Influence of betaine hydrochloride on glucocorticoid-induced stressed broiler chickens

ABSTRACT

Aim: To investigate the ameliorative effect of betaine HCl fed to broiler chickens under chronic stress conditions.
Study design: Completely Randomized Design.
Place and Duration of Study: Experimental site: Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Nigeria; Haematology Laboratory, Faculty of Medicine, Ahmadu Bello University, Nigeria, between September 2016 and October 2016.
Methodology:A total of two-hundred-and-forty (240) day-old *Arbor acre* broiler chickens were randomly allotted to four experimental treatments. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design. Dexamethasone was administered in drinking water (0, 1, 2 and 3 mg) to simulate chronic stress. Dexamethasone-treated birds were fed betaine HCl at 0.15% in their diets. Birds receiving 0 mg dexamethasone served as the control.
Results: Thermoregulatory results indicatedthat respiratory rates were similar (p= 0.16)even with increasing doses of dexamethasone. Rectal temperaturewas alsosimilar (p=0.97) across all the treatment groups indicating the positive effect of betaine HCl on thermoregulatory responses of broiler chickens. Birds in the controlgavebetter (p< 0.05) final body weight, daily weight gain, daily feed intake, with feed conversion ratio being similar with some dexamethasone containing groups.Dexamethasone did not influence (p>0.05) haematological indices of broiler chickens, with betaine HCl demonstrating positive effect on all blood indices. Both serum and haematological indices were similar (p>0.05) with the control. Thigh and drumstick weights were still negatively affected (p< 0.05) by dexamethasone. Betaine HCl positively (p>0.05) improved robusticity index whiletibia weight, length, and weight/length index were negatively (p< 0.05) influenced by dexamethasone despite betaine supplementation.
Conclusion: Betaine HCl improved thermoregulatory and blood indices of dexamethasone-stressed broiler chickens and showed positive effect on tibia bone strength.

Keywords: Stress; Dexamethasone; Betaine HCl; Glucocorticoids; Broilers

1. INTRODUCTION

Chronic stress conditions will continue to impact negatively on livestock production, particularly broiler chicken production; affecting behavior, feed intake, growth and overall performance. While the intensity of chronic heat stress is relatively weak, the duration of stress is relatively long, hence will occur more frequently (Mignon-Grasteau, 2015) in the face of climate change and rising global temperatures. Basal metabolism of broiler chickens has been reported to reduce under chronic heat stress with an increase in additional heat from metabolizable energy (Sayed & Downing, 2015; Tesseraud et al., 1999 Glucocorticoids, chiefly corticosterone in poultry, are involved in the endocrine regulation of several physiological processes such as metabolism, growth, hydromineral balance, and the

immune system (Mommsen et al., 1999).Dexamethasone, a synthetic glucocorticoid has been used to replicate physiologic stress in farm animals to evaluate growth performance (Mehaisen et al., 2017, Ademu et al., 2018), stress-related signaling pathways (Calefi et al., 2016) carcass traits and immune function (Vicuña et al., 2015). Many studies have explored nutritional strategies to alleviate the adverse effects of heat stress with dietary supplementation with betaine HCl helping to reduce the negative effects. Several studies showed that dietary betaine supplementation could improve bird performance and carcass traits by improving cell osmoregulation (Leng et al., 2016; Mendoza et al., 2017; Chen et al., 2018). Attia et al. (2009) also showed that the impact of severe heat stress could partially be overcomed by adding betaine (1g/kg of diet) to the diet ofin slow-growing broilers. Betaine has been reported to enhance the digestion and absorption of nutrients, improve feed conversion ratios, breast meat yield and meat quality and reduce carcass fat (Eklund et al., 2005; Metzler-Zebeli et al., 2009). This study aims to investigate the effect of betaine HCl under chronic heat stress conditions using dexamethasone as a stressor.

2. MATERIAL AND METHODS

2.1 Experimental Design, Diets and Management of Birds

Two-hundred-and-forty-day old *Arbor acre* broiler chicks were used in this experiment. They were randomly allotted to four experimental treatments. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design. A maize/soybean meal-based broiler starter and finisher dietswereas formulated according to NRC (1994) nutrient requirement for broiler chickens (Table 1) and fed to all birds. Birds administered with 1, 2 and 3 mg dexamethasone in drinking water were given diets supplemented with betaine HCl at 0.15% (1.5g/kg). Daily doses of dexamethasone (1, 2 and 3 mg) were administered by dissolving in 1 litre of water from when the birds attained 14 days of age. Birds receiving no dexamethasone (0 mg) in their drinking water served as the control. The birds 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*. All routine and management practices were strictly adhered to. Initial weight was taken at the start of the experiment at day 1 while feed intake and weight gain were taken weekly. Mortality records were also taken as they occurred.

2.2 Thermoregulatory Measurements

Rectal temperatures and respiratory rates were measured by placing a digital thermometer in the rectum and counting of respiration (breath/minute) with the aid of a stopwatch, respectively.

2.3 Haematological and Serum Analyses

Brachial vein blood samples (4 ml) were collected from two birds per replicate on day 49 for haematological, and serum analysis. Blood samples collected into collection tubes containing EDTA (Ethylenediaminetetraacetic acid) were analyzed for haematological indices using an auto haematology analyzer (HA-17600). Whole blood was collected in tubes containing no EDTA for serum metabolites. All samples were run in duplicate with kit calibrators and controls included in each analysis. The haematological and serum analyses were carried out at the Haematology Laboratory of the Faculty of Medicine, Ahmadu Bello University, Zaria.

2.4 Organ Collection and Examination

On day 49 of the experiment, three birds per replicate were slaughtered and eviscerated. Live weights, as well as carcass cuts, weight of organs including liver, kidneys, gizzard, and heart were measured using a sensitive digital scale. Relative weights of each organ and cut part expressed as percentage of live weight were determined.

Table 1: Ingredient composition and calculated analysis of experimental diets

Ingredients	Starter	Finisher
Maize	55.00	62.5
Soyabean cake	24.00	18.00
Groundnut cake	14.00	15.00
Maize offal	2.00	-
Limestone	1.00	0.50
Bone meal	3.00	3.00
Premix	0.30	0.30
Lysine	0.20	0.20
Methionine	0.20	0.20
Total	100.00	100.00
Calculated Analysis		
ME Kcal/kg	2860	2950
Crude protein	23.06	21.05
Ether extract	4.62	4.48
Crude fibre	4.25	3.77
Calcium	1.22	1.03
Available phosphorus	0.52	0.51
Lysine	1.24	1.09
Methionine	0.60	0.58

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg.

2.5 Tibia Geometric Properties

During carcass analysis at day 49, the left tibia of three birds per replicate was removed. Tibia were then labeled and immersed in boiling water (100 °C) for 15 minutes according to

the procedure described by Applegate and Lilburn (2002) for complete tissue removal. Length of each bone was measured using a meter rule. The distance from proximal to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing balance. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor et al., 1991). The Robusticity index was determined using the formula described by Reisenfeld (1972).

$$Robusticity\ Index\ =\ \frac{Bone\ length}{Cube\ root\ of\ bone\ weight}$$

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to the procedure described by AOAC (1990). The percentage ash was then determined relative to dry weight of the tibia.

2.6 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 15.1.0 (2020). Variability in data was expressed as standard error of means (SEM), with $p < 0.05$ considered to be statistically significant. Where the resultss of ANOVA were as statistically significant, Tukey HSD test for multiple comparisons was performed to compare means of all groups.

3. RESULTS AND DISCUSSION

Thermoregulatory Parameters

The effect of betaine HCl on respiratory rate of broiler chickens underdexamethasoneinduced stressis shown in Fig. 1. Birds administered with dexamethasone exhibited similar ($p > 0.05$) respiration rates across all treatment groups. The effect of dexamethasone on rectal temperature of the broiler chickens (Fig. 2) were also not significant ($p > 0.05$). Thermoregulatory data from this study points to the ameliorative effect of betaine HCl under induced stress brought by dexamethasone. Heat stress whether cyclic or chronic will continue to impact broiler chicken performance particularly in tropical climates; especially in the face of rising global temperatures. While increased respiratory rate is a consequence of the impact of heat stress (Etches, 2008), this was not obtained in this study. Under heat stress conditions, broiler chickens will alter their behavior by panting, stretching of wings, reduction in feed intake (Lisanne et al., 2016), increasing contact area with cooler surfaces and delay feathering. Signals from the peripheral thermoreceptors in the skin or changes in blood temperature are sent to the anterior hypothalamus to initiate heat loss by triggering vasodilation and panting (Rastogi, 2007). Aengwanich (2007) who administered dexamethasone at 1-5 mg/kg to broiler chickens reported an increase in respiratory rates. Rectal temperature is an indicator of metabolic rate in broilers and this rises when birds are exposed to stressors like high temperature and corticosterone analogues like dexamethasone.Our findingagrees with Hassan et al. (2011) and Nofal et al. (2015) who concluded that betaine supplementation in diets significantly reduced rectal temperature. As an osmolyte, betaine may have a stabilizing function on cells subjected to osmotic stressors, (Klasing et al., 2002) by regulating the water balance, resulting in the stability of tissue metabolism especially in the gastro-intestinal tract (Lipinski et al. 2012) protecting them from heat shock.

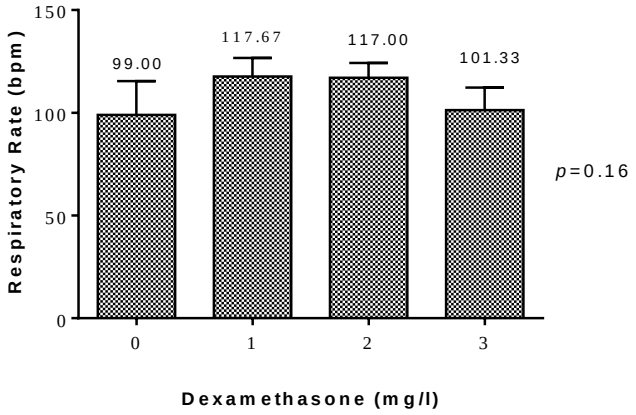

Figure 1: Respiratory rates of broiler chickens fed betaine Hcl under dexamethasone induced stress

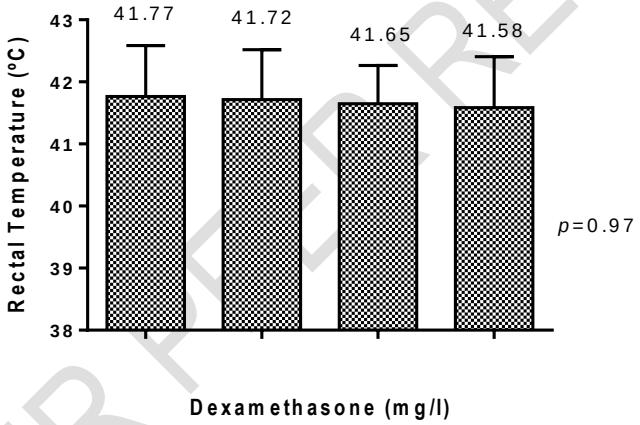

Figure 2: Rectal temperatures of broiler chickens fed betaine HCl under dexamethasone induced stress

Growth performance indices

Growth performance of broiler chickens fed betaine HCl under dexamethasone-induced stress is presented in Table 2. Final weight differences were significant ($p < 0.05$) with birds in the control having higher final weights compared to dietary treatments receiving betaine HCl under dexamethasone induced stress. Similar trends were observed across other growth indices (feed intake, weight gain). Feed conversion ratio results were similar ($p > 0.05$) for birds in control, 1mg and 3mg dexamethasone groups. The effect of dexamethasone on feed intake in birds may be dose-dependent and this appears to be more severe at higher doses. Decreased feed intake is a physiological response to minimize intrinsic heat production and to maintain the thermal homeostasis, thus bringing down feed efficiency (Faria Filho et al., 2007). However, Awad et al. (2014) reported that feeding of betaine at the rate of 1.5 g/kg like in this study resulted in a significantly higher feed intake compared to the control group. This trend was not observed in this study. The decreasing

trend observed for final weight is a consequence of reduced feed intake and weight gain decline. Attia et al. (2005) and Dunsheaet al. (2007) reported that the addition of betaine to poultry diet improved body weight significantly. In all the studies cited above, stress was not induced using dexamethasone. Under heat stress conditions however, supplementation of broiler diets with 0.1% betaine have been reported to improve weight gain, compared with control birds (Farooqi, et al., 2005).

Table 2: Growth performance of broiler chickens fed betaine HCl under dexamethasone induced stress

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters	0	0.15	0.15	0.15	SEM	P value
Initial weight (g/b)	115.83	115.83	115.83	115.00	0.72	0.8018
Final weight (g/b)	2088.79^{a}	1690.35^{b}	1541.67^{c}	1657.52^{bc}	30.45	<.0001
Weight gain (g/b/d)	46.97^{a}	37.49^{b}	33.95^{c}	36.73^{bc}	0.73	<.0001
Feed intake (g/b/d)	104.81^{a}	89.21^{b}	84.19^{b}	87.27^{b}	1.58	<.0001
FCR (g/g) (kg/kg)	2.23^{a}	2.38^{ab}	2.48^{b}	2.38^{ab}	0.03	.0041

$^{a,\ b,c}$Means with different superscript on the same row differ significantly ($P < 0.05$);

g/b/d= gram/bird/day, SEM: Standard error of the mean

Blood Indices

Blood indices results (Table 3) indicate that all serum indices (Glucose, cholesterol, triglycerides and total protein) measured were similar ($p > 0.05$) across all treatment groups.A similar trend was observed for all the hematological indices measured, indicating the alleviating properties of betaine HCl on blood indices, even under dexamethasone stress conditions.Blood is an important and reliable medium for assessing the physiological and health status of individual animals (Egbe-Nwiyiet al., 2000). Administration of corticosterone has been shown to increase glucose absorption (Nasir et al., 1999). Primarily, stress-induced metabolic alterations seem to be focused on the mobilization or production of glucose for energy needed to maintain homeostasis in the presence of the stressor. Under conditions of simulated chronic stress, physiological stress induces a higher glucose level in blood, which is second only to corticosterone (Odihamboet al., 2006; Olanrewaju et al., 2006; Lin et al., 2007).Abnormal lipid profiles and dyslipidemia are known to occur in both animals and humans following dexamethasone administration, resulting in elevated levels of serum cholesterol and triglycerides (Bruder et al., 2004). Again this phenomenon was not

observed in this study. Betaine supplementation may act by limiting lipase activity which catabolizes triacylglycerol to yield energy (He et al., 2015). Previous studies investigating stressors like dexamethasone and high temperatures reported its negative effect on haematological and immune factors (Aengwanich, 2007, Al-Sagan et al., 2021). With our findings showing levels similar with the control, this indicates further the efficacy of betaine supplementation in mitigating the adverse effects of dexamethasone induced stress.

Table 3: Blood indices of broiler chickens fed betaine HCl under dexamethasone induced stress

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters	0	0.15	0.15	0.15	SEM	P value
Glucose (mg/dl)	172.56	176.33	176.22	184.00	7.15	0.6939
Cholesterol (mg/dl)	150.40	135.19	131.73	127.94	15.41	0.0668
Triglycerides (mg/dl)	97.30	84.99	84.67	99.49	18.53	0.4254
Total Protein (g/dl)	4.67	4.77	5.40	5.50	0.28	0.1578
PCV (%)	37.74	40.83	39.57	41.89	1.61	0.2593
Haemoglobin (g/dl)	11.60	12.57	12.50	13.06	0.47	0.1912
WBC ($x10^9$/l)	79.54	82.89	85.89	86.31	3.74	0.4360
RBC ($x10^{12}$/l)	2.58	2.71	2.67	2.85	0.14	0.3098
Heterophil(%)	1.93	2.28	1.79	2.19	0.55	0.9012
Lymphocyte (%)	91.46	90.43	90.89	90.78	1.07	0.8883
H:L	0.02	0.03	0.02	0.02	0.01	0.9017

[ab]Means with different superscript on the same row differ significantly ($p<0.05$); SEM: Standard error of the mean; H:L=Heterophil-lymphocyte ratio

Carcass characteristics

Results on carcass cut parts and organs weights of dexamethasone stressed broiler chickens (Table 4) indicate that dexamethasone did not affect ($p > 0.05$) breast weights of broiler chickens fed betaine HCl. It however significantly ($p<0.05$) affected thigh and drumstick weights of broiler chickens despite betaine HCl supplementation.Liver, gizzard, heart and intestine weights were not adverselyaffected by dexamethasone administration.

Kidney weights were significantly (p < 0.05) higher with increasing levels of dexamethasone.Betaine supplementation showed a positive effect on breast weights. Akter et al. (2021) previously reported that dexamethasone decreased the individual weight of breast in broiler chickens. Waldroup and Fritts (2005) reported no improvements in breast meat yield of broilers fed diet containing 0.1% betaine. This disagrees with the reports of the improved breast yield that was reported by McDevitt et al. (2000) and Waldroup et al. (2006) when 0.5% betaine was supplemented in broiler diets.Again these studies didn't induce stress with dexamethasone. While dexamethasone negatively impacts muscle buildup, betaine may improve breast yields under chronic stress situations, comparable to control levels as demonstrated in this study. Dexamethasone has been shown to cause muscular dystrophy in not just breast weights but other carcass cuts like thigh and drumstick as was observed in this study and other works (Aengwanich, 2007; Fappi et al., 2019; Akter et al., 2021).

Table 4: Carcass cut parts and organ weights of broiler chickens fed betaine HCl under dexamethasone induced stress

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters (% LW)	0	0.15	0.15	0.15	SEM	P value
Breast	21.30	21.84	21.39	21.15	0.66	0.8879
Thigh	10.82[a]	9.63[b]	10.06[ab]	9.88[b]	0.23	0.0109
Drumstick	5.27[a]	4.70[b]	4.72[b]	4.63[b]	0.11	0.0025
Organs						
Liver	2.03	2.16	1.95	2.21	0.14	0.5561
Gizzard	2.14	2.51	2.44	2.39	0.10	0.1078
Heart	0.42	0.46	0.41	0.40	0.02	0.2806
Kidney	0.41[b]	0.53[ab]	0.45[ab]	0.61[a]	0.04	0.0201
Intestine	5.31	5.59	5.05	5.86	0.35	0.4124

[ab]Means with different superscript on the same row differ significantly (p<0.05); SEM: Standard error of the mean

Tibia Geometry

Tibia geometric properties (Table 5) show that tibia weights of birds in the control had higher (p < 0.05) tibia weight compared with the other treatment groups which were similar(p > 0.05). For tibia length, birds in the control also recorded the highest (p < 0.05) tibia length with the dexamethasone treated groups being similar. Robusticity and ash indices were similar (p > 0.05) for all groups.A balance between bone formation and resorption is an important mechanism that maintains healthy bone structure and function (Malkawi et al., 2018). Maddahian et al. (2017) stated that betaine supplementation increased tibia length of birds under heat stress. However, Konca et al. (2008) reported tibia length and weight were not improved by dietary betaine as observed in this study. Chronic stress conditions created by dexamethasone may be responsible for this. Dexamethasone acts by suppressing the adrenal glands and reduces sex hormone production which inhibits osteoblast activity (Xia et al., 2014). Robusticity index is a measure of bone strength, and a low robusticity index indicates a strong bone structure. Rath et al. (2000) found that dexamethasone decreased bone strength of turkeys. With robusticity unaffected across all treatment groups,betaine supplementation may have a positive effect on bone strength.

Table 5: Tibia geometry of broiler chickens fed betaine HCl under dexamethasone induced stress

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Tibia Compositions	0	0.15	0.15	0.15	SEM	P value
Tibia weight (g)	6.36^{a}	5.03^{b}	4.58^{b}	4.49^{b}	0.28	0.0004
Tibia length (cm)	9.50^{a}	8.32^{b}	8.20^{b}	8.32^{b}	0.11	0.0001
TWLI (g/cm)	0.67^{a}	0.60^{ab}	0.56^{b}	0.54^{b}	0.03	0.0158
Robusticity index (cm/g^{3})	5.15	4.86	4.94	5.04	0.07	0.0576
Ash (%)	35.74	40.92	43.52	35.32	3.61	0.3587

^{ab}Means with different superscript on the same row differ significantly (p<0.05); SEM:

Standard error of the mean; TWLI= Tibia weight/length index

4. CONCLUSION

Betaine HCl under chronic stress conditions had positive effect on thermoregulatory and serum indices, including some carcass indices of broiler chickens including maintaining bone strength. However, supplementation levels will need to account for chronic stress conditions to maintain optimum growth performance. Current nutritional strategies to combat the

challenge of rising global temperatures exacerbated by climate change will need to be reevaluated and remodeled to meet these new challenges.

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