

Quality of organic eggs of hybrid and Italian breed hens

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ABSTRACT The quality of eggs laid by 2 hybrid and 2 Italian dual-purpose genotypes of hens reared under organic farming system was studied. Hens belonging to Hy-Line Brown (brown eggshell, HLB), Hy-Line White (white eggshell, HLW), Ermellinata di Rovigo (brown eggshell, E), and Robusta maculata (brown eggshell, R) genotypes were reared from 24 to 43 wk of age. The trial was carried out from July to December, with environmental temperature ranging from 25°C ($\pm 5^\circ\text{C}$, summer) to 13°C ($\pm 7^\circ\text{C}$, autumn). The HLB eggs were heavier ($P < 0.01$) than HLW (62.9 vs. 60.4 g), and R eggs were heavier ($P < 0.01$) than E (56.5 vs. 54.4 g). The albumen weight differed ($P < 0.01$) among HLB, HLW, R, and E (40.7, 38.3, 32.7, 34.1 g, respectively). The E and R yolk weights were similar (16.2 g) and higher ($P < 0.01$) than hybrids; HLW yolk was higher ($P < 0.05$) than HLB (15.8 vs. 15.5 g). The HLB showed the highest ($P < 0.01$, 6.74 g) shell weight and

E had the lowest ($P < 0.01$; 5.43 g). The yolk cholesterol content was higher ($P < 0.01$) in the Italian eggs than in the hybrids (258 vs. 219 mg/yolk). The HLB yolk had the lowest ($P < 0.01$) saturated fatty acids (33.8 vs. 34.9%), and R yolks showed the lowest ($P < 0.01$) monounsaturated fatty acids (36.3 vs. 38.0%) and the highest ($P < 0.01$) polyunsaturated fatty acids (28.7 vs. 27.4%) than the other groups. The HLW yolk showed the highest ($P < 0.01$) n-6/n-3 ratio (13.7) in comparison to the other 3 groups (12.8). During 21 d of storage (at 21°C and 62% RH), the E eggs showed the lowest ($P < 0.01$) quality (albumen height, 4.93 vs. 5.56 mm; Haugh units, 71 vs. 74). A sensory profile of boiled eggs showed differences ($P < 0.05$) in odor and flavor sensations and in certain yolk and albumen texture properties according to genotype. The quality of organic eggs from different genotypes differs in relation to the strain but also the interaction with the environmental conditions has to be considered.

Key words: laying hen, organic, strain, egg quality, sensory profile

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INTRODUCTION

During the last few years, consumer demand has gradually focused more on product quality, which includes both intrinsic characteristics and the procedures of the complete productive process. Animal organic products (European Union, 1999) are obtained without the use of synthetic molecules in the feed or allopathic therapy in the case of pathologies, and the animals are allowed to live under more natural conditions. Organic procedure (European Union, 1999) requires that the animals be reared in open spaces, having access to outdoor areas for physical activity and solar radiation. Open spaces present a high variability in weather and thermal hygrometric conditions and in type of pasture. In fact, the organic procedure for hens requires an outdoor space of at least 4 m²/bird for at least one-third of their life. So

when the birds are reared in outdoor spaces for a longer time than that indicated by the procedure, the pasture can be very poor and the animals can eat a negligible quantity of forage or, when the grass is present, it can widely differ in vegetable species composition and living insects. The rearing environment should guarantee the animals better conditions for both welfare and health. The majority of consumers consider the animal welfare as the most distinctive factor for this type of product; moreover, they perceive organic products more genuine and wholesome, even if the quality of the product is not the main goal of organic procedure (Hidalgo et al., 2008; Horsted et al., 2010).

The genotypes currently being reared worldwide and intensively are hybrids resulting from intensive work in genetic improvement, highly productive under controlled environmental conditions. Few indications exist regarding productive performance and the quality of the eggs of organic hybrid laying hens (Hammershøj and Steinfeldt, 2005; Hidalgo et al., 2008; Horsted et al., 2010; Rizzi and Chiericato, 2010), while the indications available for purebred productive performance

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are scarce (Rizzi and Chiericato, 2010). Among Italian breeds, there are some double-purpose genotypes that were widely present in country farms 50 or more years ago, which are currently reared only in a few nonintensive farms. Italian breed genotypes not presently used in intensive production may exhibit satisfactory productive performance in alternative production systems (Rizzi and Chiericato, 2010).

The aim of the present work is to compare the quality of eggs of 2 hybrid lines and 2 Italian breeds in the first half (24–43 wk of age) of the oviposition cycle in hens reared under organic conditions.

MATERIALS AND METHODS

Birds and Environmental Conditions

Hens belonging to 2 hybrid genotypes and to 2 Italian breeds were reared. The hybrids were Hy-Line White (**HLW**; 70 hens) with white feathers and white eggshells and Hy-Line Brown (**HLB**; 70 hens) with brown feathers and brown eggshells (Hy-Line Int., West Des Moines, IA). Italian breeds were Ermellinata di Rovigo (**E**; 70 hens) and Robusta maculata (**R**; 70 hens) (Veneto Agricoltura, Legnaro, Padova, Italy). They were created in Northern Italy (Veneto region) during the 1950s and they were described in literature (De Marchi et al., 2005, 2006): E hens, which originate from Sussex and Rhode Island, have erminate plumage (white plumage and black with white edge hackle and saddle; black main tail feathers and white primaries with black extremity) and produce eggs with a light-brown shell. The R hens, which originate from Brown Orpington and White America, have silver plumage (black tail and white breast feathers with black extremity) and produce eggs with a brown shell. The purebred birds were provided by the regional farm, which preserves these breeds with financing from the Veneto regional government, and they have been maintained as pure breeds in the conservation program (Zanetti et al., 2007) using, since 2000, a marker-assisted conservation program with random mating within families.

The birds were 1 d of age at their arrival at the trial station. During the first 4 wk, the birds were kept indoors, on litter (straw and wood shavings), under infrared radiation lamps, with a decrease from 32 to 24°C over a 4-wk period; at 8 wk of age, the birds had free access to outdoor spaces. The birds were subjected to the same prophylaxis procedures, rearing conditions (temperature, photoperiod), and feeding from the time of hatching until the end of the experimental period. The hens were experimentally reared from 24 to 43 wk of age (July–December). Each genotype had access to outdoor (4 m²/bird) and indoor (0.20 m²/bird) spaces, divided by netting. The indoor spaces were provided with wooden nests and perches; the floor was covered by a mixture of straw and wood shavings.

The birds showed similar behavior regarding the use of their outdoor area which was initially in good condi-

tion until a few weeks before starting the experimental period. Modification of the outdoor area was caused by continuous foraging and walking on the same area by the birds and by climatic conditions: at the start of summer when rain levels are low in Northern Italy, the grass began to die and did not regrow, and this situation lasted until autumn. In such conditions, insects and worms were either not present or not broadly available to the birds in these areas. The feed (CP = 19.5% and ME = 2,830 kcal/kg) given to the birds throughout the first 15 wk of life contained no additives but did contain some nonorganic ingredients. An organic feed was fed the birds 6 wk before the start of the experimental period throughout the conversion period (European Union, 1999). Thereafter, the birds were fed *ad libitum* the organic crumbled feed (Table 1).

Throughout the experimental period, a thermo-hygrometer (model TIG-ITH, LSI, Milano, Italy) checked the environmental temperature and the relative humidity levels. The indoor and outdoor thermal-hygrometric conditions followed the same trend. The mean temperature was 25 ± 5°C and 13 ± 7°C, and the mean relative humidity was 62 ± 6% and 73 ± 9%, respectively in summer (from July 24th until September 20th) and in autumn (from September 21st until December 4th). The birds had free-access to the outdoor space and used it throughout the day and stayed indoors for laying eggs and during the night; in autumn, on rainy or foggy days, the hens spent less time outdoors. The photoperiod was initially natural and, when it began to decrease according to the geographical position of the trial station (Northern Italy), it was complemented by artificial light (one incandescent bulb, 40 W) inside the rooms where the birds spent the night to achieve a constant lighting period (16L:8D). For in-house conditions, light intensity ranged from 20 to 90 lx according to the time of day and to the weather conditions; the minimum values were recorded at the beginning and at the end of the day when the light was on.

Egg Weight, Color, and pH Measurements

At regular 4-wk intervals (30, 34, 38, and 42 wk of age), samples of 30 eggs for each genotype and from a whole day's production were collected per group, excluding the defective eggs (double yolk, abnormal shell). Samples were analyzed for weight of egg, yolk (manually separated from the albumen 3 times, by means of a separator composed of the top cup designed to retain the yolk while letting the albumen slide to the bottom cup), and eggshell (after drying at room temperature over a period of 3 d and at 60°C for an additional 3 d) and albumen (calculated as the difference between the egg weight and that of the yolk and eggshell; Scott and Silversides, 2000) and yolk color. Yolk color was measured on intact yolk by a Chroma Meter CR 300 (Minolta Co Ltd., Osaka, Japan), using the CIE (Commission International de l'Eclairage, 1976) scale (D65). The L, a*, and b* values reflect lightness

Table 1. Formulation, chemical composition, and major fatty acid profile of the diet

Ingredient	%	Fatty acids ¹	%
Corn	58.0	C12:0	0.04
Wheat bran	11.0	C14:0	0.57
Soybean	13.0	C15:0	0.06
Sunflower	10.0	C16:0	15.1
Calcium carbonate	7.50	C16:1	0.31
Dicalcium phosphate	0.50	C17:0	0.12
Chemical composition ²		C17:1	0.09
DM	89.8	C18:0	3.68
CP (N × 6.25)	18.8	C18:1 n-9	24.0
Ether extract	6.99	C18:1 n-7	1.47
Crude fiber	4.06	C18:2 n-6	46.5
Ash	11.3	C18:3 n-3	3.61
Ca	3.67	C20:0	0.32
Total P	0.88	C20:1 n-9	0.32
Calculated ME (MJ/kg) ³	11.80	C20:2 n-6	0.07
		C20:4 n-6	0.09
		C20:5 n-3	0.08
		C22:5 n-6	0.01
		C22:5 n-3	0.10
		C22:6 n-3	0.42

¹Of total fatty acids.²On a DM basis.³By NRC feed values (NRC, 1994); as-fed basis.

(0 = black, 100 = white), redness (−100 = green, 100 = red), and yellowness (−100 = blue, 100 = yellow) of the samples, respectively. The instrument was calibrated against a white standard plate. The albumen pH was measured by means of pH-meter (Hanna Instruments, Inc, Woonsocket, RI). Additional samples of 30 eggs of each genotype were stored at room temperature (at 21°C and 62% RH) until 3 wk before being analyzed for egg weight, albumen height (by means of a micrometer, Mitutoyo Co, Kawasaki, Japan), and albumen pH. Egg weight and albumen height were used for calculating the Haugh Units (HU; Zeidler, 2002a).

Chemical and Fatty Acid Composition of Diet and Egg Yolk

At 35 and 43 wk of age, samples of feed and samples of 30 eggs per genotype were collected. Ten yolk samples (3 yolks/sample; each pooled sample was homogenized by grounding after freeze-drying) per genotype were obtained and separately frozen in aluminum containers at −20°C up to 20 d for further analyses.

The crumbled feed was ground (1-mm mesh screen, Grindomix ZM 200, Retsch, GmbH, Haan, Germany) and then chemically analyzed for DM (method 950.46, AOAC International, 2006), protein (method 981.10, AOAC International, 2006), lipid (method 991.36, AOAC International, 2006), and ash (method 920.153, AOAC International, 2006). Yolk DM, protein, and ash were determined following the ISTISAN methods (Istituto Superiore di Sanità, 1996). Energy content was calculated based on the diet formulation and based on the chemical composition of each ingredient given by the NRC (National Research Council, 1994).

Lipid Extraction. Total lipids were extracted from 1 g of feed, yolk (freeze-dried and ground) according to

the method of Folch and Sloane-Stanley (1957). Each sample, placed in a glass tube provided with a sintered glass filter (a pore size of 40–60 µm) at the bottom, was homogenized (ART MICCRA D8, Mullheim, Germany) with chloroform-methanol (2:1, vol/vol), and filtered 3 times (30 mL/time); the aliquots were placed into a funnel-separator where a saline solution (0.88% KCl, vol/vol) was added (1/4 of the extracted volume). The lipid fraction was separated (after 6 h) and the bottom liquid was discharged from a funnel-separator and washed with methanol (1:1, vol/vol); after 30 min, it was filtered through paper filters (fast, ashless 41, Whatman Ltd., Maidstone, UK) topped with anhydrous sodium sulfate. Filters with anhydrous sodium sulfate were washed with 5 mL of chloroform. The bottom liquid and the chloroform wash were put into a rotating evaporator (BÜCHI Rotavapor, Fawil, Switzerland) kept in a water bath at 37°C until it reached constant weight and then dried in an oven at 105°C for 10 min.

Fatty Acid Analyses. The extracted lipids were then trans-esterified: samples of 20 to 40 mg were put into 5-mL Pyrex tubes, and 2 mL of internal standard, methylnonadecanoate-hexane (1:1, wt/vol; Fluka, Sigma-Aldrich, Steinheim, Switzerland) and 100 µL of sodium methoxide (1 M) were added. The samples were shaken (ASAL 717, ASAL, Milano, Italy) for 10 min, 150 µL of ossalic acid-diethyl ether (1 g/30 mL; Carlo Erba, Milano, Italy) were added, shaken again for 30 s, and then centrifuged (Sigma Laborzentrifugen, 3K 15, Braun Biotech International, Melsungen, Germany) at 800 × g for 10 min. The supernatant was placed in vials for gas chromatography analyses. Individual fatty acids were separated by a gas chromatograph (model 8000 Series Top, Carlo Erba Instruments, Milano, Italy), equipped with a capillary column Omega Wax

250 (30-m length, 0.25-mm internal diameter; Supelco, Bellefonte, PA) with an injection split ratio of 100:1. The operating conditions of the gas chromatograph were as follows: the initial temperature was 140°C, increasing by 4°C/min to 220°C. The temperature of the injector and the detector remained stable at 250 and 260°C, respectively. Helium was used as the carrier gas at a flow rate of 1.6 mL/min in the column. The fatty acid percentage was calculated with respect to the total fatty acids mass expressed as total area. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the standard sample (F.A.M.E. mix C4-C24, Lipid Standard, 18919-1AMP, Sigma-Aldrich, St. Louis, MO).

Cholesterol Determination. Tubes containing freeze-dried yolk (100 mg) samples had 5 mL of ethanol and 2 mL of KOH (50%, vol/vol) added and then were placed in a water bath (ASAL TRM 750) at 70°C shaking for 10 min. After cooling at room temperature, 1 mL of internal standard, pregnenolone-ethanol (45 mg/100 mL, Sigma Chemical Co., St. Louis, MO) and 35 mL of hexane-ethyl ether (1:1, vol/vol) were added. After the addition of 20 mL of deionized water, and after being shaken for 1 min, centrifugation (ALC centrifuge 4227, Milano, Italy) took place ($450 \times g$ for 20 min). An aliquot, 25 mL, of the supernatant organic phase was taken and dried in a rotating evaporator kept in a water bath at 35°C. The sample was obtained and 5 mL of the mobile phase (isopropanol/hexane, 7%, vol/vol) was analyzed by HPLC (LC Pump Series 410; UV spectrometer detector LC90 operating at 208 nm; Perkin Elmer, Waltham, MA; flow rate: 0.8 mL/min) equipped with a Bondclone column (10 m, Silica, 300 $\times$ 3.9 mm; Phenomenex, Torrance, CA).

Sensory Profile

At 43 wk of age, eggs were collected for sensory evaluation following the procedure (International Standards Organization, 2003). A trained descriptive analysis panel ($n = 8$) was used in this study. Panelists were trained on the specific matrix. During orientation sessions for this study, the lexicon and panel performance were validated through sampling, discussions, and reference materials. During training, the most adequate preparing and cooking procedures were defined to make the evaluation objective and to highlight the characteristics of the product.

Sensory attributes and definitions used by the descriptive panel to evaluate boiled eggs are described in Table 2. Odor, flavor, and texture terms represented different phases of evaluation. The lexicon of odor, aroma, and texture terms had been previously developed by the trained descriptive sensory panel (Warren and Ball, 1991; Gonzalez-Esquerre and Leeson, 2000). Samples of 30 eggs of each genotype produced within 24 h were transferred to the laboratory, where they were boiled for 15 min and then cooled for 10 min. This cooking time was necessary to ensure the adequate cooking of

the yolk of the heavier eggs. A sole cooking time was established because the egg weight interval was not overly different among groups, despite the fact that differences in yolk and albumen weights occurred among genotypes. Cooked samples were warmed at 45°C (15 min) in a pan before preparation for the sensory analysis to better sense the olfactory and aroma characteristics. Samples were placed in prewarmed glass custard dishes that were nested in coded Styrofoam cups so as to maintain the serving temperature (45°C). The eggs were presented to the panelists after being taken out of the shell and divided into 2 parts after warming and being placed in Styrofoam. Sample containers were coded with 3-digit numbers and presented to the panelists in individual sensory workstations equipped with computers for data collection using FIZZ Network software (Biosystèmes, Couternon, France). The test was performed in cabins lit with red light so as to eliminate any bias on the part of the panelists due to slight differences in yolk color.

Panelists used one-half of the egg to test for odor and flavor, the other half being used to test texture. The panelists were asked to evaluate the intensity of the sample characteristics and to mark their responses based on 10-point scales (Table 2). The sample cooking and presentation to panelists followed a monadic sequence at 20-min intervals to allow for fatigue recovery between sampling. Sensory evaluations were repeated 4 times. Sample order presentations to panelists were randomized across sessions.

Statistical Analysis

All data on the egg quality were subjected to 2-way ANOVA as a completely randomized design, with genotype and layer age as main effects, using the GLM procedure of SAS (SAS Institute, 2001). Data on the quality of stored eggs were subjected to 2-way ANOVA with genotype and storage time, and layer age and storage time, as main effects (SAS Institute, 2001). Significant differences among the means were determined using Duncan's multiple range test (SAS Institute, 2001).

Data on sensory evaluation were analyzed using FIZZ statistical calculation (FIZZ-Biosystèmes, Couternon, France) and SYSTAT 10 (SPSS Inc., Chicago, IL). This multivariate analysis procedure is used in sensory profiling to analyze genotype effect, panelists' effect, and attribute performance. Significant differences among the means were determined using Fisher's least significant difference using SYSTAT 10.

RESULTS AND DISCUSSION

Egg Yields

Before discussing the results, it is worth remembering egg yields of the studied genotypes. The HLB and HLW have better egg yields than E and R birds that are dual-purpose genotypes and have higher body gain

Table 2. Sensory attributes, definitions, and evaluation method used to profile boiled eggs

Attribute	Definition	Evaluation method	Score
Odor sensations			
Odor intensity	Force of stimulation felt over the sample when it is near the nose.	Cut the egg into two halves inside the glass, immediately smell the sample and evaluate the force of the felt stimulation.	1–10
Flavor sensations			
Aroma intensity	Force of total stimulation felt at level of odor bulb. This stimulation is due to the aromatic compounds produced during chewing and directed into the nose by respiration.	Chew the sample (3–5 chewings, eating same quantity of albumen and yolk) until aromatic compounds are felt.	1–10
Taste and texture properties evaluation			
Yolk/albumen hardness	Force required to compress the sample with molars.	Put the sample between molars and compress uniformly evaluating the force at initial deformation.	1–10
Yolk astringency	Perception caused by lubricant power loss of saliva.	Evaluate the perception of contraction and dryness of mouth mucose surface (similar to that caused by tannins of fruits or red wine).	1–10
Yolk adhesivity	Force requested to remove a sample sticking to palate and teeth by using the tongue. Sensation due to rapid disgregation of the sample into the saliva.	Chew the sample. After 4–8 chewings evaluate the force required to remove the sample from the teeth and particularly from the gums and palate by using the tongue.	1–10
Yolk moisture	Perception of moisture of a sample.	Evaluate the degree of moisture felt by mouth during the phase of texture properties evaluation.	1–10
Yolk solubility	Sensation felt when a sample quickly dissolves into saliva.	Place the whole sample into the mouth without chewing, and evaluate the time required to dissolve into saliva. Compare the dissolving time of the yolk sample with that of an analogous piece of meringue.	1–10

and a lower laying rate as reported in a previous work (Rizzi and Chiericato, 2010). The peak of oviposition was reached at 33 to 36 wk of age for E (63%), at 37 to 40 wk for HLW (91%), HLB (95%), and R (65%), and then a light decrease of production was observed.

Egg Quality

The chemical composition of the diet is found in Table 1. The fatty acid profile of the dietary lipids of feed, which was the main nutritive intake during the experimental period, is typical of diets based on corn, soybean, and sunflower seeds, where palmitic, oleic, and linoleic acids are highly represented (Galobart et al., 2002; Pesti et al., 2002).

The characteristics of the eggs are found in Table 3. Egg weight significantly differed among genotypes. Hybrid hens (61.7 g) produced heavier ($P < 0.01$) eggs than the Italian breeds (55.5 g). The HLB (62.9 g) and R (56.5 g) egg size were higher ($P < 0.01$) than HLW (60.4 g) and E (54.4 g) egg size, respectively. The albumen weight showed the same trend according to genotype. The yolk weight was higher ($P < 0.01$) in local breed eggs than in the hybrid eggs (E, 16.3 g; R, 16.1 g; HLB, 15.5 g; HLW, 15.8 g). The latter were heavier ($P < 0.05$) than HLB yolks. Our data on hybrid yolks agree with the results of Scott and Silversides (2000), whereas Bean and Leeson (2003) stated that the yolk weight of brown eggshell was higher than that of white eggshell. It is to be noted that the results obtained by other authors refer to other genotypes and different laying period and environmental conditions. The

shell weight was significantly different both between hybrid groups and purebred groups: it was higher in HLB (6.74 g) and R (6.28 g) in comparison ($P < 0.01$) to HLW (6.24 g) and E (5.43 g) birds, respectively. Jones et al. (2010) found similar results on the egg and shell weights between brown and white eggs. In hybrid eggs, the albumen percentage was higher ($P < 0.01$) than in Italian eggs, whereas the yolk percentage was lower ($P < 0.01$). The HLB eggs presented higher ($P < 0.01$) albumen (64.7) and lower ($P < 0.01$) yolk (24.6) percentage in comparison to HLW (63.5; 26.2) eggs. In local eggs, the percentage of albumen and yolk were similar. The shell percentage was different among the 4 genotypes: R (11.1) eggs showed a higher ($P < 0.01$) percentage than HLB eggs (10.8%). The latter showed a higher ($P < 0.01$) percentage in comparison to HLW (10.4%) and E (9.98%), which showed the lowest ($P < 0.01$) value.

The yolk:albumen ratio differed ($P < 0.01$) among the 4 genotypes: in Italian eggs it averaged 0.49 and the E (0.50) eggs showed a higher ($P < 0.01$) value than R (0.48), whereas in hybrid eggs, the average was 0.40 and the lower ($P < 0.01$) value was observed in HLB (0.38). Our results agree with those found by Suk and Park (2001), who observed higher yolk:albumen ratio in eggs of a dual-purpose purebreed of the Korean native chicken compared with a commercial egg-type genotype (ISA Brown). These authors found a negative correlation between yolk:albumen ratio and egg weight. Egg size is largely correlated to albumen weight (Scott and Silversides, 2000). Genetic improvement on the egg weight permitted the attainment of an egg with a

Table 3. Effects of genotype on egg weight, egg components, and yolk color

Item	Genotype ¹				SE ²
	HLB	HLW	E	R	
Egg weight, g	62.9 ^A	60.4 ^B	54.4 ^D	56.5 ^C	0.362
Albumen weight, g	40.7 ^A	38.3 ^B	32.7 ^D	34.1 ^C	0.276
Yolk weight, g	15.5 ^{c,C}	15.8 ^{b,BC}	16.3 ^{a,A}	16.1 ^{ab,AB}	0.110
Shell weight, g	6.74 ^A	6.24 ^B	5.43 ^C	6.28 ^B	0.054
% of egg:					
Albumen	64.7 ^A	63.5 ^B	60.2 ^C	60.5 ^C	0.164
Yolk	24.6 ^D	26.2 ^C	29.9 ^A	28.4 ^A	0.146
Shell	10.8 ^B	10.4 ^C	9.98 ^D	11.1 ^A	0.080
Yolk:albumen ratio	0.38 ^D	0.42 ^C	0.50 ^A	0.48 ^B	0.0035
L	62.4 ^b	62.9 ^{ab}	62.8 ^{ab}	63.2 ^a	0.238
a*	0.25 ^{b,B}	-0.09 ^{c,B}	1.03 ^{a,A}	1.15 ^{a,A}	0.1240
b*	51.6 ^B	50.7 ^B	52.1 ^B	54.2 ^A	0.470

^{a-c}Means within same rows followed by a different superscript are significantly different ($P < 0.05$).

^{A-D}Means within same rows followed by a different superscript are significantly different ($P < 0.01$).

¹HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

²HLB: n = 120; HLW: n = 123; E: n = 114; R: n = 99; color data: HLB: n = 119; HLW: n = 117; E: n = 108; R: n = 92.

higher albumen weight (Silversides and Budgell, 2004) without altering the yolk weight: the hybrid eggs can be therefore more proteic and they do not contain more lipids. This difference in egg composition can affect the protein and fat contents of fresh egg pasta (Alamprese et al., 2011). As indicated by Washburn (1990), the albumen weight has a medium-high heritability value, whereas the yolk weight has a low value; it should be noted that, within a genotype, the yolk size depends on the weight of the hen and on its nutrient intake (Coon, 2002). It is well worth remembering that the decrease in albumen/yolk ratio during aging of the hen significantly affects pasta cooking behavior (Alamprese et al., 2011).

In our study, the E (2,415 g) and R (2,636 g) hens presented the highest ($P < 0.01$) BW whereas the HLB (1,845 g) and HLW (1,574 g) weights were lower. In the interval between 24 to 43 wk of age, the heavier genotypes laid smaller eggs with lower albumen weight than the hybrids. The yolk color differs significantly among genotypes: lightness was higher ($P < 0.05$) in R yolk in comparison to HLB (63.2 vs 62.4), whereas HLW and E were intermediate. The a* parameter (green-red) in Italian eggs (1.09) was higher ($P < 0.01$) than hybrid eggs (0.08) and HLW presented more greenish ($P < 0.05$) yolks. The R yolks (54.2) showed a higher ($P < 0.01$) b* value (yellowness) compared with the other groups (51.5). In our trial, the main factor responsible of different yolk color is probably the effect of dilution of pigments into egg production (Coon, 2002), evident in hybrids as they presented a higher hen-day egg production in comparison to the purebreds (Rizzi and Chiericato, 2010) and a lower feed intake to egg mass ratio (2.26 vs. 4.37 at 41–43 wk of age) given that there was no availability of pigments from grass or insects for the conditions of the ground. It is good to remember that the experimental period started in summer when the grass was poorly present on the ground and then absent, as previously indicated, and thus there was not

a direct effect of grass and insects ingestion on pigments of eggs. If we consider the differences among the strains for the yolk color parameters, it is possible to hypothesize an effect of laying rate and a possible effect of genotype on the capacity of utilization of lipids and pigments from the feed and from mobilization of body lipids and pigments to the yolk; anyway, this aspect needs more knowledge.

Yolk composition is reported in Table 4: the eggs of the 4 groups showed similar DM and protein contents. The E yolks presented the highest content of lipid ($P < 0.05$) and ash ($P < 0.01$). Cholesterol content was different among genotypes: Italian-breed eggs (258 mg/egg) showed a higher ($P < 0.01$) content compared with hybrid eggs (219 mg/egg), both when referred to DM (+ 12.5%) and to a single egg (+ 17.3%); HLB showed lower cholesterol content than HLW (216 vs. 222 mg/egg; $P < 0.05$). Cholesterol values of this trial are higher than values present in literature (184 mg/egg, USDA, 2011), especially in the purebred eggs. Cholesterol may be affected by breed or strain, and it decreases with the age of the hen (Elkin, 2006). In the present situation, the birds were in the first half of the oviposition cycle and the Italian breeds showed a higher age at first egg (Rizzi and Chiericato, 2010), and thus, the yolk cholesterol of their eggs may be higher because of the physiological state of the birds. Some authors have stated that an inverse relationship exists between egg yolk cholesterol content and both the rate of egg production and yolk size (Elkin, 2006). In this trial and according to previous indications, the cholesterol content was lower in eggs laid by genotypes with a high rate of oviposition. It also decreased with age (43 vs. 34 wk), when referring to 100 g of DM (3,033 vs. 2,623), but when referring to a single yolk, it did not change, with the exception of the R eggs, where a 5% decrease was observed. It is important to mention that in the present study, the aging of the birds was concomitant to the decreasing environmental tem-

Table 4. Effects of genotype on chemical composition and cholesterol content of egg yolk

Item	Genotype ¹				SE ²
	HLB	HLW	E	R	
DM, %	49.9	50.2	50.5	50.0	0.236
Protein, ³ %	20.0	20.5	20.0	20.0	0.157
Lipid, ³ %	28.1 ^b	28.0 ^b	28.6 ^a	28.2 ^{ab}	0.183
Ash, ³ %	1.67 ^B	1.72 ^B	1.80 ^A	1.71 ^B	0.0219
Cholesterol, ³ mg/100 g	2,655 ^B	2,662 ^B	2,954 ^A	3,044 ^A	28.2
Cholesterol, ⁴ mg/egg	216 ^{c,B}	222 ^{b,B}	256 ^{a,A}	259 ^{a,A}	2.44

^{a,b}Means within same rows followed by a different superscript are significantly different ($P < 0.05$).

^{A,B}Means within same rows followed by a different superscript are significantly different ($P < 0.01$).

¹LB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

²HLB: n = 16; HLW: n = 19; E: n = 20; R: n = 15.

³On DM basis.

⁴On wet basis.

perature from about 30 to 5°C as naturally occurs from summer to autumn in Northern Italy. The photoperiod did not change, as it was supplemented with artificial light, which could have interfered with lipid metabolism. Hypocholesterolemic effects of the fiber content of the diet have been observed on other animal species, and in the laying hen, the dietary fiber may influence its metabolism by decreasing cholesterol absorption by binding with bile salts in the intestinal tract, thus reducing intestinal transit time and increasing fecal sterol excretion (Ayerza and Coates, 2000). In this trial, a lack of effect of lipid and fiber intake originating from grass ingestion was observed with respect to cholesterol content and this result was due to the absence of grass on the ground during the experimental period.

The fatty acid profile of yolk lipids is found in Table 5. The HLB yolks (33.8%) presented the lowest ($P < 0.01$) SFA. The results obtained on the hybrid eggs correspond to those reported by Ayerza and Coates (2000), which showed a lower content of SFA in brown eggs in comparison to white eggs. The MUFA were higher ($P < 0.01$) in HLB (38.6%) than in HLW (37.3%) and reached the lowest ($P < 0.01$) percentage in R (36.3%) birds. The higher percentage of MUFA in brown eggs in comparison to white eggs had also been observed by Ayerza and Coates (2000). The PUFA level significantly ($P < 0.01$) differed between E (27.2%) and R (28.7%) yolks, whereas hybrid eggs (27.5%) were similar; R yolks showed the highest ($P < 0.05$) levels. The PUFA n-6 are mainly represented by linoleic and arachidonic acid: the former is directly present in the diet based on corn, whereas arachidonic fatty acid derives from desaturation and elongation processes (Scheidegger et al., 1998). The highest ($P < 0.01$) percentage of PUFA n-6 and n-3 was reached in the R eggs (26.6%; 2.12%) and the lowest ($P < 0.01$) PUFA n-3 in HLW (1.88%). The PUFA n-3 were represented mainly by linolenic acid, whereas a small percentage of EPA and DHA was detected, as they were poorly present in the diet and because the hens have a limited ability to elongate and desaturate linolenic acid (Elkin, 2006). Factors capable of enhancing PUFA n-3 content of eggs might be fresh vegetables, insects, or worms (Simopoulos and

Salem, 1992). Under our conditions, as stated above, the rearing in open spaces did not allow the birds to eat them as to increase PUFA n-3 content. The imbalance between PUFA n-6 and PUFA n-3 may cause pathologies such as cancer, cardiovascular diseases, and allergy (Simopoulos, 2000). The n-6:n-3 ratio was different among the genotypes, with values being more ($P < 0.01$) favorable for HLB, E, and R eggs (12.7%) in comparison to HLW (13.6%). At any rate, the values are higher than those suggested (5:1; British Nutrition Foundation, 1999; Simopoulos, 2000) to prevent certain pathologies. The HLW and E showed less favorable ($P < 0.05$) values of SFA/PUFA and SFA/n-3 when compared with the other groups.

Furthermore, it is to be noted that the acidic profile and the ratio between groups of fatty acids observed in this and in other trials (Hidalgo et al., 2008) do not differ much from those of eggs produced intensively; this result indicates that to modify the fatty acid composition by means of ingestion of worms or fiber (Simopoulos and Salem, 1992) requires adequate ground conditions. So it may be hypothesized that the positive effect of worms, insects, and grass ingestion on the fatty acid composition of lipids mentioned by other authors may be reached only when outdoor environmental and weather conditions allow their life and growth. In many Italian regions, dry weather conditions often do not allow a pasture with a presence of grass, insects, and worms when the bird density is only 4 m²/bird at least, as required by European Regulations (European Union, 1999), without an adequate rotation.

Effect of Storage on Egg Quality

In Table 6, some characteristics of the eggs during storage are summarized. The weight of the stored eggs followed the same trend of fresh eggs among the genotypes; E showed different values ($P < 0.01$) for albumen pH (9.03), albumen height (4.93 mm), and HU (70.8) in comparison to the other strains (8.95, 5.56, 73.8, respectively). The egg weight decreased ($P < 0.01$) after 3 wk of storage (57.5 vs. 55.7 g) at 21°C, whereas the albumen pH increased ($P < 0.01$) after 1 wk of storage

Table 5. Effect of genotype on fatty acid profile¹ (% of total fatty acids) of yolk lipids

Item	Genotype ²				SE ³
	HLB	HLW	E	R	
SFA	33.8 ^{c,B}	35.1 ^{a,A}	34.7 ^{b,A}	35.0 ^{ab,A}	0.111
MUFA	38.6 ^{a,A}	37.3 ^{b,B}	38.1 ^{a,AB}	36.3 ^{c,C}	0.200
PUFA	27.4 ^B	27.6 ^B	27.2 ^B	28.7 ^A	0.220
PUFA n-6	25.6 ^{b,B}	25.7 ^{b,AB}	25.2 ^{b,B}	26.6 ^{a,A}	0.203
PUFA n-3	2.00 ^B	1.88 ^C	1.97 ^B	2.12 ^A	0.0179
n-6/n-3	12.8 ^B	13.6 ^A	12.8 ^B	12.6 ^B	0.072
SFA/PUFA	1.23 ^b	1.28 ^a	1.28 ^a	1.22 ^b	0.0112
SFA/n-3	17.0 ^{b,BC}	18.7 ^{a,A}	17.6 ^{b,B}	16.7 ^{b,C}	0.186

^{a-c}Means within same rows followed by a different superscript are significantly different ($P < 0.05$).

^{A-C}Means within same rows followed by a different superscript are significantly different ($P < 0.01$).

¹SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

²HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

³HLB: n = 16; HLW: n = 19; E: n = 20; R: n = 15.

(8.16 vs. 9.25). Albumen height (7.42–4.08) and HU (86.6–61.5) showed a progressive decrease ($P < 0.01$) throughout storage.

In Table 7, the effect of storage time on the egg quality of each genotype is reported. The albumen pH showed an increase ($P < 0.01$) and then a decrease throughout the storage at 21°C, and the peak was reached at a different storage time according to the genotype (HLB, 9.29; HLW, 9.27; E, 9.37); only R eggs showed an initial increase ($P < 0.01$) and then the values did not changed (8.16 vs. 9.22). The albumen height gradually ($P < 0.01$) decreased until 21 d of storage in the HLB and HLW hybrids which showed a similar trend (8.06–4.11; 7.97–3.94, respectively). The albumen height of E and R purebreds showed lower ($P < 0.05$) differences between 14 and 21 d (4.21 vs. 3.79; 4.86 vs. 4.38, respectively). The HU gradually decreased ($P < 0.01$) in hybrids (HLB, 88.8–58.5; HLW, 88.7–58.0) and E (83.1–59.2); R showed a less marked decrease ($P < 0.05$) from 14 to 21 d of storage (68.8 vs. 64.9). Cer-

tain factors such as the eggshell condition (thickness, membranes, and pore diameters) may be involved in the egg aging process (Lakins et al., 2009). The E eggs had an eggshell with the lowest thickness when compared with the other 3 groups (0.326 vs. 0.356 mm). The thick layer of albumen is made up of firm protein with gel characteristics that may be attributed to the large amount of ovomucin which is almost completely absent in the thin layers (Zeidler, 2002b). It is known that the quantity of thick albumen differs according to genetics (Zeidler, 2002a) and that the percentage of thin albumen increases along with egg age (Zeidler, 2002a; Kempes et al., 2010). As eggs age, H₂O and CO₂ are released through the pores of the eggs and this release causes the pH to increase with the eggs, causing rapid deterioration to the albumen quality and albumen proteins (Lakins et al., 2009).

As indicated by other authors, the Haugh units are useful in identifying the standard for quantifying the quality of the egg albumen (Jones and Musgrove, 2005),

Table 6. Effects of genotype and storage time (d) on egg weight, albumen pH, albumen height, and Haugh units

Item	Egg weight, g	Albumen pH	Albumen height, mm	Haugh units ¹
Genotype ²				
HLB	60.9 ^A	8.96 ^{AB}	5.71 ^A	74.0 ^A
HLW	58.7 ^B	8.93 ^B	5.57 ^{AB}	73.7 ^A
E	53.1 ^D	9.03 ^A	4.93 ^C	70.8 ^B
R	55.2 ^C	8.97 ^B	5.39 ^B	73.7 ^A
Storage				
1 d	57.9 ^A	8.16 ^{c,C}	7.42 ^A	86.6 ^A
7 d	57.0 ^A	9.25 ^{a,AB}	5.45 ^B	73.5 ^B
14 d	57.4 ^A	9.28 ^{a,A}	4.65 ^C	66.3 ^C
21 d	55.7 ^B	9.20 ^{b,B}	4.08 ^D	61.5 ^D
SE ³	0.301	0.0158	0.0546	0.452
Genotype × storage	ns	**	**	**

^{a-c}Means within same columns followed by a different superscript are significantly different ($P < 0.05$).

^{A-D}Means within same columns followed by a different superscript are significantly different ($P < 0.01$).

¹Zeidler (2002b).

²HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

³Genotype: HLB: n = 390; HLW: n = 387; E: n = 261; R: n = 351. Storage: 1 d: n = 422; 7 d: n = 362; 14 d: n = 249; 21 d: n = 356.

** $P < 0.01$.

Table 7. Effects of storage time (d) on albumen pH, albumen height, and Haugh units of each genotype

Item	Genotype ¹			
	HLB	HLW	E	R
Albumen pH				
1 d	8.11 ^C	8.11 ^D	8.23 ^D	8.16 ^B
7 d	9.29 ^A	9.12 ^C	9.37 ^A	9.25 ^A
14 d	9.28 ^A	9.27 ^A	9.29 ^B	9.18 ^A
21 d	9.20 ^B	9.24 ^B	9.27 ^C	9.21 ^A
SE ²	0.0147	0.0145	0.0173	0.0287
Albumen height, mm				
1 d	8.06 ^A	7.97 ^A	6.77 ^{a,A}	7.00 ^{a,A}
7 d	5.86 ^B	5.61 ^B	5.02 ^{b,B}	5.35 ^{b,B}
14 d	4.86 ^C	4.80 ^C	4.21 ^{c,C}	4.86 ^{c,BC}
21 d	4.11 ^D	3.94 ^D	3.79 ^{d,C}	4.38 ^{d,C}
SE ²	0.0946	0.1020	0.1010	0.1361
Haugh units ²				
1 d	88.8 ^A	88.7 ^A	83.1 ^A	84.0 ^{a,A}
7 d	74.4 ^B	73.5 ^B	70.7 ^B	73.2 ^{b,B}
14 d	66.2 ^C	66.5 ^C	63.2 ^C	68.8 ^{c,C}
21 d	58.5 ^D	58.0 ^D	59.2 ^D	64.9 ^{d,C}
SE ³	0.764	0.781	0.940	1.07

^{a-d}Means within same columns followed by a different superscript are significantly different ($P < 0.05$).

^{A-D}Means within same columns followed by a different superscript are significantly different ($P < 0.01$).

¹HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

²Zeidler (2002b).

³HLB: 1 d: $n = 112$; 7 d: $n = 100$; 14 d: $n = 78$; 21 d: $n = 100$. HLW: 1 d: $n = 112$; 7 d: $n = 100$; 14 d: $n = 77$; 21 d: $n = 98$. E: 1 d: $n = 112$; 7 d: $n = 85$; 14 d: $n = 66$; 21 d: $n = 88$. R: 1 d: $n = 86$; 7 d: $n = 77$; 14 d: $n = 28$; 21 d: $n = 70$.

whereas the pH value is more suitable in indicating the freshness of the egg (Silversides and Scott, 2001; Silversides and Budgell, 2004). It is worth pointing out that in our trial, pH values lightly differed among genotypes and they differently changed during storage according to the strain, so it is possible to hypothesize that characteristics of albumen or shell could be involved (Zeidler, 2002a,b; Lakins et al., 2009), and indications of egg freshness have to be considered particularly during the first week of storage. Also Jones et al. (2010) did not find differences in HU values between brown and white eggs as well as in albumen height.

Sensory Profile

In Table 8, sensory parameters of boiled eggs are reported. Odor intensity significantly differed among genotypes: hybrid and R eggs showed lower scores ($P < 0.05$) in comparison to the E group. Aroma scores were significantly ($P < 0.05$) lower in HLW than in R, whereas HLB and E were intermediate. It should be noted that egg odor and aroma reflect chemical composition, particularly the amino acid and lipid composition (Cherian et al., 2002) and how they have been processed before use. The odor sensation is generally induced by volatile compounds of low molecular weight, whereas water-soluble compounds influence flavor (Aliani and Farmer, 2005). The volatile compounds of yolk that originate from the oxidative processes of boiling depend on the lipid composition of the hen diet (Cherian et al., 2002). Under the present conditions, interaction between diet and genotype and environmental

conditions may have occurred and may have affected lipid metabolism and thus the lipid profile of eggs and some of their sensory characteristics. The yolk taste did not present differences as sourness, bitterness, sweetness, and saltiness characteristics were similar among genotypes. The HLB presented lower ($P < 0.05$) adhesivity, whereas R showed the highest ($P < 0.05$) score; the other groups were intermediate. Yolk astrincency did not differ among genotypes; nor did hardness. Yolk solubility presented higher ($P < 0.05$) values in HLB and lower ($P < 0.05$) in R. Moisture scores did not differ among the groups. Further research is needed to elucidate possible changes in the quantity of fatty acids having a different polarity and hydrophobicity which could be partially responsible for variations in physical properties of the eggs (Watkins et al., 2003).

As far as albumen characteristics are concerned (Table 8), albumen hardness did not present significantly different scores among the 4 groups. The HLB hens laid eggs with higher albumen weight and incidence but with similar albumen height when compared with HLW hens. The variation in albumen sensory response might be in relation to its protein component and content rather than boiling time. Among protein components of albumen, ovomucin is responsible for albumen height; it is not clear whether there is a relationship between the height of albumen and its functional characteristics (Silversides and Scott, 2001). The present trial gives indications on the quality of eggs laid by hens belonging to 2 widely used hybrids and 2 Italian breeds reared under organic production system conditions during the first phase of the oviposition cycle. It is worth re-

Table 8. Sensory profile of boiled eggs

Item	Genotype ¹				SE ²
	HLB	HLW	E	R	
Egg odor and flavor sensations					
Odor	4.88 ^b	4.13 ^b	6.62 ^a	3.63 ^b	0.5534
Aroma	5.07 ^{bc}	3.93 ^c	5.58 ^{ab}	6.60 ^a	0.4922
Yolk taste and texture properties					
Sourness	5.05	5.28	4.83	3.88	0.8792
Bitterness	5.36	4.27	4.85	3.97	0.9170
Sweetness	5.83	4.80	5.62	5.45	0.4291
Saltiness	5.66	4.62	5.73	6.02	0.4746
Adhesivity	5.38 ^b	6.22 ^{ab}	6.05 ^{ab}	7.12 ^a	0.4117
Astringency	1.33	1.65	0.37	2.97	0.7059
Hardness	3.84	3.88	4.50	4.92	0.4356
Solubility	6.44 ^a	3.67 ^{bc}	6.23 ^{ab}	3.00 ^c	0.8779
Moisture	6.83	6.03	5.78	4.87	0.6145
Albumen texture properties					
Hardness	3.01	4.25	2.88	3.05	0.5326

^{a-c}Means within same rows followed by a different superscript are significantly different ($P < 0.05$).

¹HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta maculata.

²n = 8.

membering that the positive effects of outdoor rearing for the birds was limited only to physical activity and solar radiation, as foraging in our conditions was not available and the positive effect of high ingestion of carotenoids, α -tocopherols, and polyphenols (Castellini, 2005) occurred only before and in the first part of the experimental period. The different genetic assessment of 2 hybrid strains selected for table-egg production and 2 double-purpose Italian breeds affected the egg quality as it concerns the egg components (eggshell, albumen, and yolk weight), yolk composition, and egg sensory profile. The careful consideration of some quality parameters has suggested the possibility to identify 2 kinds of eggs according to their different use. The purebred eggs, laid by hens with an oviposition rate lower than that of the hybrids due to their double-purpose characteristics, have larger and more pigmented yolks and higher cholesterol content and yolk:albumen ratio. Therefore, it may be stated that the eggs laid by the different genotypes may have different functional properties, as indicated by Alamprese et al. (2011), and also different employments either for direct consumers or egg products because their constituents contribute to the appearance, texture, and flavor of many food products.

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