Scanning Electron Microscopic Comparison of Different Poultry Species' Eggshells

Farklı Kanatlı Türlerine Ait Yumurta Kabuklarının Tarama Elektron Mikroskobik Karşılaştırması

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ABSTRACT Objective: The aim of this study is to investigate the eggshells of different poultry species such as goose, duck, turkey, chicken and quail by scanning electron microscopy (SEM). **Material and Methods:** After collecting the eggs, their width and length were measured for shape index ratios. Then they were broken and left in distilled water for a night and the remaining membranes were detached and the eggshells were left to dry. Then the samples were covered with gold palladium and observed by using scanning electron microscope. SPSS 16.0 was used for statistical analysis. **Results:** Cross-section micrographs of species derived from SEM showed that the layers from the outer surface to the inner surface were cuticle, vertical crystal, palisade layer, mammillary layer and eggshell membrane, respectively. Hollow vesicles were observed in the palisade layer of the eggshells of all species. Passages were observed among the walls of hollow vesicles of quail eggshell. It was observed that basal caps of the mammillary bodies took place on the eggshell membrane in all species. The shape indices of eggs from geese (64.74 ± 3.3%) and quails (80.40 ± 4.5%) were significantly different from each other and were also different from the other three species' eggs. However, regarding the eggshell thicknesses, each species of goose and quail were significantly different from the other four species. **Conclusion:** Although there are some differences among the microstructure of avian eggshells, it can be said that the structure of their eggshells are generally similar to each other.

Key Words: Egg shell; geese; mamillary bodies; quail

ÖZET Amaç: Bu çalışmanın amacı, taramalı elektron mikroskobu (SEM) ile kaz, ördek, hindi, tavuk ve bıldırcın gibi farklı kanatlı türlerinin yumurta kabuğunu incelemektir. **Gereç ve Yöntemler:** Yumurtalar toplandıktan sonra şekil indeksi oranlarını hesaplamak için yumurtaların en ve boyları ölçüldü. Kırılan yumurtaların kabukları bir gece boyunca distile su içinde tutuldu ve kalan zarlar uzaklaştırıldıktan sonra kurumaya bırakıldı. Daha sonra altın palladium ile kaplanan örnekler taramalı elektron mikroskop ile incelendi. İstatistiksel analizler için SPSS 16.0 programı kullanıldı. **Bulgular:** SEM ile elde edilen tüm türlere ait enine kesitten alınan mikrograflarda dıştan içe doğru sırasıyla kütikül, dikey kristal, süngerimsi katman, mememsi katman ve yumurta kabuğu zarı tabakaları görüldü. Tüm türlerin yumurta kabuğunun süngerimsi katmanında gözenek vezikülleri gözlendi. Bıldırcın gözenek veziküllerinin duvarları arasında geçişler olduğu ve tüm türlerde meme gibi cisimlerin uç kısımlarının yumurta zarının üzerine oturduğu görüldü. Yumurtalar şekil indeksi oranları bakımından karşılaştırıldığında kaz (64,74 ± %3,3) ve bıldırcın (80,40 ± %4,5) arasında istatistiksel açıdan anlamlı fark olduğu ve bu türler ile diğer türler arasında da istatistiksel farkın anlamlı olduğu bulundu. Yumurta kalınlığı bakımından kaz ve bıldırcın türlerinin her biri diğer dört türden istatistiksel olarak farklıydı. **Sonuç:** Kanatlı yumurta kabuklarının ultrastrüktürel yapıları arasında bazı farklılıklar olmasına rağmen genel olarak farklı türlere ait yumurta kabuğu yapılarının birbirine benzer olduğu söylenebilir.

Anahtar Kelimeler: Yumurta kabuğu; kaz; mememsi cisimler; bıldırcın

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The eggshell is known as a respiratory organ for embryos and its structure has some barriers to exchange gases which the embryo needs.[1] At the same time, the eggshell protects the egg from bacteria, predation, dehydration and mechanical effects by small animals.[1-4] The majority of the eggshell structure is made of mineral. The avian eggshells include

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layers which extend from the innermost to the outermost; the inner and outer membrane, the mammillary, the palisade, the vertical crystal and the cuticle layers.[5] Avian eggshell includes more calcium than other elements.[5,6] The egg consists of a shell membrane, shell, albumen and a yolk.[7] The eggshell membrane, which is the first layer of eggshell, surrounds the albumen as mentioned before.[8] The shell membrane is divided into two layers, an inner and an outer membrane, and the outer membrane sticks to the basal caps of a mammillary bodies layer that is the second layer of the eggshell.[9] The palisade layer of the eggshell is located above the mammillary layer, and the cuticle is located above the palisade layer.[9,10] Mineralization of eggshell occurs in the uterus. The protein and ion composition of the uterus gradually change during this process because the epithelium and mucosa cells of the uterus secrete all required components for eggshell mineralization.[5,11] This eggshell mineral organization should be strong enough to protect the contents of the egg and also the embryo during hatching.[12] The avian egg has a stable shape because of the hard eggshell.[13] There are different egg shapes among species of avian eggs. The shape differences are characterized by the shape index. There are three kinds of shapes; one is sharp if the shape index has a value less than 72%, the second is normal if the shape index has a value between 72% and 76% and the third is round if the shape index has a value greater than 76%.[14] Many structural studies have been conducted on avian eggshells, but the eggshell of the chicken species is usually chosen in these kinds of studies, because a chicken egg is an important nutritious food for humans; as a result, few studies have dealt with the eggshell of other species.[1]

This study aims to investigate the eggshells of different poultry species such as goose, duck, turkey, chicken and quail by scanning electron microscopy (SEM) in order to reveal the structural differences among the eggshells of these species.

MATERIAL AND METHODS

Within the scope of the study, two eggs of each species were investigated by SEM and eight eggs of each species were used to measure the thickness of the eggshells and the shape index of the eggs. The eggs of geese (*Anser anser*), native Turkish ducks, turkeys (*Meleagris gallopavo*) and chickens (*Gallus gallus domesticus*) were provided from the producers in Kars who breed local strains while the eggs of Japanese quail (*Coturnix coturnix japonica*) were provided from the farm of the Veterinary Faculty at Kafkas University. After the eggs were broken, their eggshells were put in distilled water and the membranes of the eggshells were detached by hand. The eggshells were left in distilled water over night and the remaining membranes were detached. Then, the eggshells were left to dry for about 24 hours. For scanning electron microscopic investigation, 0.5 cm^2 sized eggshells were taken from the equatorial areas of the eggs. In order to obtain conductivity, the samples were covered with gold palladium in an EMS 550 Sputter Coater for 3 minutes. The samples were observed by using scanning electron microscope (Zeiss EVO 50) between 15 kV and 30 kV. The width, length and the thickness in the equatorial areas of the eggshells were measured by a 0.02 mm sensitive caliper. **Statistical comparisons:** One-way ANOVA of the SPSS 16.0 package program was used when the species were compared in terms of eggshell thickness in the equatorial area and shape index (width/ lengthx100) ratio. Because of the homogeneity variances, Dunnett's T3 post hoc test was used for comparison of eggshell thickness and Bonferroni post hoc test was used for comparison of shape index ($P<0.05$).

RESULTS

Micrographs of a cross-section of the eggshells of all species were derived from scanning electron microscopy which showed that the layers from the outer to the inner include cuticle, vertical crystal, palisade, mammillary and eggshell membrane, respectively. A rectangular appearance was found by chance in one side of a mammillary body of a turkey eggshell (Figure 1). Hollow vesicles (spherical voids) were observed in the palisade layer of the eggshells of all species. Passages were seen between the hollow vesicle walls of quail eggshell and their hollow vesicles were larger than those of

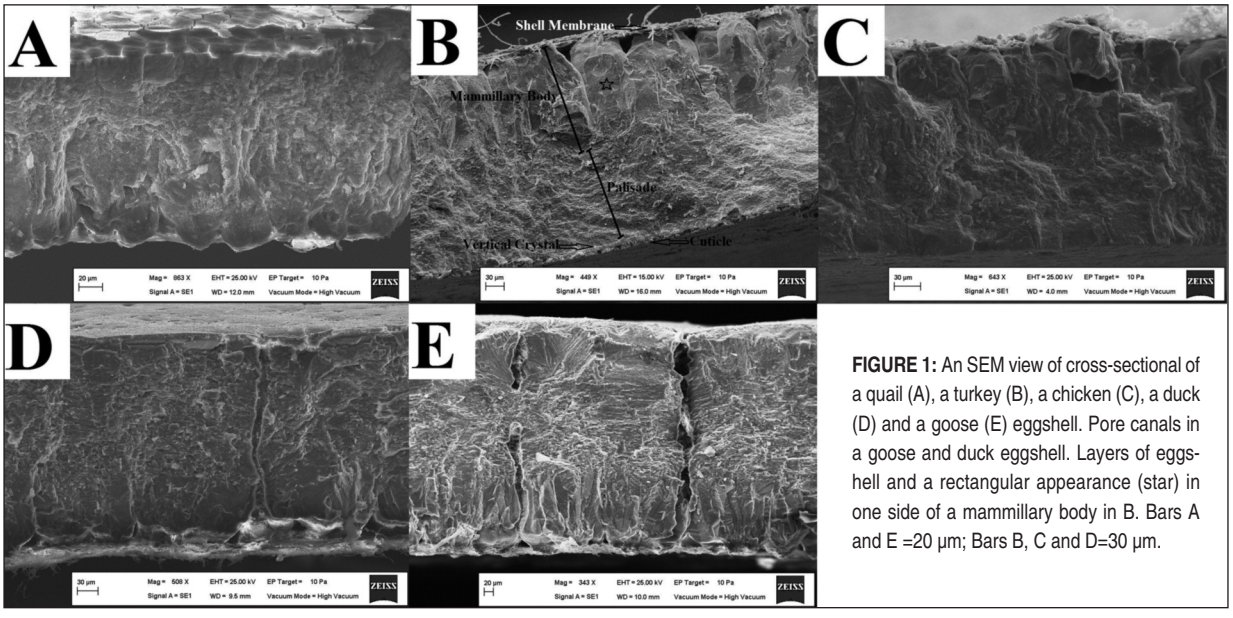

FIGURE 1: An SEM view of cross-sectional of a quail (A), a turkey (B), a chicken (C), a duck (D) and a goose (E) eggshell. Pore canals in a goose and duck eggshell. Layers of eggshell and a rectangular appearance (star) in one side of a mammillary body in B. Bars A and E =20 µm; Bars B, C and D=30 µm.

other species. Moreover, the boundaries of the hollow vesicles in this species were distinctively flatter than those of other species and their walls were transparent in quails (Figure 2). Structural similarity in mammillaria layers, palisade layers and pore canals of duck and goose eggshells were remarkable. It was observed that the appearance of mammillaria and palisade layers and hollow vesicles of chicken and turkey eggshells were similar to each other (Figures 1, 2). It was seen that the basal caps of mammillary bodies of all the species eggshells investigated were perfectly placed on the eggshell membrane and each tip of the basal caps was attached to the membrane in all species (Figure 1). It was seen that the eggshell membrane had more than one membrane layer which included fibers. These membrane fibers of eggshells were composed of a tight reticular structure intertwined in all directions which were parallel to the eggshell surface. It was shown that fibers of the membrane did

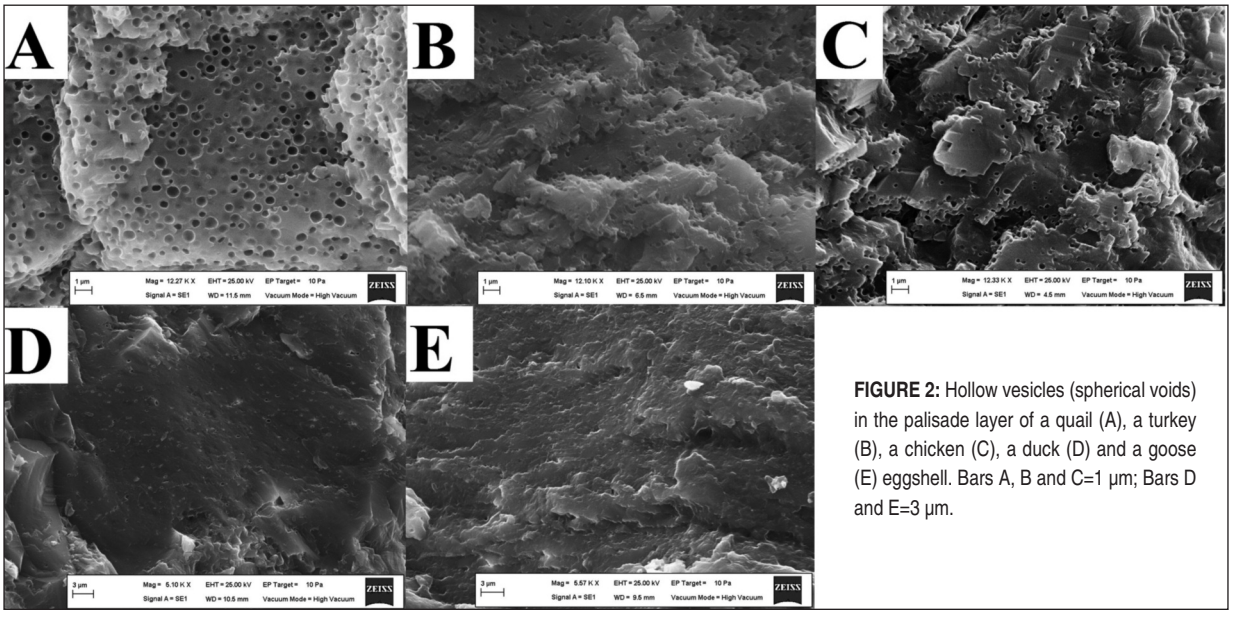

FIGURE 2: Hollow vesicles (spherical voids) in the palisade layer of a quail (A), a turkey (B), a chicken (C), a duck (D) and a goose (E) eggshell. Bars A, B and C=1 µm; Bars D and E=3 µm.

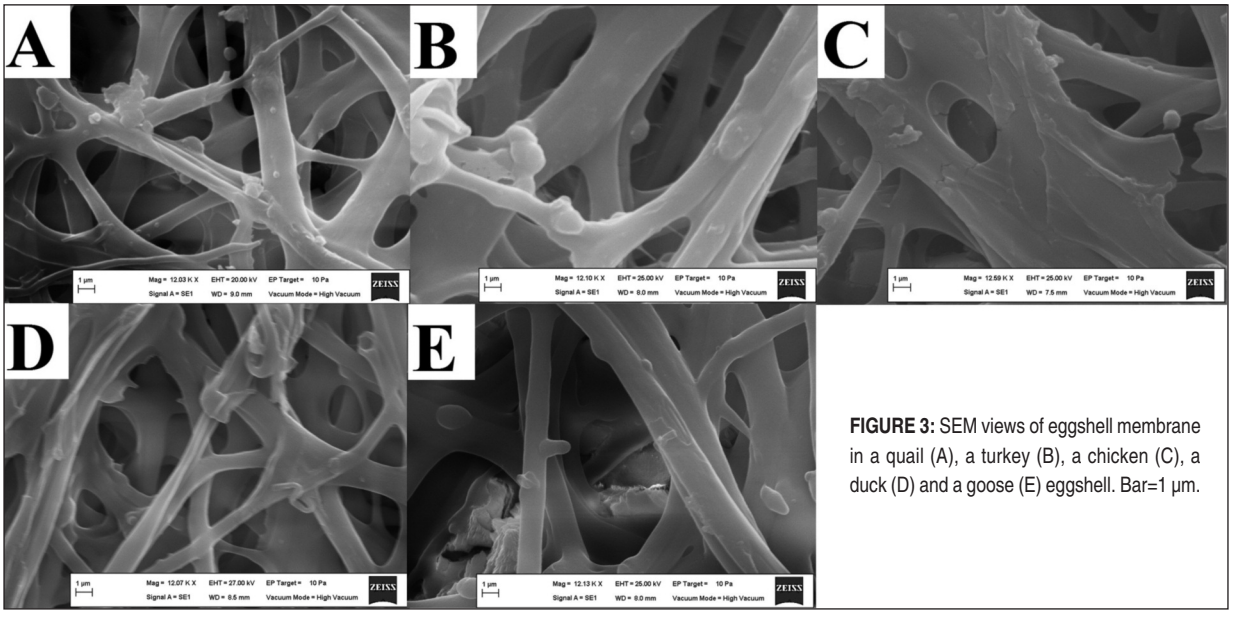

FIGURE 3: SEM views of eggshell membrane in a quail (A), a turkey (B), a chicken (C), a duck (D) and a goose (E) eggshell. Bar=1 µm.

not cover all parts of the inner surface in all species' eggshell, and there were some interspaces among the fibers. The broken fiber was like a tubule which had an empty inside and was covered by a mantle layer was observed (Figure 3). On the outer surfaces of all species' eggshells, similar structural features were seen. The cuticle layer covered every part of the outer surface of the eggshell and a network of fissures was shown on the cuticle in all species. There were more networks of fissures on the cuticles of quail and turkey eggshells than those of other species. Pore openings on the cuticle were seen but there were very few pore openings in all species' eggshell because many of them were covered by cuticle (Figure 4). Significant statistical differences were determined when the shape index ratios of the poultry species eggs were compared. The shape index ratios of eggs of the geese (64.74 ± 3.3%) and quails (80.40±4.5%) were different from each other and at the same time both of

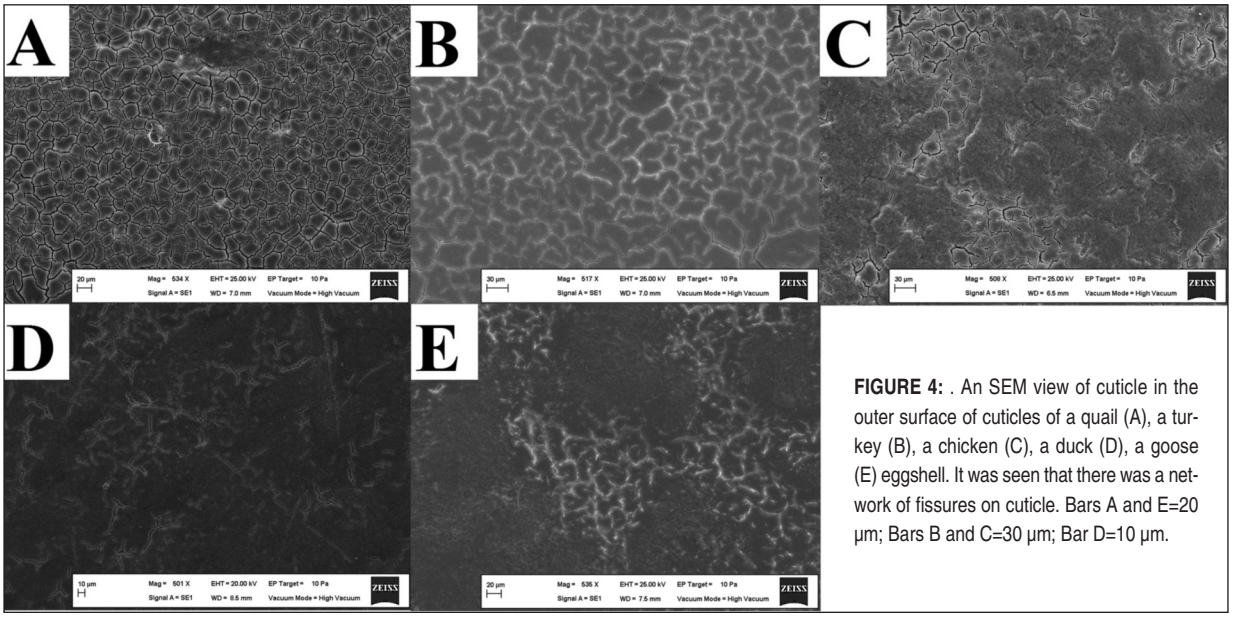

FIGURE 4: . An SEM view of cuticle in the outer surface of cuticles of a quail (A), a turkey (B), a chicken (C), a duck (D), a goose (E) eggshell. It was seen that there was a network of fissures on cuticle. Bars A and E=20 µm; Bars B and C=30 µm; Bar D=10 µm.

TABLE 1: Comparison of shape index ratios of the eggs among the poultry species.

Poultry Species	n	Shape Index of the eggs (%) Mean ± SD
Goose	8	64.74 ± 3.3[a]
Duck	8	74.54 ± 2.3[b]
Turkey	8	72.13 ± 2.9[b]
Chicken	8	74.72 ± 3.0[b]
Quail	8	80.40 ± 4.5[c]

Values of shape index are mean ± SD (Std. Deviation), and those with different letters (a,b and c) are significantly different (*p<0.05).

TABLE 2: Comparison of eggshell thickness among the poultry species.

Poultry Species	n	Thickness of the eggshell (mm) Mean ± SD
Goose	8	0.607 ± 0.045[a]
Duck	8	0.390 ± 0.018[b]
Turkey	8	0.400 ± 0.018[b]
Chicken	8	0.325 ± 0.017[c]
Quail	8	0.212 ± 0.018[d]

Values of eggshell thicknesses are mean ± SD (Std. Deviation), and those with different letters (a,b,c and d) are significantly different (*p<0.05).

them were different from those of the three other species' eggs. It was found that the eggs of ducks (74.54 ± 2.3%), turkeys (72.13 ± 2.9%) and chickens (74.72 ±3.0%) were statistically similar in terms of their shape index ratios, but they were different ($p<0.05$) from those of geese and quails (Table 1). However, regarding the average of the eggshell thickness, the species of geese, chickens and quails were significantly different from those of the other four species. The average of the eggshell thickness of ducks (0.390 ± 0.018 mm) and turkeys (0.400 ± 0.018 mm) were statistically similar to each other but thicker than the eggshells of chickens (0.325 ± 0.017 mm) and quails (0.212 ± 0.018 mm) but thinner than the eggshell of goose (0.607 ± 0.045 mm), ($p<0.05$) (Table 2).

DISCUSSION

The aim of this study was to investigate the eggshells of different poultry species such as goose, duck, turkey, chicken and quail by scanning electron microscopy (SEM) to compare the eggshell thicknesses and shape indices of these species' eggs.

Panheleux et al. stated that the eggshell structures of various poultry species were similar but this similarity was quite clear in the mammillaria layers of geese and ducks eggshells.[10] We found that the eggshell structure of five species was generally similar to each other but there was some similarities between geese and ducks, and between chickens and turkeys. We thought that these similarities were dependent on their families because geese and ducks are members of the *anseriformes* family (known as waterfowl), and chickens and turkeys are members of the *galliformes* family. Shen and Chen concluded that hollow vesicles in the palisade layer were compact in the ducks eggshells and there were also many of them in the chickens' eggshells.[15] In the current study, hollow vesicles were seen in compact form apart from those of the quails eggshells. We could not find in any literature anything concerning these hollow vesicle passages which we found between those hollow vesicles in the palisade layer of the quails eggshells. According to us, although quail is a member of the *galliformes*, its eggs are smaller than those of others, so it has different shape of hollow vesicles from others. In a study, Mao et al. reported that eggshell membranes consist of the inner and the outer layers parallel to the inner surface of the eggshell.[16] Tan et al. mentioned that the fibers of the eggshell membrane had mantle layer and they were tubelike.[17] In the current study, our results were parallel with results of Mao et al. and Tan et al. regarding eggshell membrane and their fibers in all species.[16,17] Rodriguez-Navarro et al. indicated that there were fissures on the cuticle of chicken eggshell and the cuticle covered pore openings of the outer most layer of eggshell.[18] Fraser and Cusack concluded that there were cracks on the SEM examination of the cuticle because of dry conditions.[19] In our study, we found that micro cracks on cuticle of all species' eggshell and cuticle covered every part of the outer most layer of eggshell. Ozcelik indicated that the average shape index of Japanese quail eggs was 80.40% and the thickness of their eggshells was 0.23 mm.[20] These values were close to the values measured in the current study. Sarica and Erensayin mentioned three kinds of

shape index and according to their classification, our results about shape index included three kinds of them.[14] The quails eggs were round, geese eggs were sharp and eggs of chickens, turkeys and ducks were found to be normal in the current study. Nedomova and Buchar showed that the average shape index of geese eggs was 65% and their result was almost the same as the values in our study.[21] Saatci et al. and, Tilki and Inal found that the shape index of geese eggs was greater than that of the current study.[22,23] Kokoszynski et al. reported that the thickness of duck eggshell was between 0,379 and 0,391 mm.[24] It was similar to our results and they found that the shape index of duck eggs' values were similar to the values of this study. Dodu indicated that the shape index of turkey eggs was 74% and the eggshell thickness' values of turkey eggs were between 0.352 and 0.444 mm.[25] The shape index average was greater than that of this study but the eggshell thickness' values were similar to the values of the current study.

CONCLUSION

In conclusion, there are some differences among the microstructure of avian eggshells but it can be said that the structure of their eggshells are generally similar to each other. We found that the quails have the thinnest eggshells and a different shape of hollow vesicles from the other species so we concluded that the fetus breaks it easily at birth. We suggest that hollow vesicles of different species' eggshell should be studied with every aspect of their structure.

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