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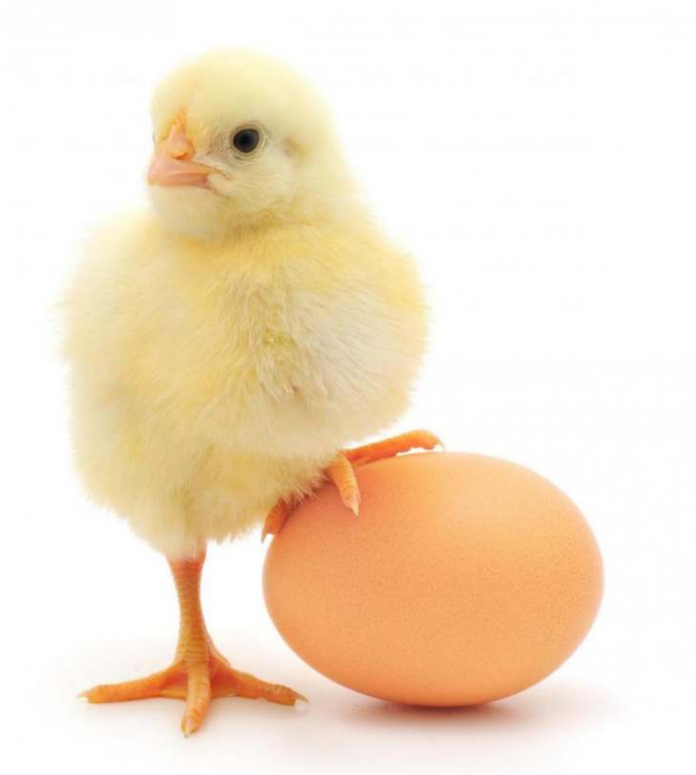

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# Assessment of Eggshell Quality Before and After Incubation from 29 and 46 Weeks-old Leghorn Hens

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**Abstract:** Eggshell quality is based mainly upon two important characteristics; its thickness and weight. This study has as an objective to determine the changes in eggshell quality before and after incubation and it consisted of two experiments with 29 and 46 weeks-old leghorn type hens respectively. In both experiments a group was worked upon before incubation (group 1) and another group after incubation (group 2). Experiment 1, group 1 consisted of 40 eggs and group 2 of 100 eggs; both groups had eggs and eggshell without membranes weighed and the percentage was obtained; thickness was measured and the amount of calcium was established. Experiment 2, group 1 had 25 eggs and group 2 had 75 eggs, eggs and eggshell without membranes were weighed and the percentage was obtained, as well as thickness, density and amount of calcium that was present. After that the data were subjected to statistical analysis to establish if the differences were statistically significant. Group 1 had significant differences ($p<0.05$) in eggshell weight, eggshell percentages and thickness and in experiment 2 there was significant differences ($p<0.05$) in eggshell weight, eggshell percentages, thickness and eggshell density. There were no differences found in both studies in relation to calcium levels. The fact that there were no differences found in calcium levels could be associated to the sample size, the use of mixed samples or to a non-detected incubation problem. At the end of the study it was concluded that values for eggshell weight, thickness, percentages and density diminish after incubation.

**Key words:** Eggs incubation, eggshell, thickness, density, calcium

## INTRODUCTION

Currently, the poultry industry is interested in obtaining high productivity lines and in the case of egg production attention is given to number, size, eggshell quality and feed consumption, among other outstanding factors (Guerra *et al.*, 2004), without forgetting that an adequate eggshell thickness is very important during the incubation process (Leeson and Summers, 2000). Appropriate quality of eggshell is important to the poultry industry due to economic losses incurred when eggs degrade, since it is equivalent to 7 or 8% of total production (Garcia *et al.*, 2001), which requires that knowledge of its characteristics and the techniques to determine them be increased constantly.

Eggshell quality is based mainly upon two important characteristics, its thickness and its weight; likewise, other characteristics are also to be taken into account such as volume, specific gravity and surface (Narushin, 1997; Ingram *et al.*, 2008; Sanchez, 2009). Harm to eggshells is directly related to their strength; which in turn is determined by their thickness (calcium carbonate content) and its matrix organization (Moyle *et al.*, 2008; Butcher and Miles, 2011).

In order to measure eggshell quality, there are several methods such as measuring the thickness of the shell by means of a micrometric screw and specific gravity tests that determine eggshell density, amongst other methods (Lera, 2005; Sanchez, 2009; Butcher and Miles, 2011). Eggshell thickness measuring is carried out by the use of a micrometer on the equator area of the eggshell, nevertheless, this may have errors due to variations in eggshell thickness and therefore it is recommended that several zones in this area be measured, in order to obtain an average (Hunton, 1987; Leeson and Summers, 2000). Specific gravity is the relationship between the weight of an object as compared to the weight of an equal volume of water that is displaced (Butcher and Miles, 2011). When this is done with the complete egg, it indicates the amount of eggshell in relation to other egg components. The eggshell, generally is reduced as the age of the hen increases, likewise there is a correlation between specific gravity and eggshell thickness (Bennett, 1993; Narushin, 1997; Garcia *et al.*, 2001; Moyle *et al.*, 2008; Butcher and Miles, 2011). There are two methods to obtain specific gravity of the egg: the Archimedes

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method and the saline solution method (Moyle *et al*., 2008).

The Archimedes method implies weighing the eggs individually and then weighing the water displaced when the egg is submerged in order to establish their relationship. Nevertheless, due to the fact that eggs must be weighed individually, this method is rarely used. (Moyle *et al*., 2008) The salt water bath uses several containers of water, each one of them has a greater salt concentration than the previous one and the egg floats in the solution that corresponds to the specific gravity (Bennett, 1993; Moyle *et al*., 2008; Butcher and Miles, 2011). This method is based on the fact that the eggshell has approximately 2.2 times the density of water (Guerra *et al*., 2004). This method is popular since it allows a rapid measurement of a large number of eggs, providing precise and efficacious information in a short amount of time (Narushin, 1997; Moyle *et al*., 2008; Butcher and Miles, 2011).

Likewise, more elaborate methods have been developed but many times they are very costly or not practical enough, such as for example the techniques based on compression or percussion of the eggshell until rupture is achieved (Butcher and Miles, 2011).

In poultry, during incubation, the developing embryo mobilizes a large amount of calcium from sources external to the embryo since it must cover its own metabolic needs of bone growth, neuromuscular activities and other physiological functions (Tuan and Ono, 1986). It has been reported that the eggshell is the main source of calcium for the embryo, indicating that around 100 to 150 mg or 70 to 80% of its requirements come from this source (Tuan and Ono, 1986; Rowlett, 1991; Packard, 1994; Yoshizaki and Saito, 2002) This is a one-way process and is facilitated by several millivolts voltage that is present throughout the egg (Stern, 1991; Gabrielli and Accili, 2010), as well as by pH changes in albumin and the concentration of ions that are present (Stern, 1991). Associated to this, it has been shown that this is a highly calcium specific active process, that is regulated basically by vitamins K and D and the availability of calcium to be transported (Gabrielli and Accili, 2010). In several published studies, it has been shown that vitamin D activates Calbindin, a calcium transportation protein (Ono and Tuan, 1991).

In the first place this process serves as a source of calcium and magnesium for the embryo, especially during osteogenesis and bone mineralization, also weakening the eggshell structure to facilitate hatching (Stern, 1991).

Calcium is present in several structures of the egg and in different amounts, 372.88 mg/g of eggshell, 1.40 mg/g of yolk and 0.11 mg/g of albumin (Dijkstra *et al*., 2006).

The objective of this study was the evaluation of eggshell quality changes of fertile eggs from hens of two different ages, by means of two experiments before and after hatching.

## MATERIALS AND METHODS

**Experiment 1:** One-hundred and fifty commercial fertile eggs were used from a 29 weeks-old flock of Bovans White line hens. The eggs weighed on average 51.7 grams with a range of 48.1 to 55.2 grams. The eggs were selected and 40 were chosen for the prior study (group 1) and 100 for incubation (group 2). Selection was carried out looking for ideal incubation characteristics to insure maximum hatching as possible.

Forty eggs were taken and weighed individually in a properly calibrated digital scale. By carefully cutting the eggshell, content was extracted taking care of maintaining the eggshell as integral as possible. Each eggshell had both testaceous membranes eliminated, was dried and weighed.

To determine eggshell thickness a properly calibrated Mitutoyo brand digital micrometer was used. Previously, both methods as described in the literature were tested in order to establish the most ideal form of carrying out the test, one measured the equator and both poles, in the other test only the equator was measured. After that it was decided to determine only the equator values.

Once the eggs were selected to be used for incubation, they were weighed and later incubated in 48 eggs capacity Brinsea brand incubator machines, each one with an automatic process of temperature and humidity control using a constant temperature of 37.7°C during the first eighteen days and during the last three days a temperature of 37.2°C was used. Relative Humidity (RH) of 55% was handled in a constant manner during the 21 incubation days. In all machines, turning was carried out every 12 hrs in 45° angles during the first 18 incubation days.

At 21 days, 48 eggshells of the pipped birds were collected, dried and weighed and then the measuring of the eggshell was performed by means of the procedure described above. Eggshells were weighed and measured prior to as well as after incubation and calcium analysis was carried out by the calcium oxalate precipitation method having placed the samples in six sets from each group (prior to and after incubation). This study was carried out in the Nutrition and Biochemistry Department of the UNAM.

In the final stage an average of each data was obtained as well as its standard deviation and then the results were analyzed to verify their statistical value through the Student's T test and the Mann-Whitney test, according to the nature of each data, the program that was used was SPSS version 17.0.

**Experiment 2:** One-hundred fertile eggs from a flock of 46 weeks-old hens of the Bovans White line were used. The eggs weighed on average 59.64 grams with a range of 55.0-64.7 grams. These eggs were selected and 25 were chosen for a prior study (group 1) and 75 for incubation (group 2).

For the first stage, 25 eggs were taken, weighed individually in a properly calibrated digital scale and then the eggshell was carefully cut and the content extracted, taking care of maintaining the eggshell as integral as possible. Each eggshell was dried and weighed and the testaceous membranes eliminated. Also, by the use of a digital micrometer, the thickness of each shell was quantified for each eggshell in three different regions of its equator (Leeson and Summers, 2000).

The eggshell specific gravity was established by the Archimedes Method, A constant amount of one gram per eggshell, previously weighed in a digital scale, was placed in a graduated cylinder and the amount of displaced water was recorded in order to obtain its volume. Since specific gravity of water depends on temperature, a correction factor based on water temperature must be multiplied by the weight of the displaced water before specific gravity of the eggshell may be determined (Kell, 1975). This was established using the modified equation: specific gravity = weight of the eggshell in air (weight of displaced water x temperature correction factor) (Ingram *et al.*, 2008).

Once the eggs were selected for incubation, they were weighed and then incubated in incubating machines of 48 eggs each one, at a constant temperature of 37.7°C during the first eighteen days and during the last three days the temperature was 37.2°C. Relative Humidity (HR) was handled at a constant 55% during the 21 days of incubation. In all machines, turning was carried out twice a day at 45° angles.

At 21 days, eggshells of pipped out chicks were collected, taking 30 eggshells; they were dried and weighed and then they were measured by the procedure as described above.

Specific gravity was determined for these 30 eggshells by the same method described above.

Four samples, comprising several eggshells in each one, were taken from each group to determine calcium through atomic absorption test, in the Facultad de Química of the UNAM where each sample was worked in triplicate.

Later on the results were statistically analyzed through the SPSS program version 17.0, using Student's T and Mann-Whitney's tests.

## RESULTS

**Experiment 1:** In group 1 (before incubation) the average weight of eggs was 52.8 with SD of 1.14, also, average eggshell weight was 5.52 grams with SD 0.31, representing a percentage of 10.41%, while in group 2 (incubated) the egg had a weight of 50.7 g with SD of 1.53, the eggshell had a weight of 4.90 grams with SD of 0.41, which is equivalent to 9.59%. In reference to eggshell thickness, in the previous study when comparing the measuring technique at the equator and at both poles or only at the equator, there was no difference observed when the data was evaluated (Abarca *et al.*, 2011) and therefore for this study it was decided that eggshells would be measured only at the equator. Eggshell thickness at the level of the equator, in group 1 showed a mean of 0.350 mm SD 0.01, while this value for eggs from group 2 was 0.321 mm with SD of 0.02. Calcium analysis means corresponded to 34.82% (348.21 mg/g) SD 2.10 for group 1, while for group 2 the level of calcium was 33.81% (338.10 mg/g) SD 2.57 (Table 1) During the statistical analysis significant differences were found for both groups in eggshell weight, percentages and thickness, but there were no differences in calcium percentages in both groups.

Table 1: Results of measurement averages prior to incubation (group 1) and after incubation (group 2)

| Measurement | Group 1 | Group 2 |
|---|---|---|
| Eggshell weight | 5.52 g$^{a}$ | 4.90 g$^{b}$ |
| Eggshell percentage | 10.41%$^{a}$ | 9.59%$^{b}$ |
| Eggshell thickness | 0.350 mm$^{a}$ | 0.321 mm$^{b}$ |
| Calcium | 348.21 mg/g$^{a}$ | 338.10 mg/g$^{a}$ |

$p<0.05$

Table 2: Results of measurement averages during experiment 2 prior to incubation (group 1) and after incubation (group 2)

| Measurement | Group 1 | Group 2 |
|---|---|---|
| Eggshell weight | 5.13 g$^{a}$ | 4.61 g$^{b}$ |
| Eggshell percentage | 8.58% | 7.74% |
| Eggshell thickness | 0.336 mm$^{a}$ | 0.302 mm$^{b}$ |
| Eggshell density | 2.15 g/ml$^{a}$ | 2.02 g/ml$^{b}$ |
| Calcium | 358.11 mg/g$^{a}$ | 358.22 mg/g$^{a}$ |

$p<0.05$

**Experiment 2:** In group 1 the eggs had an average weight of 59.75 grams, with a SD of 1.65, while the eggshell weighed 5.13 grams, with a SD of 0.39, which represented an 8.58% of the total egg weight. The eggshell at the equator had a thickness of 0.336 mm with a SD of 0.02; likewise, density was 2.15 g/ml with a SD of 0.20. Group 2 had an egg weight of 59.56 grams, with a SD of 2.86; eggshell weight was 4.61 grams, with a SD of 0.50, which represented 7.74% of total egg weight and eggshell thickness at the equator of 0.302 mm, with a SD of 0.02. This group also had eggshell density of 2.02 g/ml, with a SD of 0.16. For the calcium analysis, group one had 358.11 mg/g with a SD of 10.73 and group two 358.22 mg/g with a SD of 17.17 (Table 2). Statistical differences ($p<0.05$) were found in eggshell weight, thickness and density, yet not in the amount of calcium that was present.

## DISCUSSION

Eggshells are made up of 93.5% calcium carbonate, 1.2% magnesium carbonate, 0.5% tricalcium phosphate, 3.3% organic matter and 1.5% humidity (Buxade, 2000) and this represents close to 11% of the total egg weight although it is known that it diminishes as the hens age until they are between 8 and 9% of the egg weight. This factor is used as an indicator of eggshell quality (Safaa *et al.*, 2008; Quintana, 2011). In experiment 1, group 1 (prior to incubation) eggshell weight that was obtained was 5.52 grams, representing a 10.41% while group 2 (incubated) had an eggshell weight of 4.9 grams, which is equivalent to 9.59%; while in experiment 2, weight in group 1 represented 8.58% of the total, while those of group 2 had 7.74%. In reference to the difference in eggshell weights of both groups, eggshell loss during pipping of the chick must be taken into consideration as well as the fact that during incubation there is humidity loss of the egg (Smart, 1991; Mauldin, 2002), which in a certain manner could contribute to this effect on eggshell weight. It has been reported that the embryo takes approximately 100 mg of eggshell calcium to cover it physiological needs (Rowlett, 1991), which could contribute to this difference in weights.

The simplest and most representative method to determine eggshell thickness is the use of the micrometer that even though it might not be ideal it does offer a real result, since others that may be more elaborate, have greater probabilities for error (Hunton, 1987); given that there may be error due to eggshell thickness variations, the equator was measured several times and an average was obtained, since in a prior study it was shown that it was enough if measurements were taken from this zone, even though other authors report preferring measuring both poles as well as the equator (Hunton, 1987; Leeson and Summers, 2000; Chowdhury and Smith, 2001; Star *et al.*, 2008; Abarca *et al.*, 2011), contradicting what is mentioned by Buxade (2000) who states that the equator is the thinnest area of the eggshell. Thicknesses obtained prior to incubation coincide with statements by Buxade (2000); also Guerra *et al.* (2004) cite Lopez *et al.* (1997) that mention an ideal eggshell between entre 0.36 a 0.43 mm at the equator in incubation eggs of broiler breeders, while other authors mentioned that eggshells should have around 0.3 mm in thickness and thickness is increased up to values close to 0.4 mm by the cuticle and membranes (Leeson and Summers, 2000). Likewise Mortola and Al Awam (2010) found eggshell thickness of 0.365 mm in eggs that weighed less than 55 grams and 0.357 mm in eggs with more than 62 grams. This is relevant since when the eggshell is too thick, gaseous exchange is more difficult which in turn harms embryo development and if the eggshell is too thin bacterial invasion and fractures are more likely (Leeson and Summers, 2000). In the first experiment, eggshell thickness at the equator in group 1 had a mean of 0.350 mm, while this value for eggs from group 2 was 0.321 mm, likewise in the second experiment eggshell thickness was 0.336 mm for group 1 and in group 2 it was 0.302 mm.

The study by Mortola and Al Awam (2010) reported eggshell density of 2.03 g/ml in non-incubated eggs with less than 55 grams in weight and in eggs above 62 grams it was 1.98 g/ml. Group 1 presented a density of 2.15 g/ml, while group 2 had a density of 2.02 g/ml; in this case, the lowest density was also obtained from the thinnest eggshells. It has been generally accepted that the specific gravity of eggs is a sufficient estimate of eggshell quality, since it has been demonstrated that there is a positive correlation between specific gravity and eggshell thickness (Ingram *et al.*, 2008; Butcher and Miles, 2011). It is known that when specific gravity diminishes, eggshell ruptures increase, therefore differences in specific gravity of eggs with similar weights are due mainly to variations in the amount of calcium deposited in the eggshell (Butcher and Miles, 2011).

The process of calcium taking from the eggshell has been described as beginning around day 10 and that it reaches a peak around day 17 of incubation (Gabrielli and Accili, 2010) and that this would be the main source of calcium for the embryo, receiving around 100-150 mg or that 70-80% of its physiological requirements come from this source (Tuan and Ono, 1986; Rowlett, 1991; Stern, 1991; Packard, 1994; Yoshizaki and Saito, 2002) and some authors have indicated some of the important characteristics of this process (Ono and Tuan, 1991; Gabrielli and Accili, 2010). Yet the results of both studies did not show significant differences in calcium levels, nevertheless in the first experiment by the calcium oxalate precipitation technique, a numeric difference was obtained, although this was below what has been reported (approximately 55 mg); in the second assay, using the atomic absorption technique there weren't any differences since group one had 358.11 mg/g with a SD of 10.73 and group two 358.22 mg/g with a SD of 17.17. These results could have been affected by diverse factors, amongst which the number of samples could be mentioned, the possibility that when membranes were removed also non determined amounts of calcium could also have been removed (Packard, 1994) or some other non-detected problem that might have happened during the incubation process, since it is known that low humidity can affect the incubation process generating low calcium metabolism, as well as fluctuations in relation to the natural incubation temperature (Kuehler and Good, 1990; Yoshizaki and Saito, 2002; Merino, 2009) and turning problems may influence the development of the chorioallantoic membrane which is considered responsible for the calcium taking process from the eggshell (Yoshizaki and Saito, 2002). Also, due to the mixed samples that were used with several eggshells per sample, to carry out the calcium analysis, that could have had repercussions on the results due to individual differences among them. It is suggested that the next studies quantify calcium in yolk prior to and after incubation to establish the degree of calcification in the chick.

**Conclusion:** It is concluded that the values of weight, thickness, percentage and eggshell density diminish after incubation.

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