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Effect of different levels of Adrenocorticotropic hormone (ACTH) on physiological performance of broiler under heat stress conditions

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Abstract

This experiment was holdup in A-Faris poultry farms from 1st March to 11 of Aprile 2019. (ACTH) hormone infusion was tested in this experiment on acid-base regulation in broiler chickens. For 7 days, osmotic pumps dispensed 8 IU of ACTH in saline/kg of BW/d, or the same volume of saline as in ACTH at 1 l/h. On days 0 and 14, after the beginning of the infusions, blood samples were obtained to establish a baseline. The plasma concentrations of Na+, K+, and Cl- were decreased, whereas the partial pressure of CO2, anion gap, corticosterone, mean corpuscular hemoglobin concentration, and blood concentrations of hemoglobin and HCO - were all elevated due to the ACTH administration. When given ACTH, neither blood pH nor plasma Ca2+ levels changed. High levels of glucose, cholesterol, high-density lipoprotein, and triglyceride were also seen in the plasma after the ACTH infusion. All blood components examined were similar to those seen in healthy controls. As shown by the data, plasma acid-base status and other blood metabolic variables were altered following infusion of ACTH. However, consistent blood pH shows that acid-base balance was being maintained despite the ACTH administration. The birds' erythropoiesis increased in response to the increased oxygen demand for glycogenic energy generation. With the help of this adaptive reaction, the body produced more erythrocytes, which in turn increased the quantity of hemoglobin circulating in the blood and consequently the amount of oxygen available for metabolism.

Keywords: ACID-BASE EQUILIBRIUM, ANXIETY, ACTH, BROILER CHICKEN.

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Introduction

When an organism is threatened, it responds by activating its defense mechanisms (a process characterized by the stress concept), which may be thought of as a response to the stress stimulus (stressor) (Muhammad & Al-Hassani ,2022). It's possible that the birds' habitat as a whole is stressful for them. A few examples are extreme temperatures (below freezing and above boiling), unfavorable environmental conditions (a lack of air circulation, bright lights, and moist litter), and an insufficient nutrition (shortages of nutrients, feed intake problems), The physical (capturing, immobilizing, injecting, and transporting), social (overcrowding, uneven body weight), physiological (rapid growth, sexual maturation), psychological (fear, harsh caretakers, noise), and pathological (exposure to infectious agents) causes of animal distress are just some of the many that exist(Muhammad & Al-Hassani ,2022). Possibility of causing stress, which has deleterious consequences on animal health and performance (Freeman, 1987; Scheele, 1997; Feltenstein et al., 2003; Cheng and Muir, 2004; Corzo et al., 2005; Mousa et al., 2022). At high enough levels of stress, stress syndromes may emerge. These diseases can be divided into neurogenic (alarm reaction), endocrine-mediated (fight-or-flight response), and psychogenic (resistance to adaptation, metabolic depletion) subtypes (the stage of exhaustion). After being triggered by adrenocorticotropic hormone, birds create corticosterone (CS) and other corticosteroids (ACTH). Increased cortisol levels have been linked to several chronic stress symptoms, including heart disease (arteriosclerosis, ascites), and changes to the immune system (Grandin, 1998). Poultry farmers are interested in controlling environmental factors like temperature and humidity so their birds maintain their physiological prime and produce the most eggs and meat possible. The acid-base balance of an organism is susceptible to a wide variety of physiological and environmental influences. These stressors and their effects are what will decide the shape of the change. Depending on the rate and condition of metabolism, respiration, and H+ equivalent exchange. No other factor outside acid-base variables, especially pH, affects metabolic processes as much as the rate at which energy is consumed. Turnover Multiple studies have established a connection between changes in the body's acid-base balance and stress on its physiological systems (Aguilera-Tejero et al., 2000; Jochem, 2001; Sandercock et al., 2001; Derjant, Li et al., 2002; Parker et al., 2003; Borges et al., 2003; Yalcin et al., 2004; Kaim et al., 2022). However, there are a variety of perspectives on acid-base chemistry. Preexisting problems with balance might be much more challenging to correct if the patient is under stress. Different species and stressors cause changes in a number of critical factors that often act in opposition to one another. In the absence of a suitable model, research on physiological and adaptive Birds, including chickens, have been studied in regards to how they react to stress. just not enough to suffice. Mini-osmotic pumps are used in the method proposed by Puvadolpirod and Thaxton (2000a, b) to supply ACTH constantly. There were 42 recorded responses to ACTH, including elevations in the heterophil:lymphocyte ratio, circulating concentrations of CS, glucose, cholesterol, triglycerides, and lipoproteins, and other measures of metabolic adaption. When this paradigm was used on adult chickens, Odihambo et al. (2006) found that, in addition to the responses shown in young birds, hens stopped reproducing as a result of atrophying egg sacs in the ovaries. It was not noted by Puvadolpirod and Thaxton that ACTH infusion altered blood gases or electrolytes (2000a, b, c, d). However, heat stress causes well-documented alterations in avian electrolytes. When a person's body temperature and respiration rate increase to dangerous levels, hyperthermia sets in and causes a decrease in blood CO2 partial pressure (pCO2). An acid-base imbalance, brought on by a decline in pCO2, can make breathing difficult. Alkalosis (Raup and Bottje, 1990; Macari et al., 1994). (Raup and Bottje, 1990; Macari et al., 1994; Mohammed and Al-Gharawi, 2022). According to many sources (Raup and Bottje, 1990; Macari et al., 1994).

Material and Methods

Study Birds and Condition

A total of 360 male chicks, from Cobb 500 commercial strains, were selected from a commercial hatchery and randomly distributed throughout six separate, climate-controlled rooms (60 chicks per chamber). There were 60 chicks total, 30 in each of the two enclosures in each room (2 replicates of 30 chicks in each chamber). In the hatchery, vaccines against Marek's, Newcastle, and infectious bronchitis were given to the chicks. There were seven nipples in the watering system and fresh pine shavings in each cage. Diets based on maize and soybean meal and designed to meet or exceed NRC (1994) nutritional guidelines were administered throughout the duration of the 42-day feeding regimen (days 1-15 for the starting phase, days 16-28 for the growth phase, and days 29-42 for the finisher phase). Crumbles were used as a starter feed, whereas full pellets were used for maintenance and growth. The animals in the research were provided with food and water on an as-needed basis, and they were always under constant illumination of around 10 lx. The birds were kept in an environment with a constant ambient temperature of 33°C for the first few days of the experiment and then gradually cooled to a more comfortable 21°C by the time they were 35 days old and beyond .Each of the six chambers included 12 treated birds (6 each pen), for a total of 48 birds. Additional birds were stashed away in each compartment. A total of three chambers were chosen at random for each treatment (ACTH, saline). The additional 48 birds in each cage helped researchers establish physiological baselines and reduce social interactions. All of the birds in each cage were weighed collectively on day 35. Once the 12 birds in each compartment had been implanted with a mini-osmotic .Pumps were filled with either ACTH or salt water. ACTH (Sigma-Aldrich Fine Chemicals, St. Louis, MO) was pumped into the animals at a rate of 8 IU per kg of BW.d for 7 days. For 7 days in a row, CON birds got pumps that administered the same volume of saline as ACTH-treated birds. All pumps averaged a delivery rate of 1 L/h. Pumps were detailed in detail by Paradiploid and Thaxton (2000a).

Blood and Biochemical Analysis:

To ensure the pumps were functioning properly, four more birds were selected at random (2 from each pen repeated inside each chamber) and bled just before the implantation process began. These birds were taken out of the experiment after blood samples were taken. Physiological parameters were determined from the analysis of these blood samples. After implanting the pumps, we bled and withdrew from the experiment a total of 14 birds: 4 from each chamber (2 from each pen replication) on days 4, 7, and 14. We took blood samples from the patient's heart using a cardiac stab and put them in heparinized tubes. Prefilled monovete syringes (50 international units per milliliter). The needle was implanted between the avian's third and fourth true ribs when the bird was lying on its right side. Since the blood is a brighter crimson when drawn from the left ventricle, we made it a point to only ever prick that chamber. Within 45 seconds of capture, all birds had been bled to death. A blood gas and electrolyte analyzer (ABL-77, Radiometer America, Westlake, OH) was used to immediately measure pCO2, pO2, pH, hematocrit, hemoglobin, and electrolytes (Na+, K+, Ca2+, HCO3-, and Cl-) after syringes were inserted into patients' veins. A 41.5 C body temperature was used to adjust the pH, pCO2, pO2, and HCO3 values (Burnett and Noonan, 1974; Fedde, 1986). With the use of the canonical formula, we were able to determine the average McHb concentration. After removing the needles from the monovette syringes, the needle ports were sealed and the syringes containing the blood samples were put in a bowl of ice. Once all of the birds were bled, the samples were quickly frozen and sent to a facility where they were centrifuged at 4,000 g for 20 minutes to separate the plasma from the red blood cells. Each monovette's plunger was removed, and the syringe was used to hold the residual plasma. By following these steps, we were able to guarantee that the plasma samples were shielded from any possible contamination from the air. We froze the plasma samples at -20 degrees Celsius for further chemical analysis. CS, CHOL, GLU, TRI, and high-density lipoprotein levels were determined in each thawed plasma sample (HDL). With the exception of CS, the concentrations of all other chemical components in plasma were measured by auto analyzer (Ektachem model DT 60). Elliott's stated enzymatic processes are used in this analyser (1984). The CS concentration in plasma was determined using an ELISA-CS Kit and a universal microplate spectrophotometer (BioTek Instrument Inc., Winooki, VT) (Assay Designs Inc., Ann Arbor, MI). Agent assay test kits from Assay Designs Inc., performed in accordance with the manufacturer's protocols.

STATISTICAL ANALYSIS

Each day of sample collection was treated as a separate block and the results were analyzed using a randomized full block method. Data were combined for readability and ran through the GLM method in SAS (SAS Institute, 1990). Six sets of chambers were used to calculate the means and standard deviations for each treatment (i.e., 3 chambers per trial). The LSD was used to measure differences in treatment means, and ≤P 0.05 was used to determine statistical significance.

RESULTS AND DISCUSSION

From Table1. That Shown the Changes in pH, HCO3 - , pCO2, and pO2 in the circulation of broilers following administration of adrenocorticotropic hormone (ACTH) in Information is shown as a mean standard error of the mean. Time is tracked from the moment a pump is installed.

Table 1. Changes in pH, HCO3 - , pCO2, and pO2 in the circulation of broilers following administration of adrenocorticotropic hormone (ACTH)

VARIABLES	TIMES (DAYS) OF ADRENOCORTICOTROPIC HORMONE (ACTH)				SIG
	0	4	7	14	
pH	7.35±0.02	7.39±0.02	7.39+0.02	7.32±0.02	*NS*
HCO3 – (mmHg)	30.2±1.06	36.3±1.05*	35.3±1.06*	29.2±1.06	*
pCO2	61.2±1.22	67.3±1.25*	67.5±1.26*	59.2±1.23	*
pO2	72.3±1.33	65.2±1.32*	65.2±1.33	65.2±1.33*	*
SIG	*NS*	*	*	*	

Data are presented as the mean ± SEM. Time measurement corresponds to time of pump placement.

***Differs (*P* < 0.05) from corresponding control mean**.

Table 2 displays the effects of continuous ACTH infusion on pH and blood gases. There was no effect of ACTH administration on blood pH on any of the days that readings were taken. On no day of monitoring did ACTH administration impact blood pH readings. Blood HCO3- and pCO2 concentrations were raised by ACTH infusion on days 4 and 7, but pO2 was lowered on both days and on day 14. ACTH-treated birds had higher hematocrit, McHc, and Hb values compared to controls 4 and 7, but Ca2+ levels were unaltered (Table 3). Na+ levels in the blood dropped on days 4 and 7, but rose again on day 14. The anion gap narrowed on days 4, 7, and 14, while blood concentrations of K+ and Cl- narrowed on days 4 and 7. (Table.4). In addition, plasma concentrations of GLU, CHOL, HDL, and TRI were all significantly raised after the ACTH infusion (Table 4). None of the tested blood components were different between the CON groups. Plasma CS concentrations over time are shown in Table 4. Over the course of the three days of testing, the average CS concentration in both control (CON) and baseline (baseline) birds was 11-10 ng/mL. On days 4, 7, and 14, the CS concentrations in the ACTH-treated birds were increased by around 18-, 26-, and 7-fold, respectively. These findings suggest that physiological stress in broiler chickens may be induced by the continuous infusion of ACTH at 8 IU/kg of BW/d for 7 days at a rate of 1 uL/h through a mini-osmotic pump. This outcome is consistent with prior research (Latour et al., 1996; Puvadolpirod and Thaxton, 2000a, b, c, d). In the current investigation, post-implant plasma CS, GLU, CHOL, HDL, and TRI were all shown to be higher after continuous ACTH treatment. Metabolic alterations in response to stress as measured by plasma concentrations of CS, GLU, CHOL, and TRI are consistent with these findings (Mickey et al., 1996; Puvadolpirod and Thaxton, 2000a, b). It is well established that elevated amounts of glucocorticoids in the bloodstream cause gluconeogenesis, leading to elevated levels of glucose, fructose, and glucose-6-phosphate dehydrogenase in the blood, as well as a rise in the heterophil: lymphocyte ratio (Siegel, 1961, 1971). There is a wide variety of stress-related GLU increases. Hyperglycemia is brought on by the increased catecholamine secretion that occurs in response to physical stress (Bell, 1971). Further, in many bird species, neurogenic amines including adrenaline (epinephrine), noradrenaline, and glucocorticoids cause the liver to convert glycogen into GLU, resulting in elevated blood GLU (Bell, 1971; Assenmacher, 1973). Primary actions of glucocorticoids on metabolism include stimulation of gluconeogenesis from proteins in skeletal muscle, lymphatic tissue, and connective tissue. Whether the chicks are given ACTH (Davis and Siopes, 1989), CS (Siegel and Van Kampen, 1984; Donker and Beuving, 1985), or subjected to a feeding restriction, the stress response causes plasma concentrations of CS to rise (Weber et al., 1990). In turn, elevated CS levels in the blood operate on the intermediate metabolism of carbs, protein, and lipids, resulting in a boost in energy levels. Indicated by an uptick in nonprotein nitrogen and an accompanying elevation in excretory uric acid, corticosterone is linked to ACTH-mediated gluconeogenesis from labile protein (Halliday et al., 1977; Siegel and Van Kampen, 1984). Birds given ACTH had lower amounts of oxygen, potassium, and sodium, and greater levels of carbon dioxide and bicarbonate. Additionally, neither pH nor Ca2+ levels shifted. In this study, we found that both fast- and slow-growing strains of broilers had arterial blood pH and blood gas levels within the same stated limits (Buys et al., 1999; Malan et al., 2003). Our findings are supported by fetal arterial blood gas and acid-base status data from lambs treated with intravenous ACTH (Carter et al., 2002).Enhanced metabolic activity to fulfill the energy demands for maintenance and development may be linked to increases in Hct and Hb, as well as decreases in pO2, under relatively extreme stressful situations. The higher levels of Hct, Hb, and McHc also point to enhanced erythropoiesis as a compensatory response to tissue hypoxia. It has been shown that ACTH increases plasma HCO3- in domestic chicken by stimulating adrenal steroid release (Kutas et al., 1970). Skeletal muscle anatomical, metabolic, and functional factors can be negatively impacted by broiler selection programs that prioritize rapid development and high muscle production, leading to conditions like spontaneous myopathy (Soike and Bergmann, 1998). Reductions in systemic arterial pO2, saturation of hemoglobin with oxygen (HbO2), and increases in partial pressures of carbon monoxide cause respiratory acidosis because of the buildup of hydrogen ions in the blood (Julian and Mirsalimi, 1992; Wideman and Tackett, 2000; Wideman et al., 2000, 2002). During the stressful period, plasma TRI levels rose.

Table 2. Changes in Hct , Hb , McHc , and Ca^{2+} in the circulation of broilers following administration of adrenocorticotropic hormone (ACTH)

VARIABLES	TIMES (DAYS) OF ADRENOCORTICOTROPIC HORMONE (ACTH)				SIG
	0	4	7	14	
Hct(%)	22.5±1.03	26.2±1.02*	27.3±1.02*	25.2±1.03	*
Hb (g/dl)	7.3±1.25	8.4±1.26*	8.6±1.25*	7.8±1.26	*
McHc (%)	31.92±1.35	32.25±1.32*	32.19±1.32*	31.98±1.35	*
Ca^{2+}(mEq/L)	3.02±0.22	3.00±0.25	3.02±0.25	3.02±0.23	*NS*
SIG	*NS*	*	*	*NS*	

Data are presented as the mean ± SEM. Time measurement corresponds to time of pump placement.
***Differs (*P* < 0.05) from corresponding control mean.**

Table 3. Changes in Na^{*} , K^{+} , Cl^{-}, and Anion gap in the circulation of broilers following administration of adrenocorticotropic hormone (ACTH)

VARIABLES	TIMES (DAYS) OF ADRENOCORTICOTROPIC HORMONE (ACTH)				SIG
	0	4	7	14	
Na (mEq/L)	146.5±1.15	139.2±1.12*	140.6±1.12*	154.2±1.15	*
K^{+}(mEq/L)	5.32±0.25	5.55±0.26*	5.53±0.25*	5.58±0.26	*
Cl^{-} (mEq/L)	112.02±1.21	99.82±1.22*	104.25±1.22*	119.88±1.22	*
Anion gap(mEq/L)	11.32±0.02	10.50±0.05*	9.82±0.05*	10.32±0.03	*
SIG	*NS*	*	*	*NS*	

Data are presented as the mean ± SEM. Time measurement corresponds to time of pump placement.
***Differs ($P < 0.05$) from corresponding control mean.**

Table 4. Changes in Glucose, Cholesterol, Corticosterone, HDL, and triglyceride in the circulation of broilers following administration of adrenocorticotropic hormone (ACTH)

VARIABLES	TIMES (DAYS) OF ADRENOCORTICOTROPIC HORMONE (ACTH)				SIG
	0	4	7	14	
Glucose (mg/mL)	210.2±2.25	1198.2±2.25*	1165.3±2.25*	210.2±2.25	*
Cholesterol (mg/mL)	125.33±1.25	249.52±1.26*	285.53±1.25*	125.38±1.26	*
HDL(mg/mL)	82.22±1.62	165.23±1.65*	126.625±1.62*	74.38±1.22	*
Triglyceride(mg/mL)	125.32±1.62	254.30±1.65*	126.32±1.65*	74.32±1.63	*
Corticosterone (mg/mL)	3.23±0.05	22.42±0.06*	30.23±0.06*	9.62±0.05*	*
SIG	*NS*	*	*	*	

Data are presented as the mean ± SEM. Time measurement corresponds to time of pump placement.
***Differs ($P < 0.05$) from corresponding control mean.**

Still unproven is whether this is an adrenaline impact or the result of some other stress-reactive system. While epinephrine has been shown to boost TRI lipase activity (Norris, 1985), glucocorticoids have been shown to decrease the production of TRI from nonesterified fatty acids (Bentley, 1998; Remage-Healey and Romero, 2001). However, these findings warrant more investigation because lipid appears to be the predominant source of energy for most birds (Blem, 1990; Klasing, 1998). The higher HDL and TRI levels at 4 and 7 days are consistent with the results obtained by Puvadolpirod and Thaxton in their earlier studies (2000a,b,c,d). When extracellular fluid volume is reduced, some of the water in the body is lost, and with it, some of the Na+. It is believed that the shift in Na+ and K+ caused a corresponding reduction in Cl- concentration. Cl- ions are the most numerous osmotically active solutes, and changes in Cl- are subsequent to changes in Na+. However, the most crucial element is the concentration of Na+ in the extracellular fluid. Due to Na+'s pivotal role in volume homeostasis, it is regulated by several mechanisms. Injection of ACTH into chickens causes them to drink and urinate more than usual both during times of stress and when they are recovering (Puvadolpirod and Thaxton, 2000d). This is because the body needs to flush away metabolic uric acid and extra electrolytes (Siegel and Van Kampen, 1984). It is generally known that chickens' kidneys produce erythropoietin, and that this hormone may stimulate erythroblast synthesis in the bone marrow (Samurut and Nigon, 1976; Pain et al., 1991; Wickeramasinghe et al., 1994). Furthermore, it has been demonstrated that glucocorticoids are essential for the continued proliferation of erythroid progenitor cells (Bauer et al., 1999). Accordingly, the fact that we observed increased levels of Hct and Hb in the ACTH-treated birds is suggestive of the activation of erythropoiesis. This erythropoietic adaptive alteration may be permanent because pO2 concentration, Hct, and Hb were all still high on day 14 (i.e., 7 days after the infusion of ACTH was discontinued). If you want to know if your Hct and Hb will go back to their pre-ACTH values or if there have been new set points, you'll need to do further study. Stress hormones called glucocorticoids stimulate glucose production in the body. Non-carbohydrate moieties are GLU-converted in this process. For gluconeogenic conversion into GLU, amino acids are the most common substrate. Each enzymatic step in the generation of GLU by glycolysis and gluconeogenesis uses one mole of oxygen for every mole of ATP that is needed. Therefore, gluconeogenesis is not only expensive from the aspect of energy generation, but it is also expensive in terms of O2 utilization. Note that gluconeogenesis can be continued so long as the animal keeps its protein reserve (Klasing, 1998), has enough oxygen (King, 2006), and doesn't use up its electrolyte stores, so disrupting its homeostatic acid-base balance (Julian and Mirsalimi, 1992; Wideman and Tackett, 2000; Wideman et al., 2000, 2002). Acute physiological stress generated by continuous infusion of ACTH was observed to increase CS levels, as well as GLU, CHOL, HDL, and TRI blood levels, while decreasing pO2 and increasing pCO2 and HCO3- in chickens. Furthermore, electrolyte shifts occurred constantly to keep acid-base balance constant. These modifications appear to be crucial for handling stress, since they make it possible to generate the vitality necessary for adapting to a stressful circumstance.

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