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Laying hen biodiversity: the effect of genotype and age on the yield performance and egg quality of two Italian purebred chickens

Chiara Rizzi*

University of Padua, Department of Agronomy, Food, Natural resources, Animals and Environment – Viale dell'Università, 16, 35020 Legnaro (Padua), Italy

Yield performance and external and internal quality traits of eggs laid by two Italian purebreds, Ermellinata di Rovigo (ER) and Pepoi (PP) were studied at 36 and 50 weeks of age. Daily egg production and egg traits were evaluated by a factorial model (2 x 2) with genotype and age as main effects and their interaction. The daily laying rate was similar between the breeds and the hen-day egg mass was higher (p<0.01) for ER than for PP. The ER eggs differed (p<0.01) from PP eggs with higher egg weight, yolk and albumen weight, and a more coloured eggshell with lower L, higher a* and b*, and lower eggshell thickness. PP showed more (p<0.05) inclusion of albumen than yolk in comparison to ER. With increased hen age, egg weight, eggshell lenght, width, lightness and thickness increased (p<0.01), whereas shape index and a* and b* did not change. Throughout the laying period, the eggs were classified as small (PP) and medium size (ER). With increased hen age, PP eggs were more spherical, ER eggs were more ovoid; yolk and albumen weight increased (p<0.01) in ER, whereas yolk and eggshell weight increased (p<0.01) in PP. The yolk:albumen ratio increased (p<0.01) along with hen's age and the Haugh Units and egg inclusions decreased (p<0.01). The ER and PP eggs differed for many egg external traits, which allow to distinguish the eggs by weight, shape, and eggshell colour and for the eggshell thickness and the albumen traits.

Keywords: laying hen, local breed, eggshell trait

1 Introduction

In the European Union egg production industry, there are multiple different farming methods, including enriched cages (49.5%), barn (32.5%), free range (11.8%) and organic (6.2%) production systems according to the commission implementing regulation (EU) 2017/1185 (European Commission, 2020). The production utilizes hybrid hens, mainly reared under the first two methods, but in recent years interest in purebred hens has progressively increased. There are many reasons for the growing interest in local and purebred genotypes: they are linked to local traditions and represent historical and traditional products, show many phenotypes that may be appreciated by an aesthetic point of view and they represent biodiversity. Biodiversity is a source of genetic resources, regarding anatomical and physiological traits, but also behavioural and production quality traits, which can be useful. The increasing interest shown by consumers in animal welfare and rearing systems, and for the typical and regional products, besides the perspective by the producer of counteracting the drugs uses in the intensive production systems and the body abnormalities of the highly productive selected hybrids, have induced a progressive request to genotypes whose productivity can be enhanced in less intensive production systems, such as free-range and organic. Furthermore, dual-purpose purebreds can counteract the problem of culling one-day old male birds of laying strains, given that the males can be used for meat production and the females for egg production (Giersberg et al., 2020). The knowledge of the anatomical and physiological traits as well as the production traits of a breed is important for the breeding activity, which can obtain F1 crossed birds with a specific production profile.

* **Corresponding Author**: Chiara Rizzi. University of Padua, Department of Agronomy, Food, Natural resources, Animals and Environment – Viale dell'Università, 16, 35020 Legnaro (Padua), Italy E-mail: chiara.rizzi@unipd.it.

Previous studies on local laying hen breeds have been carried out in many countries (González Ariza et al., 2019 a,b, Lordelo et al., 2020) and some indications exist for the Italian breeds (Rizzi & Chiericato, 2010, Rizzi & Marangon, 2012, Di Rosa et al., 2020, Rizzi, 2020).
In order to increase the knowledge of the productive performance and egg quality of local laying hen breeds, a trial was carried out to study the effect of genotype and age on yield performance and egg quality of two Italian purebreds, at 36 and 50 weeks of age.

2 Material and methods

2.1 *Animals and data collection*

The eggs used in the present study came from two Italian breeds, Ermellinata di Rovigo (white plumage and black with white edge hackle and saddle; black main tail feathers and white primaries with black extremities, single comb - ER) and Pepoi (golden plumage with black breast and tail feathers, and brown primaries, hackle and saddle, single comb - PP). The ER is a dual-purpose (meat and egg, adult body weight of female: 2.2-2.4 kg) breed and PP is a small (adult body weight of female: 1.0-1.1 kg) breed. They originate from Veneto (Italy): ER was bred in Veneto during the 1950s using Sussex and Rhode Island breeds and PP is an old autochthonous breed (Cassandro et al., 2014).

The hens of each genetic group were reared on the same farm of the Veneto region in Northern Italy from hatching to pubertal age and throughout the laying period, which started in autumn. The newly hatched chicks were kept indoors during the first 4 weeks of life, in litters under infra-red radiation lamps, with environmental temperature decreasing from 32 to 24°C. At 2 months of age, the birds were given free access to outdoor spaces from spring until autumn and winter. Each genotype (60 hens) had free access to indoor (0.20 m^2/bird) space, mainly used for laying eggs and at night, and outdoor (4 m^2/bird) space, where they stayed throughout the day; the area (indoor and outdoor) available to each genotype was divided by netting. Feeding, rearing conditions (temperature, photoperiod), and prophylaxis procedures were the same for the two groups from the time of hatching until the end of the tested period. The animals were given two commercial feeds *ad libitum*, which met the nutrient requirements for the growing and laying phases. The egg production was checked daily, throughout two periods, from 29 until 36 (autumn) and from 43 until 50 weeks of age (winter). At the start of laying (29 weeks of age, autumn), the photoperiod was natural and decreased according to the season and the geographical position of the trial station in Northern Italy (11L:13D). For the second period it was gradually incremented with artificial light (inside the indoor space) (16L:8D). The external environmental temperature was 10±3.2°C and 11±3.5°C, respectively, for the two stages.
At 36 and 50 weeks of age, samples of 15-20 eggs (depending on the daily production of each genotype) from a whole day's production per each genotype were collected throughout two weeks. The eggs were weighed, the eggshell colour was tested with a colorimeter (Chroma meter CR 300 Minolta Co Ltd., Osaka, Japan), using the CIE scale: L, a* and b* values reflect lightness (0 = black, 100 = white), redness (-100 = green, 100 = red) and yellowness (-100 = blue, 100 = yellow), respectively. The hen-day egg production was calculated as daily number of eggs/number of live hens x 100 and the hen-day egg mass was calculated as hen-day egg production x daily egg weight. The width and the length of each egg was measured with callipers, and the shape index was calculated as egg width/egg length x 100.
For internal quality, yolks were manually separated from the albumen, weighed and the albumen weight was calculated as the difference between the weight of the egg and the sum of the weight of yolk and eggshell (after drying at 50°C for 12 h). For Haugh Units (HU) measurements, the eggshell was broken along the equatorial axis and the yolk and albumen were put on a glass plate to measure the albumen height by means of a micrometer (0.01 mm) (Mitutoyo Co, Kawasaki, Japan). The eggshell thickness was measured with digital callipers (0.001 mm) (Mitutoyo, Japan). Egg weight and albumen height were used for calculating HU (Zeidler, 2002). Blood and meat spots were visually evaluated on yolk and albumen, respectively.

2.2 Statistical analysis

The hen-day egg production (on a weekly basis) and mass (on a daily basis), and the external (egg weight, egg length, egg width, shape index, eggshell colour and thickness, and eggshell percentage) and internal egg quality (weight and percentage of yolk, albumen, yolk:albumen ratio, Haugh Units) were evaluated by ANOVA, following a factorial model (2 x 2) considering genotype and age as main effects, and their interaction and using the proc GLM of SAS (SAS Institute Inc., Cary, NC, USA). Significant differences among least squares means were tested using Tukey's test. Significant differences between breeds for egg inclusion percentages and between blood and meat spots for each genotype were tested by χ^2 test (SAS Institute inc., Cary, NC, USA).

3 Results and discussion

The effects of genotype, age, and genotype x age interaction on hen-day egg production, hen-day egg mass and egg weight of ER and PP hens are shown in Table 1. The two breeds did not differ significantly for oviposition rate. However oviposition was affected by age. Hen-day egg mass was significantly affected by genotype and age as a consequence of different egg weights (Table 1).

Table 1 Significance of the effects of genotype (G), age (A), and genotype x age interaction (G x A) on yield performance and egg weight of Ermellinata di Rovigo (ER) and Pepoi (PP) hens

	G	A	G x A	RMSE	*p*-value
Hen-day egg production	0.2756	0.0060	0.8494	11.51	0.0314
Hen-day egg mass	<0.0001	<0.0001	0.0028	4.54	<0.0001
Egg weight	<0.0001	<0.0001	0.3591	3.71	<0.0001

RMSE = Root Mean Square Error.

In Figure 1 the hen-day egg productions of ER and PP at 29-36 and 43-50 weeks of age are shown. The laying rates of ER and PP hens did not differ, showing high variability, whereas they increased ($p<0.01$) with age. The laying rates were lower than those of hybrid strains, both brown-eggshell and white eggshell (Hy-Line International, 2020), intensively reared throughout the same weeks of productive cycle.

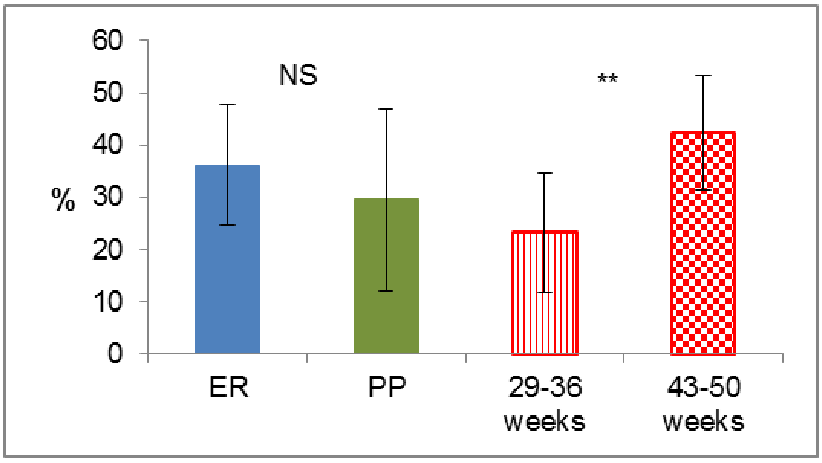

Figure 1 Effect of genotype (Ermellinata di Rovigo - ER and Pepoi - PP) and age (29-36 and 43-50 weeks) on the hen-day egg production (means ± SD)

NS: no statistical significance between genotypes; **: different values ($p<0.01$) between ages.

Observations: n = 4 / genotype / time period.

The ER and PP breeds have not been selected for high laying rates and show slow body growth and descrete muscle growth, especially ER, as indicated in previous research (Rizzi & Chiericato, 2010). For this reason they generally start laying later than hybrids (Hy-line International, 2020). It is well known that reproduction and laying activity in birds depend on hormones, nutrients, and environmental conditions (Hadinia et al., 2020). The oviposition rate of these breeds was also affected by photoperiod and temperature, given that the birds were reared outdoors, and until 36 weeks of age the photoperiod was natural and decreasing, and from 40 weeks of age the photoperiod was artificially increased. Such lighting management may be adopted when the birth of offspring occurs in the early spring and the birds reach sexual maturity by the end of the summer. With the aim of having the egg production period of these birds under outdoor conditions, it would be more appropriate to avoid artificial lighting the first months of laying activity throughout the autumn, thus allowing the birds to complete their growth and store energy reserves for the cold winter months. It is well known that the start of laying activity is affected by the photoperiod. In the present study, only ER and PP hens started laying, whereas other purebreds of same age did not show any laying activity (Padovana and Polverara) or a very low (Robusta Maculata and Robusta Lionata) oviposition rate. The reason for this might be possible differences in responses of the hens to the natural decreasing photoperiod and their body conditions. In Figure 2 the effect of genotype on the hen-day egg mass production at 35-36 and 49-50 weeks of age is shown. The ER daily egg mass was higher (p<0.01) than for PP; age positively affected (p<0.01) the daily egg mass in both genotypes (Table 1). It is worth noting that, as a consequence of a not stable laying activity, the two genotypes may not be differentiated, as it occurs between ER-36w and PP-50w.

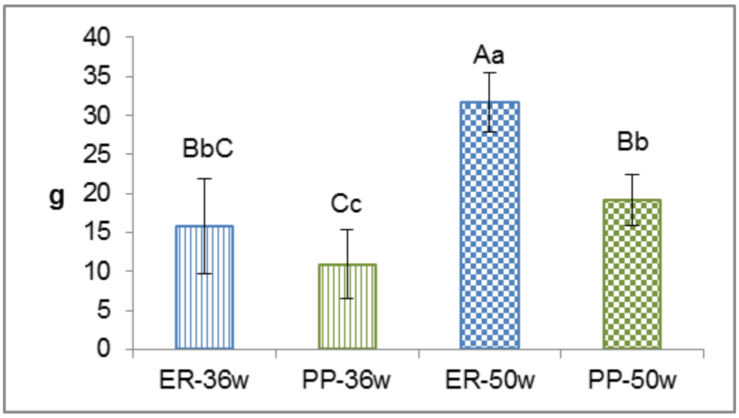

Figure 2 Hen-day egg mass (means ± SD) of Ermellinata di Rovigo (ER) and Pepoi (PP) at 36 (-36w) and 50 (-50w) weeks of age

Different letters among columns indicate different values. a,b,c: p<0.05; A, B, C: p<0.01.
Observations: n = 14 / genotype / time period.

The egg weight and size class percentages of ER and PP eggs at 36 and 50 weeks of age are shown in Figure 3.

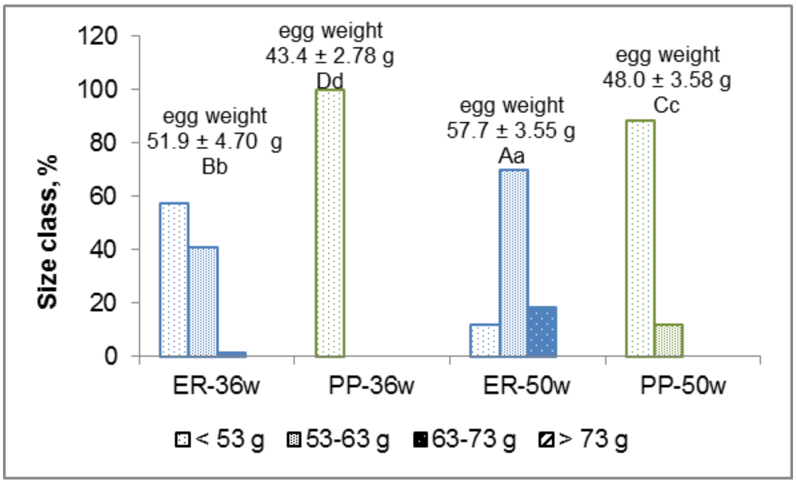

Figure 3 Egg weight (means ± SD) and size class of Ermellinata di Rovigo (ER) and Pepoi (PP) at 36 (-36w) and 50 (-50w) weeks of age

Different letters among genotypes indicate different average egg weights. a,b,c,d: p<0.05; A, B, C, D: p<0.01.
Observations: n = 61 ER (n = 36 at 36 weeks, n = 25 at 50 weeks), n = 66 PP (n = 40 at 36 weeks, n = 26 at 50 weeks).

The egg weight was higher (p<0.01) for ER than for PP and it increased (p<0.01) with age. According to the EU classification of egg size (Commission Regulation EC, 2008), the ER and PP eggs were classified as small (< 53 g), medium (53-63 g) and large (63-73 g), but not as very large (> 73 g). The

ER-36w egg sizes ranged from small (57.6%) to medium (40.9%) and large (1.5%), which changed their percentages at 50 weeks, as small size decreased (11.7%) and medium (70.0%) and large (18.3%) sizes increased; the PP hens laid eggs of small size (100%) at 36 weeks, and at 50 weeks only 11.7% were medium size, as showed in Figure 3. The ER hen can be classified as a medium-egg breed, excluding the first months of egg production. The PP eggs had increased weight with age, but given the low % of medium sized eggs, this genotype can be classified as a small-egg breed throughout the entire laying period. The knowledge of the egg size throughout the laying period is important for both the producer and the consumer in a market perspective.

The effect of genotype and age and their interaction on the eggshell traits are shown in Table 2. The two genotypes showed significant differences for all traits, with the exception of eggshell weight. The age effect was significant for the egg dimensions, as well as for eggshell lightness, thickness and weight. The shape index, a* and b* values and eggshell percentage did not change with age. The interaction was significant for the eggshell lenght, shape, colours and percentage. The eggshell length and width differed between the genotypes, being higher (p<0.01) for ER than for PP and increased (p<0.01) with age. As shown in Table 2, the egg length increased (p<0.01) for ER, whereas the egg width increased for both ER (p<0.05) and PP (p<0.01). As a result, at 50 weeks of age, the ER eggs were more ovoid (p<0.01), and PP eggs were more spherical (p<0.05).

Table 2 Eggshell traits of Ermellinata di Rovigo (ER) and Pepoi (PP) hens at 36 (-36w) and 50 (-50w) weeks of age

	ER-36w	PP-36w	ER-50w	PP-50w	G	A	G x A	RMSE
Length, mm	5.46 ^{Bb}	5.17 ^{Cc}	5.77 ^{Aa}	5.27 ^{Cc}	<0.0001	<0.0001	0.0027	0.19
Width, mm	4.12 ^{ABb}	3.85 ^{Cd}	4.22 ^{Aa}	4.03 ^{Bc}	<0.0001	<0.0001	0.0684	0.12
Shape index,%	75.6 ^{Aab}	74.6 ^{ABbc}	73.2 ^{Bc}	76.5 ^{Aa}	0.0160	0.6317	<0.0001	2.69
L	70.5 ^{Cc}	85.0 ^{Aa}	77.4 ^{Bb}	84.3 ^{Aa}	<0.0001	<0.0001	<0.0001	3.68
a*	10.6 ^{Aa}	1.53 ^{Cd}	7.77 ^{Bb}	3.16 ^{Cc}	<0.0001	0.1255	<0.0001	2.11
b*	22.3 ^{Aa}	12.9 ^{Bc}	21.0 ^{Aa}	15.2 ^{Bb}	<0.0001	0.4329	0.0021	3.16
Thickness, µm	301 ^{Bc}	318 ^{ABbc}	321 ^{AaBb}	337 ^{Aa}	0.0015	0.0002	0.8885	27.00
Weight, g	4.41 ^{ABbc}	4.14 ^{Bc}	4.88 ^{Aa}	4.80 ^{Aab}	0.1331	<0.0001	0.4009	0.51
Ratio[1], %	8.51 ^{Bc}	9.40 ^{Ab}	8.44 ^{Bc}	10.0 ^{Aa}	<0.0001	0.0654	0.0185	0.63

RMSE = Root Mean Square Error.

Different letters among columns indicate different values. a,b,c,d: p<0.05; A, B, C: p<0.01.
Observations: n = 61 ER (n = 36 at 36 weeks, n = 25 at 50 weeks), n = 66 PP (n = 40 at 36 weeks, n = 26 at 50 weeks).
[1] Ratio = eggshell weight/egg weight x 100.

The eggshell lightness was lower (p<0.01) for ER than for PP. From 36 until 50 weeks of age, it increased (p<0.01), but only ER showed significantly higher (p<0.01) values. Concerning redness index, the ER eggshells showed higher a* values (p<0.01) than PP. With age, no significant difference was found as the trend was opposite for the two breeds. The ER eggshells showed decreasing (p<0.01) values, whereas PP showed increasing (p<0.05) values. The b* values were higher (p<0.01) for ER eggs than for PP, and it only increased with age (p<0.05) for PP. Regarding eggshell thickness, it was lower (p<0.01) for ER eggs than for PP, and increased (p<0.05) for both genotypes with age. Genotype did not affect eggshell weight, but ER eggshell percentages were lower (p<0.01) than those of PP. The eggs laid by older hens showed higher eggshell weights, both for ER (p<0.05) and PP (p<0.01). Age did not affect the incidence of eggshell on the egg weight as only PP showed an increase (p<0.05). It is known that with increased hen age, egg weight increases along with a decrease of the surface to area volume ratio. This may have a positive effect on egg storage, as it may counteract weight loss. Eggshell colour and thickness are the two main factors affecting weight loss as stated by a previous research on *Galliformes* (Nowaczewski et al., 2013). For ER, the eggshell thickness values slightly exceeded 300 µm, which is considered the minimum value for an acceptable eggshell, both for its resistance and for the exchanges between internal and external environment. It is well known that the egg shape may contribute to the resistance of the eggshell as the more spherical an egg is, the more it is resistant to mechanical insults (Altuntaș & Șekeroğlu, 2008). A pigmented

eggshell may counteract the influence of the external environment on the internal egg quality, besides an adequate eggshell thickness (Nowaczewski et al., 2013). The PP eggs showed eggshells that were less coloured than those of ER, but with higher eggshell thickness. In a previous research, the ER eggshell thickness showed lower values in comparison to Robusta Maculata purebreed, and white-eggshell and brown-eggshell hybrid strains (Rizzi & Chiericato, 2005). Negative changes in eggshell pigmentation according to the age of highly productive hybrid hens were stated by other authors (Odobasi et al., 2007). This trial examined the first weeks of the laying period when the uterus activity and the calcium deposition may not be settled completely and interactions between internal physiological signals and external environmental factors may occur. As stated by other authors (Casiraghi et al., 2005, Hidalgo et al., 2008) the egg weight affects the shell percentage as it may be lower in larger eggs. The production system may affect this (Philippe et al., 2020). Genotype seems to be an important factor influencing the eggshell formation and mineral deposition, but more knowledge is needed to evaluate which may be the environmental factors that are able to influence the eggshell thickness.
In Table 3 the effects of genotype, age, and interaction on internal egg quality are shown. The effect of genotype was only significant for yolk and albumen weight, and for albumen ratio. Age significantly affected all the studied traits. The interaction effect was only significant for yolk weight.

Table 3 Internal egg quality traits of Ermellinata di Rovigo (ER) and Pepoi (PP) hens at 36 (-36w) and 50 (-50w) weeks of age

	ER-36w	PP-36w	ER-50w	PP-50w	G	A	G x A	RMSE
Yolk, g	14.8 ^{Bb}	12.7 ^{Cc}	17.8 ^{Aa}	14.5 ^{Bb}	<0.0001	<0.0001	0.0410	1.37
Albumen, g	32.5 ^{Bb}	26.9 ^{Cc}	35.1 ^{Aa}	28.7 ^{Cc}	<0.0001	<0.0001	0.4503	2.32
Yolk:albumen	0.46 ^{Bb}	0.48 ^{AaBb}	0.51 ^{Aa}	0.51 ^{Aa}	0.1963	<0.0001	0.1252	0.04
HU	113 ^{Aa}	112 ^{Aa}	100 ^{Bb}	97.7 ^{Bb}	0.0907	<0.0001	0.3832	5.00
Yolk, %	28.6 ^{Bb}	29.4 ^{AaBb}	30.8 ^{Aa}	30.2 ^{AaB}	0.8189	0.0002	0.0712	1.71
Albumen, %	62.9 ^{Aa}	61.2 ^{ABb}	60.8 ^{Bbc}	59.8 ^{Bc}	0.0008	<0.0001	0.3361	1.66

RMSE = Root Mean Square Error.

Different letters among columns indicate different values. a,b,c: $p<0.05$; A, B, C: $p<0.01$.
Observations: n = 42 ER (n = 17 at 36 weeks, n = 25 at 50 weeks), n = 42 PP (n = 17 at 36 weeks, n = 25 at 50 weeks).

The ER eggs showed yolk and albumen weights higher ($p<0.01$) than those of PP eggs, and age caused an increase for both genotypes. The ER-50w yolk weights were the highest ($p<0.01$) and PP-36w yolk weights were the lowest ($p<0.01$). The ER-36W and PP-50W were intermediate. Concerning albumen weights, ER eggs increased with age ($p<0.01$), whereas PP did not signifcantly change and showed the lowest values. The yolk to albumen ratios were similar among breeds and only ER significantly increased with age ($p<0.01$). The two breeds showed similar HU, which changed ($p<0.01$) with age for both ER and PP, reaching the lowest ($p<0.01$) values at 50 weeks of age. The yolk percentages were similar between genotypes and a significant increase with age was only found for ER ($p<0.01$), exhibiting the lowest and the highest values at 36 and 50 weeks of age, respectively. The albumen percentage showed differences ($p<0.01$) between the genotypes, being higher for ER than for PP, especially at the first age period ($p<0.05$). From 36 to 50 weeks of age, it significantly decreased for both ER ($p<0.01$) and PP ($p<0.05$). The three egg components are very important for their nutritional and functional roles. The lowest yolk weight was shown by PP-36w eggs, but not at 50 weeks, when it was similar to that of ER at 36 weeks. The lowest albumen weight was shown by PP eggs throughout the entire studied laying period. A comparison with other trials is only possible for some traits, because it is difficult for others due to different environmental conditions during rearing and storaging of the eggs. At the beginning of egg production, the yolk:albumen ratio was similar to those of hybrid eggs (Philippe et al., 2020, Rizzi, 2020), where yolk is less than 50% albumen weight, but with age the ratio increased. Eggs laid by other Italian and Portuguese hen breeds showed higher yolk percentages and lower albumen percentages than those of this trial (Di Rosa et al., 2020, Lordelo et al., 2020). The HU values were high, but they are referred to eggs laid within 24 h and at low environmental temperatures; it is well known that HU values decrease according to the age of the hens and to the environmental conditions where the eggs are laid and stored. Unlike this trial, the ER eggs, produced using organic production systems and high environmental temperatures, showed lower HU values (Rizzi & Marangon, 2012).

The effects of genotype and age on total (blood and meat) egg inclusions and differences between blood and meat spot percentages in eggs laid by ER and PP hens at 36 and 50 weeks of age are shown in Figure 4 and Figure 5, respectively. No statistical differences were found between ER and PP. With increased age, the quality of yolk and albumen increased (p<0.01), as blood and meat inclusions (30.7 vs. 12.9%) decreased. The egg inclusions detected in these breeds are higher than those reported for hybrid strains, which have been selected for this trait. In a previous trial carried out on ER and Robusta Maculata hens (Rizzi, 2020), the blood and meat spots were similar to those of this study. Eggs laid by other breeds showed egg inclusions similar or higher to those of this trial (Lordelo et al., 2020).

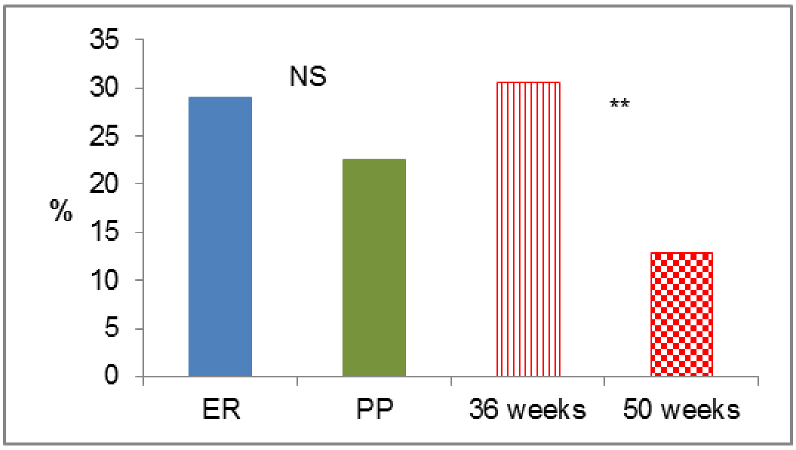

Figure 4 Effect of genotype (Ermellinata di Rovigo – ER; Pepoi – PP) and age on total (blood and meat) egg inclusions

NS = no statistical significance between genotypes; **: different values (p<0.01) between ages.
X^2 (p–value): genotype = 1.46 (0.203), age = 8.75 (0.003).
Observations: n = 183 ER (n = 148 at 36 weeks, n = 35 at 50 weeks), n = 115 PP (n = 80 at 36 weeks, n = 35 at 50 weeks.

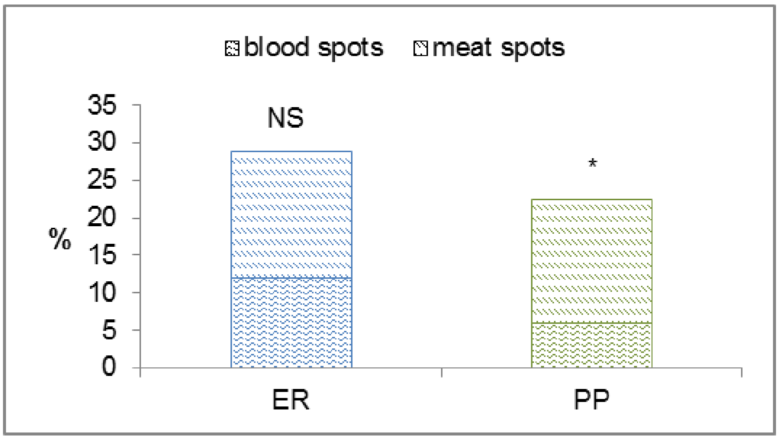

Figure 5 Effect of genotype (Ermellinata di Rovigo – ER; Pepoi – PP) on total (at 36 and 50 weeks of age) blood and meat spots

NS = no statistical significance between blood and meat spots; *: different values (p<0.05) between blood and meat spots.
X^2 (p–value): ER = 1.79 (0.172), PP = 6.24 (0.020).
Observations: n = 183 ER, n = 115 PP.

For ER no significant difference was found between blood (16.9%) and meat (12.0%) spots, whereas PP eggs meat spots were higher (p<0.05) than blood spots (16.5 vs. 6.0%). Literature states that the white-eggshell strains show egg inclusions, especially blood spots in yolk, lower than those of brown-eggshell strains (Zeidler, 2002): although PP eggs showed a small presence of eggshell pigmentation, the eggshell is not completely white, thus this breed is considered a coloured eggshell breed.

4 Conclusions

The results gave first indications for the two Italian breeds of Veneto region, with phenotypic differences for plumage colour and body size. The hen-day egg mass was higher for ER than for PP, at 36 and 50 weeks of age. The ER and PP eggs differed for many egg external traits, which allow to distinguish the eggs by weight, shape and eggshell colour. Until 50 weeks of age, the PP hens produced eggs of small size, whereas ER eggs were classified as small, medium and large size, with prevailing medium size. With age, ER eggs were more ovoid, whereas PP eggs were more spherical. The eggshell colour changed with age, but the two breeds differed for the redness index, which was higher for ER and for the eggshell thickness, which was higher for PP. The results of the egg constituents indicate the need of more knowledge regarding eggshell thickness for the ER eggs, and for the albumen traits for the PP eggs.

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