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ORIGINAL ARTICLE

The Effect of *In Ovo* Serotonin Pretreatment on Hatchability of Heat Stressed Broiler Eggs

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Abstract

Thermal stress is a major challenge in poultry production, particularly affecting embryonic development and post-hatch performance. Although thermal manipulation during embryogenesis has been recognized as an effective strategy for improving thermo-tolerance and *in ovo*, injection of bioactive compounds has shown success in modulating physiological responses. Additionally, research has indicated that serotonin plays an important role in embryonic development through epigenetic mechanisms. This study investigated the combined effects of thermal manipulation and *in ovo* serotonin injection on hatchability in Ross chicken eggs. In this study, 60 fertile eggs were divided into three groups (control, 10 µg and 20 µg serotonin), each containing 20 eggs. Each group was further subdivided into a normal temperature (37.5 °C) and heat stress (39.5 °C) group. Findings showed that *in ovo* administration of 10 and 20 µg serotonin did not have an enhancing effect on hatchability under normal temperature nor stress condition. These findings showed that serotonin injection has not positive effect on hatchability in poultry embryos, suggesting that appropriate serotonin doses may enhance this effect by modifying stress response pathways.

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Introduction

The poultry industry plays a crucial role in the global agricultural sector, serving as a significant source of animal protein. In recent decades, poultry production has experienced substantial growth, with increases in both chicken meat and egg production (1). Compared to other livestock species, poultry has experienced the most significant increase in production (2). However, the environment of industrial poultry production is often characterized by various stressors, including external factors such as temperature, light, social conditions, and human-animal interactions, as well as internal factors such as pathogens and toxins. Poultry responds to these challenging conditions with behavioral and physiological adaptations to maintain internal homeostasis. Therefore, an animal's success in adapting to its environment depends on the intensity of stressors and its physiological capacity to respond appropriately. Coordinating and managing the neuroendocrine and autonomic stress systems are essential for restoring and maintaining homeostasis (3). Both the nervous and endocrine systems are critical for maintaining internal balance or homeostasis in the body. In this context, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis regulate physiological responses to stress in both mammals and birds (4). The ANS releases epinephrine (adrenaline) from the adrenal glands, thereby activating metabolic resources and coordinating the fight-or-flight response. Meanwhile, the HPA axis produces glucocorticoids, including corticosterone (CORT) in birds, which are involved in the regulation of long-term stress (5).

Incubation conditions significantly impact embryonic development, hatchability, chick quality, and post-hatch performance, with hatchability and chick quality being crucial factors determining hatchery success (6, 7). Several studies have identified temperature manipulation as one of the most effective strategies for improving these factors, showing that controlling the temperature during incubation can enhance hatchability and chick quality by improving the embryo's physiological and biological functions. Temperature is considered the most critical incubation condition as it significantly affects the biological functions and behaviors of birds (6, 8, 9). Research has demonstrated that controlling temperature during incubation influences embryonic organ development, hatching parameters, and chick quality (6, 10, 11). Optimizing temperature conditions during incubation is critical, as the optimal range and duration of exposure depend on the specific poultry breed. By adjusting temperature, hatcheries can produce healthier, more robust chicks that exhibit higher growth rates, improved thermal tolerance, stronger immune systems, and lower mortality rates (6, 11, 12).

Thermal manipulation (TM) during incubation has been shown to significantly improve birds' metabolism, enhance post-hatch thermoregulation, and reduce the negative effects of high ambient temperatures, while also benefiting their development and productivity (13, 14). TM involving elevated incubation temperatures, such as 38.5–39.5 °C for 6–8 hours per day during embryonic days (EDs) 10–18, has been well studied and demonstrated to improve thermo-tolerance in post-hatch chicks and chickens of broiler strains (15, 16).

In addition to TM, the use of bioactive compounds such as serotonin (5-HT) has garnered attention as a potential strategy for improving hatching outcomes. 5-HT is a secondary metabolite of tryptophan (TRY), and in addition to being a potent antioxidant, it acts as a central neurotransmitter that influences neuroendocrine function and physiological status while regulating aggressive and anxious behaviors in animals (17). Dysregulation of the serotonergic system can lead to various neurodevelopmental disorders, including depression, anxiety, and disorders of energy metabolism (18). Studies have demonstrated a significant negative correlation between the level of 5-HT in the peripheral blood and brain and the frequency of aggressive behavior in laying hens (19, 20). Exogenous supplementation of TRY could alleviate anxiety and aggressive behavior in laying hens, including feather pecking and cloacal pecking (21). Peripheral 5-HT has also been reported as a positive welfare indicator (22). Furthermore, TRY supplementation significantly increased hypothalamic 5-HT content and decreased plasma norepinephrine (NE) concentrations, accompanied by a reduction in aggressive behavior (23). It has been reported that higher concentrations of CORT and abnormal secretion of NE in laying hens exacerbated feather pecking (24). The NE concentration was closely associated with serotonin-based regulation of anxiety and aggressive behavior. These hormones, secreted by the hypothalamic-pituitary-adrenal (HPA) axis, can significantly influence the behavior of adult laying hens. (25).

One promising approach to mitigating heat stress in poultry is the manipulation of embryonic development through *in ovo* techniques. The *in ovo* injection of 5-HT has been proposed as a means to enhance embryonic development and improve hatching outcomes in chicks. 5-HT plays a significant role in regulating various physiological processes, including mood, behavior, and embryonic development (26). Additionally, 5-HT possesses antioxidant properties that may help reduce oxidative stress during incubation, thereby further enhancing embryo development and improving resistance to environmental stressors. Studies suggest that the administration of 5-HT during incubation could alter gene expression and enhance the resilience of embryos to thermal stress (27). The objective of this study is to investigate the synergistic effects of *in ovo* 5-HT injection and TM on the hatching outcomes of chicken embryos. The findings from these studies could provide valuable insights into practical strategies for optimizing poultry management under challenging environmental conditions.

Materials and Methods

Experimental Design and Sample Preparation

A total of 60 fertilized eggs (Ross 308; weighing 55 ± 2 g) were obtained from the Mahan Chicken Meat Production Complex, Kerman, Iran, and placed in an incubator (Cocks Machine Co., Ltd., Iran). The incubator automatically rotated the eggs at a 45-degree angle every two hours throughout the incubation period and maintained a constant temperature of 37.5 °C with a relative humidity of 55–65%. Temperature and humidity were continuously monitored using digital sensors with an accuracy of ±0.1 °C and ±1% RH, respectively, in accordance with the Ross 308 breeder guidelines. The eggs were divided into three groups, each consisting of 20 eggs: the control group received 50 µl of normal saline (0.9% NaCl) (Darou Pakhsh, Pharmaceutical Mfg. Co., Tehran, Iran) per egg; the second group was administered 10 µg of 5-HT (Sigma-Aldrich) in 50 µl of saline per egg; and the third group received 20 µg of 5-HT in 50 µl of saline per egg. Injection of either 5-HT or saline solution into the egg albumen was performed before incubation (28, 29). After disinfection, a small hole was made at the larger end of each egg, and either sterile saline or 5-HT solution was injected to a depth of 25 mm using a 1-ml disposable syringe with a 25-gauge needle. Following injection, the holes were immediately sealed with Scotch tape, and the eggs were returned to the incubator. Unfertilized eggs were identified by candling and discarded on the seventh day of incubation (30).

On day 13 of incubation, each group was subjected to either heat stress or normal temperature conditions. The heat stress groups were initially exposed to 39.5 °C for 2 hours, with the duration of heat stress increasing by 2 hours daily until day 17, reaching a total of 10 hours of heat stress on day 17. These days were selected based on the establishment of the pituitary-adrenal axis and the initiation of the hypothalamic-pituitary-thyroid axis (31). Twenty eggs from each group (10 maintained at normal temperature and 10 under heat stress) were kept in the incubator until the end of the incubation period. Hatchability was defined as the percentage of fertilized eggs that successfully hatched. Eggs were checked daily for hatching, and the number of hatched chicks was recorded.

These parameters were selected to provide a comprehensive assessment of the impact of the experimental treatments on embryonic development and hatching success.

Statistical Analysis

Statistical analyses were conducted using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). Differences in hatchability percentages among groups were analyzed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

Results

Effects of the *in ovo* injection of 5-HT (10 and 20 µg per egg) under normal and heat stress conditions on hatchability are shown in Figure 1. The experimental treatments did not significantly influence hatchability.

The control group showed hatchability rates of 90% under normal temperature (NN) and 70% under heat stress conditions (SN). The 10 µg 5-HT group demonstrated higher hatchability rates (80%) in the normal temperature situation (NSr10) compared to the stress condition (50%) (SSr10).

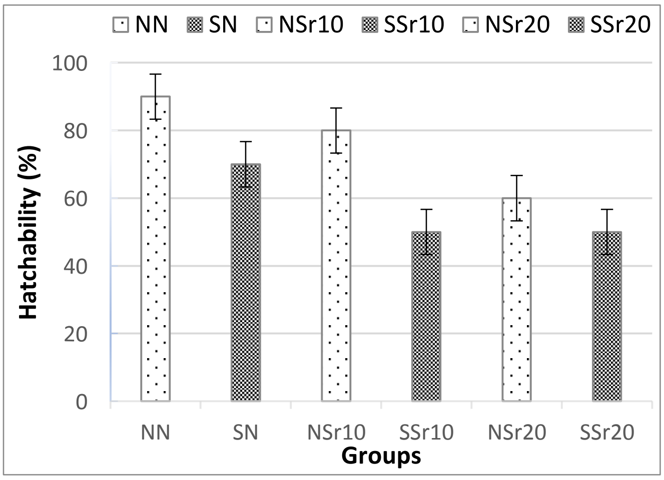

Figure 1. Hatchability (%) (Mean ±SEM) in different groups. (NN: Normal temprature+Normal Saline, SN: Heat Stress+Normal Saline, NSr10: Normal Tempraturel+Serotonin 10 µg/egg, SSr10: Heat Stress+Serotonin 10 µg/egg, NSr20: Normal Tempraturel+Serotonin 20 µg/egg, SSr20: Heat Stress+Serotonin 20 µg/egg)

The 20 µg 5-HT group exhibited the lowest hatchability rates, with 60% under normal temperature (NSr20) and 50% under heat stress (SSr20), suggesting a potential dose-dependent effect on embryonic development and survival.

Discussion

Findings of the present study indicate that the 10 µg *in ovo* injection of 5-HT improved hatchability compared to the 20 µg 5-HT in normal temperature conditions. In heat stress conditions, hatchability decreased compared to normal temperature conditions, and 5-HT did not have a positive effect on improving the hatching percentage.

Heat stress is a major challenge in poultry production, triggering complex physiological responses at both the cellular and molecular levels. It has been shown that heat stress induces oxidative stress and reduces antioxidant status at the molecular level (32). TM appears to be most effective during the critical period of hypothalamic-pituitary-thyroid or adrenal axis development, or both (16). Therefore, the timing of TM must be aligned with the onset of the hypothalamic-pituitary-thyroid axis to alter the thermotolerance threshold capacity (33). Studies have reported that TM birds exhibit enhanced thermotolerance (34), improved hatchability, growth performance, muscle development, immunocompetence, and overall welfare (35).

It has been shown that TM during incubation, particularly between days 12 and 18, can significantly improve hatchability, post-hatch growth, hatching parameters, and post-hatch thermo-tolerance. Early-life programming through TM can also reduce various physiological and biological challenges (36). Positive effects of this process include improvements in embryonic development indicators, such as embryonic weight, yolk sac utilization, and cardiovascular system development (37). These findings are consistent with previous studies indicating that TM can improve hatch parameters by optimizing embryonic development under various temperature conditions (37). The beneficial effects of TM depend on factors such as timing, temperature level, and treatment duration, all of which influence embryonic outcomes (37).

Moreover, it was found that TM positively influenced hatching parameters, including hatch time and the number of pipped eggs, reducing hatch time by 3.3% and decreasing the number of pipped eggs by 46.7% (36). Additionally, TM during embryogenesis induces various favorable characteristics in hatching, particularly in the hatching process, which is attributed to its positive effects on embryonic physiological performance (6). Similarly, it was reported that temperature manipulation between 16 and 18 days 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 reduced metabolic rate(33, 38). TM during egg incubation is considered a mild heat shock exposure during embryogenesis, which improves tissue stability, oxidative stress response, and immunological adaptation to heat stress (8). These findings suggest that TM during embryogenesis may reduce the intensity of hepatic energy metabolism, thereby decreasing heat production (39).

A novel aspect of our research is the integration of serotonergic modulation with TM. We hypothesize that these two interventions may act synergistically to improve heat tolerance and hatching success. 5-HT plays crucial roles during embryogenesis, acting as a neurogenic, trophic, and morphogenetic factor. 5-HT is present in fertilized eggs before incubation, and 5-HT receptors are expressed during the early stages of development (40). The observed increase in 5-HT levels and its metabolites from EDs 12 to 20 indicates that the serotonergic system progressively develops throughout embryogenesis (41). The presence of 5-HT in fertilized eggs and its receptors during early developmental stages highlights its essential role in shaping various physiological processes critical for embryonic development (40). Moreover, another study suggested that 5-HT levels, particularly in response to TM, may influence the hypothalamic-pituitary-thyroid axis, which is vital for regulating heat tolerance in poultry (33). This metabolic pattern may facilitate the optimal accumulation of 5-HT in hypothalamic regions, likely strengthening stress adaptation mechanisms. Additionally, thyroid hormones T3 and T4, which may be affected by temperature changes during incubation, play a crucial role in the hatching process (42). These hormones influence embryonic metabolism, the final maturation of tissues, and physiological integrity during hatching (43). The combination of TM and serotonergic modulation appears to influence the hypothalamic-pituitary-thyroid axis, likely enhancing long-term heat tolerance (33). 5-HT, by modulating stress response pathways, may improve heat tolerance and lead to better developmental outcomes in poultry, particularly under challenging thermal conditions. However, the results also indicate that 5-HT's effects are dose-dependent, with higher doses (20 µg) leading to negative outcomes, such as reduced hatchability. This finding emphasizes the importance of optimizing 5-HT dosage to achieve the desired effects on embryonic development and hatchability.

The epigenetic implications of our findings are particularly intriguing. It was observed that a high dose of 5-HT (15 µg) leads to a reduction in the methylation status of the hypothalamic serotonin receptor (5-HTR1A) (44). This change results in decreased production of mRNA for this gene. In other words, this dose of 5-HT reduces the activity of this receptor, which in turn affects the growth and behavior of birds. These epigenetic changes may serve as a mechanism through which embryonic exposure to 5-HT influences post-hatch behavior and development (28,44). This epigenetic modification may represent a novel mechanism through which 5-HT and TM influence developmental outcomes in poultry.

Other investigations have shown that TM significantly impacts chick quality, particularly in terms of weight, length, activity, appearance, navel area, membrane remnants, and yolk residual. This method led to a 7.1% increase in chick weight, a 17.4% increase in chick length, as well as a 10% improvement in chick activity (36). Similarly, it was reported that chick weight and length in groups subjected to TM during embryogenesis were significantly higher compared to control groups (11, 35). Additionally, TM can affect hormonal control centers such as the hypothalamus, which in turn influences some growth hormones and chick weight (45).

Chick weight at hatch can serve as a criterion for evaluating chick quality. Various TM protocols have been shown to significantly increase chick body weight (46, 47). The increase in embryonic weight during incubation, influenced by thermal stimulation during embryogenesis, is reflected in the increased weight at hatch (6).

Conclusion

The findings of the present study suggest that 5-HT injection does not enhance hatchability in poultry embryos, especially under heat stress conditions.

To gain new insights into the interaction between TM and serotonergic regulation during embryonic development in poultry, various doses of 5-HT should be examined to evaluate their effects on chicken embryonic development, survival, and hatching rates. Future studies should focus on elucidating the specific epigenetic pathways involved and refining the optimal combination of TM and 5-HT administration to maximize benefits in poultry production.

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Author Contributions

Hamed Khasti: Formal analysis, investigation, writing the original draft. **Ladan Emadi:** Conceptualization, formal analysis, investigation, methodology, supervision, validation, visualization, review & editing. **Hadi Tavakoli:** Conceptualization, formal analysis, investigation, methodology, supervision,

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

All procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals by the National Academy of Sciences (National Institutes of Health Publication No. 86-23) and after receiving institutional approval from the animal handling committee of the University of Kerman.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Consent for Publication

Not applicable.

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