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Original

Visual scale evaluation as a measure of internal quality and freshness of commercial eggs

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ABSTRACT

Objective. We aimed to evaluate the use of visual assessment scale of internal egg quality and its relationship with other quality variables during storage time. **Materials and methods**. The analyses were carried out in the Egg Quality Laboratory of Promitec Santander. To the study, 527 commercial table eggs from five regions of Colombia were used. Eggs were stored between 28 and 30°C. On days 3, 6, 8, 12, and 17 of storage, front and top view photographic records were made for visual scale scoring, and physicochemical measurements were performed. ANOVA and TUKEY test were used to compare means for each variable between the different measurement times. The correlations between the internal quality variables were analysed using correlation coefficient and linear regression. **Results.** Albumin and yolk pH increased and the visual scale and the yolk index decreased through the storage time ($p>0.05$). A negative correlation was observed between the pH of albumin with the yolk index and the visual scale. On the other hand, a positive correlation between the yolk index and the visual scale was found ($p<0.05$). **Conclusions.** Storage time at room temperature affects the physicochemical variables measured for quality, and also decreases the score on the visual evaluation scale; which allowed to identify and score observable physical chemical changes in the internal components of the egg.

Keywords: Egg deterioration; egg properties; layer chickens; storage conditions (*Sources: Agrovoc, CAB*).

RESUMEN

Objetivo. El objetivo del presente estudio fue evaluar el uso de una escala de valoración visual de calidad interna de huevo comercial y su relación con otras variables de calidad durante el almacenamiento. **Materiales y métodos**. Los análisis se realizaron en Laboratorio de Calidad de Huevo de Promitec Santander, se utilizaron 527 huevos comerciales de cinco regiones de Colombia. Los huevos se almacenaron entre 28 y 30°C. Los días 3, 6, 8, 12 y 17 de almacenamiento se realizaron registros fotográficos de vistas frontal y superior para la puntuación de escala visual y se llevaron

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a cabo mediciones fisicoquímicas. Se utilizaron pruebas de ANOVA y TUKEY para comparar medias de cada variable entre los diferentes tiempos de medición. Las correlaciones entre las variables de calidad interna se analizaron mediante coeficientes de correlación además de regresión lineal. **Resultados.** valores de pH de la albumina y la yema presentaron un aumento significativo ($p<0.05$) durante el tiempo de almacenamiento; por el contrario, el índice de yema decreció ($p <0.05$) en el tiempo. Se observó correlación negativa entre el pH de la albumina con las variables de índice de yema y la escala visual. Por su parte la correlación entre el índice de yema y la escala visual fue positiva ($p<0.05$). **Conclusiones.** El tiempo de almacenamiento a temperatura ambiente afecta las variables fisicoquímicas medidas de calidad, además disminuye la puntuación en la escala de evaluación visual; la cual permitió identificar y puntuar cambios físicos químicos observables en los componentes internos del huevo.

Palabras clave: Condiciones de almacenamiento; deterioro del huevo; gallinas de postura; propiedades del huevo (*Fuente: Agrovoc, CAB*).

INTRODUCTION

Commercial egg companies in Colombia have tried to differentiate themselves in the market appealing to quality and freshness as a differentiating attribute between brands. In the commercial laying hen industry, the production of eggs with good quality characteristics, both external and internal, is vital to reduce losses due to egg discarding and, besides, to bring fresher and quality eggs to the final consumer who is increasingly demanding with the freshness of the food that arrives at their table (1).

In the useful life of the egg overtime, from the laying of the egg until it reaches the final consumer, several physicochemical changes can occur. These changes have shown to modify several characteristics such as the egg flavor, texture and, in general, the egg palatability. These changes are generated by a series of chemical reactions related to the transfer of carbon dioxide through the eggshell in which the dense albumen becomes liquid. This process involves carbonic acid (H_2CO_3) as one of the components of the buffering system of albumen that dissociates in water and carbon dioxide (CO_2) altering the total solids content and the pH inside the egg (2).

There are several indicators of internal egg quality and freshness, some of them associated with the albumen such as the Hauhg units (HU), and others associated with the yolk such as yolk index. In addition, other variables dependent on storage time, oxygen and CO_2 exchange such as the pH, can be evaluated to assess the degree of loss of the quality characteristics of the internal components of the egg and, therefore, its freshness (3, 4). However, the above-mentioned method application at a commercial or consumer level is not easy to implement. Thus, new quality and freshness assessment methods, that can be used quickly and easily by any link of the commercial egg production chain, must be developed. The objective of the present study was to evaluate the use of a visual assessment scale of internal quality of commercial egg-laying hen eggs and its relationship with other quality variables during storage time at room temperature.

MATERIALS AND METHODS

Characteristics of the study. The present study, was retrospective observational. A total of 527 eggs (white and brown) of commercial laying hens were included with an average weight of 60 ± 6.2g, from productive farms located in the departments of Valle del Cauca, Cauca, Antioquia, Caldas, Cundinamarca and Santander, in Colombia. The eggs were transported the same day they were collected in the farm, packed in 30 units plastic trays using expanded polystyrene plates as a means of protection. The eggs were received and analyzed from March 29, 2019 to January 14, 2020, in the Egg Quality Laboratory of Promitec, Santander, Colombia.

The samples from different commercial egg producing companies were taken in the afternoon after they had been classified in the respective distribution centers and dispatched to the laboratory in such a way that they did not exceed 3 days of laying upon arrival. Once the samples were received, visual inspection and ovoscope testing was performed for shell integrity, discarding those with macroscopic breaks. The eggs selected as viable to be analyzed were stored in 30 units plastic trays at room temperature ranging between 28 and 30°C with relative humidity between 58 to 64%.

The internal quality analysis of the eggs was carried out on days 3, 6, 8, 12 and 17 of storage, being the day zero the egg laying day. For the analysis, four eggs were randomly taken from each tray on each of the established days. Each egg unit was weighed using a digital scale with precision of two decimal places before removing its shell; then, the contents were deposited in an 11.5 cm in diameter and 1 cm height transparent acetate container. Finally, the components of each egg (shell, albumen and yolk) were mechanically separated and weighed individually. These measures were expressed as absolute weight and relative weight, which indicates the percentage that each component of the total weight of the egg represents. In addition, by dividing the relative weight of the yolk and the egg white, the yolk to albumen ratio was obtained.

Yolk and albumen pH. Using the internal components of the egg already separated, the pH of each one was of the internal fractions was evaluated using a Lutron® PH-207HA digital pH meter.

Yolk index (YI). Using a mediaPad M3 tablet with an 8-megapixel resolution, two photographic records were taken: a front one (6 cm distance between the camera and the egg), and an upper one (23 cm between the camera and the egg) (Figure 1). These pictures were used to determine the yolk height and diameter measured in millimeters by the free image processing software ImageJ®. Regarding the yolk measurements, the following formula was used:

$$YI = \frac{Yolk\ height\ (mm)}{Yolk\ diameter\ (mm)}$$

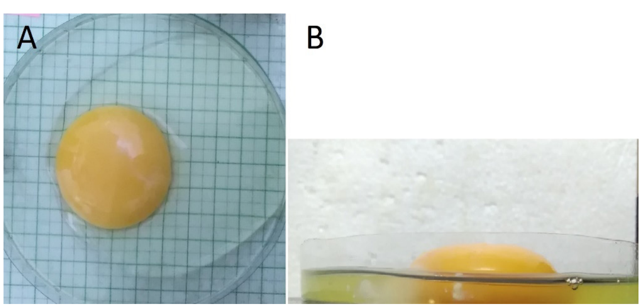

Figure 1. Top (A) and front (B) views used to determine the yolk index and the score of the internal egg quality evaluation scale.

Internal egg quality visual scale. The visual internal egg quality evaluation scale used in this research was developed based on the modification of the freshness measurement scales of the United States Department of Agriculture (USDA) referenced by Sastre (5) in 2002 and the scale reported by Van A. and Wilgus (6) in 1935**.**

Two photographs of each egg were used, one from the front view and the other from the top view (Figure 1) to determine the score of the eggs on the visual evaluation scale and these images were evaluated independently by three people from the egg quality laboratory. Each of the two views were scored separately within the visual scale, obtaining both frontal and top score values. Subsequently, the values of each egg were averaged to obtain a visual scale score for each of the eggs.

The visual evaluation scale was made up of ten categories with integer values from zero to ten where ten represented the best internal quality (Figure 2). For the scoring of the eggs, the front and top view images of each egg were used, each of which was classified into one of five main categories of the scale represented by the even points (2,4,6,8 and 10) which describe visible structural changes in physical characteristics of both albumen and yolk. The following characteristics were defined for each score:

10 Points: the dense and fluid albumen fractions are evident in the top view. Moreover, the yolk is centered in the dense fraction. In the lateral view, dense albumen is distinguished from the fluid, the yolk protrudes from the albumen, and its shape is convex.

8 points: the two albumen fractions are evident in the top view; their differentiation being less marked. In the lateral view there is no presence of dense albumen. On the other hand, the yolk protrudes from the albumen and maintains the convex shape.

6 points: In the top view the dense fraction of the albumen is diffuse, the yolk increases in diameter and loses its location in the center of the dense albumen and begins to move to the sides. The albumen fractions are not differentiated in the lateral view and the yolk loses height with respect to the albumen and is slightly flattened.

4 points: The albumen fractions are not differentiated in the top view. Regarding the yolk, a marked increase in diameter is evident.

In lateral view, the level of the yolk is flat and its height is low without protruding from the albumen.

2 points: Yolk break

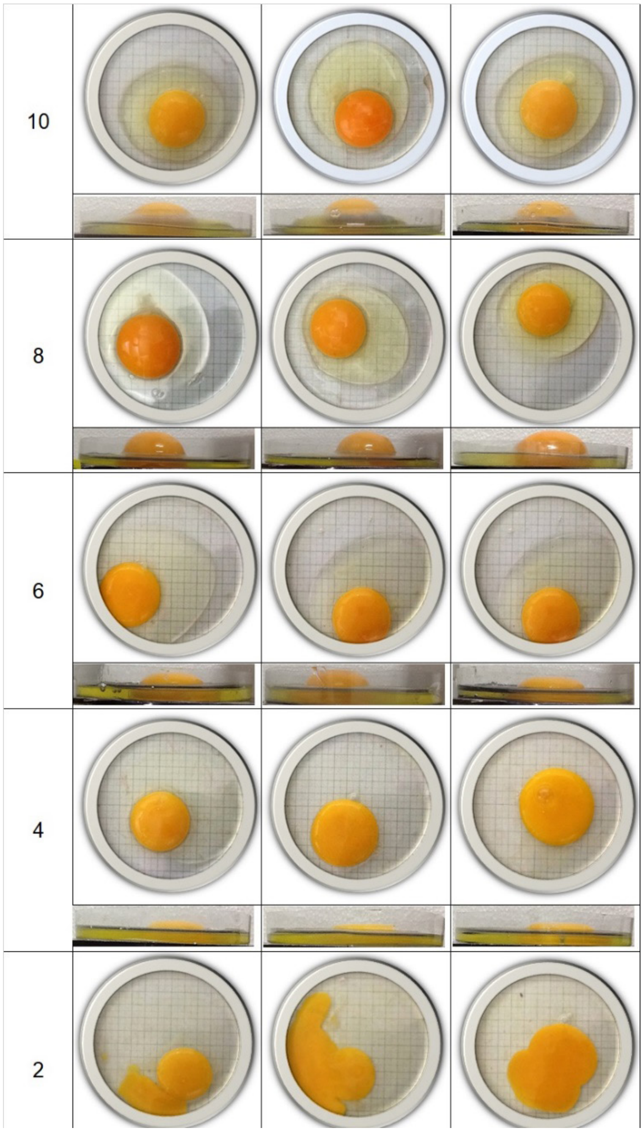

Figure 2. Visual scale of internal egg quality. Main categories of the visual scale, where three images of the front and top views of each of the categories can be seen to exemplify the physical characteristics described for each of the categories

Finally, the score of the two photographs of each egg was averaged to obtain the average visual scale score which represents the internal quality status of each egg. In this procedure, eggs classified in the intermediate scores were obtained represented by the odd categories of the scale (3,5,7 and 9) that constituted transition points between the main categories of the scale in which the physicochemical changes of the egg were appreciated alone in one of the two views of the egg.

Statistical analysis. The present study was retrospective observational. The data were analyzed using the STATA 16 licensed statistical package. The data for each of the variables were tabulated to obtain descriptive statistics. The ANOVA and TUKEY tests were used to compare the means of each variable of weight and internal quality between the different measurement times. The visual scale, as it is a categorical variable, was described using the median and mode at each of the times. The correlations between the internal quality variables (yolk index, yolk pH, albumen pH and visual scale) were analyzed using the Pearson and Spearman correlation coefficient procedure in the case of the visual scale, and their association was also determined using a linear regression model (Eq.1) after checking the assumptions and verification of the reliability of the model was carried out.

Eq.1. Linear regression model

Visual scale=B0+B1(Yolk index)+B2(albumen pH)+B3(Yolk pH+B4 (measurement month)+ε

RESULTS

Egg components weight. After analyzing the effect of storage time at room temperature on the weight of the egg components, a significant decrease ($p<0.05$) was observed in both the absolute weight and the relative weight of albumen, that showed a reduction close to 10% on day 17 compared to the initial absolute weight. On the other hand, the shelf life storage did not affect the absolute weight of the shell or of the yolk, while a significant increase ($p<0.01$) was observed in the relative yolk weight, in addition to a higher yolk albumen proportion ratio on day 17 of storage compared to the initial day of measurement) (Table 1). No significant differences ($p>0.05$) were observed regarding the origin of the egg or the month in which the measurement was made.

Table 1. Weight of commercial laying hen egg components and their variation during storage time at room temperature.

Time (days)	Weight (g)				Relative weight (%)			
	Egg	Shell	Albumen	Yolk	Shell	Albumen	Yolk	Yolk: albumen
3	61.13 b	7.32	38.46 c	15.35	11.98	62.91 b	25.02 a	39.77 a
6	60.90 b	7.39	37.38 ab	16.12	12.17	61.32 b	26.53 b	43.26 ab
9	60.23 ab	7.16	36.95 abc	16.15	11.88	61.31 ab	26.80 b	43.71 ab
13	58.22 ab	7.13	35.59 ab	15.49	12.28	61.13 ab	26.49 b	43.33 ab
17	57.37 a	7.01	34.63 a	15.65	12.25	60.36 a	27.27 b	45.17 b
SEM	0.34	0.05	0.23	0.13	0.01	0.01	0.04	0.34
P value	0.002	0.077	0.000	0.417	0.082	0.000	0.000	0.004

Letters a, b and c for significant differences (p<0.05) between times determined by ANOVA and TUKEY tests. n = 105 eggs per measurement day.

Internal quality of the egg. Regarding the variables measured as markers of internal egg quality, a significant effect (p<0.05) of the storage time at room temperature was observed on the internal quality variables of yolk index (albumen pH and yolk pH) (Table 2). When analyzing the pH changes of the main internal components of the egg during the storage time, an increased albumen and yolk pH were observed. Similarly, the yolk index showed a significant decrease (p<0.05) from the first days of egg storage.

Table 2. Internal quality variables of commercial laying hen egg and its evolution in storage time at room temperature.

Time	pH yolk	pH albumen	Yolk index
3	6.10 a	8.91 a	0.42 a
6	6.09 a	9.19 b	0.34 b
9	6.11 a	9.20 b	0.32 c
13	6.21 b	9.32 bc	0.31 c
17	6.25 b	9.38 c	0.26 d
SEM	0.01	0.01	0.00
P value	0.001	0.001	0.001

Letters a, b, c and d for significant differences (p <0.05) between times determined by ANOVA and TUKEY tests. n = 105 eggs per measurement day.

When comparing the egg scores following the visual evaluation scale, a statistically significant decrease (p<0.05) was observed in all the days evaluated, except for days 9 and 13. As shown in Table 3, the visual scale scores at the first time point were found to be between nine and ten points, while the scores reached a mode and a median of four points on day 17, a score that represents, in physical characteristics, the increase in the fluidity of the albumen that is known to be perceived as a main characteristic of a low quality egg (Table 3)

Table 3. Behavior of scores of visual assessment scale of commercial laying hen eggs during storage time at room temperature.

Day	Average visual scale		Top view score		Front view score		*CV
	Me	M	M	Mo	Me	M	
3	8	10	8	10	8	8	0.07
6	6	6	6	6	6	6	0.04
9	5	5	6	4	4	6	0.05
13	5	5	6	4	4	6	0.04
17	4	4	4	4	4	4	0.02

Me= Median; M=Mode; *CV Coefficient of variation between the average visual scale score of the three evaluators for each of the evaluation days. n = 105

Correlation between internal egg quality variables. Table 4 shows the results of the correlation and linear regression tests between the different variables. When performing the Spearman correlation analysis between the different variables of internal egg quality, a negative correlation was observed between the pH of albumen with the variables of yolk index and visual scale. In turn, there was a positive correlation between the yolk index and visual scale, with a correlation index of 0.84. Regarding the yolk pH, no significant (p>0.05) correlation coefficients were found.

Table 4. Correlation coefficient between variables of internal egg quality.

Quality variable	Visual scale	Yolk index	Albumen pH	Yolk pH
Visual scale	1			
Yolk index	0.84**	1		
Albumen pH	-0.44*	-0.51*	1	
Yolk pH	-0.33	-0.29	0.60*	1

Correlation coefficient values. Model reliability (R^2 = 0.74). Association determination by linear regression (R^2 = 0.73). P value of the association in the linear regression model between pairs of variables * p <0.05, ** p <0.01.

When the 95% confidence intervals of the yolk index and albumen pH variables were determined for the main values on the scale of visual evaluation of egg quality (Table 5), yolk index ranges from 0.42 to 0.44 were observed on score ten of the scale while, when the score was four in the scale, a category in which the physical characteristics of the egg describe the loss of fluidity of the egg white, the yolk index falls to values between 0.24 and 0.26 while the albumen pH increases reaching values between 9.39 and 9.49.

Table 5. 95% mean and confidence intervals of yolk index and albumen pH values in each level of the visual scale.

Visual scale	Yolk index	Albumen pH
4	0.25 (0.24 – 0.26)	9.44 (9.39 – 9.49)
6	0.33 (0.32 – 0.35)	9.24 (9.19 – 9.30)
8	0.41 (0.39 – 0.44	9.07 (9.01 – 9.19)
10	0.42 (0.41 – 0.44)	8.92 (8.75 – 9.09)

DISCUSSION

Visual assessment scale. In Van A. and Wilgus (6) methodology in 1935, the authors assigned a visual scale with values ranging from 1 to 5 with intervals of 0.5, using both the front and top views, and describing the position and distribution around the yolk, being 5 the lowest score where no differences were observed between the two albumen fractions.

The visual evaluation scale used in the present research was developed based on the previous studies of Wagenen and Wilgus (6) and Sastre et al (5) which were related to other internal quality indicator variables such as yolk index. It was found that, with yolk indices greater than 0.38, the eggs are in higher categories of the visual evaluation scale (>8), while in indicative scores of poor quality (<5), the yolk index adopted values less than 0.26. In this way, it was observed that the visual evaluation scale used in the research revealed evidence of physicochemical changes that occurred in the components of the egg in relation to its quality and freshness. On the other hand, it is necessary to evaluate the visual egg scale relationship with other egg quality measures such as the HU (Haugh Unit) in future research, in addition to the influence of the age of birds on the different egg quality indices, including the visual measurement scale.

Regarding the variation between the scores made by the three evaluators of the eggs within the average visual scale, coefficients of variation between 0.08 and 0.03 were found, thus finding small differences in the scores among the evaluators. These discrepancies, due to the subjectivity of a visual evaluation, become less noticeable in the last days of evaluation when there were more eggs rated between categories 2 and 4 which presented more easily distinguishable visual characteristics such as total loss of dense albumen (category 4) and breakage of the yolk (category 2). There were no differences of more than two points in the evaluation of the same egg by the different experts, so the coefficient of variation remained at low levels throughout the test.

Egg component weight and internal egg quality. In the present study, the absolute and relative weight loss of albumen during storage time was observed, which is related to what was observed by Pissinati et al (7) in 2014, who reported a loss in albumen relative weight close to two percentage points in a period of 21 days of storage at room temperature that had an average of 25ºC. On the other hand, Akter et al (8) in 2014 showed a significant loss ($p<0.01$) of four percentage points in the relative weight of albumen at 21 days of storage at 28°C with a reduction greater than that found in the present study. In the work carried out by Jin et al (9) in 2011, they exposed eggs to different storage temperatures (5, 21 and 29ºC) and found that both the increase in temperature and the storage time, negatively affected the weight of albumen.

In 2014 Akter et al (8) reported that egg weight loss depends on temperature, finding that eggs stored at 4°C showed significantly ($p<0.05$) less weight loss than at room temperature, explaining that this may be due to the lesser loss of water and other gaseous products in the egg content when the room temperature is lower (8). The loss of humidity and gases, from the interior through the pores of the eggshell, begins from the moment of laying and is exacerbated with the storage time and some factors in addition to the aforementioned temperature, including the relative humidity and the flow of air to which the eggs are subjected during storage and which determine the degree of weight loss presented by the egg and especially albumen loss (10).

In the present study, the pH of the albumen showed significant growth ($p<0.05$) during the storage time, which is consistent with the results of other authors who carried out similar measurements (11, 12). As it has been reported, the pH of the albumen acts as an indicator of the freshness of the egg due to its relationship with the chemical processes that take place inside it (7), where the eggs exposed to the environment present a gas exchange in which the egg loses CO2 that passes through the pores of the shell from the dissociation of carbonic acid; This is part of the egg albumen buffering system (1).

On the other hand, these chemical changes that occurred inside the egg due to the normal gas exchange, are reflected in pH changes, and are related to the sensorial quality of the egg and the characteristics of the egg albumen. The entry of oxygen into the egg causes an increase in the size of the air chamber (1, 10) while the increase in the pH of egg albumen leads to the loss of protein complexes so that the albumen loses viscosity and, with it, to the loss of the dense fraction of the egg albumen (1, 10).

These chemical reactions with negative consequences on the internal quality of the egg and on sensory characteristics such as the viscosity of egg albumen and the presence of a dense albumen layer surrounding the yolk, could be evidenced and measured by means of the visual scale used in the present study, where the average score of the visual scale decreased with the time of egg storage as did the yolk index, finding in turn a significant correlation ($p < 0.05$) of 0.84 ($R^2 = 0.73$).

In conclusion, this study has shown that the storage time at temperatures varying from 29 to 32°C negatively affects the relative weight of the egg albumen and the parameters of internal egg quality such as the yolk index and the pH of albumen, hence, decreasing the score on the visual assessment scale. The visual scale used allowed identifying observable physical changes in the internal components of the egg (yolk and egg albumen) and to score them on a scale that permits identifying the degree of degradation. The behavior of the scale against other internal egg quality variables should be further evaluated.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented.

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