

## Effect of egg weight and amount of protoporphyrin and biliverdin in the egg shell on hatching characteristics and embryonal mortality in pheasants (*Phasianus Colchicus*)

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**Summary:** This study was performed to investigate the effects of egg weight and amount of protoporphyrin and biliverdin in the eggshell on hatchability and embryonic mortality in pheasants. A total of 1908 eggs obtained from 48 weeks old pheasants were used in the study. The eggs were divided into three colour groups (dark brown, light brown and green) and two weight groups (up to 32 g and above 32 g). For the weight groups, there were significant differences in the hatchability of fertile eggs ( $P<0.01$ ) and late period embryonic mortality (LPEM) ( $P<0.05$ ). The highest hatchability of fertile eggs and the lowest embryonic mortality were determined in light eggs ( $\leq 32$  g). In terms of eggshell colour groups, there were significant differences in the hatchability of total eggs ( $P<0.01$ ); hatchability of fertile eggs ( $P<0.01$ ); and early (PEM) ( $P<0.01$ ), middle (MPEM) ( $P<0.05$ ) and late period (LPEM) ( $P<0.05$ ) embryonic mortality. In present study, the highest hatchability and the lowest embryonic mortality rate (except for MPEM) were in the dark brown eggshell group. For all groups, the differences in fertility rate were insignificant ( $P>0.05$ ). The highest hatching characteristics and the lowest embryonic mortality rates were determined in the dark brown eggs. Based on these results, to achieve higher a hatchability of total eggs, hatchability of fertile eggs and a lower embryonic mortality rate, it may be beneficial selection of dark brown pheasant eggs.

Keywords: Biliverdin, egg, hatching characteristics, pheasant, protoporphyrin.

### Sülünlerde (*Phasianus Colchicus*) yumurta ağırlığı ve yumurta kabuğundaki protoporfirin ve biliverdin miktarının kuluçka özellikleri ve embriyonal ölüm üzerine etkisi

**Özet:** Bu araştırma, sülünlerde yumurta ağırlığı ve yumurta kabuğundaki protoporfirin ve biliverdin miktarının kuluçka özellikleri ve embriyonal ölüm üzerine etkisini araştırmak amacıyla yapılmıştır. Araştırmada 48 haftalık sülünlerden elde edilen toplam 1908 yumurta kullanılmıştır. Yumurtalar üç renk grubu (koyu kahve, açık kahve ve yeşil) ve iki ağırlık grubu ( $\leq 32$  g ve  $32 < g$ )'na ayrılmıştır. Ağırlık gruplarında, çıkım gücü ( $P<0.01$ ) ve geç dönem embriyonal mortalite (LPEM) ( $P<0.05$ ) ortalamaları arasındaki farklılıklar önemli bulunmuştur. En yüksek çıkım gücü ve en düşük embriyonal ölüm hafif yumurtalarda ( $\leq 32$  g) tespit edilmiştir. Yumurta kabuk rengi grupları bakımından, kuluçka randımanı ( $P<0.01$ ); çıkım gücü ( $P<0.01$ ); erken (PEM) ( $P<0.01$ ), orta (MPEM) ( $P<0.05$ ) ve geç dönem (LPEM) ( $P<0.05$ ) embriyonal ölüm ortalamaları arasındaki farklılıklar önemli bulunmuştur. Araştırmada, en yüksek kuluçka randımanı ve en düşük (MPEM hariç) embriyonal ölüm oranı koyu kahve kabuklu yumurtalarda bulunmuştur. Tüm gruplarda, fertilitite oranı ortalamaları arasındaki farklılık önemsiz ( $P>0.05$ ) bulunmuştur. En yüksek kuluçka performansı, kuluçka oranı ve en düşük embriyonal ölüm oranı koyu kahve kabuklu yumurtalarda bulunmuştur. Bu sonuçlara bağlı olarak, daha yüksek bir kuluçka randımanı, çıkım gücü ve daha düşük bir embriyonal ölüm oranı elde etmek için, koyu kahverengi sülün yumurtalarının seçimi yararlı olabilir.

Anahtar sözcükler: Biliverdin, kuluçka özellikleri, protoporfirin, sülün, yumurta.

### Introduction

In poultry breeding, profitability and productivity are closely related to the numbers of chicks obtained from hatching eggs, and obtaining the maximum number of chicks is dependent on a successful hatching period. The physical characteristics of the egg play an important role in the process of embryo development and successful hatching (28). Egg weight and eggshell colour are two physical factors that influence hatchability. Their effects

on hatchability have been studied for hens (1, 31, 34, 37), wild birds (25, 26) and pheasants (4, 14, 21, 22).

Effect of egg weight on hatchability is an important economic trait in domestic poultry. It was reported that smaller and excessively big eggs tended to have lower hatchability of fertile eggs (2, 18, 41). Senapati et al. (33) reported positive correlations between egg weight and hatchability. However, Deeming (7) found that hatchability of ostrich eggs decreased with increasing egg weight.

Eggshell color is determined by levels of the pigments protoporphyrin and biliverdin which are the products of hem metabolism. Biliverdin and protoporphyrin, which are released by the eggshell gland, are included in the structure of the eggshell when it begins to form. Protoporphyrin is red and causes brown colouring and biliverdin causes green colouring (16, 17, 27, 30). Depending on the amount of protoporphyrin, eggshell colour can range from light brown to dark brown. Biliverdin was reported from blue and green eggshells of domesticated fowl (19, 32).

As in the breeding of all poultry species, the effects of physical characteristics of eggs on successful hatching are among the most important influences for pheasants (8, 21). It is known that Ring-necked pheasants (*Phasianus Colchicus*) live at an altitude 400 m in Marmara and Black Sea Regions in Turkey (38). Ring-necked pheasants are one of the endangered species in natural conditions in Turkey. However, the pheasants are unleashed from generating station of pheasant to natural life in Marmara and Black Sea Region by Ministry of Forest and Water Management (6). In pheasant breeding, obtaining of fertile eggs, high hatchability and lowest embryonal mortality are important for continuity of herd. For this purpose, the effect of egg weight, length of storage, stock age, different breeding regimes on hatchability performance has been studied for pheasants (4, 9, 20).

Pheasant eggs can be different colours, including grey-white, blue, olive green, light brown and dark brown. In studies of the hatchability of pheasant eggs of different colours, it was reported that fertility, hatchability and hatchability of fertilized eggs were higher in brown eggs than in eggs of other colours (21, 22). Shafey et al. (34) reported that differences in hatchability and embryonal mortality might be related to differences in pigment density in the eggshell and that eggshell pigmentation could play a key role in shielding the egg's contents from harmful radiation and hence successful embryonic development. Nevertheless, reasons for an association between hatchability and eggshell colour are unclear. Therefore, association between hatchability and colour pigment of pheasants eggshell should be conceived. However, research on the relationship between hatchability and the amount of protoporphyrin and biliverdin in the eggshells of pheasants is rather limited.

As a contribution to efforts to increase the efficiency of pheasant breeding, the current study aimed to investigate the effects of egg weight, amount of protoporphyrin and biliverdin in eggshell on hatchability and embryonal mortality.

## Materials and Methods

*Animals, housing and designation of groups:* A total of 1908 eggs (1728 for hatching and 180 for pigment

analyze) laid by 48 weeks old pheasants were obtained from the Gelemen Pheasant Breeding Centre of the Forest and Water Ministry of Turkey. The study was approved by Ethical Committee for Experimental Animals of Ondokuz Mayıs University (HADYEK/85). The eggs were collected from small breeding flocks having one male and seven females kept in open cages of 4 m × 5 m. The pheasants were fed ad libitum with 14.70% crude protein and rations that contained 2665 kcal/kg ME. A preliminary study was performed before planning the present study. It determined that the mean weight of pheasant eggs obtained in the previous production period was 32 g. They generally had shell colours of dark brown, light brown or green, which were visually determined. On the basis of the results of the preliminary study, eggshell colour groups (dark brown, light brown, and green) and egg weight categories of light (up to 32 g) and heavy (above 32 g) were designated. After these classifications, a total of six groups was constructed, with each colour group divided into two weight subgroups. The six groups were designated as dark brown shelled-light (Dbrw-light), dark brown shelled-heavy (Dbrw-heavy), light brown shelled-light (Lbrw-light), light brown shelled-heavy (Lbrw-heavy), green shelled-light (Grn-light) and green shelled-heavy (Grn-heavy).

*Hatchery conditions and evaluation of incubation results:* The selected pheasant eggs were placed in trays and kept at 18°C and relative humidity of 75% for seven days and then transferred to a 2500 egg capacity, cupboard type incubator (Çimuka-T series). The eggs were incubated at 37.70°C and 65% moisture for 21 days in the development section, and at 37.50°C and 90% moisture for last 3 days (5). At the end of the 24 day incubation period, the eggs which had not hatched were broken one by one and observed with the naked eye. In that macroscopic examination, the stage of embryo development at death was classified in terms of 4 possible death periods. The classification was done as follows; EPEM: early period embryonic mortality (eyes developed, feathered developed); MPEM: middle period embryonic mortality (feathers developed, more of yolk sack external to the body); LPEM: late period embryonic mortality (2/3 or whole of yolk sack in the body of embryo) and LDM: wholly developed embryo in the cracked eggshell (last day mortality) (11). Fertility, hatchability of total eggs and hatchability of fertile eggs was calculated as the fertile eggs / total eggs, chick number of hatched / total eggs and chick number of hatched / fertile eggs respectively. Also, EPEM, MPEM, LPEM and LDM was calculated as early period embryo mortality / fertile eggs, middle period embryo mortality / fertile eggs, late period embryo mortality / fertile eggs and last day mortality / fertile eggs respectively (2).

*Analysis of protoporphyrin and biliverdin in eggshells:* Extraction from samples: For the analysis of protoporphyrin and biliverdin pigments, 30 eggs were selected from each group, with a total of 180 eggs examined. Eggshell colour were visually determined for biliverdin and protoporphyrin analyses. From each egg 100 mg of crushed eggshell was placed in a polypropylene eppendorf centrifuge tube and 0.5 ml of disodium EDTA (pH 7.2, adjusted with NaOH) (Merck, Darmstadt, Germany) solution (100 mg/ml) was added and allowed to sit for 5 min, followed by stirring for 1 min in a vortex. The stopper of the tube was released carefully. After the foaming disappeared, the tube was centrifuged for 2 min at 15000 rpm and the supernatant was removed. These stages were repeated three times. In the final stage, 1 ml of acetonitrile-acetic acid (v/v; 80:20) was added and mixed for 2 min in a vortex. The stopper of the tube was uncapped after 30 seconds to free liberated CO<sub>2</sub>. Then the tube was centrifuged for 4 min at 15000 rpm/min. The supernatant was filtered through a 0.45 µm PTFE disc (Isolab, Wertheim, Germany) into a 1.5 ml amber vial, and 20 µl of the filtered supernatant was injected into a HPLC system (Prominence LC - 20A, Shimadzu, Kyoto, Japan) (12) for determination of the the amounts of protoporphyrin and biliverdin.

*HPLC operating conditions:* Calibration solutions were prepared with protoporphyrin IX and biliverdin standards (Sigma-Aldrich Co. LLC. St. Louis, MO, USA). A Lichrosorb RP-8-10 µm column (250 × 4 mm, Merck, Darmstadt, Germany) was used for separation. The mobile phase solvents A and B were 100 mM ammonium acetate (pH 5.5), 2-methoxyethanol and methanol (v/v; 45:5:50); and 2-methoxyethanol and methanol (v/v; 5:95), respectively. The flow gradient was changed at a linear rate from 100% solvent A to 100% solvent B in 11 min. After that, it was changed from 100% solvent B to 100% solvent A in 4 min. Total analysis time was 15 min and the flow speed was 1.4 mL/min. The protoporphyrin and biliverdin amounts were determined at 400 nm and 376 nm, respectively, using a DAD detector (Shimadzu, SPD M20A, Kyoto, Japan) (17).

*Statistical analysis:* The Chi-square test was used for the comparison of fertility, hatchability of total eggs, hatchability of fertile eggs, early period embryo mortality, middle period embryo mortality, late period embryo mortality and last day mortality values having the proportional values among and inside the research groups. Least square variance analysis was performed for the comparison of egg weight, amounts of protoporphyrin and biliverdin in the different weight, colour and colour × weight groups, and determination of the significance of differences between the groups was done with the Duncan test (3).

## Results

*Hatchability and embryonic mortality:* The means of egg weight and eggshell colour groups are presented in Table 1, with egg weight ranging from 30.04 to 34.76 g. Differences among egg weight (P<0.001) were significant in this study. However, differences among eggshell colour groups were no significant (P>0.05).

Table 1. Mean of egg weight in pheasant groups (Mean± S.E.).  
Tablo 1. Gruplardaki yumurta ağırlığı ortalamaları (Ortalama± S.E.).

Characters	Total eggs	Mean egg weight
Weight		***
Light	768	30.24±0.06
Heavy	960	34.57±0.05
Colours		NS.
Dbrw	672	32.53±0.09
Lbrw	672	32.24±0.09
Grn	384	32.43±0.12
Grand mean	1728	32.40±0.04

\*\*\*: P<0.001, NS: not significant.

\*\*\*: P<0.001, NS: önemsiz

The mean values for hatchability and embryonic mortality rate of pheasant eggs for weight, colour and different colours in weight groups are given in Table 2. The highest fertility among egg weight groups was determined for the heavy groups. In contrast, the highest hatchability of total eggs and hatchability of fertile eggs (P<0.01) were for the light groups. Therefore, fertility increased with increasing egg weight, but hatchability of total eggs and hatchability of fertile eggs increased with decreasing egg weight. The lowest embryonic mortality rate among egg weight groups was in the light groups; that is, embryonic mortality increased parallel to increasing egg weight. In the present study, the highest embryonic mortality rate for both of egg weight groups was for late period embryo mortality (P<0.05). Furthermore, it was determined that the early embryonic mortality decrease with increasing egg weight while middle embryonic mortality and last day mortality increased with increasing egg weight.

In terms of eggshell colour, there were significant differences for hatchability of total eggs (P<0.01) and hatchability of fertile eggs (P<0.01) but not for fertility (P>0.05). Moreover, the highest fertility, hatchability of total eggs and hatchability of fertile eggs were determined for dark brown eggs. When based on the dark brown eggshell colour value, the difference between dark brown and light brown eggs were 3.11%, 6.35%, 3.35% and difference between dark brown and green eggs were 4.02%, 14.06% and 10.46% for fertility, hatchability of total eggs and hatchability of fertile eggs respectively. The lowest early period embryonic mortality (P<0.01), last

period embryonal mortality ( $P<0.05$ ) and last day mortality ( $P>0.05$ ) were for the dark brown eggshell group, whereas the lowest middle period embryonal mortality ( $P<0.05$ ) was for green eggshells.

For weight, colour and different colours in weight groups, the differences of fertility, hatchability of total eggs, hatchability of fertile eggs and all of embryonal

mortalities for light eggs were not significant ( $P>0.05$ ). However, the differences for hatchability of total eggs ( $P<0.01$ ), hatchability of fertile eggs ( $P<0.01$ ) and late period embryonal mortality ( $P<0.01$ ) were significant for heavy eggs, while fertility, early period embryonal mortality, middle period embryonal mortality and last day mortality were not significant ( $P>0.05$ ).

Table 2. Hatching characteristics and embryonic mortality rates for pheasant eggs.

Tablo 2. Sülün yumurtalarında kuluçka özellikleri ve embriyonik ölüm oranları.

Weight	Colour	Total eggs n	Fertile eggs n	Hatchability n	Fertility %	HR %	HFE %	EPEM %	MPEM %	LPEM %	LDM %
Light		768	633	556	82.42	72.39	87.83	1.82	1.17	6.25	0.78
Heavy		960	822	665	85.62	69.27	80.90	3.22	1.04	9.27	1.77
					3.291 <sup>NS</sup>	2.010 <sup>NS</sup>	12.746 <sup>**</sup>	3.327	0.067	5.334 <sup>*</sup>	3.181
	Dbrw	672	578	503	86.01	74.85 <sup>a</sup>	87.02 <sup>a</sup>	1.64 <sup>b</sup>	2.68 <sup>a</sup>	6.10 <sup>b</sup>	0.74 <sup>b</sup>
	Lbrw	672	560	471	83.33	70.09 <sup>a</sup>	84.10 <sup>a</sup>	2.23 <sup>b</sup>	1.34 <sup>a</sup>	6.70 <sup>b</sup>	1.49 <sup>b</sup>
	Grn	384	317	247	82.55	64.32 <sup>b</sup>	77.91 <sup>b</sup>	4.95 <sup>a</sup>	0.52 <sup>b</sup>	10.68 <sup>a</sup>	2.08 <sup>a</sup>
					2.822 <sup>NS</sup>	13.238 <sup>**</sup>	12.603 <sup>**</sup>	11.162 <sup>**</sup>	7.661 <sup>*</sup>	8.023 <sup>*</sup>	3.544 <sup>NS</sup>
Light	Dbrw	288	244	218	84.72	75.69	89.34	1.04	1.39	6.25	0.34
	Lbrw	288	233	209	80.90	72.56	85.69	1.74	1.40	4.17	1.38
	Grn	192	156	129	81.25	67.18	82.69	2.10	0.70	6.25	0.34
					1.693 <sup>NS</sup>	4.179 <sup>NS</sup>	5.140 <sup>NS</sup>	1.016 <sup>NS</sup>	0.674 <sup>NS</sup>	1.588 <sup>NS</sup>	3.004 <sup>NS</sup>
Heavy	Dbrw	384	334	285	86.97	74.22 <sup>a</sup>	85.32 <sup>a</sup>	2.80	1.40	8.00 <sup>b</sup>	1.38
	Lbrw	384	327	262	85.16	68.23 <sup>a</sup>	80.12 <sup>a</sup>	3.50	2.10	14.90 <sup>a</sup>	2.10
	Grn	192	161	118	83.85	61.46 <sup>b</sup>	73.29 <sup>b</sup>	4.60	0.00	8.00 <sup>b</sup>	2.40
					1.130 <sup>NS</sup>	10.117 <sup>**</sup>	10.400 <sup>**</sup>	1.272 <sup>NS</sup>	5.666 <sup>NS</sup>	10.021 <sup>**</sup>	0.850 <sup>NS</sup>

EW: Egg Weight, HR: Hatchability Rate, HFE: Hatchability of Fertile Eggs Dbrw: Dark brown shelled eggs, Lbrw: Light brown shelled eggs, Grn: Green shelled eggs, EPEM: Early Period Embryonic Mortality, MPEM: Middle Period Embryonic Mortality, LPEM: Late Period Embryonic Mortality, LDM: Last Day Mortality.

\*: $P<0.05$ , \*\*: $P<0.01$ , NS: not significant.

EW: Yumurta ağırlığı, HR: Kuluçka Randımanı, HFE: Çıkım Gücü, DBrw: Koyu kahve kabuklu yumurtalar, Lbrw: Açık kahve kabuklu yumurtalar, Grn: Yeşil kabuklu yumurtalar, EPEM: Erken Dönem Embriyonik Ölümler, MPEM: Orta Dönem Embriyonik Ölümler, LPEM: Geç Dönem Embriyonik Ölümler, LDM: Son Gün Ölümleri.

\*: $P<0.05$ , \*\*: $P<0.01$ , NS: önemsiz.

Table 3. Biliverdin and protoporphyrin amounts in pheasant egg groups (Mean± S.E.).

Tablo 3. Sülün yumurtalarında biliverdin ve protoporfirin miktarları (Ortalama± S.E.).

Main effects	Total eggs n	Biliverdin ( $\mu\text{mol/g}$ )	Protoporphyrin ( $\mu\text{mol/g}$ )
Weight		**	NS
Light	89	4.88±0.13	11.28±0.30
Heavy	85	4.24±0.13	10.72±0.31
Color		***	***
Dbrw	59	5.24±0.16 <sup>a</sup>	14.87±0.38 <sup>a</sup>
Lbrw	55	3.72±0.17 <sup>c</sup>	9.44±0.39 <sup>b</sup>
Grn	60	4.72±0.16 <sup>b</sup>	8.68±0.37 <sup>b</sup>
Interactions		**	NS
Light	Dbrw	5.66±0.23 <sup>a</sup>	15.15±0.53
	Lbrw	3.58±0.23 <sup>b</sup>	9.38±0.54
	Grn	5.39±0.23 <sup>a</sup>	9.29±0.53
Heavy	Dbrw	4.82±0.23 <sup>a</sup>	14.59±0.54
	Lbrw	3.85±0.25 <sup>b</sup>	9.49±0.57
	Grn	4.05±0.23 <sup>b</sup>	8.07±0.53
Grand mean	174	4.56±0.09	11.00±0.22

Dbrw: Dark brown shelled eggs, Lbrw: Light brown shelled eggs, Grn: Green shelled eggs.

\*\*\*: $P<0.01$ , \*\*\*\*: $P<0.001$ , NS: not significant.

Dbrw: Koyu kahve kabuklu yumurtalar, Lbrw: Açık kahve kabuklu yumurtalar, Grn: Yeşil kabuklu yumurtalar.

\*\*\*: $P<0.01$ , \*\*\*\*: $P<0.001$ , NS: önemsiz

**Biliverdin and protoporphyrin amounts:** The mean values and standard deviations for protoporphyrin and biliverdin amounts are provided in Table 3. For egg weight, biliverdin ( $P<0.01$ ) and protoporphyrin ( $P>0.05$ ) amounts in light eggs were higher than in heavy eggs. Moreover, the differences between light and heavy eggs for biliverdin and protoporphyrin were 13.11% and 4.96% respectively. HPLC-DAD chromatograms for dark-brown, light-brown and green eggshells are shown in Figures 1, 2 and 3, respectively.

In the present study, the protoporphyrin and biliverdin amounts of  $14.87\mu\text{mol/g}$  and  $5.24\mu\text{mol/g}$ , respectively, in the dark brown eggshells were higher than in the other colour groups. For the eggshell colour groups, the differences in the mean values for both of protoporphyrin and biliverdin were significant ( $P<0.001$ ). In terms of the colour  $\times$  weight groups, the differences in protoporphyrin amount were not significant while differences in biliverdin amount in the pheasant eggshells were significant

( $P<0.01$ ). When based on the dark brown eggshell colour value, the differences of biliverdin and protoporphyrin between dark brown and light brown eggs were 29.00% and 36.50%, respectively, 9.92% and 41.62% for dark-brown and green eggs, respectively.

## Discussion and Conclusion

In the present study on pheasant eggs, there was no significant effect of eggshell colour on egg weight. Mean egg weight was 32.53, 32.24 and 32.43 g for brown, light brown and green shelled eggs, respectively, (Table 1), with egg weight ranging from 30.04 to 34.76 g. Egg weight values were similar to those reported by Krystianiak (21) namely, 31.70, 31.13 and 31.17 g, and by Kozuszek et al. (22), namely 33.99, 32.88 and 31.97 g, for brown, light brown and green shelled eggs, respectively. Also, Kırıkçı et al. (20) reported mean weights of 31.89 and 31.16 g for brown and green eggs, respectively.

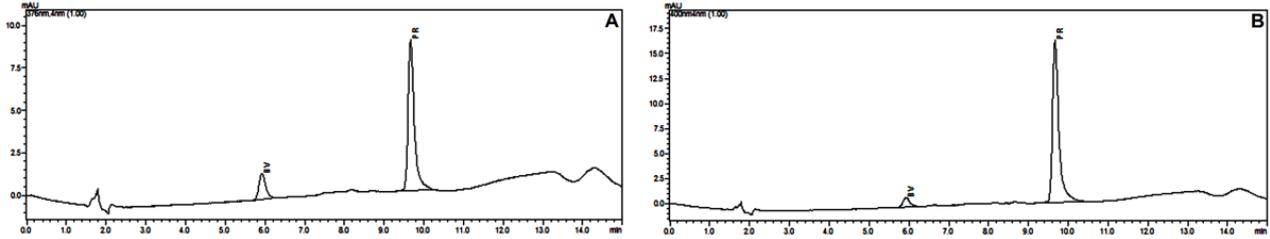


Figure 1. The HPLC-DAD chromatograms for dark-brown egg shells at 376 nm (A) and 400 nm (B).

Şekil 1. Koyu kahve kabuklu yumurtalarda 376 nm (A) ve 400 nm (B) HPLC-DAD kromatogramları.

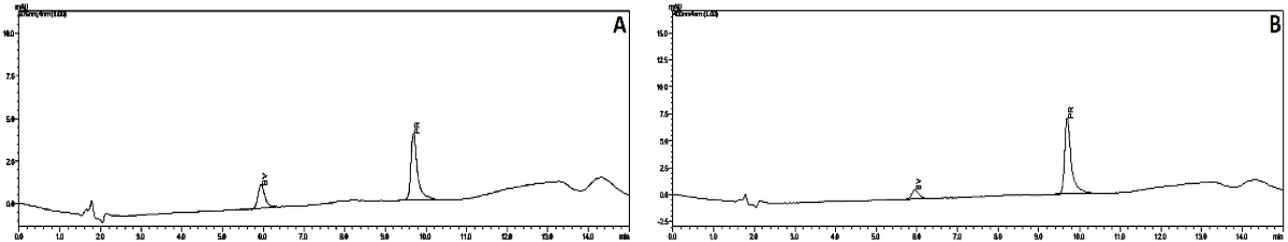


Figure 2. The HPLC-DAD chromatograms for light Brown eggshells at 376 nm(A) and 400 nm (B).

Şekil 2. Açık kahve kabuklu yumurtalarda 376 nm (A) ve 400 nm (B) HPLC-DAD kromatogramları.

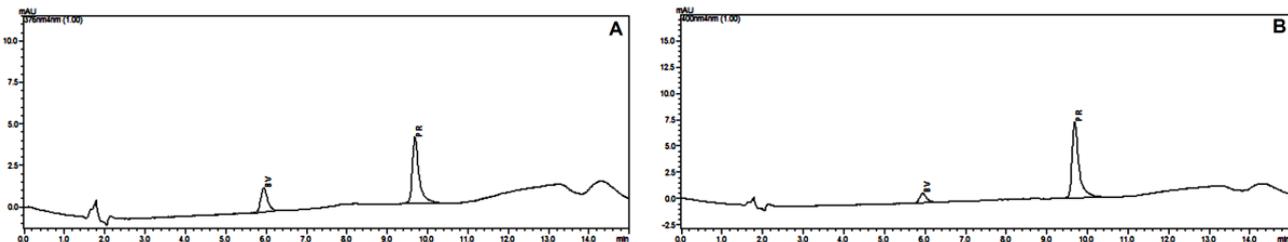


Figure 3. The HPLC-DAD chromatograms for dark-brown eggshells at 376 nm (A) and 400 nm (B).

Şekil 3. Yeşil kabuklu yumurtalarda 376 nm (A) ve 400 nm (B) HPLC-DAD kromatogramları.

It was determined that the fertility of light eggs was lower than that of heavy eggs but the difference was not significant ( $P>0.05$ ) in this study (Table 2). Likewise, Çağlayan et al. (4) reported that the fertility for up to 32 g pheasant eggs was lower than for eggs above 32 g. When the weight groups were compared in terms of hatchability of total eggs and hatchability of fertile eggs, light eggs tended to have higher hatchability of total eggs and hatchability of fertile eggs than heavy eggs (Table 2). However, the hatchability of total eggs in light eggs for broilers was lower than in heavy eggs (1, 37). Furthermore, contrary to the results for hatchability of total eggs and hatchability of fertile eggs in the present study, Çağlayan et al. (4) reported that hatchability of total eggs and hatchability of fertile eggs for the up to 32 g pheasant eggs was lower than for the above 32 g eggs. In the current study, when the weight groups were compared for embryonic mortality, the total embryonic mortality rate in the heavy eggs was higher than in the light eggs (Table 2). Likewise, the total embryonic mortality rate for heavy eggs was higher than for light eggs (31, 36). Especially, the late period embryonic mortality rates (9.27%) higher than other embryonic mortality rate for the heavy eggs (Table 2). Therefore, the loss of hatchability of total eggs and hatchability of fertile eggs for heavy eggs were attributable to an increase in late period embryonic mortality.

Pheasant eggs generally have dark brown, light brown or green eggshell colour. Eggshell colour is determined by levels of the pigments protoporphyrin and biliverdin (14). The biliverdin had increased anti-oxidant activity (10, 26), while protoporphyrin had photo-dynamic and anti-bacterial effects (15). It was reported that there was the effects of antioxidants on fertility and hatchability of total eggs (13). In that study, protoporphyrin and biliverdin amounts of 14.87  $\mu\text{mol/g}$  and 5.24  $\mu\text{mol/g}$  respectively, in the dark brown eggs were higher than in the other colour groups. In the present study, the higher amount of protoporphyrin in the dark brown eggs was comparable with the results of published studies (39, 42). As in the results of the present study, Kozuszek et al. (22) reported that the hatchability of total eggs and hatchability of fertile eggs in dark brown eggs were higher than in light brown and green eggs. In the present study, when the fertility, hatchability of total eggs and hatchability of fertile eggs were compared for colour groups, they were higher in the dark brown eggs than in the light brown and green eggs (Table 2). Therefore, the fertility, hatchability of total eggs, hatchability of fertile eggs and embryonic life for dark brown eggs might be related to the amounts of protoporphyrin and biliverdin in these eggs. In the present study, when the levels of embryonic deaths were compared for the colour groups, the total embryonic

mortality rates were the lowest value for the dark brown eggshells. Also, the total embryonic mortality rates for light eggs were lower than those of heavy eggs (Table 2). The positive effects of dark brown eggshell colour on hatchability of total eggs, hatchability of fertile eggs and embryonic life were also reported in stock broilers (28). Also, the total embryonic mortality rates for light eggs were lower than those of heavy eggs (Table 2). Solomon (35) reported that the size of egg does not affect the amount of pigment. Therefore, the amount of pigment per unit area is higher in light eggs than in heavy eggs and may affect the hatchability of total eggs, hatchability of fertile eggs and embryonic mortality rate. Supporting this assertion, Shafey et al. (34) reported that improved hatchability of total eggs and reduced embryonic mortality with increasing pigment density in the eggshell. Thus, in this study it was reported that biliverdin and protoporphyrin amount for light and dark brown eggs were higher than those of other eggs. Concordantly, the reduced late embryonic mortality for light eggs and dark brown were attributable to an increase in eggshell pigmentation. However, in that study, dark brown eggshells had the highest MPEM rate. Leeson et al. (23) have suggested that MPEM was sensitive indicator of stock diet deficiencies because mortality was normally very low during this period. The National Research Council (29) reported as 15% crude protein requirements for stock pheasants. In this study, it was reported as 14.7% crude protein. Therefore, it was determined that crude protein was no effective on middle period embryonic mortality. However, it was reported that ambient heat absorption by dark eggshells was higher than by light coloured eggshells (24). Also, the avian embryos produced more radiant heat during growth in the hatching period. Avian embryos can be very tolerant of hypothermia, but are far less tolerant of hyperthermia (40). Therefore, the higher MPEM rate in brown shelled eggs might be due to exceeding the lethal core temperature value.

Consequently, in this study, the hatchability rate and hatchability of fertile eggs were higher in dark brown eggs, while the number of embryonic deaths was lower, except for MPEM. Based on these results, to achieve higher a hatchability rate and hatchability of fertile eggs, and a lower embryonic mortality rate, it may be beneficial to select dark brown pheasant eggs for incubation.

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## References

1. **Abiola SS, Meshioye OO, Oyerinde BO, et al.** (2008): *Effect of size on hatchability of broiler chicks*. Arch Zootec, **57**, 83-86.
2. **Aksoy FT** (1999): *Tavuk Yetiştiriciliği*. Üçüncü Baskı. Şahin Matbaası, Ankara.
3. **Anonymous** (1993): *SPSS Statistical package in social science for windows*. Statistical Innovations Inc. Chicago, USA.
4. **Çağlayan T, Alaşahan S, Çetin O, et al.** (2010): *Effects of egg weight and length of storage period on chick weight and hatchability performance of pheasant (Phasianus Colchicus)*. JFAE, **8**, 407-410.
5. **Çetin O, Kırıkçı K** (2000): *Alternatif kanatlı yetiştiriciliği. Sülün-Keklik*. Selçuk Üniversitesi Yayınları, Konya.
6. **Çetin O, Tepeli C, Kırıkçı K** (1997): *Sülünlerin (P. Colchicus) entansif ortam ve karasal iklimde yetiştirilme imkânlarının araştırılması: I. Yumurta verimi ve kuluçka özellikleri*. Vet Bil Derg, **13**, 5-10.
7. **Deeming DC** (1996): *Production, fertility and hatchability of ostrich (Struthio Camelus) eggs on a farm in the United Kingdom*. Anim Sci, **63**, 329-336.
8. **Demirel S, Kırıkçı K** (2009): *Effect of different egg storage times on some egg quality characteristics and hatchability of pheasants (Phasianus Colchicus)*. Poult Sci, **88**, 440-444.
9. **Esen F, Özbey O, Genç F** (2010): *The effect age on egg production, hatchability and egg quality characteristics in Pheasants (Phasianus Colchicus)*. J Anim Vet Adv, **9**, 1237-1241.
10. **Falchuk KH, Contin JM, Dzedzic TS, et al.** (2002): *A role for biliverdin IXa in dorsal axis development of Xenopus laevis embryos*. Proc Nat Acad Sci, **99**, 251-256.
11. **Fant RJ** (1957): *Criteria for aging pheasant embryos*. J Wildl Manage, **21**, 324-328.
12. **Gorchein A, Lim CK, Cassey P** (2009): *Extraction and analysis of colourful eggshell pigments using HPLC and HPLC/electrospray ionization tandem mass spectrometry*. Biomed Chromatogr, **23**, 602-606.
13. **Hennig A, Marckwardt E, Richter G** (1986): *Relation between vitamin E supply and the fertility of laying hens [abstract]*. Archiv für Tierernahrung, **36**, 519-529.
14. **Hullett RM, Flegal CJ, Carpentier GH, et al.** (1985): *Effect of eggshell color and thickness on hatchability in Chinese Ring-Necked Pheasants [abstract]*. Poult Sci, **64**, 235-237.
15. **Ischikawa S, Suzuki K, Fukuda E, et al.** (2010): *Photodynamic antimicrobial activity of avian eggshell pigments*. FEBS Letters, **584**, 770-774.
16. **Ito S, Tsudzuki M, Komori M, et al.** (1993): *Celadon: An eggshell colour mutation in Japanese quail*. J Hered, **84**, 148-150.
17. **Kaur H, Hughes MN, Green CJ, et al.** (2003): *Interaction of bilirubin and biliverdin with reactive nitrogen species*. FEBS Letters, **543**, 113-119.
18. **Kalita N** (1994): *Effect of egg weight, storage period and position of eggs on hatchability*. IJPS, **9**, 281-283.
19. **Kennedy GY, Vevers HG** (1976): *A survey of avian eggshell pigments*. Comp Biochem Physiol, **55B**, 117-123.
20. **Kırıkçı K, Günlü A, Garip M** (2005): *Some quality characteristics of pheasant (Phasianus Colchicus) eggs with different shell colors*. Turkish J Vet Anim Sci, **29**, 315-318.
21. **Krystianiak S, Kozuszek R, Kontecka H, et al.** (2005): *Quality and ultrastructure of eggshell and hatchability of eggs in relation to eggshell colour in pheasants*. Anim Sci Pap Rep, **23**, 5-14.
22. **Kozuszek R, Kontecka H, Nowaczewski S, et al.** (2009): *Storage time and eggshell colour of pheasant eggs vs. the number of blastodermal cells and hatchability results*. Folia Biol (Kraków), **57**, 121-130.
23. **Leeson S, Reinhart BS, Summers I D** (1979 b): *Response of White Leghorn and Rhode Island Red breeder hens to dietary deficiencies of synthetic vitamins. 2. Embryo mortality and abnormalities*. Can J Anim Sci, **59**, 569-575.
24. **Magige FJ, Moe B, Roskaft E** (2008): *The white colour of the Ostrich (Struthio Camelus) egg is a trade-off between predation and overheating*. J Ornithol, **149**, 323-328.
25. **Morales J, Velando A, Moreno J** (2008): *Pigment allocation to eggs decreases plasma antioxidants in a songbird*. Behav Ecol Sociobiol, **63**, 227-233.
26. **Moreno J, Morales J, Lobato E, et al.** (2005): *Evidence for the signaling function of egg color in the Pied Flycatcher Ficedula Hypoleuca*. Behav Ecol, **16**, 931-937.
27. **Murray RK, Kurt I** (2004): *Porfirinler ve safra pigmentleri*. In, Murray RK, Granner DK, Mayes PA, Rodwell WW(Ed) Dikmen N, Özgünen T (Çeviri Ed): Harper Biyokimyası, 359-373, Nobel Matbaacılık, İstanbul.
28. **Narushin VG, Romanov MN** (2002): *Egg physical characteristics and hatchability*. World's Poult Sci J, **58**, 297-303.
29. **NRC** (1994): *Nutrient Requirements of Poultry*. National Research Council, Washington D.C.
30. **Poole HK** (1965): *Eggshell pigmentation of Japanese quail: Genetic control of the white egg trait*. J Hered, **55**, 136-138.
31. **Reinhart BS, Hurnik GI** (1984): *Traits affecting the hatching performance of commercial chicken broiler eggs*. Poult Sci, **63**, 240-245.
32. **Schwartz SW, Raux A, Schacter BA, et al.** (1980): *Loss of hereditary uterine protoporphyria through chromosomal rearrangement in mutant Rhode Island Red hens*. Int J Biochem, **12**, 935-940.
33. **Senepati PK, Das K, Mondal KG, et al.** (1996): *Relationship between egg weight, shape index and fertility and hatchability of Japanese quail eggs*. Environ Ecol, **14**, 574-577.
34. **Shafey TM, Al-Batshan HA, Ghannam MM, et al.** (2005): *Effect of intensity of eggshell pigment and illuminated incubation on hatchability of brown eggs*. Br Poult Sci, **46**, 190-198.
35. **Solomon SE** (1987): *Egg shell pigmentation*. In, Wells RG, Belyavin CG (Ed): Egg Quality Current Problems and Recent Advance, 147-158. London, Butterworths.
36. **Szczerbinska D, Zubrecki A** (1999): *The quail egg weight and their storage period vs.hatching success and rearing performance*. Adv Agr Sci, **6**, 91-100.
37. **Şekeroglu A, Duman M** (2011): *Etlik piliç ebeveynlerinde kuluçkalık yumurta kabuk renginin kuluçka sonuçları, piliç performansı, karkas özellikleri, iç organ ağırlıkları ve bazı*

- stres indikatörlerine etkisi (in Turkish)*. Kafkas Üniv Vet Fak Derg, **17**, 837-842.
38. **Turan N** (1990): *Türkiye'nin evcil ve yaban hayvanları: Kuşlar*. Orman Genel Müdürlüğü Eğitim Dairesi Başkanlığı Yayınları, Ankara.
39. **Wang XT, Zhao CJ, Li JY, et al.** (2009): *Comparison of the total amount of shell pigments in Dongxiang brown-shelled eggs and Dongxiang blue-shelled eggs*. Poult Sci, **88**, 1735-1739.
40. **Webb DR** (1987): *Tolerance of avian embryos: A Review*. Condor, **89**, 874-898.
41. **Wilson HR** (1991): *Interrelationships of egg size, chick size, posthatching growth and hatchability*. World's Poult Sci J, **47**, 5-20.
42. **Zhao R, Xu GY, Liu ZZ, et al.** (2006): *A study on eggshell pigmentation: Biliverdin in blue-shelled chickens*. Poult Sci, **85**, 546-549.

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