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Evaluation of the physical quality of eggs from laying hens raised in the cage-free system during the storage

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Abstract. This study assessed the effects of storage periods at room temperature on the physical quality of eggs from laying hens reared in the Cage-Free system. A total of 210 integral eggs from laying hens at 58 weeks of age from the Isa Brown lineage were used for the experiment. The eggs were distributed in a completely randomized design (DCC) comprised of five treatments based on storage period (eggs of 0, 7, 14, 21 and 28 days), in 7 repetitions of 6 eggs each. The variables of the study were: egg weight and weight loss; shape index; Haugh unit; gem index; shell, white and yolk percentages; and shell thickness. The data obtained were subjected to analysis of variance using the SISVAR 5.8 statistical package and the means obtained were compared using Tukey's test at 5% significance level. The shape index and shell thickness variables did not show significant differences (P>0,05) between storage periods. However, the remaining variables studied were significantly influenced (P≤0,05) by storage periods, it is verified that the storage periods at room temperature had influences on the parameters related to the physical quality of the eggs, with the exception of the shape index and thickness of the shell, and eggs of 0 days showed better physical quality when compared to stored eggs. It is concluded that the eggs began to lose marked quality after 14 days of storage.
Keywords: physical analysis, egg storage, physical quality, room temperature, storage time.

Introduction

Under the conditions of rearing laying hens in cages to intensify the egg production process, as well as to facilitate sanitary management, reduce labor costs and allow for greater breeding density (Pavan, 2005), birds cannot express important behaviors such as stretching their wings, taking a sand bath, or moving properly.

In this way, the topic of animal welfare has been gaining attention from the international community of researchers and professionals in the layer poultry sector. At the same time, concern about the poor conditions of laying hens raised in cages has increased, suggesting a series of changes in the facilities and management of these birds (Thimotheo, 2016).

From this concern, among other alternative systems, Cage-Free emerged, which allows birds to be raised outside of cages, giving them opportunities to walk, establish hierarchical and social bonds, and express behaviors intrinsic to their nature. species, such as laying eggs in nests, perching, scratching and dispersing heat through the opening of the wings (Alves, 2006).

Such conditions create an environment that harmonizes with the idea of producing food using natural principles, which has been established as essential for good nutrition (Thimotheo, 2016).

About the product generated by laying birds, the egg, is a food of great importance in human nutrition, due to its nutritional composition of relevant quality, pleasant flavor and great diversity in the form of consumption. It has the advantage of being a low-

cost protein source with high biological value, in addition to being easily available in the diet of all social classes in Mozambique.

However, it is a food that can be nutritionally complete, due to the fact that it is the exclusive means of development of the future chick, therefore it is necessary that it contains all the nutrients in its composition for the complete growth and formation of the chick. embryo (Vieira & Phopal, 2000).

On the other hand, egg quality does not last forever, the drop in the egg's qualitative potential can be influenced by several factors, whether related to the production system or even the management adopted after the egg leaves the aviary. Therefore, it is essential that this food is in good storage condition so that it maintains its nutritional qualities until it reaches the consumer (Rego, 2012).

Regarding this fact, it is believed that during storage, changes may occur in the physical, chemical and functional characteristics of egg proteins, depending not only on time and temperature, but also on the relative humidity of the air (Alleoni & Antunes, 2001). When these changes occur, the egg will reduce its original nutritional value, meaning that several quality attributes of the albumen and yolk will be lost.

So, it becomes an object of interest for researchers to rationally elucidate under which specific conditions these losses occur. Therefore, there is great concern about the external and internal quality of the egg depending on the storage time and temperature.

Due to scientific research, the internal and external quality of the egg can be evaluated based on the size of the air chamber, height of the albumen, yolk index, pH of the albumen and yolk, Haugh unit, shell thickness, specific gravity and weight. of the egg (Baptista, 2002).

In view of the above, the present study aimed to evaluate the physical quality of eggs produced by layers raised in the Cage-Free system stored for up to 28 days at room temperature.

Material and methods

Place and Period

The experiment was conducted at the production unit of Miss Porcínia Massunguine, based in the municipality of Vilankulo in the Aeroporto neighbourhood. Egg collection was carried out from a single batch of production birds raised in the aviary under the Cage-Free system. The analyses were carried out in the Food Chemistry and Biochemistry laboratory of the Department of Agricultural Production, UEM, ESUDER-Vilankulo campus. And the evaluations took place from 10/31/2022 to 11/28/2022, in the summer season.

Sample

210 eggs were used, with brown shells, from laying hens of the Isa Brown lineage, aged 58 weeks, raised in the Cage-Free system.

Experimental Design

The experimental design used was completely randomized (DCC) composed of five treatments: eggs with 0, 7, 14, 21 and 28 days of storage, with 7 replications of 6 eggs each.

Experimental Procedures

During the experimental period, the birds had free access to water and feed. The feed was formulated to meet the nutritional requirements of layers, as can be seen in table 1.

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Table 1. Nutritional Composition of the Feed (Forte A5) Provided to Layers During the Experimental Period. Source: Company MEREC INDUSTRIES, SA.

Nutrients	Composition
Protein	14,5%
Metabolizable energy	2700 kcal/kg
Calcium	3,5%
Phosphor	0,5%

Characterization of the production system (Cage-Free) applied at the unit

The breeding system used meets the recommended by CERTIFIED HUMANE BRASIL for the production of laying hens under the Cage-Free regime (CHB, 2022).

Therefore, the layers were housed in an aviary built from reeds, with two full walls and two partially filled walls on the sides. These walls had plastic curtains that were used depending on the need for less or more heat.

Furthermore, the aviary was equipped with drinkers and manual feeders (in a ratio of 1:50 birds). The birds were raised on a floor covered with sawdust, which served as bedding and provided a soft surface.

The minimum available floor area (not counting the inclusion of nests and perches) was 80 m². However, the birds had access to nests (at a minimum ratio of 1:5 birds), and perches (with a minimum space of 20 cm/bird) located throughout the aviary.

However, the birds had complete freedom of movement within the aviary and demonstrated behaviours such as pecking and scratching the litter,

resting, bathing in sawdust, perching, stretching and flapping their wings, laying eggs in the nests, and therefore, such behaviours were in accordance with HFAC (2014).

Egg Collection

The collection of eggs for analysis was carried out in a single batch of birds in production. All eggs came from the same batch, collected directly from the aviary itself, early in the morning, ensuring eggs laid during the day. After acquisition, they were packaged in cellulose honeycombs with a capacity of 30 eggs each, then housed in cardboard boxes and transported immediately to the Food Chemistry and Biochemistry Laboratory on the ESUDER Campus.

In the laboratory, all eggs were identified and weighed, then 168 of these eggs were sent for storage in the Veterinary Laboratory room at room temperature (±26,74ºC) and 42 were separated for evaluation on the day (eggs aged 0 days). of storage).

The experimental treatments consisted of five evaluation periods, namely: 0-day eggs and eggs stored for 7, 14, 21 and 28 days. During the evaluation period, the maximum and minimum temperatures and relative humidity of the storage room were recorded using a thermohygrometer, daily, three times a day.

Data collection methods

Every seven days of storage, seven half-dozens were separated to evaluate the following characteristics: weight and percentage of weight loss of eggs; shape index; Haugh unit; gem index; percentages of shell, white and yolk; and shell thickness.

a) Weight of Eggs

The weight of the eggs was obtained by weighing them individually on a semi-analytical digital scale with a precision of 0,01g.

b) Weight Loss

To evaluate weight loss (%), eggs were weighed before being stored and at the end of each storage period (7, 14, 21 and 28 days). Based on the difference between the initial weight and that obtained during the respective storage time, the weight loss was measured in grams, later converted into a percentage using the following formula: %PP = 100-[(Pf*100)/Pi]. Where: %PP – percentage of weight loss; Pf – final weight (g); and Pi – Initial weight (g).

c) Form Index

The shape index was obtained by taking measurements of the southern and equatorial regions of the eggs, with the aid of a calliper and through the relationship between the width and height of the egg, according to the equation: IF = (LO/AO)*100, described by Silva *et al*., (2018), where, IF – shape index; LO – egg width (mm) and AO – egg height (mm).

d) Haugh Unit

In the case of the Haugh Unit, after weighing, the eggs were broken onto a flat disposable plate on a flat and level marble table, and using a calliper, the height of the white was measured.

Three measurements were taken at the midpoint between the end of the yolk and the outer end of the dense egg white, avoiding chalazae. The average of the three white height points was then calculated and the individual values for each egg were applied to the following formula: $HU = 100\log(h+7,57-1,7W^{0,37})$, described by Alcobia (2018), where, UH – Haugh unit; h – clear height (mm); W – egg weight (g).

e) Gem Index

The gem index was obtained by measuring and the egg white and yolk were weighed separately. the height and width of the gem with the aid of a calliper. The values found were applied to the following formula: GI = (AG/LG), described by Thimotheo (2016), where, GI – yolk index; AG – bud height (mm); LG – bud width (mm).

f) Percentages of Shell, White and Yolk

In the case of the percentages of shell, white and yolk, it was first necessary to manually separate each of the components, the white and chalaza attached to the yolk were removed.

Therefore, the shell was washed in running water and dried at room temperature for 72 hours, for subsequent weighing.

The percentages of white, yolk and shell were calculated using the following formula:
% Shell = (PCa/PO)*100; %Clear = (PCl/PO)*100; %Gem = (PG/PO)*100.
Where: PCa – Shell Weight; PCl – Clara's weight; PG – Yolk Weight and PO – Egg Weight.

g) Shell Thickness

For shell thickness, three points were measured on the midline of the egg with the aid of a calliper in the first phase and then the arithmetic mean of the three previously measured points was calculated in order to obtain the shell thickness value. Egg shell thickness was measured without removing the internal shell membranes and was expressed in millimetres.

Statistical Analysis

For data consistency and analysis of variance (ANOVA), the SISVAR 5.8 statistical package (Build 92) was used and the means of the results obtained were compared using the Tukey test at 5% significance.

Results and discussion

The averages for weight, weight loss, shape index, Haugh unit, yolk index, percentage of shell, white and yolk and eggshell thickness are presented in table 2.

Table 2. Averages Obtained for Weight, Weight Loss, Shape Index, Haugh Unit, Yolk Index, Percentage of Shell, White and Yolk and Shell Thickness of Egg Layers Raised in the "Cage-Free" System During Storage.

Variables		0 days old eggs	Storage period (days)				CV (%)
			7	14	21	28	
Weight (g)	Iw	58,76 a1a2	60,32 a2a3	62,66 a3	59,87 a1a2a3	56,44 a1	9,62
	Fw	58,76 a2	59,42 a2	61,17 a2	57,92 a2	53,93 a1	9,68
Weight loss (%)		0,00 a1	1,42 a2	2,36 a3	3,17 a4	4,52 a5	21,29
Shape Index		79,01 a1	79,35 a1	80,76 a1	77,91 a1	77,71 a1	10,22
Haugh Unit		95,49 a4	81,22 a3	66,17 a2	56,68 a1	55,07 a1	7,95
Egg yolk Index		0,45 a5	0,37 a4	0,27 a3	0,22 a2	0,18 a1	13,78
Bark (%)		9,64 a1	9,88 a1a2	9,90 a1a2	10,04 a1a2	10,38 a2	8,96
Egg white (%)		64,90 a4	61,12 a3	57,97 a2	56,85 a2	55,02 a1	4,41
Egg yolk (%)		24,76 a1	27,66 a2	30,35 a3	31,64 a3	33,28 a4	8,56
Shell Thickness (mm)		0,35 a1	0,35 a1	0,35 a1	0,34 a1	0,34 a1	7,50

Means followed by the letter "a" and different numbers in the lines differ significantly from each other using the Tukey test (P≤0,05); CV: coefficient of variation of the plot; Iw: Initial weight; Fw: Final weight.

Is important to highlight that for the present study, egg classes in terms of weight (S, M, L and of eggs between treatments was done randomly without discrimination of the initial weights identified on the day of laying. However, in this research, when the weight of the egg is mentioned, it refers to its final weight (except 0-day-old eggs), which was identified on the day of analysis of each storage period.

However, based on the results obtained for initial weight (Table 2), there were no significant differences (P≤0,05) between 21 day old eggs from the other treatments. Higher and lower weights were observed in the 14 and 28 day treatments respectively.

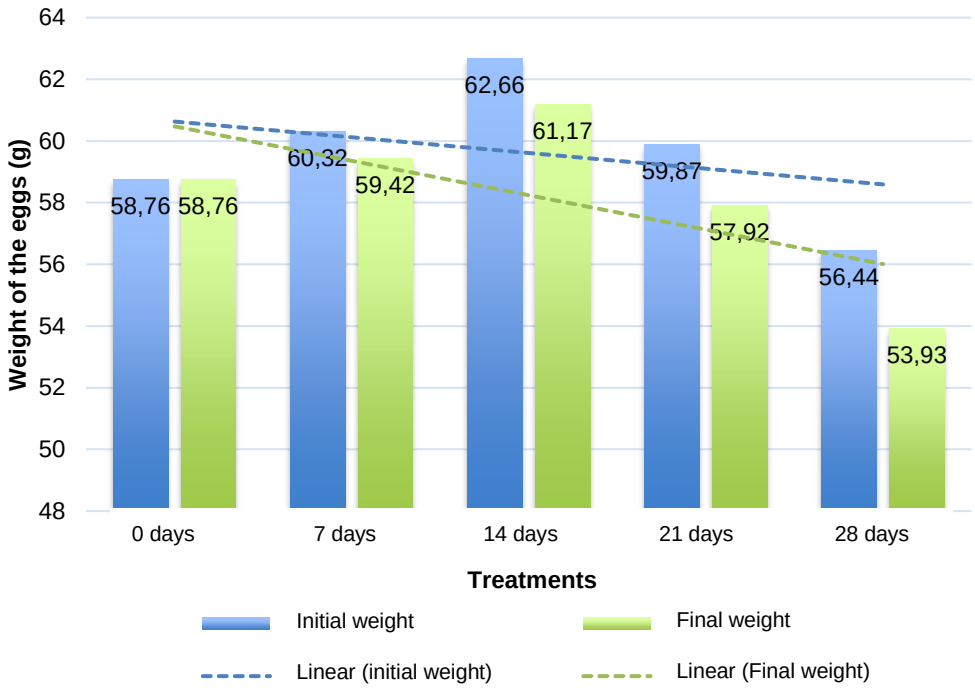
Initially, the ISA HENDRIX GENETICS COMPANY (IHGC) (2007) manual establishes an average weight of 63,7 g/egg for layers aged 58-60 weeks.

Therefore, it is clear that the average weight obtained in this study (59,60 g) does not comply with what is stipulated in Isa Brown's manual. In fact, according to the eggs obtained in this study, they are classified as "Medium (M)", which are those with a standard weight between 53-62 g, a good size for commercialization in natura according to the European Community classification (Alcobia, 2018).

However, about the averages obtained for the final weight of the eggs (table 2), they show that there were no statistical differences (P≤0,05) for eggs aged 0 days and eggs kept for up to 7, 14 and 21 days. of storage, this may be due to the random distribution of eggs between treatments without considering their initial size. However, there was an increasing reduction in the weight of stored eggs, with 28-day-old eggs differing statistically (P≤0,05) from the other treatments.

The results observed in the present study for egg weight corroborate those reported by Mendes *et al*. (2014) and Thimotheo (2016), who found a decline in egg weight when stored for long periods due to the evaporation of water present in the white into the environment.

For the weight loss variable, significant differences (P≤0,05) were observed between treatments (table 2), with egg weight loss being linear and increasing with increasing storage time (graphic 2).



Graphic 1. Graphical representation of egg weight depending on treatments.

The results obtained in the present research were in accordance with Mendes et al. (2014), who, when evaluating the weight loss of sanitized commercial eggs, experimentally contaminated with *Pseudomonas aeruginosa*, concluded that eggs kept at refrigerated temperatures showed less weight loss, indicating better nutritional quality. And the increase in the egg storage period, regardless of the storage temperature, caused continuous loss of egg weight. In the same context, according to Carvalho *et al*. (2006), Barbosa *et al*. (2008), Ramos *et al*., (2010) and Thimotheo (2016), when evaluating the effects of temperature and storage time on egg quality, concluded that increasing storage time, as well as storage at room temperature, promote a continuous decline in egg weight.

In the results observed in the present study, it is noted that throughout storage the weight loss was linear and increasing, eggs with 14, 21 and 28 days of storage were approximately double, triple and quadruple the loss presented with 7 days, respectively.

In this way, eggs stored for up to 14 days are in accordance with recommendations by FAO (2003), which considers a loss of 2% to 3% in egg weight to be considered viable for consumption. However, according to Santos *et al*. (2009), the weight loss of eggs during the storage period occurs due to the transfer of water from the white to the environment, through the pores in the shell. In addition, according to Seibel *et al*. (2005), the chemical reactions that occur inside the eggs, during storage, can cause weight loss by denaturing ovalbumin, promoting the dissociation of the ovomucin-lysozyme complex with the destruction of the ovomucin gel.

Furthermore, the magnitude of weight loss also varies depending on the thickness of the shell (Stadelman & Cotteril, 1994). Thinner eggs tend to have more pores in the shell and result in a greater degree of dehydration of the egg, thus reducing its mass. Although many authors state that the greater the thickness of the shell, the better its quality, on the other hand, according to Arazi *et al.* (2009) states that, although increasing the thickness of the shell improves its resistance to rupture, it could also affect the exchange of gases and water in the shell.

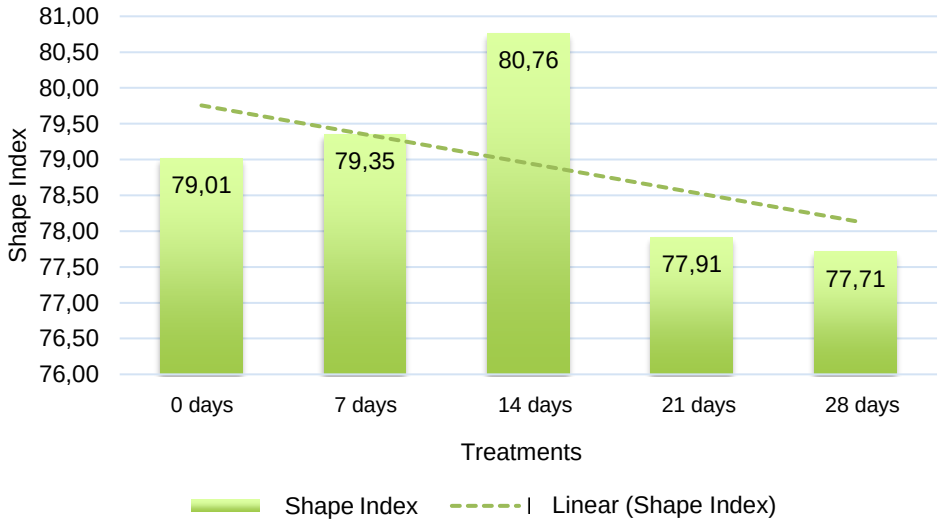
About the averages obtained for shell thickness (table 2), they show that the eggs are thicker than 0,33 mm which, according to Samli *et al*., (2006) are eggs with great resistance to breakage and consequently have fewer pores in the shell, thus hindering the exchange of gases and water with the environment during storage.

Graphic 2. Graphical Representation of Weight Loss Depending on Treatments.

Based on the results obtained for the shape index (table 2), no statistical differences (P>0,05) were observed between treatments. In this way, it is possible to verify that there was no effect of storage time on this geometric parameter of the egg, keeping its shape stable until the end of each experimental period.

According to Silva *et al*. (2018) evaluating the shape of the egg is important for standardizing packaging and for product acceptance on the market. Thus, there is a standard established by Altuntas & Sekeroglu (2008), which classifies the egg shape index as pointed for those with values lower than 72, normal for those with values between 72 and 76 and rounded for those with values greater than 76.

Therefore, the average values obtained in the present study reveal that the eggs evaluated had a rounded shape. Results similar to these were reported by Thimotheo (2016), when evaluating the physical, chemical and microbiological quality of eggs kept under the same conditions as the present experiment.



Graphic 3. Graphical Representation of the Form Index as a Function of Treatments.

According to the means obtained for UH (table 2), statistical differences (P≤0,05) were observed between treatments, but eggs aged 21 and 28 days did not differ statistically (P≤0,05) from each other, This fact may be the result of the low and constant temperature that occurred in the last week of the experiment (± 24ºC from 21st to 28/11).

It is also possible to observe that during periods of storage at room temperature there were linear reductions in HU values in eggs depending on storage time (graphic 4).

HU values were significantly higher (P≤0,05) in 0-day-old eggs than in stored eggs. The 0-day-old eggs presented an average of 95,49 HU and at the

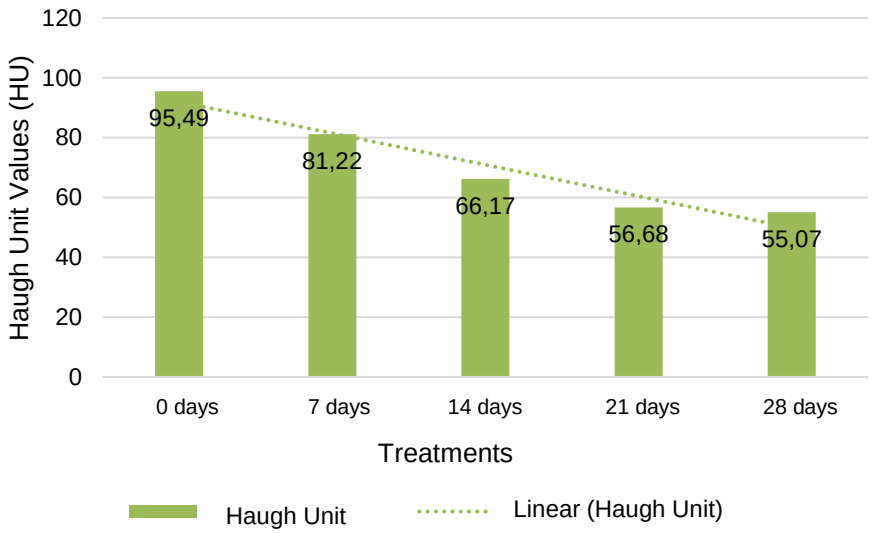
end of the experiment, after 28 days of storage, they began to present average values of 55,07 HU.

Mathematically, the HU is a measurement resulting from the relationship between the height of the white and the weight of the egg. Thus, according to Thimotheo (2016), the linear reduction of HU depending on storage periods may be the result of reactions occurring in the egg white, which leads to a decrease in its height, making it liquefied, a process that is accelerated by temperature. environment.

In the same context, according to Xavier *et al*. (2008), when studying the quality of eggs for consumption subjected to different storage conditions, concluded that the HU values of eggs decrease according to the storage time at room temperature in a more pronounced way than with the storage time under refrigeration.

In this way, it is possible to conclude that two whole eggs can have the same weight, but with differences in HU values due to the difference in storage period that both have, regardless of the temperature of the medium in which they are inserted.

However, according to the United States Department of Agriculture's egg classification manual (USDA, 2012), considering the results presented for the Haugh unit, eggs aged 0 and 7 days fall into the "AA" class, those stored for up to 14 days are class "A" and those stored for up to 21 and 28 days belong to class "B", thus indicating eggs of excellent, average and inferior quality respectively.



Graphic 4. Graphical Representation of the Haugh Unit as a Function of Treatments

For the present study, according to table 2, the means obtained regarding the yolk index indicate that there were significant differences (P≤0.05) between the treatments, with 0-day-old eggs showing higher yolk index values than those stored eggs.

The linear behaviour of this parameter (graph 5) was similar to that of UH (graph 4) as its values decreased throughout the storage period, as the diameter of this component tends to increase with a consequent reduction in its height.

Furthermore, the decrease in the yolk index can be explained by the fact that the enzymes that act on the white proteins during egg storage hydrolyse the amino acid chains and release the water that is linked to the protein molecules. By osmosis, the water that is released in the egg white crosses the vitelline membrane and is retained by the yolk, as it is more concentrated. This accumulation of water in the yolk causes the yolk membrane to weaken, causing stretching and flattening to occur, contributing to a decrease in the yolk index (Souza, 1997) Apud Thimotheo (2016).

Thus, it is clear that the storage period of eggs at room temperature has mainly negative influences on this parameter.

In particular, this variable is considered important in determining egg quality when compared to HU and pH, as these can present flaws and instability (Spada *et al*., 2012). And according to the same author, eggs that have an index above 0,25 can be considered quality for consumption.

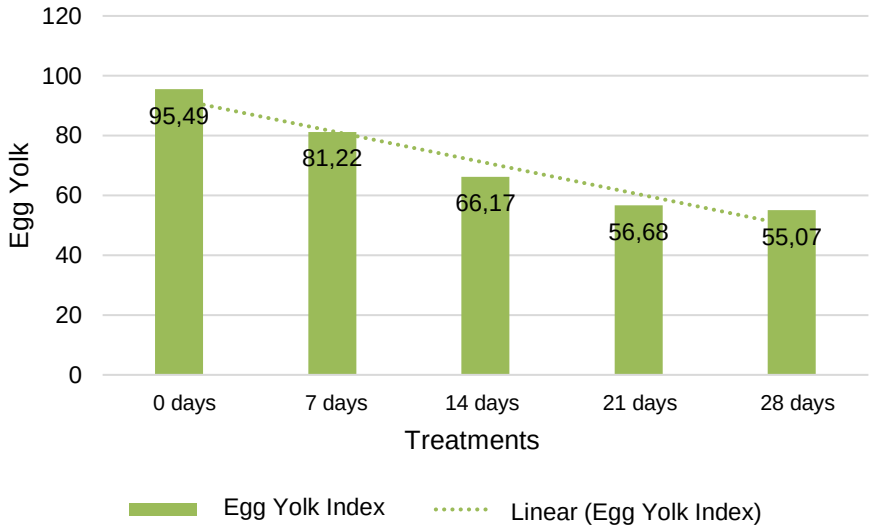
Similar recommendations corroborate with Mertens *et al*. (2011), who established that at the highest levels of egg quality, a good quality egg has a yolk index of approximately 0,45.

Therefore, according to the recommended indices, the eggs evaluated in this study, up to at 14 days they were acceptable for consumption, whereas eggs stored for up to 21 and 28 days presented lower values than recommended, thus making them unacceptable for consumption.

Controversial results to those obtained in this study, for the yolk index, were reported by Thimotheo (2016), when studying the duration of egg quality under the same conditions as the present experiment, he obtained approximate averages of 0,26 yolk index in eggs stored for 21 days.

In the same context, it was observed by Alves (2015), who, when studying the internal and microbiological quality of the eggshell of commercial

laying hens coated with propolis and stored for different periods, obtained values around 0,29 for the yolk index of eggs stored for 28 days. Thus, demonstrating that during all storage periods the eggs maintained their yolk index values at acceptable levels for consumption.



Graph 5. Graphical Representation of the Yolk Index as a Function of Treatments.

Based on the averages obtained for shell percentage (table 2), they reveal that there were significant differences (P≤0,05) between 0-day-old eggs and eggs stored for up to 28 days, however, these two treatments did not show significant differences in relation to eggs stored for up to 7, 14 and 21 days. This fact meant that the trend line remained constant in all treatments, although there were slight numerical differences, especially increasing ones between treatments (graph 6).

Approximate results were reported by Thimotheo (2016) in which he observed that differences in shell percentage occurred between 0-day-old eggs and eggs stored for up to 28 days, with the highest value of the variable being found in storage periods (9,86% at 7 days; 9,78% at 14 days; 9,96% at 21 days and 10,25% at 28 days) than in 0-day eggs (9,32%).

However, the increase in the percentage of shell throughout the storage period may be due to the reduction in the weight of the egg and white throughout storage caused by the hydrolysis of proteins and the consequent transfer of water and carbon dioxide present in the white to the environment.

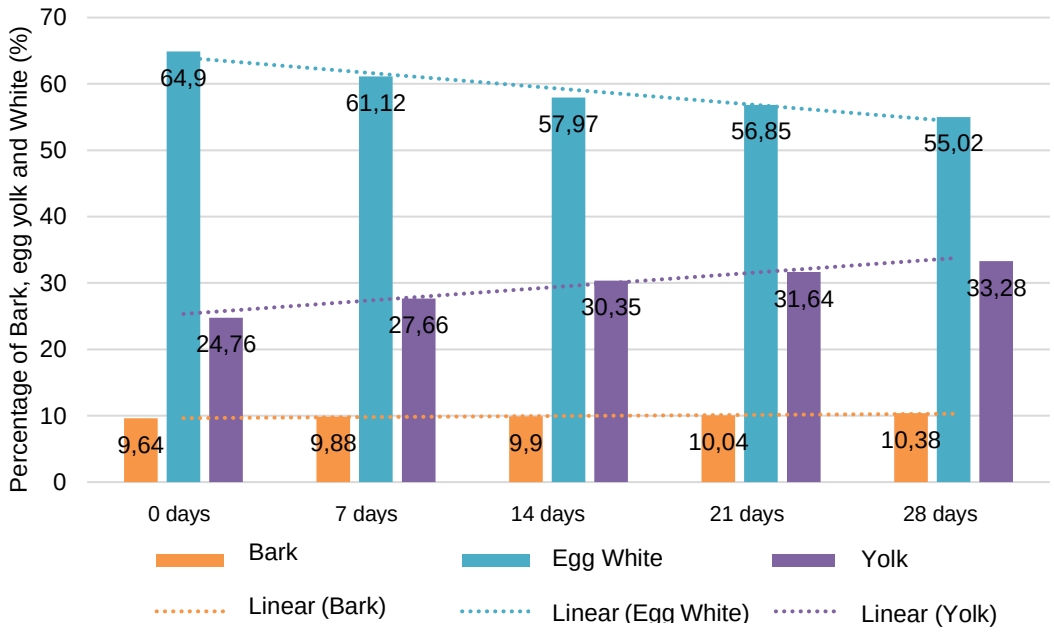
Data regarding the percentage of white and yolk were almost homologous between the storage periods, as for both variables significant differences were observed between treatments (P≤0,05), but eggs aged 14 and 21 days did not differ statistically from each other.

The difference between these two parameters was verified in their behaviour during the storage periods, as a linear reduction in the percentage of white and a consequent increase in the percentage of yolk was observed (graph 6).

These results corroborate those found by Scott & Silversides (2000), Alves (2015), Thimotheo (2016) and Suszek *et al*. (2020), who reported that the percentage of egg whites decreases as eggs are stored, and as a consequence the percentages of yolk and shell increase depending on the reduction in egg weight and the percentage of whites.

The reduction observed in the percentage of white over the storage period, and increase in the percentage of yolk can be explained by the transfer of water from the white to the yolk.

Due to the main physical-chemical changes that affect egg whites immediately after laying, two processes can be measured, including the loss of carbon dioxide and water through the evaporation of the external fluid white; and biochemical modifications of proteins and loss of water to the yolk, through the internal fluid of the white (Austic & Nesheim, 1990), consequently determining an increase in the volume of the yolk, leading to the weakening of the vitelline membrane (Moreng & Avens, 1990).

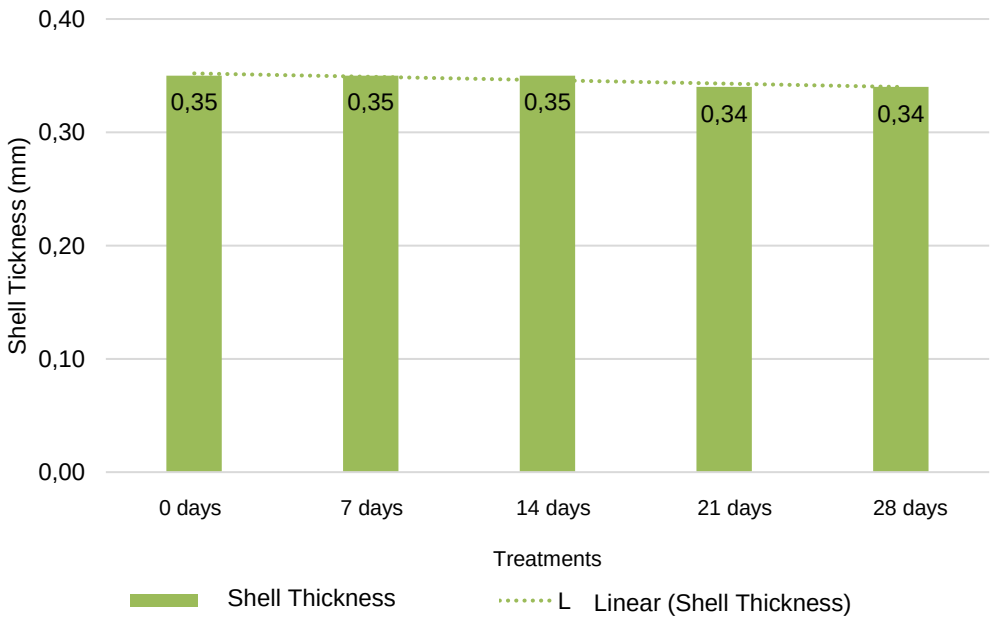


Graphic 6. Graphical Representation of the Percentages of Shell, White and Yolk Depending on the Treatments.

The means obtained for shell thickness show that there were no statistical differences (P>0,05) between treatments. Thus, it is possible to state that storage periods at room temperature had no influence on this variable.

Similar results were reported by Oliveira (2006), Thimotheo (2016) who, when studying the influence of temperature and storage time on the physical qualities of eggs, did not observe significant effects on thickness depending on temperature and storage time. Regarding the quality of the shell, according to Thimotheo (2016), the variable thickness of the shell is an indicator of fragility, therefore, the thinner the shell, the greater the ease of breaking, however, the thicker it is, the greater it will be difficult to break.

However, according to Samli *et al*. (2006), eggs with thicknesses greater than 0,33 mm are highly resistant to breakage. However, in the present experiment the average thickness value was 0,35 mm, thus reflecting that they are resistant to physical impacts and suitable for being transported when packaged.



Graphic 7. Graphical Representation of Shell Thickness as a Function of Treatments.

Conclusion

Under the conditions of the present experiment, the eggs began to lose marked quality after 14 days of storage.

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