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Responses of Broiler Chickens Under Dexamethasone-Induced Stress with or Without Seleno Methionine and Vitamin E Supplementation

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Abstract

Heat stress poses a significant threat to the health and productivity of both humans and animals, with modern commercial broilers being particularly susceptible. This study explored the physiological responses of broiler chickens under chronic heat stress conditions with antioxidant supplementation. A total of 135 unsexed Arbor acre broiler chicks were randomly assigned to three experimental groups: control, dexamethasone (DEX) only, and dexamethasone with selenomethionine and vitamin E supplementation (DEX+SeMet+Vit.E) and replicated three times in a completely randomized design. The birds were exposed to daily doses of dexamethasone for 14 days. Temperature-Humidity Index (THI) measurements revealed severe heat stress in the afternoons, impacting ($p<0.05$) respiratory rates and body temperatures. Supplementation with selenomethionine and vitamin E improved ($p<0.05$) body temperature of the broiler chickens. DEX significantly ($p<0.05$) affected all growth performance indices (feed intake, weight gain and feed conversion ratio), despite antioxidant supplementation. SeMet and Vitamin E showed significantly ($p<0.05$) positive effect on leukocytes in spite of DEX administration. Intestinal morphometry revealed negative effects on villi area, perimeter, and width due to dexamethasone. It was concluded that dexamethasone influenced thermoregulation, growth, blood and intestinal indices in broiler chickens, with antioxidant supplementation with SeMet and Vitamin E partially ameliorating these effects.

Keywords: Broiler chickens, Heat stress, Seleno methionine, Vitamin E, Serum Biochemical Analysis

INTRODUCTION

The health of humans as well as animals is increasingly threatened by heat stress, which has become a more common environmental stressor (Arifwidodo & Chandrasiri, 2020; Chauhan *et al.*, 2021). The severity of the rising global temperatures has only made this worse. The constrictive heat loss capacity, rapid metabolic rates, heat production, hypoplasia of the sweat gland, and rigorous genetic selection of modern commercial broilers make them particularly susceptible to heat stress in high ambient temperatures (Deeb & Cahaner, 2002; Nawab *et al.*, 2018). Chickens' blood corticosteroid content is well-established as a stress marker, and dexamethasone, a glucocorticoid, has a long history of being used to induce cell-mediated immunosuppression (Coutinho and Chapman, 2011), being able to mimic stress and produce a heat stress response (HSR).

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Heat stress impairs the welfare and growth performance of broilers (Hamidi *et al.*, 2022), causes enormous economic loss in the commercial broiler industry (Mohammadi, 2021), impairs liver function (Chen *et al.*, 2021), affects some morphometric parameters of the small intestinal mucosa (Santos *et al.*, 2015). Intestinal barrier function can be adversely affected by dexamethasone administration in feed (Vicuña *et al.*, 2015), resulting in increased intestinal inflammation-associated permeability. Being close to endogenous corticosteroids, dexamethasone induces effects similar to elevated concentrations of corticosterone and triggers stress-related signalling pathways (Calefi *et al.*, 2016). The application of dexamethasone as a stress protocol in animal studies is well-established (Aengwanich, 2007; Guerrero *et al.*, 2011; Ademu *et al.*, 2018).

Antioxidant defence against heat stress may be improved by selenium reserves found in the body and supplemented via diet (Zheng *et al*. 2021). Se is involved in the protection against oxidative stress and regulation of cell growth, apoptosis, and modification of cell signalling systems and transcription factors (Surai *et al.*, 2018). Due to their inability to synthesise selenium (SeMet), chickens must receive it through diet as one of their primary defences against stresses that are relevant to the chicken industry. There are no recommendations for additional dietary supplementation in case of chronic exposure to HS. Higher Se levels (from 1 to 3 mg/kg) have however been used in other studies (Pappas *et al*., 2012; Habibian *et al*., 2016) to improve oxidative stability. Vitamin E (α-tocopherol and its derivatives) is the major fat-soluble antioxidant found in animal cells. As an essential free radical scavenger, it is beneficial by limiting lipid peroxidation by protecting cells from oxygen radicals, both *in vivo* and *in vitro*. Thus, the aim of this research was to provide an insight into the behavioural, blood and gut morphometric alterations induced by dexamethasone with or without antioxidant supplementation using an avian model.

MATERIALS AND METHODS

Experimental Design, Diets, and Management of Broiler Chicks

This experiment was performed at the Teaching and Research farm of the Department of Animal Production and Health, Federal University Wukari, Nigeria. One-hundred-and-thirty-five-two-week-old *Arbor acre* broiler chicks (unsexed) were used in the experiment. They were randomly allotted to three experimental treatments; control, 1 mg dexamethasone (DEX) only, and 1 mg dexamethasone + 0.1 g/kg of selenomethionine +125 mg/kg of vitamin E (DEX+SeMet+Vit.E). In a completely randomized design, each treatment was replicated three times, with fifteen birds per replicate. A maize/soybean meal-based broiler starter and finisher diet was formulated according to NRC (1994) nutrient requirement for broiler chickens (Table 1) and fed to all the treatment groups. Daily doses of dexamethasone (1 mg) were administered by dissolving it in a litre of water and supplied for fourteeen days.

Table 1: Feed Composition of Broiler starter and finisher diets

Ingredients	Starter (kg)	Finisher (kg)
Maize	55.0	64.0
Soybean cake	38.0	31.5
Maize offal	2.0	-
Limestone	1.0	0.5
Bone meal	3.0	3.0
Common salt	0.3	0.3
Vitamin premix	0.3	0.3
Lysine	0.2	0.2
Methionine	0.2	0.2
Total	100.0	100.0

The DEX+SeMet+Vit.E treatment containing SeMet and vitamin E, supplemented in the diet was administered until the end of the experiment. The broiler chicks were raised on deep litter and housed in 2.5 m x 1.96 m bird pens for each replicate with feed and water provided *ad libitum* using conical feeders and drinkers. All routine and management practices were strictly adhered to. The initial weight was recorded on day 1 of the trial, while feed intake, weight gain and feed conversion ratio were recorded weekly.

Thermoregulatory Measurements

Indoor temperature and relative humidity readings were recorded daily using an electronic digital thermo-hygrometer (HTC-1). Both readings were taken in the mornings (8.00 am) and afternoons (3.00 pm) throughout the experimental period and used to calculate the morning and afternoon Temperature-Humidity-Index (THI). Individual temperatures and respiratory rates were also measured using an infrared digital thermometer and counting of respiration (breath/minute) with the aid of a stopwatch, respectively. THI was determined using the formula described by Tao and Xin (2003). Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression functions by Stull (2011). Heat stress was classified as the absence of heat stress (<27.8), moderate heat stress (27.8 -28.8), severe heat stress (28.9 -29.9), and very severe heat stress (>30.0) (Marai *et al*., 2001).

Blood Analyses

At the end of the trial on day 42, three chickens were randomly selected from each replicate for haematological and serum analyses. The sampled chickens were bled by severing the jugular vein to obtain 4 mL of blood from each bird. 2 mL each was collected into bottles containing Ethylene Diamine Tetra Acetate (EDTA) and no EDTA for haematological and serum chemistry assays respectively. Haematological analysis was carried out using a Mindray BC3600 hematology analyzer. Commercial kits were used for determining anti-oxidative indices and liver enzymes.

Organ Collection and Examination

On day 42 of the experiment, three birds per replicate were bled and eviscerated. Intestinal weights and length were recorded. For histomorphometry, samples of the jejunum were harvested and fixed in 10% formal saline. Fixed tissues were histologically processed according to the method of Bancroft & Stevens (2008).

Data Analysis

All data collected from the experiment were subjected to one-way analysis of variance (ANOVA) using the Fit Y by X function of JMP Pro 16. Where the result of ANOVA was statistically significant, Tukey's post-hoc test for multiple comparisons was performed to compare the means of all groups. Graph for the THI was prepared using GraphPad Prism 6.

RESULTS AND DISCUSSION

Thermoregulatory Indices

The indoor THI (Fig. 1) of the poultry house during the experimental period in the mornings averaged 26.3 and 32.6 in the afternoons. Results indicated the absence of heat stress in the morning and the presence of severe heat stress in the afternoon as reported by Marai *et al.* (2001). THI is a reliable indicator of thermal comfort. Body temperature (Table 2) of the experimental birds indicate that broiler chickens in the control group had higher ($p<0.05$) body temperature, being similar ($p>0.05$) with the DEX only group. Respiratory rate across

the treatment groups varied ($p<0.05$) with birds in the DEX groups showing higher ($p<0.05$) respiratory rates compared to the control group. Birds administered SeMet+Vit E showed similar ($p>0.05$) respiratory rates with the DEX-only group.

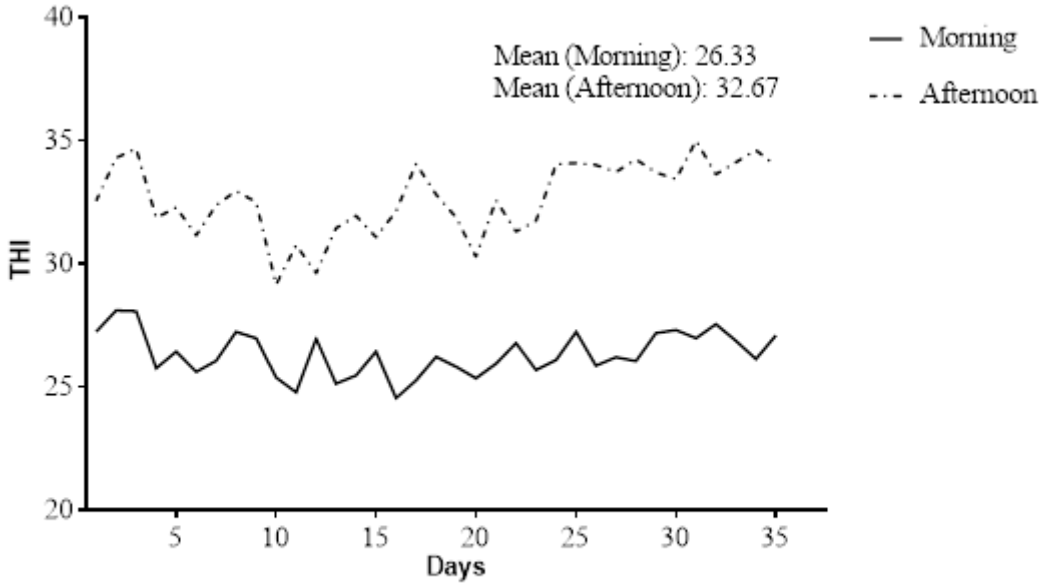

Figure 1: Daily Temperature-humidity index inside the poultry house

Chronic heat stress, induced by dexamethasone, adversely affected respiratory rates, body temperature, and overall thermoregulatory indices in broiler chickens. Chronic heat stress poses a significant threat to animal health and productivity by negatively influencing their ability to dissipate heat and altering overall behaviour. The respiratory rate of broiler chickens administered dexamethasone experienced elevated respiratory rates compared with the control group. This pattern was maintained despite SeMet and Vit. E supplementation. This confirms the understanding that chronic heat stress influences thermoregulation negatively in poultry. Heat stress causes an increase in the respiratory rate, resulting in glycogen depletion and a negative energy balance (Emami *et al.*, 2021). This finding is also consistent with the work of Yadav *et al.* (2015) that reported an increase in respiratory rate as a physiological response to stress in chickens.

In broilers supplied dexamethasone, Aengwanich (2007) and Ademu *et al.* (2018) reported a decrease in rectal temperature. This is due to the birds' successful efforts to re-establish homeostasis and mitigate the effects of dexamethasone. While this is contrary to the findings of Ndubuisi *et al.* (2021), it is in agreement with Khan *et al.* (2014) who reported a decrease in body temperature as a response to antioxidants in broiler chickens. An increase in respiratory rate and a decrease in body temperature also validates successful heat loss by panting employed by the birds to combat heat stress.

Table 2: Thermoregulatory indices of broiler chickens under chronic heat stress

Parameters	Control	DEX	Dex+ SeMet+ Vit E	SEM	p-value
Body Temperature (°C)	36.7^{a}	36.6^{a}	36.5^{b}	0.03	0.0053
Respiratory Rate (bpm)	108.2^{b}	113.4^{a}	111.9^{a}	0.76	0.0007

^{a,b} means with different superscripts on the same row differ significantly ($p<0.05$)

Growth Performance

Growth performance results indicate that broilers fed the control diet had higher ($p<0.05$) final weight, daily feed intake, daily weight gain and FCR compared with the DEX-containing groups which were similar ($p>0.05$) for all the indices (Table 3). Similar to heat stress, dexamethasone also depresses feed intake and this is a physiological response to minimize innate heat production to maintain the thermal homeostasis (Lin *et al.*, 2004; Hanafy & Khalil, 2015). Overall, birds eat less in order to avoid excess heat load. As a consequence, feed efficiency is adversely affected (Onagbesan et al., 2023). This has a direct effect on protein catabolism which is further reflected in the significantly suppressed body weight gain and decreased feed efficiency in broiler chickens (Zaboli *et al.*, 2019). Muscular dystrophy and reduced growth rate is a common outcome of DEX administration (Akter *et al*, 2021). While it is expected supplementation with seleno methionine and vitamin E should alleviate the effect of heat stress caused by DEX, this was not observed. This however may also be dose dependent.

Table 3: Growth performance of broiler chickens under chronic heat stress

Parameters	Control	DEX	Dex+ SeMet+ Vit E	SEM	p-value
Initial weight (g/b)	121.1	121.1	123.3	0.91	0.2160
Final weight (g/b)	1815.9^{a}	1301.3^{b}	1343.9^{b}	52.92	0.0008
Daily feed intake (g/b/d)	100.2^{a}	81.8^{b}	82.6^{b}	3.30	0.0124
Daily weight gain (g/b/d)	48.4^{a}	33.7^{b}	34.9^{b}	1.50	0.0008
Feed Conversion Ratio (g)	2.1^{a}	2.4^{b}	2.4^{b}	0.06	0.0148

$^{a.b}$ means with different superscripts on the same row differ significantly ($p<0.05$)

Serum Biochemical Indices

Serum biochemical indices of broiler chickens under chronic stress indicate lower glucose levels in theSeMet+Vit E group compared to the control (Table 4). AST levels were significant ($p<0.05$) with both DEX containing groups having higher levels compared with the control. A similar trend was observed for TC, globulins and total protein. Treatment with corticosteroids or synthetic glucocorticoids (such as dexamethasone) produce stress-induced metabolic alterations focused on the mobilization or production of glucose for energy (Gao *et al.*, 2008) needed to maintain homeostasis in the presence of the stressor. Dexamethasone also induces changes in liver enzymes and lipid profiles, partially mitigated by antioxidant supplementation. Liver enzymes are good indicators of corticosteroid levels. Antioxidants may help to lower liver enzyme levels by neutralizing free radicals that can damage liver cells. Results obtained in this study align with the known impact of corticosteroids on liver function (Chen *et al.*, 2021) and lipid metabolism (Akter *et al.*, 2021). Higher AST levels can be indicative of potential liver damage. Reports from previous studies involving glucocorticoids on cholesterol vary, with authors reporting an increase (Shini *et al.*, 2008), decrease (Giudetti & Gnoni, 1998) or no change (Wang *et al.*, 2012).

Table 4: Serum biochemistry of broiler chickens under chronic heat stress

Parameters	Control	DEX	DEX+ SeMet+ Vit. E	SEM	p-value
Glucose (mg/dl)	174.5[a]	137.4[ab]	82.00[b]	17.35	0.0064
AST (µ/L)	37.2[b]	42.6[ab]	52.9[a]	3.19	0.0108
ALT (µ/L)	72.5	74.9	69.3	4.12	0.6366
ALP (µ/L)	1169.9	1188.1	1247.9	53.15	0.5669
TG (mg/dl)	0.5[a]	0.3[b]	0.4[ab]	0.03	0.0146
TC (nmol/l)	2.2[b]	3.3[a]	3.6[a]	0.10	<0.0001
HDL (mg/dl)	1.0	1.4	1.2	0.3	0.6682
LDL (mg/dl)	0.8	0.8	1.0	0.24	0.7841
VLDL (mg/dl)	0.10[a]	0.06[b]	0.08[ab]	0.01	0.0033
Globulin (g/dl)	1.7[b]	3.0[a]	2.9[a]	0.21	0.0023
Albumin (g/dl)	1.6	2.1	1.6	0.21	0.2218
Total protein (g/dl)	3.3[b]	5.1[a]	4.2[ab]	0.37	0.0148

[a.b] means with different superscripts on the same row differ significantly ($p<0.05$); ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: alkaline phosphatase; TG: Triglycerides; TC: Total Cholesterol; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; VLDL: Very Low-Density Lipoprotein

Haematological Indices

DEX-only treated birds showed higher ($P<0.05$) haemoglobin, leukocyte and erythrocyte counts compared to the control group (Table 5). Birds fed DEX+ SeMet + Vit E showed reduced leucocyte counts which were comparable to the control group. Other haematological indices were similar across all treatments. Leukocytes increased significantly ($p<0.05$) in the DEX treatment. Dexamethasone increased leukocyte counts, indicating an immune-linked stress response. Previous studies (Shini *et al*., 2004, 2005; Aengwanich, 2007) show significant increases in the percentages of heterophils, and H/L ratio, as well as a significant decrease in the percentage of lymphocytes in DEX-treated chickens when compared with control chickens which is contrary to the findings of this study. The decrease in leucocyte count observed in the DEX+ SeMet + Vit E group indicates the efficacy of SeMet and Vitamin E in mitigating the immune-linked stress response caused by dexamethasone.

Table 5: Haematological indices of broiler chickens under chronic heat stress

Parameters	Control	DEX	DEX+ SeMet + Vit E	SEM	p-value
Packed Cell Volume (%)	39.1	43.6	43.1	2.03	0.2606
Haemoglobin (g/dl)	13.1[b]	14.6[a]	14.4[ab]	0.35	0.0245
Leukocytes (x 10^9/L)	5.6[b]	7.5[a]	4.2[b]	0.79	0.0309
Erythrocyte (x 10^{12}/L)	6.3[b]	7.5[a]	7.2[ab]	0.30	0.0416
Lymphocytes (%)	85.4	82.9	79.8	2.27	0.2429
Heterophils (%)	5.7	7.6	8.3	1.11	0.2668
Heterophil-Lymphocyte ratio	0.1	0.1	0.1	0.02	0.2629

[a.b] means with different superscripts on the same row differ significantly ($p<0.05$)

Intestinal Morphometry

The results of the intestinal morphometry indicates that the villi area was significant ($p<0.05$) with birds in the control having higher villi area compared to birds in the DEX + Vit E + SeMet group (Table 6). The same trend was observed for villi perimeter and villi width. Crypt length, villi/crypt ratio, and intestine length were unchanged ($p>0.05$) across the treatment groups. DEX influenced intestine weight when compared with the control group despite Vitamin E and SeMet supplementation. Chronic stress negatively impacted intestinal function, with observable changes in villi morphology and also impacted nutrient retention. Results on villi length, villi width, and surface area support existing knowledge that dexamethasone induced stress can impact intestinal function and morphology (Olsen *et al.*, 2005; Islam *et al.*, 2022).

Table 6: Intestinal morphology of broiler chickens under chronic stress

Parameters	Control	DEX	DEX+ SeMet+ Vit. E	SEM	p-value
Villi Area (μm²)	50432.0^{a}	43832.8^{ab}	30508.5^{b}	5108.90	0.0330
Villi Perimeter (μm)	1653.6^{a}	1434.8^{ab}	1173.3^{b}	97.54	0.0073
Villi length (μm)	650.2^{a}	655.7^{a}	545.2^{b}	35.94	0.0435
Villi width (μm)	269.3^{a}	149.1^{ab}	114.9^{b}	39.67	0.0269
Crypt length (μm)	307.6	310.5	276.0	23.42	0.5226
Villi/crypt ratio	2.3	2.2	2.0	0.22	0.6081
Intestine weight (g)	70.0^{a}	44.3^{b}	49.7^{b}	4.73	0.0039
Intestine length (cm)	199.7	179.8	200.5	10.87	0.3408

$^{a.b.}$ means with different superscripts on the same row differ significantly ($p<0.05$)

Villi length is a widely regarded measure of the intestine's ability to absorb nutrients from feed. The reduction of the area of the villi might also explain the lower weights of the duodenum and cecum due to reduced nutrient absorption. Corticosterone administration slows down intestinal epithelial cell proliferation and thus decreases intestinal villi length and crypt depth which impairs nutrient absorption in the intestines of broilers (Berrocoso *et al.*, 2017). Under chronic stress conditions, supplementation with Vitamin E and SeMet were unable to reverse the trend observed for the morphology indices.

CONCLUSION

In this study dexamethasone-induced chronic heat stress adversely affected thermoregulation, growth performance indices, blood parameters, and intestinal morphometry in broiler chickens. Supplementation with SeMet and Vitamin E partially ameliorated these effects, particularly body temperature and leucocyte count. Our findings may be specific to the experimental conditions and may not fully represent all real-world scenarios as dexamethasone was employed as a constant and quantifiable stressor. It does however provide insights for researchers studying the impact of chronic heat stress and corticosteroid administration in broiler chickens and offer guidance for managing broiler chickens under potential heat stress conditions, with considerations for antioxidant supplementation.

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