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Genetic Differences in Eggshell Ultrastructural Properties of Saudi Native Chicken Breeds Kept at High Ambient Temperatures

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ABSTRACT

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Egg quality and eggshell ultrastructural properties of four Saudi native chicken breeds were compared to detect genetic differences. A total of 480 laying hens at 28 weeks of age, representing four Saudi native breeds of chicken (black, black-barred, gray, and brown) kept under hot environmental conditions (34 ± 1°C) were randomly assigned to the current experiment. Laying hens were housed in individual cages in an open-sided house during hot summer season. A total of 360 intact eggs were randomly collected (90 from each breed) for egg quality assessment. Eggshell samples were prepared to evaluate ultrastructural measurements using a scanning electron microscope. No significant variations between the breeds concerning egg weight, egg-shape index, yolk index, albumen (%), yolk (%), shell (%), and shell thickness traits were detected. Brown breed showed significantly higher Haugh units compared to the other breeds. Moreover, brown breed scored the best for eggshell breaking strength trait (the highest crack resistance), followed by gray, black, and black-barred breeds, respectively ($P < 0.005$). Total ultrastructural score for mammillary layer measurements was significantly higher in black-barred breed if compared with black, brown, and gray breeds. Gray, brown, and black Saudi native breeds had an outstanding eggshell ultrastructure and, in turn, stiffer and stronger eggshell comparable with black-barred counterpart under high ambient temperatures. At the national level, for cross-breeding and selection programs aimed at producing good quality table and hatching eggs the use of gray, brown, and black breeds can be recommended due to excellent ultrastructural properties of their eggshell.

Keywords: eggshell strength; egg quality; mammilliary layer; total score

Native chicken breeds play an important role in poultry production sector of developing countries, especially in rural areas. In rural sector, poultry farmers prefer the local strains for their vital characteristics such as high viability, disease resistance, heat tolerance, and meeting the local consumer

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preferences. Therefore, conservation of native breeds as animal genetic resources is emphasized by many organizations worldwide such as FAO.

Local consumers appreciate the eggs produced by native hens for delicious taste and darker yolk than produced by commercial layers. However, the reproductive performance of Saudi hens is considerably low. The selection of local breeds has been targeted more at adaptation to harsh tropical environments and resistance to diseases rather than production enhancement (Minga et al. 2004). Native chickens are self-reliant and gained the capacity to withstand harsh weather condition and adaptation to an adverse environment (Ajayi 2010). Moreover, local poultry species represent valuable resources for livestock development because their extensive genetic diversity allows for rearing poultry under varied environmental conditions, providing a range of products and functions. Thus, great genetic resources embedded in the local poultry await full exploitation that will provide basis for genetic improvement and diversification to produce breeds that are adapted to local conditions for the benefit of farmers in developing countries (Horst 1988). These types of chickens need to be maintained for the purpose of conserving the wide gene pool for further genetic improvement.

It is obvious that beneficial egg quality traits are of immense importance to poultry breeding industries (Bain 2005). The avian eggshell is a unique mineralized structure produced by birds (Chien et al. 2009). The eggshell is a complex and highly calcitic structure. Approximately 94% of eggshell minerals represent calcium carbonate, with other inorganic minerals such as magnesium carbonate, calcium phosphate, and magnesium phosphate (Rodriguez-Navarro et al. 2015). As known, the eggshell plays an important role in the resistance of eggs to physical and microbial invasion. At the same time, the eggshell must permit the exchange of gas and water and serve as a source of calcium for the growing embryo. For producers, cracked and broken eggshells are also regarded as a major source of economic loss. An eggshell cracks if the strength of the shell is lower than the strength of the environmental force to which it is exposed (Tumova and Ebeid 2005; Bain et al. 2006; Kemps et al. 2006). There exist genetic differences in eggshell characteristics among breeds and strains (Silversides and Scott 2001; Tumova et al. 2007; Fathi et al. 2016). Specific eggshell traits and ultrastructural measurements should be included in chicken selection programs to improve eggshell quality and reduce broken eggs (Solomon 2010). The structural and external appearance of eggshells is of considerable economic importance.

The eggshell ultrastructure examination has greatly enhanced understanding of the eggshell architecture and has reinforced the view that the mechanical properties of eggshell cannot be defined by a simple thickness measurement. To our knowledge, no previously conducted research has investigated the differences in eggshell ultrastructural properties in Saudi native chicken strains. Therefore, the goal of this study was to detect the ultrastructural differences of eggshell and to assess the egg quality characteristics of four Saudi local chicken breeds raised under high ambient temperature.

MATERIAL AND METHODS

Birds, housing, and management. Four Saudi local breeds of chicken were genetically divided according to plumage colour into four groups (black, black-barred, gray, and brown) and raised in an open-sided house at the Poultry Research Farm under hot environmental conditions in Qassim, Saudi Arabia. The maximum high ambient temperature during the experimental period was 34 ± 1°C. All birds were provided with the same environmental and hygienic conditions. A total of 480 laying hens at 28 weeks of age representing all the breeds were used in this experiment (120 from each breed). Laying 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. The lighting schedule was maintained at 17 h of daylight and 7 h of darkness throughout the study. The diet was formulated to contain approximately 17.5% of crude protein and 2875 ME kcal/kg in a typical layer diet (NRC 1994) and was mixed every three weeks. The procedures of the experiment were conducted in accordance with the guidelines of Qassim University, Saudi Arabia.

Egg quality traits. At 28 weeks of age, 360 intact eggs (90 eggs from each studied breed) were col-

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lected to assess internal and external egg quality. All eggs were individually weighed to the nearest 0.01 g using an electronic digital balance. Egg length and width were individually measured by using a digital caliper. Egg-shape index was calculated as (width/length) × 100.

Following collection, the breaking strength for intact eggs was determined in kg/cm^2 using an Egg Force Reader™ (Orka Food Technology Ltd, USA). Also, Haugh units were measured automatically using an Egg Analyzer™ (Orka Food Technology Ltd). The liquid contents were put aside and the shell plus membranes were washed under running water to remove adhering albumen. The wet eggshell was left to dry for 24 h and then weighed to the nearest 0.01 g. The relative weight of dry eggshell was calculated on the basis of egg weight. To measure shell thickness, pieces from three different regions (two poles and equator) of each eggshell with intact membranes were measured with a dial gauge micrometer to the nearest 0.01 mm. Using the individual weight of each egg and the weight of its components, yolk percentage and albumen percentage were determined.

Scanning electron microscopy (SEM) technique. Forty samples (10 per breed) were chosen randomly and prepared to evaluate ultrastructural measurements using SEM. 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 fibres, each sample was then immersed overnight in 6% sodium hypochlorite, 4.12% sodium chloride, and 0.15% sodium hydroxide. Afterward, the shells were rinsed with water and left to dry at room temperature for 48 h according to the methodology described by Radwan et al. (2010).

Following these preparative treatments, two samples from each egg were mounted in inner side uppermost and in vertical manner on aluminum stubs, coated with gold for 3 min in an Emscope Sputter Coater. These samples were examined using a JEOL JSM-T330A scanning electron microscope (JEOL, USA) at 15 kV. The incidence of ultrastructural variants at the mammillary layer level was assessed according to the methodology and terminology developed by the Poultry Research Unit, University of Glasgow (Bain 1990, 1992) and later modified by Darnell-Middleton (1999). Briefly, twelve mammillary variants including confluence, fusion, cuffing, alignment, type Bs, erosion, caps, type As, depression, cubic, aragonite, and changed membrane were evaluated (Fathi 2001).

The cross-sections of palisade and mammillary layers were directly measured in µm using a scaling software provided with the SEM at ×200 magnification. Total thickness of each specimen was measured as the distance from its outermost surface to the point where the basal caps were 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 gave the resulting palisade thickness and/or effective thickness (Bain 1990). Triplicate measures were performed in each case and the mean values were statistically analysed. The ultrastructural integrity of each egg was summarized by means of a total ultrastructural score as outlined by Fathi (2001).

Statistical analysis. Data were subjected to one-way analysis of variance using JMP software (Version 11, 2013) with breed as a fixed effect. The model was as follows:

$$Y_{ij} = \mu + L_i + e_{ij}$$

where:

Y_{ij} = trait measured

μ = overall means

L_i = breed effect (i = 1, 2, 3, and 4)

e_{ij} = experimental error

All data were presented as means and the pooled standard error of the means. Significant differences among means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Data presented in Table 1 show the internal egg quality measurements for four different Saudi native chicken breeds investigated. Data revealed no significant variations between breeds for egg weight, yolk index, albumen and yolk percentages. Indeed, egg weight of Saudi native strains was lower than in commercial egg producing strains and this might be attributed to the fact that these chickens were not subject to any genetic improve-

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Table 1. Means ± standard error (SEM) of internal egg quality measurements for different Saudi native chicken breeds

Trait	Breed				SEM	*P*-value
	black	black-barred	gray	brown		
Egg weight (g)	37.86	38.16	36.91	37.58	0.21	ns
Yolk index	46.51	47.48	46.50	47.82	0.48	ns
Albumen (%)	58.28	58.22	57.36	58.42	0.34	ns
Yolk (%)	31.06	31.21	31.86	30.86	0.31	ns
Haugh units	80.40[b]	78.97[b]	80.23[b]	86.68[a]	0.50	0.0001

ns = not significant
[a,b]values with different superscripts are statistically different within the same row

ment programs. These results are in agreement with Al-Yousef and Najib (1997) who elucidated that egg weight in Saudi native chickens was lower than in their counterpart, the White Leghorn. As shown in Table 1, brown breed exhibited exceptionally superior Haugh units compared to the other genetic groups of chickens (black, gray, and black-barred). Silversides (1994) reported that Haugh units (as a measurement of the thickness of thick albumen of a freshly broken egg) is the accepted commercial and research standard for measuring albumen quality of chicken eggs. Likewise, albumen quality traits exhibited highly significant differences between the breeds (Saiful-Islam and Dutta 2010). In the same direction, Cook and Briggs (1977) documented that the breed, strain, and age of hens directly influence the size and composition of eggs.

External egg quality parameters were presented in Table 2. The data revealed no significant variations for egg-shape index, shell weight, shell percentage and thickness between the breeds. In this concern, Tharrington et al. (1999) found no significant differences in the percentage of eggshell among single comb White Leghorn strains (SCWL – CS5, CS7, and CS10) even with the increased egg size of these commercial strains. Similarly, there were no significant differences between experimental cross (SLU-1329) and Lohmann Selected Leghorn regarding the shell deformation and shell percentage (Abrahamsson and Tauson 1998). On the other hand, Al-Moshawah (2014) reported a highly significant difference in egg-shape index due to strain impact. Recently, Ketta and Tumova (2016) have stated that eggshell thickness is related to the length of eggshell formation and is more affected by genotype in comparison with eggshell weight and probably it is a more reliable indicator of eggshell quality than eggshell weight.

As concerns eggshell strength, brown breed had the highest shell breaking strength (higher crack resistance), followed by gray, black, and black-barred breeds, respectively (Table 2; $P < 0.005$). These results are in accordance with Hocking et al. (2001) and Anderson et al. (2004) who presented strong evidence that genetic variation for eggshell strength does exist. In fact, the strength of eggshell is actually due to a combination of material and structural variables (Fathi 2001; Bain 2005). Although there was no significant difference between

Table 2. Means ± standard error (SEM) of external egg quality measurements for different Saudi native chicken breeds

Trait	Breed				SEM	*P*-value
	black	black-barred	gray	brown		
Shape index	78.02	77.94	77.98	77.93	0.002	ns
Shell weight (g)	3.70	3.78	4.08	4.14	0.08	ns
Shell (%)	10.32	10.48	11.42	11.40	0.19	ns
Shell thickness (μm)	0.376	0.382	0.392	0.402	0.006	ns
Shell strength (kg/cm^2)	4.30[c]	4.12[d]	4.45[b]	4.70[a]	0.04	0.005

ns = not significant
[a–d]values with different superscripts are statistically different within the same row

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Table 3. Major mammillary layer measurements of eggshell for Saudi native chicken breeds

Trait	Breed				SEM	*P*-value
	black	black-barred	gray	brown		
Confluence	2.60[ab]	3.40[a]	2.43[ab]	1.80[b]	0.225	0.05
Fusion	1.80[b]	3.40[a]	1.29[b]	1.80[b]	0.255	0.01
Cuffing	2.20	2.20	1.86	2.20	0.217	ns
Alignment	2.60[ab]	3.40[a]	1.57[b]	2.60[ab]	0.235	0.03
Type Bs	2.60	2.60	2.43	3.00	0.214	ns
Erosion	1.80[b]	1.80[b]	1.57[c]	3.00[a]	0.218	0.08
Caps	3.00[ab]	3.80[a]	2.43[b]	1.80[c]	0.273	0.05
Type As	1.40	1.40	1.00	1.00	0.126	ns
Depression	1.80[c]	4.00[a]	2.57[b]	1.00[c]	0.375	0.03
Cubic	1.40	1.00	1.00	1.00	0.091	ns
Aragonite	1.00	1.00	1.00	1.00	0.000	ns
Changed membrane	1.00	1.00	1.86	1.00	0.188	ns
Total score	23.20[b]	29.00[a]	21.00[b]	21.20[b]	0.939	0.002

SEM = standard error of the means, ns = not significant

[a–c]values with different superscripts are statistically different within the same row

the studied genetic groups in eggshell thickness, the brown breed eggshell was thicker than the others (Table 2). Shell thickness is the main factor contributing to the mechanical strength of an eggshell; thicker eggshells do not guarantee stiffer or stronger eggs (Bain 2005). Genetics plays a very important role in determining eggshell characteristics (Silversides and Scott 2001; Tumova et al. 2007; Fathi et al. 2016). Generally, all Saudi native breeds (especially brown and gray) had high and acceptable breaking strength under the prevailing high ambient temperature throughout the experimental period (Table 2), while it was documented that eggs produced from the commercial strains had lower eggshell weight, shell thickness, and shell strength at higher ambient temperatures (Roberts 2004; Franco-Jimenez et al. 2007). Therefore we may state that getting such benefits in eggshell quality characteristics of native breeds is a necessity under heat stress condition.

Table 3 illustrates the ultrastructural parameters which affect the strength and microstructure of eggshell. It is clear that brown breed had the least significant values of confluence, caps, and depression in comparison with the other breeds, while black-barred breed recorded the highest and significant values for confluence, fusion, alignment, caps, and depression parameters compared to other genetic groups, which means deteriorated eggshell microstructure qualities related with the black-barred breed (Figure 1). Erosion criterion was significantly higher in brown local breed followed by black and black-barred chicken eggs (Table 3, Figure 2), while gray breed was the best one for erosion measurement (lower estimated value). Good confluence in brown breed was observed, meaning that the mammillary caps made a good attachment with membranes (Figure 3). No significant differences between the studied breeds as regards cuffing, type A, type B, cubic, aragonite and changed membrane were observed. Figure 4 showed type B and a poor confluence in

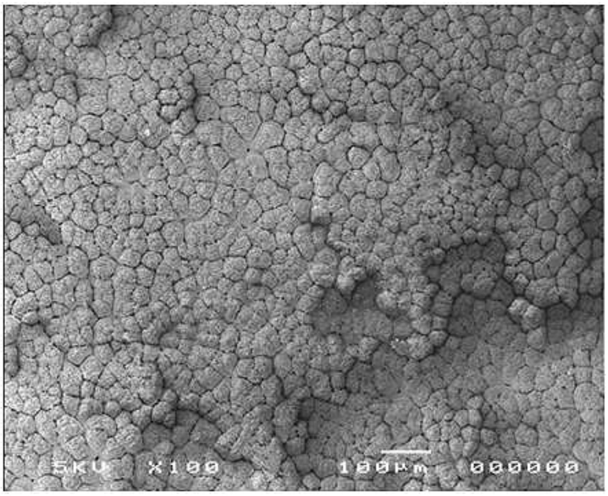

Figure 1. Depression – black-barred breed eggshell

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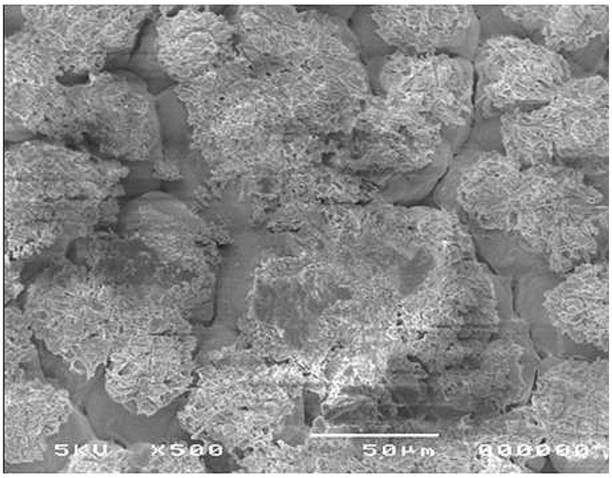

Figure 2. Erosion – black breed eggshell

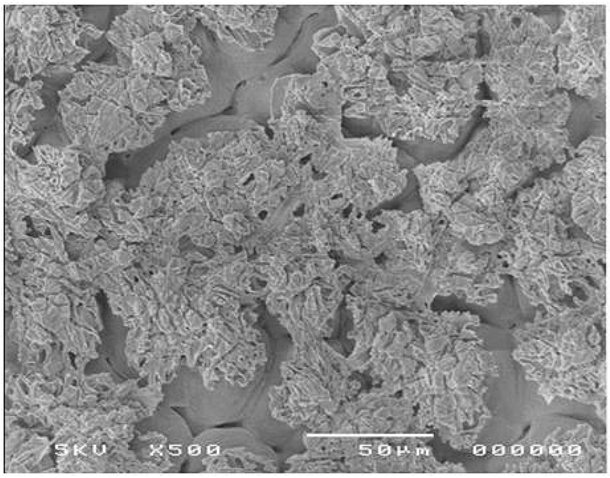

Figure 3. A good confluence in brown breed eggshell

gray breed, suggesting poor ultrastructural construction. Nascimento et al. (1992) stated that the aberrant crystal forms of type B bodies provided no meaningful contribution to the palisade layer and poor cap modifications do not offer any anchorage points. Also, it is well known that abnormalities in mammillary knob layer are directly related to eggshell stability (Bain et al. 2006; Solomon 2010). Collectively, total ultrastructural score value was significantly higher in black-barred breed (meaning bad quality), suggesting lower microstructure quality for eggshell related with this breed, comparing with gray, brown, and black breeds, respectively with low total score indicating better shell quality. Consequently, from ultrastructural point of view, the eggs produced from those birds had a stronger eggshell as compared to the black-barred ones, although there was no significant difference between them for eggshell thickness.

Table 4 presents data on cross-sectional lengths of eggshell ultrastructure layers (mammillary and palisade). Of all the Saudi native breeds studied, the palisade layer was significantly the longest in gray breed, followed by brown and black breeds, while it was noteworthy shorter in black-barred breed ($P < 0.05$). According to Bain (1992), the palisade layer reinforces the shell, thereby imparting strength to the egg. Thus, a reduction in its relative thickness could compromise strength, leading to a higher incidence of breakage during incubation and a subsequent reduction in embryo viability. Improved shell ultrastructure reflected good attachment to membranes and caps. Regarding the mammillary layer length, it was the greatest in brown breed, while black breed recorded the lowest value, both black-barred and gray showed intermediate lengths ($P < 0.05$; Table 4). Concerning total thickness, it could be noted that both brown and gray breeds attained the highest values compared to black and black-barred ones, respectively ($P < 0.05$; Table 4). As shown in Table 4, no significant variations between the four breeds were observed for both mammillary and palisade percentages. The mammillary layer does not contribute to the stiffness of shell but late fusion of adjacent palisade columns is related to fracture toughness (Bain 1992; Fathi 2001). Another powerful point to increase resistance to eggshell cracking is columnar density. It is noteworthy that the lowest columnar density

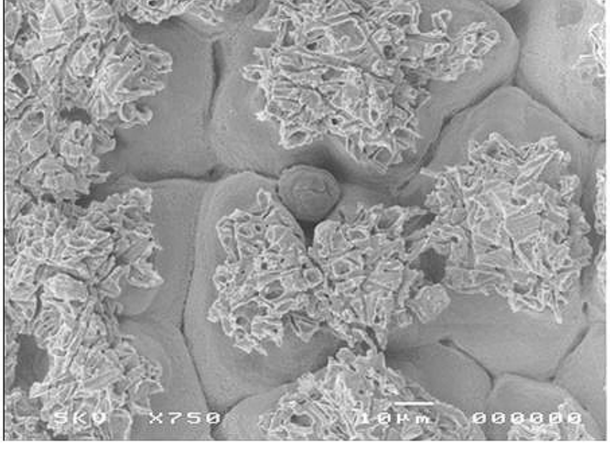

Figure 4. Type B and a poor confluence in gray breed eggshell

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

Layer	Breed				SEM	*P*-value
	black	black-barred	gray	brown		
Palisade	286.64[b]	277.11[c]	294.53[a]	289.02[b]	3.33	0.05
Mammillary	45.87[c]	49.13[b]	47.75[b]	56.15[a]	1.55	0.05
Total	332.51[b]	326.24[c]	342.29[a]	345.17[a]	3.30	0.05
Palisade (%)	86.2	84.9	86.1	83.7	0.87	ns
Mammillary (%)	13.8	15.1	13.9	16.3	0.87	ns

SEM = standard error of the means, ns = not significant

[a–c]means without the same superscripts in each row significantly differ

Palisade (%) = Palisade/Total × 100, Mammillary (%) = Mammillary/Total × 100

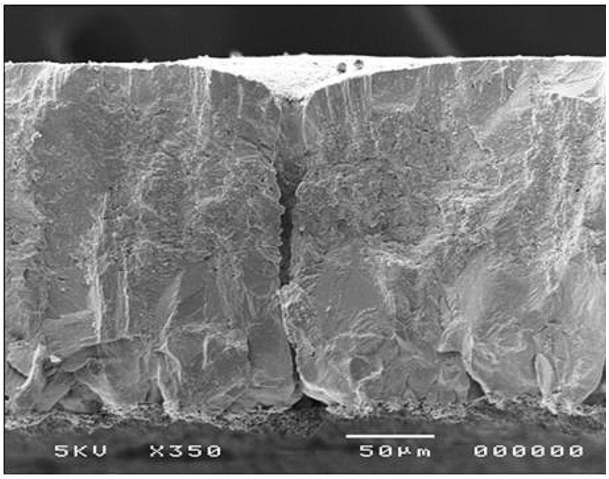

Figure 5. Columnar low density – black-barred breed eggshell

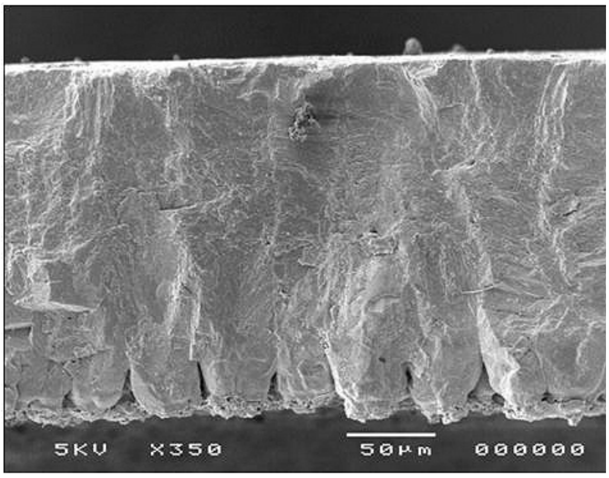

Figure 6. Columnar high density – brown breed eggshell

(decreased ultrastructural quality of eggshell) was detected in black-barred breed (Figure 5), whereas the highest columnar density (increased ultrastructural quality of eggshell) was observed in brown breed (Figure 6). Several previous studies illustrated that there is a strong correlation between crystallographic texture and eggshell strength (Carnarius et al. 1996; Fathi 2001).

CONCLUSION

The gray, brown, and black Saudi native breeds studied showed an outstanding eggshell ultrastructure and stiffer and stronger eggshell if compared with the black-barred counterpart, even under high ambient temperatures. At national or regional level, for crossbreeding and selection programs aimed at producing good quality table and hatching eggs the gray, brown, and black breeds can be recommended due to excellent ultrastructural properties of eggshell.

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