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## INFLUENCE OF DIETARY BETAINE ON HAEMATOLOGY AND SERUM BIOCHEMISTRY OF HEAT-STRESSED BROILER CHICKENS

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### ABSTRACT

Heat stress has been negatively implicated in the physiological changes of farm animals, including respiratory alkalosis, endocrine problems, electrolyte imbalance among others. Betaine has been used to mitigate the impact of high ambient temperatures on the performance and health status of broilers. This study investigated the haematological and serum biochemical response of broilers fed different levels of dietary betaine for 56 days during dry season using 180 day-old broiler chicks randomly assigned to five dietary treatments with 36 birds per treatment, each treatment was replicated thrice with 12 birds per replicate in a completely randomized design. Treatment 1 was the control diet without betaine while diets 2, 3, 4 and 5 contained 1, 2, 3, and 4g of betaine/kg of feed respectively. Results revealed that haematological parameters of the birds were not significantly affected by dietary betaine, except platelet which was significantly affected ( $p < 0.05$ ) with increasing level of betaine. Chickens on diet 5 had significantly superior ( $p < 0.05$ ) platelet counts ( $165.50 \times 10^3/\mu\text{L}$ ) when compared with those on other diets. Platelet counts in chickens on  $T_4$  ( $147.00 \times 10^3/\mu\text{L}$ ),  $T_3$  ( $135.50 \times 10^3/\mu\text{L}$ ) and  $T_2$  ( $130.50 \times 10^3/\mu\text{L}$ ) were similar. Serum albumin concentrations were significantly higher ( $p < 0.05$ ) in broilers in other treatments compared with those on control diet. Chickens on treatment 3 had superior ( $p < 0.05$ ) albumin value of 1.55 compared with those on other diets. Albumin in treatments 2, 4, and 5 were statistically similar. Serum electrolytes were significantly reduced ( $p < 0.05$ ) as dietary betaine increased. Other serum parameters were not significantly ( $p > 0.05$ ) affected.

**Keywords:** Betaine, haematology, Broilers, Serum Biochemistry, Heat stress

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### INTRODUCTION

The poultry sub-sector in Nigeria employs about 14 million Nigerians in direct and indirect employment (Alawode, 2015). It has proven to be one of the best and most important aspect of farming and also a very profitable business opportunity for job seekers. The purpose of breeding and raising broiler chickens is to produce meat (Damerow, 2019). They are developed and raised to grow quickly so that they can produce more tender meat than native birds and reach a market weight of 1.8 to 4 kg in about 6 to 8 weeks of age. In addition to having a low degree of activity, they have good feed conversion efficiency. This is possible if there is a conducive environment and also if high grade nutrients are supplied. Broiler industry is a source of nutrition, provides rural employment and fulfills all nutritional standards. Therefore, chickens and eggs are inexpensive sources of protein among all animal products.

However, the inability of chickens to discharge body heat due to their feather covering and sparse sweat glands makes them particularly susceptible to thermal stress (Brugaletta *et al.*, 2022). One major obstacle to the production of poultry is heat stress, particularly during the dry and hot months of the year. Heat stress has a negative impact on physiological changes of farm animals, including respiratory alkalosis, endocrine problems, electrolyte imbalance, and systemic immunological dysregulation (Rostagno, 2020).

Utilizing betaine is one way to mitigate the impact of high ambient temperatures on the performance of broiler chicken. In hot or thermoneutral temperatures, betaine regulates gut health, prevents heat and osmotic stress, and increases feeding effectiveness in animal production (Lehr, 2021). It was reported that when betaine was introduced to the diet of broiler chicks, their weight gain and feed conversion ratio rose, and their rectal temperatures decreased from 43.2 to 41.9°C (Attia *et al.*, 2009),

but available information on the impact of betaine on the health status of broilers are scanty, particularly in Nigeria. This study therefore aimed at investigating the influence of dietary betaine on the haematology and serum biochemical parameters of broiler chickens.

## MATERIALS AND METHODS

A total of 180 1-day-old broiler chicks of the Arbor Acres strain were used for the experiment that lasted for 56 days. Five dietary regimens with a total of 36 birds each were given to the chicks. Twelve (12) chicks per replicate made up of three (3) replicates of each treatment. A completely randomised experimental design (CRD) was used. The treatments consisted of five different trial diets. Both the starter and finisher phases used the betaine as an additive. Broiler medication regimens were meticulously followed throughout the research. Table 1 displays the overall composition of the experimental diets for both stages before the addition of betaine. The first treatment was a control diet devoid of betaine, while the remaining four treatments contained betaine at doses of 1, 2, 3, and 4g, respectively, per kilogram of feed.

### Formulation of Experimental Diets

Betaine was sourced for from Agrited Limited in Ibadan, Nigeria and used for this experiment at the following inclusion levels:

**Treatment 1:** Control diet (without betaine)

**Treatment 2:** Control diet + 1g of betaine/kg of diet

**Treatment 3:** Control diet + 2g of betaine/kg of diet

**Treatment 4:** Control diet + 3g of betaine/kg of diet

**Treatment 5:** Control diet + 4g of betaine/kg of diet

**Table 1. Composition of Experimental Diets (g/100kg)**

<b>Ingredients</b>	<b>Starter</b>	<b>Finisher</b>
Maize	53.2	58.1
Soyabean meal	19.5	13.0
Groundnut cake	12.0	18.0
Fish meal	5.0	3.5
Di-calcium phosphate (195)	5.0	0.2
Limestone	1.0	2.0
Corn bran	3.5	4.5
Vitamin-mineral premix	0.3	0.25
Methionine	0.1	0.1
Lysine	0.1	0.1
Common Salt	0.3	0.25
<b>Total</b>	<b>100</b>	<b>100</b>
<b>Calculated Nutrients</b>		
Energy (MEKcal/kg)	3000	3100
Crude protein (%)	23	20
Crude fibre (%)	5	8

### Collection of blood samples

A random selection was made of three birds from each treatment after the feeding. Blood was drawn from the wing vein using a 2 ml needle syringe, placed into vacutainer vials containing ethylene diamine tetra acetic acid (EDTA), covered, and gently mixed for roughly a minute by repeated inversion for haematological testing. Packed cell volume, red blood cell, white blood cell, platelet, haemoglobin concentration, mean cell volume, mean cell haemoglobin concentration, and differential counts of white blood cells were among the haematological parameters examined. The serum was decanted for use in biochemical studies after a portion of the blood collected from the wing vein was

placed in a second set of vacutainer bottles without EDTA and let to stand in a slanting position for around 6 hours. Total protein, albumin, globulin, cholesterol, total glucose, creatinine, aspartate aminotransferase, alanine amino transferase, alkaline phosphatase, catalase, malondialdehyde and superoxide dismutase were examined.

### Hormonal Assay

The tube-based enzyme immune assay (EIA) approach was used to conduct the test for hormonal parameters in the blood as stated in the Kit's instructions of BioCheck ELISA Assay, USA (Micaleft *et al.*, 1995). Thyroxine and Corticosterone were the hormones evaluated.

### Electrolytes Panel

The electrolyte panel was used to assess the blood's levels of calcium, sodium, potassium, and phosphorus.

### Statistical Analysis

Analysis of variance (ANOVA) techniques from Minitab (2007) statistical software version were used to statistically evaluate all the data collected. When the group standard errors of means were significant, the Duncan Multiple Range Test (DMRT) was employed to compare the treatment means with the group averages at 0.05.

## RESULTS AND DISCUSSION

Table 2 shows the haematological characteristics of broilers fed diets supplemented with betaine.

While dietary betaine did not significantly ( $p>0.05$ ) affect the packed cell volume, haemoglobin, white blood cells, red blood cells, lymphocytes, monocytes, eosinophils, heterophils, or basophil components in the blood of experimental broilers, statistical analysis of the data revealed a significant increase ( $p<0.05$ ) in the platelet count of the chickens examined. When compared to broilers receiving other treatments, broilers receiving treatment 5 showed considerably higher ( $p<0.05$ ) platelet counts of  $165.50 \times 10^3/\mu\text{L}$ . The platelet counts in T2, T3, T4 and T5 were statistically similar ( $p>0.05$ ).

**Table 2: Haematology Indices of Broiler Chickens fed Varied Levels of Dietary Betaine**

Parameters	Betaine Levels					SEM
	T <sub>1</sub> Control	T <sub>2</sub> 1g/kg	T <sub>3</sub> 2g/kg	T <sub>4</sub> 3g/kg	T <sub>5</sub> 4g/kg	
RBC( $\times 10^6$ ) uL	2.69	3.04	3.08	2.84	2.87	0.04
HB(g/dL)	9.15	9.65	9.85	9.90	9.92	0.14
PCV(%)	28.00	30.00	31.00	32.00	32.00	0.38
Platelets( $\times 10^3$ ) uL	129.50 <sup>b</sup>	130.50 <sup>b</sup>	135.00 <sup>b</sup>	147.00 <sup>ab</sup>	165.50 <sup>a</sup>	2.27
WBC( $\times 10^3$ ) uL	15.40	15.78	17.50	14.05	15.00	0.12
BAS%	0.00	0.50	0.00	1.00	0.00	0.02
EOS%	2.50	4.50	4.50	3.00	4.50	0.22
MON%	4.50	2.50	2.50	3.50	2.00	0.06
HET%	31.00	30.00	24.00	43.00	36.50	0.50
LYM%	62.00	62.00	69.00	49.50	57.00	0.66
MCHC(%)	32.67	32.17	32.77	30.94	31.00	0.02
MCH (pg)	34.01	31.74	31.98	34.86	32.57	0.01
MCV ( $\mu^3$ )	10.41	9.87	10.05	11.27	11.15	0.02

a, b, means on the same row with different superscripts are statistically different ( $p<0.05$ ).

SEM = Standard Error of Means. WBC = White Blood Cells, Packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (HB), Lymphocyte (LYM), Monocyte (MON), Eosinophil (EOS), Basophil (BAS), Heterophils (HET), Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), Mean Cell Volume (MCV).

The haematological parameters of the birds indicate that their health status was not compromised by the dietary betaine. The platelet counts were significantly higher in experimental chicken compared to

control birds. As a result, it suggests that chicken fed a diet enriched with betaine could be considered haemorrhage-free.

Table 3 shows the effects of betain on serum parameters of broiler chicken. Broilers fed diets containing dietary betaine exhibited albumin concentrations that were considerably greater than those of broilers fed diets lacking dietary betaine. This is in line with the findings of Pack and Kim (2019), who discovered that broilers fed diets high in betaine (1.2g/kg) compared to broilers fed diets low in betaine had higher total serum and albumin concentrations. The ability of betaine to donate methyl groups, which is a characteristic that is generally consistent in protein metabolism, may help to explain this. Results also show that broilers fed diets containing betaine had concentrations of SOD, AST, ALT, ALP, catalase, and MDA that were noticeably greater than those of broilers on diets devoid of dietary betaine (Table 4). According to Yang *et al.* (2022), betaine boosted total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, decreased hydroxyl radical production, and increased the total antioxidant capacity's (T-AOC) activity in the chest muscle. Feeding betaine increased levels of SOD, glutathione, and GSHPx activities but decreased levels of malondialdehyde (MDA) in the pectoral muscles of broilers under heat stress (Saleh *et al.*, 2024). However, MDA levels in broilers serum fed nutritional betaine in this study were significantly greater than those in the control group. An earlier study (Alirezai *et al.*, 2012) assumed that the higher ability of betaine to scavenge free radicals was responsible for the antioxidant effects of betaine. Antioxidant levels were higher in the betaine treatments in the current investigation. Betaine possesses antioxidant properties which as a result, may protect the organ systems and cells of broiler chickens from oxidative damage. Also, broiler hormone concentrations of thyroxine and corticosterone were reportedly higher and lower, respectively (Table 5). This indicates that supplementing feeds with betaine at a rate of 1 g/kg and above will result in an increase in thyroxine and a decrease in corticosterone in the birds. According to Singh *et al.* (2015), supplementing chickens with betaine can boost their thyroid activity. In hot conditions, the thyroid hormone's activity decreases, which has an adverse effect on bird growth performance. Betaine will help reduce the elevated levels of corticosterone hormone that heat stress causes in chicken blood. Statistical examination of the data revealed a considerable reduction in the blood calcium, sodium and potassium levels in the experimental chickens. The results of this study disagree with those of Willingham *et al.* (2020) and Park and Kim (2019), who reported that betaine may alleviate heat stress by maintaining blood electrolyte levels and stabilizing osmotic pressure. When the amount of betaine was increased in this investigation, the electrolytes decreased rather than increasing. The environment, a feed ingredient, the type of bird, the geography (temperate or tropical), the animal's genetic makeup, and betaine sources are only a few of the many elements that may be the causes for decreased electrolytes synthesis.

**Table: 3. Serum Metabolites of Broiler Chickens fed Varied Levels of Dietary Betaine**

Parameters	Betaine Levels					SEM
	T <sub>1</sub> Control	T <sub>2</sub> 1g/kg	T <sub>3</sub> 2g/kg	T <sub>4</sub> 3g/kg	T <sub>5</sub> 4g/kg	
Total Protein (g/dL)	3.59	3.59	3.83	3.85	3.89	0.07
Albumin (g/dL)	1.49 <sup>b</sup>	1.50 <sup>b</sup>	1.55 <sup>a</sup>	1.50 <sup>b</sup>	1.47 <sup>b</sup>	0.01
Globulin (g/dL)	2.10	2.09	2.28	2.35	2.42	0.06
Triglyceride (mg/dL)	88.53	113.94	123.45	121.31	105.74	3.66
CHOL (mg/dL)	143.97	147.47	130.35	127.24	104.66	2.59
Glucose (g/dL)	195.31	198.92	200.36	195.32	151.26	2.89
Creatinine (mg/dL)	1.20	1.35	1.20	1.25	1.25	0.01

a, b, means on the same row with different superscripts are statistically different (p.0.05)

SEM=Standard Error of Means. CHOL=Cholesterol

**Table 4: Serum Enzymes and Antioxidant properties of Broiler Chickens fed Varied Levels of dietary Betaine**

Parameters	Betaine Levels					SEM
	T <sub>1</sub> Control	T <sub>2</sub> 1g/kg	T <sub>3</sub> 2g/kg	T <sub>4</sub> 3g/kg	T <sub>5</sub> 4g/kg	
AST (i. u/L)	147.11	104.04	140.57	145.25	152.87	2.49
ALT (i. u/L)	24.01	24.50	25.00	25.20	25.60	0.81
ALP (i. u/L)	27.38	29.04	45.23	63.09	64.55	3.46
SOD (u/mL)	135.16	140.69	146.17	135.41	151.30	0.67
MDA (u/mL)	28.55 <sup>b</sup>	28.60 <sup>b</sup>	30.99 <sup>ab</sup>	32.20 <sup>a</sup>	33.37 <sup>a</sup>	0.09
Catalase (u/mL)	171.45	176.17	175.77	176.11	189.98	1.04

a, b, means on the same row with different superscripts are statistically different ( $p < 0.05$ ).

SEM=Standard Error of Means. AST= Aspartate amino transferase, ALT=Alanine amino transferase, ALP=Alkaline Phosphatase, SOD=Superoxide dismutase, MDA=Malondialdehyde.

**Table 5: Hormonal Responses of Broiler Chickens fed Varied Levels of Dietary Betaine**

Parameters	Betaine Levels					SEM
	T <sub>1</sub> Control	T <sub>2</sub> 1g/kg	T <sub>3</sub> 2g/kg	T <sub>4</sub> 3g/kg	T <sub>5</sub> 4g/kg	
Thyroxine (iu/L)	62.50	64.00	64.50	66.00	67.00	0.62
Corticosterone (iu/L)	13.25	13.00	12.10	11.50	11.00	0.24

SEM=Standard Error of Means.

In Table 6, the serum electrolytes of chickens fed diets containing betaine are shown.

Statistical examination of the data revealed a considerable reduction in the blood calcium, sodium and potassium levels in the experimental chickens. The results of this study disagree with those of Nutautaite *et al.* (2020) who reported that betaine may alleviate heat stress by maintaining blood electrolyte levels and stabilizing osmotic pressure. When the amount of betaine was increased in this investigation, the electrolytes decreased rather than increasing. The environment, a feed ingredient, the type of bird, the geography (temperate or tropical), the animal's genetic makeup, and betaine sources are only a few of the many elements that may be the causes for decreased electrolytes synthesis.

**Table 6: Serum Electrolytes of Broiler Chickens fed Varied Levels of Dietary Betaine**

Parameters	Betaine Levels					SEM
	T <sub>1</sub> Control	T <sub>2</sub> 1g/kg	T <sub>3</sub> 2g/kg	T <sub>4</sub> 3g/kg	T <sub>5</sub> 4g/kg	
Calcium (mmol/L)	11.20 <sup>a</sup>	6.50 <sup>b</sup>	6.16 <sup>b</sup>	7.71 <sup>b</sup>	7.82 <sup>b</sup>	0.06
Sodium (mmol/L)	119.27 <sup>a</sup>	119.20 <sup>a</sup>	117.90 <sup>ab</sup>	114.54 <sup>b</sup>	114.55 <sup>b</sup>	0.12
Potassium(mmol/L)	50.83 <sup>a</sup>	49.84 <sup>a</sup>	46.49 <sup>b</sup>	46.70 <sup>b</sup>	45.77 <sup>b</sup>	0.07

a, b, means on the same row with different superscripts are statistically different ( $p < 0.05$ ).

SEM = Standard Error of Means.

In conclusion, the results of this study suggest that employing synthetic betaine HCL at 2g of betaine/kg of feed as a method to improve broiler performance under heat stress is a viable option which did not compromise the health status of the birds. The albumin concentration and platelet levels in the birds subjected to temperature stress throughout the experimental feed supplementation with betaine HCl enhanced the metabolic processes, and sustained improved development rates. Therefore, to lessen the negative effects of thermal stress on broilers, betaine should be supplemented up to 2g of Betaine/kg of feed.

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