Exogenous insulin could alter stress response during slaughter in broiler chicken

By:

Arfanul Saif

Roll No: 17/109

Reg No: 01885

Intern ID: 80

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Exogenous insulin could alter stress response during slaughter in broiler chicken

Dr. Md Ridoan Pasha
Assistant Professor
Department of Physiology Biochemistry and Pharmacology

Faculty of Veterinary Medicine
Chattogram Veterinary and Animal Sciences University
Khulshi, Chattogram – 4225, Bangladesh

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Statement of Author

I, Arfanul Saif, certify unequivocally that I have performed all the tasks detailed in this report. The data was gathered from books, national and international periodicals, and other sources. All citations have been properly acknowledged. Consequently, I am solely responsible for collecting, manipulating, preserving, and publishing all data compiled in this report.

The Author

List of Abbreviations and Symbols Used

Abbreviation and Symbols	Elaboration
%	Percentage
H:L ratio	Heterophil : Lymphocyte ratio
HPA Axis,	Hypothalamic-Pituitary-Adrenal Axis
IU	International unit
ACTH	Adrenocorticotropic hormone
No, N	Number
Hb	Hemoglobin
T1	Treatment group 1
T2	Treatment group 2
ng	Nanogram
mg	Milligram
e.g.,	Example
etc.	Et cetera
et. al	And his associate
CVASU	Chattogram Veterinary and Animal Sciences University

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Abstract

The process of slaughtering animals is known to induce significant stress, which subsequently triggers a wide array of physiological and metabolic alterations. Amongst avian species, this stress often leads to changes in the secretion of various hormones, particularly insulin and cortisol. This study aimed to investigate the role of insulin by administering synthetic insulin during the slaughter of broiler chicken. Fifteen broiler chickens were randomly divided into three groups, comprising a control group receiving subcutaneous saline (s/c saline) and two treatment groups, with one receiving 4 IU of insulin subcutaneously (T1) and the other receiving 8 IU of insulin subcutaneously (T2). The research protocol involved collecting blood samples both prior to and subsequent to the slaughtering process. These blood samples were then analyzed to measure the levels of serum glucose and cortisol, as well as to assess hemoglobin concentration and determine the Heterophil:Lymphocyte (H:L) ratio. The administration of insulin, 30 minutes before the slaughtering process, leads to a significant decrease in serum glucose levels in the treatment groups. In contrast, the hemoglobin levels remained relatively stable, showing no significant changes following insulin administration. Equally significant was the observation that the H:L ratio, which indicates stress in avian species and serum cortisol, exhibited marked reductions after insulin administration. These results strongly suggest that insulin may have a role in reducing stress levels in broiler chickens during the slaughter process. These findings point could contribute to enhanced animal welfare practices in the live bird market.

Keywords: Broiler, insulin, cortisol, stress, H:L ratio

Chapter 1. Introduction

Stress is defined as an animal's behavioral, physiological, and emotional state in response to a situation that it believes hampers the normal function of its body or mind (Désiré *et al*., 2004; Terlouw, 2005). According to Veissier and Boissy (2007), the ideas of well-being and stress are like opposites. This means that if an animal is stressed, it cannot be in a state of well-being, and vice versa.

The slaughtering process is a complex one that frequently involves several possible sources of stress. Stress can arise from physical factors like long and rough transportation, lack of food, improper environmental conditions (such as temperature, air quality, or water quality), aggression, or physical shocks, leading to fatigue, hunger, discomfort from temperature, respiratory distress and pain. Additionally, stress can be triggered by psychological factors, such as disruptions in social interactions or the presence of fear (Terlouw *et al*., 2008).

It is important to assess stress in broilers during slaughter because stress doesn't just lead to decreased meat production and quality. However, it also affects animal welfare, which, in turn, can significantly impact consumer acceptance of poultry products (Scanes, 2016).

The Hypothalamic-Pituitary-Adrenal (HPA) axis involves the release of neuropeptides from the hypothalamus, the secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland, and the synthesis of corticosterone by the adrenal glands. (Orbell *et al.*, 2013).

Insulin has notable effects on the Hypothalamic-Pituitary-Adrenal (HPA) Axis, both centrally and peripherally, independent of its role in regulating blood sugar levels. The hypothalamic-pituitary-adrenal (HPA) axis is responsible for responding to imminent or perceived threats and stressors through a response that involves the release of glucocorticoids. Glucocorticoids are also crucial in that they regulate energy balance and glucose homeostasis. Comparing the peak pituitary-adrenal responses to different HPA activators, such as hyperinsulinemia, restraint, and hypoglycemia, reveals that diabetic animals with HPA axis defects do not respond effectively to stress challenges, with their peak stress response remaining significantly lower than that of normal animals (Chan *et al*., 2005). Most research on the central interactions between insulin

and the HPA axis has centered on their roles in maintaining energy balance. Generally, it is understood that excessive glucocorticoids can create an unfavorable environment, leading and insulin resistance (Baskin *et al*., 1999).

Although there have been limited studies examining the effect of insulin on pituitary-adrenal function in human subjects, research by Fruehwald-Schultes *et al*. (1999) demonstrated that intravenous infusion of high insulin doses into human subjects under euglycemic conditions increases the concentrations of plasma ACTH and cortisol. Conversely, lower insulin doses do not significantly impact pituitary-adrenal activity. These studies suggest that insulin's stimulatory effect on the HPA axis appears to be most effective when insulin levels are elevated.

Notably, cortisol, a stress hormone, is released during the slaughter of animals, including birds (Ismail *et al.,* 2019). It's important to highlight that insulin has an opposing effect on cortisol secretion, as reported by Varlamov *et al.* in 2021.

However, many comprehensive researches have been conducted, but there is a significant gap in our understanding of the role of insulin in modulating stress responses in broiler chickens. This study is therefore crucial as it seeks to bridge this knowledge gap by investigating the impact of exogenous insulin administration on the stress responses of broiler chickens. By doing so, we hope to contribute to the development of more humane slaughtering practices and improve the overall quality of poultry products. This could have significant implications for the poultry industry, animal welfare, and consumer satisfaction.

This study aims to explore if exogenous insulin administration could affect stress response in broiler chickens.

The objectives were-

1. To assess the effect of insulin on stress by hematobiochemical parameters.
2. To evaluate the effect of different doses of insulin on stress responses of chickens.

Chapter 2. Materials and Methods

2.1 Study area and animals

A total of fifteen broiler chickens were used for this study. The apparently healthy broiler birds were collected from the live bird market, having not more than a 10% difference in body weight after checking their general health condition. The birds were kept in a cage in the Physiology laboratory of CVASU before slaughter. A one-hour resting time was allowed before the slaughter.

2.2 Collection of samples

The blood sample was collected by proper restraining and via venipuncture of the jugular vein in an aseptic condition between 8 AM and 10 AM. The broiler chickens were separated into three groups: the control group, T1 group and T2 group, each comprising five broiler chickens. The control group received 0.9% NaCl solution subcutaneously. Insulin (Ansulin 30/70, Square Pharmaceuticals Ltd. Bangladesh) was given to two treatment groups of birds subcutaneously, where 4 IU insulin was administered to the T1 group and 8 IU insulin was administered to the T2 group. Thirty minutes after the insulin administration, slaughter was performed by halal slaughter method and blood was collected in a vacutainer without anticoagulant and with EDTA for hemoglobin estimation with a unique identification number. The vacutainers without anticoagulant were kept in a rack for 2 hours for smooth coagulation of blood. After that the vacutainers containing clotted blood were centrifuged and serum was collected. The serum samples were kept in aliquots in an eppendorf tube and marked accordingly. A direct blood smear was also made to evaluate the heterophil: lymphocyte ratio.

2.3 Storage of samples:

The serum samples were stored at -20 °C until analysis.

2.4 Evaluation of biochemical and hormone level

The level of glucose was analyzed by a commercial kit (Glucose-GOD PAP) using a biochemical analyzer (Humalyzer-3000, Germany). The hemoglobin level was checked by Sahli hemoglobinometer, H:L ratio from the direct smear of blood, and cortisol was analyzed using a commercially available cortisol ELISA kit using an ELISA reader (Thermofisher Scientific, USA).

2.5 Data analysis

The data obtained in the study was stored in MS Excel 2007. Data were then sorted, cleaned, and exported to STATA-11. Then the level of glucose, hemoglobin, and cortisol was expressed as mean, standard error and compared against insulin-administered (T1 and T2 group) and non-administered (Control) groups.

Chapter 3. Result

3.1. Effect of preslaughter insulin on blood cortisol level

Cortisol level was increased in the T1 group (4 IU) compared to the control group, whereas the T2 group (8 IU), while still showing higher cortisol levels than the control group, had lower cortisol levels than the T1 group (Figure 1). These finding suggest that the effect of insulin on cortisol levels is dose-dependent. While higher insulin dosage still elevates cortisol levels, the increase is less pronounced compared to the T1 group. The results indicate that insulin administration is associated with increased cortisol levels in broilers, and the magnitude of this increase appears to be influenced by the insulin dosage, with 4 IU leading to a more significant increase than 8 IU. These results may have implications for understanding the stress response and potential physiological effects of insulin treatment in broilers.

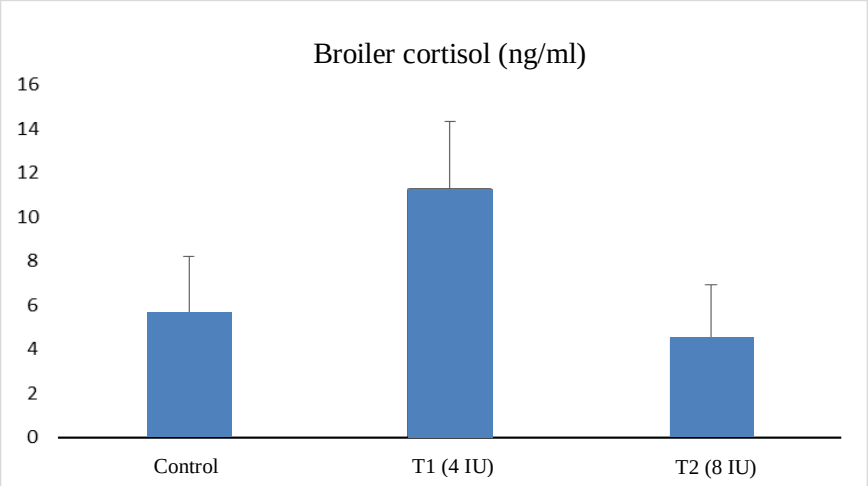

Figure 1: Cortisol level in broiler chicken after insulin administration

3.2. Effect of insulin on H:L ratio

H:L ratio decreased after insulin administration at both dose levels (Figure 2). The H/L (Heterophil-to-Lymphocyte) ratio serves as a pivotal biomarker for assessing stress levels in broiler chickens. The control group exhibited the highest H/L ratio, indicating higher stress levels in broiler chickens. In contrast, T2 group (8 IU) showed a

substantial reduction in stress levels. The T1 group (4 IU) demonstrated a slightly higher H/L ratio compared to the T2 group, though it remained significantly lower than the control group.

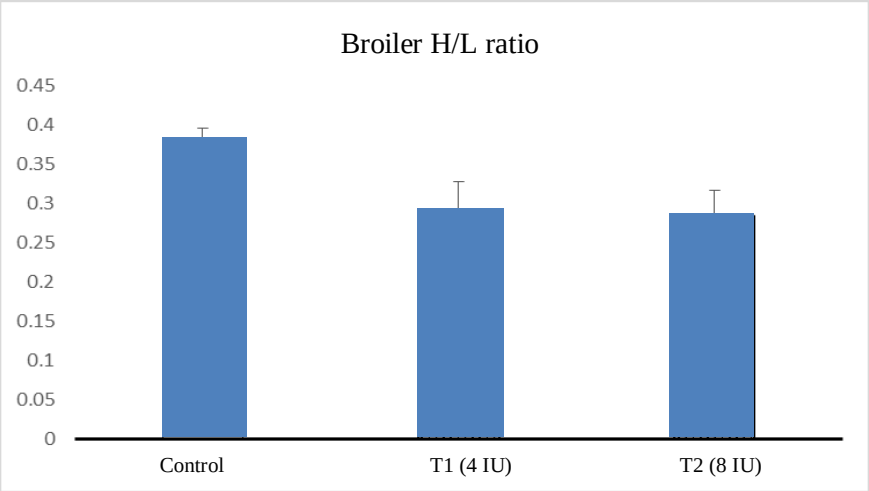

Figure 2: H:L ratio in broiler chicken after insulin administration

3.3. Effect of insulin on Glucose level

Glucose levels decreased both T1 (4 IU) and T2 group (8 IU) after insulin administration (Figure 3). The results of this study demonstrate a clear dose-dependent effect of insulin treatment on broiler glucose levels. Notably, the administration of 4 IU of insulin led to a significant reduction in blood glucose, indicating the effectiveness of this dosage in improving glucose regulation. However, the effect was even more pronounced with an 8 IU insulin treatment, which resulted in the lowest glucose levels.

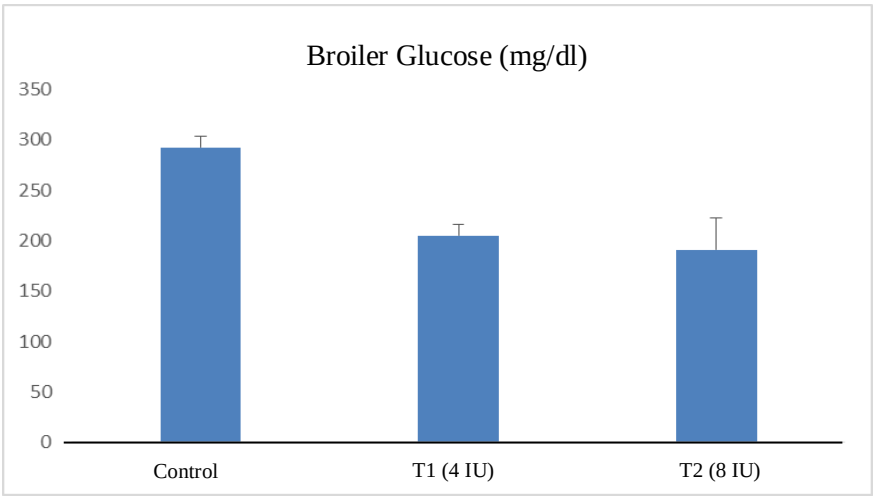

Figure 3*:* Glucose level in broiler chicken after insulin administration

3.4. Effect of insulin on Hemoglobin level

After insulin administration, T1 group (4 IU) showed a slightly lower Hemoglobin (Hb) level compared to the control group (Figure 4). In contrast, T2 group (8 IU) exhibited a notably higher Hb level. The findings suggest that insulin treatment in broiler chickens, particularly at a higher dose of 8 IU, had a significant and positive effect on Hb levels. This outcome may have important implications for broiler management, as elevated Hb levels could potentially be associated with improved health and growth performance.

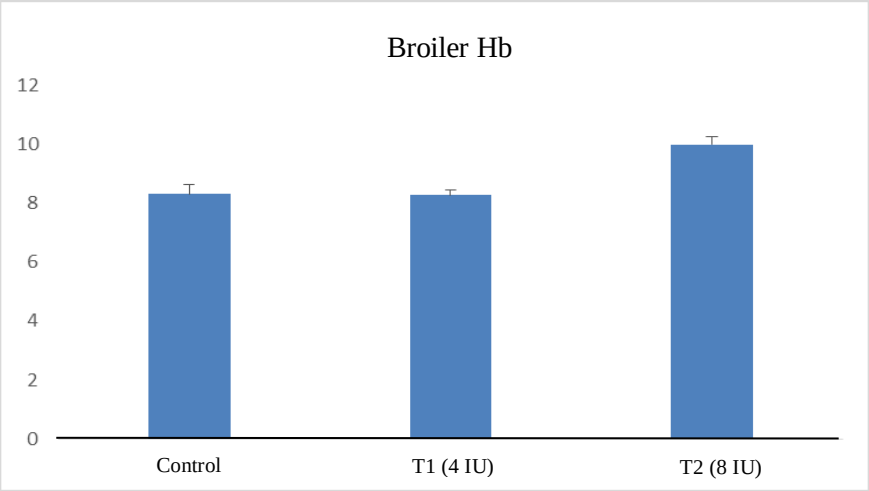

Figure 4: Hb level in broiler chicken after insulin administration

Chapter 4. Discussion

This study revealed that there has been a good interplay between insulin and cortisol in broiler chickens. The study observed significant changes in cortisol levels, heterophil-to-lymphocyte (H:L) ratios, glucose levels, and hemoglobin levels following insulin administration. However, this effect of decreasing stress by insulin administration at the studied dose level (4IU/8IU) may not be successful.

Cortisol is the main adrenal glucocorticoid in poultry, but it is produced very minimally (Kalliecharan and Hall, 1974). enhance the release of corticotropin-releasing hormone and adrenocorticotropic hormone (ACTH), which raise plasma concentrations of cortisol. Effectively managing stress and cortisol levels is vital for upholding the health and productivity of broiler chickens. Recent research has presented emerging evidence suggesting that insulin, primarily renowned for regulating glucose levels, might potentially reduce stress and cortisol levels in broiler chickens (Sohail *et al*., 2010).

While insulin is predominantly acknowledged for its role in maintaining glucose balance and regulating carbohydrate and lipid metabolism, recent studies indicate that it could also influence the stress response in diverse animal models. Notably, insulin appears to interact with cortisol through multiple pathways, implying a potential regulatory role. Administering insulin has been shown to reduce cortisol production by influencing the activity of key enzymes involved in cortisol synthesis and metabolism. Furthermore, insulin may indirectly impact cortisol levels by enhancing glucose uptake in tissues, thereby preventing an excess of glucose that could otherwise stimulate cortisol secretion (Vijayan *et al*., 1997). Experiments conducted in rodents have demonstrated that insulin administration can temper the stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, the controller of cortisol production. By modulating the HPA axis response, insulin may assist in curbing the release of cortisol in response to stressors (Janssen, 2022).

The results of this study revealed that insulin administration had a significant impact on cortisol levels in broiler chickens. Specifically, the administration of 4 IU of insulin

led to a substantial increase in cortisol levels compared to the control group, while the 8 IU insulin group exhibited lower cortisol levels than the control group. This suggests a dose-dependent relationship between insulin dosage and cortisol levels.

It is widely acknowledged that stress causes noticeable changes in the H:L ratio in birds (Davis *et al.*, 2008). This parameter has been growing interest as a stress indicator for poultry (e.g., Bedanova *et al.*, 2006; Prieto and Campo, 2010; Al-Aqil *et al*., 2013; Najafi *et al.*, 2015). The elevation in the Heterophil-to-Lymphocyte (H:L) ratio due to stress can be partially attributed to the impact of stress on increasing cortisol levels. In our study, we observed a decrease in the Heterophil-to-Lymphocyte (H:L) ratio following the administration of insulin at both dosage levels. The higher insulin dose resulted in a more significant reduction in the H:L ratio.

Insulin controls blood glucose levels in mammals and, through the hypothalamus, is a major regulator of appetite. In contrast, chicken exhibits unusual glucose homeostasis, exhibiting comparatively higher blood glucose levels, decreased glucose sensitivity of pancreatic beta cells, and significant resistance to exogenous insulin (Proszkowiec-Weglarz *et al.*, 2017). Stress triggers the release of hormones like cortisol and epinephrine (adrenaline), which can elevate blood glucose levels. This is part of the "fight or flight" response, where the body releases energy stores to deal with a perceived threat. Elevated stress hormone levels can counteract the glucose-lowering effects of insulin. Our study findings are consistent with the role of insulin in regulating blood glucose levels, even in chickens with unusual glucose homeostasis characteristics. It indicates that insulin treatment can effectively lower blood glucose in a dose-dependent manner, with higher doses resulting in more significant reductions in blood glucose levels.

There is evidence that stressors can affect another hematological parameter by triggering blood hemoglobin concentrations to either increase or fall. On the one hand, pre-slaughter stressors like shackling lead to drops in hemoglobin concentrations in the bloodstream and an increase in cortisol (Bedanova *et al.*, 2006). Findings from our study suggest that insulin treatment in broiler chickens, particularly at a higher dose of 8 IU, had a significant and positive effect on hemoglobin levels.

A comprehensive understanding of stress responses during the slaughter process is crucial, and its significance extends beyond ethical considerations. The way chickens respond both behaviorally and physiologically to slaughter conditions can have a direct impact on both pre-and post-mortem muscle metabolism, which, in turn, significantly affects the quality of chicken meat. (Terlouw *et al*., 2008).

To enhance animal welfare during slaughter process, there is a crucial need for advancement on various fronts. Gaining deeper insights into animal emotions, understanding reactivity profiles in stress responses, and investigating the molecular-level consequences of stress can all contribute to our understanding of animal welfare in the context of slaughter.

Chapter 5. Limitations

The primary limitation of this study is the relatively small sample size, with only 15 broiler chickens divided into three groups. A larger sample size would provide more statistically robust results and a better representation of the population. The study examined two specific doses of insulin (4 IU and 8 IU) but did not explore a broader range of insulin doses. Future research with a larger sample size, a more comprehensive spectrum of insulin doses, and more controlled environmental conditions is needed to generalize these findings.

Chapter 6. Conclusion

The findings of this study shed light on the complex interplay between insulin and cortisol in broiler chickens, particularly concerning their response to the stress of slaughter. Notably, the results indicated that insulin administration, both at 4 IU and 8 IU dosages, had discernible effects on various physiological parameters, including glucose, cortisol, hemoglobin, and Heterophil: Lymphocyte (H:L) ratio.

While the primary objective of this study was not to directly assess stress during the slaughter process, the implications are significant. The results suggest that insulin administration has the potential to modulate the physiological stress response in broiler chickens, albeit in a dose-dependent manner. The impact on cortisol levels indicates that insulin, a hormone traditionally associated with glucose regulation, may play an essential role in stress management in poultry.

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Biography of Author

This is Arfanul Saif, son of Minhaz Uddin Mahmud and Ferdous Akter, doing his graduation on Doctor of Veterinary Medicine (DVM) at Chattogram Veterinary and Animal Sciences University under the Faculty of Veterinary Medicine. He passed the Secondary School Certificate Examination (SSC) in 2014 from Collegiate School, Chattogram, and got a GPA 5.00 and then the Higher Secondary Certificate Examination (HSC) in 2016 from Govt. Hazi Muhammad Mohsin College, Chattogram, and earned a GPA 5.00 out of 5.00. Presently, he is engaged in a yearlong internship. Arfanul is deeply passionate about his field of study and is dedicated to developing foundational skills from day one, striving to acquire practical knowledge to meet the demands of the modern scientific era.