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The Effect of Non-Genetic Parameters on Sex Determination Running Title: Gender Determination by Shell Quality Characteristics

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Abstract

From commercial, ethical and scientific perspectives, the culling of male chicks, particularly obtained from egg-laying breeders, is the most fundamental problem to be solved in the commercial poultry industry. This study aimed to determine the gender of the embryo based on characteristics of shell quality related to the weight of hatching eggs from broiler breeders. A total of 60 hatching eggs were divided into two groups: one weighing 55.50 g and the other weighing 66.50 g. The egg weight, width and length of air space, and width and length of each egg before incubation, and the water loss of the eggs during incubation was measured. At hatching, the weight of the chicks categorised by gender was measured, as well as the residual shell thickness, pore number, width and length of the pores. The difference in chick weight by gender is due to egg weight. Male and female chicks in the 66.50 EW group were heavier than those in the 55.50 EW group. Male chicks that hatched from heavier eggs were heavier than females. Male chicks hatched from thinner eggshells. Male chicks hatched from eggs with higher shape index, wider air space, smaller pore width at the pointed end and shorter pore lengths at the blunt and equatorial ends of lighter eggs. In contrast, female chicks hatched from eggs with lower shape index, higher shell thickness and wider pore width at the blunt, equatorial and pointed ends of lighter eggs, and with smaller air space width of heavier eggs. In conclusion, the gender of the embryo can be determined before or during embryo development based on various shell quality characteristics resulting from differences in egg weights obtained from broiler breeders of the same genotype and age.

Introduction

Poultry have faster reproductive capacity and are more productive than other farm animals. Chickens are the most important poultry species in animal food production, ranking first in both egg and meat production. Their importance is further increased by the high nutritional value (100% in eggs) of these products, which are main sources of animal protein. However, the sustainability of these products depends on the development of embryos in fertilised hatching eggs obtained from broiler and layer breeders.

In the commercial poultry industry, male chicks hatched from the eggs of meat-type breeders are raised because they perform better than female chicks (Cygan-Szczegielniak and Bogucka, 2021). In contrast, male chicks hatched from eggs obtained from layer breeders are subject to culling through the implementation of specific methodologies (Krautwald-Junghanns *et al.*, 2018). The culling of male chicks in egg production, in particular, poses a major problem both industrially and ethically, leading to the rearing of male chicks for meat production; however, this has been considered a very costly method. This is because male laying chicks do not possess sufficient performance values in terms of meat quality and yield when compared to broiler chickens (Leenstra *et al.*, 2011). Therefore, it is necessary to ensure that gender determination of chicken embryos can be performed as early as possible using a fast, practical, and accurate method. Numerous studies have been conducted on this subject, and these studies have been carried out at three different stages: before

embryo development begins in the egg, during the embryo development stage, and at hatching (Göğer, 2017). The interaction between maternal effect genes transferred to the egg cell, depending on the maternal endocrine structure, and genes located in the zygote determines the gender of the embryo (Werren and Hatcher 2000). Before incubation, gender of the embryo can be predicted by chromosome differences in the DNA content of blastoderm cells (Dunislawska *et al*. 2021). However, in the first stage these genetic methods are difficult and expensive for determining gender. In the second stage of gender determination, which is the embryo development stage, gender can be determined through gender chromosomes in samples taken from the allantoic fluid at different embryonic ages (Xu et al. 2025). Recent studies demonstrated that the gender of an embryo can be identified by its gonads or blood rather than in the allantoic fluid (Lv et al. 2025). In the final stage, the gender of daily chicks is determined using methods based on phenotypic characteristics. However, it has become essential to determine the gender at an early stage of incubation, before reaching the final stage. The gender of the embryo can also be predicted before or during incubation by examining characteristics such as egg weight and shell quality. Egg weight is the most fundamental quality characteristic of hatching eggs (Şekeroğlu and Altuntaş 2008; Stadelman *et al*., 2017). Furthermore, egg weight is significantly and positively correlated with hatchling weight, with 64% of egg weight corresponding to hatchling weight (Shanawany, 1987). Egg weight increases linearly with breeder age, and this also causes significant changes in shell quality characteristics (Roque and Soares, 1994). For example, heavier eggs from older flocks have thinner shells and more pores, that can vary in number, length, size and shape (Roque and Soares 1994; Tserveni-Gousi and Yannakopoulos, 1990). Water loss from eggs occurs through the pores in the shell, and the average water loss during incubation is 10–15%, meaning a fertilised egg loses an average of 400–450 mg of water per day (Etches 1998). Water loss during egg storage and incubation is affected by the surface area/egg weight, and larger eggs lose less water (Roque and Soares 1994, Etches 1998; Babacanoğlu *et al*., 2007; Campo and Ruano 1995). As a result, chick weight/egg weight is higher (Babacanoğlu *et al*., 2007).

A study reported that the gender of the embryo can be predicted based on the characteristics of the egg and egg shell. For instance, it was indicated that female chicks hatched from turkey eggs with high shell thickness (49.48 µm), while male chicks hatched from eggs with low shell thickness (47.63 µm) (Eratalar, 2023). Another study found that female chicks hatched from eggs with high shell thickness (0.32 mm) and male chicks from eggs with low shell thickness (0.28 mm) (Tunç and Babacanoğlu Çakır, 2024). The male-biased gender ratio was 40% of chicks hatched from eggs with high shape index (74.21%), while 60% of chicks hatched from eggs with low shape index (72.42%) had female-biased gender ratio (Tunç and Babacanoğlu Çakır, 2024). This result confirmed that male chicks hatched from eggs with high shape index (71.22% male-biased), while female chicks hatched from eggs with low shape index (70.89% female-biased) (Eratalar, 2023). Therefore, the objective of the present study was to ascertain the shape index and air space area of eggs, in addition to the quality characteristics of the shell, including thickness, pore area and pore count, in diverse regions of residual shells. This research is based on weights of hatching eggs obtained from broiler breeders, as well as the gender of the resulting broiler chicks.

Materials and Methods

Experimental design

A total of 60 hatching eggs weighing an average of 61.50 g were used as experiment material. These eggs were obtained from 37-week-old breeding flocks of the Ross 308 genotype. Before incubation, the egg storage room, incubation and hatching machines, egg trays, and eggs were disinfected. At the onset of the experiment, the initial egg weights were determined for each egg using a scale with a sensitivity of 0.001 g, and each egg was numbered. Egg weight groups were formed based on the calculated average of all egg weights, taking into account the weights falling between the highest and lowest egg weight classifications, resulting in a light group (weighing 55.50 and 61.50 g) and a heavy group (weighing 61.50 and 66.50 g). Thus, the experimental design was established. In the incubator, the egg weight groups were incubated for the first 18 days at 37.7 °C temperature and 60% humidity in two separate trays (Image 1). On the 18th day of incubation, fertility checks were performed, infertile eggs were removed, and fertilised eggs were transferred to the hatching unit, which was set at 37.2 °C and 68% humidity. Eggs from each group were placed in individual compartments created in the hatching boxes (Image 1). Since the egg from which each individual chick hatched was known, the effects of all examined characteristics on gender were determined. Hatched chicks were removed from the hatcher after drying for 2 hours, and gender determination was made based on wing feather length. Hatching results were determined according to the following formulas:

Hatchability (%) =hatched chicks/fertilised eggs×100

Unfertilised egg rate (%) =unfertilised eggs/fertilised eggs×100

Embryo mortality (%) = dead embryos/fertilised eggs×100

Gender ratio (%) = female chicks/total chicks×100; male chicks/total chicks×100

Images of individual egg arrangement in hatching trays and hatching boxes arranged according to egg weight groups (Image 1).

Features examined and measurements

Egg weight and water loss

At the onset of the experiment, each egg was weighed with a scale accurate to 0.001 g to determine the initial egg weights. Then, after analysing the average weight value of all eggs and the distribution of each weight, egg weight groups (light 55.50 g and heavy 66.50 g) were formed. The individual weight of the eggs in each group was measured on days 3, 6, 9, 12, 15, and 18 of incubation.

Width and length of air space and shape index

Before incubation, the width and length of the egg were measured using digital callipers and the shape index of the egg was calculated as follows. The width and length of the air space were measured using digital callipers in a dark room (Image 1).

Shape index = width of egg mm/length of egg, mm ×100

Image 1. Images of individual egg arrangement in hatching trays and hatching boxes arranged according to egg weight groups

Shell thickness and number and diameter of pores

Shell thickness, pore count, and pore diameter were determined at the blunt, equatorial and pointed ends of residual eggshells from newly hatched male and female chicks in each group. Shell thickness was measured at 10 different areas in each of the 3 regions using an LCD micrometre (Mitutoyo, Japan), and the regional average was taken.

After preparing a dye containing methylene blue, each shell region was treated with the dye (Babacanoğlu, 2018). The pore count values in the blunt, equatorial, and pointed areas of the stained eggshell were read with 10 repetitions in a 0.25 cm² area (Peebles and Mcdaniel, 2014). Micrographs of the pore width and length measurements for each shell region were obtained using a camera (Leica Microsystems, Switzerland, 2010) at 1024×magnification (EZ4 HD stereomicroscope and ICC50 HD) (Image 2).

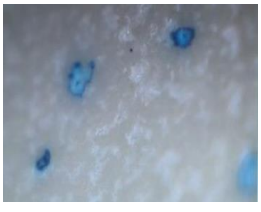

Image 2. Microscopic image of pores

Gender determination and Chick weight

The sex of broiler chicks hatched from hatching eggs obtained from Ross 308 broiler breeders, which possess a genotype enabling sex determination in day-old chicks based on wing feather length, has been determined with high accuracy. At hatching, gender determination was based on wing feather length, and the live weights of newly hatched female and male chicks of daily age in each group were individually measured.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyse the data related to the traits under examination (SAS 2009). The egg weight and gender and the interaction between these two factors were modelled as the main effects. Comparisons between means were performed using the Tukey test at significance level of $P < 0.05$.

Results

Hatchability was found to be 86.67 % in both weight groups, with a total mortality rate of 6.66%. Early embryonic death was the cause of mortality in the 55.50 EW group, with an infertile egg rate of 6.66%. In the 66.50 EW group, the early mortality rate was 3.33%, with an infertile egg rate of 9.99%. The gender ratio at hatching was consistent across each of egg weight groups, at 54% male and 46% female.

Egg weight

Upon examination, the egg weights measured at the onset of incubation and at 3-day intervals throughout incubation were found to be highly significant within the egg weight (EW) groups. The 66.50 EW group was found to have heavier egg values than the 55.50 EW group. No significant effect of gender was observed on egg weights measured at different embryonic ages. However, the interaction between EW and gender on egg weights measured at the onset of incubation and on days 3, 6, 9, 12, 15 and 18 of incubation was significant (Table 1). Accordingly, on each measurement day, the eggs of each gender in the 66.50 EW group were heavier than those of the females and males in the 55.50 EW group.

Egg water loss

The effects of the EW group, gender, and the interaction between the EW group and gender were not significant with regard to egg water loss during incubation on days 3, 6, 9, 12, 15 and 18 (Table 2).

Pore number

No significant main effects were detected in the number of pores determined at the blunt and equatorial ends of the egg (Table 3). Similar results were found for the average pore count measured and calculated at the

Table 1. Average egg weight according to egg weight and gender at 3-day intervals during incubation

	Embryonic age, day					
	3	6	9	12	15	18
Egg weight groups (EW)	Egg water loss, %					
55.50	2.19±0.08	4.53±0.18	6.37±0.24	8.35±0.31	10.24±0.37	11.89±0.53
66.50	2.39±0.08	4.80±0.19	6.09±0.24	7.91±0.31	9.60±0.37	11.99±0.53
P value	0.059	0.308	0.425	0.331	0.240	0.905
Gender						
Female	2.31±0.08	4.67±0.17	6.29±0.24	8.24±0.30	10.00±0.36	12.21±0.51
Male	2.26±0.08	4.89±0.19	6.18±0.25	8.02±0.32	9.84±0.34	11.68±0.55
P value	0.685	0.867	0.759	0.633	0.775	0.485
EW * Gender						
55.50 * Female	2.47±0.11	4.94±0.25	6.63±0.33	8.66±0.42	10.62±0.51	12.24±0.72
55.50 * Male	2.31±0.12	4.67±0.28	6.12±0.36	8.03±0.46	9.87±0.55	11.74±0.78
66.50 * Female	22.15±0.11	4.35±0.27	5.95±0.33	7.81±0.42	9.38±05.51	12.18±0.72
66.50 * Male	2.21±0.12	4.71±0.28	6.24±0.36	8.01±0.46	9.82±0.55	11.62±0.78
P value	0.347	0.249	0.264	0.358	0.274	0.966

[ab]Different letters in each row indicate significant differences at the P<0.05 level.

Table 2. Egg water loss according to egg weight and gender at 3-day intervals during incubation

	Pore widths			Pore lengths		
	Blunt end	Equatorial end	Pointed end	Blunt end	Equatorial end	Pointed end
Egg weight groups (EW)	µm	µm	µm	µm	µm	µm
55.50	0.146±0.01	0.133±0.02	0.119±0.00	0.184±0.02	0.174±0.02	0.141±0.00
66.50	0.151±0.01	0.157±0.02	0.128±0.01	0.185±0.02	0.171±0.02	0.155±0.01
P value	0.824	0.147	0.667	0.704	0.927	0.792
Gender						
Female	0.156±0.01	0.145±0.02	0.150±0.01[a]	0.194±0.02	0.188±0.02	0.158±0.01
Male	0.140±0.01	0.128±0.02	0.108±0.01[b]	0.175±0.02	0.157±0.02	0.138±0.01
P value	0.507	0.412	0.028	0.549	0.373	0.252
EW * Gender						
55.50 * Female	0.187±0.02[a]	0.185±0.03[a]	0.143±0.01[a]	0.236±0.03[a]	0.217±0.03[a]	0.165±0.01
55.50 * Male	0.105±0.02[c]	0.117±0.02[c]	0.095±0.01[c]	0.132±0.03[c]	0.134±0.04[c]	0.117±0.01
66.50 * Female	0.126±0.02[c]	0.127±0.02[bc]	0.127±0.01[b]	0.152±0.02[b]	0.160±0.03[b]	0.151±0.01
66.50 * Male	0.176±0.01[b]	0.138±0.02[b]	0.122±0.00[b]	0.218±0.02[a]	0.183±0.02[ab]	0.159±0.01
P value	0.018	0.034	0.014	0.021	0.034	0.105

[ab] Different letters in each row indicate significant differences at the P<0.05 level.

Table 3. Number of pores in the residual eggshell according to egg weight and gender at hatching

	Pore number			
	Blunt end	Equatorial end	Pointed end	Mean
Egg weight groups (EW)				
55.50	23.58±2.42	22.08±3.16	17.18±1.21	20.92±1.29
66.50	23.13±1.93	19.83±2.53	19.57±1.51	20.84±1.03
P value	0.867	0.592	0.231	0.966
Gender				
Female	23.58±1.93	17.58±3.16	18.42±1.51	19.86±1.29
Male	23.13±2.42	24.33±2.52	18.23±1.21	21.90±1.03
P value	0.887	0.129	0.926	0.248
EW * Gender				
55.50 * Female	26.50±3.74	18.50±3.89	20.50±2.34[ab]	21.83±1.99[ab]
55.50 * Male	20.67±3.06	25.67±3.99	13.67±1.91[b]	20.00±1.63[b]
66.50 * Female	20.66±3.06	16.67±3.99	16.33±1.91[b]	17.89±1.63[b]
66.50 * Male	25.60±2.37	23.00±3.09	22.80±1.48[a]	23.80±1.26[a]
P value	0.116	0.920	0.007	0.043

[ab] Different letters in each row indicate significant differences at the P<0.05 level.

Table 4. Pore widths and lengths of residual eggshells according to egg weight and gender at hatching

	Pore widths			Pore lengths		
	Blunt end	Equatorial end	Pointed end	Blunt end	Equatorial end	Pointed end
Egg weight groups (EW)	µm	µm	µm	µm	µm	µm
55.50	0.146±0.01	0.133±0.02	0.119±0.00	0.184±0.02	0.174±0.02	0.141±0.00
66.50	0.151±0.01	0.157±0.02	0.128±0.01	0.185±0.02	0.171±0.02	0.155±0.01
P value	0.824	0.147	0.667	0.704	0.927	0.792
Gender						
Female	0.156±0.01	0.145±0.02	0.150±0.01^{a}	0.194±0.02	0.188±0.02	0.158±0.01
Male	0.140±0.01	0.128±0.02	0.108±0.01^{b}	0.175±0.02	0.157±0.02	0.138±0.01
P value	0.507	0.412	0.028	0.549	0.373	0.252
EW * Gender						
55.50 * Female	0.187±0.02^{a}	0.185±0.03^{a}	0.143±0.01^{a}	0.236±0.03^{a}	0.217±0.03^{a}	0.165±0.01
55.50 * Male	0.105±0.02^{c}	0.117±0.02^{c}	0.095±0.01^{c}	0.132±0.03^{c}	0.134±0.04^{c}	0.117±0.01
66.50 * Female	0.126±0.02^{c}	0.127±0.02^{bc}	0.127±0.01^{b}	0.152±0.02^{b}	0.160±0.03^{b}	0.151±0.01
66.50 * Male	0.176±0.01^{b}	0.138±0.02^{b}	0.122±0.00^{b}	0.218±0.02^{a}	0.183±0.02^{ab}	0.159±0.01
P value	0.018	0.034	0.014	0.021	0.034	0.105

^{abc} Different letters in each row indicate significant differences at the P<0.05 level.

pointed end of the egg. Here, main effects of EW and gender were detected, and the interaction between these two traits was significant (P = 0.007 and P = 0.043). The pore counts at the pointed end and the average pore count for the whole shell were significantly higher for male chicks in the 66.50 EW group than for female chicks in the same group and for male chicks in the 55.50 EW group (Table 3).

Pore width and length

The effects of EW and gender on the pore length and width values at the blunt, equatorial and pointed ends of the residual egg shells were found to be insignificant, while the effect of gender on the length of the pointed end was found to be significant. According to this significant finding, the length of the pointed end of shells hatching male chicks was shorter than that of shells hatching female chicks (Table 4). The highest pore widths at the blunt, equatorial, and pointed ends, as well as the longest pore length at the blunt end, were obtained for males in the 66.50 EW group and females in the 55.50 EW group. The lowest values were found for males in the 55.50 EW group. The pore lengths at the equatorial end pore ends were highest for females from the 55.50 EW group and differed from that of females from the 66.50 EW group. Conversely, equatorial end pore length was lowest in males from the 55.50 EW group. The interaction effect among the main effects on the pointed end pore length was insignificant (*P = 0.105*; Table 4).

Air space width and length

The effect of EW on the width and length of the air space was found to be significantly greater in the 66.50 EW group (21.01 ± 0.21 mm and 21.52 ± 0.25 mm) than in the 55.50 EW group (15.59 ± 0.21 mm and 19.53 ± 0.26 mm), respectively (P < 0.001). The effect of gender on air space width (P = 0.926) and length (P = 0.915) was not significant. The interaction between egg weight and gender had a significant effect on egg air space width (P = 0.043).

This significance was sourced from male chicks hatched from heavier eggs (66.50 EW) having greater egg air space width than female chicks in the same group and both genders hatched from eggs in the 55.60 EW group (Figure 1). Similarly, a significant interaction between egg weight and gender was found in relation to changes in air space (P = 0.043).

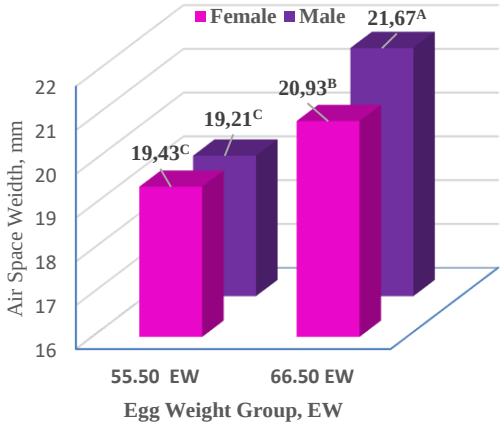

Figure 1. Egg air space width variations depending on the interaction between egg weight and gender (P=0.043); ^{ABC} Different letters in each row indicate significant differences at the P<0.05 level.

This interaction was found to be significant because male chicks hatched from heavy group eggs had greater air space length than both genders hatched from the light group eggs (Figure 2).

Shape index

The effects of egg weight (P = 0.106) and gender (P = 0.916) on the shape index were insignificant. Eggs hatching male chicks in the 66.50 g weight group had lower shape index than those hatching male chicks in hatching male chicks was higher than that of eggs

hatching female chicks in the 55.50 g weight group, indicating a significant interaction between egg weight and gender (Figure 3). According to this result, the highest shape index was found for eggs hatching males from lighter weight eggs, while the lowest shape index was found for eggs hatching males and females from the heavier egg group (P = 0.053).

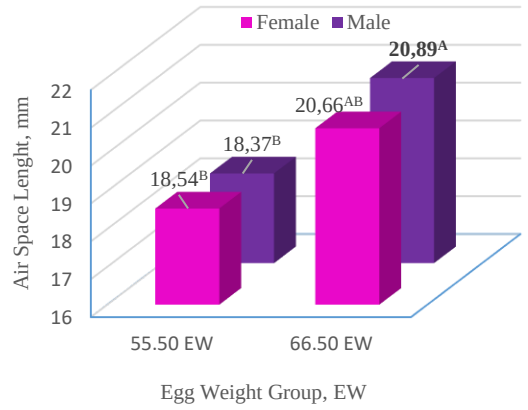

Figure 2. Egg air space length variations depending on the interaction between egg weight and gender (P=0.043); ABDifferent letters in each row indicate significant differences at the P<0.05 level.

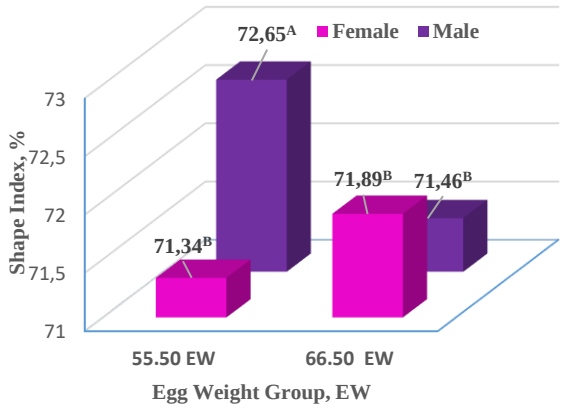

Figure 3. Shape index variations according to the interaction between egg weight and gender (P=0.053); AB Different letters in each row indicate significant differences at the P<0.05 level.

Shell thickness

At hatching, the effect of EW was not significant (P = 0.312). The average shell thickness in the three regions of the residual shells was 0.379 ± 0.03 mm for the 55.50 EW group and 0.392 ± 0.03 mm for the 66.50 EW group. The effect of gender was significant for the average shell thickness in the three regions of the residual shells. Eggs hatching female chicks had average shell thickness of 0.387 ± 0.01 mm, while eggs hatching male chicks had average shell thickness of 0.373±0.01 mm (P = 0.024).

The interaction between EW and gender was significant for the blunt end (P = 0.046), the equatorial end (P= 0.038) and the pointed end (P = 0.033), respectively. The fundamental source of this interaction for each shell region was that male chicks in the 55.50 EW group had lower shell thicknesses compared to female chicks in the same group and to both genders in the 66.50 EW group (Figure 4).

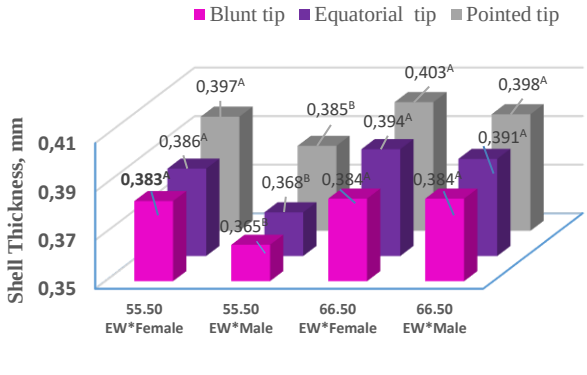

Figure 4. Variations in thickness of residual shells according to the interaction between egg weight and gender (P=0.024); AB Different letters in each row indicate significant differences at the P<0.05 level.

Chick weight

At hatching, chick weight was 37.18±0.42 in the 55.50 g EW group and 44.55±0.42 in the 66.50 g EW group, and the group effect was found to be significant (P < 0.001). The effect of gender on chick weight was found to be insignificant (P = 0.778), while the interaction between EW group and gender was significant (P = 0.046). The source of this interaction was that newly hatched female and male chicks were heavier when hatched from heavy eggs and lighter when hatched from light eggs (Figure 5). However, egg weight was the main reason why this interaction was found to be significant on chick weight.

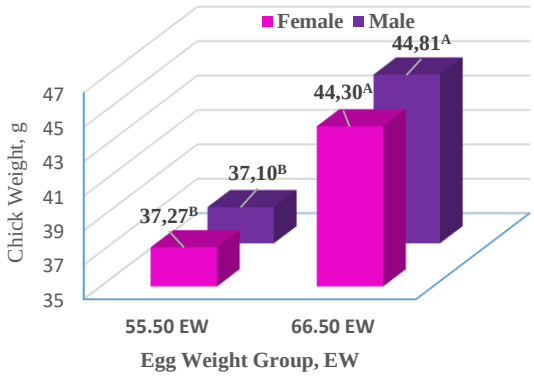

Figure 5. Chick weight variations according to the interaction between egg weight and gender (P=0.046); ABDifferent letters in each row indicate significant differences at the P<0.05 level.

Discussion

In poultry, it is the female parent that determines the gender ratio, which is estimated to be 50:50 female to male. An important result of this study is the finding that the gender ratio of chicks hatched from eggs of different weights obtained from breeding hens was similar, at 54% male and 46% female. This similar conclusion in both egg weight groups is attributed to similarity in genotype and breeder age. This finding is important for improving our understanding of the characteristics related to egg quality that could affect gender. Once all the results are examined, it may be possible to conclude that gender differences are caused by egg quality characteristics that vary depending on egg weight. The expression of hundreds of genes in the early stages of an embryo's life is shaped by the influence of the maternal structure (Formanek *et al.,* 2009). One of these is gender. However, through these maternal effects, the gender ratio can be converted to favour females (Luo *et al*. 2024). In this study, although gender had no effect on egg weights measured at different embryonic ages, the fact that female and male chicks that hatched from heavy eggs were heavier than female and male chicks hatched from lighter eggs reflects the impact of egg weight before incubation on chick weight.

This is because 64% of egg weight corresponds to hatchling weight (Shanawany, 1987) due to a significant positive correlation between egg weight and hatchling weight (Shanawany 1984; Wilson 1991). This study also confirmed the relationship between chick weight and egg weight, with day-old broiler chicks of both genders hatching from light and heavy eggs. Furthermore, it is evident that female chicks that hatched from light eggs had lower live weight than those hatched from heavy eggs. The same result was obtained for male chicks, which is again due to egg weight. In this study, even with different egg weight classifications belonging to the same genotype and breeder age, chick weights varied even within the same gender, which is related to egg weight (Aslam *et al*., 2013). Egg water loss occurs within the optimum range every three days during incubation, depending on egg weight. More importantly, the average water loss level of 12% on the 18th day, which is the optimal level, was not affected by whether the eggs belonged to female or male chicks.

The study concluded that eggs with high shape index hatched male chicks, while those with lower index hatched females. Another study confirmed this result, reporting that male chicks hatched from eggs with high shape index (71.22% male-biased gender), while female chicks hatched from eggs with low index (70.89% female-biased gender) (Eratalar, 2023). However, the study showed that the shape index of eggs from which male and female chicks hatched was similar as the weight of the eggs increased. Taking the results of these studies into account, it can be concluded that egg weight indirectly affects the shape index, which also influences gender. This is because the effect of egg weight on gender occurs at lower egg weights. The reason for this is a high correlation between shape index and gender (r = 0.78) (Kayadan and Uzun, 2023). In this study, the number of pores at the pointed end of the residual shells from heavy eggs was significantly higher when male chicks hatched from these eggs than when female chicks hatched in the same group, and compared to when male chicks hatched from lighter eggs. The same pattern of results was reflected in the total pore number of the shells. It was reported that no difference in pore number was found at the blunt end, while significant differences in pore number were observed at the equator and pointed ends of eggs classified according to their shape index (Kayadan and Uzun 2023). This may be due to the relationship between the shape index and the variation in the pore number at the pointed end of the egg shell (Arslan *et al*., 2023), which affects egg weight and gender.

A significant gender-related difference was observed in the length of the pointed end of the residual egg shells. A similar gender-related difference was observed in the pore width at the pointed end of shells from which male chicks hatched; this was smaller than for shells from which female chicks hatched. When egg weight is taken into account, shells of male chicks hatched from the lighter group had lowest pore widths and lengths at the blunt and equatorial ends. Also, shells of male chicks hatched from the heavier group of eggs had wider air space than shells of female chicks. As no previous studies examining these traits have been found, these results can be considered initial findings.

A study found that female chicks (49.48 µm) hatch from eggs with high shell thickness, while male chicks (47.63 µm) hatch from eggs with low shell thickness (Eratalar, 2023). Another study observed a similar result, with female chicks hatching from eggs with high shell thickness (0.32 mm) and male chicks from eggs with low shell thickness (0.28 mm) (Tunç and Babacanoğlu Çakır, 2024). These results are similar to those of (Araújo *et al*., 2017). Studies show that chicks that hatch from lighter eggs with thinner shells are more likely to be male. This study obtained the same results as previous research, but only for the effect of egg weight. This could be because the shell thickness of the eggs gradually increased from the blunt end to the pointed end, especially in lighter eggs.

Conclusions

The results of this study in relation to the characteristics examined are as follows: the primary source of differences in chick weight by gender was egg weight. The effects of egg weight and water loss during incubation on gender was insignificant, with an average water loss of 12% found in eggs from both groups, which is within the optimal range. Male embryos/chicks were obtained from eggs weighing 66.50 g which had large air cell and high number of pointed-end pores on the shell.

Furthermore, male embryos/chicks were obtained from 55.50 g eggs with high shape index and low shell thickness at each end. These eggs also had low pore width at the blunt and pointed ends, as well as low pore length at the equatorial end. Heavier males and females were obtained from heavy eggs compared to light eggs, with males in the heavy eggs having higher chick weights than females. Consequently, the gender of the embryo can be predicted without breaking the egg using highly sensitive and specific measuring devices based on characteristics of the egg and shell quality, which are shaped according to egg weight before incubation. Therefore, gender could also be determined before or during incubation by considering certain phenotypic characteristics using non-genetic methods.

Ethics approval and consent to participate

The study was approved by the Committee for the Ethical Care and Use of Animals of the Van Yuzuncu Yil University (Approval number: 2024/12-08).

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