Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.1 / 001-010

http://dx.doi.org/10.1590/1806-9061-2023-1647

Original Article

Assessing Differences in the Quality Properties and Ultrastructure of Eggshell as Affected by Chicken Strain and Flock Age During Incubation Period

■Author(s)

Rayan GN[I,II] https://orcid.org/0000-0001-7677-123X
Galal A[II] https://orcid.org/0000-0002-3576-4743
Fathi MM[II,III] https://orcid.org/0000-0001-9207-3861
Alawaid S[I] https://orcid.org/0000-0002-5288-0017
Shehata W[IV] https://orcid.org/0000-0001-8426-3868
El-Attar AH[II] https://orcid.org/0000-0002-0781-9399

[I] Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, 31982, Saudi Arabia.
[II] Department of Poultry Production, Faculty of Agriculture, Ain Shams University, Hadayek Shoubra, Cairo 11241, Egypt.
[III] Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, Qassim University, Al-Qassim 51452, Saudi Arabia.
[IV] Department of Agricultural Biotechnology, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, 31982, Saudi Arabia.

■Mail Address

Corresponding author e-mail address
Gamal Rayan
Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, Al-Hofof, Al-Ahsa, 31982, Saudi Arabia.
Phone: 00966 546975218
Email: gahmed@kfu.edu.sa

■Keywords

Eggshell Quality, Ultrastructure; Strain, Age, Incubation.

Submitted: 16/March/2022
Approved: 13/September/2022

ABSTRACT

Three hundred hatching eggs from two layer breeder flocks (Hy-Line Brown and hy-Line W-36; 150 each) were obtained at three different periods of laying cycle (early, middle and late stages). The hatching eggs were randomly taken to assess the impact of chicken strain, age, and hatching time on some eggshell measurements and structures. Results indicate that egg weight, shell percentage and shell thickness were significantly affected by strain, breeder age, and incubation time. Increasing rate of calcium absorption and utilization by the embryos during the embryonic stage clearly reflects the decrease occurred in eggshell strength. The differences of eggshell breaking strength during incubation time periods were 0.41 and 0.55 kg/cm^2, the differences due to the incubation time may be due to the higher demand for calcium utilization in the late incubation period (18 days), observed on the10th day of incubation. Brown eggs recorded significant higher calcium percentage in eggshell compared to the white ones. The brown breeder hens recorded significant higher total pores per egg compared to the white egg. Concerning eggshell ultrastructure, total thickness of the brown eggshell was significantly higher than those of the white eggshell by about 3.7%. All parameters, except for total thickness, were significantly affected by the interaction between strain and breeder age. Finally, we conclude that some traits including egg weight, shell percentage, shell thickness, calcium percentages in eggshell, and total pores per egg were significantly affected by strain, breeder age, and incubation time. Eggshell ultrastructure of brown egg was better compared to the white ones.

INTRODUCTION

Egg production is the most important selection standard in layer breeding (Preisinger, 2018). Eggshell structure is a highly ordered bio-ceramic complex because of controlled interactions between both mineral and organic matrix constituents. Deterioration of eggshell qualityconsiders a major factor affecting egg production industry, particularly at late laying cycle (Ren *et al.*, 2018; Fathi *et al.*, 2019). Eggshell strength, however, is also an important trait in the result of selection programs. Shell strength should be determined not only by shell-breaking strength but also by shell microscopic properties. Mertens *et al.* (2006), reported that percentage of shell is one of the criterions to estimate the eggshell quality. Edmond *et al.* (2005), compared breaking strength at the beginning of lay (25 wks old), peak production (31 wks old), mid-lay (45 wks old) and end of lay (57wks old). They stated a significant reduction in breaking strength between weeks 25 and 57.Particularly, there is a deterioration of skeletal integrity as hen ages, almost 2 grams of calcium are transported for eggshell formation from the diet and the skeleton (Alfonso-Carrillo *et al.*, 2021). Minerals are related to changes of the arrangement pattern of shell membrane fibers in relation to the eggshell structure. Calcium (Ca) supplementation is key for eggshell quality. Each shell comprises up to 3 g of Ca, thus the hen's diet must contain sufficient amount of Ca in efficiently utilizable form (Roberts, 2010).

Another factor affecting egg quality and embryonic development is pores. The egg of a hen has up to 7,500 pores. Shell pores are located on the shell surface and extend through the calcified regions. Pores in the shell permit the exchange of water vapor, ions, and gases necessary for life of the growing bird. All gas exchange between the embryo and external environment in the avian egg occurs by diffusion through thousands of microscopic pores in the chorionallantoic membrane (Wagner-Amos & Seymour, 2002; 2003; Nys *et al.*, 2004). Pores provide a means for release of water vapors and the exchange of gases. Oxygen diffuses in and carbon dioxide waste from fetal respiration diffuses out. Gases exchange and water is essential to the development of the embryo. Air inside the shell increases as water vapor escapes from the egg as the embryo develops. Eggshell porosity could directly determine the incubation period (Massaro & Davis, 2005).

Lunam & Ruiz (2000), reported that significant variations in the relative thickness of the individual calcified layers occurred with hen age. The thickness of the calcified region did not differ significantly over the 30 weeks. Mammillary layer was thickest at 28 weeks and significantly thinner at 43 and 48 weeks of age. The relative thickness of the vertical crystal layer was lowest at the beginning of the egg production period, followed by a significant increase at 38 weeks of age. Bain (2005), studied the relationship between stiffness and ultrastructural of eggshell, where the relationship between stiffness characteristics of shell, its thickness and palisade layer have the greatest influence on stiffness characteristics of an eggshell. The finding of material removed from the outer surface of egg and the change in stiffness, suggested that, even within the palisade layer, the contribution to the shell's stiffness is not uniform. Bain *et al.* (2006), stated that the term of effective thickness (palisade layer) must conserve as a tool for selection programs in both broiler breeders and parent stock of layers. Karlsson & Lilja (2008), reported that the number of mammillary cones affects the level of Ca absorption of the developing embryo. Fathi *et al.* (2016), reported that good eggshell quality should have mammillary bodies that are even in size and distribution and rounded for maximum attachment to the fibers of the outer membrane. Few studies look at the effect of flock age and strain on eggshell quality and ultrastructure during different incubation time performed. Thus, the current study was conducted to investigate if these differences are affected by chicken strain and flock age.

MATERIALS AND METHODS

Hatching eggs and experimental design

Three hundred hatching eggs from two Hy-Line strains (150 Brown or 150 W-36) obtained from three-layer breeders differed in laying stages (early; 25 wks, mid; 47 wks, and late; 61 wks). Each stage represented by fifty hatching eggs per strain. The hatching eggs were randomly taken to assess the impact of chicken strain, age, and hatching time on some eggshell measurements and structures. Sixty hatching eggs (ten eggs for each of laying stage from each strain) were taken for internal and external egg quality measurements evaluation. All eggs were identified and numbered before hatching. The eggs were individually weighed to the nearest 0.01 g using an electronic digital balance, before hatching, on the10th and 18th days of incubation. Eggshell quality measurements was conducted using 60 eggs (ten eggs from each laying stage within breeder strains).The strength of the eggshell was determined according to Attar & Fathi (2014) using eggshell strength apparatus in the following manner. The equation was consequently derived to calculate the correction factor "f" as follows:

f = 0.0109 e 5.73* I

Where: I = egg length / egg width

We can obtain the actual strength of eggshell to breakage from the following equation:

Pa = f. pr

Where: Pa = actual strength in kg/cm^2

Pr = registered gauge reading.

Eggshell quality

Subsequent to eggshell cracking, the fractured egg was opened to isolate the entire eggshell after emptying the egg's contents, the shell fragments or pieces were maintained. All eggshell pieces cleaned from the albumen remaining were rinsed free of external debris or internal egg contents, washed under distilled water and air-dried at room temperature. In addition, shell membranes were preserved. Eggshell weight (to the nearest 0.01 gram) were measured using second decimal scales. The shell percentage was calculated according to following equation:

Shell percentage = Wet shell weight / Egg weight x 100.

Shell thickness (mm) with membranes was measured at three different points in the middle part of the egg (the equator) using a dial gauge micrometer. The shell thickness without membranes (mm) was measured after removing the shell membranes manually, using a dial gauge micrometer and by recording the thickness of three different points in the middle part of the egg. The thickness of the eggshell membranes was calculatedby subtracting the shell thickness with membranes minus the shell thickness without membranes. At the 10th and 18th days of egg incubation, eggs were candled using collective candling lighted tray. Eggshell measurements were conducted using 60 eggs (10 eggs from each of laying stages either for Brown or W-36 layer breeder strain) which were randomly taken from each stage at 10 days of egg incubation and at 18 days of incubation. These measurements included eggshell thickness, shell percentage and the thickness of shell membranes.

Number of pores in eggshell at 0, 10 and 18 days of incubation were calculated using the following equation:

Pores Number (N) = 304 $M^{0.767}$(Rahn & Paganelli, 1990)

Where: M = Egg weight.

On the 18th day of incubation, the remaining eggs with apparently living embryos were transferred from the incubator to the hatchery trays in a separate individual egg box. After hatching, all chicks were removed at 21.5 days of incubation, whereas some measurements conducted on eggshell that included shell weight, shell thickness (with and without membranes) and shell membranes thickness.

Chemical determination of calcium and phosphorus in eggshell

Samples of eggshell were prepared in a solution form according to the methods of association of analytical chemists. Chemical analysis for eggshell was evaluated for each laying stage within each strain (before hatching, 10th, 18th days of incubation and after hatching). Calcium and phosphorus of eggshell extraction determined by the available commercial kits (SPINREACT, S. A. Ctra. Santa Coloma, 7- E- 17176 SANT ESTEVE DE BAS – (Girona) SPAIN).

Eggshell ultrastructural evaluation

At 0, 18th days of incubation and post-hatching,48 samples of eggshell were randomly taken from Hy-Line Brown and W-36 layer breeder flocks at early (25 weeks of age) and late laying stage (61 weeks of age) within each of breeder strain (16 each) to investigate ultra-structural variations. The samples were prepared for ultrastructural determination using scanning electron microscopy according to the methods of Bain, 1990 and 1992. The cross-sectional lengths of palisade and mammillary layers directly measured in µm using scaling software provided with the SEM at a magnification of x200. The total thickness of each specimen measured as the distance from its outermost surface to the point where the basal caps inserted into the shell membranes. The thickness of mammillary layer also assessed, this being the distance from the basal caps to the point at which the palisade columns first fused. Subtraction of these two measures provided a length of the palisade thickness or effective thickness (Bain, 1990). Triplicate measures performed in each case and the mean values were used for the statistical analysis. The incidence of ultrastructural variants at the level of the mammillary layer assessed according to the methodology and terminology developed by the Poultry Research Unit, University of Glasgow (Bain, 1990; 1992). Ultrastructural integrity of each egg summarized by means of a total ultrastructural score as outlined by (Fathi, 2001).

Statistical Analysis

Data were subjected to analysis using three-way analysis of variance with breeder Strain, layer breeder Age, Incubation time and their interaction using General Linear Model (GLM) procedure of SAS (2004), as the following model:

Yijkl= µ + Si + Aj + Tk + (S*A)ij + (S*T)ik + (A*T)jk + (S*A*T)ijk + eijkl

Where:

Y_{ijkl} = Trait measured,

µ = Overall means,

S_i = breeder Strain effect (i= 1, 2),

A_j = layer breeder flock Age effect (three levels of age),

T_k = Incubation time (three or four levels of time),

$(S*A)_{ij}$ = Interaction between Strain and Age,

$(S*T)_{ik}$ = Interaction between Strain and Incubation time,

$(A*T)_{jk}$= Interaction between Age and Incubation time,

$(S*A*T)_{ijk}$ = Interaction between Strain, Age and Incubation time,

e_{ijkl} = Experimental error.

Duncan multiple range tests were used to detected differences among treatment means.

RESULTS AND DISCUSSION

Eggshell quality

Eggshell quality as affected by strain, breeder age, incubation time and their interactions are presented in Table (1). It could be noticed that egg weight was significantly affected by strain, breeder age, and incubation time; whereas the brown layer breeder hens produced significantly heavier egg weight compared to the white ones. In addition, present results showed egg weight increased as the breeders age progressed. On the contrary, the egg weight decreased after 10 or 18 days of incubation period. This is a logical result due to egg weight loss during incubation period. In addition, (Zita *et al.*, 2009) emphasized that egg weight increased with the layer's age. The egg weight of layer breeder hens were not significantly affected by interaction between age and strain. Egg weight was significantly affected by interaction between both strain x breeder age and breeder age x incubation time. Shell percentage can be used to estimate the eggshell quality (Mertens *et al.*, 2006). Shell percentage was significantly affected by strain, breeder age, and incubation time, where the brown eggs had significantly higher shell percentage when compared with white ones (Table 1). Scott & Silversides (2000), reported similar trend. Likewise, (Silversides *et al.*, 2001) noticed that shell, as a percentage of egg weight, decreased more in ISA-White eggs with increasing age than it did in ISA-Brown eggs. With respect to age and incubation time effects, we found that shell percentage decreased with advancing of hen's ages and incubation time. Shell thickness with membranes was significantly affected by strain, breeder age, and incubation time. The brown eggs recorded significantly higher shell thickness with membranes compared to the white ones. The differences of shell thickness between the two strains were 0.019mm. The shell thickness decreases with advancing hens' ages. Our results agreed with those reported by (Peebles & Brake, 1987), they found that young hens produce eggs with thicker shells and longer pores than older hens i.e. the eggshell generally gets thinner with age. With reference to incubation time effect, shell thickness significantly decreased as incubation time increased. Since early stage of incubation some researchers found that thickness of paired shell membranes are approximately 70μm. Previous studies indicated that the amount of shell membrane is related to shell strength and the age of the bird. The present results indicated that there was no significant difference between strains for shell membranes thickness. Inversely, shell membranes thickness significantly affected by breeder age, where shell membranes thickness significantly decreases as age increased.

Concerning eggshell breaking strength, data showed breaking strength of the brown shells was higher than those of white ones, however results

Table 1 – Eggshell quality as affected by strain, breeder age, incubation time and their interactions.

		Trait				
		Egg weight (g)	Shell percentage	Shell thickness with membranes (mm)	Shell membranes thickness (mm)	Eggshell breaking strength (Kg/cm2)
Strain (S)	Brown	53.46[a]	9.92[a]	0.338[a]	0.022	3.49
	W-36	50.67[b]	9.42[b]	0.319[b]	0.023	3.33
Breeder age (A)	25	47.58[b]	9.92[a]	0.336[a]	0.024[a]	3.78[a]
	47	54.20[a]	9.71[b]	0.325[b]	0.022[b]	3.30[b]
	61	54.42[a]	9.37[b]	0.317[c]	0.020[c]	3.16[b]
Incubation time (T)	0	56.77[a]	10.25[a]	0.348[a]	0.021	3.87[a]
	10	51.88[b]	10.08[ab]	0.338[b]	0.023	3.46[b]
	18	47.54[c]	9.90[b]	0.320[c]	0.022	2.91[c]
	A.H	------	8.50[c]	0.312[d]	0.022	------
SEM		0.49	0.07	0.002	0.0006	0.08
Probability						
Strain (S)		0.0001	0.0001	0.0001	NS	NS
Age (A)		0.0001	0.001	0.0001	0.05	0.003
Incubation time (T)		0.0001	0.0001	0.0001	NS	0.0001
S*A		0.05	NS	NS	NS	NS
S*T		NS	NS	NS	NS	NS
A*T		0.005	NS	NS	0.02	NS
S*A*T		NS	NS	NS	NS	NS

[a, b, C and d] Means within each affecting factor with different letters are significantly differed.

NS= Non-significant, A.H = after hatching.

confirmed that no significant difference between strains for eggshell breaking strength. On the contrary, eggshell breaking strength significantly affected by breeder age, where the eggshell breaking strength decreases with advancing of hen's ages. Several reasons have been advanced to explain this change in shell strength with age. It has been proposed that the amount of Ca hen absorbs in the body and retains and the skeletal Ca available for shell calcification decrease with age. Results of the present study aimed to confirm this theory. Likewise, (Ousterhout, 1981) observed that egg weight increased at a faster rate than shell weight resulting in a decrease in the amount of shell to cover the egg. In addition, when stressed with inadequate Ca, old hens were able to maintain shell strength as well as young hens, the increasing egg size with progressing age without a concomitant increase in shell weight as an important contributing factor to decrease shell strength with age. From results of the present study, we can agree with them in this opinion. With reference to incubation time effect, we noticed that eggshell strength was significantly affected by incubation time, where eggshell breaking strength decreased as incubation time increased. Such results can referred to the increasing rate of calcium absorption and utilization by the embryos during the embryonic stage. Therefore, reflecting on the decreasing the eggshell breaking strength. The differences for the eggshell breaking strength were 0.41 and 0.55 kg/cm^2, the differences due to the incubation time may be due to a higher demand for calcium utilization in the late incubation period (18 days).

Chemical analysis of shell

Percentages of calcium (Ca) and phosphorus (P) in eggshell as affected by strain, breeder age, incubation time and their interactions are summarized in Table (2). Eggshell contains inorganic (almost 95 %calcium carbonate); it weighs about 5 g and contains 2.2 g calcium, which represents about 38 % of its weight (Nys *et al.*, 1999). If the calcium from the shell was removed, the organic matrix material would be left behind. Data showed that brown eggs recorded significantly higher calcium percentage in eggshell in comparison with the white eggs. The data revealed that there was no significant difference to breeder strains due to the percentage of phosphorus in eggshell, however, a noticeable variation for the percentage of phosphorus. The brown eggs were higher than the white eggs. Regarding breeder age effect, calcium and phosphorus percentages significantly decreases as age increased. This has been explained in earlier studies that state that as the hen aged, the increase in egg weight with no proportionate increase in shell deposition was the major factor that explained the age-related decline in shell quality of commercial egg-type laying hens.

Table 2 – Percentages of calcium (Ca), phosphorus (P) in eggshell and total pores per egg as affected by strain, breeder age, incubation time and their interactions.

		Trait		
		Calcium (%)	Phosphorus (%)	Total pores per egg
Strain (S)	Brown	23.28^a	0.36	6449.91^a
	W-36	20.36^b	0.33	6303.57^b
Breeder age (A)	25	23.57^a	0.40^a	5958.23^c
	47	21.93^b	0.34^b	6535.96^b
	61	19.97^b	0.31^b	6667.21^a
Incubation time (T)	0	30.88^a	0.43^a	6657.85^a
	10	25.83^b	0.39^a	6335.48^b
	18	15.51^c	0.29^b	6004.63^c
	A.H	15.05^c	0.28^b	-------
SEM		0.56	0.01	20.44
Probability				
Strain (S)		0.0001	NS	0.0001
Age (A)		0.0001	0.0003	0.0001
Incubation time (T)		0.0001	0.0001	0.0001
S*A		NS	NS	NS
S*T		NS	NS	NS
A*T		NS	NS	0.03
S*A*T		NS	NS	NS

[a, b and c] Means within each affecting factor with different letters are significantly differed.

NS= Non-significant, A.H = after hatching.

With reference to incubation time effect, we noticed that calcium and phosphorus percentages significantly declined with advancing of incubation time. Such results confirm our previous speculation that the embryo efficiently utilized the calcium and phosphorus content of eggshell at the later stage of incubation (10-18) days rather than in the earlier stage of incubation period (0-10) days. In addition, the continuous utilization of the calcium carbonate in the eggshell as the development of embryos through the incubation period. About 80% of calcium in chick's body is obtained from the shell (Ono & Wakasugi, 1984) at a late stage of development.

Total pores per egg

Thousands of microscopic pores in the chorionallantoic membrane are responsible for gas exchange between the embryo and the external environment in the avian egg by diffusion (Wagner-Amos & Seymour, 2002; 2003; Nys *et al.*, 2004). Data in Table (2) showed that the total pores per egg was significantly affected by strain, whereas the brown breeder hens recorded significantly higher total pores per egg in comparison with the white ones. Regarding age effect, total pores per egg significantly increases with advancing of breeder ages. The increasing in total number of pores associated with advancing of breeder ages could be attributed to weight and volume of eggwhich dramatically increased with age. The percentage increments of the total pores per egg due to advancing breeder age for brown eggs were 10.9 and 10.7 % for the mid and late breeder age relatively to earlier age, while the white eggs-were 9.24 and 11.63 % for the two ages, respectively. With respect to incubation time effect, total pores per egg significantly decreased as incubation time increased. Concerning interaction effect, the present results revealed that total pores per egg was significantly affected by the interaction between breeder age and incubation time only.

Scanning electron microscopy (SEM) technique

Ultrastructural studies have demonstrated that the eggshell is comprised of three morphologically distinct calcified layers. Data presented in Table (3) showed that absolute and relative thickness of individual egg layer is affected by strain, breeder age, incubation time and their interactions. Results showed that total thickness of brown eggshell was significantly higher than those of white eggshell by about 3.7%. Similar trend wasn't observed for absolute palisade thickness, whereas there was no significant difference between strains. However, the relative palisade thickness of white eggshell was significantly higher than that of brown eggshell. Therefore, it is likely that alterations in the thickness of the palisade layer, independent of structural reorganization of the palisade columns, could affect shell strength. In contrast, the brown eggshell was significantly thicker in both absolute and relative cap thickness compared to white eggshell. With respect to age effect, we observed that all absolute parameters (total, palisade and cap thickness)

Table 3 – Absolute and relative thickness of individual egg layer as affected by strain, breeder age, incubation time and their interactions.

		Trait				
		Total thickness, µm	Palisade thickness, µm	Cap thickness, µm	Palisade, %	Cap, %
Strain (S)	Brown	257.53[a]	212.21	45.32[a]	82.40[b]	17.60[a]
	W-36	247.94[b]	211.96	35.98[b]	85.49[a]	14.51[b]
Breeder age (A)	25	258.09[a]	214.70[a]	43.48[a]	83.39	16.68
	61	247.29[b]	209.47[b]	37.83[b]	84.99	15.01
Incubation time (T)	0	271.79[a]	219.59[a]	52.20[a]	80.88[c]	19.14[a]
	18	256.40[b]	212.78[a]	43.63[b]	83.01[b]	17.00[b]
	A.H	230.02[c]	203.99[b]	26.13[c]	88.71[a]	11.30[c]
SEM		4.76	4.08	2.70	0.79	0.79
Probability						
Strain (S)		0.001	NS	0.001	0.001	0.001
Age (A)		0.001	0.02	0.001	NS	NS
Incubation time (T)		0.0001	0.01	0.0001	0.0001	0.0001
S*A		NS	0.02	0.01	0.05	0.04
S*T		NS	0.02	0.01	0.05	NS
A*T		0.05	0.01	0.01	0.03	0.01
S*A*T		NS	0.05	NS	NS	0.02

[a, b and c] Means within each affecting factor with different letters are significantly differed.

significantly reduced with advanced age. Increased erosion of the calcium reserve assembly region of the mammillary layer as the breeder hen ages. Concerning incubation time, the results indicated the absolute total, palisade and cap thickness were significantly reduced with advanced hatching time. Similar trend noticed for relative cap thickness (Table 3). Photo (1) presented eroded mammillary caps of eggshell in Hy-Line brown strain after 18 days of incubation. The erosion of cap thickness was due to that this layer gives rise to the calcium reserve assembly, which provides 80% of the total calcium requirement of the developing embryo (Deickert *et al.*, 1989), a reduction in thickness of the mammillary layer may be detrimental to embryonic development. Relative palisade thickness significantly increased with advanced incubation time. With reference to interaction effect, we observed that all parameters, except of total thickness, significantly affected by interaction between strain and breeder age. Also all parameters, except of total thickness and relative cap thickness, were significantly affected by interaction between strain and incubation time. While all parameters, were significantly affected by interaction between breeder age and incubation time. Results showed no significant difference between strains, breeder age and incubation time for all parameters, except that of palisade thickness and relative cap thickness. Bain (2005), described twelve structural variations in the mammillary layer of weak and poor quality eggshells. In the current study the depression, cubics, aragonite, caps and changed membrane did not significantly affected by strain, age of breeder and their interactions. Both white and brown eggs had shells with similar values of confluence. Photo (2) showed the confluent area in eggshell of Hy-Line brown strain at early stage. Solomon (1999), found good shell ultrastructural beneficial high confluence reflects good attachment with membranes and caps. Data presented in Photo (3) indicated the late fusion in eggshell of Hy-Line W-36 strain at early production cycle. The eggs produced from older breeders have owned fusion value higher than that produced from younger breeders. Increased late fusion of mammillary columns declined fracture toughness of eggshell (Bain, 1990). With respect to erosion trait, data presented in Photos (4, 5, 6, 7 and 8) indicated the different erosion trait according to strain and incubation time, whereas the present result indicated that the little erosion in mammillary caps was observed in Hy-Line W-36 eggshell (at early embryonic mortality) (Figure 4). In addition, the erosion degree increased with advancing the incubation time.

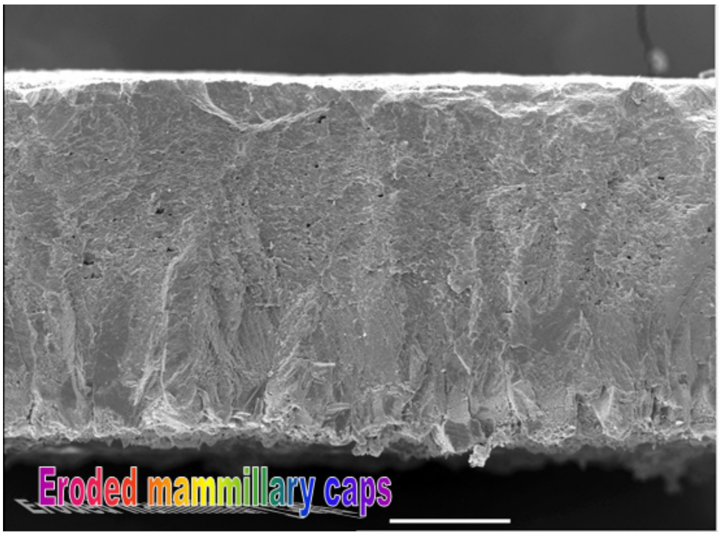

Photo 1 – Eroded mammillary caps of eggshell in Hy-Line Brown strain after 18 days of incubation.

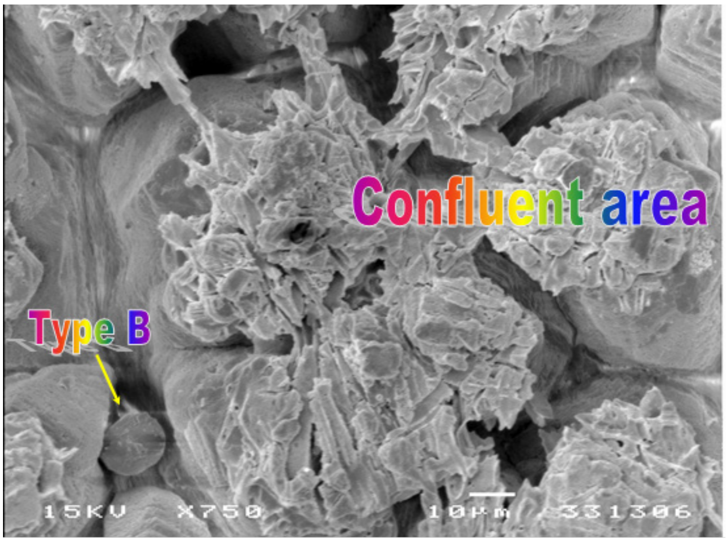

Photo 2 – Confluent area in eggshell of Hy-Line Brown strain (early).

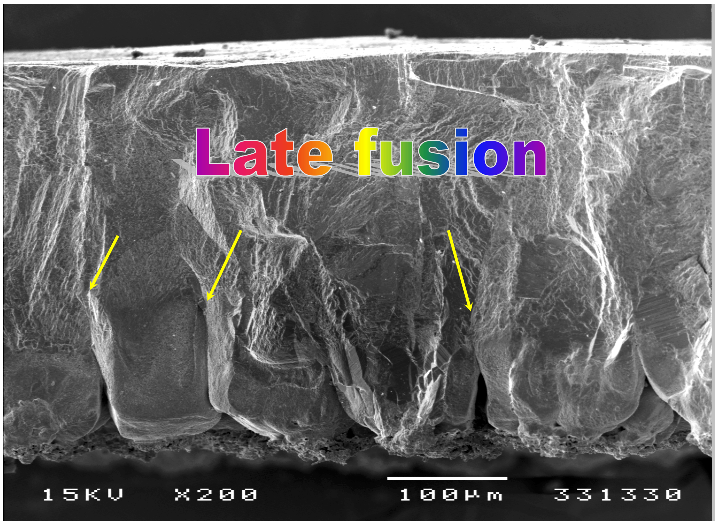

Photo 3 – Late fusion in eggshell of Hy-Line W-36 strain at early production cycle.

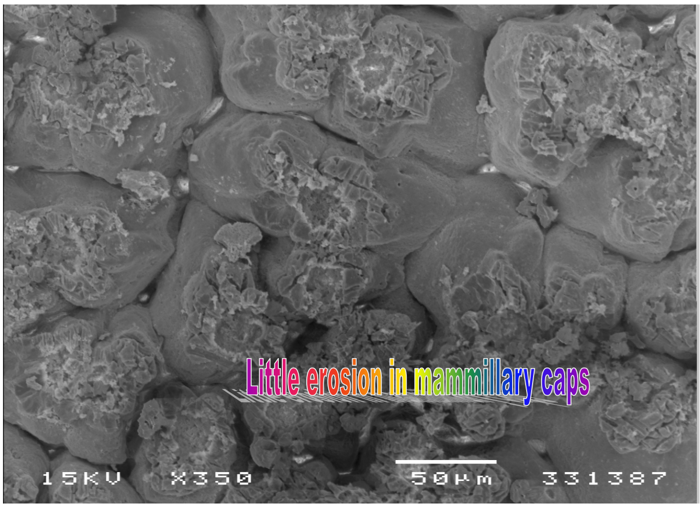

Photo 4 – Little erosion in mammillary caps of Hy-Line W-36 eggshell (early embryonic mortality).

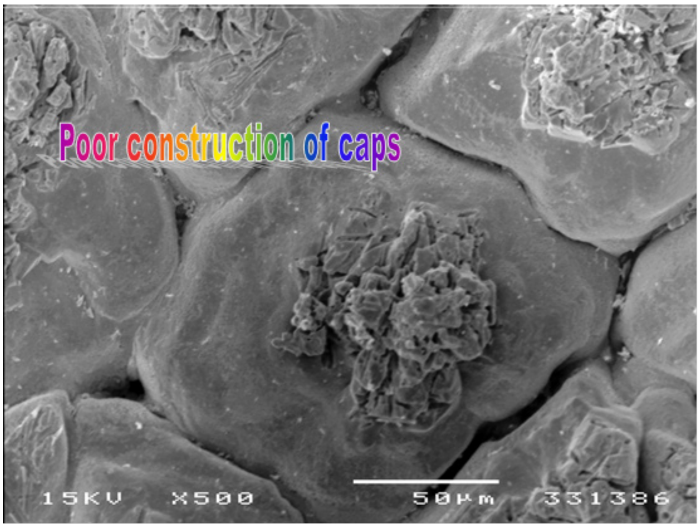

Photo 5 – Poor construction of mammillary caps in early embryonic mortality of Hy-Line W-36 strain.

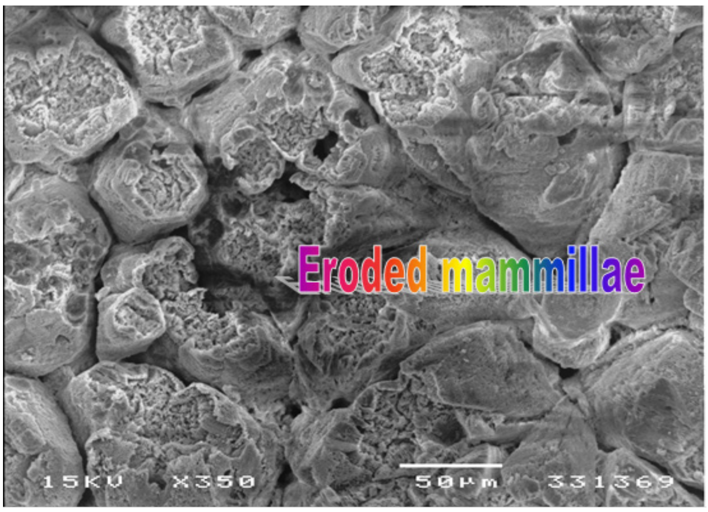

Photo 6 – Moderate eroded mammillary caps after 18 days of incubation in eggshell of Hy-Line Brown strain (late).

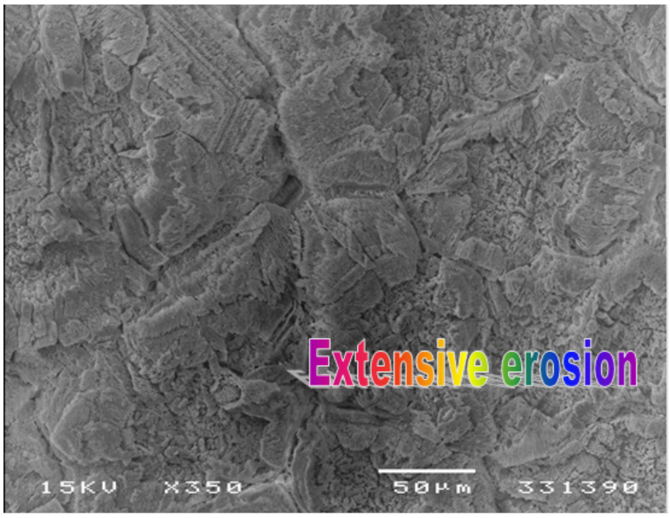

Photo 7 – Extensive erosion resulting from calcium withdrawn via embryo after 18 days of incubation in eggshell of Hy-Line W-36 strain (late embryonic mortality).

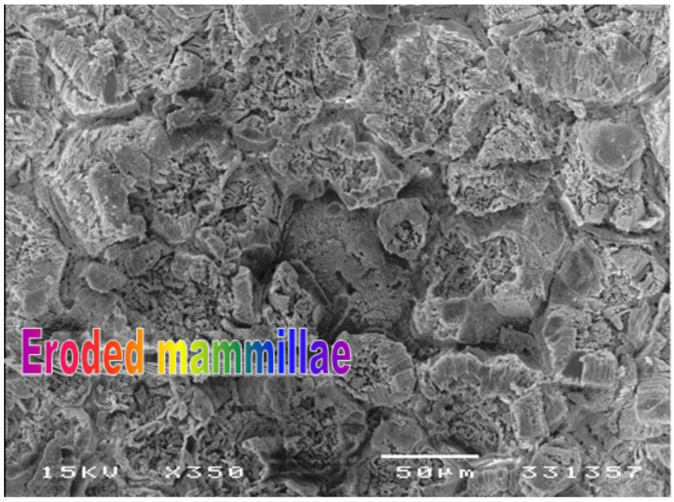

Photo 8 – Eroded mammillary caps in eggshell of Hy-Line Brown strain (late embryonic period).

CONCLUSION

Finally, our results show that some traits including egg weight, shell percentage, shell thickness, calcium percentages in eggshell, and total pores per egg were significantly affected by strain, breeder age, and incubation time. Eggshell ultrastructure of brown egg was better compared to white eggs. Palisade thickness and relative cap thickness were significantly affected by interaction between strains, breeder age and incubation time.

ACKNOWLEDGMENTS

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. GRANT180]'.

CONFLICTS OF INTEREST

All the authors declare that they have no conflict of interest.

REFERENCES

Alfonso-Carrillo C, Benavides-Reyes C, Los Mozos J de, Dominguez-Gasca N, Sanchez-Rodríguez E, *et al.* Relationship between bone quality, egg production and eggshell quality in laying hens at the end of an extended production cycle (105 weeks). Animal 2021;11:623.

Attar M, Fathi M. Non-destructive acoustic resonance method for evaluating eggshell strength and thickness. International Journal Biophysics 2014;4:9-15.

Bain M. Eggshell strength: a mechanical/ultrastructural evaluation [thesis]. Glasgow (UK): University of Glasgow; 1990.

Bain M. Eggshell strength: a relationship between the mechanism of failure and the ultrastructural organisation of the mammillary layer. British Poultry Science 1992;33:303-19.

Bain M. Recent advances in the assessment of eggshell quality and their future application. World Poultry Science Journal 2005;61:268-77.

Bain M, MacLeod N, Thomson R, Hancock, JW. Microcracks in eggs. Poultry Science 2006;85:2001-8.

Deickert J, Dieckert M, Creger C. Calcium reserve assembly: a basic structural unit of the calcium reserve system of the hen egg shell. Poultry Science 1989;68:1569-84.

Edmond A, King L, Solomon S, Bain M. Effect of environmental enrichment during the rearing phase on subsequent eggshell quality in broiler breeders. British Poultry Science 2005;46:182-9.

Fathi M. Ultrastructural evaluation of eggshell quality produced from two different genetic groups of chicken. Egyptian Poultry Science 2001;21:263-76.

Fathi M, Galal A, Ali U, Abou-Emera O. Physical and mechanical properties of eggshell as affected by chicken breed and flock age. British Poultry Science 2019;60:506-12.

Fathi M, El-Dlebshany A, El-Deen MB, Radwan L, Rayan G. Effect of long-term selection for egg production on eggshell quality of Japanese quail (Coturnix japonica). Poultry Science 2016;95:2570-5.

Karlsson O, Lilja C. Eggshell structure, mode of development and growth rate in birds. Zoology 2008;111:494-502.

Lunam C, Ruiz J. Ultrastructural analysis of the eggshell: contribution of the individual calcified layers and the cuticle to hatchability and egg viability in broiler breeders. British Poultry Science 2000;41:584-92.

Massaro M, Davis LS. Differences in egg size, shell thickness, pore density, pore diameter and water vapour conductance between first and second eggs of Snares Penguins Eudyptes robustus and their influence on hatching asynchrony. Ibis 2005;147:251-8.

Mertens K, Bamelis F, Kemps B, Kamers B, Verhoelst E, De Ketelaere B, *et al.* Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. Poultry Science 2006;85:1670-7.

Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol 2004;3:549-62.

Nys Y, Hincke M, Arias J, Garcia-Ruiz J, Solomon S. Avian eggshell mineralization. Poultry and Avian Biology Reviews 1999;10:143-66.

Ono T, Wakasugi N. Mineral content of quail embryos cultured in mineral-rich and mineral-free conditions. Poultry Science 1984;63:159-66.

Ousterhout L. The effects of phased feeding protein and calcium on egg weight and shell quality with four strains of White Leghorn hens. Poultry Science 1981;60:1036-42.

Peebles E, Brake J. Eggshell quality and hatchability in broiler breeder eggs. Poultry Science 1987;66:596-604.

Preisinger R. Innovative layer genetics to handle global challenges in egg production. British Poultry Science 2018;59:1-6.

Rahn H, Paganelli CV. Gas fluxes in avian eggs: driving forces and the pathway for exchange. Comparative Biochemistry and Physiology 1990;95:1-15.

Ren Z, Piepenburg A, Bütz D, Claus J, Cook M. Vaccine to fibroblast growth factor 23 peptides increases eggshell strength. Poultry Science 2018;97:882-9.

Roberts JR. Factors affecting egg shell and internal egg quality. Journal Poultry Science 2010;3:161-77.

SAS. SAS/STAT user guide. Version 9.1. Cary: SAS Institute; 2004.

Scott T, Silversides FG. The effect of storage and strain of hen on egg quality. Poultry Science 2000;79:1725-9.

Silversides F, Scott, TA. Effect of storage and layer age on quality of eggs from two lines of hens. Poult. Sci. 2001;80:1240-5.

Solomon S. An egg ist ein ei, es un huevo, est un oeuf. British Poultry Science 1999;40:5-11.

Wagner-Amos K, Seymour RS. Effect of regional changes to shell conductance on oxygen consumption and growth of chicken embryos. Respiration Physiology 2002;129:385-95.

Wagner-Amos K, Seymour RS. Effect of local shell conductance on the vascularisation of the chicken chorioallantoic membrane. Respiratory, Physiology & Neurobiology 2003;134:155-67.

Zita L, Tůmová E, Štolc L. Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. Acta Veterinaria Brno 2009;78:85-91.