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Effect of eggshell temperatures on hatching performance, egg production, and bone morphology of laying hens

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Abstract: The present study aims to evaluate the effect of lower or higher eggshell temperatures from days 7 to 21 on hatching performance, bone and blood parameters, and egg production along with the age of laying chickens. A total of 3150 eggs obtained from a white egg-type breeder were divided randomly into 3 groups of 1050 eggs. From 7 to 21 days, eggs were incubated at one of three eggshell temperatures: Control (IC) 37.5 °C, low (IL) 36.9 °C, and high (IH) 38.5 °C. At hatch, chicks were weighed, and blood was collected to measure total Ca, inorganic P, and ALP level weights, dimensions, and ash contents of femur, humerus, and tibia were obtained. A total of 240 chicks from each incubation temperature were reared up to 58 weeks. The results showed that hatchability was similar among the eggshell temperature groups. The IL chicks had the heaviest chick weight and yolk free chick weight. The IH group had reduced chick weight compared to IL and IC but increased lengths of tibia and humerus, ash contents of tibia and femur, and blood Ca levels at the day of the hatch. Laying hens from the IH group had impaired body weight during the laying cycle. At 58 weeks, there was no effect of eggshell temperature on egg production, bone length, and width. IH hens had lower tibia ash and serum Ca but higher P and ALP levels compared to IL and IC groups. However, bone mineral content and mineral density were similar for hens from different eggshell temperature groups. In conclusion, it appeared that although chicks from the IH group had reduced chick weight, they had improved bone morphology and ash content of bones compared to IC and IL at hatch. However, IH hens could not maintain the higher ash content during the grow-out and laying periods. The results indicate that the positive effect of IH incubation temperature on bone morphological measurements and ash content of tibia would not be long term.

Key words: Incubation temperature, laying chick, laying hen, bone, egg production

1. Introduction

Incubation temperature is one of the most important factors affecting embryo development [1,2]. Recent studies in broilers showed that the temperatures higher or lower than optimum incubation temperatures influence embryonic development and bone parameters [3,4]. Our previous studies [1] showed that cyclic (6 h/day) high or low incubation temperatures between either from days 0 to 8 or from days 10 to 18 decreased leg bone lengths of broiler embryo; however, this effect did not exist on the day of the hatch. Oviedo-Rondon et al. [5] reported that high temperature from days 18 to 21 reduced the relative weights of femur and shank of broilers. Recently, Güz et al. [6] noted that eggshell temperature 1.1 °C higher than optimum from 8 to 14 days of incubation stimulated tibia morphological and mechanical traits at slaughter age, while 1.1 °C lower temperature from day 15 to day 21 negatively affected tibia traits. However, the effects of variation in incubation temperature on laying breeder embryo and chicks have not been clearly demonstrated. Hammond et al. [2] showed that 1°C higher incubation temperature between days 4 and 7 of the embryonic period increased tibia and tarsus bones length of White Leghorn chicks. Sgavioli et al. [7] reported that bone mineralization of laying chicks was negatively influenced when incubated at 39 °C up until 21th day.

Due to excessive use of bone Ca for eggshell production, selection for egg production has led to increased egg

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production but decreased bone quality of laying hens [8]. Poor bone quality and decreased mineralization lead to increased fragility and fracture in laying hens [8]. Humerus, sternum, ischium, and keel bones were found to be the most commonly broken and fractured bones [9] and femur and tibia are known as breaking frequently [10,11]. Moreover, keel bone damages were significantly correlated with tibia bone ash content and strength in laying hens [12]. Because the skeleton system of laying hens contributes to eggshell Ca during the most intense period of shell formation [13], establishing and maintaining a strong skeleton is of paramount importance for both egg production and animal welfare. Bone quality can be characterized by bone ash, mineral density, bone volume, and length [14]. Bone mineralization, which is correlated with bone ash [15], is considered a reflection of bone health [16], while the bone length is correlated with bone volume [14].

Bone mineralization and skeletal growth require Ca and P. Bone ash, bone mineral density, and alkaline phosphate (ALP) are reliable indicators of bone metabolism and formation [17,18]. Because bone formation starts during embryonic development, primarily between 14 and 18 days [2,19], we hypothesized that exposure to low or high eggshell temperatures may affect bone development, which in turn would affect bone parameters of laying chicks later in life. During incubation, there is a rapid increase in mineralization between 7th and 21th days. Therefore, the experiment reported here was designed to evaluate the impact of lower or higher than recommended eggshell temperatures from days 7 to 21 on hatching performance, bone mineralization, morphological measurements, blood Ca, P, and ALP levels, and egg production along with the age of the laying chickens.

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 Poultry Research Institute (22.04.2016, 07).

2.1. Incubation conditions

A total of 3150 eggs were obtained from Atabey, a white egg-type breeder (produced by Poultry Research Institute, Ankara, Turkey) at 45 weeks of age. The eggs were stored at 18 °C and 65% relative humidity for 3 days before setting. The eggs were numbered, weighed, divided randomly into 3 groups of 1050 eggs, and incubated at 37.5 °C eggshell temperature from days 0 to 6. One Pas Reform, Smart (The Netherlands) incubator with a maximum capacity of 115,200 eggs was used. The incubator was divided into three modular setters by a curtain such that temperature and humidity could be digitally controlled. From days 7 to 21, eggs were incubated at one of three eggshell temperatures: Control (IC) eggshell temperature (37.5 °C), low (IL) eggshell temperature (36.9 °C), and high (IH) eggshell temperature (38.5 °C). Relative humidity was kept at 60%. The eggs in each temperature group were placed in the middle part of one trolley. There were 9 replicated egg trays and 116–117 eggs in each egg tray/temperature. At 18th day, the eggs were transferred to a hatcher basket and placed as replicate groups until 21th day.

2.2. Parameters measured during incubation

The eggshell temperature was measured on preidentified 20 eggs/tray/temperature using the Termoscan instrument (Braun, Kronberg, Germany) every day from days 7 to 21 at the same time. The mean eggshell temperature was calculated, and the temperature was adjusted accordingly. The variation in eggshell temperature was between 0.05–0.1 °C. At 18th day, 20 eggs from each group were weighed to determine egg weight loss.

At hatching, chicks/eggshell were pulled out after drying. A total of randomly selected feather sexing 12 female chicks/temperature were weighed at feather dryness, killed by decapitation, and blood was sampled from the jugular vein into tubes without heparin to measure total Ca, inorganic P, and ALP activity. The weights of the residual yolk sac, left femur, humerus, and tibia were obtained and relative weights were calculated. The length and width of bones were measured in mm with an electronic caliper. All sampled bones were stored at –20 °C for later determination of ash content. Unhatched eggs were broken out for macroscopic determination of infertility and early (from 0 to 7 days), mid (from 8 to 14 days), and late-term (from 15 to 18 days) embryonic mortalities, and piped but unhatched embryo. Hatchability and embryonic mortalities were expressed as the percentage of fertile eggs. Because the experiment was started at 7 days of incubation, mid and late-term mortalities represented the total embryonic mortality.

Blood was centrifuged at 1500 × *g* for 15 min; serum samples were kept at –20 °C until analyzed. Serum ALP, Ca, and P levels were determined by commercially available colorimetric assay kits (ERBA, Germany) using a biochemical analyzer (ERBA XL 600, Meinheim, Germany). Serum samples were diluted 1:1 (indicated as dilution factor 2) before serum Ca analysis. The intraassay CVs for Ca were 0.89% and 0.43% and the interassay CVs were 2.05% and 2.26%, respectively, for sample 1 (n = 20) and sample 2 (n = 20). The limit of quantification of the Ca determination assay was 0.6 mg/dL. The intraassay CVs of P determination were 0.94% and 1.22% and the interassay CVs were 2.61% and 1.12%, respectively, for sample 1 (n = 20) and sample 2 (n = 20). The limit of quantification of the P determination assay was 0.2 mg/dL. Serum samples were diluted 1:3 (indicated as dilution factor 4) before serum ALP analysis. For serum ALP determination, the

intraassay CVs were 0.87% and 1.07% and the interassay CVs were 2.68% and 2.66%, respectively, for sample 1 (n = 20) and sample 2 (n = 20). The limit of quantification of the ALP determination assay was 3.2 U/L. Tibias were ashed for 12 h at 550 °C to determine percent ash [(ash weight/ tibia dry weight) × 100)].

2.3. Rearing and laying periods conditions

A total of 720 female chicks (240 from each incubation temperature) were reared at 135 cm^2/chick stocking density from 0 to 3 weeks and 270 cm^2/chick stocking density from 4 to 16 weeks in cages in an environmentally controlled house. The cage dimensions were 123 × 66 cm. There were 4 replicated cages/incubation temperature with 60 chicks/cage from 0 to 3 weeks and 8 replicated cages/ incubation temperature with 30 chicks/cage from 4 to 16 weeks. The temperature was kept at 33 °C for the first 3 days, reduced by 4 °C per week until reaching 21 °C on day 21, and maintained until the end of the experiment. Feed and water were offered ad libitum. Chicks were fed a diet containing 19%, 18%, and 16% protein and 2900, 2800, and 2700 kcal/kg energy from weeks 0 to 3, 4 to 10, and 11 to 16, respectively. The lighting program started with 23:1 light:dark, reduced gradually to reach 10:14 light:dark until 4 weeks and this regime was kept until 16 weeks.

At 16 weeks, 192 randomly selected pullets from each incubation temperature were transferred to the enriched cages (240 × 60 cm). There were 12 replicated cages per incubation temperature with 16 pullets in each cage, providing approximately 900 cm^2 of floor space per bird. Cages were equipped with feeders, nipple drinkers, nest boxes, a perch area, and a scratch pad.

From 17 to 58 weeks, pullets received a laying hen diet containing 4.2% Ca, 16% protein, and 2700 kcal/kg ME. The temperature and humidity of the laying house were maintained at 20 ± 3 °C and 65%, respectively. The daily photoperiod was set at 14:10 light:dark during the laying period.

In each period, replications from each incubation group were represented at all cage levels.

2.4. Parameters measured during the laying period

The body weights of hens were recorded at 18, 28, 48, and 58 weeks by weighing randomly selected one or two hens (a total of 20 hens/incubation temperature) from each replication. Daily mortality was recorded, and livability was calculated as a percentage of mortality. Egg production and broken eggs were recorded daily on a replication basis. The egg weight was recorded at 18, 28, 48, and 58 weeks by weighing all eggs laid on two consecutive days.

At 18 and 58 weeks, blood was collected between 10:00–12:00 h from randomly selected 12 hens (one hen/ replicate/incubation temperature) in tubes without heparin for repeat Ca, P, and ALP analyses. Then, the hens were killed by cervical dislocation and the left tibiae, femur, and humerus were taken to measure length and width. Tibia samples were kept frozen in plastic bags at −20 °C until analysis for bone mineral density and ash content. Bone mineral density analysis (BMD) was performed using dual-energy x-ray absorptiometry (Hologic Discovery QDR, Bedford, MA, USA). Then, tibia samples were ashed at 550 °C.

2.5. Statistical analysis

Data were analyzed by one-way ANOVA using a statistical package of JMP (SAS Institute, North Carolina, NC, USA) [20]. There was no effect of incubation temperature on mid and late-term embryonic mortalities; thus, the total embryonic mortality was presented. A two-way ANOVA was applied for bone ash content of chicks obtained on the day of the hatch using a model of eggshell temperature and bone (tibia, one, and humerus) and interaction between them. Tukey's test was used to assess any significant differences at a probability level of 0.05 among the incubation temperature groups. Data were presented as LSMEANS.

3. Results

3.1. On the day of the hatch

There were no differences among the groups for the initial egg weight (Table 1). IH resulted in higher egg weight loss compared to IL and IC. The eggshell temperature did not affect total embryonic mortalities and hatchability. The heaviest chick weight was obtained for IL whereas the lightest one was obtained for IH. The eggshell temperature had no effect on residual yolk sac weight ($P = 0.083$). Chicks from IC and IH had similar yolk sac free chick weight, being lighter than IL (Table 1).

On the day of the hatch, absolute bone weights were similar among treatment groups. IH increased relative weights of tibia, femur, and humerus (Table 2). Longer tibia and humerus were obtained by IH. The ash content of bones on the day of the hatch is given in Figure 1. The ash of tibia and femur was increased by IH, while incubation temperature did not affect humerus ash content (Figure 1). The average ash content of tibia (29.72%) was higher compared to femur and humerus bones (27.48% and 16.90% for femur and humerus, respectively) ($P < 0.001$, data not shown). IH chicks had higher blood Ca but lower P and ALP levels than IC and IL chicks (Figure 2A).

3.2. Laying period

The body weight of the hens was not influenced by incubation temperature at 18 weeks. The body weight of IH hens was lighter than that of IL hens, while IC hens had intermediate body weight between IL and IH at 28 weeks (Table 3). At weeks 48 and 58, IH hens had the lightest body weight. Neither egg production nor egg weight were affected by eggshell temperature (Table 3). Livability was

Table 1. Initial egg weight, egg weight loss during incubation, and hatching performance by incubation temperature.

	Incubation temperature[1]			SEM[2]	P values
	IL	IC	IH		
Initial egg weight, g	58.0	57.5	58.3	0.3	0.160
Egg weight loss, %	11.5[b]	12.1[b]	14.9[a]	0.2	<0.001
Total embryonic mortality[3], %	10.1	11.3	9.0	1.1	0.389
Hatchability of fertile eggs, %	79.1	79.9	82.8	2.0	0.424
Chick weight, g	38.3[a]	34.7[b]	33.7[c]	0.2	0.001
Residual yolk sac weight, %	7.8	9.7	7.6	0.7	0.083
Yolk free chick weight, g	35.3[a]	31.1[b]	31.1[b]	0.7	<0.001

[1]From the day 0 to 6, incubator was set to attain an eggshell temperature of 37.5 °C. From 7th to 21th days, eggs were incubated at one of three eggshell temperature: low (IL) eggshell temperature (36.9°C), Control (IC) eggshell temperature (37.5°C), or high (IH) eggshell temperature (38.5°C).
[2] SEM: Standard error of mean
[3] Total of mid- and late-term embryonic mortalities
[a,b] Means within the same row lacking a common superscript differ significantly (P < 0.05).

Table 2. Effect of incubation temperature on weight (absolute and relative to chick weight), length, and width of tibia, femur, and humerus of chicks at the day of the hatch.

		Incubation temperature[1]			SEM[2]	P values
		IL	IC	IH		
Tibia	Weight, g	0.208	0.211	0.212	0.005	0.549
	Weight, %	0.545[b]	0.616[a]	0.648[a]	0.020	<0.001
	Length, mm	29.44[b]	29.28[b]	30.86[a]	0.44	0.025
	Width, mm	1.61	1.56	1.60	0.02	0.269
Femur	Weight, g	0.132	0.132	0.143	0.005	0.342
	Weight, %	0.345[b]	0.383[ab]	0.424[a]	0.016	0.002
	Length, mm	21.60	21.60	21.97	0.20	0.266
	Width, mm	1.68	1.64	1.69	0.02	0.133
Humerus	Weight, g	0.067	0.063	0.071	0.004	0.476
	Weight, %	0.175[b]	0.184[b]	0.212[a]	0.012	0.021
	Length, mm	15.98[b]	15.84[b]	17.01[a]	0.27	0.008
	Width, mm	0.814	0.731	0.739	0.036	0.236

[1]From the day 0 to 6, incubator was set to attain an eggshell temperature of 37.5 °C. From 7th to 21th days, eggs were incubated at one of three eggshell temperature: low (IL) eggshell temperature (36.9 °C), Control (IC) eggshell temperature (37.5 °C), or high (IH) eggshell temperature (38.5 °C).
[2]SEM: Standard error of mean
[a,b]Means within the same row lacking a common superscript differ significantly (P < 0.05).

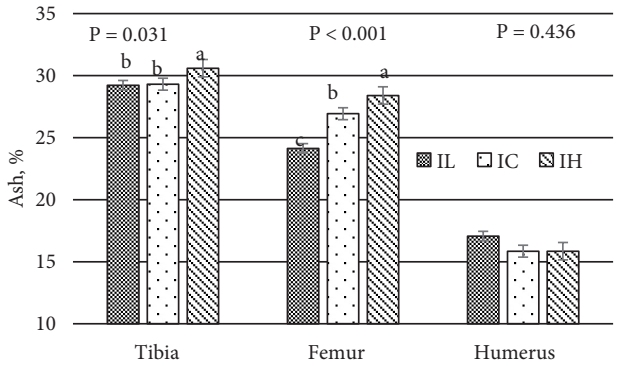

Figure 1. The effect of incubation temperature on tibia, femur, and humerus ash content of chicks on the day of the hatch (LS mean ± SEM). IL: eggshell temperature was 36.9 °C; IC: eggshell temperature was 37.5 °C; IH: eggshell temperature was 38.5 °C from 7 to 21 days of incubation. [a,b,c]Means lacking a common superscript differ significantly (P < 0.05).

similar among the groups: 97.6, 97.1, and 96.2 for IL, IC, and IH hens, respectively (P = 0.835, data not shown).

At 18 weeks, the eggshell temperature did not influence the length and width of femur and humerus bones, while tibia length and ash were influenced by incubation temperature (Table 4). IL and IH hens had longer tibia than IC hens. The ash content of tibia was higher for IL hens than for IH, similar to IC. There was no effect of incubation temperature on tibia mineral density and mineral content at 18 weeks. IH increased blood Ca, P, and ALP levels at 18 weeks (Figure 2B).

Lengths of tibia, femur, and humerus were similar among the eggshell temperature groups, while humerus widths of IL and IH were larger than of IC hens (Table 5). IH reduced tibia ash level. The eggshell temperature had no effect on tibia mineral density and mineral content. IH resulted in lower blood Ca, but higher P and ALP levels (Figure 2C).

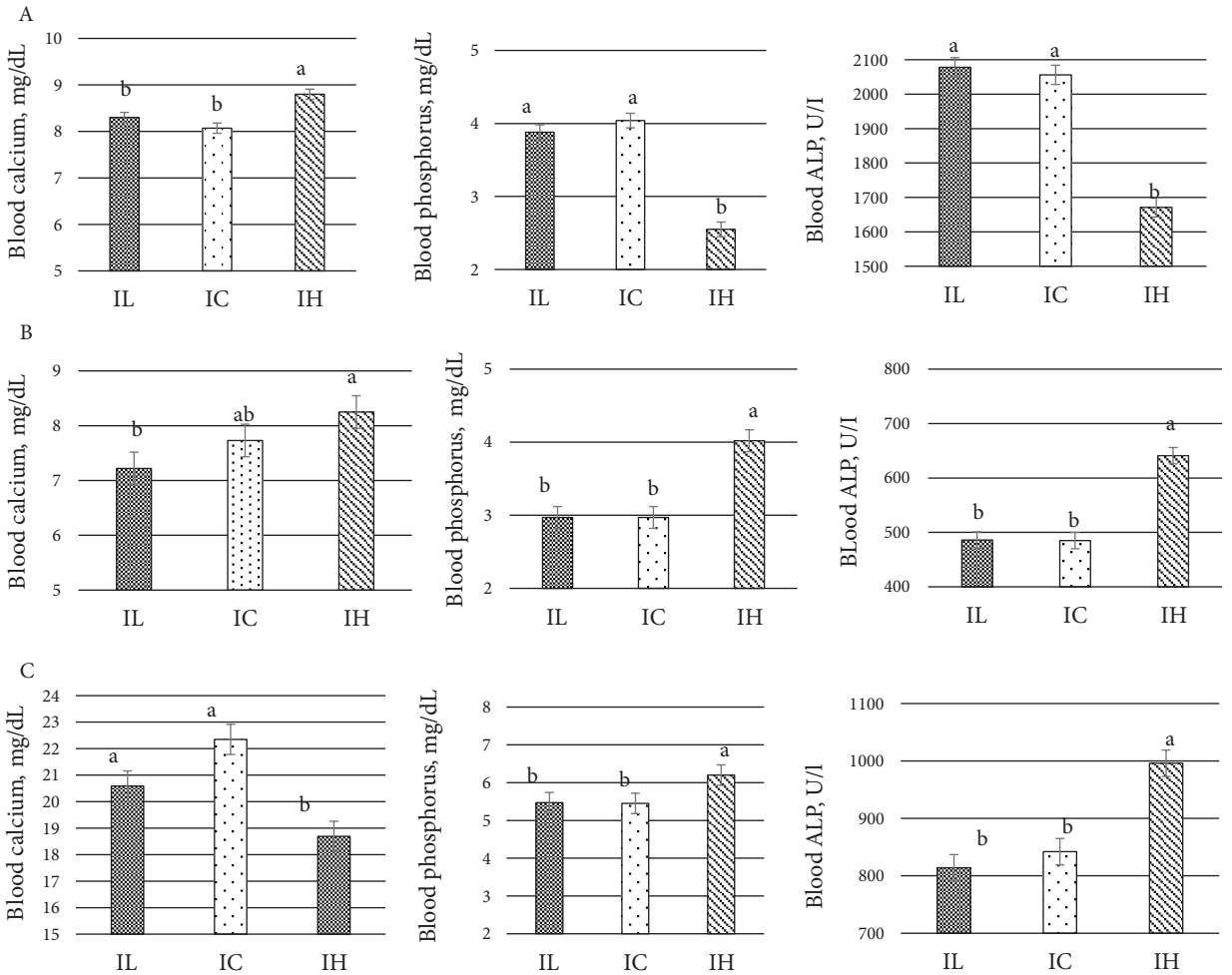

Figure 2. The effect of incubation temperature on blood calcium (A), phosphorus (B), and alkaline phosphatase (ALP) (C) levels of chicks on the day of the hatch (A), 18 weeks (B), and 58 weeks (C) (LS mean ± SEM). IL: eggshell temperature was 36.9 °C; IC: eggshell temperature was 37.5 °C; IH: eggshell temperature was 38.5 °C from 7 to 21 days of incubation. [a,b]Means lacking a common superscript differ significantly (P < 0.001).

Table 3. Effect of incubation temperature on body weight, average egg production, and egg weight of hens.

		Incubation temperature[1]			SEM[2]	P values
		IL	IC	IH		
Body weight, g	18 weeks	1162	1143	1135	17	0.661
	28 weeks	1655[a]	1571[ab]	1518[b]	35	0.032
	48 weeks	1854[a]	1785[a]	1628[b]	41	<0.001
	58 weeks	1856[a]	1790[a]	1627[b]	43	0.001
Egg production, %	Hen-day	80.9	81.4	81.3	1.3	0.956
	Hen-housed	82.1	82.7	82.2	1.5	0.954
	Broken eggs	10.2	10.5	11.1	1.0	0.803
Egg weight, g	18 weeks	46.15	45.7	45.7	0.5	0.845
	28 weeks	56.3	56.4	56.2	0.6	0.980
	48 weks	60.8	60.7	60.6	0.4	0.351
	58 weeks	62.37	62.2	60.2	0.7	0.122

[1]From the day 0 to 6, incubator was set to attain an eggshell temperature of 37.5 °C. From 7th to 21th days, eggs were incubated at one of three eggshell temperature: low (IL) eggshell temperature (36.9 °C), Control (IC) eggshell temperature (37.5 °C), or high (IH) eggshell temperature (38.5 °C).
[2]SEM: Standard error of mean
[a,b]Means within the same row lacking a common superscript differ significantly ($P < 0.05$).

Table 4. Effect of incubation temperature on length and width of tibia, femur, and humerus and ash, mineral density and mineral content of tibia of hens at 18 weeks.

		Incubation temperature[1]			SEM[2]	P values
		IL	IC	IH		
Tibia	Length, mm	115[a]	113[b]	116[a]	1	0.023
	Width, mm	6.56	5.39	6.53	0.12	0.561
Femur	Length, mm	79	78	80	0.8	0.499
	Width, mm	7.08	6.84	6.98	0.10	0.255
Humerus	Length, mm	74	73	75	0.8	0.144
	Width, mm	6.36	6.15	5.45	0.11	0.197
Tibia	Ash, %	38.9[a]	37.9[ab]	36.0[b]	0.7	0.033
	Mineral density, g/cm^2	0.243	0.241	0.240	0.005	0.932
	Mineral content, g	1.94	2.01	2.06	0.01	0.412

[1]From the day 0 to 6, incubator was set to attain an eggshell temperature of 37.5 °C. From 7th to 21th days, eggs were incubated at one of three eggshell temperature: low (IL) eggshell temperature (36.9 °C), Control (IC) eggshell temperature (37.5 °C), or high (IH) eggshell temperature (38.5 °C).
[2] SEM: Standard error of mean
[a,b]Means within the same row lacking a common superscript differ significantly ($P < 0.05$).

Table 5. Effect of incubation temperature length and width of tibia, femur, and humerus and ash, mineral density and mineral content of tibia of hens at 58 weeks.

		Incubation temperature[1]			SEM[2]	P values
		IL	IC	IH		
Tibia	Length, mm	116	116	117	1	0.272
	Width, mm	6.60	6.64	6.68	0.79	0.793
Femur	Length, mm	80	79	79	0.8	0.801
	Width, mm	6.98	7.09	6.92	0.22	0.210
Humerus	Length, mm	74	75	75	0.1	0.098
	Width, mm	6.40	6.49	6.40	0.72	0.717
Tibia	Ash, %	52.2[a]	52.1[a]	48.9[b]	0.7	<0.001
	Mineral density, g/cm^2	0.304	0.308	0.301	0.010	0.696
	Mineral content, g	2.63	2.72	2.71	0.11	0.696

[1]From the day 0 to 6, incubator was set to attain an eggshell temperature of 37.5 °C. From 7 to 21 days eggs were incubated at one of three eggshell temperature: low (IL) eggshell temperature (36.9 °C), Control (IC) eggshell temperature (37.5 °C), or high (IH) eggshell temperature (38.5 °C).
[2] SEM: Standard error of mean
[a,b]Means within the same row lacking a common superscript differ significantly ($P < 0.05$).

4. Discussion

Chickens are particularly sensitive to temperature during the incubation period [1]. It is important to keep the eggshell temperature between 37.5 and 37.8 °C for optimum hatching results [21–23]. Extensive literature suggests that deviations from the optimum temperature could affect hatching performance, posthatch growth, bone parameters of broilers [1,3,24,25], and could induce persistent effects on the physiology of broilers[26–30]. However, the embryonic days that were exposed to higher or lower than optimum temperatures and duration of temperature appear important for long-term effects [31]. In this study, we aimed to determine whether lower or higher than optimum eggshell temperatures would affect hatching performance and have long-term consequences on egg production and bone traits.

4.1. On the day of the hatch

Egg weight loss from days 0 to 18 of incubation varied between 6.5% and 14.0% [26]. Although initial egg weights were similar among the eggshell temperature groups, IH eggs had higher egg weight loss compared to the other groups. This result agreed with Sgavioli et al. [27] who reported that incubation at 39 °C resulted in greater egg mass loss in layer breeder eggs. It is known that higher eggshell temperatures accelerate embryonic growth [28]. Although we have not investigated the hatching time in this study, higher water loss may indicate a larger air cell, which may shorten incubation duration. Although hatchability was numerically higher in IH than in IC and IL, it was not statistically significant. However, numerically higher hatchability obtained for IH may have an importance for the broiler industry. No significant effect of eggshell temperature on hatchability may indicate that lower or higher constant temperatures from days 7 to 21 will not be a limiting factor for the hatching performance of layer breeder chicks. However, in the present study, IH reduced chick weight compared to IC and IL, while the heaviest chicks were obtained in IL conditions. Oviedo-Rondon et al. [5] found no effect of high or low incubation temperatures (from days 0 to 7 or 18 to 21) on broiler chick weight but reported a heavier residual yolk sac by low incubation temperature from 0 to 7 days of incubation. Our previous studies showed that cyclic (6h/d) higher temperatures between 10 and 18 days of incubation increased the hatching weight of broiler chicks [28], while no effect on hatch weight was found for laying chicks [29]. However, all these studies examined hatch weights of chicks obtained under short-term low or high incubation temperatures. It is not possible to compare these results with the present study because here, laying breeder eggs were exposed to constant high or low eggshell temperatures from days 7 to 21. In the present study, the lower chick weight obtained for IH chicks was related to neither hatching time nor utilization of yolk sac by chicks because chicks/eggshell temperature was sampled when dried, and residual yolk sac weights were similar among the eggshell groups. Moreover, chick weight without residual yolk sac was highest for the IL chicks. This result

suggested that IL applied in the present study increased the chick weight of layers. Similarly, Wineland et al. [31] found the greatest broiler chick weight when eggs were incubated at a low temperature.

The growth of leg bones of broilers is affected by age, body weight, conformation, and incubation temperature [3–5]. Bone ash content could be used as an indicator of bone mineralization [32]. In the present study, the eggshell temperature was the same for all groups from day 0 to 6. Therefore, the changes in ash content and bone morphology would be attributed to different eggshell temperatures from days 7 to 21. In spite of similar absolute bone weights of chicks from different eggshell temperature groups, IH increased the relative weights of tibia and humerus and the ash content of tibia and femur on the day of the hatch. This result suggests that IH from 7 to 21 days of incubation accelerated bone development and mineralization. Bone mineralization is formed through cartilage formation [33,34]. It seems that IH increased the mineralization of the cartilage matrix. On the other hand, ALP participates in the calcification process. The activity of ALP in chicken embryo tibias increases from days 12 to 16 [19]. An excess of thyroid hormones is associated with increased levels of ALP [35]. Indeed, higher triiodothyronine (T_3) activity in broiler chicks from cyclic high incubation temperature was found in a previous study [28]. The question remains whether lower serum ALP in IH chicks is related to T_3 level or is a reflection of complete bone growth.

Compared to IC, tibia, femur, and humerus ash were 1.29%, 1.47%, and 1% higher in IH chicks, suggesting that humerus mineralization is less sensitive to the changes in the eggshell temperature. Our results also showed that tibia ash was higher than femur and humerus ash content. This result suggested that the tibia of laying chicks ossify at a higher rate than femur and humerus or start to mineralize before femur and humerus.

The higher blood Ca levels for IH chicks showed elevated Ca absorption. The observed increase in free Ca in the blood of chicks from IH may demonstrate that there will be enough Ca available for bone development. This result was different from the findings of Sgavioli et al. [7] who reported a reduced ionized Ca in chicks from eggs incubated at 39 °C. This difference between studies may be attributed to the different eggshell temperatures in the studies.

4.2. Laying period

IH hens continued to be lighter than IL and IC hens at 18 weeks, although not significantly; moreover, IH hens had a significantly lower body weight than IL and IC hens between the 28th to 58th weeks. All hens were kept at similar environmental conditions and fed by the same diet; therefore, this result could be explained by the effect of incubation temperature. This result indicated that IH chicks were unable to compensate for the lower hatch weight. No effect of eggshell temperature on egg production and egg weight agreed with Kamanli et al. [29]. The number of broken eggs depends on rearing conditions and eggshell strength. In our study, because all hens were kept in the same conditions, we might indicate that incubation temperatures had no effect on eggshell quality. However, further research is necessary to get to a conclusion on the effect of incubation temperature on eggshell quality.

The increase in ALP activity could be induced by increased osteoblastic related skeletal problems and remodeling of bone and may decrease bone mineral density. At 18 and 58 weeks, higher serum ALP in IH hens was associated with lower bone ash content. This result demonstrates that the digestive system is not able to supply enough Ca and P to meet the needs of shell formation and loss of Ca from the bone leads to reduced bone ash. However, BMC and BMD were similar for hens from different eggshell temperature groups. This result may indicate that serum ALP plays a different role than bone ALP, in agreement with Chen et al. [35]. Because bone mechanical properties are related to BMD [16], our results show that eggshell temperatures applied in this study would not affect bone breaking strength. Although tibia and humerus lengths were shorter in IL than in IH chicks on the day of the hatch, at 18 and 58 weeks, IL hens had similar bone length and width to IH hens, indicating that bone growth was compensated.

In conclusion, the results show that the lower or higher incubation temperature used in this study does not affect hatchability. IH decreased hatch weight but increased the weights of tibia, femur, and humerus bones and the ash of tibia and femur bones of laying chicks on the day of the hatch when bone growth and remodeling are very notable. However, this positive effect of IH on bone ash did not persist. Moreover, IH chicks were unable to compensate for the lower hatch weight until 58 weeks of age. Based on the present results, we may conclude that IL and IH eggshell temperatures did not affect hatching and egg-laying performance but IH negatively affected the tibia ash content of laying hens during the egg production cycle.

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