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Effects of embryo thermal manipulation on thermotolerance of broiler chicks between 28-40 days of rearing

Winnie Luiza dos Santos Clímaco[1], **Italo Conrado Sousa Araújo**[1]*, **Kamilla Ribas Soares**[1], **Edgard Onoda Luiz Caldas**[1], **Fernanda Lima de Souza Castro**[1], **Henrique Carneiro Lobato**[1], **Mariana Cristina Vieira**[1], **Leonardo José Camargos Lara**[1]

[1] Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Zootecnia, Belo Horizonte, MG, Brasil.

***Corresponding author:**
italloconradovet@hotmail.com

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ABSTRACT - This study examined the impact of a 37.5 °C temperature on chick embryos during the hatching period (18-21 days of incubation) and its subsequent effect on hatchery results and the thermotolerance of broiler chicks from 28-40 days of rearing. For hatchery results, the study involved 16 trays per treatment. For performance, the study involved 360 broiler chicks from two distinct hatching temperature conditions: a control group (36.5 °C and 65% relative humidity (RH)) and a thermal manipulation group (37.5 °C/6 h/d from 18 to 21 days of incubation with 65% RH). The chicks were reared in a thermoneutral temperature until day 28. Subsequently, on day 28 post-hatch, the chicks were randomly assigned to a 2 × 2 arrangement to assess the differences in thermotolerance acquisition. The primary factors were embryo thermal manipulation in the hatcher and the thermal environmental rearing from day 28 until day 40 (thermoneutrality (at a constant 23.0 °C) or thermal environmental challenge (30.0 °C/6 h/d)). No effect was observed on hatchability, residual analysis of unhatched eggs, and chick quality. No interaction was observed between treatments for performance or cloacal temperature from day 28 to day 40. However, birds reared in a thermoneutral environment exhibited higher feed intake and body weight gain from day 28 to day 40 and a lower feed conversion ratio than the birds reared in a cyclic heat temperature environment. An increase of 1 °C/6 h/d in the hatcher from day 18-21 does not affect hatchery results or enhance the adaptability of broilers to a heat stress environment during the final rearing period

Keywords: broiler production, cloacal temperature, hatcher temperature, heat stress

1. Introduction

Heat stress negatively impacts poultry meat production, particularly in tropical countries. When exposed to environmental conditions beyond the thermoneutrality zone, birds undergo physiological changes and decrease their feed intake to minimize the metabolic heat produced (Cardoso et al., 2022; Tang, 2022; Khan et al., 2023). Recent studies have explored embryonic thermal manipulation as a potential strategy to enhance broilers' thermotolerance in their rearing environment (Costa et al., 2020).

Thermotolerance results from epigenetic changes, where the environment modifies the expression of specific genes (Tzschentke and Halle, 2009; Goel, 2021). However, Costa et al. (2020) suggested that the application protocols for embryonic thermal manipulation (TM), such as temperature, embryo age, and exposure time, remain unclear, leaving a significant knowledge gap. Furthermore, few studies have examined TM applied after the 18th day of incubation, a stage when the thermoregulatory system is more developed (Amjadian and Shahir, 2020).

This study examined the impact of embryonic TM, performed from the 18th to the 21st day of incubation, on the hatchery results, heat tolerance, and performance of broiler chickens. Additionally, we hypothesized that TM during embryonic development could potentially enhance the heat resilience and overall performance of broilers subjected to cyclical heat stress conditions, characterized by exposure to 30 °C temperatures for six hours daily during the rearing period from 28 to 40 days of age.

2. Material and Methods

The current experimental protocol was approved by the Ethical Principles in Animal Experimentation Committee under protocol n 253/2011. The chicks were incubated in São José da Varginha, MG, Brazil (19°42'25.3'' S and 44°33'47.1'' W), while their performance was evaluated in Belo Horizonte, MG, Brazil (19°55' S and 43°56'' W).

A total of 9,600 fertile Cobb500 eggs, sourced from a 48-week-old breeder flock, were used for incubation. The eggs were incubated in a commercial multistage setter, model CASP CMG 125E (Amparo, São Paulo, Brazil), which has a capacity of 124,416 eggs. The incubation conditions were maintained at 37.5 °C and 56% relative humidity (RH) from the start of incubation (0DE) through to the 17th day of incubation (17DE). The eggs were turned 90° every hour until the 17th day. The setter was fully loaded with eggs, including those not involved in the experiment.

After the 18th day of incubation, 500 fertile eggs with developing embryos were randomly selected. These eggs were divided into two different hatchers (CASP 19HR model; capacity of 20,736 eggs). The control group eggs, following the company's guidelines, were subjected to conditions of 36.5 °C and 65% RH from DE18 to DE21 (250 eggs). The TM eggs were subjected to conditions of 37.5 °C and 65% RH from DE18 to DE21 (250 eggs). This TM was determined based on a literature review (Costa et al., 2020).

The hatchers were filled with baskets containing eggs that were not part of the experiment. At 508 h of incubation, all chicks were counted in each basket to calculate hatchability in relation to fertile eggs (%). For the hatchability, newly hatched chick weight and residual analysis of unhatched eggs, the experiment consisted of two treatments with 16 repetitions each, with the 96-egg tray considered as one repetition. Residual analysis was performed according to Araújo et al. (2016), and chicks were weighted at hatch.

For performance evaluation, 360 broiler chicks (90 from each treatment) were randomly selected to determine thermotolerance by assessing performance and cloacal temperature. The chicks were weighed and allocated across 24 metallic rearing cages, each measuring 1 m^2. Each cage, housing six repetitions of 15 birds, was equipped with 50 cm of feeders and a single nipple drinker. For the initial 28 days of rearing, a computer-controlled environmental room was utilized to maintain a thermoneutral temperature, as recommended by Cobb (2013). On the 28th day post-housing, the chicks were randomly assigned to a 2 × 2 arrangement to assess the variance in thermotolerance acquisition among them. The primary factors considered were the thermal manipulation of the embryo in the hatcher or the thermal environmental rearing from days 28 to 40 by employing either thermoneutrality (at a constant 23.0 °C) or a thermal environmental challenge (TEC; 30.0 °C/6 h/day). The lighting program implemented throughout the rearing phase consisted of 18 h of light and 6 h of dark daily.

Performance was assessed from day 28 to 40, considering total feed intake, body weight gain, feed conversion ratio (FCR), and viability (%) at day 40. Cloacal temperature (°C) was measured in two broiler chicks per cage from day 31 to 39 using a digital thermometer and was determined during a thermal challenge from day 31 to 39 by inserting a digital thermometer into the birds' cloaca until the reading stabilized. This analysis was conducted 4 h post heat treatment.

The mash feed was formulated using corn and soybean meal, adhering to the nutritional values determined by Rostagno et al. (2011). The nutritional levels for the starter and finisher phases were set according to the levels proposed by Cobb (2013), with the feed in mash form. Both feed and water were available for *ad libitum* intake throughout all rearing periods across all groups. The starter diet (3,000 kcal metabolizable energy [ME]/kg, 22.5% crude protein [CP], and 1.21% digestible lysine [dLys]) was provided from day 1 to 21, while the finisher diet (3,146 kcal ME/kg, 19.9% CP, and 1.00% dLys) was given from days 22 to 40. The animals were consistently fed *ad libitum* from days 1 to 40.

2.1. Statistical analysis

Broiler cloacal temperature and hatchery results data were analyzed using the following fixed effect model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

in which Y is the response variable, μ is the overall mean, T_i is the fixed effect of treatment (i = control; TM), and e is the residual error. Performance data were analyzed using the following fixed effect model:

$$Y_{ij} = \mu + T^1 i + T^2 j + Y_{ij} + \varepsilon_{ijk},$$

in which Y_{ij} is the quantitative response variable; μ is the overall mean; $T^1 i$ = control, TM; $T^2 j$ = thermoneutrality or thermal challenge; Y is the effect of factors i and j, and ε is the random error.

Means were assessed for normality and homoscedasticity using the Lilliefors and Bartlett tests. Upon confirming the normality and homogeneity of all data, the performance results from 28 to 40 days and the cloacal temperature of the chickens were subjected to an analysis of variance. The averages were then compared using Tukey's test (P<0.05), and the SAGE program was utilized for data analysis.

3. Results

There was no effect of embryo TM on fertility, hatchability of fertile eggs, residual analysis, and chick weight at hatch (P>0.05) (Table 1). No interaction was observed between TM and TEC in terms of broiler performance at 40 days (P>0.05) or cloacal temperature (P>0.05). Similarly, TM had no effect (P>0.05) on performance of broiler chicks from 28 to 40 days. However, broiler chicks raised in a thermoneutral environment exhibited higher feed intake and body weight gain (P<0.05) than chicks in the thermally challenged group. The environmental rearing temperature did not affect viability or feed conversion ratio (Table 2). Throughout the entire evaluation period (31-39 days), the type of TM did not influence cloacal temperature (P>0.05). Nevertheless, the group reared under thermal stress consistently exhibited higher cloacal temperatures than the thermoneutral group on all evaluated days (P<0.05) (Figure 1).

Table 1 - Effect of thermal manipulation (TM) on incubation results

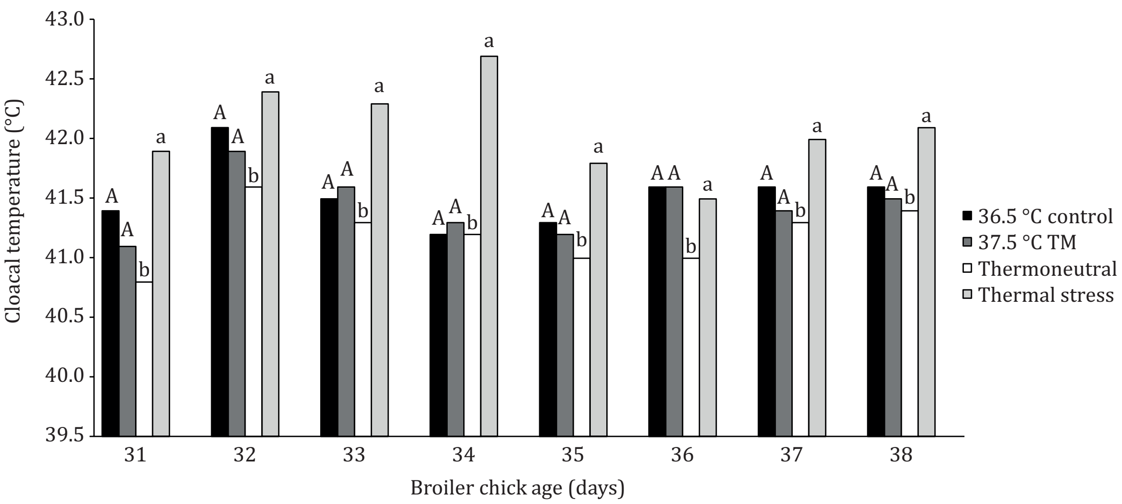
Treatment	Hatch of fertile eggs (%)	Fertility (%)	MI (%)	MII (%)	MIII (%)	CONT (%)	Chick weight (g)
36.5 °C	92.48	96.61	2.43	0.40	0.53	0.40	48.66
37.5 °C	92.05	96.61	2.35	0.20	0.53	0.60	49.49
P-value	0.903	0.955	0.890	0.900	0.988	0.910	0.840
SEM	1.38	0.90	0.45	0.12	0.20	0.50	0.91

MI - mortality from 0 to 4 days of embryo development; MII - mortality from 5 to 10 days of embryo development; MIII - mortality from 11 to 21 days of embryo development; CONT - contaminated eggs; SEM - standard error of the mean.

Table 2 - Effect of thermal manipulation (TM) and thermal environmental challenge (TEC) on broiler chick performance (28-40 days)

Treatment	Feed intake (g)	Body weight gain (g)	Feed conversion ratio	Viability (%)
Control (36.5 °C)	2,527.7	2,744.51	2.16	96.6
TM (37.5 °C/6 h/d)	2,555.8	2,705.27	2.18	100.0
TEC[1]				
Non-challenged	2,630.4a	2,763.5a	2.17	96.6
Challenged	2,453.0b	2,686.2b	2.18	100.0
P-value				
TM	0.789	0.455	0.802	0.901
TEC	0.034	0.011	0.890	0.903
TM × TEC	0.560	0.691	0.450	0.901
SEM	134.2	90.0	0.03	0.77

SEM - standard error of the mean.
[1] Non-challenged: reared in thermoneutrality (23 °C); challenged: reared under cyclic heat stress with 30 °C/6 h/d).
Means followed by different letters differed by Tukey's test (P<0.05).



Thermoneutral: constant 23 °C; thermal stress: 30 °C /6 h/d.

Figure 1 - Effects of thermal manipulation (TM) during hatcher period (18-21 days) and heat cyclic stress on broiler cloacal temperature from 31 to 38 days of age.

4. Discussion

No ideal embryonic TM protocol exists to enhance broiler chick performance (Costa et al., 2020). Nevertheless, our findings contribute to the formulation of such a protocol. We found that exposing broilers to 37.5 °C for 6 h per day from 18 to 21 DE does not induce thermotolerance when these broilers are later subjected to cyclic heat from 28 to 40 days of age.

Our treatment did not influence the incubation results and chick weight at hatch. Given the implications outlined, genetic selection programs have led to significant muscle mass gain in broiler chickens, without corresponding development of their cardiovascular and respiratory systems, crucial for efficient thermoregulation. Meat-type chickens cope with high temperatures by reducing feed intake and growth, but acute heat exposure can lead to increased morbidity and mortality (Yahav et al., 2004; Loyau et al., 2015). Recent research has focused on early TM to stimulate lasting thermotolerance

(Al Amaz et al., 2024), but our findings suggest it did not significantly influence incubation outcomes or confer thermotolerance to broiler chickens. This underscores the complexity of physiological mechanisms involved and highlights the ongoing need for comprehensive approaches in poultry production.

The performance impairment of broilers reared outside of thermoneutrality can be attributed to a decrease in feed intake, which in turn reduces the metabolic heat produced (Cardoso et al., 2022). Additionally, a diminished utilization of nutrients from the diets, an upsurge in the expression of genes associated with protein catabolism and cell apoptosis, compromised immunity and weight reduction, yield and carcass quality reduction, and an increase in mortality were observed (Tang, 2022; Brugaletta et al., 2023; Park et al., 2022). However, an attempt to enhance the chickens' thermotolerance by increasing the incubation temperature in the hatcher by 1 °C for 6 h per day did not yield positive results, leading to a further decrease in performance.

The results presented indicate that broilers struggled to maintain their body temperature within the comfort zone in heat stress environments (Cardoso et al., 2022). This outcome was unexpected, given the anticipation of some form of heat adaptation resulting from the TM conducted during incubation (Tzschentke and Halle, 2009). These findings contradict those of Saleh et al. (2020), who employed a different TM protocol (39.0 °C/18 h/d from 10-18 DE). They subjected the birds to cyclical stress challenges of 35 °C from days 28 to 35 and observed thermotolerance in the TM group. Consequently, the ability of thermally manipulated animals to lower their metabolic rate and thereby better manage their behavior under cyclic stress conditions was demonstrated.

5. Conclusions

Embryonic thermal manipulation in the hatcher (18-21 DE), at 65% RH, and 1 °C above the standard (36.5 °C), does not present a feasible solution for enhancing the thermotolerance of broiler chicks.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Clímaco, W. L. S. and Lara, L. J. C. **Data curation:** Clímaco, W. L. S.; Soares, K. R. and Caldas, E. O. L. **Formal analysis:** Clímaco, W. L. S.; Caldas, E. O. L. and Castro, F. L. S. **Funding acquisition:** Lara, L. J. C. **Investigation:** Clímaco, W. L. S.; Soares, K. R.; Caldas, E. O. L. and Castro, F. L. S. **Project administration:** Lara, L. J. C. **Supervision:** Lara, L. J. C. **Validation:** Araújo, I. C. S. **Visualization:** Araújo, I. C. S. and Castro, F. L. S. **Writing – original draft:** Clímaco, W. L. S. and Lara, L. J. C. **Writing – review & editing:** Araújo, I. C. S.; Lobato, H. C. and Vieira, M. C.

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References

Al Amaz, S.; Shahid, M. A. H.; Chaudhary, A.; Jha, R. and Mishra, B. 2024. Embryonic thermal manipulation reduces hatch time, increases hatchability, thermotolerance, and liver metabolism in broiler embryos. Poultry Science 103:103527. https://doi.org/10.1016/j.psj.2024.103527

Amjadian, T. and Shahir, M. H. 2020. Effects of repeated thermal manipulation of broiler embryos on hatchability, chick quality, and post-hatch performance. International Journal of Biometeorology 64:2177-2183. https://doi.org/10.1007/s00484-020-02012-w

Araújo, I. C. S.; Leandro, N. S. M.; Mesquita, M. A.; Café, M. B.; Mello, H. H. C. and Gonzales, E. 2016. Effect of incubator type and broiler breeder age on hatchability and chick quality. Brazilian Journal of Poultry Science 18:17-25. https://doi.org/10.1590/1806-9061-2015-0146

Brugaletta, G.; Laghi, L.; Zampiga, M.; Oliveri, C.; Indio, V.; Piscitelli, R.; Pignata, S.; Petracci, M.; De Cesare, A. and Sirri, F. 2023. Metabolic and microbiota response to arginine supplementation and cyclic heat stress in broiler chickens. Frontiers in Physiology 14:1155324. https://doi.org/10.3389/fphys.2023.1155324

Cardoso, D. M.; Cardeal, P. C.; Soares, K. R.; Sousa, L. S.; Castro, F. L. S.; Araújo, I. C. S. and Lara, L. J. C. 2022. Feed form and nutritional level for rearing growing broilers in thermoneutral or heat stress environments. Journal of Thermal Biology 103:103159. https://doi.org/10.1016/j.jtherbio.2021.103159

Cobb broiler management guide. 2013. Cobb-Vantress Inc., Siloam Springs, AR. 68p.

Costa, B. T. A.; Lopes, T. S. B.; Mesquita, M. A.; Lara, L. J. C. and Araújo, I. C. S. 2020. Thermal manipulations of birds during embryogenesis. World's Poultry Science Journal 76:843-851. https://doi.org/10.1080/00439339.2020.1823302

Goel, A. 2021. Heat stress management in poultry. Journal of Animal Physiology and Animal Nutrition 105:1136-1145. https://doi.org/10.1111/jpn.13496

Khan, R. U.; Naz, S.; Ullah, H.; Ullah, Q.; Laudadio, V.; Qudratullah; Bozzo, G. and Tufarelli, V. 2023. Physiological dynamics in broiler chickens under heat stress and possible mitigation strategies. Animal Biotechnology 34:438-447. https://doi.org/10.1080/10495398.2021.1972005

Loyau, T.; Bedrani, L.; Berri, C.; Métayer-Coustard, S.; Praud, C.; Coustham, V.; Mignon-Grasteau, S.; Duclos, M. J.; Tesseraud, S.; Rideau, N.; Hennequet-Antier, C.; Everaert, N.; Yahav, S. and Collin, A. 2015. Cyclic variations in incubation conditions induce adaptive responses to later heat exposure in chickens: a review. Animal 9:76-85. https://doi.org/10.1017/S1751731114001931

Park, J. S.; Kang, D. R. and Shim, K. S. 2022. Proteomic changes in broiler liver by body weight differences under chronic heat stress. Poultry Science 101:101794. https://doi.org/10.1016/j.psj.2022.101794

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG.

Saleh, K. M. M.; Tarkhan, A. H. and Al-Zghoul, M. B. 2020. Embryonic thermal manipulation affects the antioxidant response to post-hatch thermal exposure in broiler chickens. Animals 10:126. https://doi.org/10.3390/ani10010126

Tang, L. P.; Liu, Y. L.; Zhang, J. X.; Ding, K. N.; Lu, M. H. and He, Y. M. 2022. Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poultry Science 101:102085. https://doi.org/10.1016/j.psj.2022.102085

Tzschentke, B. and Halle, I. 2009. Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. British Poultry Science 50:634-640. https://doi.org/10.1080/00071660903186570

Yahav, S.; Rath, R. S. and Shinder, D. 2004. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. Journal of Thermal Biology 29:245-250. https://doi.org/10.1016/j.jtherbio.2004.03.002