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# Effect of laying month on egg quality in the Utrerana chicken breed (Partridge variety) during the first laying period

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**ABSTRACT**
The Utrerana chicken is an endangered egg-producing breed native to Spain with four feather varieties that display productive seasonality. To fill the gap in knowledge on the seasonal variation in egg quality, 378 eggs were collected during the entire first laying period, between March and August, from an experimental flock of hens of the Partridge variety of the Utrerana breed and subsequently analysed to assess the effect of the laying month on external and external egg quality traits. The eggs, with an average weight of 61.66 g and sharp shape (shape index: 70.77), had 31.64% yolk, 56.59% albumen, and 11.77% white-coloured shell 0.43 mm in thickness. The egg yolk was light orange in colour (Roche fan score: 10.56) with a yolk index of 37.78%, whereas the albumen was moderately firm (Haugh units: 69.12). The egg yield was seasonal and peaked in the second month of the laying period, with a hen-day egg production of 61.4%. Throughout the first laying period, egg weight and eggshell content remained invariable and yolk content increased, whereas its quality and colour intensity worsened, and albumen content and quality decreased as a consequence of aging and increasing ambient temperature. In conclusion, the Partridge variety of the Utrerana chicken breed can be considered suitable for alternative farming systems because it lays good-quality eggs at a moderate seasonal laying rate.

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## 1. Introduction

The Utrerana chicken, an endangered autochthonous breed documented by various studies (García Romero et al., 2018; León & Cabello, 2009; Ministerio de Agricultura, Pesca y Alimentación, 2023a; Orozco, 1987; Plata-Casado et al., 2023), is recognised in the Official Catalogue of Livestock Breeds of Spain (Ministerio de Agricultura, Pesca y Alimentación, 2023b; Real Decreto 527/2023, 2023) and was created in 1926 in Utrera (province of Seville, Spain) by Joaquín del Castillo (1951), a well-known poultry farmer at that time. It is a light breed part of the Mediterranean group, characterised by four feather varieties: Black, Partridge, Black-barred and White (Liaño et al., 1953; Parés-Casanova & Berenguer-Boix, 2020) and a predominant aptitude for egg production (del Castillo, 1951; González Ariza, 2021; Plata-Casado et al., 2023), having been selected from a founding animal stock made up of hens with good potential for laying large eggs, gathered from ranches and farmhouses in the Utrera countryside (del Castillo, 1951). Its good laying performance resulted in the breed being prized for commercial poultry farming until the mid-20th century (Liaño et al., 1953; Rabanal, 1948). Currently, it is reared under free-range, low input conditions, has a rustic character (García Romero et al., 2018; León & Cabello, 2009) and displays productive seasonality and only a moderate egg yield (González Ariza, Arando Arbulu, León Jurado et al., 2022), due to the fact that the selection process for egg laying undertaken in the second quarter of the 20th century (del Castillo, 1951) was later abandoned for economic reasons after improved, more productive hen strains began to be bred widely in the second half of the 20th century (Lleonart & Castelló, 1984; Tortuero, 2009).

After being discarded from intensive commercial poultry farming, research on the productive performance and egg quality of the Utrerana breed has received relatively little attention from the scientific

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community. Previous studies, mostly carried out within the framework of a recent functional characterization of the breed (González Ariza, 2021), have investigated several aspects related to laying performance and egg quality in Utrerana hens (reviewed in Plata-Casado et al., 2023). This research has focused on the laying curve and productive performance (González Ariza, Arando Arbulu, León Jurado et al., 2022), the chemical composition of eggs (González Ariza, Navas González, Arando Arbulu et al., 2021), and the relationship between egg quality traits and the commercial classification of eggs by grading them by weight (González Ariza, Arando Arbulu, Navas González et al., 2021) in the four varieties of the Utrerana breed. Additionally, studies (González Ariza, Arando Arbulu, Navas González et al., 2022) have compared the egg quality of Utrerana breed varieties with other Spanish and foreign breeds. The quality of eggs from hens of the four varieties of this breed, housed in cages under ambient conditions not specified, was also studied for a four-month period between March and June (González Ariza et al., 2019). Research has also found differences in laying curves among the breed's feather varieties, with the Partridge variety displaying more marked seasonality (González Ariza, Arando Arbulu, León Jurado et al., 2022). Moreover, differences were found between the Utrerana hen's egg and the White Leghorn egg in most quality traits, except for yolk diameter, albumen height, yolk pH, and L* trichromatic coordinate of yolk colour. Studies show that hen's age affects various egg quality traits, including eggshell weight, egg width, albumen height, and several yolk's colour parameters (L* and a* trichromatic coordinates). The effects of laying month and time of the laying period on albumen height and yolk colour have also been reported (González Ariza et al., 2019). Regarding the relationship between internal and external quality traits and the commercial classification of eggs by weight grading (Commission Delegated Regulation (EU) 2023/2465 2023), it has been reported that albumen, yolk, and eggshell weights are the most influential traits that determine differences in egg quality categories (González Ariza, Arando Arbulu, Navas González et al., 2021).

Today, the Utrerana chicken breed is reared mainly for its attractive appearance by amateur breeders, who select it for its morphological traits and rear birds for their own consumption of eggs and meat (Rodero et al., 1994; León & Cabello, 2007). However, the breed has potential for use in alternative production systems (Dávila et al., 2009; Dávila et al., 2011), and, to valorise it, it is therefore of interest to characterise it in order to improve egg productivity and evaluate its quality. Given that differences have been reported between the varieties for productive performance (González Ariza, 2021) and egg quality (González Ariza, Navas González, Arando Arbulu et al., 2021; González Ariza, Arando Arbulu, Navas González et al., 2021), and that the breed shows productive seasonality (González Ariza et al., 2019; González Ariza, Arando Arbulu, León Jurado et al., 2022), it would be of interest to characterise further, for each Utrerana feather variety, the variations in egg quality in function of the laying month during the whole laying curve, since it has only been partially studied during an incomplete period of the laying curve (González Ariza et al., 2019). In this context, this study aims to assess how the laying month affects both external and internal quality traits of eggs from the Partridge variety of the Utrerana chicken breed, specifically during the first laying period. The initial hypothesis was that the productive seasonality of this breed, particularly marked in the Partridge variety and already described in many autochthonous, not selected chicken breeds (Brito et al., 2021; Mannelli et al., 2023), could lead to variation in egg quality during the laying period.

## 2. Materials and methods

### *2.1. Housing of the hens and husbandry*

The eggs used in this study were collected from an experimental flock of hens of the Partridge variety of the Utrerana chicken breed donated by the Asociación Nacional de Criadores de Gallinas Utreranas and kept at the Research and Teaching Farm of the Higher Technical School of Agricultural Engineering of the University of Seville (Spain), located at 37° 21′ 36.3″ N latitude, 5° 56′ 23.9″ W longitude.

To ensure accuracy in egg sampling, the hens were individually housed in wire mesh battery cages (40 × 40 × 40 cm), located in a closed facility, equipped with one longitudinal feeder and one drinking cup, and subjected to natural lighting (increasing from 12 L:12D to 13 L:11D) at room temperature under static ventilation. The ambient temperature ranged between 15.4 ± 0.37 °C (mean ± SE of the minimum temperatures) and 30.1 ± 0.50 °C (mean ± SE of the maximum temperatures), and the relative humidity ranged from 50 to 70%. The housing and husbandry of the experimental flock complied with the Spanish Royal Decree 3/2002 (Ministerio de Agricultura, Pesca y Alimentación, 2002), which establishes the minimum standards for the protection of laying hens, and Directive 2010/63/EU of the European Parliament and Council (2010) on the protection of animals used for scientific purposes.

After a two-month adaptation period to housing and feed, the pullets began to lay eggs on March 1st, 2021, at an age of eight months and with an average live weight of 1886 ± 69.6 g. At the end of the experimental period, the hens weighed 1990 ± 78.2 g, and there was no mortality.

The hens were fed balanced commercial feed (Ponemax®, Cereales Maestre; Los Palacios y Villafranca, Spain). Feed and water were provided *ad libitum*. Table 1 shows the nutrient composition of the balanced feed, which met the nutritional requirements of laying hens (Santomá & Mateos, 2018) and whose main ingredients were maize, toasted soybean meal, triticale, calcium carbonate, fatty acids, and premix, according to the manufacturer. The metabolizable energy (ME) level of the balanced feed was estimated using the prediction equation from Sibbald et al. (1980) and subsequently converted to the as feed basis:

ME (kcal/kg DM) = 3951 + 54.4 × ether extract (%DM) – 88.7 × crude fibre (%DM) – 40.8 × ash (%DM).

## 2.2. Egg collection and experimental design

A total of 378 eggs were collected daily during the first laying curve, over a 24-week period, starting from March 16th, when significant hen-day egg production commenced, to August 29th, 2021, when the hens began to stop laying, aligning with the hens' seasonal laying pattern. The date of egg laying was individually recorded for each egg, and the eggs were subsequently kept at room temperature (18 – 22 °C) until they were analysed in the laboratory, which took place once a week (on Mondays) for all the eggs laid in the previous seven days. Consequently, all measurements and analyses were performed on eggs aged between one and seven days.

The laying period was divided into six four-week segments, each defined as 'laying month', to analyse the impact of laying month on egg quality. Hen-day egg production percentage for each month of the laying period was calculated by dividing the total number of eggs laid in that month by the number of hens in the flock and 28 days, then multiplying the result by 100.

**Table 1.** Nutrient composition (as feed basis) of the balanced feed supplied to the hens (Ponemax®, Cereales Maestre; Los Palacios y Villafranca, Spain)[a].

| Item | Content |
|---|---|
| Dry matter (%) | 89.1 |
| Crude protein (%) | 17.0 |
| Crude fibre (%) | 2.6 |
| Crude fat (%) | 4.2 |
| Ash (%) | 13.5 |
| Total Calcium (%) | 4.09 |
| Total Phosphorus (%) | 0.55 |
| Sodium (%) | 0.16 |
| Lysine (%) | 0.93 |
| Methionine (%) | 0.41 |
| Metabolizable energy (kcal/kg) | 2822 |

[a]Additives per kg of balanced feed contains: vitamin A: 7875 IU; vitamin $D_3$: 1575 IU; copper (cupric sulphate pentahydrate): 4.2 mg; iodine (potassium iodide): 2 mg; canthaxanthin: 2 mg; butylated hydroxytoluene (BHT): 0.231 mg; ethoxyquin: 0.034 mg; Butylated hydroxyanisole (BHA): 0.021; sepiolite: 1 g.

## 2.3. Recording and calculation of quality traits

External and internal egg quality traits were measured or calculated as follows:

### 2.3.1. External traits

Egg weight was determined using an electronic balance (precision ±0.01 g; CB Complet, Cobos, Hospitalet de Llobregat, Spain). The width and length of the eggs were measured using a digital calliper (precision ±0.01 mm; model 5900603; Comecta SA, Barcelona, Spain) and the shape index (%) was calculated as follows: egg width/egg length × 100 (Zanon et al., 2006). The eggshell colour of each egg was tested using a Minolta CM-2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in CieLab space (CIE, 1976) to record trichromatic coordinates: L* (lightness; 0 = black, 100 = white), a* (redness; −100 = green, 100 = red), and b* (yellowness; −100 = blue, 100 = yellow). The chroma (C*) and hue angle (H*) indexes were subsequently calculated as follows: $C^* = (a^2 + b^2)^{1/2}$ and $H^* = \tan^{-1}(b^*/a^*) \times 57.29$ (expressed in degrees). The trichromatic coordinates and colorimetric indexes were obtained in triplicate, and the mean values were calculated. The specific gravity ($g/cm^3$) of the eggs was tested according to Archimedes' principle, based on the egg weight measured in water in different salt solutions (range 85–120 gNaCl/l).

### 2.3.2. Internal traits

Once the external features were measured, the eggs were broken on a glass plate to analyse the internal egg traits. The albumen and yolk pH were measured using 4.5–10 pH test strips (PanReac Appli-Chem, ITW Reagents, Darmstadt, Germany). Yolk colour was measured using a Roche yolk colour fan with a 15-point scale (Roche Ltd., Basel, Switzerland), with yolk pigmentation ranging from lightest (score 1) to darkest (score 15). The trichromatic coordinates of yolk colour in the CieLab space were also measured, and the H* and C* indexes were calculated, using the same procedure as for the eggshell colour.

Yolk was manually separated from the albumen and weighed with ±0.01 g precision. After any albumen residues were cleaned and dried with a paper towel, the eggshell was weighed with ±0.01 g precision. Albumen weight was calculated by subtracting the weights of the yolk and eggshell from the total egg weight. Yolk ratio (%), albumen ratio (%), and eggshell ratio (%) were calculated as the ratio between the weight of each egg component and the total egg weight × 100. To measure the albumen and yolk heights, a tripod micrometre (precision ±0.01 mm; Baxlo Precision Co., Barcelona, Spain) was used. Yolk diameter of each egg was measured using a digital calliper. Haugh units (HU) were computed according to the method proposed by Haugh (1937) as follows: $HU = 100 \log (H + 7.57 - 1.7 \times W^{0.37})$, where H and W are albumen height (mm) and egg weight (g), respectively. The yolk index (%) was calculated as yolk height/yolk diameter × 100 (Funk, 1948).

Eggshell thickness was measured at the equatorial level using a micrometre (precision ± 0.001 mm; model 103–137, Mitutoyo Co, Kawasaki, Japan).

### *2.4. Statistical analyses*

Each external and internal egg quality trait was analysed as a dependent variable using the univariate general linear model (GLM) procedure with laying month as the fixed effect and egg weight as a covariate. The time elapsed between egg laying and its analysis in the laboratory was also considered as a covariate, since its effect on egg quality traits was linear. Fisher's least significant difference (LSD) *post hoc* test was used to separate means among the six laying months studied. Each egg was considered as a replicate.

The results of the egg quality traits were expressed as estimated marginal means, and the pooled standard errors of the means were calculated.

For all comparisons, statistical significance was set at $p < 0.05$. Analyses were performed using the Statistical Package SPSS version 15.0 (SPSS Inc., 2006).

## 3. Results

Table 2 shows hen-day egg production and the external quality traits of eggs of the Partridge variety from the Utrerana chicken breed during the first laying period.

During the first laying period, hen-day egg production averaged 46.8%, and peaked in the second laying month (61.4%). The mean egg weight was 61.66 g and did not vary ($p < 0.05$) during the first laying period. The mean egg length was 60.43 mm, which varied ($p < 0.001$) during the laying period, increasing in the last three months of the laying period compared with the second and third months, when it reached its lowest values. The mean egg width was 42.71 mm, which also varied ($p < 0.01$) during the laying period, with eggs laid in the first

**Table 2.** Hen-day egg production and estimated marginal means of external quality traits of eggs from the Partridge variety of the Utrerana chicken breed during the first laying period.

| | Laying Month$^{A}$ | | | | | | Whole period | Pooled | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | 1$^{st}$ ($n = 67$) | 2$^{nd}$ ($n = 81$) | 3$^{rd}$ ($n = 67$) | 4$^{th}$ ($n = 59$) | 5$^{th}$ ($n = 63$) | 6$^{th}$ ($n = 41$) | ($n = 378$) | SEM | $p$-value |
| Hen-day egg production (%) | 47.9 | 61.4 | 50.7 | 43.6 | 46.4 | 30.7 | 46.8 | – | – |
| Egg Weight (g) | 61.46 | 62.54 | 61.98 | 61.62 | 61.29 | 61.10 | 61.66 | 0.178 | 0.166 |
| Egg Length (mm) | 60.41$^{bc}$ | 59.34$^{d}$ | 60.15$^{c}$ | 60.82$^{ab}$ | 61.03$^{a}$ | 60.85$^{ab}$ | 60.43 | 0.088 | <0.001 |
| Egg Width (mm) | 42.45$^{c}$ | 42.66$^{bc}$ | 42.90$^{a}$ | 42.77$^{ab}$ | 42.62$^{bc}$ | 42.85$^{ab}$ | 42.71 | 0.036 | 0.003 |
| Shape Index (%) | 70.33$^{c}$ | 71.95$^{a}$ | 71.41$^{ab}$ | 70.47$^{bc}$ | 69.98$^{c}$ | 70.51$^{bc}$ | 70.77 | 0.154 | 0.001 |
| Specific Gravity (g/cm$^{3}$) | 122.19$^{a}$ | 119.09$^{b}$ | 106.55$^{e}$ | 113.98$^{c}$ | 110.19$^{d}$ | 108.99$^{de}$ | 113.50 | 0.426 | <0.001 |
| Eggshell Weight (g) | 7.44 | 7.62 | 7.26 | 8.19 | 6.71 | 6.43 | 7.28 | 0.197 | 0.178 |
| Eggshell Ratio (%) | 12.06 | 12.34 | 11.77 | 13.19 | 10.86 | 10.41 | 11.77 | 0.309 | 0.162 |
| Eggshell Thickness (mm) | 0.50$^{a}$ | 0.45$^{b}$ | 0.46$^{b}$ | 0.43$^{c}$ | 0.40$^{d}$ | 0.36$^{e}$ | 0.43 | 0.002 | <0.001 |
| Eggshell Colour: | | | | | | | | | |
| L* | 89.57$^{b}$ | 89.54$^{b}$ | 90.90$^{a}$ | 90.49$^{ab}$ | 90.87$^{a}$ | 89.45$^{b}$ | 90.14 | 0.166 | 0.011 |
| a* | 0.19$^{ab}$ | 0.26$^{a}$ | 0.21$^{ab}$ | 0.06$^{bc}$ | −0.16$^{d}$ | −0.15$^{c}$ | 0.07 | 0.029 | <0.001 |
| b* | 8.78$^{ab}$ | 8.87$^{a}$ | 7.90$^{abc}$ | 7.44$^{bc}$ | 6.63$^{c}$ | 8.32$^{ab}$ | 7.99 | 0.202 | 0.007 |
| C* | 8.80$^{ab}$ | 8.90$^{a}$ | 7.92$^{abc}$ | 7.45$^{bc}$ | 6.64$^{c}$ | 8.33$^{ab}$ | 8.01 | 0.203 | 0.006 |
| H* | 20.05$^{a}$ | 20.37$^{a}$ | 8.10$^{a}$ | −3.56$^{a}$ | −43.09$^{b}$ | −47.02$^{b}$ | −7.52 | 4.363 | <0.001 |

$^{a-e}$Estimated marginal means in the same row sharing different superscript letters are different ($p < 0.05$). SEM: Standard error of the mean.
$^{A}$Laying months: 1$^{st}$: 16 March – 11 April; 2$^{nd}$: 12 April – 9 May; 3$^{rd}$: 10 May – 6 June; 4$^{th}$: 7 June – 4 July; 5$^{th}$: 5 July – 1 August; 6$^{th}$: 2 August – 29 August.

month showing the lowest width and eggs laid in the third month the highest. The mean shape index was 70.77%, which also varied ($p < 0.01$) during the first laying period, showing higher values in the second and third months and lower values in the first and last three months of the laying period. The specific gravity averaged 113.50 g/cm$^3$, decreasing ($p < 0.001$) during the laying period, with the highest specific gravity found during the first month of the laying period, and the lowest values found in the third and sixth months.

The eggshell weight and eggshell ratio averaged 7.28 mm and 11.77%, respectively, and were invariable ($p > 0.05$) during the first laying period. The mean eggshell thickness was 0.43 mm and decreased progressively ($p < 0.001$) over the first laying period.

The L*, a*, b*, C*, and H* indices of eggshell colour varied throughout the first laying period ($p < 0.05$). The mean lightness (L*) was 90.14 and the highest values were observed during the third to fifth laying months (range: 90.49 – 90.90). The redness index (a*) averaged 0.07, peaking in the 2$^{nd}$ month (0.26), and negative values were observed from the fifth laying month. Mean yellowness index (b*) was 7.99, varied in the range 6.63 to 8.87 during the whole laying period ($p < 0.01$), peaking in the second month (8.87), and the lowest values were observed in the third, fourth and fifth months of the laying period (range: 6.63 – 7.90). The mean C* was 8.01, peaking in the second month (8.90), and the lowest values were observed during the fourth and fifth laying months (7.45 and 6.64, respectively). The H* value averaged −7.52, and decreased progressively ($p < 0.001$) from positive to negative values during the entire first laying period.

Table 3 shows the quality traits of egg yolk from the Partridge variety of the Utrerana chicken breed during the first laying cycle.

All quality traits related to egg yolk were affected by the month of the first laying period ($p < 0.05$). The mean yolk weight and yolk ratio were 19.52 g and 31.64% on average, respectively, and increased progressively during the laying period ($p < 0.001$). The mean yolk height was 17.08 mm, which decreased during the laying period, while the mean yolk diameter was 45.51 mm, which increased during the laying period ($p < 0.001$). This resulted in a mean yolk index of 37.78%, which progressively decreased during the laying period ($p < 0.001$). The mean yolk pH was 6.00, which decreased ($p < 0.001$) during the laying period.

The yolk colour of the eggs from the Partridge variety of the Utrerana chicken breed, measured using a Roche fan, had an average score of 10.56, which decreased from the fifth month of the first laying period ($p < 0.05$). All instrumental measurements of yolk colour (L*, a*, b*, C*, and H*) were affected by laying period ($p < 0.001$). Thus, the L* coordinate of yolk colour (mean: 55.70) was significantly higher from the third month of the laying period onwards, ranging between 56.00 and 56.68 between the third and sixth months. The mean redness coordinate (a*) of the yolk colour was 6.65, and in the first and second months it was significantly higher (7.49 and 7.91, respectively) than in the other months of the laying period. In fact, a progressive decrease in the redness index was observed between the second and fourth months of the laying period. The mean yellowness coordinate (b*) of yolk colour was 36.24, with higher values observed in the second and third months of the laying period (38.09

**Table 3.** Quality traits of the egg yolk from the Partridge variety of the Utrerana chicken breed during the first laying period (estimated marginal means).

| Trait | Laying Month$^A$ | | | | | | Whole period ($n = 378$) | Pooled SEM | $p$-value |
|---|---|---|---|---|---|---|---|---|---|
| | 1$^{st}$ ($n = 67$) | 2$^{nd}$ ($n = 81$) | 3$^{rd}$ ($n = 67$) | 4$^{th}$ ($n = 59$) | 5$^{th}$ ($n = 63$) | 6$^{th}$ ($n = 41$) | | | |
| Yolk Weight (g) | 18.06$^c$ | 18.41$^c$ | 19.74$^b$ | 20.08$^{ab}$ | 20.19$^{ab}$ | 20.66$^a$ | 19.52 | 0.076 | <0.001 |
| Yolk Ratio (%) | 29.23$^c$ | 29.88$^c$ | 31.99$^b$ | 32.54$^b$ | 32.74$^{ab}$ | 33.48$^a$ | 31.64 | 0.121 | <0.001 |
| Yolk Height (mm) | 18.34$^a$ | 18.46$^a$ | 17.03$^b$ | 16.92$^b$ | 16.14$^c$ | 15.56$^d$ | 17.08 | 0.066 | <0.001 |
| Yolk Diameter (mm) | 43.81$^c$ | 43.49$^c$ | 46.01$^b$ | 45.58$^b$ | 46.82$^a$ | 47.36$^a$ | 45.51 | 0.110 | <0.001 |
| Yolk Index (%) | 41.98$^a$ | 42.50$^a$ | 37.18$^b$ | 37.34$^b$ | 34.65$^c$ | 33.02$^d$ | 37.78 | 0.198 | <0.001 |
| Yolk pH | 6.13$^a$ | 5.99$^b$ | 6.01$^b$ | 5.91$^c$ | 6.00$^b$ | 5.99$^{bc}$ | 6.00 | 0.012 | <0.001 |
| Yolk Colour – Roche Fan | 11.25$^a$ | 11.22$^a$ | 11.14$^{ab}$ | 11.46$^a$ | 9.42$^{bc}$ | 8.85$^c$ | 10.56 | 0.276 | 0.038 |
| Yolk Colour: | | | | | | | | | |
| L* | 54.01$^b$ | 54.48$^b$ | 56.00$^a$ | 56.68$^a$ | 56.58$^a$ | 56.43$^a$ | 55.70 | 0.132 | <0.001 |
| a* | 7.49$^{ab}$ | 7.91$^a$ | 7.39$^b$ | 5.62$^c$ | 5.77$^c$ | 5.71$^c$ | 6.65 | 0.073 | <0.001 |
| b* | 36.56$^{bc}$ | 38.09$^a$ | 37.38$^{ab}$ | 34.02$^d$ | 36.01$^c$ | 35.37$^{cd}$ | 36.24 | 0.208 | <0.001 |
| C* | 37.35$^{bc}$ | 38.92$^a$ | 38.13$^{ab}$ | 34.48$^d$ | 36.48$^c$ | 35.84$^{cd}$ | 36.87 | 0.211 | <0.001 |
| H* | 78.32$^b$ | 78.25$^b$ | 78.79$^b$ | 80.61$^a$ | 80.90$^a$ | 80.88$^a$ | 79.62 | 0.099 | <0.001 |

$^{a-d}$Estimated marginal means in the same row sharing different superscript letters are different ($p < 0.05$). SEM: Standard error of the mean.
$^A$Laying months: 1$^{st}$: 16 March – 11 April; 2$^{nd}$: 12 April – 9 May; 3$^{rd}$: 10 May – 6 June; 4$^{th}$: 7 June – 4 July; 5$^{th}$: 5 July – 1 August; 6$^{th}$: 2 August – 29 August.

and 37.38, respectively). The mean chroma index (C*) of the yolk colour was 36.87, with the highest values for C* observed during the second and third months, while a dramatic decrease in the C* index was noted from the fourth month of laying period onwards. Finally, the hue angle (H*) of the yolk colour averaged 79.62 during the laying period, with a significant increase from the fourth month onwards, reaching a value in the range of 80.61 – 80.90 in the last three months of the laying period.

Table 4 shows the quality traits of egg albumen from the Partridge variety of the Utrerana chicken breed during the first laying period.

All quality traits related to egg albumen in the Partridge variety of the Utrerana chicken breed were affected by the month of the first laying period ($p < 0.05$). The mean albumen weight and albumen ratio were 34.94 g and 56.59%, respectively, which in general terms decreased ($p < 0.01$) during the laying period, with the highest values being reached during the first month and the lowest during the fourth month of the laying period. The mean albumen height and Haugh units were 5.35 mm and 69.12, respectively, which decreased ($p < 0.001$) up to the third month of the laying period. The mean albumen pH was 9.34, which progressively increased ($p < 0.001$) during the laying period.

## 4. Discussion

Biological diversity is a source of wealth, providing a significant amount of resources necessary for humanity. In this regard, one of the priorities of the Ministry of Agriculture, Fisheries and Food of the Government of Spain is the conservation of the rich genetic heritage of Spanish livestock breeds. To this end, it implements measures that facilitate the protection of livestock products (Ministerio de Agricultura, Pesca y Alimentación, 2023b). In order to promote the maintenance of the biodiversity of livestock breeds, this work addresses the characterization of the egg quality of the endangered Utrerana chicken breed across the seasonal laying period as a measure to protect native poultry genetic resources in Spain.

In a study to characterise the laying curve of Utrerana hens, González Ariza, Arando Arbulu, León Jurado et al. (2022) showed that the Partridge variety is the least productive of the four varieties of the breed. In the present trial, hen-day egg production peaked at 61.4% in the second month of the laying period. This coincides with the laying curve described by González Ariza, Arando Arbulu, León Jurado et al. (2022) for this variety, in which the maximum hen-day egg production was 66.2%, reached at the 7th week. In the Partridge variety, peak yield is higher and peaks earlier, but egg laying steadily decreases after the peak compared to the other three varieties of the breed due to the stronger seasonality displayed by this variety (González Ariza, Arando Arbulu, León Jurado et al., 2022). In our trial, the persistence of the laying curve was relatively good until the 5th month, with eggs laying dropping sharply in the sixth laying month (Table 2). Although the productive life of the Utrerana hen can last three years (Orozco, 1987), due to seasonality and the lack of genetic selection typical of native breeds, its laying curve is not comparable to that of commercial white and brown layers, which can maintain longer, continuous, and more profitable laying periods, with hen-day peak production as high as 95% (Hy-Line International, 2018; Hy-Line International, 2020).

The mean weight of the eggs of the Partridge variety of the Utrerana chicken breed found in this trial was at the lowest limit of the range described in 1951 by the breed's founder (62 – 64 g) (del Castillo, 1951). It was also found to be in the range reported for the breed (59.1 – 63.5 g) in a recent study on egg quality in native breeds (Utrerana, Andaluza Azul, Spanish Cara Blanca, and Andaluza Tufona) (González Ariza, Arando Arbulu, Navas González et al., 2022) and within the range described in informative publications on the breed (55 – 80 g) (García Romero et al., 2018; León & Cabello, 2009). It was also higher than that reported for eggs of commercial white layers in

**Table 4.** Quality traits of the egg albumen from the Partridge variety of the Utrerana chicken breed during the first laying period (estimated marginal means).

| | Laying Month[A] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | 1st ($n=67$) | 2nd ($n=81$) | 3rd ($n=67$) | 4th ($n=59$) | 5th ($n=63$) | 6th ($n=41$) | Whole period ($n=378$) | Pooled SEM | $p$-value |
| Albumen Weight (g) | 36.24$^{a}$ | 35.71$^{ab}$ | 34.73$^{bc}$ | 33.46$^{c}$ | 34.84$^{bc}$ | 34.66$^{bc}$ | 34.94 | 0.212 | 0.004 |
| Albumen Ratio (%) | 58.71$^{a}$ | 57.78$^{ab}$ | 56.24$^{bc}$ | 54.27$^{c}$ | 56.41$^{bc}$ | 56.11$^{bc}$ | 56.59 | 0.332 | 0.003 |
| Albumen Height (mm) | 6.69$^{a}$ | 5.84$^{b}$ | 5.01$^{c}$ | 4.83$^{c}$ | 4.73$^{c}$ | 4.97$^{c}$ | 5.35 | 0.087 | <0.001 |
| Haugh Units | 85.66$^{a}$ | 72.48$^{b}$ | 65.64$^{c}$ | 62.96$^{c}$ | 63.29$^{c}$ | 64.70$^{c}$ | 69.12 | 0.733 | <0.001 |
| Albumen pH | 9.23$^{c}$ | 9.18$^{c}$ | 9.35$^{b}$ | 9.42$^{ab}$ | 9.37$^{ab}$ | 9.48$^{a}$ | 9.34 | 0.016 | <0.001 |

[a-c]Estimated marginal means in the same row sharing different superscript letters are different ($p < 0.05$). SEM: Standard error of the mean.
[A]Laying months: 1st: 16 March – 11 April; 2nd: 12 April – 9 May; 3rd: 10 May – 6 June; 4th: 7 June – 4 July; 5th: 5 July – 1 August; 6th: 2 August – 29 August.

the first six months of the laying period (which increases from 44 to 61 g) (Hy-Line International, 2020) and that of the eggs of the commercial brown layers during the first 16 weeks of the laying period (which increases from 49 to 63 g during the first six months) (Hy-Line International, 2018). This is because the Utrerana breed was first selected for laying performance in 1926, from large-bodied hens that laid large eggs, gathered in farmhouses in the countryside and surroundings of Utrera (province of Seville, Spain) (del Castillo, 1951). The laying month did not affect egg weight in the present trial, which is in agreement with that reported by González Ariza et al. (2019) for the Utrerana breed. However, contrary to our trial, they did not study a complete laying period. This observation is contrary to the progressive increase in egg weight throughout the laying curve reported for commercial layers (Hy-Line International, 2018; Hy-Line International, 2020).

The egg length and width found in our study coincided with the values previously described for the Partridge variety of the Utrerana chicken breed (60.7 and 43.2 mm, respectively) (González Ariza, Arando Arbulu, Navas González et al., 2022), and were influenced by the laying month, as in a previous study carried out between March and June (González Ariza et al., 2019). The shape index was found to be at the lowest limit of the range described for the breed, varying between 71.3 and 75.6% (González Ariza, Arando Arbulu, Navas González et al., 2022). With a shape index of less than 72, the eggs of the Partridge variety of the Utrerana hen are sharp in shape, which can lead to unusually long eggs that do not fit well in egg cartons and are therefore more likely to be broken during shipment than standard-shaped eggs (Sarıca & Erensayın, 2009). Moreover, the decrease in the shape index of eggs from the Partridge variety of the Utrerana breed found in the present trial between the laying peak and the end of the laying period coincides with that reported for commercial Hy-Line Brown layers (Sirri et al., 2018). It has also been reported that the length and width of the Utrerana chicken eggs are less than those of the selected White Leghorn breed, except for the Partridge variety, whose eggs have the same egg length as White Leghorn eggs (González Ariza et al., 2019).

The weight of the egg components of the Partridge variety of the Utrerana chicken breed found in this trial coincides with that reported by González Ariza, Arando Arbulu, Navas González et al. (2022) for this breed, while egg component ratios were slightly better because of the higher proportions of the edible parts. Thus, our eggshell weight was close to that reported for the Partridge variety (7.74 g) and lower than that reported for the other three varieties of the Utrerana breed (8.00 – 8.38 g) and the White Leghorn (8.89 g), while eggshell ratio was lower than that reported for the Utrerana breed (12.5–13.8%; González Ariza, Arando Arbulu, Navas González et al., 2022). Albumen weight was higher than that described for the Partridge variety (33.09 g), within the range of the other varieties of the breed (30.96 – 35.93 g) and lower than that of White Leghorn (35.43 g), whereas the albumen ratio was in the upper level of the range described for the breed (52.4–56.6%; González Ariza, Arando Arbulu, Navas González et al., 2022). Yolk weight was similar to that of the Partridge variety (19.46 g) and higher than that of the other varieties of the breed (18.01 – 19.10 g) and that of White Leghorn (16.83 g), while the yolk ratio was within the range previously described for the breed (28.4–32.3%; González Ariza, Arando Arbulu, Navas González et al., 2022). González Ariza et al. (2019) also reported no effect of the month of the laying period on the weight and ratio of the egg components of the Utrerana chicken breed, while in our trial the weight and ratio of the albumen decreased and that of the yolk increased as the laying period progressed, whereas the weight and ratio of the eggshell remained invariable. Our results match those of Zita et al. (2012), who reported for ISA Brown hens that, as the hen ages during the laying period, yolk ratio increases, albumen ratio decreases, and eggshell ratio fluctuates with a decreasing tendency of the eggshell proportion towards the end of the laying period. They also fit those of Johnston and Gous (2007), who reported in Amber-Link hens that the yolk ratio increases while albumen and eggshell ratios decrease across the laying period. This is due to the fact that, as the hen ages, the increase in egg yolk occurs at the expense of albumen (Whitehead et al., 1991) and because at the beginning of the laying period, the hens are not able to synthesize large amounts of yolk in the follicle (Kaminska & Skraba, 1991).

As reported by Arthur and O'Sullivan (2005), both white-shelled and brown-shelled eggs are equally recognized in the European market. However, for consumers, eggshell colour is an important characteristic for purchasing decisions, and preferences depend on geographic areas, consumer segments, and preconceived ideas (Rondoni et al., 2020). According to the recent eggshell colour scale proposed by Wang et al. (2023), the eggs analysed in the present trial collected from the Utrerana chicken breed (Partridge variety) can be classified as white, slightly bluish, and luminous. This matches the eggshell colour described by the breed's founder (del Castillo, 1951) and permitted by the breed standard (Asociación Nacional de Criadores de Gallinas Utreranas, 2019; Liaño et al., 1953), which is white or off-white, with shades of cream colour being accepted. The lightness, redness, and yellowness shell values were within the range described by González Ariza, Arando Arbulu, Navas González et al., (2022) for eggshells in the Utrerana chicken breed and by Rizzi et al. (2023) for other autochthonous Mediterranean black or white Italian breeds such as Polverara Nera or Polverara Bianca. Differences in the trichromatic coordinates of eggshell colour between eggs from the Utrerana chicken breed and those from the White Leghorn breed have been reported, with an effect of the laying month between March and June (González Ariza et al., 2019). Hens in the present trial laid eggs with lighter coloured shells as the laying frequency increased, as evidenced by the lightness (L*) values increasing at the peak of the laying period (Bi et al., 2018). Moreover, the decrease in eggshell pigmentation was associated with a decrease in the amount of redness (a*) as the flock aged during the laying period, as was also reported by Odabaşi et al. (2007) for eggs from commercial-type Hy-Line Brown hens. Our results showed that eggshell yellowness intensity (b*) weakened from the 3rd to 5th month of the laying period, in which the laying frequency remained relatively high. However, while eggshell colour traits changed during the laying period, egg weight remained invariable. Therefore, we can suppose that in the Utrerana chicken breed (Partridge variety), egg weight has little impact on eggshell colour (Bi et al., 2018). In addition, a dramatic decrease in the eggshell hue value was observed during the laying period. These findings confirm the observations that the purity of eggshell colour of eggs from older hens of the Partridge variety of the Utrerana chicken breed is lower than that of young hens. This is probably due to changes in the amount of pigment deposited on the surface of the shell as a consequence of the increase in the hen's age (Banaszewska et al., 2019; Odabaşi et al., 2007) and the environmental temperatures during the hottest part of the laying period (Tumová & Gous, 2012). Moreover, our observations confirm that the intensity of eggshell colour is inversely proportional to laying rate (Basmacioglu & Ergul, 2005). The influence of hen's age or laying rate on the intensity of shell colour is more marked in eggs from native breeds, such as the Utrerana studied in this trial, than in commercial hybrids, whose shell color is more stable (Sokołowicz et al., 2018).

Sufficient eggshell thickness is a quality parameter that guarantees that the egg withstands transportation and handling without breaking during its commercial life (Casiraghi et al., 2005), preserves it from moisture loss, and protects it from contamination by acting as a physical barrier against pathogens (Kocetkovs et al., 2022). The mean thickness of the eggshell of the Partridge variety of the Utrerana chicken breed found in this trial was greater than the range reported by González Ariza, Arando Arbulu, Navas González et al. (2022) for eggs from hens of the same breed (0.37 – 0.40 mm), from other native Spanish breeds (0.36 – 0.38 mm) and from the White Leghorn (0.40 mm). In the present trial, the eggshell thickness of the Utrerana chicken (Partridge variety) decreased progressively throughout the laying period. It is known that eggshell thickness correlates positively with egg weight (Iqbal et al., 2017) and is influenced by mineral metabolism (Clunies et al., 1992). However, since the egg weight in the present trial remained constant throughout the laying period and the balanced feed fed to the hens (Table 1) met the nutritional recommendations of minerals for laying hens (Santomá & Mateos, 2018), the reduction in eggshell thickness could be due to the progressive increase in temperature (from 15.4 to 30.1 °C) as the laying period progressed. In fact, it is known that heat stress and length of time in production reduce eggshell thickness (Lin et al., 2004) and quality (Pope et al., 1960) as a consequence of exhaustion and imbalance of mineral metabolism involved in eggshell formation (Lin et al., 2004; Pope et al., 1960). Furthermore, eggshell thickness and specific gravity followed a parallel, decreasing trend during the laying period, in line with that reported in the literature (Hunton, 2005).

Previous studies have also reported an effect of the laying month on yolk colour measured using the Roche fan and differences between the Utrerana varieties and among this breed, other native Spanish breeds and the White Leghorn (González Ariza et al., 2019; González Ariza, Arando Arbulu, Navas González et al., 2022), revealing higher values for the Utrerana breed (11.89 – 12.85; González Ariza, Arando Arbulu, Navas González et al., 2022) than in our trial. Such differences in yolk colour between trials are partly due to the fact that yolk colour is influenced by the content and profile of pigmenting carotenoids present in the raw materials and the additives intentionally added to the balanced feed to reach the target yolk colour because yolk colour is one of the main parameters by which consumers judge the quality of eggs (Beardsworth & Hernandez, 2004). The balanced feed supplied to the hens in the present trial was supplemented with 2 mg/kg canthaxanthin only, a red carotenoid source that produces the golden-yellow colour from the yellow base in the yolk (Beardsworth & Hernandez, 2004). This relatively low amount of canthaxantin and the lack of addition of yellow pigments in the balanced feed could be responsible for the yolk colour in this trial, because it is widely accepted in the egg industry that lutein (yellow pigment) levels between 4 and 7 mg together with 2.2 to 3.5 mg of a red pigment (canthaxanthin) per kg of feed are sufficient to achieve good yolk pigmentation (>10 in the Roche fan scale) (Santomá & Mateos, 2018). The addition of yellow pigment is not necessary in feeds with at least 30–40% maize (Santomá & Mateos, 2018), although we do not know the level of maize inclusion in the feed used in this trial. In general, lightness (L*), redness (*) and yellowness (b*) of the egg yolk found in this trial were within the ranges described by González Ariza, Arando Arbulu, Navas González et al. (2022) for eggs of the Utrerana chicken breed (Partridge variety). However, the egg yolk in our trial showed slightly higher lightness values than those reported by González Ariza, Arando Arbulu, Navas González et al. (2022) (51.50) for eggs of the same Partridge variety. Moreover, differences in the trichromatic coordinates of yolk colour have been observed in eggs from the Utrerana chicken breed, other native Spanish breeds, and, particularly, the White Leghorn, whose eggs show lower yolk lightness and yellowness and greater redness than those of the Utrerana breed (González Ariza, Arando Arbulu, Navas González et al., 2022). In another study, González Ariza et al. (2019) also reported differences in the trichromatic coordinates of yolk among the varieties of the Utrerana chicken breed and between this breed and White Leghorn. In the present study, the variation in yolk colour over the laying period was characterised by the fact that, after reaching the laying period plateau (around the 3rd month), the lightness of the egg yolk increased, whereas redness and yellowness values dramatically decreased. The same observation was reported by Liu et al. (2020) in Hy-Line Brown hens, who showed that after reaching the highest laying frequency (3rd month), a decrease in brightness and an increase in purity of yellow colour of egg yolk were observed in the colour traits of the egg yolk. Moreover, González Ariza et al. (2019), studying the monthly effect between March and June, reported a variation in the trichromatic coordinates of yolk in eggs from the Utrerana chicken breed. This observation is especially important in the summer months of the laying period because, from June to August, water consumption is greater (Aswathi et al., 2019) and can influence the increase in the L* value of the yolk, whereas a decrease in the values of a* and b* can be associated with a decrease in feed consumption during summer (i.e. June to August) due to heat stress (Kirunda et al., 2001). This leads to a lower intake of the pigments responsible for yolk colour when the yolk is formed in the follicle, usually 1–2 weeks before ovulation (Aswathi et al., 2019). However, only the major alterations in C* and H* values in the plateau of the laying period observed in the present trial could be perceived by the eye in the last two months of the laying period, as revealed by the Roche fan scale data.

In addition to colour and weight, yolk shape is also a factor in yolk quality and, therefore, their acceptance of eggs by consumers, because consumers prefer yolks to be round and firm, although this attribute varies with hen genotype and age (Zita et al., 2009). It is also known that, as the egg ages and the vitelline membrane degenerates, water from the albumen moves into the yolk and gives it a flatter shape (Roberts, 2004). The yolk height of eggs from the Partridge variety of the Utrerana chicken breed decreased progressively in the present trial over the laying period, whereas yolk diameter increased, thus leading to a progressive decrease in the yolk index during the laying period. Unlike in our trial, González Ariza et al. (2019) did not observe a variation in yolk diameter between March and June in Utrerana hen eggs, but they did find differences among the varieties of the breed, with eggs of the Partridge variety showing the highest median yolk diameter. Moreover, although the literature reports differences in yolk diameter (González Ariza, Arando Arbulu, Navas González et al., 2022) and shape among breeds (Melesse et al., 2010), no difference has been reported by González Ariza et al. (2019) in yolk diameter between eggs from the Utrerana chicken breed and those from White Leghorn. The mean yolk diameter found in the present trial for the Partridge variety of the Utrerana chicken breed was higher than the values reported for this breed (42.60 – 43.82 mm), other Spanish native breeds (42.95 – 44.57 mm), and White Leghorn breed (42.05 mm) (González Ariza, Arando Arbulu, Navas González et al., 2022). The evolution of the yolk index over the laying period found in the present trial for the Partridge variety of the Utrerana chicken breed agrees with previous reports demonstrating its decrease as the laying period progresses and the hen ages (Dikmen et al., 2017).

Egg albumen quality in the Utrerana chicken Partridge variety, which is determined by the albumen height and Haugh units and is also related to the specific gravity of the egg, experienced a progressive worsening between the first and third months of the laying period and remained at a low level thereafter. This could have been caused by the increase in environmental temperatures and the increase in water consumption that occurred as the laying period progressed. In fact, it is well known that high temperatures and thermal stress increase water intake (Aswathi et al., 2019), deteriorate albumen quality and reduce the specific gravity of the egg (Allahverdi et al., 2013; Pope et al., 1960). González Ariza, Arando Arbulu, Navas González et al. (2022) also reported a decrease in the albumen height of eggs from hens of the Utrerana breed studied from the second half of March to the first half of June. The worsening of albumen quality observed in the eggs of this trial as the laying period progressed was also due to the progressive increase in hen age, an observation that is in accordance with the decrease in Haugh units in eggs from older hens reported by Marzec et al. (2019). This is due to a decrease in the albumen viscosity of normally laid eggs with hen age, which results from deteriorative changes in the oviduct (Sturkie & Polin, 1954). The mean Haugh units value found in this trial can be classified as moderately firm ('A' grade according to the USDA standard; Department of Agriculture US, 2000). However, it was lower than the range reported by González Ariza, Arando Arbulu, Navas González et al., (2022) for eggs from hens of the same breed (82.2 – 86.9), other native Spanish breeds (83.8 – 86.8), such as Andaluza Azul, Spanish Cara Blanca, and Andaluza Tufona, and White Leghorn (94.5).

The yolk pH of the Partridge variety from the Utrerana hen decreased, whereas albumen pH increased during the laying period. The yolk pH found in this trial was lower than the range reported by González Ariza, Arando Arbulu, Navas González et al. (2022) for eggs from hens of the same breed (6.22 – 6.30), other native Spanish breeds (6.24 – 6.26), and White Leghorn (6.24). In contrast, albumen pH was higher than the range reported by González Ariza, Arando Arbulu, Navas González et al. (2022) for eggs from hens of the same breed (8.41 – 8.45), other native Spanish breeds (8.46 – 8.55), and White Leghorn (8.42), as well as that reported (8.57 – 8.74) by Silversides and Scott (2001) for eggs from commercial ISA White and ISA Brown hens (with no difference between both improved strains). These differences could be due, in part, to the measurement methods used in each study (Allan et al., 2017). González Ariza et al. (2019) reported that the laying month influences the yolk pH, but not the albumen pH, of Utrerana hen eggs, whereas the Utrerana variety affects the albumen pH but not the yolk pH. Silversides and Scott (2001) reported variations in albumen pH for eggs from commercial ISA White and ISA Brown hens, depending on the hen's age, but with no clear trend during the laying period. Our results match those of Marzec et al. (2019), who reported that albumen pH in younger ISA Brown hens was lower than that in older hens. Alkalization of egg albumen with hen age, which is related to its structure, is determined by the lysozyme-ovomucin interaction (Krawczyk et al., 2021). On the other hand, the results from our trial do not agree with previous studies reporting no effect (Banaszewska et al., 2019) or an increase in yolk pH with hen's age over the laying period in ISA Brown laying hens (Marzec et al., 2019), and in several breeds included in a conservation programme in Poland (Krawczyk et al., 2021).

## 5. Conclusions and implications

This is the first study to investigate the changes over the entire first laying period in the external and internal quality traits of eggs of the Partridge variety of the Utrerana chicken breed gathered from hens reared under natural photoperiod and ambient temperature conditions. Under these rearing conditions, egg quality during the first laying period of the Partridge variety of the Utrerana chicken breed was characterised by eggs being of medium size and sharp in shape, featuring a predominantly light orange yolk and a moderately firm albumen, with a low proportion of white shell of good thickness. Throughout the first laying period, egg weight and eggshell content remained invariable, yolk content increased, although its quality and colour intensity were lower, and albumen content and quality decreased as a consequence of the hen's aging and increasing ambient temperatures. This variety of the Utrerana chicken breed lays good quality eggs during the first laying period, and although it does not show high productive performance, it maintains good laying rates in the hottest months of the year, which makes it of interest for rearing in alternative or organic poultry systems.

Information on the egg quality of this native chicken breed can contribute to the maintenance of livestock heritage in Spain, by taking it into account it in the breed's conservation and improvement programs (Plata-Casado et al., 2023). Further research is required to recover its ancestral egg production, which could focus on investigating the variations in egg quality during the complete laying period in the other three varieties of the Utrerana chicken breed and to explore the variation in successive laying periods throughout the lifespan of the four varieties of the breed. Assessment of the variation in the nutritional quality of eggs (fatty acid and cholesterol contents, antioxidant profile, etc.) throughout the laying period is also of interest. Ideally, this research should be carried out under free-range or pen-housing conditions so that the results would be more directly applicable to the current breeding conditions of Utrerana hens.

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## Author contributions

A.H. and P.G.-R. conceived the experiment, obtained the resources, performed and supervised the experimental work, analyzed the data, interpreted the results, and wrote the manuscript. Y.G.-R. and C.A.S.-R. performed the experiment, obtained and prepared the data. All authors read, reviewed, and agreed to the final version of the manuscript.

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## Data availability statement

The data supporting the findings of this study are available from the corresponding author, P.G.-R., upon reasonable request.

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