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Effect of thermal manipulation on embryonic development, hatching process, and chick quality under heat-stress conditions

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ABSTRACT Thermal stress is a risk that threatens poultry welfare and productivity. Thermal manipulation during egg incubation is considered a prevention strategy used to mitigate the detrimental effects of high ambient temperatures on birds. This study aimed to investigate the impact of thermal manipulation, applied to chicken breeder's eggs during the incubation period, on embryonic development, hatching characteristics, and chick quality, as well as posthatch thermotolerance and performance. A total of 1,200 fertile eggs were randomly and equally assigned into 2 groups of 3 replicates (200 eggs/replicate), using a randomized experimental design followed by t test. The first group eggs (**G1**) were subjected to a commercial setter temperature of 37.5°C with 55% relative humidity (**RH**) throughout the incubation period (1−18 d) and served as a control, while the second group eggs (**G2**) were treated the same commercial setter conditions until the 11th day of the incubation, then the eggs were exposed to a higher temperature of 39.5°C with 60% RH for 4 h daily from the 12th to the 18th day of incubation. All eggs in both groups were exposed to the same temperature condition of 37.2°C with 70% RH from the 19th to the 22nd days of the incubation (hatching period). Three hundred hatched female chicks per each treatment group were transferred into a closed-system house and distributed randomly into 20 floor pens (15 birds per pen). At the 8th week of age, birds were exposed to a daily heat challenge by raising the temperature to 35°C for 6 h until the 18th week of the chick's age. According to the results, thermal manipulation at 12 to 18 d of egg incubation positively ($P \leq 0.05$) affected several studied traits. It improved some embryonic development traits, such as embryonic weight and tibia length, as well as some hatching parameters, such as hatching time and pipped eggs. It also improved hatched chick quality traits, including the chick's weight, length, and activity. In addition, it enhanced the posthatch chick's thermotolerance and body weight. Hatched chicks of G2 had significantly ($P \leq 0.05$) higher total protein, albumin, IgM, glucose, calcium, total antioxidant, and T_3 than G1 chicks. They also had significantly ($P = 0.001$) higher body weight (23%) at the 18th week of age than G1, as well as a lower feed conversion ratio (20.71%) than G1 chicks at 8 to 18 wk of age. Therefore, it is recommended to apply thermal manipulation during egg incubation, particularly at 12 to 18 d, for its positive effects on the pre- and posthatch performance.

Key words: antioxidant capacity, incubation temperature, internal organs, thermotolerance, T_3/T_4 hormones

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INTRODUCTION

Incubation conditions have a substantial impact on embryonic development, hatchability, chick quality, and posthatch performance (Han et al., 2022; Rocha et al., 2022; Fares et al., 2023; Al-Zghoul et al., 2023). Hatchability and chick quality are 2 crucial factors that determine the success of a hatchery (Yassin et al., 2008). Several studies have been conducted to discover strategies to improve these factors, and one of the most effective methods is temperature manipulation (Han et al., 2022; Rocha et al., 2022; Tona et al., 2022; Al-Zghoul et al., 2023). They demonstrated that by controlling the temperature during incubation, it is possible to boost the hatchability percentage and chick quality by improving the embryo's physiological and biological functions.

Temperature, as an important environmental condition that impacts several biological functions and behavioral activities of birds, is considered the most critical incubation condition (Han et al., 2022; Rocha et al., 2022; Yalcin et al., 2022). Piestun et al. (2015), Al-Zghoul and El-Bahr (2019), and Han et al. (2022) have shown that

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controlling the temperature during incubation can influence embryonic organ development, hatching parameters, and chick quality at the hatch. Finding the optimal temperature range and exposure period is crucial, and it depends on the specific poultry breed. By optimizing the temperature conditions during incubation, hatcheries can produce healthier, more robust chicks that are better suited for commercial production. Furthermore, chicks incubated in a manipulating temperature range have a higher growth rate and thermal tolerance, as well as a stronger immune system and a reduced mortality rate (Liu et al., 2015; Ismail et al., 2016; Al-Zghoul and El-Bahr, 2019; Han et al., 2022; Ramiah et al., 2022; Rocha et al., 2022). Thermal manipulation during chicken egg incubation has been demonstrated to significantly improve birds' metabolism, posthatch thermoregulation, and reduce the adverse effect of high ambient temperature (Han et al., 2022; Ramiah et al., 2022). In addition, it can positively affect birds' development, productivity, and welfare (Loyau et al., 2016; Vinoth et al., 2018; Garvalho et al., 2020; Al-Zghoul et al., 2023).

Animal husbandry and well-being have recently experienced substantial challenges as a consequence of rising global temperatures caused by global warming which represents a significant environmental issue that affects the entire world. Under commercial rearing systems, birds are exposed to various stressful circumstances, such as changes in the ambient temperature (Goel et al., 2023). Consequently, researchers and producers must continue investigating the best surrounding incubation conditions for their strains, and hatcheries must become more efficient to fulfill the requirements of modern/improved chick genotypes. Based on the strain and age, the embryo incubation temperature requirement changes during embryogenesis (Rocha et al., 2022; Tona et al., 2022; Al-Zghoul et al., 2023). However, several studies have shown the benefits of thermal manipulation during egg incubation on broiler or layer- type chicks' quality, such as improved productive performance (Saleh et al., 2020; Han et al., 2022; Rocha et al., 2022; Al-Zghoul et al., 2023), but few have been interested in investigating this treatment on dual-purpose chicken breeders. Additionally, some research studies that addressed temperature manipulation during chicken egg incubation have shown conflicting or inconsistent findings (Ismail et al., 2016; Zaboli et al., 2017; El-Zeniny et al., 2019; Al-Zghoul et al., 2023). Therefore, the aim of the present experiment was to investigate the effects of thermal manipulation, applied to dual-purpose chicken breeder's eggs during the incubation period, on embryonic development, hatching traits, and chick quality, as well as posthatch thermotolerance and performance.

MATERIALS AND METHODS

Animal Ethics

All experiments and procedures were followed in accordance with the Experimental Animal Care Committee Ethics of Animal Production Research Institute and Alexandria University (Alex. Agri. 092308313).

Experimental Design

The experiment was conducted at El-Sabahia Poultry Research Station, Animal Production Research Institute, Alexandria (31.2001° N, 29.9187° E), during the summer season (31°C temperature, 75% humidity rate, 13 km/h wind speed, as averages). It was performed using a randomized experimental design, whereas 1,200 fertile eggs, from Mandarah breeder chickens (an Egyptian-improved dual-purpose strain) (Abdel-Ghany and Abdel-Ghany, 2011), with an average body weight of 1652.97 ± 23.58 at 30 wk of age, were randomly and equally assigned into 2 groups of 3 replicates (200 eggs/replicate). Each group was incubated in a separate incubator (S380, PTO company, Egypt) with the same specifications and conditions. The first group eggs (**G1**) were subjected to a commercial setter temperature of 37.5°C with 55% RH throughout the incubation period (1−18 d) and served as a control, while the second group eggs (**G2**) were treated the same commercial setter conditions until the 11^{th} day of the incubation, then the eggs were exposed to a higher temperature of 39.5°C with 60% RH for 4 h daily from the 12^{th} to the 18^{th} day of incubation. All eggs in both groups were exposed to the same temperature condition of 37.2°C with 70% RH and an egg turning rate of 6 times/d with a ventilation rate of 350 m^3/h from the 19^{th} to the 22nd days of the incubation (hatching period).

All hatched chicks from each replicate were weighed and considered the chicks' hatch weight. Three hundred hatched female chicks per each treatment group were transferred into a closed-system house (15 birds/2.4 m^2; 30°C temperature; 60% RH; 20 lux light intensity) and distributed randomly into 20 floor pens (each pen dimension is 2.0 m × 1.2 m × 2.0 m and is furnished with wheat straw). At the 8^{th} week of age, birds were exposed to a daily heat challenge by raising the temperature to 35°C for 6 h (from 10:00 am until 16:00 pm) and then the temperature was returned to the normal condition (27°C) within 1 h. This thermos challenge persisted until the 18^{th} week of the chick's age. Relative humidity was maintained at a constant range of 60 to 70%. Feed and water were provided ad libitum throughout the experimental period. Birds were fed a starter diet (from 1 to 8 wk) containing 2,860 kcal/kg ME and 19.5% crude protein and a grower diet (from 9 to 18 wk) containing 2,705 kcal/kg ME and 15.5% crude protein.

Evaluated Parameters

Egg Weight Loss All hatching eggs were individually weighed (g) on the 12^{th} and 18^{th} days of the incubation to obtain egg weight loss percentages for the incubated intervals (0−12, 12−18, and 0−18 d).

Embryonic Development Parameters On d 12 and 18 of incubation, 18 eggs were chosen randomly from

each experimental group (6 eggs/replicate) and weighed. They were then opened, and the embryos were separated from the remaining egg contents. The dried embryos were allowed to reach room temperature and then weighed to the nearest 0.001 g. Relative embryo weight, yolk sac weight, and eggshell weight were determined as a percentage of egg weight, and eggshell thickness was measured using the Micrometer. At the 18th day of incubation, the tibia bone and heart were weighed along with the previous measurements. At the end of the incubation, 10 hatched chicks for each group were weighed and slaughtered to determine the hatched weight, the relative weight of some internal organs (liver, spleen, heart, Fabricius gland), and the tibia bone.

Embryonic Mortality Eggs that were not successful in hatching were broken out and examined macroscopically to estimate embryonic age, which was then assigned based on death time in days. The embryonic mortality percentage was expressed as a percentage of fertile eggs set in the incubator and classified into 3 periods (0−7, 8−14, and 15−18 d).

Hatching Time, Hatchability Percentage, Pipped Eggs, and Embryonic Malposition Beginning at 465 h of incubation, the hatcher was opened, and hatching time was monitored every 6 h after the hatch of the first chick. The hatchability percentage was estimated as a percentage of the total number of hatched eggs out of the total number of fertile eggs. Pipped eggs and embryonic malposition percentages were also recorded.

Chick Quality Parameters

All hatched chicks from each group were weighed, and their weights were considered the chick hatch weight. Chick lengths were also measured. Chick quality parameters including down and appearance (clean/dirty and dry/wet), navel quality (completely closed and clean or not), remaining membrane (no membrane), remaining yolk (no yolk), and chick activity were assessed and scored within a total scale of 100 according to Tona et al. (2003).

Hematological Parameters, Biochemical Constituents, Yolk Sac, and Internal Organs' Weight of Hatched Chicks At hatch, 40 chicks for each group (2 chicks/pen) were taken randomly and slaughtered (Islamic method). Blood samples were collected to obtain serum and plasma for hematological and biochemical analyses. The red blood cells count (**RBCs**), white blood cells count (**WBCs**), hemoglobin (**Hb**), and packed cell volume (**PCV**) were the hematological parameters determined according to El-Saadany et al. (2022). Blood mean cell volume (**MCV**), mean cell hemoglobin (**MCH**), and mean cell hemoglobin concentration (**MCHC**) were calculated as per the standard formula. Blood pH values were measured using a pH meter (HI 9321, MICRO pH METER, Portugal).

Plasma was obtained by centrifuging the blood at 3,500 rpm for 20 min and it was stored at −20°C for biochemical analysis. Total protein concentration (g/dL) was determined according to Henry et al. (1974), while albumin concentration (g/dL) was estimated using Doumas et al. (1971) method. Globulin concentration (g/dL) was calculated by subtracting total protein from albumin. The plasma immunoglobulin G (**IgG**) concentration determined using Chicken IgG ELISA kit (CEA544Ga, Enzyme-linked Immunosorbent Assay Kit, Cloud-Clone Corp.), while immunoglobulin M (**IgM**) was determined using Chicken IgM ELISA kit (Immunology Consultants Laboratory, Inc.), according to the manufacturer's instructions. Liver enzymes activities (aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**)) were assayed in plasma by the method of Reitman and Frankel (1957) using a specific kit (Diamond Diagnostics Chemical Company, Cairo, Egypt). Plasma glucose concentration was measured according to the method of Trinder (1969) using the instructions of a specific kit (Diamond Diagnostics Chemical Company, Egypt). Plasma calcium and phosphorus (mg/100 mL) were determined using commercial colorimetric kits as described by Tietz (1995). Plasma total cholesterol (mg/dL) and triglycerides (mg/dL) concentrations were determined according to the manufacturers' guidelines (Diagnostic Systems Laboratories). Plasma total antioxidant capacity and malondialdehyde were determined according to Benzie and Strain (1996) and Placer et al. (1966), respectively. Plasma triiodothyronine ($\mathbf{T_3}$) and thyroxine ($\mathbf{T_4}$) were analyzed using radioimmunoassay (**RIA**) kits (Diagnostic Systems Laboratories) according to the method described by (Hollander and Shenkman, 1974). Plasma corticosterone level was assayed using corticosterone competitive ELISA kit (Bioassay Technology Laboratory, China), as described by the manufacturer.

Embryonic yolk sac, liver, spleen, heart, Fabricius gland, and tibia were removed and weighed to the nearest 0.1 g using a digital balance.

Body Weight Two hundred chicks from each group were weighed in the morning before offering feed. Birds were individually weighed biweekly from 8 to 18 wk of age. Change in body weight was calculated by subtracting the initial average live weight of a certain period from the average final live weight throughout the same experimental period.

Feed Consumption To calculate the amount of feed consumed by each experimental group during the growing period from 8 to 18 wk of age, the amount of feed remaining at the end of each interval weeks (8−10, 11−14, 15−18) was subtracted from the total feed given during the period.

Feed Conversion Ratio The feed conversion ratio was calculated by dividing the average consumed feed by the average weight gain of birds for each interval weeks (8−10, 11−14, 15−18).

Statistical Analysis

Data were statistically analyzed according to the SAS program (SAS Institute, 2018) using the general linear

Table 1. The effect of thermal manipulation at 12 to 18 d of incubation on egg weight loss and embryonic mortality.

Treatments	G1 (Control)	G2 (T.M.)	*P* value
Interval periods egg weight loss (%)			
0–12 d	5.91 ± 0.18	5.88 ± 0.19	0.821
13–18 d	4.89 ± 0.11	5.03 ± 0.16	0.093
0–18 d	10.79 ± 0.35	10.98 ± 0.33	0.098
Embryonic mortality (%)			
0–7 d	1.10 ± 0.11	1.11 ± 0.26	0.554
8–14 d	1.38 ± 0.63	1.50 ± 0.26	0.635
15–18 d	0.77 ± 0.01	0.65 ± 0.25	0.612
0–18 d	3.22 ± 0.17	3.30 ± 0.34	0.633

T.M. = temperature manipulation.

model (**GLM**) procedure. All values were presented as least-square means with an overall standard error of the mean. Significant differences between the 2 groups were subjected to *t* test. Results were considered significant at $P \leq 0.05$.

RESULTS

As a general result, thermal manipulation at 12 to 18 d of egg incubation had positive effects on several studied traits. It improved some embryonic development traits such as embryonic weight and tibia length, as well as some hatching parameters such as hatching time and pipped eggs. It also improved hatched chick quality traits including the chick's weight, length, and activity. In addition, it enhanced the posthatch chick's thermotolerance and body weight.

The effect of thermal manipulation during egg incubation on egg weight loss is presented in Table 1. It was observed that thermal manipulation has a slight impact on egg weight loss during the incubation, as there were insignificant ($P = 0.098$) differences between the 2 groups under the study. Thermal manipulation also did not affect ($P = 0.633$) the embryonic mortality (Table 1). On the other hand, thermal manipulation during embryogenesis had positive significant ($P \leq 0.05$) effects on some embryonic development parameters at the 18th day of incubation such as embryonic weight, yolk sac, tibia weight/length, and heart weight (Table 2), while there was no difference regarding initial egg weights (46.35 ± 0.80; 46.38 ± 0.77) or embryo weights (6.27 ± 0.15; 6.30 ± 0.18) on d 12.

Thermal manipulation during egg incubation significantly affected ($P \leq 0.05$) some hatching parameters such as hatching time, pipped eggs, and embryonic malposition of unhatched eggs, but it did not affect ($P = 0.100$) the hatchability percentage (Table 3).

The effects of thermal manipulation during embryogenesis on hatched chick quality traits are presented in Table 4. The thermal manipulation treatment significantly ($P = 0.001$) affected the chick's weight, length, activity, appearance, navel quality/health, remaining membrane, and remaining yolk.

The results of Table 5 showed that the thermal manipulation significantly ($P \leq 0.05$) affected some hematological traits of hatched chicks. Hatched chicks of G2 had significantly higher WBCs, lymphocyte, MCV, MCHC, and blood pH than G1 chicks. Furthermore, thermal manipulation significantly ($P = 0.001$) impacted some blood biochemical parameters of hatched chicks, as shown in Table 6. Hatched chicks of G2 had significantly higher total protein, albumin, IgM, glucose, calcium, total antioxidant, and T_3 than G1 chicks. They also had significantly ($P = 0.002$) lower AST, ALT, cholesterol, T_4, and corticosterone than G1 chicks.

The results of Table 7 showed that the thermal manipulation treatment significantly ($P = 0.010$; 0.003) affected the hatched chick's yolk sac weight and liver

Table 2. The effect of thermal manipulation at 12 to 18 d of incubation on embryonic development.

Treatments	G1 (Control)	G2 (T.M.)	*P* value
Traits at the 12th day of incubation			
Egg weight (g)	46.35 ± 0.80	46.38 ± 0.77	0.994
Embryonic weight (g)	6.27 ± 0.15	6.30 ± 0.18	0.780
Yolk sac (%)	80.43 ± 0.46	79.71 ± 0.53	0.133
Shell weight (%)	11.07 ± 0.30	10.64 ± 0.32	0.397
Shell thickness (mm)	0.33 ± 0.01	0.31 ± 0.03	0.082
At the 18th day of incubation			
Egg weight (g)	41.46 ± 0.44	41.33 ± 0.73	0.942
Embryonic weight (g)	22.88^{b} ± 0.44	24.05^{a} ± 0.65	0.009
Yolk sac (%)	23.92^{a} ± 1.45	20.25^{b} ± 1.15	0.037
Shell weight (%)	9.83 ± 0.52	9.67 ± 0.36	0.871
Shell thickness (mm)	0.30 ± 0.01	0.28 ± 0.01	0.145
Tibia length (cm)	1.09^{b} ± 0.03	1.61^{a} ± 0.04	0.003
Tibia weight (g)	0.19^{b} ± 0.01	0.21^{a} ± 0.01	0.055
Heart weight (g)	0.14^{a} ± 0.00	0.12^{b} ± 0.01	0.005

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation. % = as a percentage of egg weight.

Table 3. The effect of thermal manipulation at 12 to 18 d of incubation on hatching parameters.

Parameters	G1 (Control)	G2 (T.M.)	*P* value
Hatching time (h)	487.00^{a} ± 2.37	471.00^{b} ± 3.62	0.001
Hatchability of fertile eggs (%)	91.31 ± 1.23	93.94 ± 0.56	0.100
Pipped eggs (%)	3.75^{a} ± 0.60	2.01^{b} ± 0.50	0.038
Embryonic malposition (%)*	1.88^{b} ± 0.25	3.01^{a} ± 0.59	0.043

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation. *% = as a percentage of unhatched eggs.

Table 4. The effect of thermal manipulation at 12 to 18 d of incubation on hatched chick quality parameters.

Parameters	G1 (Control)	G2 (T.M.)	*P* value
Chick weight (g)	34.85^{b} ± 0.21	36.89^{a} ± 0.25	0.001
Chick length (cm)	13.31^{b} ± 0.19	15.62^{a} ± 0.19	0.001
Chick activity (%)	86.55^{b} ± 1.06	95.21^{a} ± 0.72	0.001
Down and appearance (%)	92.34^{b} ± 1.31	96.79^{a} ± 0.63	0.007
Navel quality (%)	89.44^{b} ± 1.17	93.34^{a} ± 0.94	0.009
No remaining membrane (%)	97.03^{b} ± 0.53	98.59^{a} ± 0.40	0.013
No remaining yolk (%)	97.37^{a} ± 0.67	91.59^{b} ± 1.24	0.001

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation.

Table 5. The effect of thermal manipulation at 12 to 18 d of incubation on some hematological traits of hatched chicks.

Traits	G1 (Control)	G2 (T.M.)	*P* value
WBCs ($\times 10^3$ /mm^3)	6.80^b ± 0.32	9.90^a ± 0.64	0.002
Monocyte (%)	6.20 ± 0.48	5.17 ± 0.58	0.223
Eosinophil (%)	3.30 ± 0.33	2.70 ± 0.33	0.119
Lymphocyte (%)	66.41^b ± 1.63	77.85^a ± 1.65	0.001
Heterophil (%)	22.40^a ± 1.22	14.10^b ± 1.26	0.005
H/L ratio	0.34^a ± 0.02	0.18^b ± 0.02	0.001
RBCs ($\times 10^6$/mm^3)	5.50 ± 0.15	5.70 ± 0.22	0.125
Hb (g/dL)	12.10 ± 0.45	12.82 ± 0.42	0.314
PCV (%)	44.22 ± 1.34	46.20 ± 2.17	0.212
MCV (μm^3)	72.50^b ± 0.83	75.30^a ± 1.00	0.043
MCH (pg/dL)	20.51 ± 0.52	21.00 ± 0.50	0.062
MCHC (%)	26.80^b ± 0.46	28.80^a ± 0.74	0.045
Blood pH	7.30^b ± 0.15	8.00^a ± 0.00	0.003

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation; WBCs = white blood cells; H/L ratio = heterophils/lymphocytes ratio; RBCs = red blood cells; PCV = packed-cell volume; Hb = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

Table 6. The effect of thermal manipulation at 12 to 18 d of incubation on some blood biochemical parameters of hatched chicks.

Parameters	G1 (Control)	G2 (T.M.)	*P* value
Total protein (g/dL)	2.80^b ± 0.12	3.88^a ± 0.09	0.001
Albumin (g/dL)	1.69^b ± 0.13	2.22^a ± 0.12	0.032
Globulin (g/dL)	1.28 ± 0 .13	1.19 ± 0.12	0.422
A/G ratio	1.59^b ± 0 .19	2.06^a ± 0.12	0.011
IgG (mg/dL)	3.52 ± 0.12	3.63 ± 0.19	0.714
IgM (mg/dL)	0.99^b ± 0.01	1.44^a ± 0.14	0.012
AST (U/L)	54.77^a ± 0.83	39.17^b ± 0.53	0.002
ALT (U/L)	19.48^a ± 0.48	16.79^b ± 0.05	0.001
Glucose (mg/dL)	101^b ± 3.49	125.80^a ± 2.78	0.001
Calcium (mg/dL)	9.91^b ± 0 .31	10.89^a ± 0.09	0.001
Phosphorus (mg/dL)	10.71^a ± 0 .58	7.60^b ± 0.20	0.002
Ca/P ratio	0.96^b ± 0 .07	1.64^a ± 0.04	0.001
Cholesterol (mg/dL)	292.57^a ± 14.91	204.12^b ± 13.79	0.002
Triglycerides (mg/dL)	157.80 ± 7.78	148.30 ± 10.81	0.180
Total antioxidant (mg/dL)	1.89^b ± 0.09	2.78^a ± 0.12	0.002
Malondialdehyde (nmol/mL)	1.67 ± 0.13	1.38 ± 0 .14	0.195
Triiodothyronine (T_3) (ng/mL)	1.37^b ± 0.15	2.03^a ± 0.11	0.002
Thyroxine (T_4) (ng/mL)	11.89^a ± 0.162	9.40^b ± 0.30	0.001
T_3/T_4 ratio	0.11^b ± 0.008	0.21^a ± 0.010	0.001
Corticosterone (ng/mL)	25.01^a ± 1.33	17.66^b ± 0.93	0.005

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation.

Table 7. The effect of thermal manipulation at 12 to 18 d of incubation on relative weight of yolk sac, some internal organs, and tibia for hatched chicks.

Traits	G1 (Control)	G2 (T.M.)	*P* value
Yolk sac weight (%)	14.78^a ± 0.71	11.62^b ± 0.84	0.010
Liver weight (%)	2.04^b ± 0.09	2.61^a ± 0.12	0.003
Spleen weight (%)	0.04 ± 0.01	0.04 ± 0.00	0.699
Heart weight (%)	6.18 ± 0.44	6.53 ± 0.27	0.701
Fabricius gland weight (%)	0.16 ± 0.02	0.15 ± 0.03	0.655
Tibia weight (%)	1.06 ± 0.04	1.07 ± 0.05	0.989

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation.

Table 8. The effect of thermal manipulation at 12 to 18 d of incubation on body weight, feed consumption, and feed conversion ratio of chicks exposed to thermal challenge at 8 to 18 wk of age.

Treatments	G1 (Control)	G2 (T.M.)	*P* value
Interval weeks body weight (g)			
At the 8th week	493.92 ± 5.56	527.46 ± 4.60	0.094
At the 10th week	670.97^b ± 5.42	755.37^a ± 6.43	0.045
At the 14th week	1050.01^b ± 10.92	1245.37^a ± 11.61	0.001
At the 18th week	1500.58^b ± 11.76	1845.92^a ± 19.41	0.001
Feed consumption (g/bird/period)			
From 8 to 10 wk	700.87 ± 7.36	711.98 ± 7.31	0.811
From 11 to 14 wk	1500.61^b ± 14.94	1550.09^a ± 12.00	0.002
From 15 to 18 wk	1800.70^b ± 11.22	1900.01^a ± 14.09	0.001
From 8 to 18 wk	4002.19^b ± 17.86	4151.09^a ± 19.38	0.001
Feed conversion ratio (g feed/g gain)			
From 8 to 10 wk	3.88^a ± 0.05	3.07^b ± 0.04	0.001
From 11 to 14 wk	3.94^a ± 0.06	3.16^b ± 0.03	0.001
From 15 to 18 wk	4.00^a ± 0.03	3.16^b ± 0.03	0.001
From 8 to 18 wk	3.96^a ± 0.03	3.14^b ± 0.03	0.001

^{a,b}Means having different letters in the same row are significantly different ($P \leq 0.05$).

T.M. = temperature manipulation.

weight. Hatched chicks of G2 had significantly lower yolk sac weight and higher liver weight than those of G1 chicks. The effect of thermal manipulation during embryogenesis on postchick weight is presented in Table 8. The thermal manipulation treatment significantly ($P = 0.001$) affected the chick's body weight at the 10th, 14th, and 18th weeks of age. G2 chicks had significantly higher body weight (23%) at the 18th week than G1. Moreover, it significantly affected the chick's feed consumption and feed conversion ratio (Table 8). G2 chicks had significantly higher feed consumption (3.72%) and lower feed conversion ratio (20.71%) than those of G1 chicks at 8 to 18 wk of age.

DISCUSSION

Considering the importance of avian welfare, thermal manipulation, as a powerful practical approach applied during the embryogenesis process, has proven to improve several physiological, antioxidative, and immunological statuses of breeder chicks, which has a positive impact on posthatch chick performance, including thermal tolerance capacity and thermoregulation. An adequate management strategy during the incubation period is necessary for guaranteeing good chick quality and improving posthatch health and productivity, since it is a crucial stage in the chick's life (El-Hanoun et al., 2019; El-Sabrout et al., 2019; Rocha et al., 2022; Fares et al., 2023).

Egg weight loss is a quantitative trait that is affected not only by the eggshell characteristics and hen genotype (strain), but also by surrounding environmental conditions (such as temperature and RH), as well as the egg management (such as the preincubation egg storage) (Khalil et al., 2016; Grochowska et al., 2019; Okasha et al., 2023). The proportion of egg weight loss is crucial for ensuring enough air cell size inside the egg for proper lung function and chick pipping (Ar and Rahn, 1980). From the results of Table 1, it has shown that thermal

manipulation did not affect the egg weight loss during the incubation. This finding could be attributed to the short duration of the thermal manipulation applied (just 4 h daily from the 12th to the 18th day of incubation). In agreement, Elsayed (2016) reported that thermal manipulation had no significant effect on egg water loss during incubation. Furthermore, the egg weight loss percentages for both studied groups were within the normal range, therefore egg weight loss did not impact embryo mortality and the hatchability percentages (Tables 1 and 3). This finding is consistent with those previously mentioned by Ismail et al. (2016), El-Zeniny et al. (2019), Amjadian and Shahir (2020), Saleh et al. (2020), and Ramiah et al. (2022).

Thermal manipulation at the 18th day of incubation had positive significant effects on several embryonic development indices, such as embryonic weight, yolk sac, tibia weight/length, and heart weight (Table 2). Nakage et al. (2003), Willemsen et al. (2010), Morita et al. (2010), and Badran et al. (2012) revealed that incubating embryos at slightly higher temperatures than usual can accelerate embryonic development by altering embryo growth, tissue metabolism, and respiration rate. They also demonstrated that slight changes in the incubation temperature could affect the avian's heart and bone development. According to Christensen et al. (2003) and Morita et al. (2016), heart weight decreases with increasing incubation temperature, and this decrease in heart weight/size may be explained by a reduction in cardiac cell development or an increase in the incidence of metabolic disorders that are associated with cardiovascular development (Leksrisompong et al., 2007; Molenaar et al., 2011). In addition, Piestun et al. (2013) and Han et al. (2022) stated that short-term (occurring at not continuous intervals) change in incubation temperature at different embryogenesis stages can improve muscle growth and development at hatch. The increase in embryonic weight was apparently mediated by the upregulation of muscle growth factor/marker genes during pre- and posthatch phases (Al-Zghoul and El-Bahr, 2019), as well as a reflection of increased yolk consumption. Likewise, thermal manipulation can improve embryonic muscle development (proliferation and hypertrophy), according to the findings of Maltby et al. (2004) and Li et al. (2017), however, it depends on the treatment period and stage. Furthermore, the thermal manipulation group, in the current study, had a nonsignificant decrease in eggshell weight (Table 2), which could indicate that the embryo was consuming enough calcium for bone formation.

Thermal manipulation during egg incubation positively affected some hatching parameters, such as hatching time and pipped eggs (Table 3). It has shortened hatching time (incubation duration) by 3.3% and decreased pipped eggs by 46.7%. Han et al. (2022) found that thermal manipulation during embryogenesis resulted in different favorable hatching features, particularly in hatching time and hatching process, due to its positive effects on embryo physiology performance. On the other hand, hatching thermal manipulation has increased the embryonic malposition of unhatched eggs in the present study, but it did not affect the hatchability percentage (Table 3). It could be attributed to applying thermal manipulation treatment in a short duration and lately after the critical first 10 d of embryogenesis. These findings are consistent with Saleh et al. (2020), but not in agreement with Zaboli et al. (2017), maybe for using different treatment protocols (temperature, duration, etc.) or different strains. However, several research studies (Morita et al., 2010; Badran et al., 2012; Piestun et al., 2013; Han et al., 2022) demonstrated the role of incubation temperature in accelerating embryonic systems' cell multiplication, which in turn boosts embryonic development. Furthermore, they mentioned that a higher temperature than the usual one can increase embryonic weight by altering respiration rate, tissue metabolism, and embryonic growth, leading to a shorter incubation duration and easier eggshell breaking. Furthermore, thyroid hormones (T_3 and T_4), which could be influenced by altering temperature during incubation, play an essential role in hatching (Decuypere et al., 1991; Ismail et al., 2016). They influence embryo metabolism, final tissue maturation, and hatching physiological integration (Decuypere and Michels., 1992; Rippamonti and Dzialowski, 2023).

Thermal manipulation significantly affected the chick's quality, particularly the chick's weight, length, activity, appearance, navel area, remaining membrane, and remaining yolk (Table 4). This temperature treatment increased the chick's weight by 7.1% and chick's length by 17.4%, as well as improved the chick's activity by 10%. Similarly, Piestun et al. (2013) and Al-Zghoul and El-Bahr (2019) reported that chick weight/length was significantly increased for the groups exposed to thermal manipulation during the embryogenesis phase as compared to the control groups. It is well known that chick weight and length at hatch are useful tools for predicting chick growth in subsequent stages. Yalcin et al. (2012) and Liu et al. (2015) found that hatch weight can be used as a measure of chick quality. Different thermal manipulation protocols resulted in a significant increase in chick body weight (Han et al., 2022; Ramiah et al., 2022). The improvement in embryonic weight during incubation, which was affected by thermal stimulation during embryogenesis, is reflected in the increase in hatch weight. Thermal manipulation can affect hormonal control centers like the hypothalamus, which in turn affects some growth hormones and chick weights (Morita et al., 2016; Goel et al., 2023). Furthermore, the amount of remaining yolk is critical for nurturing the embryo during the final stage of incubation, as several biological critical processes occur at this stage, such as embryo absorbing the yolk into its stomach and moving into hatching position (van der Wagt et al., 2020; Kuzmina, 2023).

The results of Table 5 showed that the thermal manipulation significantly affected some hematological traits of hatched chicks. Hatched chicks of G2 had significantly higher WBCs, lymphocyte, MCV, MCHC, and blood pH than G1 chicks. The increased lymphocyte

percentage and lower H/L ratio in the treated hatched chicks (G2) could indicate that more lymphocytes have proliferated from the bursa to the peripheral blood, predicting better later-life health and stress resistance (Al-Murrani et al., 2006). Furthermore, thermal manipulation significantly impacted some blood biochemical parameters of hatched chicks, as shown in Table 6. Hatched chicks of G2 had significantly higher total protein, albumin, IgM, glucose, calcium, total antioxidant, and T_3 than G1 chicks. They also had significantly lower AST, ALT, cholesterol, T_4, and corticosterone than G1 chicks. These findings reflect that thermal manipulation can positively improve some chick's hematological traits, antioxidant capacity, and immunity response. In the same manner, Ismail et al. (2016), Elsayed (2016), and Zaboli et al. (2017) found that embryonic thermal manipulation can affect some blood biochemical traits such as total protein, albumin, cholesterol, and glucose, as well as triiodothyronine and corticosterone levels. According to Christensen et al. (2001), temperature manipulation increased the embryonic plasma glucose concentration of turkey embryos and altered insulin-like growth factor concentrations. In addition, they indicated that incubation temperature can change some hormone levels, such as T_3 and T_4 (growth promoters), related to the metabolism and growth of the embryo, which might have an impact on the hatch chick quality. According to Piestun et al. (2008) and Willemsen et al. (2010), the substantial influence of thermal manipulation on several hormones, such as corticosterone, may function as an epigenetic temperature adaptation because the same mechanisms are used to cope with posthatch heat stress.

According to the findings of Tollba and Hassan (2003), Elsayed et al. (2009), Al-Zghoul and El-Bahr (2019), and Han et al. (2022), the changes happened in some hematological and biochemical traits could be due to thermal manipulation impact during embryogenesis on some chick organs (weight/function). Moreover, thermal treatment can cause an increase in the antioxidant activity of the cell membrane as well as dehydration of the bird's body, which leads to an increase in some hematological features (Meiri et al., 1991; Saleh et al., 2020). On the other hand, Yahav et al. (2004) reported that thermal treatment during embryogenesis had no effect on the corticosterone or thyroid levels at chick hatch, possibly due to the use of different treatment protocols or strains.

Incubation temperature is considered one of the most critical physical factors influencing bird embryonic development, organogenesis, and hatchability (Deeming, 2002; Maltby et al., 2004; Liu et al., 2015). Thermal manipulation implementation during embryogenesis would promote the chick's muscle development such as the tibia muscle, as well as enhance some immune organs such as the thymus and bursa (Hammond et al., 2007; Liu et al., 2015; Ismail et al., 2016). According to Table 7 findings, the temperature manipulation treatment significantly affected the weight of the yolk sac and liver in newly hatched chicks while having no significant effect on heart, spleen, Fabricius gland, or tibia weights. Hatched G2 chicks were heavier in the liver and had considerably ($P \leq 0.05$) lower yolk sac weights than G1 chicks. Likewise, Leksrisompong et al. (2007), Walstra et al. (2010), Willemsen et al. (2011), and Al-Zghoul and El-Bahr (2019) revealed that chick liver weight increased with thermal manipulation during the incubation period while yolk sac weight decreased. These changes may be due to stimulating fat absorption from the yolk sac and activating fat metabolism to produce energy, as well as increase of metabolic rate in liver tissues as stated by Moraes et al. (2003) and van der Wagt et al. (2020). Temperature changes during incubation can influence the development of several organs and systems, including the central monoaminergic system (Loyau et al., 2015; Rocha et al., 2021). This system includes the catecholamines, dopamine, norepinephrine, and serotonin, which regulate a variety of biological functions in the avian's body, such as thermal regulation, breathing, and stress (Gargaglioni et al., 2008). Thus, regulating the bird's metabolism during embryonic development may be possible to precondition it for posthatch environmental conditions (Akşit et al., 2010; Tzschentke and Rumpf, 2011). However, these effects (known as epigenetic effects) assist birds in better adapting to environmental changes (Willemsen et al., 2010; Rocha et al., 2022).

Referring to Table 8 results, thermal manipulation treatment during embryogenesis significantly affected postchick weight at the 10^{th}, 14^{th}, and 18^{th} weeks of age. G2 chicks had significantly higher body weight (23%) at the 18^{th} week than G1. The thermal manipulation also significantly affected the chick's feed consumption and feed conversion ratio (Table 8). G2 chicks had higher ($P \leq 0.05$) feed consumption and lower feed conversion ratio than those of G1 chicks at 8 to 18 wk of age. Similar results were observed by Hammond et al. (2007), Piestun et al. (2011, 2015), and Ismail et al. (2016) who found that the increased temperature during embryogenesis has a positive effect on the posthatch muscle development, feed consumption, feed conversion ratio, and body weight by altering thyroid gland activity, metabolic rate, and heat output. Additionally, Yahav and Plavnik (1999) demonstrated that thermal therapy significantly increases feed intake and body weight gain while decreasing mortality with heat tolerance. The prenatal-determined developmental trend influences adaptation to the actual posthatch environment (Janke and Tzschentke, 2010). Collin et al. (2005) mentioned that incubation temperature modification improves thermotolerance acquisition (thermoregulatory functions) in chickens, as indicated by lower body temperature at hatch and in the first days after hatch. Intermittent manipulation of incubation temperature between different embryonic ages resulted in enhanced thermoregulatory functions. In the same trend, Yahav et al. (2004) and Alkan et al. (2013) reported that temperature manipulation at 16 to 18 d of embryogenesis can result in a considerable improvement in heat resistance, which is probably related to a decrease in body temperature

and thyroid hormone levels, implying a decrease metabolic rate. To avoid an increase in stress response, the timing of thermal manipulation must be linked to the development of the hypothalamus hypophysis-thyroid axis and the development of the hypothalamus-hypophysis-adrenal axis (Janke et al., 2002; Yahav et al., 2004; Tzschentke, 2008; Ismail et al., 2016).

Thermal manipulation during egg incubation is considered mild heat shock exposure during embryogenesis, which improves tissue stability, oxidative stress response, and immunological response to heat stress (Al-Zghoul et al., 2023). Different thermal manipulation protocols resulted in a significant increase in heat shock proteins mRNA expression in the pectoral and thigh muscles (Al-Zghoul et al., 2013, 2015; Ali et al., 2022; Ramiah et al., 2022), and the same was associated with enhanced thermoregulation and thermotolerance, as previously reported by Al-Aqil and Zulkifli (2009). Likewise, Li et al. (2017) reported that thermal manipulation can promote mRNA and protein expression of growth marker genes and muscle-related genes. The gene ontology analyses indicated that cellular processes, including cell cycle, metabolism, catalytic activity, and enzyme regulatory activity may have been involved in the muscle mass affected by thermal modification (Liu et al., 2015). Transforming growth factor-β (**TGF-beta**) and insulin pathways, which are both associated with muscle development, may potentially be involved in regulating muscle mass (Liu et al., 2015). These findings could aid in understanding the physiological and biochemical mechanisms of muscle development in embryonic birds subjected to thermal treatments.

CONCLUSIONS

According to the current findings, increasing the incubation temperature to 39.5°C with 60% RH for 4 h daily from the 12^{th} to the 18^{th} days in the incubator improved some hatching traits, such as hatching time and pipped eggs, as well as hatched chick quality including the chick's weight and activity. It also enhanced the post-hatch chick's adaptation to heat stress and body weight. Therefore, it is recommended to apply thermal manipulation during egg incubation, particularly at 12 to 18 d, to achieve maximum benefit for commercial poultry production.

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DISCLOSURES

The authors declare that there is no known conflict of interest associated with this publication.

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