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Differential responses of Ross and Arian broilers to embryonic thermal manipulation and post-hatch heat stress

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Abstract This study investigated the effects of heat stress and embryonic thermal manipulation (TM) on physiological, biochemical, and heat shock protein 70 (HSP70) gene expression in two broiler strains. A total of 400 fertilized eggs were incubated under either normal (37.8 °C) or thermally manipulated (39.5 °C; embryonic day 7 to 16) conditions. Post-hatch chicks were reared under either normal (25 °C) or heat stress (32 °C; from day 21 onward) conditions. The four experimental groups were defined as follows: Arian strain subjected to embryonic thermal manipulation but not to post-hatch heat stress (ATC); Arian strain without embryonic manipulation but subjected to post-hatch heat stress (ACH); Ross 308 strain subjected to embryonic thermal manipulation but not to post-hatch heat stress (RTC); and Ross 308 strain without embryonic manipulation but subjected to post-hatch heat stress (RCH). Blood parameters, lipid profiles, thyroid hormone (T3) levels, and HSP70 gene expression were analyzed at 42 days of age. Significant differences were observed in red and white blood cell counts, hematocrit, and T3 levels among treatments. RCH exhibited higher cholesterol (157.75±2.21) and LDL levels (59.67±2.88), whereas ATC showed higher T3 levels (8.85±0.32) and spleen weight (0.17±0.02). HSP70 gene expression was highest in RCH. The feed conversion ratio (FCR) was significantly lower in RCH (1.93±0.01). In conclusion, RCH demonstrated superior FCR under heat stress, while ATC modulate the thyroidal activity and immune responses following TM. Thermal manipulation during incubation reduced cholesterol, LDL, and HSP70 levels, enhancing thermotolerance. These findings highlighted the potential of TM to mitigate the heat stress effects, emphasizing the importance of strain-specific strategies for optimizing the broiler performance in heat-stressed environments.

Keywords: Arian, heat stress, Ross, thermal manipulation

Introduction

One of the most critical environmental factors that adversely affect poultry health and production is the increase in ambient temperature (Wang et al., 2018). Heat stress, defined as the exposure of birds to temperatures above their thermoneutral zone, has been shown to disrupt physiological homeostasis, impair growth performance, and reduce overall productivity (Lara and Rostagno, 2013). Numerous studies have demonstrated the detrimental effects of heat stress on various physiological and production parameters, including the weight of internal organs and carcass traits (Zeferino et al., 2016), hematological parameters (Yahav et al., 1997), and growth performance (Liu et al., 2020). For instance, heat stress has been reported to reduce feed intake, body weight gain, and feed efficiency, while increasing mortality rates in

broilers (Lin et al., 2006).

At the molecular level, heat stress induces the transcription and expression of the HSP70 gene, which plays a crucial role in cellular protection and thermotolerance (Xu et al., 2018). HSP70 acts as a molecular chaperone, helping to refold denatured proteins and prevent cellular damage under stress conditions (Zulkifli et al., 2009). However, prolonged or severe heat stress can suppress these protective mechanisms, leading to oxidative stress, immune suppression, and reduced productivity (Akbarian et al., 2016).

To mitigate the adverse effects of heat stress, researchers have explored various strategies, including embryonic thermal manipulation (ETM). ETM involves exposing eggs to controlled temperature variations during specific periods of incubation to induce epigenetic adaptations and improve post-hatch thermotolerance (Piestun et al., 2011; Vinoth et al., 2015). Studies have shown that thermal manipulation during the incubation period can enhance climate adaptation to both cold (Aminoroaya et al., 2016) and heat stress (Piestun et al., 2011) in the post-hatch period. However, deviations from optimal incubation temperatures can have detrimental effects, such as reduced hatchability and increased embryonic mortality (Tona et al., 2008).

Thermal manipulation (TM) during the embryonic stage has been shown to improve thermotolerance, growth performance, and meat quality in broilers exposed to heat stress during the rearing period (Collin et al., 2007). For example, Piestun et al. (2011) demonstrated that TM applied between the 7th and 16th days of embryonic development, with an increase in temperature to 39.5 °C and 65% relative humidity (RH) for 12 hours per day, significantly improved thermotolerance, performance parameters, and breast muscle yield in broilers exposed to heat stress from 21 to 35 days of age. Similarly, Al-Zghoul et al. (2019) reported that Cobb and Hubbard broilers subjected to acute heat stress at 28 days of age, following embryonic TM (39 °C and 65% RH) from embryonic days 10 to 18, exhibited lower mRNA expression levels of antioxidant enzymes and reduced total antioxidant capacity compared to the control group. Recent findings also demonstrate that incubating eggs under either control conditions (37.8 °C, 56% RH) or TM conditions (39 °C, 65% RH for 18 hours per day from embryonic day 10 to 18), followed by post-hatch exposure to either heat stress (35 °C for five days) or thermoneutral environments, offers a promising strategy to improve broiler resilience to heat stress (Dahadha et al., 2025). Previous studies have shown that applying thermal manipulation (TM) during embryonic days 12 to 18 (39.5 °C, 60% RH for 4 hours daily) can enhance thermotolerance, antioxidant capacity, immune response, and semen quality in roosters exposed to heat stress. These outcomes indicated that early thermal conditioning could support physiological and reproductive resilience under high-temperature conditions (El-Prollosy et al., 2024). These findings suggested that TM could the modulate oxidative stress responses and enhance thermotolerance in broilers. The effectiveness of TM in improving thermotolerance is thought to be linked to its impact on the development of the hypothalamic–hypophyseal–thyroidal axis (thermoregulation) and the hypothalamus–hypophyseal–adrenal axis (stress response) (Yahav, 2009). During critical periods of embryogenesis, TM can alter the 'set point' or 'response threshold' of these regulatory systems, which lead to long-term improvements in thermotolerance (Piestun et al., 2008). For instance, intermittent TM (39.5 °C and 65% RH for 12 hours per day), applied from embryonic day 7 to 16, has been shown to enhance thermotolerance in broilers by influencing these axes (Piestun et al., 2008).

Despite the promising results of TM, the response to thermal manipulation may vary depending on the broiler strain. Ross and Arian are two widely used commercial broiler strains, each with distinct genetic characteristics that influence their growth rates, feed efficiency, and resilience to environmental stressors (Dozier et al., 2006). Comparative studies on the effects of TM and heat stress on these strains are limited, highlighting the need for further research to optimize thermal manipulation protocols and improve broiler resilience to heat stress. The aim of this study was to compare the responses of Ross and Arian strains to embryonic thermal manipulation and post-hatch heat stress by evaluating performance metrics, blood parameters, T3 hormone levels, and HSP70 gene expression.

Materials and methods

Experimental design and Animal management

For this research, 200 fertilized eggs from each of the Ross 308 and Arian strains were used (400 eggs in total). The eggs were divided into two incubation treatments: a control group incubated under normal conditions (37.8 °C; 56% RH), and an experimental group subjected to thermal manipulation (39.5 °C; 65% RH). Each group was placed in identical incubators, and half of the eggs from each strain were assigned to each condition. Thermal manipulation was applied from embryonic day (ED) 7 to 16, for 12 hours each day. On ED 7, infertile and undeveloped eggs were identified and removed by candling.

After hatching, chicks were assigned to a completely randomized design consisting of four predefined treatment groups based on the combination of strain (Arian or Ross 308), embryonic thermal manipulation (TM), and post-hatch heat stress (HS). Each treatment group included four replicates, with 10 chicks per replicate (pen dimension 2.0×1.0×0.8 m). The treatment groups were as follows: Arian strain subjected to embryonic thermal manipulation but not to post-hatch heat stress (ATC); Arian strain without embryonic manipulation but subjected to post-hatch heat stress (ACH); Ross 308 strain subjected to embryonic thermal

manipulation but not to post-hatch heat stress (RTC); and Ross 308 strain without embryonic manipulation but subjected to post-hatch heat stress (RCH). Chicks in each group were reared under either normal temperature (25 °C) or heat stress conditions (32 °C; RH 60-65%) from day 21 onward for 12 hours daily.

A graphical abstract summarizing the experimental design, including incubation conditions, treatment groups, and rearing conditions, is provided to enhance clarity and reader understanding (Figure 1).

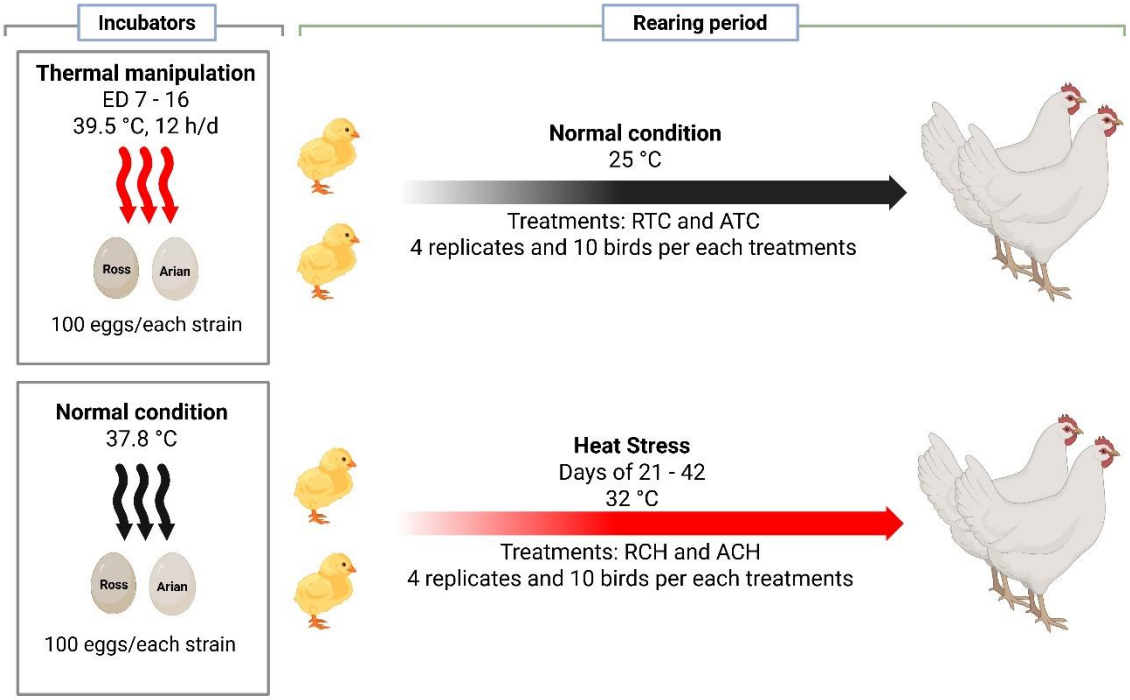

Figure 1. A graphical abstract summarizing the experimental design

The experimental procedures and animal care protocols were reviewed and approved by the Ethics Committee of Sari Agriculture and Natural Resources University (SANRU), Sari, Iran, under the approval number IR.SANRU.REC.03.1397.13, in accordance with national regulations for the care and use of laboratory animals.

Other than the intended differences in rearing temperatures, other environmental conditions (including humidity, lighting, ventilation, and management) were kept consistent across all treatment groups. Chicks were reared under uniform conditions with free access to feed and water. The management practices followed the standard protocols to minimize any external variables.

Hematological and biochemical analysis

At 42 days of age, four birds per replicate were chosen for blood sampling. Hemoglobin concentration was measured spectrophotometrically at 540 nm and reported in grams per deciliter. For hematocrit measurement, capillary tubes containing blood were centrifuged for 3 minutes at 3,000 rpm, and the hematocrit percentage was calculated based on three replicates per bird. Red blood cell (RBC) count was performed after diluting blood at a ratio of 1:200 with a formalin–citrate solution, while the white blood cell (WBC) count was conducted using a 1:20 dilution. Results were expressed as cells per microliter, equivalent to $\times 10^6$/mm³ for RBCs and $\times 10^3$/mm³ for WBCs. Glucose, total protein, albumin, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were measured photometrically using an auto-analyzer and commercial kits (Pars Azmoon Co., Tehran, Iran). Triiodothyronine (T3) levels were determined using a commercial ELISA kit (Pars Azmoon Co., Tehran, Iran). The intra- and inter-assay coefficients of variation for T3 were <3.8% and <8.8% respectively, with an analytical sensitivity for assay of <0.1 ng/ml.

RNA extraction and gene expression analysis

The RNA was extracted from four blood samples per replicate. Total RNA was extracted from 30 mg of frozen tissue using RNX Plus Solution (Sinacolon Co.) and resuspended in RNase-free water. The RNA integrity and quantities were assessed by spectrophotometric A260/A280 ratio and confirmed by 1.5% denaturing agarose gel electrophoresis. Reverse transcription was

performed using 1 mmol/L oligo(dT) primers and 4 U Omniscript RTase, following the instructions provided for the cDNA synthesis kit (Yektatajhiz Co., Iran).

Quantitative real-time PCR was performed on a Corbett 3000 instrument using SYBR Green qPCR Master Mix (Yektatajhiz Co.) and avian-specific primers targeting HSP70 mRNA (Table 1). The thermal cycling program consisted of an initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds. All samples were analyzed in duplicate. The expression of HSP70 was normalized to β-Actin as the housekeeping gene, which was confirmed to be stable under our laboratory conditions. Relative mRNA levels were calculated using the $2^{-}\Delta\Delta Ct$ method, with correction for amplification efficiency (Livak and Schmittgen, 2001).

Table 1. Primers sequences of target and house-keeping genes

Primers		Sequence (5´-3´)	Accession No.	Size (bp)
Beta-Actin	F	CACAGATCATGTTTGAGACCTT	NM_205518	101
	R	CATCACAATACCAGTGGTACG		
HSP70	F	AGCGTAACACCACCATTCC	AY288298	372
	R	TGGCTCCCACCCTATCTC		

HSP70: Heat Shock Protein 70

Statistical analysis

The experimental design comprised four predefined treatment groups (ATC, ACH, RTC, and RCH), each representing a specific combination of strain, incubation condition, and rearing environment. Data were first tested for normality test. When normality assumptions were met, data were analyzed using the General Linear Model (GLM) procedure (SAS, 2003), with treatment as the fixed factor. Differences among treatment means were assessed using the Duncan's multiple range test, and results are presented as means ± SEM. A P-value of < 0.05 was considered statistically significant.

Results

Blood parameters and thyroid hormones

The results related to RBC, WBC, hematocrit, hemoglobin, T3, glucose, triglycerides, cholesterol, total protein, albumin, LDL, HDL, and VLDL are presented in Table 2. RBC counts were significantly lower in the ATC group compared to other treatments. The RCH group exhibited the lowest WBC, which was significantly lower than that of the RTC group, whereas both Arian groups (ATC and ACH) showed elevated WBC levels. Hematocrit percentage also varied significantly among groups. Plasma triiodothyronine (T3) concentration in ATC was significantly higher than in other treatments. Triglyceride levels were significantly elevated in ATC compared to RTC. Conversely, cholesterol levels in RCH were higher than in RTC and ATC. The LDL levels were significantly lower in RCH compared to other groups, while VLDL was lowest in RTC. No significant differences were observed among treatments for hemoglobin, glucose, total protein, albumin, or HDL.

Table 2. The effect of embryonic thermal manipulation and post-hatch heat stress on blood parameters in Arian and Ross 308 broilers

Parameters	ATC	ACH	RTC	RCH	P-value
RBC (10^6/mm^3)	1.57±0.11^{b}	1.94±0.15^{a}	2.07±0.17^{a}	1.94±0.07^{a}	0.046
WBC (10^3/mm^3)	36630±1.33^{a}	36910±2.84^{a}	27550±1.14^{b}	21950±1.44^{c}	<0.0001
Hematocrit (%)	33.10±0.33^{b}	27.86±0.81^{d}	34.84±0.38^{a}	30.93±0.46^{c}	<0.0001
Hemoglobin (g/dL)	9.35±0.32	9.11±0.34	9.51±0.35	9.41±0.21	0.825
T3 (ng/dL)	8.85±0.32^{a}	6.64±0.18^{b}	6.55±0.35^{b}	5.70±0.13^{b}	0.0172
Glucose (mg/dL)	222.88±4.72	223.50±2.84	207.75±1.10	213.75±1.12	0.4629
Triglyceride (mg/dL)	64.63±2.98^{a}	50.75±2.73^{ab}	37.75±0.84^{b}	53.13±3.64^{ab}	0.1395
Cholesterol (mg/dL)	135.500±3.31^{b}	140.000±2.59^{ab}	127.750±1.37^{b}	157.750±2.21^{a}	0.0339
Total protein (mg/dL)	3.72±0.12	3.20±0.06	3.31±0.04	3.48±0.02	0.3128
Albumin (mg/dL)	3.32±0.13	3.01±0.03	3.13±0.06	2.93±0.03	0.6403
HDL (mg/dL)	85.60±1.27	90.93±2.22	90.75±1.31	87.45±2.14	0.8506
LDL (mg/dL)	36.97±2.35^{b}	38.91±1.64^{b}	29.45±1.78^{b}	59.67±2.88^{a}	0.0134
VLDL (mg/dL)	12.92±0.59^{a}	10.15±0.54^{ab}	7.55±0.16^{b}	10.62±0.73^{ab}	0.1359

$^{a–c}$ Within rows, means with common superscript (s) are not different (P>0.05)

ATC: Arian strain, with embryonic heat stress and without heat stress during rearing; ACH: Arian strain, without embryonic heat stress and with heat stress during rearing; RTC: Ross 308 strain, with embryonic heat stress and without heat stress during rearing; RCH: Ross 308 strain, without embryonic heat stress and with heat stress during rearing. RBC: Red blood cell, WBC: White blood cell, T3: Triiodothyronine, High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL).

Growth performance

The feed conversion ratio (FCR) over the entire experimental period was significantly influenced by the treatments (Table 3). The RCH group demonstrated the most favorable FCR, which was significantly lower than that of the ACH, ATC, and RTC groups. The RTC group also had a significantly better FCR than the two Arian groups (ACH and ATC).

Carcass characteristics

The effects of heat stress during rearing and incubation on live weight, carcass yield, pectoral muscle, drum+thigh, liver, and spleen are presented in Table 4. No significant differences were observed among treatments for live weight, carcass yield, pectoral

muscle, or liver weight. However, drum+thigh weight was significantly lower in RTC and RCH compared to ACH. Spleen weight was significantly higher in the ATC group than in other treatments.

Table 3. The effect of embryonic thermal manipulation and post-hatch heat stress on body weight, feed intake, and feed conversion ratio in Arian and Ross 308 broilers

Parameters	ATC	ACH	RTC	RCH	P-value
BW1(g)	983.83±17.15^{b}	1099.20±32.56^{a}	975.88±13.22^{b}	1100.88±39.75^{a}	0.008
BW2 (g)	1363.90±44.91^{a}	1324.80±27.09^{a}	1139.25±38.29^{b}	974.43±24.63^{c}	<0.0001
BW3 (g)	2347.73±49.52^{a}	2424.00±71.69^{a}	2115.13±40.50^{b}	2057.30±31.51^{b}	<0.0001
FI1 (g)	1770.73±28.32^{b}	1966.98±51.88^{a}	1751.58±25.87^{b}	1938.23±76.34^{a}	0.018
FI2 (g)	3055.60±105.53^{a}	2954.63±65.52^{a}	2449.10±80.24^{b}	2065.45±48.69^{c}	<0.0001
FI3 (g)	4826.35±109.58^{a}	4912.60±68.84^{a}	4200.65±96.16^{b}	4003.68±66.98^{b}	<0.0001
FCR1	1.80±0.01	1.79±0.01	1.80±0.02	1.76±0.1	0.047
FCR2	2.24±0.01^{a}	2.23±0.00^{a}	2.15±0.01^{b}	2.12±0.02^{c}	<0.0001
FCR3	2.05±0.01^{a}	2.03±0.01^{a}	1.98±0.01^{b}	1.93±0.01^{c}	<0.0001

$^{a–c}$ Within rows, means with common superscript (s) are not different (P>0.05)

ATC: Arian strain, with embryonic heat stress and without heat stress during rearing; ACH: Arian strain, without embryonic heat stress and with heat stress during rearing; RTC: Ross 308 strain, with embryonic heat stress and without heat stress during rearing; RCH: Ross 308 strain, without embryonic heat stress and with heat stress during rearing. BW1: body weight 1-21 days old; BW2: body weight 22-42 days old; BW3: body weight 1-42 days old; FI1: feed intake 1-21 days old; FI2: feed intake 22-42 days old; FI3: feed intake 1-42 days old; FCR1: feed conversion ratio 1-21 days old; FCR2: feed conversion ratio 22-42 days old; FCR3: feed conversion ratio 1-42 days old.

Table 4. The effect of embryonic thermal manipulation and post-hatch heat stress on carcass characteristics and relative organ weight in Arian and Ross 308 broilers

Parameters	ATC	ACH	RTC	RCH	P-value
Live weight (g)	2007.50±159.84	1857.50±36.29	2025.26±125.30	1900.00±82.38	0.655
Carcass yield (%)	65.27±1.21	65.60±0.40	70.82±3.61	71.47±2.76	0.141
Pectoral muscle yield (%)	33.70±1.33	33.26±0.83	36.69±1.74	35.67±1.87	0.325
Drum+thigh yield (%)	39.93±0.43^{ab}	32.29±1.18^{a}	28.98±1.04^{b}	28.39±1.09^{b}	0.044
Liver weight (%)	3.68±0.40	3.20±0.10	3.16±0.18	3.65±0.50	0.567
Spleen weight (%)	0.17±0.02^{a}	0.11±0.01^{b}	0.13±0.02^{b}	0.13±0.01^{b}	0.038

$^{a–c}$ Within rows, means with common superscript (s) are not different (P>0.05)

ATC: Arian strain, with embryonic heat stress and without heat stress during rearing; ACH: Arian strain, without embryonic heat stress and with heat stress during rearing; RTC: Ross 308 strain, with embryonic heat stress and without heat stress during rearing; RCH: Ross 308 strain, without embryonic heat stress and with heat stress during rearing.

Expression of the HSP70 gene

The expression of the HSP70 gene in blood was significantly affected by the experimental conditions (Figure 2). The highest level of HSP70 mRNA was observed in the RCH group, which was not subjected to embryonic TM but was exposed to post-hatch heat stress. This expression was significantly elevated compared to other groups. The groups that underwent embryonic TM (ATC and RTC) showed lower HSP70 expression.

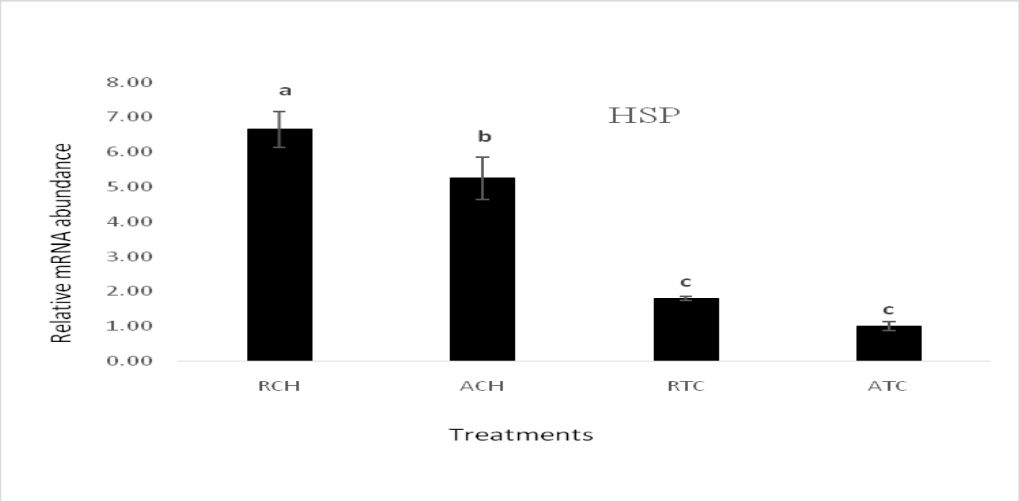

Figure 2. Relative expression of HSP70 mRNA in the blood of Araian and Ross 308 broilers under embryonic and post-hatch heat stress conditions

$^{a-c}$ Means with common superscript are not different (P>0.05). ATC: Arian strain, with embryonic heat stress and without heat stress during rearing; ACH: Arian strain, without embryonic heat stress and with heat stress during rearing; RTC: Ross 308 strain, with embryonic heat stress and without heat stress during rearing; RCH: Ross 308 strain, without embryonic heat stress and with heat stress during rearing.

Discussion

Under heat stress conditions, lipid metabolism changes in broilers. Previous studies showed that heat stress often increased plasma cholesterol concentrations in broilers. In our study, the highest cholesterol levels were observed in the RCH and ACH groups, suggestive of stress response in these treatments. In contrast, the ATC and RTC groups showed reduced cholesterol levels, which may reflect an adaptive mechanism that prioritizes energy conservation for thermoregulation over lipid synthesis. These findings are consistent with previous reports indicating that cholesterol reduction may be part of the physiological adaptation to heat stress (Zaboli et al., 2017; Iraqi et al., 2024; Ismail et al., 2016).

The LDL levels typically increase under heat stress, contributing to elevated total cholesterol and disrupted lipid transport. Rahnama et al. (2020) also reported elevated LDL concentrations in broilers exposed to thermal stress. In contrast, our results showed that thermotolerance—particularly in the ATC and RTC groups—was associated with significantly reduced LDL levels. Similarly, Iraqi et al. (2024), and Al Amaz and Mishra (2024) found that embryonic thermal manipulation reduced the LDL levels, suggesting a long-term metabolic adaptation induced during embryogenesis. The decrease in cholesterol and LDL levels following embryonic thermal manipulation may be attributed to long-term changes in lipid metabolism during embryogenesis. While this remains speculative, previous studies showed that thermal manipulation during incubation could influence endocrine signaling, particularly the hypothalamic-pituitary-thyroid (HPT) axis, which regulates the metabolic rate and lipid mobilization (Piestun et al., 2011). Altered thyroidal sensitivity in TM-treated birds may shift energy usage from lipid storage toward thermoregulation and growth. However, further research, including gene expression or enzyme activity related to lipid metabolism, is needed to confirm this hypothesis.

The highest red blood cell (RBC) count was observed in the RTC treatment. In poultry, heat stress can lead to a reduction in RBC count due to a combination of physiological, cellular, and molecular factors. Oxidative stress induced by high temperatures, damages cellular structures, including RBC membranes (Xu et al., 2018). Thermal manipulation during incubation may condition the chicks to maintain immune cell populations more effectively under post-hatch heat stress. his is supported by our data, where the lowest WBC count was found in the Ross group exposed to rearing heat stress without embryonic thermal manipulation (RCH), indicating potential immune suppression (Al-Rukibat et al., 2017). The significantly higher T3 level in the ATC group compared to RTC, ACH, and RCH suggests that ETM enhances thyroid activity in the Arian strain. TM applied during the critical period of embryonic development (e.g., days 7–16) has been shown to improve thermotolerance and metabolic efficiency in the post-hatch period by modulating endocrine responses, including thyroid hormone production (Yahav et al., 2004; Tona et al., 2008). The elevated T3 levels observed in TM-treated groups are consistent with previous findings (Piestun et al., 2008; Piestun et al., 2011), and suggest that the Arian strain may be more responsive to thermal conditioning at the thyroid level.

The improved FCR in the RCH group, despite lower body weight, was primarily driven by a proportionally greater reduction in feed intake, suggesting enhanced metabolic efficiency under heat stress. This suggests that RCH birds had a lower metabolic rate during the growth period, with energy more efficiently directed toward production. Among the TM treatments, RTC had a significantly lower FCR than ATC. Overall, our findings support the view that reduced thyroidal activity and lower T3 concentrations-observed in heat-stressed groups like RCH-are linked to reduced metabolic rates. This is consistent with previous studies reporting that high ambient temperatures suppressed the thyroid function in chickens, leading to decreased plasma T3 levels (Al-Rukibat et al., 2017; Onagbesan et al., 2023).

The expression of HSP70 serves as a critical biomarker for cellular stress responses, particularly under heat stress conditions. In this study, the highest HSP70 expression was observed in the RCH group, which was significantly elevated compared to other treatments. This finding aligns with previous research showing that acute heat stress during rearing triggered strong upregulation of HSP70 as a protective mechanism to prevent protein denaturation and maintain cellular homeostasis (Wang et al., 2013).

In contrast, the RTC and ATC groups exhibited lower HSP70 levels, suggesting that thermal manipulation during incubation induced a protective “stress memory.” This early conditioning has been shown to enhance thermotolerance and reduce the severity of stress responses later in life, thereby decreasing the need for extensive HSP70 activation (Loyau et al., 2013). These results are consistent with studies indicating that thermal manipulation during incubation induced epigenetic modifications, leading to improved cellular resilience through regulated heat shock protein expression (Zhang et al., 2012). Loyau et al. (2013) found that embryonic thermal conditioning enhances thermotolerance and reduces the need for HSP70 upregulation during later heat stress, which is consistent with our findings of lower HSP70 expression in the ATC and RTC groups. Similarly, Piestun et al. (2008) reported that broilers exposed to thermal manipulation during embryogenesis exhibited reduced HSP70 expression post-hatch.

The drum+thigh weight percentage was significantly higher in the Arian strain (ATC and ACH) compared to the Ross 308 strain (RTC and RCH), likely due to genetic characteristics that favor greater muscle development in legs. Spleen weight was also significantly higher in the ATC group compared to the other treatments. Spleen plays a critical role in stress and immune responses, and its size can serve as an indicator of immunological

activity in poultry (Quinteiro-Filho et al., 2010). The increased spleen weight in ATC suggests that embryonic heat exposure may have stimulated a stronger immune response in the Arian strain. In contrast, the lower spleen weights observed in ACH and RCH may reflect immune suppression resulting from rearing heat stress. This is in agreement with findings by Lara and Rostagno (2013), who reported that chronic heat stress suppresses immune function and reduces the size of lymphoid organs such as the spleen.

Conclusion

This study demonstrated that embryonic thermal manipulation (TM) may be an effective strategy to enhance thermotolerance by improving thyroid activity and immune response, while concurrently reducing the stress biomarkers, including LDL cholesterol and HSP70 expression. These adaptive benefits were more strongly exhibited in the Arian strain. In contrast, the Ross 308 strain demonstrated superior innate feed efficiency under post-hatch heat stress without requiring embryonic conditioning. Consequently, the efficacy of TM is highly strain-dependent, indicating that genetic background should be a primary consideration when implementing thermal conditioning protocols to optimize broiler resilience.

Conflict of interest

The author declares no conflicts of interest.

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