

PROOF

RESEARCH PAPER



Effect of Eggshell Colour on Incubation Performance in Broiler Breeders*

Emir Hisarcıklıoğlu¹ Nezih Okur^{2,*}

¹Bolu Abant İzzet Baysal Üniversitesi, Fen Bilimleri Enstitüsü, Bolu, Türkiye

²Bolu Abant İzzet Baysal Üniversitesi, Ziraat Fakültesi, Kanatlı Hayvan Yetiştiriciliği Bölümü, Bolu, Türkiye

*Master Thesis

Abstract

In this study, the effect of hatching eggshell colour on some incubation performance characteristics in broiler breeders were investigated. In the study, a total of 2400 hatching eggs obtained from two Ross 308 meat breeder flocks of the same age (29 weeks) and from different houses were used and these eggs were divided into two groups as brown (control) and white (experimental) according to their shell colours and incubated. At the end of the incubation period, the rates of losses such as embryo deaths, discards, broken chicks, contamination, brain bursts, etc. were compared with basic performance traits such as fertility, hatchability and hatchability by means of breaking analysis. As a result of the study, lower mid-term embryo mortality, higher fertility rate, hatchability of fertile eggs and hatchability values were obtained in the control group ($p<.05$). In contrast, early and late embryo mortality, the number of broken and discarded chicks, and the rates of eggs with contamination, transfer error, brain burst and fungus were similar ($p>.05$). As a result, it was determined that better incubation performance was obtained in brown-shelled eggs in terms of hatchability and therefore hatchability. It is thought that more attention should be paid to breeder poultry health for a higher and sustainable incubation performance and that similar studies including other poultry animals are needed.

Introduction

Egg shell color in breeder hens and commercial layers is a hereditary trait and not much work has been done on this subject. However, in recent years it has become a noteworthy issue due to the antioxidant properties of the pigments that give shell color (Stocker *et al.*, 1987; Kaur *et al.*, 2003).

Egg quality is an important factor affecting hatching results. Quality in both hatching and table eggs is examined in two parts as internal and external quality characteristics. While determining external quality criteria; egg weight, shape index, shell cleanliness, shell shape, shell thickness are generally taken into account; in terms of internal quality, albumen height, albumen width, yolk height, yolk diameter, flesh-blood spots and yolk color are taken into account (Karaman and

Bulut, 2018; Mahawar *et al.*, 2023; Turkoglu and Sarica, 2024; Elibol and Ozlu, 2024). Another factor affecting hatching results and chick performance is egg shell color and it has become an increasingly interesting subject due to the antioxidant properties of the pigments that give color to the shell (protoporphyrin, biliverdin and zinc biliverdin chelate) (Sparks, 2011). It has been reported that two pigments (biliverdin and zinc chelate) are present in blue-green shelled eggs and these 3 pigments are present together in blue and brown shelled eggs, the amount of protoporphyrin pigment is higher in brown eggs and depending on the density of this pigment, egg shell color varies from light cream to dark brown (Kennedy and Vevers, 1976; Schwartz *et al.*, 1980; Butcher and Miles, 1995).

Biliverdin, one of the pigments that form egg shell color, has an antioxidant; Protoporphyrin has been found to have antioxidant, antifungal and egg shell fracture resistance enhancing effects (Solomon, 1987; Kaur *et al.*, 2003; Ishikawa *et al.* 2010). In addition, it has been reported that egg yolk antibody (IgY) level is high in dark-shelled eggs of healthy hens and the quality of the hatched chicks is higher accordingly (Moreno *et al.*, 2004).

In a broiler breeder (Hybro) study (Shafey *et al.* 2005) in which egg shell color was classified as light (S1005-Y30R-S1005-Y40R), medium (S1010-Y40R-S1010-Y50R) and dark (S1020-Y40R-S1020-Y50R) using the "Natural Color System (NCS)", it was found that shell color in brown hatching eggs affected the rate of dead chicks under-shell dead and thus the hatchability ($P<0.05$), but did not affect the fertility rate and early and late embryo deaths, and that performance improved as the color of brown eggs became lighter, and that this was due to the shell color changing the effect of light. In another broiler breeder (Ross 308) study (Şekeroğlu and Duman, 2011) in which eggs were classified according to egg shell color and separated into three groups as dark ($E<80.00$), medium ($E:80.00$) and light ($E>83.50$) using E ($E=(L2+a2+b2)\frac{1}{2}$) values, it was found that fertility rate, hatchability and hatchability were higher in eggs with dark shells ($E<80.00$).

In a layer breeder study conducted on the Barred Rock-1 line, which constitutes the main line at the pure line level, within the scope of improving and standardizing egg shell color in ATAK-S, one of the Turkish layer hybrids registered in 2006 (Yurtoğulları, 2011), hatching eggs were divided into 3 groups according to shell color as dark (L: 0-59), medium (L: 60-70) and light (L: 71-78). It was found that the fertility rate in eggs with light-colored shells was low, and the mid- and late-stage embryo mortality rate was high. In a study conducted on brown egg layers (Mertens *et al.*, 2010), it was reported that there was a relationship between shell color and disease and stress, that animals exposed to heat stress and parasite infestation had lightening in egg shell color and a decrease in egg weight, that lightened shell color was an indicator of infectious bronchitis, and that shell color was a criterion that could be used to determine flock health and stress levels. This study was conducted to determine the effects of egg shell color on hatchability performance in broiler breeders by taking into account the results of the study briefly mentioned above and to obtain up-to-date and more detailed information on this subject with the help of a more practical classification system that can be easily applied in a commercial enterprise and on a commercial scale.

Materials and Methods

Hatching eggs were obtained from two 29-week-old broiler breeder flocks of the same Ross 308 genotype from a private integrated poultry company. A total of

2,400 hatching eggs were used in the trial. A private company's egg shell colour chart (The Egg Shell Color Fan, Hendrix Genetics) was used to classify the hatching eggs according to shell color. The egg handling, sorting, storage and incubation processes were also carried out in the same integrated company facilities. During the incubation phase, setters (AirStreamer™ 12S Focus, Petersime NV, Belgium) with a capacity of 57,600 eggs and hatchers (AirStreamer™ 4H Focus, Petersime NV, Belgium) with a capacity of 19,200 eggs were used.

Hatching eggs from both flocks were examined according to their eggshell colours and according to the eggshell colour fan (The Egg Shell Color Fan, Hendrix Genetics), eggs with colour codes 1 and 2 were accepted as white (experimental), and eggs with other colour codes were accepted as brown (control). The colors determined for the shell color were also measured with a chromameter (CR-400, Konica Minolta, Japan), the L (lightness), a (red/green), b (blue/yellow) values corresponding to these codes were determined, and c (chroma) and H (Hue angle) values were calculated using these values (white/trial1; L: 93.12; a: -0.66; b: 3.32; c: 3.39, H: -78.73; 2; L: 91.32; a: 0.67; b: 2.68; c: 2.76, H: 75.97; Brown/control; L: <91.32, a: >0.67, b: >3.32, H: <75.97).

After colour classification, 1200 eggs determined to have white shell colour (450 from the first flock and 750 from the second flock) were separated and placed in the setter trays with a capacity of 150 eggs each. Then, the same number of eggs determined to have brown shell colour, randomly selected from the same flocks, were separated in the same way and placed in the setter trays. Each tray in the treatment groups was accepted as a replication.

During incubation, setter machines were operated at 100°F shell temperature, 57.5% humidity and 24 turns/day, aiming for 12% egg weight loss. Transfer was performed on the 18th day of incubation and while eggs were being transferred from setter trays to hatcher baskets, eggs that were permeable to light during lamp control were considered as infertile and separated and lamp fertility rate was determined accordingly. At the end of the incubation period, eggs that did not hatch were separated and broken and after the breakout analysis, real fertility, embryo deaths (0-6 days early, 7-17 days middle/between the comb begins to grow and the egg tooth begins to appear and the head is between the legs, and 18-21 days late), broken shell but not hatched, discard, contamination, position error, transfer error, brain burst, fungus and other loss reasons were determined separately for all treatment groups and their rates were calculated (Formula 3.1). Then, classical hatchability of fertile eggs and classical hatchability values were calculated from the collected data (Formula 3.2 and 3.4). In addition, it was taken into account that cull chicks were not sent to the field on a commercial scale, and the number of discarded chicks was subtracted from the number of hatched chicks, and the values accepted and expressed as the real

hatchability of fertile eggs and real hatchability were also calculated (Formula 3.3 and 3.5).

Embryonic mortality rates was calculated using the Equation 1 given below

$$x, \% = \frac{\text{number of embryos that died for } x}{\text{total number of incubated eggs}}$$

x: number of embryos that died in the early/ middle/last period, number of chicks that broke the shell but could not hatch/discard, number of lamp fertilized/ true fertilized/contamination/position error/transfer error/brain burst/ fungus detected eggs.

Classic hatchability of fertile eggs was calculated using the Equation 2 given below.

$$\text{Classic hatchability of fertile eggs, \%} = \frac{\text{number of hatched chicks}}{\text{total number of Incubated fertile eggs}}$$

Real hatchability of fertile eggs was calculated using the Equation 3 given below.

$$\text{Real hatchability of fertile eggs, \%} = \frac{\text{number of hatched chick} - \text{number of discarded chick}}{\text{total number of Incubated fertile eggs}}$$

Classic hatchability was calculated using the Equation 4 given below.

$$\text{Classic hatchability of fertile eggs, \%} = \frac{\text{number of hatched chicks}}{\text{total number of incubated eggs}}$$

Real hatchability was calculated using the Equation 5 given below.

$$\text{Real hatchability of fertile eggs, \%} = \frac{\text{number of hatched chick} - \text{number of discarded chick}}{\text{total number of incubated eggs}}$$

In the study, randomized blocks were carried out in accordance with the experimental plan, a statistical package program (Minitab, 2013) was used to analyze the obtained data, and the t-test (Formula 3.6) was used to determine the differences between the groups.

t statistical value in the experiment was calculated using the Equation 6 given below (Kocabas *et al.*, 2013).

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{\sum d^2 x - \sum d^2 y}{(nx-1)+(ny-1)} \times \frac{nx+ny}{nx \times ny}}}$$

\bar{x} : means of control (brown eggshell colour) group,

\bar{y} : means of treatment (white eggshell colour) group,

$\sum d^2$: sum of differences from the mean, n: number of treatments.

In the study, the significance level between the treatment groups was determined as 5% ($p=0.05$) and the differences between the treatment groups with a p value less than 5% ($p<0.05$) were considered statistically significant. The results obtained in the experiments are shown as Mean \pm Standard Error of the Mean ($M \pm OSH$). In addition, Degrees of Freedom (DF) and critical t values are given.

Result and Discussion

The results obtained in the research were summarized in a table on both group and feature basis and examined and discussed separately in order to provide more detailed information (Table 1).

When hatching egg shell colours are taken into consideration, it is seen that we work with lighter coloured eggs compared to both Shafey *et al.* (2005; S502-G50Y and S0603-Y80R < S 1005-Y30R-S 1005-Y40R), Sekeroğlu and Duman (2011; E: 93.18 and 91.36 >78) and Yurtogullari, (2011; L: 93.12 91.32 > 78).

When the fertility rates were examined, it was determined that the values obtained were in accordance with the line objectives (94.00; Tullett, 2009), that lower values were obtained in the treatment groups in terms of both lamp fertility and true fertility, on a herd basis and accordingly in total, and that this situation was more evident in lamp fertility. It was observed that the results obtained in lamp fertility were similar to those in true fertility and that the study results were consistent with the results of the study (Yurtogullari, 2011; Sekeroğlu and Duman, 2011) reporting that the fertility rate in eggs with light shell colours was lower than in those with dark shell colours.

Similar to the fertility rates, early, mid and late embryo mortality rates were similar to the line objectives (5.50, 1.00 3.50, respectively; Tullett, 2009), with numerical differences between treatment groups, and higher in white-shelled eggs. However, the differences between treatment groups did not reach statistically significant levels [$t(14) = -1.87, p = .83$ and $t(14) = -2.03, p = .62$ for early and late stage respectively], except for mid-term embryo mortality, which was statistically significantly higher in the experimental group (white-shelled) eggs [$t(14) = -5.29, p = .00$]. The study results were found to be consistent with the results of studies reporting that early embryo mortality did not vary according to shell colour (Shafey *et al.*, 2005; Yurtogullari, 2011; Sekeroğlu and Duman, 2011), that mid+late embryo mortality was higher in eggs with light-coloured shells (Yurtogullari and Elibol, 2011) and that late embryo mortality was similar (Shafey *et al.*, 2005; Sekeroğlu and Duman, 2011), but inconsistent with the results of studies reporting that mid and late embryo mortality were similar (Shafey *et al.*, 2005; Sekeroğlu and Duman, 2011). In the breakage analysis, the cracked but unhatched and cull chicks and the rate of eggs with contamination, transfer error, brain burst and fungus

Table 1. Effect of eggshell colour on some hatchability performance criteria in broiler breeders

Features, %	First Group (Flock)				Second Group (Flock)				Total			
	Control 1	Treatment 1	t (DF)	P	Control 2	Treatment 2	t (DF)	P	Control	Treatment	t (DF)	P
Infertility	3.33 ± 1.02	6.22 ± 0.22	-2.77 (4)	0.050	2.80 ± 0.71 ^b	5.33 ± 0.56 ^a	-2.80 (8)	0.023	3.00 ± 0.55 ^b	5.67 ± 0.38 ^a	-4.00 (14)	0.001
Early Stage	1.11 ± 0.44	2.22 ± 0.59	-1.51 (4)	0.206	1.20 ± 0.33	2.00 ± 0.63	-1.12 (8)	0.294	1.17 ± 0.24	2.08 ± 0.43	-1.87 (14)	0.083
Mid Stage	1.11 ± 0.59	3.11 ± 0.44	-2.71 (4)	0.053	0.93 ± 0.34 ^b	2.93 ± 0.34 ^a	-4.16 (8)	0.003	1.00 ± 0.28 ^b	3.00 ± 0.25 ^a	-5.29 (14)	0.000
Late Stage	1.11 ± 0.44	2.44 ± 0.59	-1.81 (4)	0.145	2.13 ± 0.49	3.33 ± 0.70	-1.41 (8)	0.197	1.75 ± 0.38	3.00 ± 0.49	-2.03 (14)	0.062
CBU	0.00 ± 0.00	0.22 ± 0.22			0.27 ± 0.16	0.00 ± 0.00			0.17 ± 0.11	0.08 ± 0.08	0.61 (14)	0.554
Cull	0.22 ± 0.22	0.89 ± 0.44	-1.34 (4)	0.251	0.27 ± 0.16	0.27 ± 0.16	0.00 (8)	1.000	0.25 ± 0.12	0.50 ± 0.21	-1.03 (14)	0.319
Contaminated	0.44 ± 0.22	1.33 ± 0.39	-2.00 (4)	0.116	0.27 ± 0.27	0.93 ± 0.50	-1.18 (8)	0.272	0.33 ± 0.18	1.08 ± 0.33	-1.99 (14)	0.066
Malposition	0.44 ± 0.22 ^b	2.89 ± 0.18 ^a	-3.89 (4)	0.018	0.80 ± 0.33	1.47 ± 0.68	-0.88 (8)	0.403	0.67 ± 0.22 ^b	2.00 ± 0.52 ^a	-2.37 (14)	0.033
Turned failure	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.13 ± 0.13			0.00 ± 0.000	0.08 ± 0.08		
Exposed brain	0.22 ± 0.22	0.00 ± 0.00			0.27 ± 0.27	0.27 ± 0.16	0.00 (8)	1.000	0.25 ± 0.18	0.17 ± 0.11	0.40 (14)	0.639
Mould	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.000	0.00 ± 0.000		
Others	0.00 ± 0.00 ^b	2.22 ± 0.22 ^a			0.00 ± 0.00 ^b	1.47 ± 0.39 ^a			0.00 ± 0.000 ^b	1.75 ± 0.28 ^b		
Total looses	4.67 ± 0.39 ^b	15.33 ± 1.39 ^a	-7.41 (4)	0.002	6.13 ± 0.90 ^b	12.80 ± 2.09 ^a	-2.93 (8)	0.019	5.58 ± 0.62 ^b	13.75 1.41 ^a	-5.31 (14)	0.000
Candling Fertility	95.56 ± 0.97 ^a	91.56 ± 0.80 ^b	3.18 (4)	0.033	96.00 ± 0.87 ^a	92.67 ± 0.73 ^b	2.94 (8)	0.019	95.83 ± 0.61 ^a	92.25 ± 0.55 ^b	4.35 (14)	0.001
Real Fertility	96.67 ± 1.02	93.78 ± 0.22	2.77 (4)	0.050	97.20 ± 0.71 ^a	94.67 ± 0.56 ^b	2.80 (8)	0.023	97.00 ± 0.55 ^a	94.33 ± 0.38 ^b	4.00 (14)	0.001
Classic HFE	95.39 ± 0.64 ^a	84.60 ± 1.47 ^b	8.11 (4)	0.001	93.98 ± 1.01 ^a	86.74 ± 2.27 ^b	2.92 (8)	0.019	94.51 ± 0.69 ^a	85.94 ± 1.46 ^a	5.31 (14)	0.000
Real HFE	95.17 ± 0.44 ^a	83.65 ± 1.47 ^b	7.51 (4)	0.002	93.71 ± 0.91 ^a	86.46 ± 2.26 ^b	2.97 (8)	0.018	94.25 ± 0.62 ^a	85.40 ± 1.52 ^b	5.38 (14)	0.000
Classic Hatchability	92.22 ± 1.46 ^a	79.33 ± 1.02 ^b	7.25 (4)	0.002	91.33 ± 0.79 ^a	82.13 ± 2.36 ^b	3.70 (8)	0.006	91.67 ± 0.69 ^a	81.08 ± 1.62 ^b	6.28 (14)	0.000
Real Hatchability	92.00 ± 1.33 ^a	78.44 ± 1.35 ^b	7.14 (4)	0.002	91.07 ± 0.72 ^a	81.87 ± 2.38 ^b	3.70 (8)	0.006	91.42 ± 0.64 ^a	80.58 ± 1.62 ^b	6.24 (14)	0.000

were similar to or below the line objectives (1.50; 0.50; 0.50; 0.50 and 0.50; Tullett, 2009, respectively), as well as being very low or not detected at all, although numerically relatively higher rates were detected in white-shelled eggs (experimental group), the differences between the treatment groups were not found to be statistically significant for the cracked but unhatched and cull chicks, and contaminated egg and embryo with exposed brain [$t(14) = 0.61, p = .55$; $t(14) = -1.03, p = .32$; $t(14) = -1.99, p = .07$; $t(14) = 0.40, p = .64$; respectively]. After the breakage analysis, it was found that only the malposition rates were similar to the standards (1.50; Tullett, 2009), but the differences between the treatments were significant and higher in white-shelled eggs [$t(14) = -2.37, p = .03$]. The results were found to be inconsistent with studies reporting that the rate of hatched chicks was lower in light-coloured eggs (Shafey *et al.*, 2005).

When the hatchability of fertile eggs values was examined, it was found that the values obtained were similar to the line objectives (88.30; Tullett, 2009; Aviagen, 2021), and that both the classic and the real hatchability of fertile eggs were significantly lower numerically in the treatment groups on a flock basis and accordingly in total, and that these numerical differences were statistically significant [$t(14) = 5.31, p = 0.00$ and $t(14) = 5.38, p = 0.00$, respectively]. The results were found to be consistent with the results of the study reporting that hatchability was lower in eggs with lighter shell colours compared to darker ones (Şekeroğlu and Duman, 2011), but inconsistent with the results of the study reporting that shell color did not affect hatchability (Yurtoğulları, 2011) and that it was higher in lighter-colored eggs (Shafey *et al.*, 2005).

In terms of hatchability, the results were found to be similar to the line objectives (83.00; Tullett, 2009; Aviagen, 2021), higher in the control group (brown-shelled) eggs both on a flock basis and in total. Accordingly, the differences between treatment groups were statistically significant for both classic and real hatchability [$t(14) = 6.28, p = 0.00$ and $t(14) = 6.24, p = 0.00$; respectively]. A performance average was calculated. It was observed that the results are consistent with the results of the study reporting that the hatchability of eggs with lighter shell colours is lower than those with darker colours (Şekeroğlu and Duman, 2011). When the results obtained were evaluated in general, it was determined that the brown shell eggs, which were determined as the control group, had significantly lower mid-term embryo mortality rates and higher fertility rates, hatchability of fertile eggs and hatchability values compared to the white ones. On the other hand, no significant difference was found between the treatment groups in terms of early and late-term embryo mortality and the rates of eggs with broken but not hatched chick position errors, transfer

errors, brain bursts, mould and contamination. As reported by the researchers, it is thought that this situation is due to the antioxidant and antifungal properties of the pigments that give the shell its colour (Solomon, 1987; Kaur *et al.*, 2003; Moreno *et al.*, 2004; Ishikawa *et al.* 2010), and that the lightening of the shell colour is an indicator of deterioration in health.

Conclusion

In this study, the effects of brown (control) and white (experimental) shell colors of hatching eggs in broiler breeders on incubation performance were investigated through embryo deaths (early, middle and late periods), discards and unhatched chicks, contamination, brain burst, etc. determined losses rates in two consecutive experiments, fertility rate, hatchability of fertile eggs and hatchability.

As a result of the study, it was found that better values were obtained in eggs with brown shell color, shell color affected mid-term embryo death rates and higher fertility rate, hatchability and hatchability, whereas it did not affect early and late-term embryo deaths and unhatched chicks position error, transfer error, brain burst, fungus, contamination detected egg rates. It is thought that this situation may be due to the lightening of the shell color due to the health status of the breeding hens.

Therefore, in order to achieve higher hatching performances, more attention should be paid to the rate of eggs with light-colored shells and to issues that may affect the health of the breeding hens. In addition, larger-scale and more detailed studies that include other poultry animals will be beneficial and will provide a continuous and sustainable improvement in hatching performances.

Acknowledge

The authors thanks to Beypiliç® for animal material and support.

References

- Aviagen. (2021). Ross 308 parent stock: Performance objectives. Aviagen Ltd. Newbridge, Midlothian EH28 8SZ, Scotland, UK.
- Butcher, G. D. , Miles, R.D. (1995). Miles. Factors causing poor pigmentation of brown shelled eggs. Cooperative Extension Service Fact Sheet VM94. Inst. Food and Agriculture. Science., Univ. Florida, Gainesville
- Elibol, O., Ozlu, S. (2024). Embryo development and incubation. Poultry Science: Breeding, Feeding, Diseases (Ed. M. Turkoglu and M. Sarica). 1st electronic printing. 133-166p. ISBN: 978-625-97746-2-6. <http://books.turkishpublishing.com/index.php/TURSTEP/catalog/view/16/14/66>

Ishikawa, S., Suzuki, K., Fukuda, E., Arihara, K., Yamamoto, Y., Mukai, T., Itoh, M. (2010). Photodynamic antimicrobial activity of avian eggshell pigments. *FEBS Letters* 584, 770–774. <https://doi.org/10.1016/j.febslet.2009.12.041>

Karaman, M., Bulut, M. (2018). Effect of Hatching Egg Weight on the Performance of Hatchery and Post-hatching Characteristics for Japanese Quails. *Journal of Agriculture and Nature*, 21 (1), 13-19. <https://doi.org/10.18016/ksudobil.282557>

Kaur, H., Hughes, M. N., Green, C. J., Naughton, P., Foresti, R., Motterlini, R. (2003). Interaction of bilirubin and biliverdin with reactive nitrogen species. *FEBS Lett.*, 543, 113–119. [https://doi.org/10.1016/s0014-5793\(03\)00420-4](https://doi.org/10.1016/s0014-5793(03)00420-4)

Kennedy, G. Y., Vevers, H. G. (1976). A survey of avian eggshell pigments. *Comp. Biochemical Physiology* 55B, pp 117–123.

Kocabas, Z., Ozkan, M., Baspinar, E. (2013). *Basic Biometry*. Ankara University Press, Ankara.

Mahawar, M., Altuntaş, E., Güll, E. N. (2023). Mass Modelling of Eggs Based on Shape Index Using Regression Analysis. *Journal of Agriculture and Nature*, 26(1), 132-139. <https://doi.org/10.18016/ksutarimdoga.vi.992588>

Mertens, K., Vaesen, I., Ioffel, J., Kemps, B., Kamers, B., Perianu, C., Zoons, J., Darius, P., Decuyper, E., De Baerdemaeker, J., De Ketelaere, B. (2010). The transmission color value: A novel egg quality measure for recording shell color used for monitoring the stress and health status of a brown layer flock. *Poult Science*, 89 (3), 609-617. <https://doi.org/10.3382/ps.2009-00261>

Minitab 2013. Statistical Software Release, Version 17.3.1. Minitab Inc. USA.

Moreno, J., Osorno, J. L., Morales, J., Merino, S., Tomas, G. (2004). Egg colouration and male parental effort in the pied flycatcher *Ficedula hypoleuca*. *Jornal of Avian Biology*, 35, 300–304. <https://www.jstor.org/stable/3677720>

Schwartz, S., Raux, W. A., Schacter, B. A., Stephenson, B. D., Shoffner, R. N. (1980). Loss of hereditary uterine protoporphyrin through chromosomal rearrangement in mutant Rhode Island red hens. *International Journal of Biochemistry*, 12 (5-6), 935-940. [https://doi.org/10.1016/0020-711x\(80\)90188-3](https://doi.org/10.1016/0020-711x(80)90188-3)

Shafey, T. M., Al-Batshan, H. A., Ghannam, M. M., Al-Ayed, M. S. (2005). Effect of intensity of eggshell pigment and illuminated incubation on hatchability of brown eggs. *British Poultry Science*, 46 (2), 190–198. <https://doi.org/10.1080/00071660500065789>

Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., Ames, B. N. (1987). Bilirubin is an antioxidant of possible physiological importance. *Science*, 235 (4792), 1043–1046. <https://doi.org/10.1126/science.3029864>

Solomon, S. E. (1987). Egg shell pigmentation. In *Egg Quality Current Problems and Recent Advances* (Ed. R.G. Wells and C. G. Belyavin), pp. 147–158. London: Butterworths.

Sparks, N. H. C. (2011). Eggshell Pigments-from Formation to Deposition. *Avian Biology Research*, 4 (4): 162-167. <https://doi.org/10.3184/175815511X13228269481875>

Şekeroğlu, A., Duman, M. (2011). Effect of Egg Shell Colour of Broiler Parent Stocks on Hatching Results, Chickens Performance, Carcass Characteristics, Internal Organ Weights and Some Stress Indicators. *Journal of The Faculty of Veterinary Medicine, Kafkas University*, 17 (5): 837-842. <https://doi.org/10.9775/kvfd.2011.4630>.

Tullett, S. (2009). Hatchery. Ross tech: Investigating hatchery practice. Aviagen Inc. Newbridge, Midlothian EH28 8SZ, Scotland, UK.

Turkoglu, M., Sarica M. (2024). *Poultry Science: Breeding, Feeding, Diseases*. 1st electronic printing. ISBN: 978-625-97746-2-6. http://books.turkishpublishing.com/index.php/TURST_EP/catalog/view/16/14/66

Yurtogullari, S. (2011). Effect of Egg Shell Color on Some Egg Quality Traits and Hatchability (Thesis No: 299722). [Master Thesis (Supervisor: O. Elbilo)]. Ankara University Graduate School of Natural and Applied Sciences Animal Science Department]. Council of Higher Education Thesis Center