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UTLRASTRUCTURAL DIVERSITY OF EGGSHELL QUALITY IN SOME EGYPTION LOCAL BREEDS OF CHICKEN

By

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Abstract: *Ultrastructural variations of eggshells from some Egyptian local breeds (Bandara, Mandarah and Norfa) were evaluated using scanning electron microscopy (SEM). A total of 120 laying hens representing the different breeds were used in this experiment (40 each). Hens were housed in individual wire cages in an open sided house. At 48 weeks of age, two hundred and forty eggs were collected from all breeds (80 each) to measure internal and external egg quality traits at the same day of collection. In addition, ultrastructural variations of eggshells were assessed. The current results revealed that eggshells of the Norfa breed recorded the highest shell thickness ($P < 0.01$) compared to the other breeds. With respect to eggshell strength, it is of interest to note that the eggshells of Mandarah and Norfa chickens had a significantly higher strength ($P < 0.05$) compared to those of the Bandara breed. According to scanning electron microscopy (SEM)data, the incidence of certain structural variants is more common in eggshells of the Bandara breed suggesting poor shell strength. In general, the eggshells of Mandarah and Norfa chickens had a highly significant better total score ($P < 0.001$) for overall ultrastructural traits compared with those of the Bandara breed. The incidence of alignment was more prevalent in Bandara eggshells compared to the other breeds suggesting lower resistance to breakage. Also, type B abnormalities were prevailed in the Bandara breed. On the other hand, early fusion and narrow interstitial spaces of the palisade layer indicated the increased resistance to fracture in eggshells of the Mandarah and Norfa chickens. Simple correlation analysis showed that breaking strength was the best predicted by the ultrastructural characteristics and the effective thickness. In conclusion, although the shell*

thickness of Bandara and Mandarah breeds was equal, the latter recorded higher shell strength and better ultrastructural measurements of the mammillary layer. Thus, the ultrastructural measurements of the eggshell must be taken into consideration through poultry breeding programs and crossbreeding systems.

INTRODUCTION

Genetic differences in eggshell formation characteristics exist among breeds, strains and families within the species (Fathi, 2001; El-Safty, 2004; Bain, 2005; Franco-Jimenez and Beck, 2005; Bain et al., 2006a; Bahie El-Deen and Fathi, 2007). Cracked eggs are therefore, of major economic significance to those involved in the production and marketing of eggs, but equally if cracked eggs pass through the system undetected, they can also constitute a risk to food safety. Eggshell quality has always been a problem in the layer industry from an economic point of view (Hunton, 1995). Cracked and damaged eggs can account for between 6 and 8% of total production (Hamilton et al., 1979) and can be particularly problematic in older flocks. The economic losses on a worldwide basis in the egg production industry are difficult to estimate but for the UK industry an estimate can be made using the statistical data published annually. However, economic losses because of poor shell quality are estimated to be greater than $250 million per year (Bell, 1998).

Ultrastructural examination of eggshells has greatly enhanced our understanding of eggshell architecture and has reinforced the view that the mechanical properties of eggshells can not be defined by a simple thickness measurement (Bain, 2005). In this respect, Simons (1971) described the eggshell as consisting of several different layers, and proposed that each of these different layers must variously contribute to the egg's performance under load. Solomon (1991) described twelve structural variations in the mammillary layer of weak and poor quality eggshells. From these observations, the same author hypothesised that if the mammillary layer is structurally imperfect, then this would have a 'knock on effect' on the mechanical properties of the rest of the shell. A weighted scoring system was subsequently developed in an attempt to quantify these findings (Bain, 1990), such that each structural variant was given a range of possible scores to reflect whether it occurred in isolation or extensively throughout the mammillary layer of the shell. The relationship between the ultrastructural organisation of the mammillary layer and egg performance under load was then examined. This revealed that the ultrastructural organisation of the mammillae is indeed of importance with late fusion and alignment of mammillae predisposing an egg to crack whilst cuffing improved the strength of eggshells. This unique scoring system has since been applied as a quality assessment tool in many small-scale laboratory based studies but. compared to other quality assessment tools, this type of analysis is both time consuming and costly, and requires the use of specialist equipment. Therefore, the current study was performed to detect the genetic diversity of eggshell quality among some Egyptian local breeds (Bandara, Mandarah and Norfa) using scanning electron microscopy (SEM)data.

MATERIALS AND METHODS

Birds and Management

Three Egyptian local breeds of chicken (Bandara, Mandarah and Norfa) were raised in an open-sided house at the Ein Shames University farm under the same environmental and hygienic conditions. A total of 120 laying hens representing all breeds were used in this experiment (40 each). Hens were housed in individual wire cages with experimental units of 5 hens sharing access to a common feed trough. Feed and drinking water were offered to birds , whereas conventional breeding and management procedures were applied throughout the experimental period which lasted until 48 weeks of age. The lighting schedule was maintained at 17 hours of daylight and 7 hours of darkness throughout the study. The diet was formulated to contain approximately 17.5 % crude protein and 2875 ME kcal/kg in a typical layer diet (NRC, 1994) and was mixed every three weeks.

Measurements and Observations

At 48 weeks of age, a total of 80 eggs were randomly collected from each breed to assess the egg quality measurements (external and internal) at the same day of collection. The dimensions of eggs (width and length) were measured using a digital caliper to calculate shape index. Each egg was first weighed to the nearest 0.1 g. Specific gravity was measured using the flotation method. Briefly, saline solutions were prepared with NaCl and tap water to make densities from 1.060 to 1.100 g/cm^3 in increments of 0.005. Eggs were placed sequentially in saline solutions, beginning with the lowest density, until the eggs floated. The density at which each individual egg floated was recorded as its specific gravity. Each egg was broken on a table and its contents poured into a flat plate in order to measure the height of albumen and yolk using a micrometer. Subsequently, Haugh unit was calculated from the records of egg weight (g) and albumen height (mm) according to Stadelman et al. (1988).

Each egg yolk was separated from the albumen using a plastic egg separator, rolled on a tissue paper towel to remove any adhering albumen and weighed. Albumen yield was determined by subtraction of the yolk and shell with shell membranes intact from the whole egg weight. The percentage of egg components (yolk, albumen and shell) was calculated as the ratio of egg component to egg weight multiplied by 100. Yolk index (yolk height/yolk diameter) was also calculated.

Eggshell was weighed to the nearest 0.01g and the percentage of wet eggshell was calculated. The thickness (mm) of the dry shell with intact membranes was measured at three different points in the middle part of the egg using a dial gauge micrometer and the measurements was transformed to Um. The shell breaking strength (kg/cm^2) was determined using quasi-static compression method (Fathi and El- Sahar 1996).

Preparation of Samples for Ultrastructural Analysis Using SEM:

Eighteen samples of eggshell were randomly taken from the different breeds (6 each) to investigate ultrastructural variations. The specimens were prepared by cutting a piece (0.5 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 eggshell 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 ultrastructural variants at the level of the mammillary layer was assessed according to Bain (1990); and Solomon (1991). The latter was expressed as a total ultrastructural score. 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 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 and 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 ANOVA using JMP (SAS Institute, 2000) with breed as fixed effect. All data are presented as means and the pooled SEM. Significant differences among means were separated by the Tukey's test.

RESULTS AND DISCUSSION

The internal egg quality measurements of various breeds are presented in Table 1. There was no statistically significant difference among breeds for egg weight. However, the Norfa breed recorded a lighter egg weight compared with Bandara and Mandarah counterparts. This may be resulted from the heaviest body weight associated with the latter Egyptian developed breeds. With respect to egg shape index, the Bandara breed had more significant spherical shape ($P < 0.05$) than those of the Mandarah and Norfa ones. This observation may be responsible for the lowest shell strength associated with the eggs produced from Bandara breed. This finding is in agreement with results of Fathi (2001) and El-Safty (2004), who found that the elongated eggs have superior shell strength compared to spherical ones. It is well known that egg dimensions and, in turn, egg shape influences eggshell surface and strength (Fathi and El-Sahar, 1996; Fathi, 2001; Fathi et al., 2007). The albumen percentage of Bandara eggs was numerically higher than those for other breeds but not statistically significant compared with two other breeds. On the other hand, the Mandarah breed recorded the highest yolk percentage but this difference was not statistically significant. There was no significant difference among breeds for eggshell percentage. In terms of Haugh units, it could be observed that the eggs produced from the Norfa breed recorded a poorer value (80.8) compared with the other breeds (84.9, 86.2 for Bandara and Mandarah, respectively). Furthermore, the eggs of the Norfa breed recorded the lowest value of yolk index compared to the remaining breeds. These result indicated that eggs produced from both Bandara and Mandarah breeds have better internal egg quality compared with the Norfa breed.

The external egg quality measurements of different breeds are shown in Table 2. The thickness of Norfa eggshells was greater ($P < 0.01$) than that of the other breeds. On the other hand, the eggs produced from Bandara hens recorded the lowest eggshell strength ($P < 0.05$) compared to eggs of both Mandarah and Norfa breeds. In terms of specific gravity, the eggs of Norfa breed recorded the lowest figure ($P < 0.01$), although they have a stronger eggshell. This result suggests that the shell thickness and specific gravity measurements are not fully responsible for shell resistance to breakage. Shell thickness is often quoted as being synonymous with strength. However, a thicker shell is not necessarily a stiffer or stronger shell. The results of the experiment did not confirm the hypothesis that weak eggshell in Bandara breed is associated with higher specific gravity and/or greater shell thickness. There was no evidence that lower total score is markedly correlated with specific gravity. In addition, in stepwise regression analysis to determine the factors affecting ultrastructure and strength of the eggshell. Fathi et al. (2007) and El-Safty (2004) found that specific gravity had a lower ranking among eggshell quality measurements. Nevertheless, Anderson et al. (2004) reported that the differences in specific gravity of fresh eggs are due almost entirely to differences in the amount of shell percent and it appears that egg specific gravity was the best measurement of shell strength. Concerning the relationship between eggshell strength and the incidence of breakage, Charles and Strong (1988) found that specific gravity and the percentage of shell to be useful estimates of the shell quality, whereas Britton (1978) utilised shell deformation to be useful estimates of the shell quality. Total shell strength is influenced by material and structural strength. Material strength is the strength of the building units of a material and is described by the elastic modulus (EM) or Young's modulus. For eggshells, this property depends on the association of the mineral and the organic components of the shell. Structural strength, on the other hand, is related to the interaction among the building units and depends on several variables, namely size, shape, thickness, and distribution of the shell components (Bain, 1990; Bain, 2005; Kemps et al., 2006; Fathi et al., 2007). According to (SEM) observations expressed as total score in Table (2), the eggshell of both the Mandarah and Norfa breeds had a significantly ($P < 0.001$) better score (lower) for overall ultrastructural traits compared with those of the Bandara one (higher score). Similar results were observed by Afifi et al. (2007), who stated that Mandarah eggshells recorded a lower total score of ultrastructural evaluation compared to the eggshells of the remaining Egyptian breeds during the early egg production cycle.

The cross-sectional lengths of palisade and mammillary layers are given in Table 3. The eggshells produced from Mandarah and Norfa breeds recorded significantly higher palisade percentage compared to the Bandara breed. On the other hand, mammillary layer (both absolute and relative) length was significantly lower in eggshells of both Mandarah and Norfa hens compared to those of Bandara hens. These data revealed that the proportion of palisade layer could play an important role in shell stiffness in both breeds these results are in agreement with those reported by (Bain, 1990; Bain, 1992; Afifi et al., 2007; Fathi et al., 2007). 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.* (2006a) reported that the eggshell consists of several different layers, and proposed that each of these different layers must variously contribute to the eggs performance under load. Moreover, the effective thickness (palisade layer) should be used as a tool for selection programs in both broiler breeders and parent stock of layers.

Correlation coefficients between eggshell ultrastructure (total score) and the shell strength characteristics are given in Table 4. In general, a highly significant negative correlation (r = -0.72) was observed between the breaking strength and total score of the ultrastructural variants. Furthermore, the breaking strength was positively correlated with both shell thickness and total layers, but these relationships were not statistically significant. As expected, the shell thickness was positively highly correlated with the total mammillary layers (r = 0.50). Finally, it could be noticed that the correlation between the palisade layer percentage and the total score was significantly negative (r = -0.83). On the other hand, there was a positive correlation coefficient between the total score and the length of cap layer. These results are in line with those of Bain (1990; 2005) and Fathi et al. (2007) who reported that the mammillary layer (caps) was subsequently shown not to contribute to the stiffness characteristics of the eggshell; thus the effective thickness (the distance from the point of fusion of the palisade columns to the outer edge of the cuticle) is actually a more meaningful measure to make when describing the mechanical properties of eggshells. Similarly, Carnarius *et al.* (1996) found a significant correlation (P<0.01) between the effective thickness of the shell and puncture force.

The relationship between the ultrastructural organisation of the mammillary layer and egg performance under load was then examined. The strength of an eggshell is determined not just by the amount of shell that is present, but also by the quality of construction of the shell (Roberts, 2004). This revealed that the ultrastructural organisation of the mammillae is indeed of importance with late fusion and alignment of mammillae predisposing an egg to crack whilst cuffing improved the strength of eggshells (Bain, 2005). In general, it would be appear from the extensive examination of the mammillary layer that the eggshells of Mandarah and Norfa chickens had a better mammillary ultrastructure compared with those of Bandara breeds. However, eggshell of Bandara breed displayed a poor cap quality and late fusion (spacing of mammillary bodies) along with extensive alignment reflecting a lower breaking strength (Figure 1). Some of mammillary caps were conical rather than rounded with little contact with membrane fibers, thereby resembling the Type A mammillary bodies as described by Bain (1990); Solomon (1991); Fathi *et al.* (2007) and Afify *et al.* (2007). Also, rounded calcified bodies located between adjacent mammillae (Type B's) were seen (Figure 2). A good coverage with confluent appearance and flattened mammillary caps was observed in eggshells of the Mandarah breed compared to those of the Bandara one (Figure 3). It is of particular interest to note that the mammillary caps of the eggshells of the Norfa breed had early fusion with extensive confluent appearance (Figure 4).

We concluded that eggshells of both Mandarah and Norfa breeds had genetically better strength and good ultrastructural formation compared to those of the Bandara breed. Moreover, the higher eggshell thickness does not mean superior breaking strength.

Table (1): Internal egg quality measurements for different breeds.

Trait	Breed			Pooled SEM	Prob.
	Bandara	Mandarah	Norfa		
Egg weight, g	51.6	52.3	49.9	0.58	NS
Shape index	0.78 ^{a}	0.76 ^{b}	0.76 ^{b}	0.0033	*
Yolk index	0.49 ^{a}	0.47 ^{ab}	0.44 ^{b}	0.0041	**
Albumen %	56.9	55.4	55.6	0.46	NS
Yolk %	32.0	33.2	32.7	0.32	NS
Shell %	11.21	11.44	11.58	0.12	NS
Haugh unit	84.93 ^{a}	86.24 ^{a}	80.76 ^{b}	1.01	**

^{a,b} values with different superscripts are statistically different within the same row.
* P<0.05. ** P<0.01. NS=non-significant.

Table (2): External egg quality measurements for different breeds.

Trait	Breed			Pooled SEM	Prob.
	Bandara	Mandarah	Norfa		
Shell thickness, μm	351 ^{b}	347 ^{b}	370 ^{a}	8.3	**
Shell strength, kg/cm^2	4.57 ^{b}	5.06 ^{a}	4.99 ^{a}	0.41	*
Specific gravity	1.093 ^{a}	1.093 ^{a}	1.087 ^{b}	0.0010	**
Total ultrastructural score	36.60 ^{a}	25.75 ^{b}	26.60 ^{b}	1.71	**

^{a,b} Means within a row with no common superscripts are significantly different.
* P<0.05. ** P<0.01.

Table (3): Effect of breed on cross-sectional length (µm) of eggshell mammillary layer.

Layer	Breed			Pooled SEM	Prob.
	Bandara	Mandarah	Norfa		
Palisade	198.47 [b]	260.2 [a]	281.07 [a]	9.25	**
Mammillary	70.13 [a]	52.27 [b]	59.00 [b]	5.66	*
Total	268.60 [b]	312.47 [a]	340.07 [a]	12.72	**
Palisade %	73.99 [b]	83.38 [a]	82.72 [a]	1.20	**
Mammillary %	26.01 [a]	16.62 [b]	17.28 [b]	1.20	**

[a,b] values with different superscripts are statistically different within the same row.
* P<0.05. ** P<0.01.
Palisade %= Palisade /Total X100   Mammillary %= Mammillary / Total X100

Table4: Correlation coefficients between ultrastructural measurements and breaking strength of eggshell.

Item	Y1	Y2	Y3	Y4	Y5	Y6
Total score (Y1)	1.00	-0.72**	-0.20	-0.39	-0.83**	0.83**
Breaking strength (Y2)		1.00	0.47	0.32	0.70**	-0.70**
Shell thickness (Y3)			1.00	0.50*	0.27	-0.27
Total layers (Y4)				1.00	0.44	-0.44
Palisade % (Y5)					1.00	-1.00
Cap % (Y6)						1.00

* P<0.05   ** P<0.01

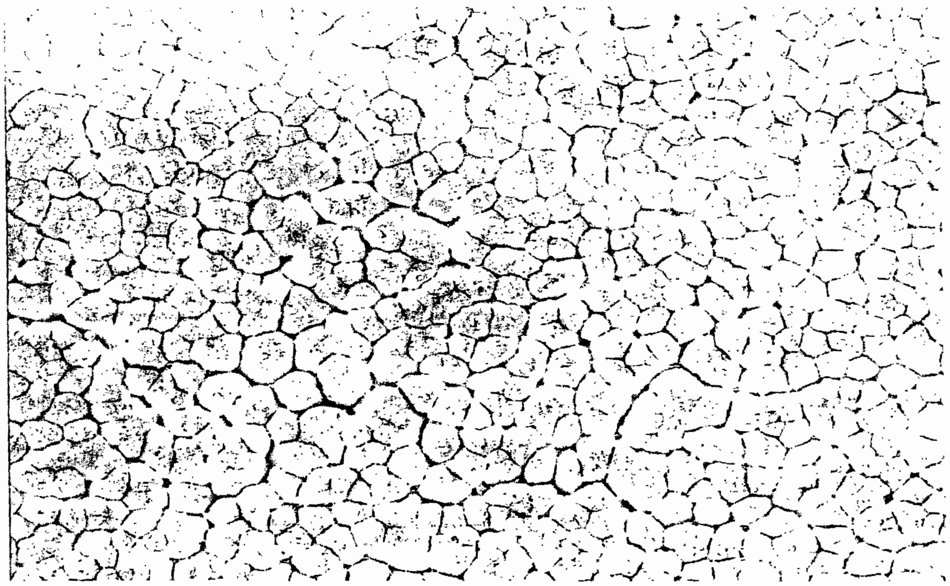

Figure. 1. Extensive aligned mammillae offer a low resistance to crack growth in Bandara breed.

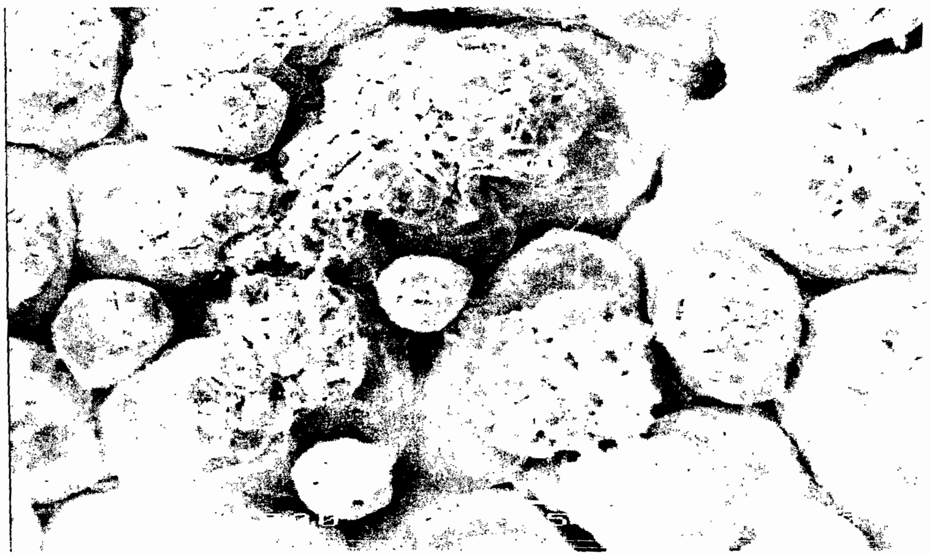

Figure. 2. Variation in shape of mammillary caps with rounded calcified bodies (Type B's) located between adjacent mammillae in eggshell of Bandara breed.

Figure. 3. Flattened mammillary caps with fair confluence (Mandarah breed).

Figure. 4. Early fusion with extensive confluent area in eggshell of Norfa b

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الملخص العربى

تنوع التركيب البنائى لجودة قشرة البيض لبعض سلالات الدجاج المحلى

معتز محمد فتحى، يسرية كمال عفيفى، صلاح عبد الرحمن الصفتى

تم تقدير الاختلافات فى تركيب قشرة البيض لبعض سلالات الدجاج المحلية (بندرة- مندرة – نورفا) مستخدما الميكروسكوب الالكترونى الماسح ٠ تم استخدام ١٢٠ دجاجة تمثل السلالات الثلاثة المستخدمة فى التجربة (٤٠ دجاجة /سلالة) ٠ ربيت الدجاجات فى اقفاص سلك فردية فى بيوت ذات النظام المفتوح٠ تم جمع ٢٤٠ بيضة عند ٤٨ اسبوع من كل السلالات (٨٠ بيضة / سلالة) لتقدير جودة البيض الخارجية و الداخلية للبيض الطازج ٠ ايضا تم تقدير التركيب البنائى لقشرة البيض ٠ سجلت النتائج ان قشرة البيض لسلالة النورفا اكثر سمكا بمعنوية(١.و) بالمقارنة بالسلالات الاخرى ٠ بالاشارة الى قوة كسر القشرة اظهرت النتائج ان قشر بيض دجاج المندرة و النورفا سجلا اعلى قيم بمعنوية (٥.و) لقوة الكسر مقارنة بقشر بيض دجاج البندرة٠

بالنسبة لبيانات الميكروسكوب الالكترونى الماسح سجل قشر بيض دجاج البندرة تباين فى تركيب القشرة و هذا يشير الى ضعف قوة الكسر ٠ عموما سجل قشر بيض المندرة و النورفا اعلى قيم بمعنوية (١..و) لصفات التركيب البنائى بالمقارنة بقشر بيض دجاجات البندرة٠

يغلب وجود الشقوق بين الاعمدة فى قشر بيض دجاج البندرة بالمقارنة بالسلالات الاخرى٠ ومن المقترح انة يقلل من مقاومتها للكسر ٠ و ايضا وجود النمط المعيب (التراكيب الكلسية التى ليس لها اصول) فى قشر بيض البندرة ٠ من ناحية اخرى يلاحظ ان الالتحام المبكر بين الاعمدة وضيق المسافات البينية لطبقات البلاسيد تزيد من المقاومة للكسر فى قشر بيض دجاج المندرة و النورفا٠اوضح تحليل الارتباط ان قوة الكسر كانت افضل بين صفات التركيب البنائى و سمك القشرة٠

و يتضح من كل ذلك انه:-

بالرغم من ان سمك القشرة لبيض دجاجات البندرة و المندرة متساوى الا ان قشرة بيض دجاج المندرة سجل مقاومة للكسر اعلى وقياسات للتركيب البنائى افضل فى طبقات القشرة٠ لذلك يجب ان يؤخذ فى الاعتبار التركيب البنائى لقشرة البيض خلال برامج التربية لسلالات البيض٠