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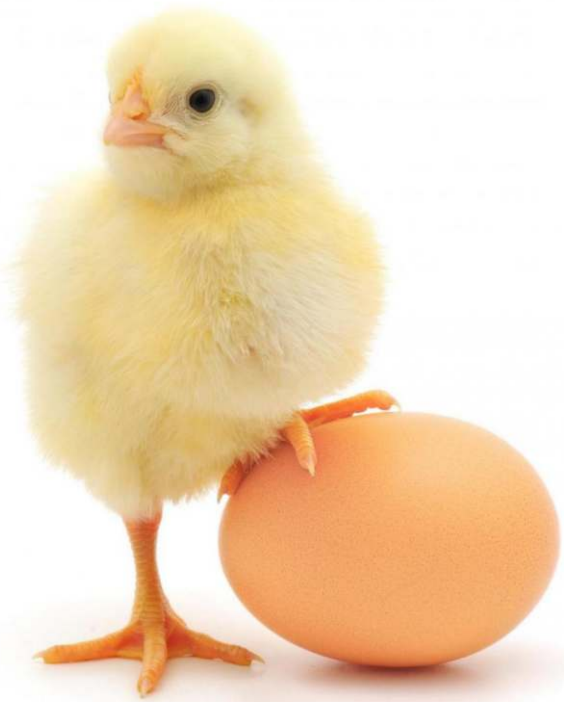

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Impact of Layer Breeder Flock Age and Strain on Mechanical and Ultrastructural Properties of Eggshell in Chicken

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Abstract: This investigation was carried out to study mechanical eggshell traits (weight, specific gravity, eggshell thickness and eggshell breaking strength) and ultrastructural properties from layer breeder hens at different ages (25, 47 and 61 weeks). The present results showed that the brown eggshell had significantly higher specific gravity, shell thickness and breaking strength compared to white eggshell. Concerning breeder flock age, it could be noticed that the age of hen significantly affected mechanical eggshell traits, where specific gravity, shell thickness and breaking strength decreased significantly as the breeder age was advance. With respect to eggshell ultrastructural properties, the brown eggshell had significantly higher effective thickness (palisade %) compared to white ones. Opposite trend was observed for relative cap thickness. With regard to layer breeder flock age effect, the results indicated that the effective thickness (palisade %) was significantly decreased as the layer breeder flock age increased. Conversely, the relative cap thickness was increased with age increased. With respect to confluence trait, the present results observed that both brown and white eggs owned shells with similar values of confluence. Significant difference observed between strains regarding fusion (early or late) trait. Type B's, type A's and alignment traits in eggshell produced from older breeders was significantly higher that of younger breeders.

Key words: Eggshell traits, breeder hen age, older hens

INTRODUCTION

The breeding programme for laying traits paid a considerable interest to the shell quality. Economic losses associated with the incidence of eggshell defects are important when evaluating the profitability of a layer operation (Bell, 1998; Flock, 1995; Seeland *et al.*, 1995). Low egg quality almost leads to a 5-8% loss in production (Krshavarz, 1994). The frequency of defective eggs may increase from 7-11% during the laying, collecting and packing stages of egg production. Thus shell quality is one of the major problems of egg production. Maintaining quality eggshells throughout the production cycle is desirable. Avian eggshell contains organic (3.5%) and inorganic (95%, almost calcium carbonate) fractions; it weighs about 5 g and contains 2.2 g calcium, which represents about 38% of its weight (Nys *et al.*, 1999). Many factors have been found to affect eggshell quality, such as disease, nutritional status of the flock, heat stress and age (Roberts, 2004). A decrease in eggshell quality of older hens has been reported by Elaroussi *et al.* (1994). Egg production rate decreases and egg weight increases as age advances, also egg composition change and shell thickness decrease with production level and age of layer (Summers and Leeson, 1983; Seeland *et al.*, 1995; Machal and Simeonovova, 2002). The decline in eggshell quality as the hens' age may in part be attributed to reduced intestinal calcium uptake as well as to increased egg size (Al-Batshan *et al.*, 1994). The increase in shell weight as hens' age was not sufficient to compensate for the increment of egg weight, so that the ratio of shell weight to egg weight decreased (Roberts and Blaney, 2000).

Ultrastructural studies have demonstrated that the eggshell is comprised of there morphologically distinct calcified layers (Solomon, 1991; Dennis *et al.*, 1996; Fraser *et al.*, 1999). Bain (1991) suggested that the organization of the palisade columns is a major determinant of shell stiffness and therefore of shell strength. Shell strength is directly related to shell thickness (Khatkar *et al.*, 1997) and the palisade layer comprises approximately two-thirds of the eggshell (Parsons, 1982). 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. Recent Transmission Electron Microscopy (TEM) studies (Dennis *et al.*, 1996; Fraser *et al.*, 1999) have revealed columnar calcite crystals in a vertically aligned matrix associated with the vertical crystal layer. Therefore, the objectives of this research were to investigate the effects of breeder hen age and strain on mechanical and ultrastructural of eggshell in laying hen breeder hens under prevailing Egyptian conditions.

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MATERIALS AND METHODS

Three hundred eggs (150 Brown and 150 White) were obtained from three layer breeder flocks differed in stage of laying season: early stage (25 wk), mid stage (47 wk) and late stage (61 wk). Each stage containing fifty eggs from each of the three laying stages either for Brown and W-36 layer breeder flocks were randomly taken to evaluate the effect of strain and breeder flock ages on mechanical and ultrastructural properties of eggshell.

Eggshell mechanical traits: Eggs were individually weighed to the nearest 0.01 g using an electronic digital balance. Eggshell quality measurements were conducted using 60 eggs (10 eggs from each laying stage within strains). Length and width of egg were individually recorded by using a digital caliper. Shape index, then, was calculated by (width/length) x 100 according to (Carter, 1968). Specific gravity was determined using the saline flotation method of Hempe *et al.* (1988). Briefly, Eggs are immersed sequentially into a series of saline solution of ascending SG. Salt solutions were made in incremental concentration of 0.005 in the range from 1.065-1.100. The SG of any egg is equal to SG of the SG of the solution in which it first floats. Egg volume was estimated by measuring the quantity of water dislodged in cm^3 after immersing the egg into known water volume. The egg surface in cm^2 was calculated by dividing shell weight on weight of 1 cm^2 area according to (Hamilton, 1978). The strength of eggshell was determined according to Fathi and El-Sahar (1996) using eggshell strength apparatus. The shell percentage was calculated according to following equation:

$$\text{Shell percentage (\%)} = \frac{\text{Wet shell weight}}{\text{Egg weight}} \times 100$$

Shell thickness (mm) was measured at three different points in the middle part of the egg (the equator) using a dial gauge micrometer. Shell index (g/100 cm^2) was calculated according to following equation:

$$\text{Shell weight (g)/Shell surface (cm}^2\text{)} \times 100$$

(Sauveur, 1988).

Scanning Electron Microscopy (SEM) technique: 48 samples of eggshell were randomly taken from Hy-Line Brown and W-36 layer breeder flocks at early and late laying stage to investigate ultrastructural variations. The specimens were prepared by cutting a piece (1 cm^2) of shell from equatorial region of each egg. The shell membranes were carefully removed by first soaking in water. The loosely adhering membranes were then gently peeled from the edge of the sample inwards. To remove the remaining tightly bound membrane fibers, each sample was then immersed overnight in 6% sodium hypochlorite, 4.12% sodium chloride and 0.15% sodium hydroxide. Thereafter the specimen was rinsed with water and left to dry at room temperature. Following these preparative treatments, two samples from each egg were mounted in inner side uppermost and in vertically manner on aluminum stubs, coated with gold for 3 min in an Emscope Sputter Coater. These samples were examined using JEOL JSM-T330A scanning electron microscopy at 15 Kv. The incidence of ultra structural variants at the level of the mammillary layer was assessed according to the methodology and terminology developed by the Poultry Research Unit, University of Glasgow (Bain, 1990,1992; Solomon, 1991). The cross-sectional lengths of palisade and mammillary layers were directly measured in µm using scaling software provided with the SEM at a magnification of x 200. The total thickness of each specimen was 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 was 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; Solomon, 1991). Triplicate measures were performed in each case and the mean values were used for the statistical analysis.

Statistical analysis: Data were subjected to a two-way analysis of variance with strain and breeder flock age using the General Linear Model (GLM) procedure of SAS User's Guide (2000) according to the following model:

$$Y_{ij} = \mu + S_i + A_j + (S*A)_{ij} + e_{ijk}$$

Where:

μ = Overall means
S_i = Strain effect (i = 1, 2)
A_j = Breeder flock age effect (j = 1, 2, 3)
$(S*A)_{ij}$ = Interaction between strain and breeder flock age
e_{ijk} = Experimental error

When significant differences among breeder flock age were found, means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Egg weight and dimension: Data summarized in Table 1 showed that the effect of strain, breeder flock age and their interaction on egg weight and dimension. The brown breeder hens produced significantly heavier egg weight compared to the white ones. Similar results were obtained by Potts and Washburn (1983); Curtis *et al.* (1985); Washburn (1990) and Badawe (2006). They reported that the brown egg strains laid heavier eggs

Table 1: Egg weight and egg dimension traits as affected by strain, breeder flock age and their interaction (Means±SE)

Trait	Strain	Breeder flock age (wk) 25	47	61	Overall	Prob. S	A	S*A
Egg weight (g)	Brown	51.34±0.48	58.86±0.58	60.19±0.73	56.80[a]			
	W-36	50.31±0.44	56.59±0.82	58.88±0.47	55.26[b]			
	Overall	50.83[c]	57.73[b]	59.54[a]		0.002	0.0001	NS
Egg shape index (%)	Brown	77.13±1.24	78.38±0.85	76.29±0.54	77.27			
	W-36	76.74±0.46	77.33±0.89	74.59±0.72	76.22			
	Overall	76.94[ab]	77.86[a]	75.44[b]		NS	0.01	NS
Egg volume (cm^3)	Brown	49.20±1.21	55.70±0.63	57.30±1.15	54.07			
	W-36	46.00±0.79	58.10±1.03	53.70±1.33	52.60			
	Overall	47.60[b]	56.90[a]	55.50[a]		NS	0.001	0.01
Eggshell area (cm^2)	Brown	68.76 ±2.75	79.98 ±2.40	86.09±1.69	78.27			
	W-36	71.06 ±1.31	86.35 ±1.23	76.66±2.63	78.02			
	Overall	69.91[b]	83.16[a]	81.37[a]		NS	0.001	0.001
Eggshell index (g/100 cm^2)	Brown	8.15±0.23	8.25 ±0.28	7.10±0.18	7.83[a]			
	W-36	7.35±0.17	6.70 ±0.29	7.05±0.27	7.03[b]			
	Overall	7.75[a]	7.48[ab]	7.08[b]		0.002	0.02	0.01

than white egg ones. With respect to breeder flock age effect, the present results showed that the egg weight increased as layer's age was advanced. Baumgartner *et al.* (2007) pointed out a significant effect of age on egg weight in the Leghorn type hens. Also, Lukáš Zita *et al.* (2008) found that egg weight increased with the layer's age. Concerning eggshell dimension traits, our results showed that the brown layer breeder hens produced eggs with higher shape index compared to the white ones. Brand *et al.* (2004) found that the egg shape index for brown Isa Waren Layers was 75.60% and this value was higher than that of white eggs produced from White Rock layers (74.10%). Monira *et al.* (2003) demonstrated to a significant decrease in egg shape index with advanced of breeder flock age. Gunlu *et al.* (2003) and Brand *et al.* (2004) reported that shape index of the eggs decreased with age because shape index is directly proportional to egg width and it is inversely related to egg length, which implies that with increasing age, the rate at which eggs becomes longer is faster than rate of being wider.

The brown eggshell had a higher volume compared to white ones. With respect to age effect, the present results showed that the egg volume significantly increased as breeder flock age increased. This result could be attributed to the egg weight dramatically increased with age. The eggshell surface plays an important role during the incubation period especially for the breeds which had comparatively thin and high porosity shells. The surface area of egg affected the porosity of shell and hence the degree of evaporation from the shell. No significant difference between strains for eggshell area was detected. Inversely, the eggshell area was significantly increases with advancing ages. The last results could be attributed to the increase egg size as the layer's age was advancing. The true eggshell area was significantly affected by interaction between strain and breeder flock age. The effect of age on true eggshell area was more pronounced in brown eggs rather than white eggs, whereas the true eggshell area of brown eggs was increases with advancing age. Similar direction did not observed in white eggs.

Results of applying eggshell index showed presence highly significant difference between strains for eggshell index trait. The brown eggs recorded significantly higher eggshell index compared to the white eggs. Concerning breeder flock age effect, it could be noticed that eggshell index was significantly affected by breeder flock age, whereas eggshell index significantly decreases as age increased. Eggshell index was highly significantly affected by interaction between strain and breeder flock age. The pervious result could be attributed to the eggshell index increased with advancing age in brown strain. Opposite trend was noticed in white strain.

Mechanical eggshell traits: Eggshell quality traits as affected by strain, layer breeder flock age and their interaction are presented in Table 2. Specific gravity has been recommended as an accurate indicator of shell strength (Well's, 1968). Despite being an indirect measure, it correlates well with direct methods but is non-destructive and has the practical advantage of speed and simplicity. Specific gravity considered as an accurate predictor of shell thickness, much more reliable for this purpose than percentage shell (Tyler and Geake, 1961). Our results showed that brown strain produced eggs associated with higher specific gravity compared to white ones. That is meant that brown eggs had a higher eggshell quality traits compared to white eggs. Concerning breeder flock age effect, it could be observed that the specific gravity decreases with advancing breeder flock ages. Similar trend was noticed by Izat *et al.* (1985) and Poggenpoel (1986). They demonstrated that hen age significantly affected specific gravity values of eggs, where specific gravity decreased significantly with increasing in hen age. Also, Butcher *et al.* (1991) reported that the egg specific gravity decrease as breeder age was advance. Such phenomena basically related to the increament on egg size were

Table 2: Eggshell quality traits as affected by strain, breeder flock age and their interaction (Means±SE)

		Breeder flock age (wk)				Prob.		
Trait	Strain	25	47	61	Overall	S	A	S*A
Specific gravity	Brown	1.070±0.002	1.070±0.001	1.070±0.001	1.070[a]			
	W-36	1.070±0.001	1.070±0.002	1.065±0.001	1.060[b]			
	Overall	1.070[a]	1.070[a]	1.067[b]		0.001	0.003	NS
Eggshell (g)	Brown	5.03±0.13	5.91±0.13	5.30±0.14	5.41[a]			
	W-36	4.73±0.09	5.23±0.21	4.87±0.14	4.94[b]			
	Overall	4.88[b]	5.57[a]	5.09[b]		0.001	0.001	NS
Eggshell (%)	Brown	10.69±0.27	10.64±0.21	9.97±0.26	10.43			
	W-36	10.60±0.26	9.93±0.28	9.65±0.45	10.06			
	Overall	10.65[a]	10.29[ab]	9.81[b]		0.001	0.001	NS
Shell thickness (mm)	Brown	0.35±0.01	0.34±0.01	0.32±0.01	0.337[a]			
	W-36	0.33±0.01	0.32±0.01	0.31±0.01	0.320[b]			
	Overall	0.340[a]	0.330[ab]	0.315[b]		0.001	0.001	NS
Eggshell breaking strength	Brown	4.79±0.60	3.65±0.26	3.44±0.22	3.96			
Kg/cm^2	W-36	4.35±0.20	3.58±0.32	3.37±0.34	3.78			
	Overall	4.57[a]	3.62[b]	3.41[b]		NS	0.003	NS

more quickly than the shell weight and also the increase in egg size relatively higher than calcium deposition in the eggshell (Curtis *et al.*, 1985). Peebles and Brake (1987) and Bennett (1992) conformed that the quality of eggshells as measured by specific gravity tends to decline with age.

In view of the fact that brown eggs had significantly heavier shell weight compared to the white eggs. With regard to breeder age, we can observed that the shell weight increased with advancing age considerably. Shell percentage can be use to estimate the eggshell quality (Mertens *et al.*, 2006). The present result showed that the shell percentage was significantly affected by breeder strain, whereas the brown eggs were significantly higher shell percentage compared to the white ones. According to Curtis *et al.* (1986) and Scott and Silversides (2000) the brown-egg strains produce eggs with greater percentage of eggshell compare to white-egg strain. Likewise, Silversides and Scott (2001) noticed that the shell, as a percentage of egg weight, decreased more for ISA-White eggs with increasing age of the hen than it did for ISA-Brown eggs. With respect to breeder age effect, the shell percentage decreases with advancing of hen ages. The brown eggs recorded significantly higher shell thickness compared to the white ones. Also, shell thickness was significantly affected by breeder flock age, whereas the shell thickness decreases with advancing of hen's ages. Such effect was more pronounced for the brown compared to white breeder strain. Roland (1976), Peebles and Brake (1987) demonstrated that young hens produce eggs with thicker shells and longer pores than older hens i.e. the eggshell generally thins with age. Also, Roland (1979) reported that the shell quality at the end of the lay season is directly related to shell quality at the beginning of lay, the reduction in eggshell thickness as the hen ages been associated with an increase in egg size without a concurrent increase in calcium carbonate deposition.

Eggshell strength one of the most important egg quality traits to be considered in a poultry breeding program is shell strength. Eggshell breaking strength of the brown eggs was higher than those of white ones, but the difference did not statistically significant. On the other hand, eggshell breaking strength was highly significantly affected by breeder flock age, whereas the eggshell breaking strength decreases with advancing of hen's ages. Several reasons have been presented to explain this change in shell strength with age. It has been proposed that the amount of calcium absorb, retains and the skeletal Ca available for shell calcification decrease with age. Roland *et al.* (1978) and 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 calcium, 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 the decreasing shell strength with age.

Eggshell ultrastructure properties: Ultrastructural studies have demonstrated that the eggshell is comprised of three morphologically distinct calcified layers. The mammillary and palisade layers comprise at least 85% of the total cross-sectional length of the eggshell (Ruiz *et al.*, 1998). Data presented in Table 3 showed that the effect of strain, breeder flock age and their interaction on total, palisade and cap thickness of eggshell. The present results indicated that the total thickness of brown eggshell was significantly higher than those of white eggshell. Similar trend was observed for palisade thickness (absolute and %), whereas the brown eggshell was significantly higher absolute and relative palisade thickness compared to white eggshell. Bain (1991) suggested that the

Table 3: Absolute and relative thickness of individual egg layer as affected by strain, breeder flock age and their interactions (Means±SE)

Trait	Strain	Breeder flock age (wk)			Prob.		
		25	61	Overall	S	A	S*A
Total thickness (μm)	Brown	286.33±9.82	272.8.21±8.21	279.44[a]			
	W-36	270.92±8.14	257.33±6.14	264.13[b]			
	Overall	278.63[a]	264.94[b]		0.001	0.001	NS
Palisade thickness (μm)	Brown	232.73±4.22	213.04±5.12	222.89			
	W-36	212.59±4.58	200.36±6.11	206.48			
	Overall	222.66	206.70		0.01	0.01	0.02
Cap thickness (μm)	Brown	53.60±1.20	59.50±2.15	56.55			
	W-36	58.33±2.12	56.97±2.10	57.65			
	Overall	55.97[b]	58.23[a]		0.001	0.001	0.01
Palisade (%)	Brown	81.28±0.74	78.17±0.66	79.73			
	W-36	78.47±0.85	77.86±0.54	78.17			
	Overall	79.88	78.02		0.001	NS	0.05
Cap (%)	Brown	18.72±0.75	21.83±0.81	20.28			
	W-36	21.53±0.77	22.14±0.54	21.84			
	Overall	20.13	21.99		0.001	0.01	0.04

organization of the palisade columns is a major determinant of shell stiffness and therefore of shell strength. Shell strength is directly related to shell thickness (Bain, 1991; Khatkar *et al*., 1997) and the palisade layer comprises approximately two-thirds of the eggshell (Parsons, 1982). 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 thinner absolute and relative cap thickness compared to white eggshell. Therefore, a reduction in the cross-sectional length of these layers may increase eggshell porosity resulting in excess water loss during incubation. With respect to breeder age effect, it could be noticed that all eggshell layer were significantly reduced as the age of breeder was advanced. Increased erosion of the calcium reserve assembly region of the mammillary layer as the breeder hen ages (Nascimento *et al*., 1992) could potentate separation of the mammillary layer and shell membranes.

Solomon (1991) and Bain (2005) described twelve structural variations in the mammillary layer of weak and poor quality eggshells. Data from Table 4 clarified the various constructions present in the interior surface of eggshell after removing shell membranes viz it being in mammillary layer which can affecting on eggshell stiffness. Our results showed that the depression, cubic, aragonite, caps and changed membrane did not significantly affected by strain, breeder flock age and their interaction. With respect to confluence trait, the present results observed that both brown and white eggs owned shells with similar values of confluence. Figure 1 presented the confluence figures for white strain at earlier age (25 wk), where white eggs owned good mammillary cap confluence and extensive confluent caps that proved good conjunction with shell membranes and consequently increases eggshell. Solomon (1999) found good shell ultrastructural beneficial high confluence reflects good attachment with membranes and caps. Concerning fusion trait, significant differences observed between strains regarding fusion (early or late). Where, white eggshell have superior value than brown eggshell (Fig. 2). Also, the eggs produced from older breeders have owned fusion value higher than that of other produced from younger breeders. Thus where fusion is late, crack propagation through the shell wall and thereafter outwards from the load point will occur more rapidly. Bain (1990) stated that the decline in fracture toughness can be explained in terms of an increase in late fusion of adjacent mammillary columns (and the types of abnormality which accompany this).

No significant differences between brown and white strain as for type B's structure. Inversely, the Type B's trait was significantly affected by breeder flock age, where Type B's in eggshell produced from older breeders was significantly higher than that of younger hens. Figure 3 showed extensive type B's in brown eggshell at late ages (61 wk), that's reduced the eggshell quality as a harmful figure disjoined adjacent columns adhesion. Nascimento *et al*. (1992) stated that the aberrant crystal forms of type B's provide no meaningful contribution to the palisade layer and like the poor cap modifications, which do not offer any anchorage point. In accordance to erosion trait, our results showed that the erosion trait value of white strain was significantly higher than that of brown ones. Also, the older breeders produced eggs have owned higher erosion trait than that other produced from younger breeders.

Type A's not significantly differed between strains. However, the type A's was significantly increased with advancing age. Figure 4 showed the Type A's of basal mammillary cones in brown eggshell produced from older hens (61 wk). Type A's appears to be associated

Table 4: Ultrastructural variants of eggshell mammillae as affected by strain, breeder flock age and their interaction

Trait	Age (wk)	Strain: Brown	Strain: White	Pooled SEM	Overall	Prob.: S	Prob.: A	Prob.: S*A
Confluence	25	4.40	4.21	0.23	4.31			
	61	2.12	2.31	0.14	2.22			
	Overall	3.26	3.26			0.01	NS	NS
Fusion	25	1.33	2.40	0.20	1.88			
	61	3.15	4.22	0.45	3.69			
	Overall	2.24	3.31			0.01	0.01	0.02
Type B's	25	1.00	1.00	0.00	1.00			
	61	4.45	4.64	0.51	4.55			
	Overall	2.73	2.82			NS	0.01	NS
Depression	25	1.00	1.00	0.00	1.00			
	61	1.00	1.00	0.00	1.00			
	Overall	1.00	1.00			NS	NS	NS
Erosion	25	1.00	1.00	0.00	1.00			
	61	2.31	3.15	0.17	2.73			
	Overall	1.66	2.08			0.01	0.001	0.05
Cubic	25	1.00	1.00	0.00	1.00			
	61	1.00	1.00	0.00	1.00			
	Overall	1.00	1.00			NS	NS	NS
Aragonite	25	1.00	1.00	0.00	1.00			
	61	1.00	1.00	0.00	1.00			
	Overall	1.00	1.00			NS	NS	NS
Caps	25	1.00	1.00	0.00	1.00			
	61	1.00	1.00	0.00	1.00			
	Overall	1.00	1.00			NS	NS	NS
Type A's	25	1.00	1.00	0.00	1.00			
	61	4.20	3.18	0.94	3.69			
	Overall	2.60	2.09			NS	0.001	0.02
Changed membrane	25	1.00	1.00	0.00	1.00			
	61	1.00	1.00	0.00	1.00			
	Overall	1.00	1.00			NS	NS	NS
Cuffing	25	4.30	2.54	0.81	3.42			
	61	1.00	1.00	0.00	1.00			
	Overall	2.65	1.77			0.01	0.01	NS
Alignment	25	1.00	1.00	0.00	1.00			
	61	3.15	4.21	0.66	3.68			
	Overall	2.08	2.61			0.02	0.001	0.02
Total scores	25	19.03	18.15	1.42	18.59			
	61	25.38	27.71	2.17	26.55			
	Overall	22.21	22.93			NS	0.001	NS

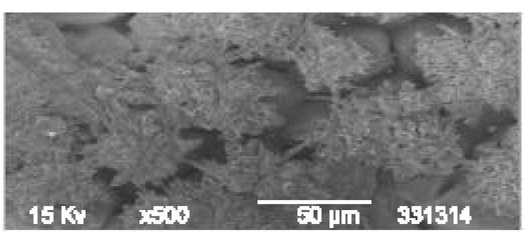

Fig. 1: Good confluence and rounded caps in eggshell of White strain at early age (25 wk)

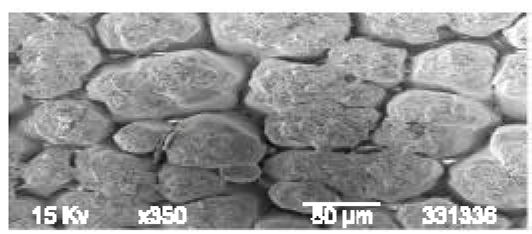

Fig. 2: Late fusion and Type A's in eggshell of white strain at late age (61 wk)

only with the basal cap crystals of the mammillae as indicated by little or no evidence of contact with the membrane fibers (Bain, 1990). There was significant difference for cuffing material between strains. Figure 5 displayed a cuffing form noticed in brown eggshell. This material had a useful job with increasing the cohesion and merging the calcified columns, thus increases eggshell strength. Cuffing appears as secondary crystallization between the cones and is believed to be formed at some point after the mammillary knobs have begun to fuse (Bain, 1990). Concerning breeder flock age effect, it could be noticed that the older hens produced eggs were significantly lower in cuffing trait compared to other produced from younger hens. The presented results showed that alignment comes out to be significantly differed between strains, where white

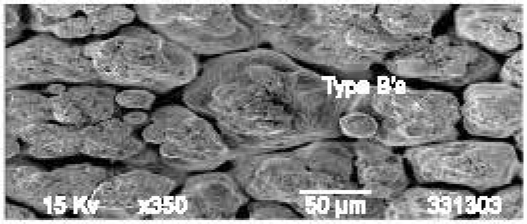

Fig. 3: Type B's (poorly constructed mammillary layer) in eggshell of Brown strain at late age (61 wk)

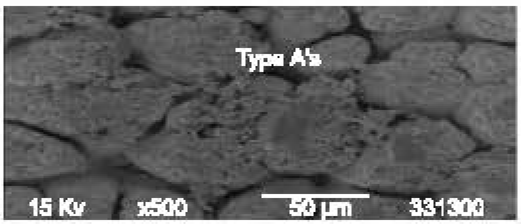

Fig. 4: Type A's in eggshell of Brown strain at late age (61 wk)

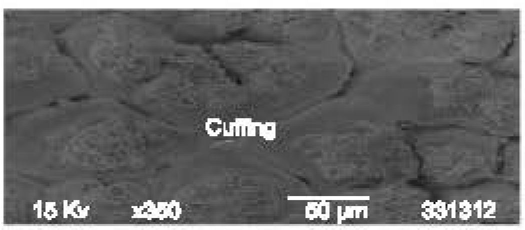

Fig. 5: Cuffing (adhesive calcium carbonate) among columns in strong eggshell of brown strain at early age (25 wk)

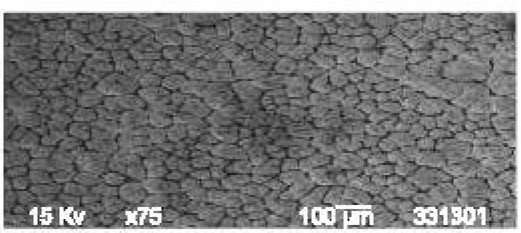

Fig. 6: Extensive alignments in Brown strain at late age (61 wk)

strain had the higher alignment value than brown ones. Also, the eggs produced from older hens had higher alignment value compared to other produced from younger hens (Fig. 6). Fathi (2001) and El-Safty (2004) reported that if an egg subjected to mechanical trauma then the crack line will tend to follow the path of least resistance (alignment) and ordered mammillae provide such a pathway.

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