

## Effect of egg weight on development, bone morphology, and breast muscle histology of embryos from fast-and slow-growing strains

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**Abstract:** The present study aimed to determine the impact of egg weight on embryonic development, breast muscle histology, and bone morphology of fast- and slow-growing embryos and chicks. For this purpose, total 192 eggs from fast-(Ross 308) (FG) and slow-growing (Hubbard JA) (SG) broiler breeders were used. Eggs were classified as medium ( $64 \pm 1$  g, Megg) and heavy ( $72 \pm 1$  g, Hegg) eggs. At 18 days of incubation, egg weight loss, embryo weight, embryo length, breast width and length, yolk sac weight, and bone morphometric parameters were measured and breast muscle histology were studied. The same measurements were repeated on the day of the hatch. Although yolk utilization of FG embryos from Hegg and Megg was similar, SG-Megg embryos were more efficient than SG-Hegg at utilizing of yolk nutrients at 18 days of the embryogenesis and the day of the hatch. On the day of hatch, Hegg chicks were heavier than Megg regardless of the strain. Embryos and chicks from FG-Hegg had more fiber area than those from SG-Hegg whereas embryos from Megg were not influenced by strain. Higher capillary number and capillary to fiber ratio was found in FG-Hegg embryos than those from FG-Megg. At hatch, the FG embryos had wider tibia and shank at 18 days and heavier tibia, femur, and shank compared to SG. Egg weight affected relative bones weight, being chicks from Megg heavier than those from Hegg. Lower tibia ash content was found in Hegg chicks. It is concluded that Hegg may be an advantage for a larger fiber area and capillary density. However, lighter femur, tibia, and shank of Hegg chicks with lower tibia ash content than those from Megg regardless of strain may indicate that heavier eggs could negatively affect bone properties of chicks.

**Key words:** Incubation, egg weight, strain, embryonic development, bone, muscle

### 1. Introduction

Genetic selection has contributed to the improvement in growth rate, breast muscle weight, and feed efficiency of broilers [1–3]. However, there are unexpected effects of genetic selection on broilers. One of the main issues is a significant predisposition in the susceptibility of broilers to leg problems [4,5]. Overweight of breast muscle has caused an unbalanced weight distribution and increased weight load on leg bones of broilers. The rapid bone formation in the fast growing broiler lines is responsible for the lower bone ash content, leading to reduced breaking strength negatively affecting broiler welfare [6,7]. Significant differences among commercial broiler strains for bone mineralization were reported [8].

Another indirect consequence of genetic selection for fast growth rate is on muscle abnormalities [9,10]. Genetic selection for fast-growing has been resulted in increased breast muscle weight. Saunderson and Leslie [11] showed that breast muscles of broilers had faster growing rate than in layer chicks. The increase in breast muscle weight is

related to increased fiber area [12,13]. Larger fiber area has reduced capillary density leading to limitation in the rate of oxygen [14] and nutrient supply and metabolic waste product displacement [15,16].

It is known that slow-growing broilers are less susceptible to leg disorders [17] and muscle myopathies [18]. Shim et al. [19] found lighter and shorter tibia and shank in the slow growing line than the fast growing line at 6 weeks, however, tibia ash of fast growing broilers were lower than those in the slow growing. It was also reported that slow-growing broilers have narrow fiber diameters and higher capillary density compared to fast-growing broilers [12,18].

Apart from the genetic line effect, it is important to understand maternal effects, such as egg weight, on muscle and bone characteristics of developing embryo as it can help to understand animal physiology. It is known that breeder age affects egg weight and consequently chick weight [20,21], bone weight, and ash content of day old chicks [22]. Recently, Yair et al. [23] reported that fast-

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growing chicks from small eggs had a higher risk for developing bone pathologies. Nowadays, the embryonic period has become a larger part of a total lifespan of broilers and a better understanding of how egg weight has shaped broiler muscle and bone development is needed. It is known that to keep egg size on the target becomes difficult as breeders age. It can be considered as a problem when egg weight exceeds 70 g [24]. However, it is common to see the egg weight variation in a breeder flock. Therefore, the present study aimed to evaluate the effect of egg weight on embryonic development, breast muscle fiber histology, and bone morphology at 18 days of embryogenesis and at the day of the hatch in fast- and slow-growing broilers.

## 2. Materials and methods

The experiment was conducted by following the Turkish guidelines for animal welfare and was approved by the Animal Ethics Committee at the Ege University (2015-011).

Eggs were obtained from fast-(Ross 308) (FG) and slow-growing (Hubbard JA) (SG) broiler breeders aged 58 weeks. All eggs from each strain were numbered, weighed and classified as medium or heavy egg. Mean egg weight for medium (Megg) and heavy eggs (Hegg) was  $64 \pm 1$  and  $72 \pm 1$  g, respectively. There were a total of 192 eggs; 48 eggs for each egg weight group/strain.

All eggs were incubated at the same standard incubation conditions (37.8 °C with 60% RH) after 3 days of storage at 18 °C and 75% of relative humidity. Eggs within each egg weight and strain were distributed in four egg trays at four different levels in the incubator (12 eggs/egg weight/strain/egg tray). At day 18, eggs were transferred to a hatcher and kept at 36.8 °C and 70% RH until hatching.

### 2.1. Measurements

On day 18, 10 eggs (2 or 3 eggs/replication) were randomly selected from each group weighed and egg weight loss (%) was calculated. Embryos were removed from the eggs, killed by cervical dislocation, dried in absorbent paper, and weighed. Relative embryo weight was obtained as the percentage of egg weight. The length of the embryos was measured from the tip of the beak to the tip of the middle-toe [25]. Breast width and length were measured using a digital caliper. The yolk sac was dissected and weighed. Relative yolk sac weight was calculated as the percentage of total embryo weight. Approximately 5 g of breast muscle was sampled for muscle histology. Right leg was dissected, cleaned from muscle, weights, lengths, and width of tibia, femur, and shank were measured. Tibia was ashed at 500 °C to determine ash content.

All chicks that had completed the hatching process were removed from the hatcher. On the day of the hatch, the same measurements were repeated on 10 chicks/strain/egg weight. Relative yolk sac weight was calculated as the ratio of weight to chick weight.

### 2.2. Muscle histology

P. major samples (1 x 1 x 1 cm) from each carcass was mounted in an embedding medium (Sigma, P0091) and kept at -80 °C. Serial transverse sections (10 µm) were cut in a cryostat (Leica, Germany) at -20 °C. The sections were brought to room temperature, fixed in Carnoy's (absolute ethyl alcohol, chloroform, and glacial acetic acid, 60%, 30%, and 10%, respectively) for 10 min, rinsed in distilled water then incubated with 1% α-amylase (Sigma, A-3176) for 30 min. After rinsing in distilled water, the sections were oxidized in 1% periodic acid (Sigma, P7875) for 10 min [14], followed by a staining with Schiff's reagent (Merck, 109 033) for 20 min and rinsing in water. The sections were then dehydrated in graded alcohols, cleared in xylol, and mounted in entellan. Images were recorded using a light microscope (Zeiss Axio Scope A1, Germany). Five photographs of different cross-sections from each muscle were taken. Photographs were analyzed morphometrically per section by computer image analysis (Zen Software, Zeiss, Germany) using a semi-automated procedure. The fiber number per mm<sup>2</sup> was counted in each area. The mean capillary density was defined as the total number of capillaries per mm<sup>2</sup>. Capillary to fiber ratio was calculated.

### 2.3. Statistical analyses

All data were tested for normality using the Shapiro-Wilk test before variance analysis. All data were subjected to two-way ANOVA with a model including strain and egg weight as main effects and their interaction using the GLM procedure of JMP [26]. Means were compared by Tukey's test. P-values less than 0.05 were considered statistically significant unless otherwise stated.

## 3. Results

There was no effect of strain and egg weight on egg weight loss at 18 days of the incubation (Table 1). A significant strain by egg weight interaction for absolute embryo weight indicated that the FG-Hegg embryos were heavier than those from FG-Megg, however, effect of egg weight was not significant for SG embryos (Table 2). Relative embryo weight was similar between strains. Effect of egg weight was significant for relative embryo weight, being embryos from Megg were heavier than those from Hegg (Table 1). Interaction for relative yolk sac weight showed that SG-Megg had the lowest relative yolk sac than SG-Hegg, while egg weight had no effect on relative yolk sac weight for FG broilers. Embryo length was not influenced by strain and egg weight.

Breast muscle width and length was not influenced by strain or egg weight at 18 days of the incubation (Table 1). The interaction for fiber area showed that the FG-Hegg embryos had the larger fiber area than SG-Hegg while strain effect was not significant for Megg (Table 2) (Figure 1). There was

**Table 1.** Effect of strain and egg weight on embryo traits on day 18 of incubation.

Traits	Strain (S)				Egg weight (EW)				S x EW
	Fast	Slow	SEM <sup>1</sup>	P	Heavy	Medium	SEM	P	P
Egg weight loss, %	9.14	10.11	0.35	0.064	9.27	9.98	0.35	0.165	0.970
Embryo, g	36.00	34.90	0.54	0.163	36.27	34.63	0.54	0.041	0.037
Embryo, % <sup>2</sup>	59.31	57.72	0.85	0.192	56.83	60.20	0.82	0.008	0.108
Yolk sac, % <sup>3</sup>	26.71	26.32	0.68	0.685	28.32	24.70	0.68	<0.001	0.024
Embryo length, cm	16.21	15.68	0.21	0.098	15.84	16.05	0.21	0.503	0.888
Breast									
Width, mm	18.16	17.42	0.37	0.165	17.71	17.87	0.37	0.752	0.388
Length, mm	18.44	17.61	0.32	0.074	17.66	18.39	0.32	0.111	0.635
Fiber area, $\mu\text{m}^2$	39.14	35.23	1.15	0.018	38.02	36.35	1.15	0.309	<0.001
Capillary number/100 $\mu\text{m}^2$	6.67	7.27	0.21	0.046	7.20	6.75	0.21	0.128	0.040
Capillary/fiber	0.0644	0.0747	0.0058	0.237	0.0682	0.0708	0.0060	0.766	0.326

<sup>1</sup>SEM: standard error of mean.

<sup>2</sup>Relative embryo weight = embryo weight without yolk/egg weight.

<sup>3</sup>Relative yolk sac weight = yolk sac weight/embryo weight with yolk.

also a significant interaction for capillary number/100  $\mu\text{m}^2$  indicating that capillary number of FG-Hegg embryos was higher than those FG-Megg embryos but capillary number of SG embryos was not influenced by egg weight (Table 2) (Figure 1).

On day 18 of embryogenesis, there was no significant effect of strain and egg weight on tibia weight, length, and ash (Table 3). FG embryos had wider tibia than SG. The Megg embryos had heavier relative femur weight compared to Hegg. There was no effect on strain and egg weight on femur length and width, and shank weight and length. Shank width was wider in FG embryos than those in SG (Table 3).

On the day of the hatch, while chick weight was similar between strains, Hegg chicks were heavier than Megg (Table 4). Chick length was not influenced by strain and egg weight. Although egg weight effect was significant for relative yolk sac weight, FG chicks from Hegg and Megg had similar relative yolk sac weight (Table 2). Breast width was not influenced by strain and egg weight. Breast length was longer for FG chicks than those SG. FG chicks have larger fiber area than SG chicks. There was a significant interaction between strain and egg weight for fiber area. Fibre area of SG chicks was not influenced by egg weight whereas chicks from FH-Hegg had larger fiber area than those FG-Megg chicks (Table 2) (Figure 2). Interaction was also significant for capillary number. Capillary number and capillary to fiber ratio was higher in FG-Hegg embryos than FG-Megg while it was vice versa for SG chicks.

Bone parameters of chicks on the day of the hatch are given in Table 5. On the day of the hatch, chicks from FG-

Megg had heavier tibia, femur, and shank. There was no significant effect of strain on the lengths of tibia, femur, and shank. Chicks from Megg had shorter femur than those chicks from Hegg. Megg resulted longer shank. Although strain had no effect on tibia width, width of femur, and shank was larger in FG strain compared to SG. Megg increased tibia width but decreased width of shank. Ash content of tibia was higher in chicks from Megg than those from Hegg.

#### 4. Discussion

During the past century, the growth rate of broilers has been improved by genetic selection. This procedure affected meat and bone quality of broilers, thereby causing welfare problems. Unlike the fast-growing broilers, slow-growing broilers do not suffer from bone and muscle problems, which may be explained by diverging pre and post-natal growth rates [18,23,27]. It is known that breeder age and egg weight is an important factor influencing broiler growth performance [20]. The growth performance of broilers from heavy eggs was found better compared to lighter eggs if both eggs have obtained from breeders with similar age [28]. However, controlling large egg size becomes difficult as birds aged and eggs weighing heavier than 70 g would be consider as a problem [24]. Information regarding bone and muscle development of embryos and chicks from the same breeder age with extreme egg weights is limited. We hypothesized that egg weight may affect embryonic growth and muscle and bone development with differing outcomes in fast- and slow-growing broilers.

**Table 2.** Effect of interaction between strain and egg weight on embryo traits at day 18 of incubation and at the day of the hatch.

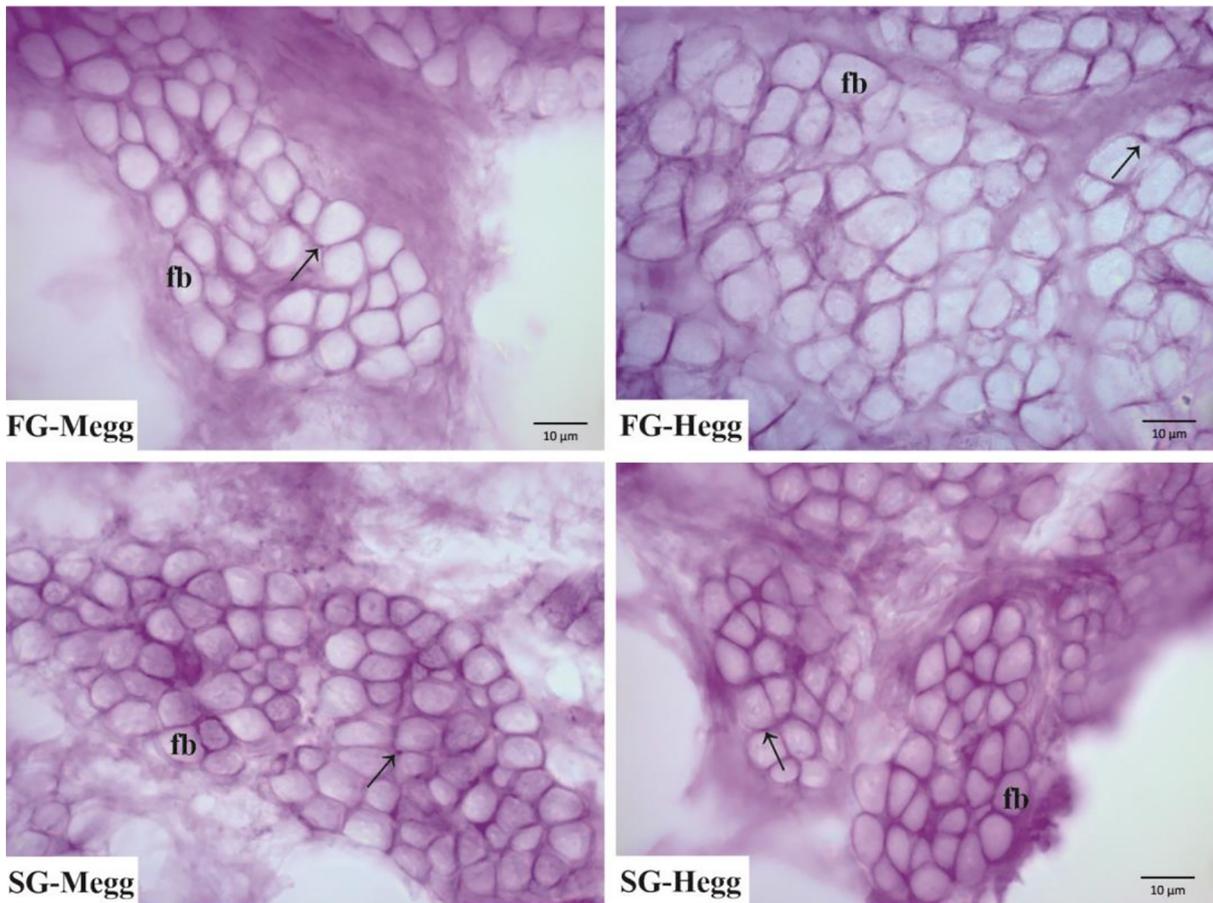
	Traits	Strain	Egg weight	
			Heavy	Medium
18 days of the incubation	Embryo weight, g	Fast	37.65 <sup>a</sup>	34.35 <sup>b</sup>
		Slow	34.16 <sup>b</sup>	34.32 <sup>b</sup>
	Yolk sac, % <sup>1</sup>	Fast	27.38 <sup>ab</sup>	26.04 <sup>bc</sup>
		Slow	29.27 <sup>a</sup>	23.36 <sup>c</sup>
	Fiber area average, µm <sup>2</sup>	Fast	43.45 <sup>a</sup>	34.83 <sup>bc</sup>
		Slow	32.60 <sup>c</sup>	37.87 <sup>b</sup>
	Capillary number/100 µm <sup>2</sup>	Fast	7.21 <sup>a</sup>	6.14 <sup>b</sup>
		Slow	7.19 <sup>a</sup>	7.35 <sup>a</sup>
At the day of the hatch	Yolk sac, % <sup>2</sup>	Fast	14.58 <sup>ab</sup>	13.53 <sup>bc</sup>
		Slow	16.34 <sup>a</sup>	12.17 <sup>c</sup>
	Fiber area average, µm <sup>2</sup>	Fast	31.15 <sup>a</sup>	26.65 <sup>b</sup>
		Slow	27.53 <sup>b</sup>	27.32 <sup>b</sup>
	Capillary number/100 µm <sup>2</sup>	Fast	7.04 <sup>a</sup>	5.86 <sup>b</sup>
		Slow	6.38 <sup>b</sup>	7.42 <sup>a</sup>
	Capillary/fiber	Fast	0.052 <sup>a</sup>	0.041 <sup>b</sup>
		Slow	0.041 <sup>b</sup>	0.051 <sup>a</sup>

<sup>ab</sup> Means with a different superscript within a trait differ significantly (p < 0.05).

<sup>1</sup>Relative to embryo weight with yolk, <sup>2</sup>Relative to chick weight.

A 6.5% to 14% egg weight loss up to 18 days of incubation is ideal for hatching [29]. Egg weight loss recorded in this experiment was between 9% and 10%, which was in a normal range. There was no effect of strain and egg weight on egg weight loss agreed with Yalçın et al. [30]. Egg yolk and lipids in yolk are the main source of nutrient for growth and development of the embryo during incubation. Gous [31] reported that yolk sac utilization can be a limiting factor for embryonic growth within small eggs. Nangsuay et al. [32] found that yolk absorption at 18 days of incubation and at hatch were higher in small eggs than in large eggs. In our study, although yolk utilization of FG embryos from Hegg and Megg was statistically similar, SG-Megg embryos utilized more than SG-Hegg at 18 days and day of the hatch. The similar embryo weight obtained

from SG-Hegg and SG-Megg on day 18 may be explained by higher yolk sac utilization of those embryos. However, at hatch, this interaction was disappeared and Hegg chicks were found heavier than Megg regardless of the strain. This result showed that SG-Hegg embryos gained more than those SG-Megg embryos between d 18 and 21. On the other hand, the heavier chick weight of Hegg than those from Megg is a common finding in avian species [21,33]. One may think that larger residual yolk sac may contribute to the heavier weights of Hegg chicks. However, yolk-free chick weight was also higher in Hegg chicks than those from Megg, indicating that small eggs may limit chick weight without respecting the strain. The heavier yolk sac weight for Hegg chicks may contribute to their post-hatch growth to meet the necessary nutrients. These differences



**Figure 1.** Muscle fibers of embryos on 18 days of the incubation. FG-Megg: fast-growing, medium egg weight, FG-Hegg: fast-growing heavy egg weight, SG-Megg: slow-growing, medium egg weight, SG-Hegg, slow-growing, heavy egg weight. Fb: fibril, arrow: capillary.

showed that FG and SG broilers have different growth pattern during prenatal stage and this pattern was affected by egg weight.

Indeed, the effect of egg weight on breast muscle fiber area was also differed between strains. The number of embryonic myofibers is set by the hatch. Scheuermann et al. [34] showed that commercial broilers had lower myofiber density and more myofiber per square millimeter than Leghorn-type chickens at 7 days old. In the present study, embryos from FG-Hegg had more fiber area than those from SG-Hegg whereas embryos from Megg were not influenced by the strain effect. Similar trends were observed on the day of the hatch. These results may indicate the advantage of heavy eggs for muscle development and growth. Moreover, the results showed that FG chicks from Hegg had a greater potential for capillary development and those from Megg. However, larger eggs have no benefit on capillary development in SG broilers. On the other hand, while embryonic breast width and length was influenced by neither strain nor egg weight, FG chicks

had longer breast muscle on the day of the hatch. This result indicated an accelerated muscle development of FG embryos during the last days of incubation. Indeed, the increase in breast length of FG chicks was necessary to obtain a heavier breast muscle.

In our experiment, better bone morphology obtained for FG embryos and day-old chicks could be expected. The wider tibia and shank of FG embryos, as well as heavier tibia, femur, and shank with wider femur and shank of FG chicks, were expected to provide additional support during the postnatal stage. Williams et al. [6] reported poor mineral content in FG lines than those SG during the growth period. Similar tibia ash content obtained for FG and SG broilers could indicate that FG chicks would have problems producing bones with sufficient mineralization during the postnatal stage.

Yair et al. [23] found that FG chicks from small eggs had inferior bone mechanical properties and were at a higher risk for developing bone problems than those SG chicks. In the present study, Megg chicks had heavier tibia,

**Table 3.** Effect of strain and egg weight on bone traits on day 18 of embryonic development.

Traits	Strain (S)				Egg weight (EW)				S x EW
	Fast	Slow	SEM <sup>1</sup>	P	Heavy	Medium	SEM	P	P
Tibia									
Weight, % <sup>2</sup>	0.90	0.88	0.038	0.652	0.89	0.89	0.038	0.946	0.250
Length, mm	28.49	28.29	0.031	0.637	28.33	28.45	0.031	0.779	0.931
Width, mm	1.97	1.84	0.04	0.029	1.91	1.90	0.04	0.880	0.578
Femur									
Weight, %	0.49	0.47	0.022	0.475	0.44	0.52	0.022	0.016	0.183
Length, mm	19.13	19.36	0.38	0.675	18.76	19.74	0.38	0.078	0.995
Width, mm	1.83	1.83	0.031	0.915	1.80	1.86	0.031	0.177	0.273
Shank									
Weight, %	2.97	2.83	0.054	0.073	2.83	2.97	0.054	0.088	0.464
Length, mm	20.74	20.55	0.22	0.537	20.68	20.62	0.22	0.842	0.145
Width, mm	3.64	3.43	0.64	0.028	3.56	3.52	0.64	0.672	0.922
Tibia ash, %	20.47	21.50	0.38	0.069	20.87	21.11	0.39	0.677	0.058

<sup>1</sup>Standard error of the mean.

<sup>2</sup>Relative to embryo weight without yolk.

**Table 4.** Effect of strain and egg weight on chick traits on the day of the hatch.

Traits	Strain (S)				Egg weight (EW)				S x EW
	Fast	Slow	SEM <sup>1</sup>	P	Heavy	Medium	SEM	P	P
Chick, g	47.60	48.74	0.60	0.192	50.32	46.01	0.60	<0.001	0.103
Residual yolk sac, % <sup>2</sup>	14.06	14.25	0.54	0.799	15.46	12.85	0.54	0.001	0.047
Yolk free chick weight, g	40.61	41.59	0.50	0.175	42.07	40.14	0.50	0.009	0.287
Chick length, cm	19.36	19.39	0.10	0.872	19.37	19.38	0.10	0.983	0.657
Breast									
Width, mm	15.03	15.12	0.19	0.717	15.05	15.09	0.19	0.884	0.166
Length, mm	21.09	19.98	0.37	0.042	20.17	20.90	0.37	0.177	0.918
Fibre area average, $\mu\text{m}^2$	28.90	27.42	0.76	0.178	29.34	26.98	0.77	0.032	0.050
Capillary number/100 $\mu\text{m}^2$	6.45	6.90	0.14	0.027	6.71	6.64	0.13	0.736	<0.001
Capillary/fibre	0.0466	0.0458	0.003	0.858	0.0465	0.0459	0.003	0.888	0.021

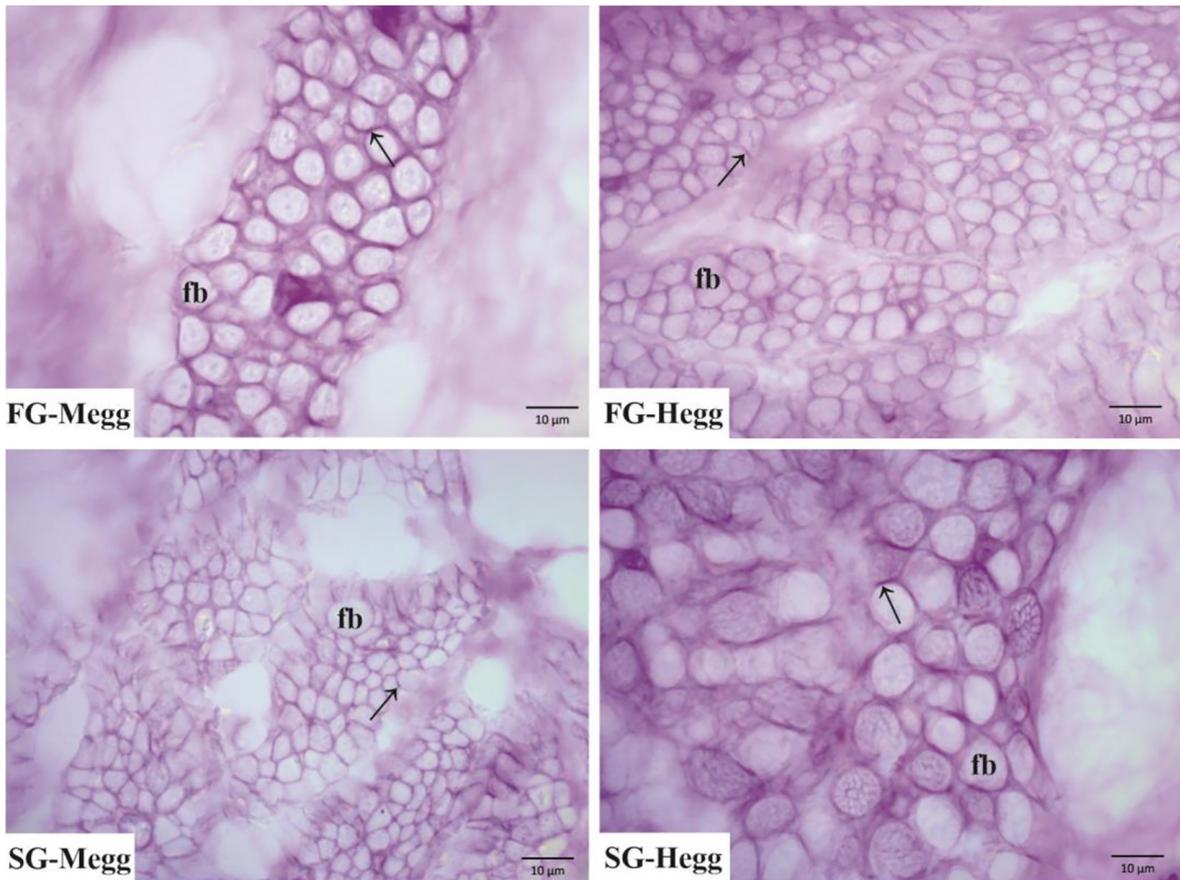
<sup>1</sup>Standard error of the mean.

<sup>2</sup>Relative to chick weight.

femur, and shank than those HE chicks. Moreover, tibia mineralization was better in Megg chicks compared to Hegg indicating that Megg chicks were better able to utilize egg calcium. Because the amount of calcium deposited in eggshell is constant during the laying cycle, larger eggs have a decreased eggshell thickness [28]. Eggshell is the primary source for bone development in embryo [35]. The limited calcium source has a negative impact on bone mineralization. Although eggshell thickness was not

measured in this study, thinner eggshell in Hegg might be a limiting factor for bone mineralization, however, this statement should be confirmed. -

In conclusion, the results showed that Hegg increased muscle fiber size and capillary number in FG chicks. Because larger fiber area is associated with larger breast weight, Hegg is an advantage for a larger breast weight. However, Hegg chicks had lighter femur, tibia, and shank with lower tibia ash content than those from Megg



**Figure 2.** Muscle fibers of chicks on the day of the hatch. FG-Megg: fast-growing, medium egg weight, FG-Hegg: fast-growing heavy egg weight, SG-Megg: slow-growing, medium egg weight, SG-Hegg, slow-growing, heavy egg weight. Fb: fibril, arrow: capillary.

**Table 5.** Effect of strain and egg weight on chick leg bone traits on the day of the hatch.

Traits	Strain (S)				Egg weight (EW)				S x EW
	Fast	Slow	SEM <sup>1</sup>	P	Heavy	Medium	SEM	P	
Tibia									
Weight, %	0.86	0.79	0.02	0.010	0.77	0.87	0.02	0.001	0.835
Length, mm	31.91	31.56	0.18	0.182	31.74	31.73	0.18	0.966	0.439
Width, mm	2.04	2.09	0.34	0.280	1.97	2.16	0.34	0.0003	0.164
Ash (%)	26.87	29.03	0.31	<0.001	27.07	28.83	0.32	<0.001	0.380
Femur									
Weight, %	0.53	0.46	0.01	0.0001	0.47	0.52	0.01	0.002	0.525
Length, mm	23.42	22.92	0.20	0.083	23.56	22.79	0.20	0.008	0.104
Width, mm	1.99	1.91	0.02	0.014	1.96	1.94	0.02	0.397	0.155
Shank									
Weight, %	2.53	2.39	0.02	0.0003	2.39	2.53	0.02	0.0004	0.080
Length, mm	22.08	22.39	0.19	0.250	21.60	22.87	0.19	<0.001	0.956
Width, mm	3.68	3.39	0.05	0.0004	3.66	3.41	0.05	0.002	0.178

<sup>1</sup> Standard error of the mean.

regardless of strain. These results showed that larger breast weight would affect bone properties negatively in chicks from Hegg. In our experiment, heavy and medium eggs in each strain were chosen from the same broiler breeder flocks. The findings suggest eggs heavier than 70 g will negatively impact bone properties of boiler chicks from

both FG and SG strains. Therefore, every effort should be devoted keeping egg weight uniformity of broiler breeder flocks.

### Conflict of interest

The authors declare that there is no conflict of interest.

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