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■ Keywords

Guinea fowl (*Numida meleagris*), breeder, eggshell color, hatchability.

Effect of Eggshell Color on the Egg Characteristics and Hatchability of Guinea Fowl (*Numida meleagris*) Eggs

ABSTRACT

The objective of this study was to determine the effects of eggshell color of guinea fowl (*Numida meleagris*) eggs on eggshell quality and hatchery results. The highest mean grey value (MGV), integrated density (ID), lightness (L*) and Hue angle (H*) values were obtained in eggs with lighter eggshell color. The effects of color difference (ΔE^*) value levels on egg characteristics were evaluated. Eggshell color presented different ($p < 0.01$) MGV, ID, L*, redness (a*), yellowness (b*), H* and Chroma (C*) values. Eggs with lighter color presented thicker eggshells and lower egg weight loss on day 25 of incubation ($p < 0.05$) compared with intermediate and darker eggs. Eggshell color did not have any significant effect on fertility and hatchability ($p > 0.05$). In conclusion, under the conditions of the present study, eggshell color influenced eggshell thickness and weight loss, but not hatching parameters of guinea fowl eggs. Further studies on this subject should be carried out.

INTRODUCTION

The size of poultry companies has increased around the world, as well as hatcheries. Therefore, it is important to achieve good breeder flock performance – which is the first stage of poultry production – and to improve hatchery results. Incubation conditions influence embryonic development, and therefore, day-old chick quality, which in turn, determines the subsequent performance of meat- and egg-producing poultry. Incubation results and the performance of different poultry species are affected by genetic and environmental factors, including genotype, egg storage time, egg internal and external quality, nutrition, breeders' body weight, breeding age, season, breeding system, male-female proportion, health status, etc. (Roberts & Nolan, 1997; Caglayan *et al.*, 2014).

Eggshell color is an external egg quality characteristic and may affect incubation results and chick performance (Shanawany, 1987). Shafey *et al.* (2005) reported that high light intensity during incubation reduced the hatchability of light-brown and medium-brown eggs, but not of dark-brown eggs in laying hens. Dark eggs from a same breeder flocks presented better incubation results than light eggs (Erensayın, 1991). In a study conducted with broiler chickens, Sekeroglu & Duman (2011) reported that eggs with dark eggshells presented better egg quality and hatchability results.

Eggshell pigmentation during egg formation can be influenced by stress or disease (Whittow, 1999), and some researchers reported that stress results in egg whitening as a consequence of premature termination of eggshell pigmentation (Nys *et al.*, 1991).

Several different color measurement techniques (reflectometry and front-face fluorescence spectroscopy) have been developed to determine eggshell color (Mertens *et al.*, 2010). Eggshell color can



also be measured using other systems, such as the CR300 Minolta Chromameter (Minolta, Osaka, Japan) that measures the L* (lightness), a* (redness), b* (yellowness) color space. The Chromatographer can easily measure brown eggshells, especially the beige eggshell of guinea fowl, but not white eggshells due to their lightness value (L*), which is 100. Therefore, grey values may be used to determine the tone of white eggshell or white transition color. Eggshell grey value may be a promising tool for estimating the lightness of white eggshells.

Two types of pigments determine eggshell color. Protoporphyrin IX, a precursor of hemoglobin, is deposited on the surface resulting in brown color (Butcher & Miles, 1995; Nys & Gautron, 2013). Biliverdin IX, derived from the bile, produces blue-green pigmentation.

Pigments are deposited in the eggshell quite late in the eggshell formation process. Therefore, problems with poor pigmentation may occur if the egg is prematurely laid (Nys *et al.*, 1991). Shafey *et al.* (2002) also reported that eggshell color differences may be due to the protoporphyrin absorption characteristics in brown eggshells when compared with the non-pigmented white eggshells (Liu *et al.*, 1998).

Chicken eggs, especially brown eggs, have been extensively studied for its eggshell color on internal and external qualities of egg and its composition; however, few information have been reported in other poultry species. Guinea fowls are wild birds that live in arid rural areas in many African countries. Its production has considerably increased in recent years in organic agriculture systems. Guinea fowl eggshell color varies between yellowish, beige speckled with ochre and brown (Le Coz-Douin, 1992).

Egg weight is an important parameter to be considered when determining incubation conditions. Literature reports average egg weight of guinea fowl as 38g (Ayorinde *et al.*, 1989), 40-45 g (Fani *et al.*, 2004), and, in between 34.0 and 45.7 g (40.1 g, on average) in Turkey (Alkan *et al.*, 2013). Guinea fowl eggs weighing 31.4 and 31.8 g presented hatchability rates of 72.8% and 73.6%, respectively, which, however, were not significantly different from each other (Naadam & Issah, 2012). An experiment was conducted to determine the effect of egg size on hatchability of guinea fowl keets and found that medium sized (39-42 g) eggs had significantly higher hatchability than small eggs (Moreki & Mothei, 2013).

The recommended storage conditions of guinea fowl eggs are 10-18°C at 70-80% relative humidity

(Binali, 1985; Belshaw, 1985; Binali & Kanengoni, 1998), for a maximum period of seven days, as their hatchability rapidly decreases with storage time. It was reported that, for each day of storage, hatchability was reduced in nearly 4% (Nwagu & Alawa, 1995).

Fertility and hatchability are major constraints in guinea fowl production. Fertility and hatchability of guinea fowl (pearl) eggs are reported as 53% and 87% respectively (Galor, 1983; Ayorinde *et al.*, 1988; Ayorinde *et al.*, 1989). An experiment on economic traits of guinea fowl production showed the hatchability and fertility of guinea fowl eggs is 75-80%, with an incubation period of 26-28 days (Fani *et al.*, 2004). Nwagu (1997) and reported the main factors that affect the hatchability of guinea fowl eggs are egg size, eggshell quality, and variation in brooding temperature.

Galor (1983) and Ayorinde *et al.* (1989) was found that the fertility of guinea fowl eggs under artificial incubation much higher than that of natural mating. The low fertility in naturally-mated stock is also associated with the monogamous sexual behavior of the guinea fowl in addition to the fertility constraints with the male (Saina *et al.*, 2005). It was shown that guinea fowl hens (pearl) are capable of laying fertile eggs throughout the year when given adequate supplementary feeds with the provision of water *adlibitum* (Konlan *et al.*, 2011).

Guinea fowl eggs exhibit low hatchability than chicken eggs mainly because of their thicker eggshells compared with chicken eggs (Yildirim, 2012). The hatchability rates of guinea fowl eggs is 67% under artificial incubation (Kabera, 1997). Hatchability of guinea fowl eggs in manual incubators are usually low due to lack of automated turning (Moreki, 2009).

There a few information in literature on guinea fowl eggshell quality and color. Therefore, light of this perspective, the present study was carried out to investigate the influence of eggshell color of hatching eggs on hatchability and eggshell quality of guinea fowl.

MATERIALS AND METHODS

A total of 763 hatching eggs collected for 14 days from a 42-week-old guinea fowl (*Numida meleagris*) flock were used in the study. Eggs were stored at 75% relative humidity (RH) and 13°C (Woodard & Morzenti, 1975; Caglayan *et al.*, 2009; González-Redondo, 2010; Reijrink *et al.*, 2010) during the egg collection period.



Breeders were reared in the Wild Animals Breeding Station affiliated to Ministry of Forest and Water Affairs in Yozgat, Turkey, situated at 34°05'–36°10'E, 38°40'–40°18'N, at 1240-m altitude. Annual temperatures range between -6.96 and 24.4 °C and average relative humidity is 66% (Anonymous, 2011). The flock was reared under natural mating system.

Digital images of eggs were obtained using a digital SLR camera fixed on a tripod. The equipment and tools used in this project are listed in Table 1.

At the end of the 14-day egg collection period, eggs were individually numbered and weighed at the

beginning and end of incubation (on d 25) to calculate moisture loss.

Spatial calibration was applied to the digital images captured by the camera (Canon EOS 60D) for converting pixels into cm. Adobe Photoshop CS5 was used to digitalized the images and measurements. The Quick Selection tool was used to select surface area of egg (projection area=PA). After selection, the measurement scale was set as custom (371 pixel=1 cm) and the Record Measurements command was applied to measure egg width, egg length, egg projection area, and egg diameters (Figure 1)

Table 1 – Imaging equipment and image properties

Imaging Equipment		Image Properties	
Camera	Canon EOS 60D	Image size	5184 x 3456
Software	Adobe Photoshop CS5	Resolution	72 Pixel per inch
Tripod	Manfrotto Professional	Focal length	135.0 mm
Flash	On	Program	Normal, Evaluative metering
Lens	EF-S18-135mm, f/3.5-5.6 IS	Exposure	1/250 sec; f/5.6; ISO 2000, auto focus
		Bit Depth	24 bits (2 ²⁴) = 16.7 million tones
		Color representation	sRGB

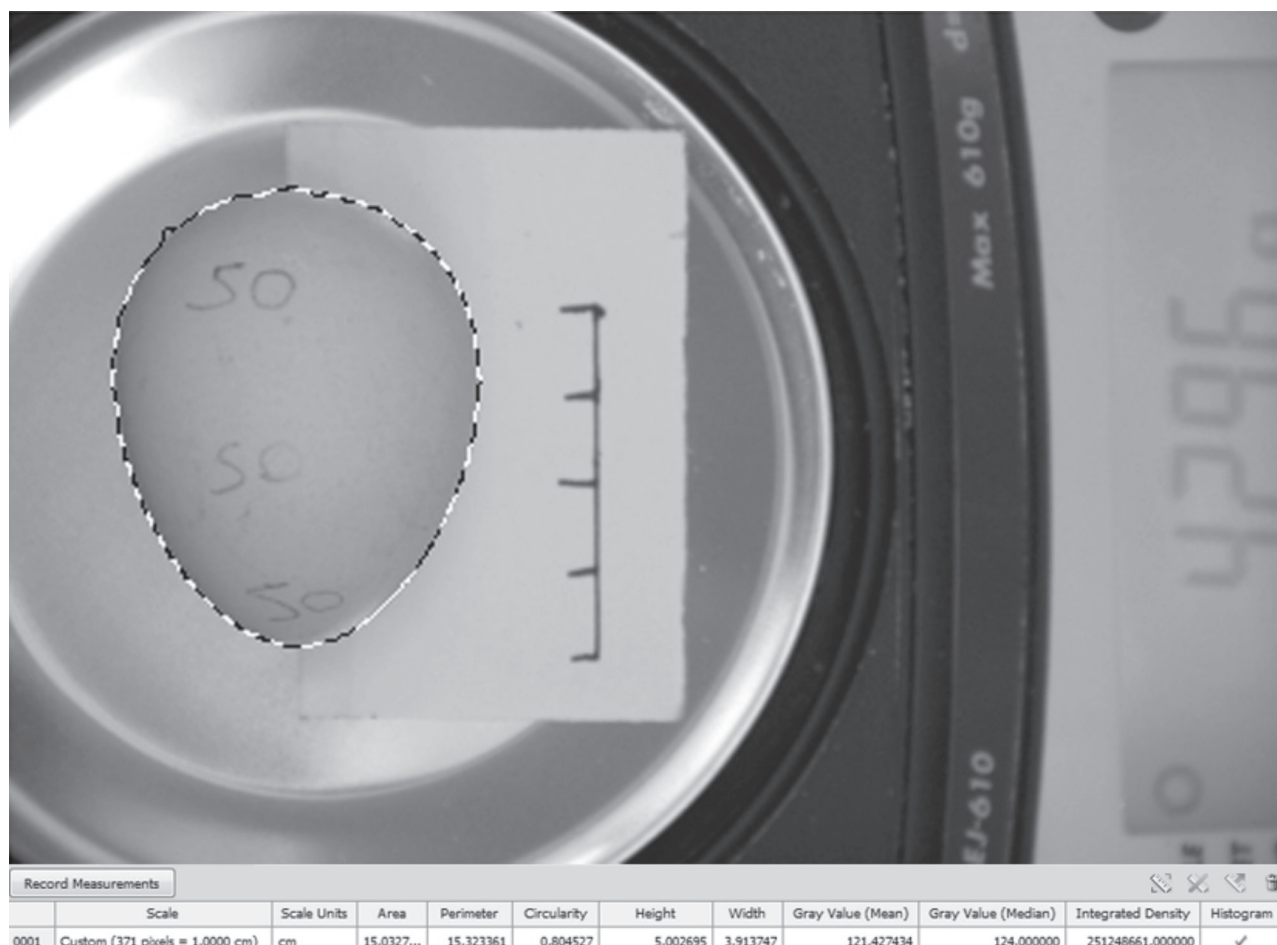


Figure 1 – The use of Adobe Photoshop CS5 to digitize the samples and measurements



Egg weight (EW) was determined in a digital scale (0.01-g accuracy; WPS 3100, Radwag, Radom, Poland). Egg weight loss was calculated as a percentage of set egg weight over the entire 0- to 25-d incubational period. Eggshell thickness of the dried eggshells without the membrane was measured using a micrometer to the nearest 1/100 mm on day 28 of incubation (at the end of incubation). The measurements were taken at three random locations at the equator, blunt and pointed edges of each eggshell without membrane. The average of the three values was recorded as the thickness of the eggshell.

Eggshell color was measured at the large end of the eggs using a colorimeter (Minolta, CR-600, Osaka, Japan). L^* , a^* , b^* , $\Delta E^*(L^{*2}+a^{*2}+b^{*2})^{1/2}$, chroma (C^* ; $\sqrt{a^{*2} + b^{*2}}$) and MGW values were determined. The L^* (measured on a scale of 0 to 100%, dark to light), a^* (on a scale of -60 to 60%, green to red), and b^* (on a scale of -60 to 60%, blue to yellow) color measurements were determined according to the CIELab color space system. The instrument was calibrated with a white reference tile ($L^* = 97.10$, $a^* = -4.88$, $b^* = 7.04$) before the measurements were taken, according to Francis (1998). ΔE^* (color differences) values were classified (Figure 2) as intermediate (78.01-81.98, $n=229$), darker (54.76-77.98, $n=414$) and lighter (82.01-94.57, $n=120$). Mean grey values classified as intermediate (80-110, $n=440$), darker (≥ 111 , $n=163$) and lighter (≤ 79 , $n=165$).

Egg surface area (ESA, cm^2) was calculated as $3.9782 \times \text{egg weight}^{0.70}$. Circularity (CI) was calculated as $4\pi(\text{PA}/\text{P}^2)$, where a value of 1.0 indicates a perfect circle. Egg shape index (SI, %) was calculated as $(\text{egg width}/\text{egg length}) \times 100$. Hue value (H^*) was calculated as $\tan^{-1} b^*/a^*$. Integrated density (ID or the sum of the values of the pixels in the selection), projection area (PA, cm^2), perimeter (P), values were calculated.

After these measurements, eggs were fumigated and set in the incubator (Petersime Model 192). On day

25 of incubation, eggs were transferred to the hatcher (Petersime Model 192). The incubator was set at 37.6 °C and a 65% RH during incubation, and at 37.4 °C temperature and 75% RH during the hatching period.

On day 25 of incubation, before eggs were transferred from the setter to the hatcher, hatching trays were partitioned into individual sections using hard boards to prevent of guinea fowl keets from mixing up during hatching.

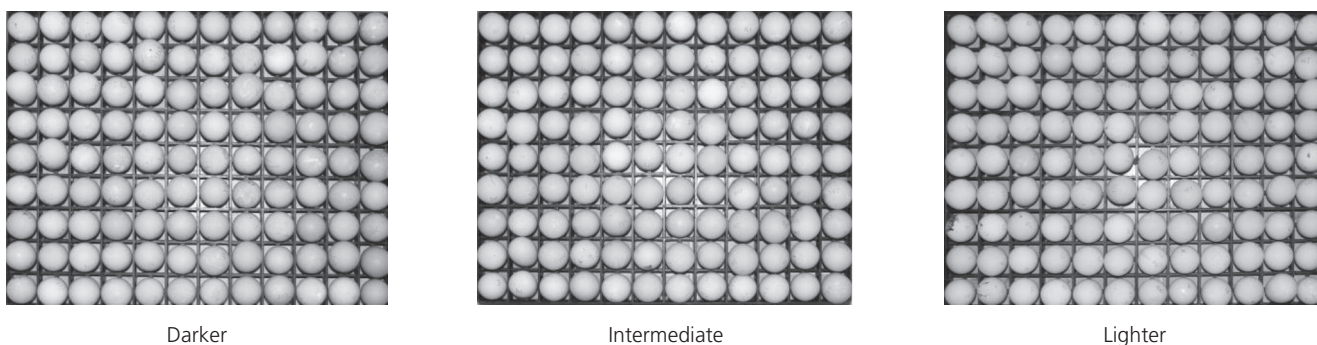
At the time keets were removed from the hatcher, all unhatched eggs were opened to distinguish unfertilized eggs from those containing dead embryos, and to determine fertility, fertile hatchability and hatchability. Unhatched eggs were examined macroscopically by a single experienced individual to embryonic mortality, which was classified as early (1 to 6 days), mid-term (7 to 24 days), or late (25 to 28 days plus pipped eggs). Fertile hatchability was calculated as the number of first-quality chicks hatched per fertile egg set.

Data were analyzed according to a completely randomized design. The analyses were conducted using SPSS 22.0 software (SPSS, 2013). Percentage data were submitted to angular transformation. Numerical data were submitted to analysis of variance. When differences were significant, means were separated using Duncan's multiple range tests at the 0.05 level of significance.

All of the experimental procedures were carried out in accordance with the permission was obtained from Cumhuriyet University Experimental Animals Ethics Committee (Protocol number:04.04.2013/39).

RESULTS AND DISCUSSION

Descriptive statistics and effects of eggshell color on hatchery results of guinea fowl eggs were separately analyzed to different parameters and results of analyses were presented in tables (Table 2, 3, 4 and 5).



Darker

Intermediate

Lighter

Figure 2 – The eggs of Guinea fowl classified into darker (54.76-77.98), intermediate (78.01-81.98) and lighter (82.01-94.57) based on ΔE^*



Table 2 – Descriptive statistics of guinea fowls eggs (*Numida meleagris*)

Parameters	Range	Minimum	Maximum	Mean	Std. Error	Std. Dev.	Variance
Weight (EW), g	18.74	29.44	48.18	39.9851	0.09745	2.69175	7.246
Height (H), cm	1.14	4.74	5.87	5.3242	0.00640	0.17674	0.031
Width (W), cm	0.71	3.79	4.49	4.1598	0.00375	0.10359	0.011
Projection area (PA), cm ²	5.49	14.02	19.51	17.0399	0.02982	0.82381	0.679
¹ Egg surface area (ESA), cm ²	11.55	28.06	39.61	34.7458	.05942	1.64124	2.694
² Circularity (CI)	0.18	0.69	0.86	0.8074	0.00039	0.01073	0.000
³ Shape index (SI), %	19.40	67.55	86.95	78.1891	.09045	2.49831	6.242
Perimeter (P)	3.50	14.79	18.29	16.2818	0.01553	0.42911	0.184
⁴ Mean grey value (MGV)	101.75	42.95	144.70	94.5498	0.66143	18.27020	333.800
⁵ Integrated Density (ID)	1,87x10 ⁸	0,94x10 ⁸	2,81 x10 ⁸	1,86 x10 ⁸	0,01x10 ⁸	0,36 x10 ⁸	
L *	38.82	49.56	88.38	74.3759	0.20042	5.53611	30.648
a *	16.10	-2.03	14.07	4.6050	0.11450	3.16284	10.004
b *	31.35	9.48	40.83	20.7018	0.12829	3.54375	12.558
⁶ H *	39.83	61.32	101.15	78.51	0.26	7.26	52.72
⁷ C *	3.14	-1.57	1.57	1.1686	0.02559	0.70696	0.500
⁸ ΔE *	40.01	54.56	94.57	77.5538	0.16254	4.48975	20.158

¹ESA=3.9782 x egg weight^{0.70}, ²CI=4pi(PA/P²), A value of 1.0 indicates a perfect circle, ³SI=(Egg width/egg length) x 100, ⁴MGV=This is a measurement of brightness,

⁵ID=The sum of the values of the pixels in the selection. ⁶H*=tan⁻¹ b*/a*, ⁷C*=√(a*² + b*²), ⁸ΔE*=(L²+a²+b²)^{1/2}

Table 3 – Effects of ΔE value levels on guinea fowls egg characteristics

Parameter	Groups of ΔE* ¹ value			SEM ²	p value
	Darker	Intermediate	Lighter		
Weight (EW), g	40.13	39.95	39.57	0.097	0.128
Height (H), cm	5.34	5.33	5.27	0.006	0.072
Width (W), cm	4.17	4.15	4.14	0.004	0.063
Projection area (PA), cm ²	17.11	17.03	16.81	0.030	0.052
³ Egg surface area (ESA), cm ²	34.83	34.72	34.49	0.059	0.132
⁴ Circularity (CI)	0.818	0.807	0.807	0.001	0.638
⁵ Shape index (SI), %	78.20	77.98	78.56	0.090	0.122
Perimeter (P), cm	16.31	16.28	16.18	0.016	0.055
⁶ Mean grey value (MGV)	85.68 ^c	100.55 ^b	113.72 ^a	0.661	0.001
⁷ Integrated Density (ID)	1,69x10 ^{8c}	1,97x10 ^{8b}	2,20 x10 ^{8a}	1,30x10 ⁶	0.001
L *	70.4091 ^c	77.3504 ^b	82.3851 ^a	0.20042	0.001
a *	6.6769 ^a	2.9933 ^b	0.5323 ^c	0.11450	0.001
b *	22.5895 ^a	19.7021 ^b	16.0972 ^c	0.12829	0.001
⁸ H *	73,8615 ^c	81,5454 ^b	88,7793 ^a	0.263	0.001
⁹ C *	23.64 ^a	16.17 ^c	19.97 ^b	0.144	0.001

¹ΔE*=(L²+a²+b²)^{1/2}, (Darker= 54.56 – 77.98, Intermediate= 78.01 – 81.98, Lighter= 82.01 – 94.57) ²SEM=Standard error of the mean ³ESA=3.9782 x egg weight^{0.70}, ⁴CI=4pi(PA/P²), A value of 1.0 indicates a perfect circle, ⁵SI=(Egg width/egg length) x 100, ⁶MGV=This is a measurement of brightness, ⁷ID=The sum of the values of the pixels in the selection

⁸H*=tan⁻¹ b*/a*, ⁹C*=√(a*² + b*²) ^{abc} Means within row followed by different superscripts are significantly different(p<0.01).

Table 4 – The effects of ΔE* value levels on hatching results of Guinea fowls

Parameters	Groups of ΔE* ¹ value			SEM ²	p value
	Darker	Intermediate	Lighter		
Egg weight loss, g	5.00 ^{ab}	6.43 ^a	4.31 ^b	0,290	0.049
Eggshell thickness, mm	37.30 ^b	39.48 ^a	40.86 ^a	0.290	0.001
Embryonic mortality %					
Early (1-6 days)	13.8	10.2	14.5	0,076	0.404
Mid (7-24 days)	12.9	21.1	13.2	0,098	0.404
Late (25-28 days plus pipped)	2.2	0.8	2.6	0,133	0.404
Hatchability traits					
Fertility, %	54.1	55.9	63.3	0.490	0.200
Fertile Hatchability, %	71.0	68.0	69.7	0.055	0.404
Hatchability, %	38.4	38.0	44.2	0.067	0.308

¹ΔE*=(L²+a²+b²)^{1/2}, (Darker= 54.56 – 77.98, Intermediate= 78.01 – 81.98, Lighter= 82.01 – 94.57). ²SEM=Standard error of the mean. ^{ab}Means within row followed by different superscripts are significantly different(p<0.05).



Table 5 – The Effects of MGCV value levels on hatching results of Guinea fowls

Parameter	Groups of MGCV ¹ value			SEM ²	p Value
	Darker	Intermediate	Lighter		
Egg weight loss, g	4.09 ^c	5.88 ^b	5.93 ^a	0.261	0.025
Eggshell thickness, mm	37,72	38,65	39,28	0.331	0.296
Embryonic mortality %					
Early (1-6 days)	11.8	12.8	13.9	0,119	0.739
Mid (7-24 days)	12.9	14.5	19.8	0,073	0.739
Late (25-28 days plus pipped)	1.2	2.5	1.0	0,117	0.739
Hatchability traits					
Fertility, %	55.5	55.0	63.9	0.018	0.062
Fertile Hatchability, %	74.1	70.2	65.3	0.055	0.474
Hatchability, %	38,2	38.6	41.8	0.067	0.318

¹MGV= Mean grey value (Darker= >111, Intermediate= 80-110, Lighter= <79)

²SEM==Standard error of the mean.

In this study, guinea fowl eggs presented average 39.99g egg weight, 5.32 mm egg height, and 4.16 mm width (Table 2). The descriptive statistic results of guinea fowl eggs obtained this study are consistent with other reports. For example, egg weight was similar to that observed by Ayorinde *et al.* (1989) and within the ranges reported by other researchers (Fani *et al.*, 2004; Naadam & Issah, 2012; Alkan *et al.*, 2013).

Relative to color measurements, descriptive egg parameters were numerically affected by mean ΔE^* value, and statistically different ($p < 0.01$) as a function of mean MGCV, ID, L^* , a^* , b^* , H^* and C^* values. The highest MGCV, ID, L^* and H^* values were obtained in eggs with lighter eggshell color. Eggshell color presented significantly different MGCV, ID, L^* , a^* , b^* , H^* and C^* values.

As shown in Table 4, eggshell thickness and egg weight loss on day 25 were significantly affected by ΔE^* values. Darker eggshells were thinner ($p < 0.01$), and eggs with lighter eggshells presented lower weight loss on day 25 of incubation ($p = 0.049$). Interestingly, the higher eggshell thickness observed in lighter eggs contrasts with earlier findings in broiler parent stocks (Sekeroglu & Duman, 2011).

However, neither embryonic mortality nor hatchability results were affected by ΔE^* values ($p > 0.05$). The embryonic mortality results presented in Table 4 and 5 are similar to those reported by Sekeroglu & Duman (2011).

Hatchability and fertility rates obtained in the present study were within range or slightly lower than those reported by previous researchers (Galor, 1983; Ayorinde *et al.*, 1988; Ayorinde *et al.*, 1989 and Alkan *et al.*, 2013). In contrast, the fertility rate of around 60% (Table 4 and 5) is lower than that observed by Fani *et al.* (2004), who obtained 75-80% fertility. The

lower fertility in present study could be explained by differences in sex ratio, which was higher in the present study. Because guinea fowl present monogamous sexual behavior, high male to female ratios in the breeding flock may have a positive effect on fertility.

The average hatchability observed in present study was close to those obtained by several authors (Saina *et al.*, 2005; Nwagu & Alawa, 1995; Galor, 1983; Binali & Kanengoni, 1998; Nwagu & Alawa, 1995; Bessin *et al.*, 1998), but and lower than the findings of Fani *et al.* (2004). The obtained hatchability may be explained by the average egg of 39.99 g, which is consistent with the findings of Moreki & Mothei (2013), who reported higher hatchability of medium-size (39-42 g) eggs.

In conclusion, under the conditions of the present study, eggshell color influenced eggshell thickness and weight loss, but not hatching parameters of guinea fowl eggs. Further studies on this subject should be carried out.

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