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Using Scanning Electron Microscopy to Detect the Ultrastructural Variations in Eggshell Quality of Fayoumi and Dandarawi Chicken Breeds

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Abstract: An experiment was conducted to evaluate egg quality and ultrastructural measurements of eggshell in two Egyptian local breeds of chicken (Fayoumi and Dandarawi). A total of 162 females representing the two breeds were randomly assigned to the current experiment. They were housed in individual cages placed in an open-sided house under the same environmental, managerial and hygienic conditions. Maturation measurements including body weight and age at sexual maturity were determined. Also, egg production (number and weight) was recorded for the first 3 months of production cycle to calculate egg mass. To assess egg quality parameters (internal and external), a total of 90 eggs were randomly collected from each breed at 26 weeks of age. In addition, eggshell samples were prepared to investigate ultrastructural variations using scanning electron microscopy (SEM). The current results revealed that Fayoumi breed had a better performance at sexual maturity compared to Dandarawi one. Inferior egg production parameters during the first 3 months of laying cycle were associated with Dandarawi breed. With respect to internal egg quality, there were no significant differences between Fayoumi and Dandarawi breeds for liquid components as a percentage of egg weight. In addition, Haugh unit and yolk index as indicators to the quality of albumen and yolk, did not exhibit a difference between breeds. Regarding eggshell quality traits, it could be noticed that Fayoumi breed significantly increased specific gravity and eggshell strength compared to Dandarawi one. In terms of ultrastructural measurements of eggshell, SEM inspection revealed that eggshell of Fayoumi breed had a higher effective thickness (palisade layer) compared to Dandarawi one. On the other hand, the length of mammillary layer (absolute or relative) was significantly lower in eggshells of Fayoumi hens compared to those of Dandarawi counterparts. Type B bodies, which are rounded and located among mammillary caps, were more frequent in eggshell of Dandarawi breed resulting in poor shell quality. However, Fayoumi breed had good rounded caps and early fusion as compared to Dandarawi one. The incidence of mammillary alignment and erosion were more common in eggshell of Dandarawi breed suggesting poor shell strength. In conclusion, Fayoumi breed had genetically not only better egg production and egg quality but also good ultrastructural formation of eggshell compared to Dandarawi breed.

Key words: Eggshell ultrastructure, shell strength, scanning electron microscopy, Fayoumi, Dandarawi

Introduction

Genetic differences in eggshell formation characteristics exist between breeds, strains and families within the species (Fathi, 2001; Anderson *et al.*, 2004; El-Safty, 2004; Bain, 2005; Hunton, 2005; Bain *et al.*, 2006a; Afifi *et al.*, 2007). Eggshell quality is receiving much attention from consumers, egg producers and poultry breeders. From selection stand point of view, the eggshell quality is one of many characteristics, which needs to be recorded, statistically analyzed and kept at a competitive level by suitable selection programs (Flock, 1991; Afifi *et al.*, 2007; El-Diebshany *et al.*, 2007). The commercial production and marketing of eggs expose them to insults that cause a high rate of broken or cracked eggshells and are responsible for major economic losses to the egg producer. 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 are difficult to estimate but for the UK industry, an estimate can be made using the statistical data published annually. In 2005, for example, the total eggs passing through UK packing stations was estimated to be 24 741 000 cases (360 eggs per case). Assuming an average breakage of 5%, then the losses sustained by the UK industry in 2005 could have amounted to as much as £16.7 million, based on an average packer to producer price of 45 pence/dozen (Bain *et al.*, 2006b). Numerous studies have been conducted on measuring eggshell strength and thickness through traditional methods, but few deals with that affecting actual egg breakage (ultrastructural measurements).

In general, eggshell strength methods can be subdivided into direct methods and indirect methods (Hamilton, 1982; De Ketelaere *et al.*, 2004; Bain, 2005).

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Indirect methods are used to measure eggshell strength on the assumption that the indirect values are correlated with the direct values. Direct methods include quasi static compression test and Instron tensile test machine. 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 and Afifi *et al.*, 2007). In addition, the details of fracture resistance have become known. Solomon (1991 and 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.*, 2006a). However, most recent research has identified the ultrastructure of eggshell with resulting opportunities for industrial remedies and possible new selection criteria, to ensure maximum shell quality throughout the egg industry (Hunton, 1995 and 2005).

Numerous studies have been performed on Egyptian breeds of chickens (particularly, Fayoumi and Dandarawi) to evaluate eggshell quality using traditional indirect and direct methods. Therefore, the current study was designed to detect the ultrastructural variations of eggshell for these breeds using scanning electron microscopy.

Materials and Methods

Flock management and observations: Two Egyptian local breeds of chicken (Fayoumi and Dandarawi) were raised at the Poultry Breeding Farm, Ain Shams University under the same environmental, managerial and hygienic conditions. At 16 weeks of age, a total of 162 females (97 Fayoumi and 65 Dandarawi) were randomly assigned to the current experiment. They were housed in individual cages placed in an open-sided house. They were fed a laying diet containing 16.3% CP and 2924 kcal ME/kg. Feed and drinking water were offered to birds *ad libitum*, whereas conventional breeding and management procedures were applied throughout the experimental period which lasted 50 weeks of age. The lighting schedule was maintained at 16 hours of daylight and 8 h of darkness throughout the experiment.

Maturation traits including body weight and age at sexual maturity were determined. Also, egg production (number and weight) was recorded for the first 3 months of

production cycle to calculate egg mass. To assess egg quality parameters (internal and external), a total of 90 eggs were randomly collected from each breed at 26 weeks of age. The dimensions of eggs (width and length) were measured using a digital calliper to calculate shape index. Each egg was first weighed to the nearest 0.1 g and broken open. The height of albumen and yolk was measured using a micrometer mounted on a stand with a platform on which the liquid content was placed. 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. Haugh units were calculated according to Stadelman *et al.* (1988). Wet eggshell was weighed before drying to the nearest 0.01g and then reweighed after drying. The percentage of both wet and dry eggshell was calculated. The thickness (mm) of the shell with intact membranes was measured at three different 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. The eggshell area in cm^2 was calculated by dividing shell weight on weight of 1 cm^2 area. Egg volume was estimated by measuring the quantity of water dislodged in cm^3 after immersing the egg into known water volume.

Preparation of samples for ultrastructural analysis using SEM:

At 26 weeks of age, twelve samples of eggshell were randomly taken from Fayoumi and Dandarawi breeds (6 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 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 the methodology and terminology developed by the Poultry

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Table 1: Means±SE of egg production characteristics for Fayoumi and Dandarawi breeds

Trait	Breed		Prob.
	Fayoumi	Dandarawi	
Body weight, g	1277.3 ^a ±25.69	1174.0 ^b ±21.93	0.001
Age at sexual maturity, d	154.7±2.85	159.2±3.09	NS
Egg number	59.64±0.81	57.59±0.97	NS
Egg production %	66.27±0.90	63.99±1.08	NS
Egg weight, gm	41.08 ^a ±0.30	38.81 ^b ±0.49	0.0002
Egg mass, gm	2450.57 ^a ±39.01	2235.56 ^b ±48.69	0.001

^{a,b}values with different superscripts are statistically different within the same raw

Table 2: Means±SE of internal egg quality measurements for Fayoumi and Dandarawi breeds

Trait	Breed		Prob.
	Fayoumi	Dandarawi	
Egg weight, gm	41.90 ^a ±0.55	38.22 ^b ±0.54	0.0001
Haugh unit	90.38±0.63	90.33±0.90	NS
Yolk index	4.90±0.06	4.75±0.06	NS
Albumin weight, gm	23.86 ^a ±0.34	22.04 ^b ±0.37	0.001
Albumin %	56.97±0.40	57.66±0.54	NS
Yolk weight, gm	12.81 ^a ±0.22	11.45 ^b ±0.24	0.0001
Yolk %	30.58±0.39	29.93±0.41	NS

^{a,b}values with different superscripts are statistically different within the same raw

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 ×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; 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 GLM (SAS Institute, 2001) with breed as fixed effect. Data of ultrastructural traits are presented as means and the pooled SEM.

Results and Discussion

Egg production parameters: The effect of breed on egg production traits is presented in Table 1. It could be observed that the body weight at the onset of laying of Fayoumi females was significantly heavier as compared to Dandarawi counterparts. Moreover, Fayoumi breed reached sexual maturity earlier than Dandarawi breed but the difference was not significant. The previous results are in congruent with the findings of Hussen (2000) and Yakoub (2006). The same trend was

observed for egg production percentage, where Fayoumi breed recorded higher percentage compared to Dandarawi one. Concerning egg weight and egg mass traits, it could be obviously noticed that Fayoumi hens laid significantly heavier egg weight and in turn had a higher egg mass than that of Dandarawi ones during the first 90 days of laying period. This result may be attributed to heavier body weight associated with Fayoumi breed and consequently had a heavier egg weight resulting from the positive relationship between body weight and egg weight. Similar results were reported by Hussen (2000) and El-Full *et al.* (2005).

Internal and external egg quality: Results presented in Table 2 showed that, in general, there were no significant differences between Fayoumi and Dandarawi for liquid components as a percentage of egg weight. Likewise, Haugh unit and yolk index as indicators to the quality of albumen and yolk, did not exhibit a difference between breeds. The mean values of external egg quality measurements for Fayoumi and Dandarawi breeds are shown in Table 3. Generally, Fayoumi breed had the highest figures of eggshell quality measurements compared to Dandarawi one. It could be noticed that Fayoumi breed significantly increased shell strength and specific gravity. Also, there were a slightly increase in shell thickness with membranes, wet shell% and dry shell % in Fayoumi eggs compared to those of Dandarawi breed. The higher dry eggshell % associated with Fayoumi breed could be attributed to compact eggshell material compared to Dandarawi eggs. In other words, calcite material and structure of eggshell in Fayoumi were more condensed than Dandarawi. The last observation may interpret the higher eggshell strength of Fayoumi eggs. Similar findings were found by Kul and Seker (2004). They explained that there were significant positive correlations among egg weight, shell weight, shell thickness and shell strength. However, these correlations could be used to predict shell strength (force required to break egg). Regarding egg shape index, there was slightly increased associated with Dandarawi eggs compared to Fayoumi one. This result was supported by Kul and Seker (2004), where the shape index was not referred to be a good estimator for the shell thickness and the shell ratio. Conversely, both eggshell area and egg volume were increased in Fayoumi breed compared to Dandarawi one.

Ultrastructural measurements of eggshell: Effect of breed on cross-sectional length (µm) of eggshell mammillary layer is shown in Table 4. The eggshell of Fayoumi breed recorded significantly higher palisade percentage compared to Dandarawi breed. On the other hand, mammillary layer (absolute or relative) length was significantly lower in eggshells of Fayoumi hens (Fig. 1) compared to those of Dandarawi counterparts. These

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Table 3: Means±SE of external egg quality measurements for Fayoumi and Dandarawi breeds

Trait	Breed		Prob.
	Fayoumi	Dandarawi	
Specific gravity	1.096 ^a ±0.001	1.094 ^b ±0.001	0.01
Egg shape index	76.89±0.38	78.03±0.49	NS
Shell strength, kg/cm ²	4.70 ^a ±0.18	3.93 ^b ±0.23	0.0001
Shell thickness with membranes, mm	0.39±0.01	0.38±0.01	NS
Wet shell %	12.44±0.19	12.41±0.25	NS
Dry shell %	10.31±0.21	10.10±0.27	NS
Shell area, cm ²	59.35 ^a ±0.88	56.38 ^b ±0.74	0.01
Egg volume, ml	38.41 ^a ±0.54	34.52 ^b ±0.52	0.0001

^{a,b}values with different superscripts are statistically different within the same raw

Table 4: Effect of breed on cross-sectional length (µm) of eggshell mammillary layer

Layer	Breed		Pooled SEM	Prob.
	Fayoumi	Dandarawi		
Palisade	262.61	248.42	15.29	NS
Mammillary	55.38 ^b	80.46 ^a	6.49	0.02
Total	318.00	328.83	15.87	NS
Palisade %	82.52 ^a	75.35 ^b	2.01	0.03
Mammillary %	17.49 ^b	24.66 ^a	2.00	0.03

Palisade % = Palisade/Total×100 Mammillary % = Mammillary /Total×100

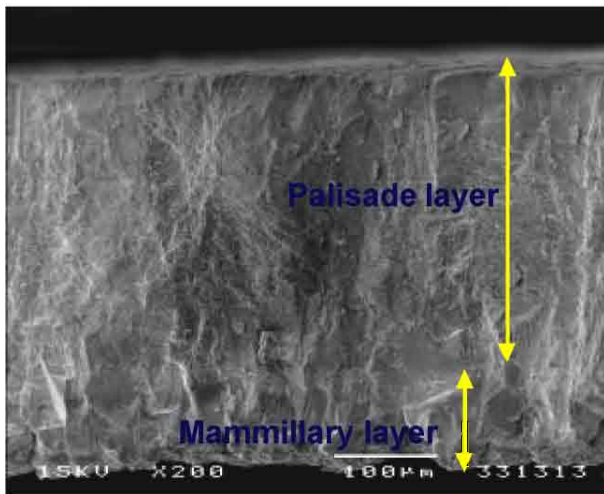


Fig. 1: Cross-sectional length of palisade and mammillary layers of the eggshell

data revealed that the proportion of palisade layer could be played an important role in shell stiffness in both breeds. 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 is consist of several different layers and proposed that each of these different layers must variously contribute to the eggs performance under load.

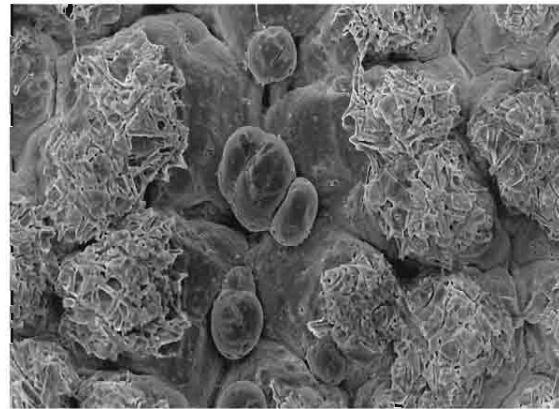


Fig. 2: Type B bodies in the eggshell of Dandarawi breed

Moreover, the term of effective thickness (palisade layer) must conserve as a tool for selection programs in both broiler breeders and parent stock of layers. According to scanning electron microscopy (SEM) observations, there was a significantly difference in fusion, alignment, erosion and good caps between the two breeds. Fayoumi breed had satisfied good caps and early fusion as compared to Dandarawi one. So, the effective thickness of the shell increased for Fayoumi compared to Dandarawi. Mammillary layer does not contribute to the stiffness of shell but late fusion of adjacent palisade columns is related to fracture toughness (Bain, 1991; Fathi, 2001; El-Safty, 2004). Traditionally, eggshell strength has been considered to depend almost completely on the properties of the crystalline part.

Type B's which are rounded and makes no contribution to palisade layer formation and so result in the formation of a weaker shell and more subjected to breakage. From scanning electron microscopy inspection, it could be detected that type B's were rarely found in Fayoumi breed compared to Dandarawi one (Fig. 2). Therefore, there were poor cap modifications, which do not offer any anchorage points in Dandarawi eggshell and in turn lower eggshell strength associated. This observation is fully agreed with the findings of Nascimento *et al.* (1992).

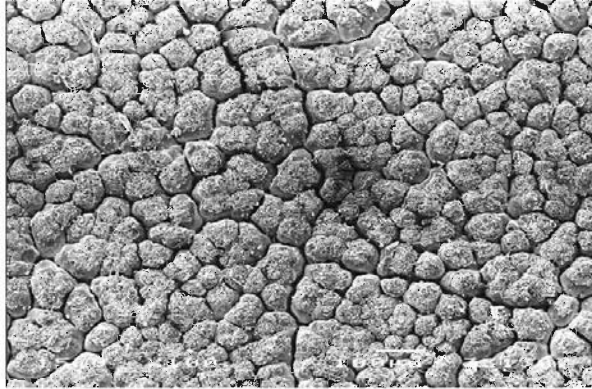


Fig. 3: Mammillary alignment encourages crack growth in Dandarawi breed

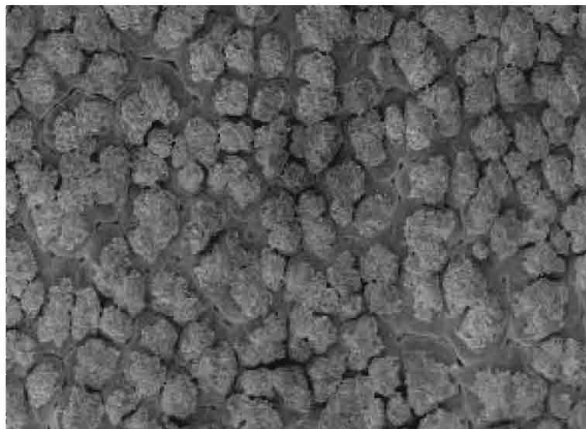


Fig. 4: Rounded good caps and early fusion of the eggshell in Fayoumi breed

Alignment is one of the ultrastructural features which decrease the eggshell resistance to breakage. Fig. 3 illustrated that the alignment was significantly increased in Dandarawi mammillae compared to Fayoumi breed. Thereby, the mammillae appear to “line up”, resulting in a long continuous groove between the cones. Alignment of the mammillae in Fayoumi breed was found to be correlated with eggshell strength. This may reflect the fact that if an egg is subjected to mechanical trauma, then the crack line will tend to follow the path of least resistance and ordered mammillae provide such a pathway (Bain, 1990; Nascimento *et al.*, 1992; Fathi, 2001). There was considerable variation in shape and spacing of mammillary bodies. Some were conical rather than rounded with little contact with membrane fibers, thereby resembling the type A mammillary bodies as described by Bain (1990) and Solomon (1991). The lack of establishment between shell and membrane fibers at this point obviously creates the ideal portal crack and bacterial penetration. This type of

ultrastructural variants was more frequent in Dandarawi eggshells. An overview of the mammillary caps in Fayoumi breed is given in Fig. 4. It could be noticed that the rounded mammillary caps with extensive confluence make a good attachment with membranes. A good eggshell quality should have mammillary bodies that are even in size and distribution and rounded so that there can be maximum attachment to the fibers of the outer membrane (Roberts and Brackpool, 1994). The last result explains that Fayoumi eggshell had high force required to break it and increased shell strength compared to Dandarawi breed.

It can be concluded that Fayoumi breed had genetically not only better egg production and egg quality but also good ultrastructural formation of eggshell compared to Dandarawi breed.

References

- Afifi, Y.K., S.A. El-Safty and M.M. Fathi, 2007. Assessment of eggshell quality measurements in some Egyptian local breeds of chicken using scanning electron microscopy. 1-Early egg production cycle. Proceedings of 4th World's Poultry Conference, Sharm El-Sheikh, Egypt, pp: 219-233.
- Anderson, K.E., J.B. Tharring, P.A. Curtis and F.T. Jones, 2004. Shell characteristics of eggs from historic strains of Single Comb White Leghorn chickens and the relationship of egg shape to shell strength. *Int. J. Poult. Sci.*, 3: 17-19.
- Bain, M.M., 1990. Eggshell strength: a mechanical /ultrastructural evaluation. Ph.D. Thesis, University of Glasgow, UK.
- Bain, M.M., 1991. A reinterpretation of eggshell strength, in Solomon, S.E. Egg and eggshell quality, Wolf publishing Ltd. London, pp: 131-144.
- Bain, M.M., 1992. Eggshell strength: A relationship between the mechanism of failure and the ultrastructural organization of the mammillary layer. *Br. Poult. Sci.*, 33: 303-319.
- Bain, M.M., 2005. Recent advances in the assessment of eggshell quality and their future application. *World's Poult. Sci. J.*, 61: 268-277.
- Bain, M.M., N. Macleod, R. Thomson and J.W. Hancock, 2006a. Microcracks in eggs. *Poult. Sci.*, 85: 2001-2008.
- Bain, M.M., I.C. Dunn, P.W. Wilson, N. Joseph, B. De Ketelaere, J. De Baerdemaeker and D. Waddington, 2006b. Probability of an egg cracking during packing can be predicted using a simple non-destructive acoustic test. *Br. Poult. Sci.*, 47: 462-469.
- De Ketelaere, B.D.E., F. Bamelis, B. Kemps, E. Decuyper and J.D.E. Baerdemaeker, 2004. Non-destructive measurements of the egg quality. *World's Poult. Sci. J.*, 60: 289-302.

- El-Diebshany, A.E., S.A. El-Safty, M. Bahie El-Deen and M.M. Fathi, 2007. Ultrastructural evaluation of eggshell in Japanese quail genetically selected for higher egg production. Proceedings of 4th World's Poultry Conference, Sharm El-Sheikh, Egypt, pp: 207-217.
- El-Full, E.A., A. Abdel-Wraith, H.A. Abdel-Latif and M.A. Khalifa, 2005. A comparative study on pause and clutch size traits in relation to egg production traits in three local breeds of chickens. *Egypt. Poult. Sci.*, 25: 825-844.
- El-Safty, S.A., 2004. Stepwise regression analysis for ultrastructural measurements of eggshell quality in two local breeds of chicken. *Egypt. Poult. Sci.*, 24: 189-203.
- Fathi, M.M., 2001. Ultrastructural evaluation of eggshell quality produced from two different genetic groups of chicken. *Egypt. Poult. Sci.*, 21: 263-276.
- Fathi, M.M. and E.A. El-Sahar, 1996. Determining the strength of eggshell by using an appropriate apparatus and an equation to calculate egg surface depending on its dimensions. *Egypt. Poult. Sci.*, 16: 285-303.
- Flock, D.K., 1991. Shell quality is an important tool in breeding. *World Poult.*, 7: 26-27.
- Hamilton, R.M.G., 1982. Methods and factors that affect the measurement of eggshell quality. *Poult. Sci.*, 61: 2022-2039.
- Hamilton, R.M.G., K.G. Hollands, P.W. Voisey and A.A. Grunder, 1979. Relationship between eggshell quality and shell breakage and factors that affect shell breakage in the field: a review. *World's Poult. Sci. J.*, 35: 177-190.
- Hunton, P., 1995. Understanding the architecture of the eggshell. *World's Poult. Sci. J.*, 51: 140-147.
- Hunton, P., 2005. Research on eggshell structure and quality: An historical overview. *Brazilian J. Poult. Sci.*, 7: 67-71.
- Hussen, Sh. H., 2000. Interrelationship between some environmental factors and Different genotypes in some Egyptian local strains of chickens. Ph.D. Thesis, Fac., of Agri. Ain Shams Univ., Egypt.
- Kul, S. and I. Seker, 2004. Phenotypic correlations between some external and internal egg quality traits in the Japanese quail (*Coturnix coturnix japonica*). *Int. J. Poult. Sci.*, 3: 400-405.
- Nascimento, V.P., S. Cranstoun and S.E. Solomon, 1992. Relationship between shell structure and movement of *Salmonella Enteritidis* across the eggshell wall. *Br. Poult. Sci.*, 33: 37-48.
- Nys, Y., J. Gautron, J.M. Garcia-Ruiz and M.T. Hincke, 2004. Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. *C.R.Palevol*, 3: 549-562.
- Roberts, J.R. and C.E. Brackpool, 1994. The ultrastructure of avian egg shells. *Poult. Sci. Rev.*, 5: 245-272.
- Rodriguez-Navarro, A., O. Kalin, Y. Nys and J.M. Garcia-Ruiz, 2002. Influence of the microstructure and crystallographic texture on the fracture strength of hen's eggshell. *Br. Poult. Sci.*, 43: 395-403.
- Ruiz, J. and C.A. Lunam, 2000. Ultrastructural analysis of the eggshell: contribution of the individual calcified layers and the cuticle to hatchability and egg viability in broiler breeders. *Br. Poult. Sci.*, 41: 584-592.
- SAS Institute, 2001. SAS/STAT User's Guide: Statistics. Ver.8.2, SAS Institute Inc., Cary, NC.
- Solomon, S.E., 1991. Egg and eggshell quality, Wolf Publishing Ltd., London.
- Solomon, S.E., 1999. An eggs ist ein ei, es un huevo, est un oeuf. *Br. Poult. Sci.*, 40: 05-11.
- Stadelman, W.J., V.M. Olson, G.A. Shemwell and S. Pasch, 1988. Egg and Poultry Meat Processing. Ellis-Horwood Ltd, Chichester, UK.
- Yakoub, H.A.M.A., 2006. Immunogenetic measurements as a tool for selection in some local chicken breeds. M. Sc. Thesis, Fac., of Agri. Ain Shams Univ., Egypt.