bosco et al. (2012) in chickens of Ancona breed. Indeed, these authors reported that pure breeds diverged from selected hybrids for some estimated indices of FA metabolism. In particular, slow-growing genotypes showed higher activity of elongase, thioesterase, and $\Delta5/\Delta6$ desaturase, which are involved in the synthesis of long-chain PUFA n-6 and n-3 starting from their respective precursors (linoleic acid and linolenic acid), coupled with a lower $\Delta9$ activity which is responsible for the MUFA synthesis (Dal Bosco et al., 2012). Also Boschetti et al. (2016) observed a significant effect of the chicken genotype on the long-chain PUFA content, coupled with differential expression of FADS2 and FADS1 genes, as well as to $\Delta6$ and $\Delta5$ activity.

In conclusion, the present study showed significant differences in egg quality parameters between Romagnola chicken breed and a commercial hybrid. Romagnola's eggs presented a higher yolk/egg ratio, carotenoids and cholesterol content, and a lower egg weight and eggshell breaking strength. The yolk of Romagnola hens resulted characterized by lower PUFA n-6 content and PUFA n-6/n-3 ratio, showing a better nutritional balance among long-chain FAs. These results highlighted several valuable quality traits of eggs from Romagnola chicken which might be taken into account for the conservation and the exploitation of this underutilized Italian purebred.

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