Variability and interaction of some egg physical and eggshell quality attributes during the entire laying hen cycle

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ABSTRACT The aim of this study was to investigate the variability and relationships between some egg physical (egg weight, width, length, shape index, and surface area) and eggshell parameters (weight and percentage, thickness, breaking strength, and L*, a*, and b* values) during the entire laying hen cycle. A total of 8,000 eggs was collected every 5 wk, from 30 to 81 wk of hen age (10 samplings of 400 eggs/house), in 2 identical poultry houses equipped with enriched cages. For the statistical analysis, ANOVA, Bivariate Correlation, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis were used. An increase of egg weight, length, and eggshell lightness (L*) associated with a reduction of eggshell percentage, breaking strength, and redness (a*) was observed as the hen aged ($P < 0.05$). Overall, the coefficients of variation resulted in <5% in width, length, shape index, and egg surface area; from 5 to 10% of egg weight, shell weight, shell percentage, shell thickness, L*, and b*; and >10% of eggshell breaking strength and a*. According to the PCA, the highest changes during the laying cycle are related to egg physical parameters (32%) and to eggshell breaking strength, percentage, and thickness (26%). The egg physical parameters appeared to be strongly correlated to each other, whereas a slight correlation between eggshell breaking strength and color attributes were evidenced (-0.231 and 0.289, respectively, for L* and a*; $P < 0.01$). Hierarchical cluster analysis, based on principal components of the overall egg attributes, is hereby considered, and evidenced dissimilarities for eggs laid from peak production up for 39 wk of hen age from the eggs laid afterwards. The latter group could also be divided into 2 subgroups, one comprising eggs laid from 44 and 53 wk of hen age and the other from 58 wk to the end. In conclusion, the large dataset created in this study allowed to extrapolate some robust information regarding the variability and correlations of the egg physical and eggshell quality attributes throughout the entire laying hen cycle.

Key words: eggshell attributes, egg quality, hen, breaking strength, color

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INTRODUCTION

The eggshell is a complex structure with important biological functions for the protection of the inner content of the egg after deposition as well as for the chicken embryo development. During both egg storage and incubation, it controls water and gas exchanges through the pores and assures a good resistance to mechanical impacts while impeding microbial invasion (Nys et al., 2004; Solomon, 2010; Samiullah et al., 2014).

Eggshell strength is a major concern for the egg industry, as during egg collection, sorting, and transportation, a high rate of cracked or damaged eggshell occurs. Eggs that are damaged during routine handling and transport to retail outlets cause major financial losses to the egg industry (Hunton, 1995), since for table egg consumption, clean and intact eggshells are required by the legislation in force. It has been estimated that loss from damaged eggshell accounts for 8 to 11% of total egg loss (Dunn et al., 2009). Eggshell integrity is influenced by a wide range of factors, including hen genotype and age, nutrition and feeding, rearing systems and environment, and handling and processing (Nys, 1986; Nys, 2001; Mabe et al., 2003; Roberts, 2004; Hidalgo et al., 2008; Valkonen et al., 2010; Świątkiewicz et al., 2015). In addition to eggshell strength, another main quality attribute for the table egg market is the eggshell color profile, which represents an important trait for consumer perception, being that brown eggs are generally preferred more than the white ones in some markets throughout the world (United Kingdom, Italy, Portugal, Ireland, Southeast Asia, Australia, and New Zealand) (Odabasi et al., 2007). One of the main factors of color variation in brown eggs is related to the hen age. Older hens lay lighter colored eggs, and this phenomenon is the result of the increased egg size

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since no proportional change in the amount of pigment deposited over the increased shell surface occurs (Odabasi et al., 2007; Samiullah et al., 2014).

The possibility to identify some non-invasive eggshell quality attributes that correlate with its resistance to its breaking nature is of great interest for the egg industry. A significant correlation between eggshell color and breaking strength has been reported for Yangzhou chicken eggs (Yang et al., 2009). Moreover, dark shell color has been linked to the higher specific gravity of eggs laid by broiler breeder hens (Joseph et al., 1999), whereas conflicting evidence has been observed in pheasant eggs (Richards and Deeming, 2001). However, no extensive and longitudinal studies on the same flock have been carried out to investigate the relationships between eggshell quality attributes, with particular regard to eggshell color and breaking strength in modern laying hen genotypes.

The present study aimed at investigating the variations of the main egg physical and eggshell quality attributes and their interactions by using descriptive and multivariate data analysis during the entire laying hen cycle.

MATERIALS AND METHODS

A total of 8,000 eggs was collected in 2 identical commercial poultry houses equipped with 5-story enriched cages (providing: at least 750 cm^2 of cage area per hen, 600 cm^2 of which shall be usable; a nest; a littered area for scratching and pecking; 15 cm of perch and 12 cm of feed trough per hen; and a claw shortening device) of 10 hens each arranged in 5 rows of double cages. In both houses, 80 cages corresponding to the sampling units were selected (8 on each side of the row) to be equally and homogeneously distributed inside the house, clearly identified, and used throughout the study. Five eggs in each of the selected points, for a total of 400 eggs/house/sampling time, were collected. Eggs were sampled from peak production (occurred at about 28 wk of age) onwards at the following hen ages: 30, 35, 39, 44, 49, 53, 58, 72, 76 and 81 weeks. The hens, belonging to the Hy-Line Brown strain, received in both houses the same commercial feed formulated according to the nutrient specifications advised by the breeding company.

Egg and Eggshell Analysis

The eggs were individually candled in order to identify those showing clear defects in the eggshell structure (e.g., broken or cracked eggs) that were subsequently excluded from the analysis. Egg weight (**EW**) was recorded and expressed in g, and width (**W**) and length (**L**) were measured with a digital caliper and reported in mm. Egg shape index (**SI**) was calculated as a ratio between W and L, whereas the egg surface (**ES**) area was determined using the formula proposed by Sauveur (1988) [ES, $cm^2 = 4.68 \text{ x } EW^{2/3}$, where EW = egg weight]. Eggshell color measurements were assessed by a reflectance colorimeter (Minolta CR-300, Minolta Italia S.p.a., Milano, Italy), and the results were expressed as lightness (L^*), redness (a^*), and yellowness (b^*) (CIE, 1978). Eggshell breaking strength (**EBS**) was determined 24 h after deposition at room temperature by quasi-static compression using an Instron testing machine equipped with a 2 kN load cell (Mabe et al., 2003). Eggs were placed horizontally between 2 flat parallel steel plates and compressed at a speed of 5 cm/min. Breaking strength represents the minimum force required to fracture the egg, and it was expressed in kg. Eggshell weight (**SW**) was obtained after removing the internal components of the egg and drying the eggshell overnight at a temperature of 80°C and given in g. Eggshell percentage (**SP**) was calculated as [(eggshell weight/egg weight) x 100]. Finally, eggshell thickness (**ST**) was estimated using the following formula: [ST, mm = SW / (ES x d)], where SW = eggshell weight, ES = egg surface area, and d = material density (2.3 g/cm^3 for calcium carbonate) (Mabe et al., 2003).

Statistical Analysis of Data

Significant differences ($P < 0.05$) between sample mean values during the laying hen cycle were explored by using the Analysis of Variance (ANOVA) within each egg physical and eggshell quality attributes. The Kruskal–Wallis test was used when data did not conform to the principles of normality and, in the case of significance, of the Levene test. The parametric Tukey HSD test or the non-parametric Tamhane test was considered for multiple comparisons ($P < 0.05$). A quantitative analysis of the sample variability within the sampling time was discussed in terms of the variation coefficient. According to the distribution, bivariate correlation analysis was performed on the explored independent variables, and Pearson's or Spearman's correlation coefficients were calculated and discussed (SAS Institute, 1988).

In order to improve data interpretation, the original variables were modeled and reduced by using the Principal Component Analysis (**PCA**) on scaled data. The independent variables' influence and their relationships were discussed and the Leave One Out cross validation was used to validate the model. The extracted significant latent variables were then used to perform the Hierarchical Cluster Analysis (Ward method) with the aim of discriminating among mean values according to the hen age (CAMO, 2007).

RESULTS AND DISCUSSION

In Table 1, the mean values of the physical properties of the egg according to the hen age are reported. As expected, the EW constantly increased from the

Table 1. Egg physical properties evaluated throughout the entire laying hen cycle.

	Parameter[1]				
Sampling time	EW, g	W, mm	L, mm	SI	ES, cm^2
30	$61.2^{a} \pm 4.0$	$44.0^{a} \pm 1.1$	$55.7^{a} \pm 1.7$	$0.79^{a} \pm 0.02$	$72.8^{a} \pm 3.2$
35	$61.9^{b} \pm 4.2$	$44.2^{b} \pm 1.1$	$56.1^{b} \pm 1.7$	$0.79^{a,b} \pm 0.02$	$73.2^{a} \pm 3.3$
39	$62.8^{c} \pm 4.3$	$44.3^{b,c,d} \pm 1.1$	$56.5^{c} \pm 1.7$	$0.79^{b} \pm 0.02$	$73.9^{b} \pm 3.4$
44	$63.7^{d} \pm 4.5$	$44.5^{c} \pm 1.3$	$57.1^{d} \pm 1.7$	$0.78^{c} \pm 0.02$	$74.6^{c,e} \pm 3.5$
49	$63.6^{d} \pm 4.4$	$44.4^{b,c} \pm 1.2$	$57.3^{d} \pm 1.8$	$0.78^{d} \pm 0.02$	$74.6^{c} \pm 3.4$
53	$63.9^{d,e} \pm 4.6$	$44.4^{b,c} \pm 1.3$	$57.7^{e} \pm 1.9$	$0.77^{e} \pm 0.03$	$74.7^{c,d} \pm 3.6$
58	$63.4^{c,d} \pm 4.5$	$44.1^{a,d,e} \pm 1.2$	$57.8^{e} \pm 1.9$	$0.77^{e,f} \pm 0.03$	$74.3^{b,c} \pm 3.5$
72	$64.5^{e} \pm 5.1$	$44.3^{b,c,e} \pm 1.5$	$58.4^{f} \pm 2.0$	$0.76^{g} \pm 0.03$	$75.2^{d,e} \pm 4.0$
76	$64.6^{e} \pm 4.9$	$44.4^{b,c} \pm 1.5$	$58.4^{f} \pm 2.3$	$0.76^{f,g} \pm 0.04$	$75.3^{d} \pm 3.8$
81	$64.5^{e} \pm 4.9$	$44.2^{b,e} \pm 1.3$	$58.5^{f} \pm 2.0$	$0.76^{g} \pm 0.03$	$75.2^{d} \pm 3.8$

[1] EW: egg weight; W: width; L: length; SI: shape index; ES: egg surface area.

[a-g] Means within a column not sharing a common superscript are significantly different ($P < 0.05$).

Table 2. Eggshell quality attributes evaluated throughout the entire laying hen cycle.

	Parameter[1]						
Sampling time	SW, g	SP, %	ST, mm	EBS, kg	L^*	a^*	b^*
30	$6.23^{a} \pm 0.46$	$10.16^{a} \pm 0.60$	$0.372^{a} \pm 0.02$	$4.781^{a} \pm 0.55$	$57.2^{a} \pm 3.2$	$20.4^{a} \pm 2.0$	$29.7^{a} \pm 1.9$
35	$6.28^{a,b} \pm 0.46$	$10.17^{a} \pm 0.55$	$0.373^{a} \pm 0.02$	$4.610^{b} \pm 0.59$	$58.9^{b} \pm 3.5$	$17.8^{b} \pm 2.1$	$31.2^{b} \pm 1.7$
39	$6.33^{b,c} \pm 0.44$	$10.10^{a,b} \pm 0.60$	$0.373^{a} \pm 0.02$	$4.587^{b} \pm 0.62$	$59.1^{b} \pm 3.7$	$17.8^{b} \pm 2.2$	$30.8^{c} \pm 1.9$
44	$6.39^{c} \pm 0.48$	$10.04^{b,c} \pm 0.59$	$0.372^{a} \pm 0.02$	$4.185^{c} \pm 0.57$	$59.0^{b} \pm 3.7$	$17.4^{c} \pm 2.1$	$29.9^{a,e} \pm 1.6$
49	$6.37^{c} \pm 0.44$	$9.99^{c} \pm 0.61$	$0.371^{a} \pm 0.02$	$4.353^{d} \pm 0.60$	$58.9^{b} \pm 3.5$	$17.2^{c} \pm 2.0$	$30.4^{d} \pm 1.5$
53	$6.38^{c} \pm 0.50$	$10.00^{b,c} \pm 0.67$	$0.371^{a} \pm 0.02$	$3.803^{f} \pm 0.62$	$58.9^{b} \pm 3.9$	$17.4^{c} \pm 2.1$	$30.2^{d} \pm 1.8$
58	$6.13^{d} \pm 0.51$	$9.68^{d} \pm 0.72$	$0.358^{b} \pm 0.02$	$3.962^{e} \pm 0.63$	$61.7^{c,d} \pm 4.0$	$16.3^{d} \pm 2.4$	$30.5^{d} \pm 1.9$
72	$6.42^{c} \pm 0.56$	$9.98^{b,c} \pm 0.72$	$0.371^{a} \pm 0.02$	$3.898^{e,f} \pm 0.64$	$61.1^{c} \pm 4.3$	$15.6^{e} \pm 2.9$	$29.1^{f} \pm 2.1$
76	$6.40^{c} \pm 0.56$	$9.94^{c} \pm 0.75$	$0.370^{a} \pm 0.03$	$3.893^{e,f} \pm 0.64$	$62.9^{e} \pm 4.5$	$15.7^{e} \pm 3.8$	$30.2^{a,e} \pm 2.4$
81	$6.22^{a,d} \pm 0.57$	$9.68^{d} \pm 0.82$	$0.360^{b} \pm 0.03$	$3.628^{g} \pm 0.62$	$62.2^{d,e} \pm 4.7$	$15.3^{e} \pm 2.9$	$29.3^{f} \pm 2.3$

[1] SW: shell weight; SP: shell percentage; ST: shell thickness; EBS: eggshell breaking strength.

[a-g] Means within a column not sharing a common superscript are significantly different ($P < 0.05$).

beginning to the end of the laying cycle with values ranging from 61.2 to 64.5 g ($P < 0.05$). The EW increment is mainly related to the increase of egg length (ranging from 55.7 to 58.5 mm; $P < 0.05$) rather than egg width (ranging from 44.0 to 44.5 mm; $P < 0.05$). Indeed, the increments of EW, width, and length through the laying cycle are, respectively, 5.6, 0.45, and 5.0%. Accordingly, the SI showed a decline over the period considered in relation to the increase of the hen age (0.79 to 0.76; $P < 0.05$).

It is well known that the EW is strictly related to the hen age (Tůmová and Gous, 2012), and its increase is one of the main production aspects kept under strict control by producers to avoid large eggs, which are more prone to eggshell ruptures during collection, transport, and packing than medium egg size (Dunn, 2013). As the hen ages, the SI of the egg decreases in accordance with the results obtained by Molnar et al. (2016), and, as emerged in the present study, it is mainly due to changes in egg length rather than in width.

In Table 2 the eggshell physical properties are given. The SW did not exhibit a constant trend during the laying phase with values ranging from 6.13 to 6.42 g. On the contrary, the SP decreased from 30 wk of hen age onwards (from 10.16 to 9.68%, respectively, for 30 and 81 wk; $P < 0.05$) Our results confirm those obtained by Samiullah et al. (2017) who found a significant decrease in SP from 44 to 73 wk of hen age.

The calculated values for ST did not exhibit significant differences throughout the laying cycle with the exception for values of 58 and 81 wk, which resulted slightly lower ($P < 0.05$). EBS decreased from 30 wk to 81 wk (4.781 vs. 3.628 kg, respectively; $P < 0.05$) in agreement with the findings of Kemps et al. (2006).

As for the color profile of eggshell, lightness values (L^*) increased during the considered period by about 8.7% ($P < 0.05$), whereas redness (a^*) greatly decreased by about 33% ($P < 0.05$). Yellowness (b^*) did not show considerable changes over time with values ranging from 29.7 to 30.8. Changes in eggshell color during the laying cycle also have been reported by Odabaşi et al. (2007) who, respectively, observed a linear increase and decrease in lightness (L^*) and redness (a^*) values over time with no changes in yellowness (b^*). Our data confirmed the results obtained in that study, although the authors used a very limited number of eggs per sampling, despite the large variation that usually affects these color attributes.

The mean values of egg physical and eggshell quality attributes, with the exception of EBS, sharply declined at 58 wk of hen age, in relation to the peak of the summer temperatures, and then recovered to normal values for the specific laying period.

Table 3. Coefficients of variation (%) of egg physical and eggshell quality attributes throughout the entire laying cycle.

	Parameter[1]											
Sampling time	EW	W	L	SI	ES	EBS	SW	SP	ST	L^*	a^*	b^*
30	6.5	2.5	3.0	3.0	4.4	11.4	7.3	5.9	5.6	5.7	10.0	6.5
35	6.8	2.5	3.0	3.0	4.6	12.8	7.3	5.4	5.2	5.9	11.6	5.3
39	6.8	2.5	3.1	3.0	4.6	13.5	7.0	6.0	5.4	6.2	12.3	6.1
44	7.1	2.9	3.0	3.2	4.7	13.6	7.6	5.8	5.5	6.2	12.3	5.2
49	6.9	2.6	3.2	3.1	4.6	13.7	6.9	6.1	5.1	6.0	11.9	4.9
53	7.2	3.0	3.3	3.5	4.8	16.3	7.8	7.7	6.2	6.6	12.2	5.9
58	7.1	2.8	3.4	3.6	4.7	15.8	8.3	7.4	7.0	6.5	14.7	6.2
72	8.0	3.5	3.5	3.8	5.4	16.4	8.6	7.2	6.7	7.0	18.6	7.1
76	7.7	3.5	4.0	5.0	5.1	16.4	8.7	7.5	7.1	7.2	24.2	7.8
81	7.6	2.9	3.5	3.6	5.1	17.2	9.1	8.5	8.0	7.5	19.2	7.9
Δ max-min	1.5	1.0	1.0	2.0	1.0	5.8	2.1	3.1	2.9	1.8	14.2	3.0

[1] EW: egg weight; W: width; L: length; SI: shape index; ES: egg surface area; EBS: eggshell breaking strength; SW: shell weight; SP: shell percentage; ST: shell thickness.

Table 4. X-loadings for the egg physical and eggshell quality attributes obtained by Principle Component Analysis (PCA).

	Principal component[1]			
	PC1	PC2	PC3	PC4
X-exp[2]	32%	26%	16%	13%
Parameter[3]				
EW	0.496	− 0.020	0.130	− 0.017
W	0.406	0.076	0.256	− 0.327
L	0.397	− 0.188	− 0.085	0.384
SI	− 0.071	0.241	0.283	− 0.630
ES	0.495	− 0.019	0.133	− 0.018
EBS	− 0.015	0.413	0.012	− 0.204
SW	0.380	0.335	− 0.192	0.052
SP	− 0.071	0.452	− 0.396	0.087
ST	0.112	0.464	− 0.362	0.082
L^*	0.092	− 0.293	− 0.433	− 0.346
a^*	− 0.094	0.305	0.444	0.334
b^*	− 0.039	0.142	0.321	0.242

[1] PC1: Principal component 1; PC2: Principal component 2; PC3: Principal component 3; PC4: Principal component 4.

[2] Percentage of total variance of the overall parameters explained by each principal component.

[3] EW: egg weight; W: width; L: length; SI: shape index; ES: egg surface area; SW: shell weight; SP: shell percentage; ST: shell thickness; EBS: eggshell breaking strength.

In Table 3, the coefficients of variation (**CV**) of the measured and calculated parameters of eggs and eggshells are shown. According to the magnitude of the variation, the parameters can be grouped into 3 categories: with a CV lower than 5% (width, length, SI, and ES area), ranging from 5 to 10% (EW, SW, SP, ST, L^*, and b^*) and higher than 10% (EBS and a^*). Moreover, it can be noted that the majority of the parameters show differences between the minimum and maximum values, observed at the beginning and at the end of the laying period, respectively, ranging between 1.0 and 3.1 with the exception of EBS and eggshell redness (a^*), which exhibit higher values (5.8 and 14.2, respectively).

In Tables 4 and 5, X-loadings for the first extracted significant principle components and the correlation coefficients for the eggshell and egg physical properties are reported, respectively. As shown, the first 4 principal components explained 86.8% of the total variance of the overall parameters. In particular, the first extracted latent variable, describing 32% of the total variance, appeared to model mainly the attributes related to the physical characteristics of the egg (EW, width, length, and ES area having absolute loading values higher than 0.4) (Table 4). These parameters are highly positively correlated (0.809 and 0.720 for EW vs. width and length, respectively; $P < 0.01$) as also shown in Table 5. Lin et al. (2004) found similar correlation coefficients for the same parameters in eggs of 60-week-old hens reared in thermoneutral conditions, whereas a lower correlation with length for eggs obtained from heat-stressed hens. The second extracted components, explaining 26% of the total variance, described mainly attributes related to the eggshell physical traits (EBS, SP, and ST having absolute loading values higher than 0.4). EBS exhibited a slight significant and positive correlation coefficient vs. SP and ST (0.450 and 0.456, respectively; $P < 0.01$) (Table 5). Yan et al. (2014) reported similar correlation coefficients for EBS and measured ST in eggs of 40-week-old Lohman brown hens. Color attributes (mainly L^* and a^*) and SI are explained, respectively, by the third (16% of total variance) and fourth (13% of total variance) extracted component (Table 4). Eggshell lightness (L^*) values appeared highly negatively correlated with redness (a^*) ones (-0.852; $P < 0.01$), whereas both of these color attributes showed mediocre correlation with the EBS (-0.231 and 0.289, respectively, for L^* and a^*; $P < 0.01$) (Tables 4 and 5). Yang et al. (2009) reported a significant negative correlation in Yangzou chicken eggs between shell strength and shell color measured with a reflectometer taking a percentage reading between black and white. Based on the correlation coefficients associated with the large variability of EBS and a^* values, as emerging from our results, shell color attributes are not reliable parameters for estimating the eggshell strength in brown eggs, at least for the hen strain tested in this study.

In Figure 1, the dendogram resulting from hierarchical cluster analysis obtained by using the extracted 4 latent variables of PCA is shown. According to the figure, 2 big clusters can be observed, the first one containing the values of egg samples collected from 30 (peak production) to 39 wk of hen age. The second cluster is

Table 5. Correlation coefficients of egg physical and eggshell quality attributes.

Parameter[1]	EW	W	L	SI	EBS	SW	SP	L*	a*	b*	ES	ST
EW	1.000											
W	0.809**											
L	0.720**	0.365**										
SI	– 0.080**	0.411*	– 0.691**									
EBS	– 0.097**	0.047**	– 0.344**	0.364**								
SW	0.602**	0.484**	0.376**	0.006	0.305**							
SP	– 0.356**	– 0.287**	– 0.321**	0.086**	0.450**	0.517**						
L*	0.099**	0.034**	0.182**	– 0.149**	– 0.231**	– 0.063**	– 0.181**					
a*	– 0.101**	– 0.034**	– 0.195**	0.163**	0.289**	0.034**	0.164**	– 0.852**				
b*	– 0.025*	0.003	– 0.070**	0.072**	0.085**	– 0.022	0.010	– 0.305**	0.344**			
ES	0.999**	0.809**	0.718**	– 0.075**	– 0.097**	0.601**	– 0.358**	0.093**	– 0.091**	– 0.030*		
ST	– 0.012	– 0.009	– 0.079**	0.067**	0.456**	0.789**	0.923**	– 0.159**	0.132**	– 0.003	– 0.014	1.000

[1] EW: egg weight; W: width; L: length; SI: shape index; EBS: eggshell breaking strength; SW: shell weight; SP: shell percentage; ES: egg surface area; ST: shell thickness.

*: $P < 0.05$; **: $P < 0.01$.

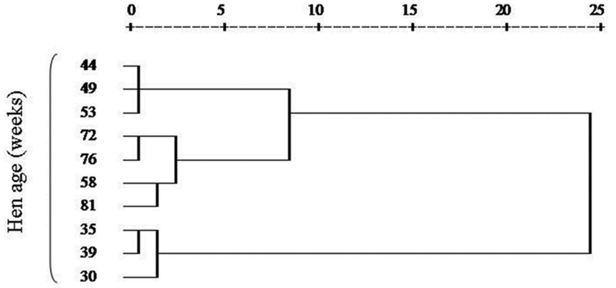

Figure 1. Hierarchical cluster analysis of the first 4 principal components of egg physical and eggshell quality attributes during the entire laying hen cycle.

divided into 2 sub-clusters: one discriminating sample obtained from 44 to 53 wk of hen age, and the other, eggs collected from 58 to 81 weeks. In particular, this analysis clearly showed that eggs can be divided into 2 different main groups according to their physical and quality attributes, showing that they can be considered very similar up to 39 wk of hen age and then markedly different. Furthermore, the eggs laid from 44 to 53 wk, although with less statistical power, can be considered different from those laid from 58 to 81 weeks. Therefore, the cluster analysis allowed to classify the eggs laid in different hen ages according to the overall quality attributes and their relative changes occurring during the entire deposition cycle.

In conclusion, from this study, carried out on a very large number of brown eggs obtained from a modern hen genotype throughout the entire laying cycle, it was possible to create a big dataset that allowed to extrapolate some important information regarding the variability of the measured attributes but also their relationships and contribution to the eggshell quality traits. In particular, it was clearly evidenced that the attributes exhibiting the largest coefficients of variations are EBS and redness values (a*). In general, the knowledge of the variability of the measured parameters is of great importance to determining the sampling dimension when setting up experimental design aimed at evaluating or comparing these traits. The present study provides a robust estimation of the variability of some egg physical and eggshell quality attributes throughout the entire laying hen cycle.

The cluster analysis, based on the principal components, and in which the overall egg attributes are hereby considered, evidenced dissimilarities for eggs laid up to 39 wk of hen age from the eggs laid afterward. The latter group also could be divided into 2 subgroups, one comprising eggs laid from 44 and 53 wk of hen age and the other, eggs laid from 58 wk to the end. Although it is recognized that a decline in egg physical and eggshell quality attributes occurs as the hen ages, through the cluster analysis, it was possible to better understand and consequently to group the eggs laid at different hen ages according to their physical and eggshell quality characteristics.

The EBS, a very important quality attribute for the egg industry, appeared slightly correlated with other eggshell quality traits, and in particular with color values (L* and a*). These outcomes suggest that eggshell robustness cannot be easily detected by measuring other egg and eggshell quality parameters requiring non-invasive techniques.

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