Kafkas Univ Vet Fak Derg
22 (6): 937-943, 2016
DOI: 10.9775/kvfd.2016.15668

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Comparison Between Goose *(Anser anser)* and Chicken *(Gallus gallus domesticus)* Eggshells During Embryonic Development by Scanning Electron Microscopy [1] [2]

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[1] *This study was supported by the Scientific Research Fund of Kafkas University (Project No: 2011-AHSYO-37)*
[2] *Summary of this study was presented in "II. International VETistanbul Group Congress 2015" 7-9 April, 2015, Saint-Petersburg, Russia*

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Article Code: KVFD-2016-15668 Received: 16.03.2016 Accepted: 29.07.2016 Published Online: 01.08.2016

Abstract

The purpose of this study was to examine the comparative differences of eggshell structure between geese and chickens during embryonic development by SEM. Goose eggs and chicken eggs were used for the study. Eggs of each species were divided into three groups: The eggs within first group were used subsequent to collection, those of second group were collected during the mid-point of incubation and those of third group were collected at the end of the incubation. The eggshells samples with dimensions of 0.5 cm^2 were taken from the equatorial region of eggshells. After these samples were passed through routine process, they were examined via SEM for structural changes of eggshells and rate of elements. In the examination, there were not changes of eggshell structure between two species for days of the incubation. Eggshells thickness of each species and the rate of calcium, oxygen, nitrogen and carbon of eggshells were not statistically different among groups. It was concluded that structural changes of eggshell did not have significant effect on low yield for hatching of goose.

Keywords: *Calcium, Chicken, Eggshell, Embryo, Goose*

Kaz *(Anser anser)* ve Tavuk *(Gallus gallus domesticus)* Yumurta Kabuğunun Embriyonik Gelişim Sırasında Taramalı Elektron Mikroskop ile Karşılaştırılması

Özet

Bu çalışmada, taramalı electron mikroskop kullanılarak embriyonik gelişim sırasında kaz ve tavukların yumurta kabuğundaki değişiklikleri karşılaştırmak amaçlanmıştır. Bu çalışmada kaz ve tavuk yumurtası kullanılmıştır. Her iki türe ait yumurtalar üç gruba ayrıldı. Birinci gruba ait yumurta kabukları kuluçkaya konulmadan, ikinci gruba ait yumurta kabukları kuluçka süresinin ortasında ve üçüncü gruba ait olanlar ise kuluçkanın son günü olan çıkım gününde toplandı. Her bir yumurta kabuğunun ekvator kısmından 0,5 cm^2 ebatında örnekler alındı. Bu örnekler rutin prosedürlerden geçirildikten sonra taramalı elektron mikroskobunda yapısal değişiklikler ve element oranı bakımından incelendi. Elektron mikroskop incelemesinde, kaz ve tavuk türlerinin kuluçka günlerine bağlı olarak yumurta kabuğu katmanlarında belirgin bir değişikliğin olmadığı tespit edildi. Her iki türün kendi içinde kuluçka günlerine göre yumurta kalınlığında ve kabuklardaki elementlerin oranlarında istatistiksel olarak benzerlik olduğu görüldü. Yumurta kabuğunun embriyonik gelişim boyunca uğradığı yapısal değişikliklerin kaz kuluçka veriminin düşük olmasında önemli bir etkiye sahip olmadığı sonucuna varıldı.

Anahtar sözcükler: *Embriyo, Kalsiyum, Kaz, Tavuk, Yumurta Kabuğu*

INTRODUCTION

Poultry lay and incubate their eggs in the nest which is made by them on the ground or in a tree in dry environments. Growth of the embryo is completed in the eggshell during the development process [1]. Poultry eggs have a specific structure and function which prevent the embryo from external infection, exchanges of heat and

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physical condition, and provide what the embryo needs to develop. This complex structure both regulates exchanging water and metabolic gases, and provides calcium for the embryo [2]. It is required that poultry eggs are rotated during the development stages. Any mistakes during this process may cause of a decrease in oxygen uptake, delayed extra embryonic membranes, vascularization and embryonic development [3,4]. Generally, it is known that the yield of incubation rate of waterfowl is lower than that of chicken. High embryonic mortality rate is seen during incubation in geese [5]. The yield of incubation in geese was between 22% and 29% in the Kars region [6]. Conversely, the yield of incubation in chickens is between 83% and 97% [7]. Studies on incubation technique include preheating before placing eggs in the incubator [8], disinfected eggshell with ultra-violet pre and during incubation [9], and the effects of temperature and humidity [8,10]. Eggshell consists of some layers of calcium carbonate ($CaCO_3$). Elements of $CaCO_3$ were based on different sources. Calcium is the most common element in the eggshell structure [11,12]. Carbon and oxygen in this compound are obtained from digestion material rather than stored in tissue [13]. It is shown clearly that eggshell of during embryonic development would be used to determine oxygen and carbon source of species [14]. There are limited studies about nitrogen in eggshells but it is known that nitrogen is found in eggshell [15]. Interaction between organic and inorganic compounds of eggshell has been revealed with regard to the structure and function. Eggshells include four layers in sequence from the inside of the eggshell mammillary layer, palisade layer, vertical crystal layer and cuticle [16]. The mammillary layer is a basic layer of calcium source to support the embryo by providing calcium during the embryonic development. Nearly 80% of calcium, which is needed by embryo during incubation, is provided by the eggshell. While the mammillary layer consists of a lot of structures which are similar to cones, the palisade layer is extended vertically on the mammillary layer. It is known that palisade layer organization is an important index in the strength and the hardness of eggshells [17]. The vertical layer is narrow and vertical, and extends from the palisade layer to the cuticle. Cuticle, which can be seen clearly from out of eggs, is the outermost layer of an eggshell [17,18]. The rate of egg defection changes between 7% and 11% during incubation, collecting and packing the eggs [12]. The eggshell quality is the most important problem in egg production [12,19] because low eggshell quality always causes yield losses of between 5% and 8% [20].

The purpose of this study was to reveal whether there is an important effect of eggshell structure in geese hatching by examining the comparative differences of eggshell structure between geese (a species of waterfowl); with a low yield for hatching, and chickens; with a high yield for hatching, during embryonic development by scanning electron microscopy (SEM).

MATERIAL and METHODS

This study was approved by Kafkas University, Local Ethics Commission of Experimental Animals (Decision no: KAU-HADYEK/2011-14).

In this study, a total of 15 geese eggs and 15 chickens eggs were used; each egg belongs to a different goose and a different chicken, and all of the eggs were obtained from local farms in Kars. The eggs of each species were divided into three groups. Eggs within the first group were used subsequent to collection and therefore not placed in an incubator, those of the second group were collected during the mid-point of incubation (the 16th day of study for goose and the 11th day of study for chicken) and those of the third group were collected at the end of the incubation process (the 31st day for goose and the 21st day for chicken). The eggshells were soaked in distilled water and the shell membranes were removed and then peeled from the samples. Following the removal of the shell membrane remnant, each sample was immersed in 6% of sodium hypochlorite, 4.12% of sodium chloride and 0.15% of sodium hydroxide solution overnight [21]. Later, the eggshells were immersed and removed from distilled water and were left to dry for 24 h. The eggshells samples with dimensions of 0.5 cm^2, were taken from the equatorial region of each eggshell. These samples were positioned on stubs and placed in the Coater, forming a platinum coating. These samples were examined via SEM (Zeiss/ Supra 55 FE-SEM) for structural changes of eggshells and the same samples were examined via BRUKER QUANTAX EDS (Energy Dispersive X-ray Spectroscopy) in SEM for determining the percentage of elements in eggshell. SEM images of all samples were obtained at 5kV, at 500X and 15.000X magnifications, and at 11 mm working distance. The thickness of the eggshell was measured by using ImageJ software (ImageJ 1.46r). To calculate the percentage of each element was used by following formula because of removing rate of platinum which is used for coating;

Final Percentage of the Element = (Percentage of the Element X 100)/(100 - Percentage of the Platinum)

All data of elements rate and eggshells thickness were statistically analyzed using SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA was used for determining differences and P-value was < 0.05.

RESULTS

From the eggshells of the first and second group; mammillary layer, palisade layer, vertical layer and cuticle were seen normal view with the SEM. For each species, on the ends of the mammillary knobs in the eggshells of the third group were seen to be partially flattened. In the SEM examination of goose and chicken eggshells

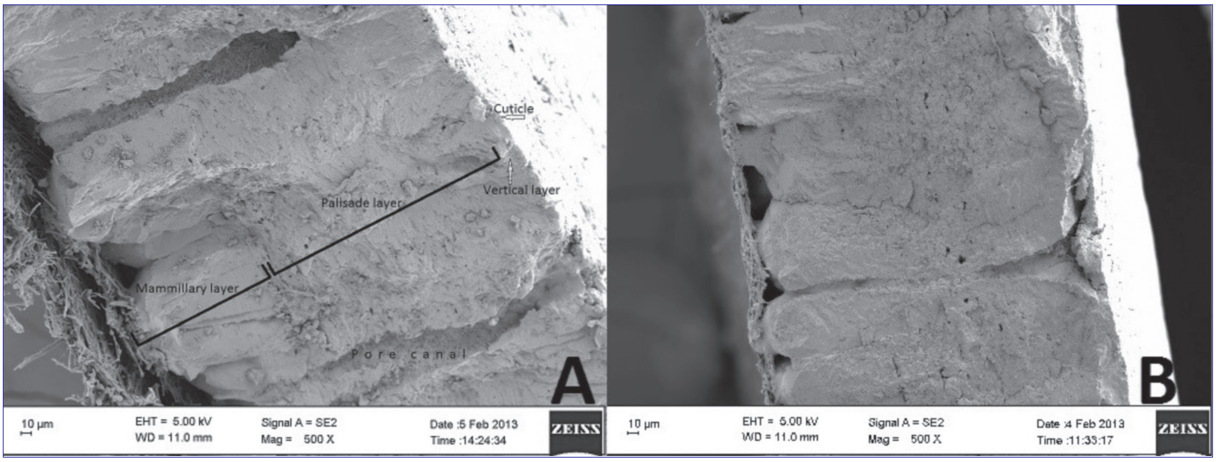
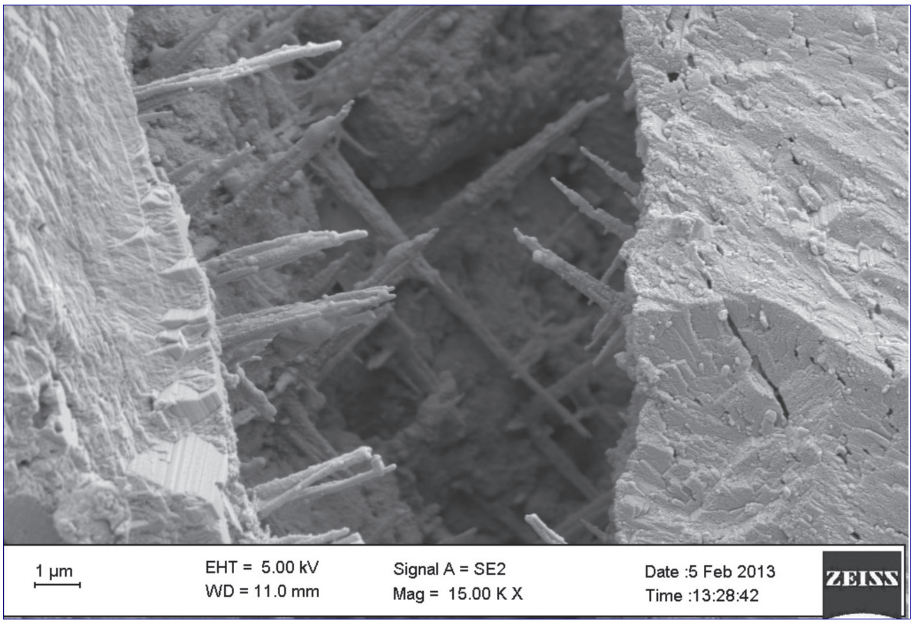


Fig 1. Cross-sectional SEM views of a goose (A) and a chicken (B) eggshells, before incubation. x500, Bar 10 µm

Şekil 1. Kuluçkaya konulmadan alınan kaz (A) ve tavuk (B) yumurtası kabuğu enine kesitinin SEM görünümü. x500, Bar 10 µm

Fig 2. A micrograph of the goose eggshell before incubation, a pore canal and some spire calcite structures which taper to center of pore canals. x15.000, Bar 1 µm

Şekil 2. Kuluçkaya konulmadan alınan kaz yumurtası kabuğundaki kanalın içine uzanan dikensi kalsiyum çıkıntıları mikrografı. x15.000, Bar 1 µm



were observed that the structure views of both species eggshells were similar to each other during embryonic development. The existence of pore canals in both goose and chicken eggshells drew attention to ultrastructural view in the cross section *(Fig. 1)*. It was found that the pore canals extended between the inner and the outer surface of both goose and chicken eggshells. The spire calcite structures which taper to center of the pore canals in the palisade layer of geese eggshells were visible *(Fig. 2)*. However, they were not seen in those of the chicken eggshells. In both species and in each group, the pore canals orifices on the inner surface of the eggshells were observed ultrastructural *(Fig. 3)* but those of outer the surface of the eggshells were usually coated by cuticle which is the outermost layer of the eggshell *(Fig. 4)*.

It was observed that palisade layer of the eggshells in both species contained many hollow vesicles disorderly in all groups *(Fig. 1)*. It was found that at the mid-point of the incubation and at the end of the incubation process in both geese and chickens eggshell membrane was able to be peeled from eggshell easier than that of first group of them *(Fig. 5)*. When examined with SEM, the inner surface of basal caps of mammillary layer was partially flattened in the eggshell at the end of the incubation process in both species. Apart from mammillary layer, the other layers of the eggshell were similar in appearance in all species from before incubation until the end *(Fig. 6)*. After analysis, it was found that the ratio of calcium, carbon and oxygen was not significantly different in both the mammillary and palisade layers in terms of incubation days among all groups *(Table 1)*. It was found that the ratio of nitrogen in both mammillary and palisade layers of geese and chickens eggshells were at a level of approximately 5% when result of EDS analysis in the SEM was considered. In these eggshell layers of both species, there was not significantly difference when

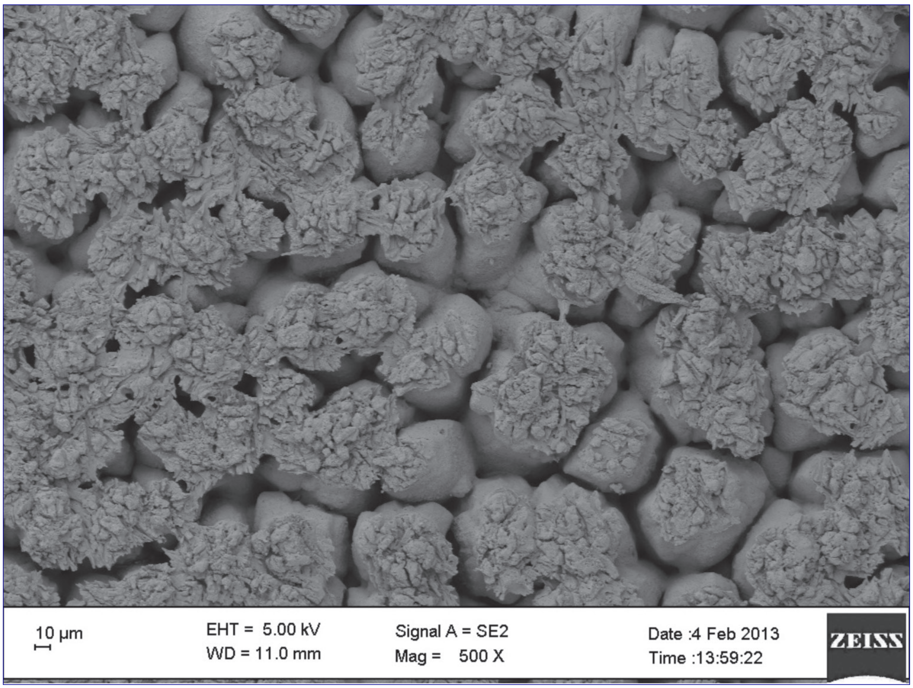


Fig 3. An SEM view of pore canals in inner surface of a chicken eggshell on the 11th day of incubation. x500, Bar 10 µm

Şekil 3. Kuluçkanın 11. gününde tavuk yumurta kabuğunun iç yüzünde gözenek kanallarının SEM görünümü. x500, Bar 10 µm

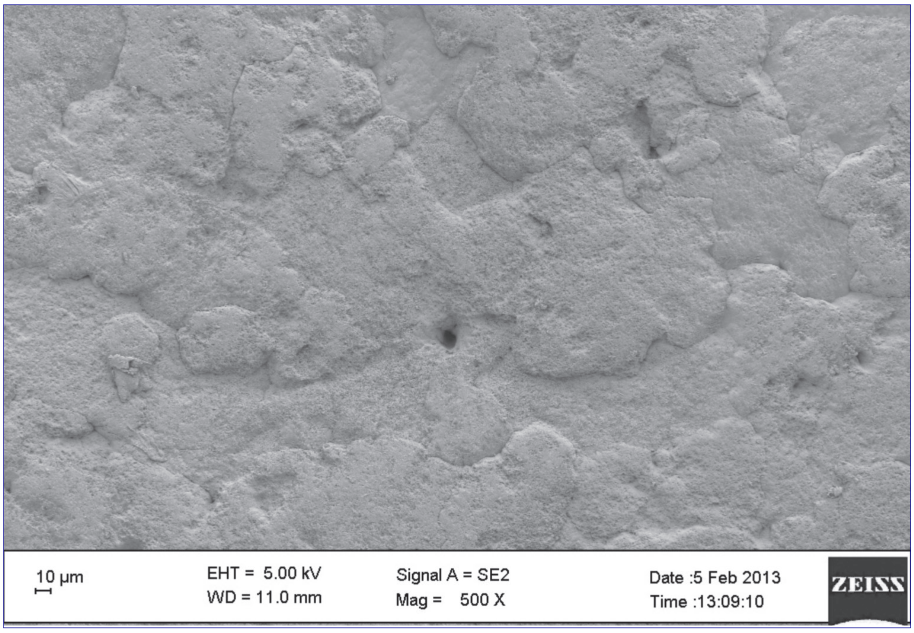


Fig 4. An SEM view of the outer surface of a chicken eggshell on 21st day of incubation. A few outer orifices of pore canals are seen in the micrograph. x500, Bar 10 µm

Şekil 4. Kuluçkanın 21.gününde tavuk yumurta kabuğunun dış yüzünün SEM görünümü. Mikrografta bir kaç gözenek kanalının girişleri görülmektedir. x500, Bar 10 µm

nitrogen ratio was analysed statistically for incubation days *(Table 1)*. Elemental analysis of the eggshell showed that the percentage of magnesium and sodium was less than 1% in both the mammillary and the palisade layers of both species for all groups. Furthermore, it was identified that the mammillary and the palisade layers of eggshells included calcium, oxygen, carbon, nitrogen and traces of magnesium and sodium in this study. It was calculated that the average thickness of chicken eggshell was 340.8±26.2 µm and that of goose eggshell was 495.8±22.2 µm. It was found that the thickness of chicken eggshells was not statistically significantly different among chicken groups for incubation days and that of geese eggshells was not significantly different in terms of statistics among geese groups for incubation days *(Table 2)*.

DISCUSSION

The purpose of this study was to examine and compare the differences in the structure of eggshells between geese; with low yield for hatching, and chickens; with high yield for hatching, during embryonic development by using SEM.

Parsons [22] reported that eggshells contained; shell membrane, mammillary layer, palisade layer, vertical crystal

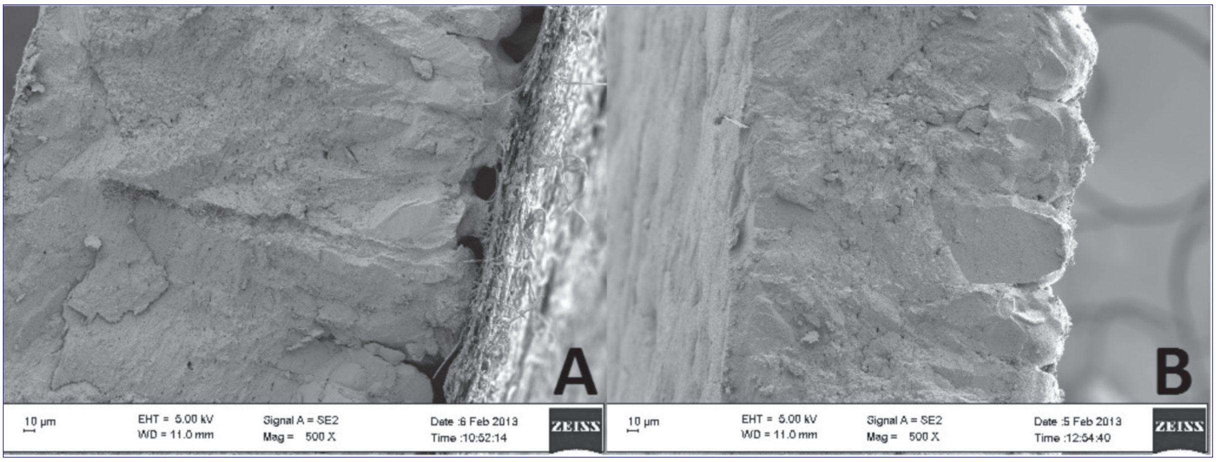


Fig 5. SEM views of cross-section of a goose (A) on the 16th day and a chicken (B) eggshell on the 11th day of incubation. x500, Bar 10 µm

Şekil 5. Kuluçkanın 16.gününde ki kaz (A) ve 11.gününde ki tavuk (B) yumurta kabuğunun enine kesitinin SEM görü-nümü. x500, Bar 10 µm

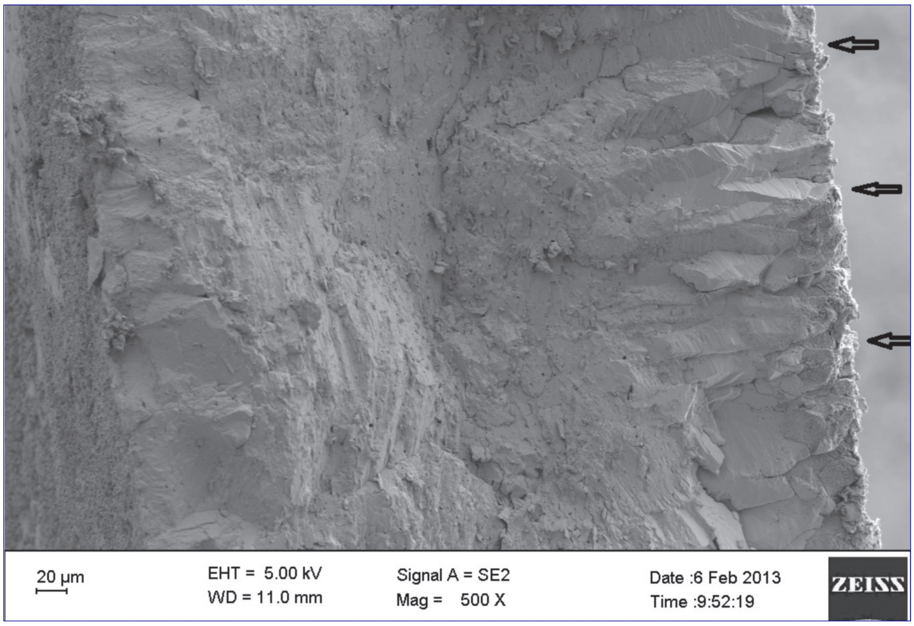


Fig 6. An SEM view of a goose eggshell at the end of incubation. It is seen that the inner surface of mammillary layer was partially flattened *(arrows)*. x500, Bar 20 µm

Şekil 6. Kuluçkanın sonunda kaz yumurta kabuğunun SEM görünümü. Mammillari katmanının kısmen düzleştiği *(oklar)* görül-mektedir. x500, Bar 20 µm

layer and cuticle. According to Abdel-Salam et al.[23], embryo consumes calcium and other elements from the mammillary layer during embryonic development in the incubation period. Ruiz and Lunam [18] reported that the eggshell quality was not significantly decreased during the hatching period. In the present study, we have identified that the basal caps of the mammillary layer on the last days of incubation were partially flattened. Besides that, it was observed that in all layers of eggshell during incubation there were not any major differences between goose and chicken eggshells. Simons and Wiertz [24] reported that in the outer orifice of the pore canal that traversed the eggshell was filled with cuticle material. In this study, it was drawn to attention that there were pore canals which extended between the mammillary layer and cuticle in both species. It was observed that the inner orifice of the pore canal was open, however the outer orifice was filed with cuticle material in all groups as mentioned earlier. Carnarius et al.[25] mentioned that the palisade layer was the most important layer for the eggshell in terms of strength. Although the spire calcite structures, taper to the center of the pore canals in the palisade layer, were observed in geese eggshell, they were not seen in those of chicken eggshell. These structures within the pore canal were seen in samples before incubation, so we considered that they were a feature of goose species and related to the strength of the eggshell and they were not related to the incubation period. Bönner et al.[26] reported that the thickness of average of goose eggshell was 530 µm, Gualhanone et al.[27] stated that the thickness of average of chicken eggshell was 370 µm and Yamak et al.[28] informed that the thickness of average of chicken eggshell was 380 µm. In this study, there was a big difference between the thickness of average of goose eggshells and that of chicken so we compared the thickness of an

Table 1. *Comparison levels of per element in both the mammillary and the palisade layers of eggshells in relation to six groups of incubation days (P<0.05)*
Tablo 1. *Kuluçka günlerine gore altı gruba ait yumurta kabuklarının mammillari ve palisade katmanlarında her bir elementin düzeylerinin karşılaştırılması (P<0.05)*

Species	Incubation Days (D)	n	Calcium (%) ± Std. Dev	F	Oxygen (%) ± Std. Dev	F	Carbon (%) ± Std. Dev	F	Nitrogen (%) ± Std. Dev	F
Mammillary Layer										
Chicken	0th D	5	64.64±8.5	1.19	22.58±9.1	1.14	9.14±3.4	0.60	6.57±0.7	1.47
	11th D	5	54.20±10.4		31.66±10.7		9.64±1.8		5.13±0.9	
	21st D	5	59.83±10.0		27.91±6.8		8.49±2.1		5.44±1.1	
Goose	0th D	5	65.73±12.3		21.00±10.2		7.90±3.1		5.31±0.7	
	16th D	5	56.44±7.3		28.74±7.2		9.75±1.6		4.92±1.3	
	31st D	5	62.59±6.4		23.90±6.6		8.13±0.7		5.34±1.0	
Palisade Layer										
Chicken	0th D	5	57.04±11.6	1.03	29.00±10.0	0.85	10.96±3.0	1.53	5.82±0.3	1.08
	11th D	5	49.98±2.0		34.65±2.9		10.22±0.7		5.53±1.4	
	21st D	5	52.81±4.1		32.19±4.7		9.64±0.5		4.87±1.1	
Goose	0th D	5	57.78±7.2		27.35±7.1		9.69±1.4		5.13±0.8	
	16th D	5	52.66±12.9		31.67±12.7		11.15±1.4		4.46±1.4	
	0th D	5	57.04±11.6		29.00±10.0		10.96±3.0		5.82±0.3	

Table 2. *Comparison of thickness of eggshells among groups of each species for incubation days (P<0.05)*
Tablo 2. *Grup içinde kuluçka günlerine göre yumurta kabuğu kalınlığının karşılaştırılması (P<0.05)*

Species	Incubation days (D)	n	Thickness of Eggshell (μm)	Std. Deviation	F
Chicken	0th D	5	349.80	30.4	0.40
	11th D	5	336.00	13.4	
	21st D	5	336.60	33.9	
Goose	0th D	5	482.60	31.9	1.45
	16th D	5	501.00	16.9	
	31st D	5	504.00	8.9	

average eggshell statistically among the groups within each species. The average thickness of goose eggshells was found to be 495 μm and that of chicken eggshells was found to be 340 μm when they were calculated by adding the three groups of each species. It was found that the thicknesses of goose and chicken eggshells were not significantly different among the groups of each species for incubation days. Turkyilmaz et al.[29] reported that eggshell thickness did not have a significant effect on the incubation period. Nys and Gautron [30] and Marie et al.[31] mentioned that approximately 95% of eggshell was made from $CaCO_3$ and the rest of eggshell was made from organic material which consisted of eggshell membrane. Nakano et al.[15] identified that there was less nitrogen ratio in eggshell than other elements. It was considered that the ratios of calcium, oxygen and carbon in EDS analysis were higher than those of other elements in an eggshell due to the fact that they take place in the $CaCO_3$ compound in eggshells. In addition, it was observed that the ratio of nitrogen in the mammillary and the palisade layer of geese and chickens eggshells were at a level of approximately 5%, and there was not statistically significant difference in each layer between species during the incubation period in terms of nitrogen ratio.

We concluded that when the indication of SEM results of goose eggshells were compared with those of chicken eggshells, the structural changes of an eggshell in SEM, identified elemental distribution in eggshells, and the thickness of eggshells did not have a clear significant effect on low yield for the hatching of goose eggs during embryonic development.

Acknowledgment

The SEM studies were performed at Mersin University, Advanced Technology, Research and Application Center.

REFERENCES

1. Yoshizaki N, Saito H: Canges in shell membranes during development of quail embryos. *Poult Sci*, 81, 246-251, 2002. DOI: 10.1093/ps/81.2.246

2. Rose MLH, Hincke MT: Protein constituents of the eggshell: Eggshell-specific matrix proteins. *Cell Mol Life Sci,* 66, 2707-2719, 2009. DOI: 10.1007/s00018-009-0046-y

3. Deeming DC: Failure to turn eggs during incubation: Development of the area vasculosa and embryonic growth. *J Morphol*, 201, 179-186, 1989. DOI: 10.1002/jmor.1052010207

4. Van Schalkwyk SJ, Cloete SWP, Brown CR, Brand Z: Hatching success of ostrich eggs in relation to setting, turning and angle of rotation. *Brit Poultry Sci*, 41, 46-52, 2000. DOI: 10.1080/00071660086394

5. Bednarczyk M, Rosinski A: Comparison of egg hatchability and in vitro survival of goose embryos of various origins. *Poultry Sci,* 78, 579-585, 1999. DOI: 10.1093/ps/78.4.579

6. Arslan C, Saatci M: Egg yield and hatchability characteristics of native geese in the Kars region. *Turk J Vet Anim Sci*, 27, 1361-1365, 2003.

7. Abiola SS, Meshioye OO, Oyerinde BO, Bamgbose MA: Effect of egg size on hatchability of broiler chicks. *Arch Zootec*, 57, 83-86. 2008.

8. Mahmud A, Pasha TN: Effect of strorage, pre-heating and turning during holding period on the hatchability of broiler breeders eggs. *Pakistan Vet J*, 28, 153-154, 2008.

9. Al-Shammari KIA, Batkowska J, Gryzinska MM: Assessment of ultraviolet light effect in hatching eggs disinfection on hatchability traits of two breeds of quails and chickens. *Acta Sci Pol Zootechnicat,* 14, 33-34, 2015.

10. Meir M, Ar A: Compensation for seasonal changes in eggshell conductance and hatchability of goose eggs by dynamic control of egg water loss. *Brit Poultry Sci*, 32, 723-732, 1991. DOI: 10.1080/00071669108417398

11. Arias JL, Fink DJ, Xiao SQ, Heuer AH, Caplan AI: Biomineralization and eggshells: Cell-mediated acellular compartments of mineralized extracellular matrix. *Int Rev Cytol*, 145, 217-250, 1993.

12. Nys Y, Hincke JL, Arias JM, Garcia-Ruiz JM, Solomon SE: Avian eggshell mineralization. *Poult Avian Biol Rev*, 10, 143-166, 1999.

13. Schaffner FC, Swart PK: Influence of diet and environmental water on the carbon and oxygen istopic signatures of seabird eggshell carbonate. *B Mar Sci,* 48, 23-38, 1991.

14. Maurer G, Portugal SJ, Boomer I, Cassey P: Avian embryonic development does not change the stable isotope composition of the calcite eggshell. *Reprod Fert Develop,* 23, 339-345, 2011. DOI: 10.1071/RD10138

15. Nakano T, Ikawa NI, Ozimek L: Chemical composition of chicken eggshell and shell membranes. *Poult Sci*, 82, 510-514, 2003. DOI: 10.1093/ps/82.3.510

16. Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT: Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. *CR Palevol*, 3, 549-562, 2004. DOI: 10.1016/j.crpv.2004.08.002

17. Simkiss K: Calcium metabolism and avian reproduction. *Biol Rev Camb Philos,* 36, 321-367, 1961.

18. Ruiz J, Lunam CA: Ultrastructural analysis of the eggshell: Contribution of the individual calcified layers and the cuticle to hatchability and egg viability in broiler breeders. *Brit Poultry Sci,* 41, 584-592, 2000.

19. Hunton P: Understanding the architecture of the eggshell. *World Poultry Sci J,* 51, 141-147, 1995.

20. Keshavarz K: Laying hens respond differently to high dietary levels of phosphorus in monobasic and dibasic calcium phosphate. *Poult Sci*, 73, 687-703, 1994. DOI: 10.3382/ps.0730687

21. Fathi MM, Afifi YK, El-Safty SA: Ultrastructural diversity of eggshell quality in some egyption local breeds of chicken. *Egypt Poult Sci*, 30, 813-827, 2010.

22. Parsons AH: Structure of the eggshell. *Poult Sci*, 61, 2013-2021. 1982. DOI: 10.3382/ps.0612013

23. Abdel-Salam ZA, Abdou AM, Harith MA: Elemental and ultrastructural analysis of the eggshell: Ca, Mg and Na distribution during embryonic development via LIBS and SEM techniques. *Int J Poult Sci*, 5, 35-42, 2006.

24. Simons PCM, Wiertz G: Notes on the structure of shell and membranes of the hen's egg: A study with the scanning electron microscope. *Ann Biol Anim Biochim Biophys*, 10, 31-49, 1970.

25. Carnarius KM, Conrad KM, Mast MG, Macneil JH: Relationship of eggshell ultrastructure and shell strength to the soundness of shell eggs. *Poult Sci*, 75, 656-663, 1996.

26. Bönner BM, Lutz W, Redmann T, Jager S, Reinhardt B, Wissing J, Knickmeier W, Kaleta EF: Morphometric and allometric studies on eggshells and embryos of free-living Canada geese (Branta c. Canadensis Linnaeus, 1758). *Eur J Wildl Res*, 50, 179-186, 2004. DOI: 10.1007/s10344-004-0061-0

27. Gualhanone A, Furlan RL, Fernandez-Alorcon MF, Macari M: Effect of breeder age on eggshell thickness, surface, temperature, hatchability and chick weigh. *Braz J Poult Sci,* 14, 9-14, 2012.

28. Yamak US, Sarıca M, Boz MA, Önder H: The effect of egg shell thickness of some hatching traits of broiler breeders. *Kafkas Univ Vet Fak Derg*, 21, 421-424, 2015. DOI: 10.9775/kvfd.2014.12485

29. Turkyılmaz MK, Dereli E, Sahin T: Effects of shell thickness, shell porosity, shape index and egg weight loss on hatchability in Japanese quail *(Coturnix coturnix japonica)*. *Kafkas Univ Vet Derg*, 11, 147-150, 2005.

30. Nys Y, Gautron J: Structure and formation of the eggshell. **In,** Lopez-Fandino R, Anton M, Schade R (Eds): Bioactive Egg Compounds. 99-102, Springer-Verlag, Berlin, 2007.

31. Marie P, Labas V, Brionne A, Harichaux G, Hennequet-Antier C, Nys Y, Gautron J: Quantitative proteomics and bioinformatic analysis provide new insight into protein function during avian eggshell biomineralization. *J Proteomics*, 113, 178-193, 2015. DOI: 10.1016/j.jprot.2014.09.024