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Mechanical and Ultrastructural Properties of Eggshell in Two Egyptian Native Breeds of Chicken

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Abstract: The objective of the study was to assess mechanical and ultrastructural properties of eggshell in Dandarawi and Fayoumi chicken breeds. At 30 weeks of age, 180 eggs (90 of each breed) were randomly chosen. The results showed that the Fayoumi eggs had significantly higher specific gravity and mechanical properties values (thickness and breaking strength of eggshell) than that of Dandarawi ones. With respect to ultrastructural traits, it could be noticed that the relative palisade layer (effective thickness) of Fayoumi eggs was significantly higher than that of Dandarawi ones. Opposite trend was noticed for relative cap layer. Concerning ultrastructural variants, the Fayoumi eggs owned shells with significantly higher values of confluence and cuffing traits. Conversely, the Dandarawi eggs have superior values of fusion, alignment and type B's traits than Fayoumi ones.

Key words: Native breeds, eggshell, mechanical traits, ultrastructural

INTRODUCTION

Native chickens play an important role in the household food supply in rural Africa and recently have been raised in semi-intensive systems with more efficient output per bird. In many developing countries, the local gene pool still provides the basis for the poultry sector. The genetic resource base of the indigenous chickens could form the basis for genetic improvement and diversification to produce breeds adapted to local condition. However, breeding programs for native breed chickens will be difficult to set-up because of the competition with commercial breeding companies which have access to technology advantages and economics of scale. As known, the commercial layers produce high egg production but the eggshell strength was less compared with local breed. Low eggshell 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 in the egg 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).

The structure of strong and week eggshells is compared by Scanning Electron Microscopy (SEM), which suggests that shell strength is dependent on both the thickness of the palisade layer and the organization of calcite crystals in this layer. Such evidence supports the conjecture that not only the density and thickness but also the ultrastructure has an important influence on shell strength. Extensive studies were conducted to evaluate the ultrastructural variations in eggshell quality among breeds and species of poultry (Bain, 1990; Solomon 1991; Solomon, 1999; Ruiz and Lunam, 2000; Fathi, 2001; Afifi *et al.*, 2007) In addition, the details of fracture resistance have become known. Solomon (1991;1999) 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; therefore, it is likely that alteration in the thickness of the palisade layer, independent of structural reorganization of the palisade columns could affect shell strength (Ruiz and Lunam, 2000). The mechanical properties of eggs depend on geometric variable such as the shape and thickness of the eggshell combined with the eggshell's fundamental material properties. The material properties of the eggshell depend on its microstructure and chemical composition, both of which vary through the shell thickness (Rodriguez-Navarro *et al.*, 2002; Nys *et al.*, 2004; Bain *et al.*, 2006). There are few studies analyzing the influence of structure on shell strength in local breeds. Therefore the present study was designed to evaluate the mechanical and ultrastructural of eggshell properties in two native breeds of chicken.

MATERIALS AND METHODS

This experiment was conducted at poultry breeding farm, Poultry Production Department, Faculty of Agriculture, Ain Shams. Two Egyptian native breeds of female chicken (125 Fayoumi and 125 Dandarawi) were used. They were brought from El-Takamouly project, at Fayoum

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government. These chicks were weighed to the nearest gram and brooded in electrical brooding batteries and rearing cages from 1 day to 14 weeks. Then, they were housed in suitable individual cages (four Fayoumi or Dandarawi) from 16 weeks of age up to the end of the experiment. All birds were reared under similar environmental, managerial and hygienic conditions.

Mechanical eggshell traits: To assess eggshell quality parameters, a total of 90 eggs were randomly collected from each breed at 30 weeks of age. The dimensions of eggs (width and length) were measured using a digital calliper to calculate shape index. The thickness (mm) of the shell with intact membranes was measured at three deferent points in the middle part of the egg using a dial gauge micrometer. The shell breaking strength (kg/cm^2) was determined according to Fathi and El-Sahar (1996). Specific gravity was determined by the flotation method using salt solution with specific gravity ranging from 1.060 to 1.100 at increments of 0.005. Eggshell area was estimated by shell weight per unit of surface area (mg/cm^2), which is an indicator of shell quality and egg surface. The egg surface in cm^2 was calculated by dividing shell weight on weight of 1 cm^2 area. Shell index (g/100 cm^2) was calculated according to following equation: (Shell weight (g)/Shell surface (cm^2) x100 (Sauveur, 1988). The shell percentage was calculated by (shell weight/egg weight) x100.

Preparation of samples for ultrastructural analysis using SEM: At 30 weeks of age, twenty samples of eggshell were randomly taken from Fayoumi and Dandarawi breeds (8 each) to investigate ultrastructural variations. The specimens were prepared by cutting a piece (1 cm^2) of shell from the 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 innerside 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 ultrastructural 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 x200. 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 the 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 in the statistical analysis.

Statistical analysis: Data were subjected to a one-way analysis using GLM (SAS Institute, 2001) with breed as fixed effect.

RESULTS AND DISCUSSION

Mechanical eggshell traits: Effect of breed on egg weight and mechanical eggshell traits are summarized in Table 1. There was no significant difference between breeds for weight and dimension of eggs. Conversely, the Fayoumi eggs recorded higher significantly higher specific gravity value compared to Dandarawi ones. 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. It has also been shown to be an accurate predictor of shell thickness, much more reliable for this purpose than percentage shell (Tyler and Geake, 1961). With respect to thickness and breaking strength of eggshell, the present results indicated that the Fayoumi eggs were significantly higher thickness and breaking strength of eggshell compared to Dandarawi ones.

Eggshell ultrastructural properties: Data presented in Table 2 showed that the effect of breed on cross-sectional length (absolute and %) of eggshell layer. Our results indicate that the total thickness of Fayoumi breed was significantly higher than that of Dandarawi one. Similar trend was observed for absolute and % of palisade thickness (effective thickness), where the Fayoumi breed produced eggs associated with significantly higher palisade thickness compared to Dandarawi one. According to Bain (1991) and Ruiz and Lunam (2000), the palisade layer provides the stiffness characteristics of the shell and thereby shell strength. Thus, a reduction in its relative thickness could compromise shell strength leading to a higher incidence of breakage. In addition, Bain *et al.* (2006) reported that the eggshell is consist of several different layers and proposed that each of these different layers must variously contribute to the eggs performance under load. Concerning cap thickness, it could be noticed that the Fayoumi breed was significantly thinner cap thickness

Table 1: Eggshell quality traits of Dandarawi and Fayoumi breeds at 30-weeks of age

Trait	Breed: Dandarawi	Fayoumi	Prob.
Egg weight (g)	41.25±0.530	40.13±0.420	NS
Egg shape index (%)	78.36±0.350	77.80±.280	NS
Eggshell area (cm^2)	55.70±0.480	54.71±0.380	NS
Egg volume (cm^3)	38.41±0.500	37.46±0.390	NS
Eggshell index (g/100 cm^2)	9.30±0.120	9.56±0.110	NS
Specific gravity	1.076±0.001	1.083±0.001	0.0001
Shell thickness with membrane (mm)	0.37±0.004	0.39±0.004	0.05
Eggshell breaking strength (kg/cm^2)	3.98±0.140	4.59±0.130	0.002

Table 2: Cross-sectional length of individual eggshell layers (absolute or %) in Dandarawi and Fayoumi breeds at 30-week of age

Trait	Breed: Dandarawi	Fayoumi	Prob.
Total thickness (µm)	300.62±5.38	343.48±7.12	0.001
Palisade thickness (µm)	211.81±4.15	261.80±5.92	0.001
Cap thickness (µm)	88.81±2.01	81.68±2.07	0.01
Palisade (%)	70.48±1.55	76.22±1.53	0.01
Cap (%)	29.52±1.14	23.78±1.50	0.001

(absolute and %) compared to Dandarawi one. The mammillary layer is the major source of calcium for the developing embryo; any variation in its thickness may affect the amount of calcium available. Approximately 80% of the calcium requirements of the chick by the time of hatch are derived from the eggshell (Simkiss, 1961). According to Dieckert *et al.* (1989), 222 mg of calcium is present in the CRA region of the mammillary layer adjacent to the chorioallantoic membrane. The embryo is expected to recover approximately 142 mg of calcium in the CRA region by the time of hatch. By using equations given by Romanoff and Romanoff (1949) and Dieckert *et al.* (1989), a mammillary layer approximately 75 µm in thickness or 21% in relative shell proportion can provide adequate calcium to the chick embryo.

Solomon (1991) and Bain (2005) described twelve structural variations in the mammillary layer of weak and poor quality eggshells. Watt and Solomon (1985) found that there were a high proportion of structural abnormalities in the cone layer of those eggs which were cracked or broken. Information from Table 3 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. Data revealed that there were no significant differences between Dandarawi and Fayoumi breeds regarding depression, erosion, cubics, aragonite, type A's and changed membranes. Nascimento *et al.* (1992) noticed that the cubics, aragonite and type A's were equal found in both Fayoumi and Dandarawi breeds in either parents or offspring flock. While, the changed membranes was equal existed in both Fayoumi and Dandarawi breeds in offspring only. The present result, also showed that eggshell of both breeds was significantly differed in confluence, fusion,

Table 3: Ultrastructural variants of eggshell mammillae for Dandarawi and Fayoumi breeds

Trait	Breed: Dandarawi	Fayoumi	SEM	Prob.
Confluence	3.00	5.40	0.003	0.01
Fusion	4.00	2.00	0.02	0.01
Type B's	2.00	1.00	0.01	0.01
Depression	1.00	1.00	0.00	NS
Erosion	1.00	1.00	0.00	NS
Cubics	1.00	1.00	0.00	NS
Aragonite	1.00	1.00	0.00	NS
Caps	4.30	1.40	0.04	0.001
Type A's	1.00	1.00	0.00	NS
Changed membrane	1.00	1.00	0.00	NS
Cuffing	4.30	1.80	0.02	0.001
Alignment	3.00	1.00	0.001	0.01
Total scores	26.00	18.40	0.14	0.001

alignment, caps and total score, where Dandarawi breed owned the most higher values of these parameters, except of confluence trait, compared to Fayoumi ones (i.e. the most stiffer shell was belongs Fayoumi breed). The eggshell of Fayoumi breed recorded bigger cone and low density of mammillary layer (Fig. 1), while Dandarawi breed feature pointed cone and high density of Mammillary layer (Fig. 2). The large size of cone may be high resistance of broken, so the Fayoumi breeds significant high force egg broken.

Respecting confluence trait, data showed that the Fayoumi eggs owned shells with significantly higher values of confluence. Figures (3, 4 and 5) presented the confluence figures for both breeds, where Fayoumi breed showed good mammillary cap confluence and extensive confluent caps, that proved good conjunction with shell membranes and consequently increases eggshell, compared to Dandarawi one. Solomon (1999) found good shell ultrastructural beneficial high

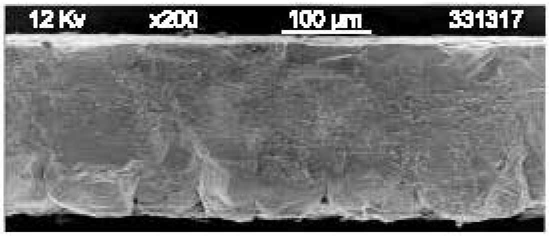

Fig. 1: Big cone for Fayoumi breed

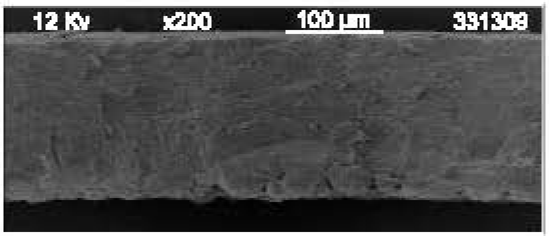

Fig. 2: Pointed caps for Dandarawi breed

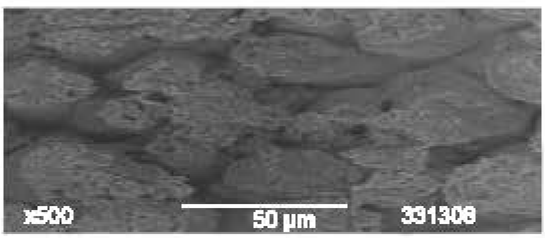

Fig. 3: Poor confluences for Dandarawi breed

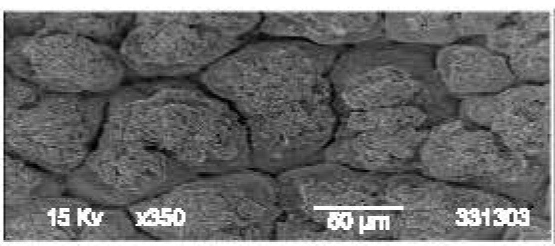

Fig. 4: Good confluence and rounded caps for Fayoumi breed

confluence reflects good attachment with membranes and caps. Concerning fusion trait, significant differences observed between Dandarawi and Fayoumi eggshells regarding fusion (early or late), where Dandarawi eggs have superior values than Fayoumi ones. Crack growth to be link with structural organization of the mammillary layer. 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).

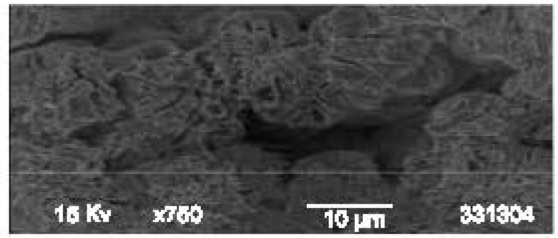

Fig. 5: Extensive confluences for Fayoumi breed

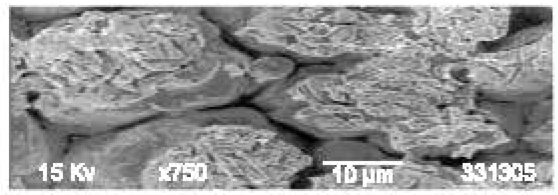

Fig. 6: Type B's (poorly constructed mammillary layer) in eggshell of Dandarawi breed

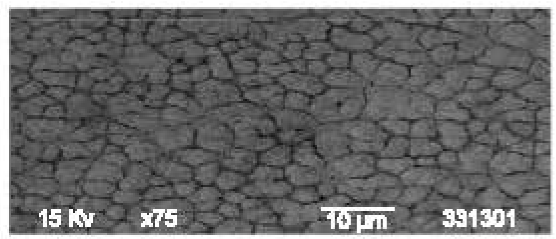

Fig. 7: Extensive alignments in Dandarawi breed

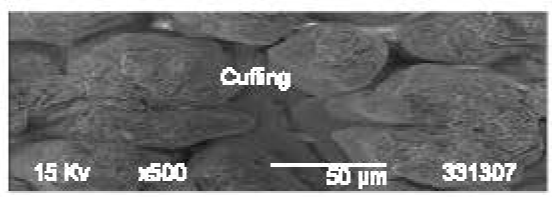

Fig. 8: Cuffing (adhesive calcium carbonate) among columns in strong eggshell of Fayoumi breeds

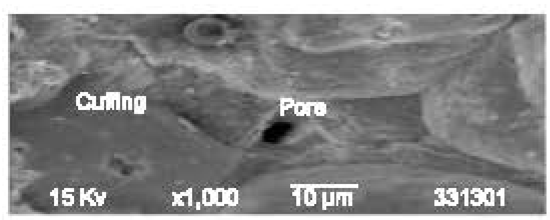

Fig. 9: Cuffing rounded pore in Fayoumi breed

A significant difference between Dandarawi and Fayoumi eggs was observed for type B's structure. Figure 6 showed type B's in Dandarawi eggshell, 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 depression and erosion traits, both local breed chickens didn't significantly differed in these traits. Concerning caps layer, it appears to be significantly different between both local breed chickens, where Dandarawi chickens owned higher values of caps compared to Fayoumi ones.

With respect to alignment trait, the presented results showed that alignment comes out to be significantly differed between Dandarawi and Fayoumi breeds, where Dandarawi breed had the higher alignment value than Fayoumi one. This clearly presented in Fig. 7, where Dandarawi breed owned extensive alignment figures among columns compared to Fayoumi one, that produced eggs have low mammillary alignment. There was significant difference for cuffing material between Dandarawi and Fayoumi breeds. Figures (8 and 9) displayed a cuffing form noticed in Fayoumi breed eggshells. 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).

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