RESEARCH ARTICLE



Antioxidant status, hematology, performance, organ and carcass evaluation of heat-stressed broiler chickens fed with dietary *Bambusa vulgaris* leaf meal

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ABSTRACT

Objectives: The objective of this study was to evaluate the inclusion of bamboo leaf meal (BLM) in broiler chicken feed to alleviate heat stress.

Materials and Methods: This investigation was conducted following institutional policies guiding the handling of animals as approved for scientific research. 150 heat-stressed Arbor Acres broiler chickens were divided randomly into five dietary treatments, T1–T5, of 30 per treatment and 10 per replicate, to determine the consequence of feeding BLM on health and performance. Birds in T1–T4 were fed 0%, 1%, 1.5%, and 2% BLM-included diets, respectively, while T5 had a 0.2% vitamin C-included diet.

Results: Increasing dietary BLM positively impacted body weight gain, feed intake, feed conversion rate (FCR), and stress indices levels. Broiler chickens had better feed intake (267 gm), weight gain (1,504 gm), and FCR (3.64) in T4. Serum glutathione peroxidase and malondialdehyde levels were not statistically different with increasing dietary BLM, while corticosterone levels were lower for chickens fed with dietary BLM. The superoxide dismutase index levels did not follow a particular pattern as dietary BLM increased. The hematology, carcass, and organ quality were unaffected by dietary BLM inclusion.

Conclusion: BLM inclusions up to 2% in the broiler diet ameliorate heat stress conditions and improve performance without imposing any detrimental impact on the birds.

ARTICLE HISTORY

Received December 14, 2022 Revised March 15, 2023 Accepted March 17, 2023 Published April 25, 2023

KEYWORDS

Stress indices; broiler chicken; leaf meal; hematology; serum; *Bambusa vulgaris*



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Introduction

Broiler chickens have assumed great global recognition among poultry meats because the production cycle is short, and the meat is universally acceptable. Broiler chicken is white meat with a high mineral, vitamin, and protein content but a low saturated fatty acid content. Over the decades, genetic tools have been used to develop broiler chickens to meet demands [1], such that broiler chickens now attain a much higher slaughter weight within a shorter rearing period. However, heat stress, especially in the tropics, has constrained the effectiveness and efficiency of broiler chicken production [2]. Heat stress is a physiological state whereby an animal is subjected to very high temperatures, resulting in concurrent physiological difficulties such as low feed intake, impaired digestive functions, poor growth, poor yield, compromised immunity, the spread of diseases, and death [2,3]. The adverse effect of heat stress could be reduced through nutritional interventions; incorporating leaves from different plants in animal feed has shown a positive effect on body weight gain and various health indicators. Plant materials such as *Moringa oleifera*, bamboo, pawpaw, and neem leaf have been used as dietary supplements, antioxidants, or feed additives in broiler chicken production [4–6].

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How to cite: Okeniyi FA, Adeniran BM, Animashahun RA, et al. Antioxidant status, hematology, performance, organ and carcass evaluation of heatstressed broiler chickens fed with dietary *Bambusa vulgaris* leaf meal. *Vet Res Notes*. 2023;3(4):29–35. doi:10.5455/vrn.2023.c25.

The leaves of the bamboo plant, which is native to many parts of the world, have been used in herbal therapy to treat fever, hypertension, renal issues, and epilepsy since ancient times [7], as well as to enhance food flavor, color, and taste [8]. The plant is rich in nutrients, antioxidants, and bioactive compounds vital to the body's physiology and biochemical reactions. Bamboo leaf was reported to increase antioxidant capacity and lower serum cholesterol in hyperlipidemic mice; it increases vascular mobility by regulating endothelial cells, reduces vascular epithelial factor adhesion, scavenges reactive species in vitro, and enhances the effectiveness of antioxidant enzymes in vivo [9]. The bamboo leaf's important biological and therapeutic properties include antioxidant, antimicrobial, anti-inflammatory, anti-helminthic, anti-diabetic, and anti-ulcer properties, which have been confirmed by *in vitro* and *in* vivo studies [9,10].

The heat stress-relieving characteristics of bamboo plants are related to the variety of its chemical components, such as polysaccharides, polyphenols, flavonoids, and sodium chlorophyllin, which are active ingredients in the bamboo leaves with anti-inflammatory and antioxidant properties that could lower lipids, thereby reducing heat stress in both animals and humans [9–11]. The existing research on bamboo leaf usage in broiler chicken production focuses mainly on broiler growth performance and meat quality. There is, however, a scarcity of information on bamboo leaf meal (BLM) usage in alleviating thermal stress in broiler chickens under the hot conditions of the dry season in Nigeria. This study, therefore, investigated the use of BLM as a feed additive in combating heat stress in broiler chickens when exposed to high environmental temperature conditions.

Materials and Methods

All aspsects of the fieldwork and laboratory experiments were performed following the Institutional Policies on the Handling of Animals as approved for scientific research by the Ethical Review Committee, Landmark University, Omu-Aran, Nigeria (LUAC/2021/0016A).

The University's Teaching and Research Farm (poultry section) was the study location. A total of 150 un-sexed broiler chickens at 1 day old were distributed, using a completely randomized design, into five treatments of three replicates each. The birds were given standard vaccinations and medications and kept in an electrically heated pen house with wood shavings as litter material. The birds were fed broiler starter diets for 3 weeks, after which they were fed the experimental broiler finisher diets from week 4 to week 8. The birds were exposed to heat between 12 and 4 pm at 50°C ± 1°C from days 29 to 56. Thermometers were provided in each pen to measure the heat generated. All experimental birds were placed under the same environmental conditions. At the same time, a thermometer was used to monitor the body temperature by insertion into the rectum through the cloaca, with the readings taken within 2 min of insertion. The investigation was carried out following institutional policies on the handling of animals as approved for scientific research.

BLM was incorporated at levels of 0%, 1.0%, 1.5%, and 2.0% into the finisher rations for birds in treatments 1–4, respectively. In comparison, the broiler finisher diet for birds in treatment 5 contained 0.2% (200 mg/kg) vitamin C (Table 1). The proximate analysis of broiler diets was conducted using the AOAC procedure [12], and photochemical screening: alkaloids, phytate, flavonoids, saponins, and tannins were carried out by standard procedure

Ingredients (%)	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% vitamin C (T5)
Maize	64.70	63.70	63.70	63.70	64.50
SBM	30.00	30.00	29.50	29.00	30.00
BM	3.00	3.00	3.00	3.00	3.00
Fish meal	1.50	1.50	1.50	1.50	1.50
Bambusa vulgaris	0.00	1.00	1.50	2.00	-
Vitamin C	-	-	-	-	0.20
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
^a Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100

Table 1. Composition of BLM diets (DI

^aVit A 10,000 IU, Vit D3 1,500 IU; Vit E 10,000 mg, Vit k3, 1,500 mg, Vit B1 1,600 mg, Vit B2 4,000 mg, Nicotinic acid 2.5 mg; Magnesium 56 mg; Pentothenic acid 10 mg Chlorine 3.5 mg; Folic acid 1 mg; Lysine 1 mg; Iron 20 mg; Cobalt 1.25 mg; Zinc 50 mg; Biotin 7.5 mg Copper 30 mg, Manganese 40 mg, Selenium 2 mg Antioxidant 1,250 mg. SBM = Soya bean meal, BM = Bone meal.

[13]. Water and feed leftovers were deducted from the water, and feed was offered each day to determine intake, while each week, individual broiler chickens were weighed before feeding the birds. Data were collected on feed intake, water consumption, and weight gain.

Blood sampling was performed by randomly selecting two birds per replicate on day 56. Blood samples were collected through the brachial veins of the birds. Hematology, superoxide dismutase (SOD), serum glutathione peroxidase (GSH-Px), corticosterone (CORT), and malondialdehyde (MDA) were determined using the method described previously [14,15]. All chemical analyses were done in triplicates. Two birds from each treatment were randomly selected based on the weight of the average group after the trial, fasted overnight, weighed, bled, dressed, and then eviscerated. Carcass parts were weighed, and the values for the dressed weights were expressed as percentages. The means of the samples were compared using analysis of variance, and Duncan's multiple range tests were used to separate the means. In contrast, the Statistical Analytical Software program was used to conduct this analysis (version 9.4).

Results

Based on the results of the experimental diet's proximate composition (Table 2), diet T4 had higher (p < 0.05) crude protein (CP), crude fiber (CF), and ash values (187.21, 375.50, and 110.01 gm, respectively). In contrast, diets T5, T1, and T5 had the lowest (p < 0.05) CF (261.55 gm), CP (135.01 gm), and ash (105.20 gm) values, respectively. The levels of CP, CF, and ash seemed to increase as the level of BLM incorporation in the diet increased. Results of the phytochemical analysis of the diets (Table 2) revealed that the values of phytate, flavonoids, tannin, and saponin increased as the quantity of BLM in the broiler feeds increased. Diet T1 had a low flavonoid content (p >

Table 2. Proximate and phytochemicals composition (g/kg DM) of the BLM diets.

0.05), while diets T4 and T5 had higher but comparable flavonoid contents.

Table 3 shows the performance level of the experimental birds fed BLM diets. The feed intake and weight gains of the birds increased as BLM was increased in the diet; birds on T1 had the lowest (p < 0.05) weight gain and feed intake, while birds fed 2% BLM had the highest (p < 0.05). Water intake was lower for birds fed BLM-included diets; however, water intake was not affected by BLM levels in the diets. The feed conversion rate (FCR) values for all the chickens were similar (p > 0.05), with the best FCR coming from birds on T4. The dietary intake of BLM, as shown in Table 4, did not influence (p > 0.05) the carcass and organ parameters of the birds. Table 5 shows the effect of the varied BLM levels on the rectal temperature of broiler chickens on different days during the period of heat exposure. BLM had varying effects on rectal temperature. The stress indicators were similar (p > 0.05) for serum GSH-Px, CORT, and MDA in the experimental birds (Table 6). However, the ranges of GSH-Px and MDA reported in this study are 226.5-419.6 U/ml and 1.2854-1.7618 nmol/ml, respectively. Serum SOD enzyme is higher (p < 0.05) for birds fed with 0% BLM (4,477.6 U/ml) and 0.20% vitamin C (8,955.2 U/ml) than birds fed other diets.

Discussion

Production of free radicals induced by heat stress results in metabolic disruption and cell death owing to reactive oxygen species (ROS) concentrations that are higher than the antioxidant capacity of animal body cells. Heat stress decreases feed intake and, consequently, weight gain; however, the high phytochemical contents in the BLMincluded diets appear to have positively impacted broiler performance and alleviated heat stress in the birds. This observation agrees with earlier reports [11,16,17]. The weight gains and FCR of the experimental animals were improved by adding BLM, thereby confirming the heat

Proximate	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% vitamin C (T5)	p-value	SEM
СР	135.01°	137.80°	157.20 ^b	187.21ª	160.02ª	0.0122	5.72
CF	350.04 ^b	355.00 ^b	360.01ª	375.50°	261.55°	0.0220	4.95
Ether extract	79.82ª	79.80 ^b	80.70ª	82.80ª	91.05ª	0.0170	1.88
Ash	105.20°	107.57 ^b	109.57ª	110.01ª	107.24 ^b	0.0203	1.76
Phytochemical							
Phytate	39.93°	40.22 ^b	42.99ª	43.02ª	40.11 ^b	0.0081	4.00
Flavonoids	50.99°	70.64 ^b	72.85 ^b	74.90ª	75.10ª	0.0254	2.32
Saponin	20.90 ^c	21.82°	38.95ª	39.31ª	36.05 ^b	0.0040	3.92
Tannin	0.15°	1.20 ^b	4.30ª	4.32ª	0.99 ^b	0.0045	0.17

a.b.cAcross dietary treatments, means with varied superscripts differ significantly (p < 0.05). SEM = Standard error of the mean.

Table 3.	Productivity	of broiler	chickens fee	diets	containing BLM.
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Parameters	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% Vit. C (T5)	p-value	SEM
Initial weight (gm)	630.00	623.33	633.33	622.22	620.00	0.7136	1.22
Final weight (gm)	1,714.17°	1,882.04 ^{bc}	1,999.26ªb	2,125.23ª	2,087.04ª	0.0040	3.39
Daily WG (gm)	51.943°	59.463 ^{bc}	65.203 ^b	71.573ª	69.857ª	0.0027	1.58
Total WG (gm)	1,074.17°	1,248.71 ^{bc}	1,355.93ªb	1,503.01ª	1,457.04ª	0.0027	3.32
Average FI (gm)	235.02 ^b	255.99ª	259.25ª	266.66ª	260.93ª	0.0007	0.82
Average WI (I)	4.09ª	3.98 ^b	4.02 ^b	3.92 ^b	4.13ª	0.0047	0.10
FCR (%)	3.95 ^b	4.54ª	4.50ª	3.64 ^b	3.74 ^b	0.0046	0.18
Mortality (%)	0.93	1.97	0.93	2.09	2.78	0.7485	0.48

a.b.c. Across dietary treatments, means with varied superscripts differ significantly (*p* < 0.05). Standard error of mean = SEM. Weight gain = WG, feed intake = FI, water intake = WI, feed conversion ratio = FCR.

Table 4. Relative organs and carcass evaluation of broiler chickens fed with bamboo leaf meal (BLM) diets.

Parameters (gm)	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% Vit. C (T5)	p-value	SEM
Live weight	1,400	1,600	1,750	1,800	1,650	0.8622	6.65
Carcass weight	747.4	857.5	965.5	974.7	913.4	0.8195	4.90
Dressing %	53.49	53.90	54.17	55.69	55.15	0.9747	0.67
Head	3.020	2.985	2.765	2.955	2.995	0.6213	0.14
Neck	6.040	5.955	6.220	6.605	6.045	0.5877	0.22
Shank	4.240	3.930	4.035	4.535	4.135	0.2654	0.17
Back	10.650	10.88	11.37	11.56	11.46	0.6215	0.28
Breast	20.73	20.54	20.50	20.60	20.41	0.9987	0.36
Drumstick	12.21	11.90	11.51	12.26	11.07	0.3418	0.26
Thigh	11.64	11.78	11.88	12.24	11.95	0.7825	0.23
Wing	8.455	9.235	8.860	9.420	8.335	0.2504	0.24
Liver	3.060	3.180	3.115	3.215	3.165	0.2306	0.09
Intestine	132.6	133.1	141.7	155.1	152.7	0.0257	0.53
Heart	0.565	0.560	0.565	0.565	0.570	0.9849	0.05
Caecum	0.965	0.895	1.035	1.055	1.015	0.5907	0.11
GPC	3.875	3.915	3.945	3.920	3.870	0.6929	0.09

GPC = Gizzard, proventriculus, crop, SEM = Standard error of the mean.

Periods	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% Vit. C (T5)	<i>p</i> -value	SEM
Day 1	41.10 ^a	40.67 ^{ab}	40.03 ^{bc}	40.17 ^{bc}	39.87°	0.0185	0.21
Day 4	41.10 ^a	40.70 ^{ab}	40.03 ^b	40.50 ^{ab}	40.00 ^b	0.0477	0.22
Day 8	41.88	40.72	40.70	41.25	41.33	0.0612	0.23
Day 12	42.18ª	40.72°	40.75°	41.87ª	41.42 ^b	<0.0001	0.16
Day 16	41.17°	41.67ª	41.48 ^b	41.74ª	41.65ª	0.1323	0.17
Day 20	42.95°	42.95°	42.90ª	42.52 ^b	42.95°	0.0223	0.13
Day 24	43.00	42.97	42.98	42.93	42.98	0.3882	0.07

^{a,b,c}Across dietary treatments, means with varied superscripts differ significantly (p < 0.05). SEM = Standard error of the mean.

stress-alleviating effect of bamboo leaf due to the presence of phytochemicals and other antioxidants in the leaf. The bamboo leaf contains bioactive compounds [10]; these antioxidants and phytochemicals enhance growth,

Table 6. Hematology and stress indices level of experimental chickens fed with BLM diets.

Parameters	0% BLM (T1)	1% BLM (T2)	1.5% BLM (T3)	2% BLM (T4)	0.2% Vit. C (T5)	p-value	SEM
RBC (×10 ¹² /l)	2.44	1.40	2.04	2.25	2.39	0.3915	0.25
Hgb (gm/dl)	9.75	4.85	7.65	8.25	9.80	0.3079	0.51
MCV (fl)	133.2	132.2	141.9	133.2	133.3	0.7543	0.96
MCH (gm/dl)	39.95	33.20	37.20	36.50	41.00	0.1526	0.55
MCHC (gm/dl)	30.00	25.00	26.25	27.45	30.85	0.1426	0.49
PCV (%)	32.50	18.90	28.80	30.20	31.85	0.5051	0.95
WBC (×10 ⁹ /l)	256.5	232.8	240.0	259.0	273.1	0.5617	1.67
GSH-Px (U/ml)	244.0	301.7	226.5	230.3	419.6	0.6609	4.1
MDA (nmol/ml)	1.747	1.531	1.742	1.762	1.285	0.6818	0.21
SOD (U/ml)	8,955ª	1,493°	1726°	1,866°	4,478 ^b	0.0019	9.81
CORT (U/ml)	2.583	1.732	1.717	3.487	1.525	0.0548	0.25

a-b-cAcross dietary treatments, means with varied superscripts differ significantly (p < 0.05), SEM = Standard error of the mean, WBC = White blood cell, PCV = Packed cell volume, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration, MCV = Mean cell volume, Hgb = Hemoglobin, RBC = Red blood cell, GSH-Px = Glutathione peroxidase, SOD = Superoxide dismutase, MDA = Malondialdehyde, CORT = Corticosterone.

reduce the risk of chronic diseases, stimulate the immune response, and have antibacterial effects. Phytochemicals function as antioxidants by neutralizing free radicals and their cell-damaging effects. The benefits derived from bamboo leaves have been linked to the presence of flavonoids, particularly isoorientin, orientin, isovitexin, and vitexin [18]. These results reveal the positive effects of the bamboo leaf since it possesses a wide range of pharmacological potentials such as anti-inflammatory, anti-cancer, and antioxidant properties. It was reported that bamboo leaf extract exerts potent antioxidant activity, such as free radical scavenging, and inhibits low-density lipoprotein oxidation [9].

The increase in feed intake and weight gain with increasing BLM in the diet is of positive value since the inclusion of BLM at 2% improved the appetite of the broiler chickens with a resulting increase in weight gain compared with and better than results from broiler chickens fed with a 0.2% vitamin C-included diet. This finding concurs with a study by He et al. [15], who reported that flavonoids in bamboo leaves improved gains in body weight by over 17% in broiler chicken diets. It was speculated that flavonoids would behave like animal growth hormones since aglycone hydroxyl groups in flavonoids are positioned in the same manner as estrogen's hydroxyl groups [16]. This study indicated that dietary inclusion of BLM at a 2% level is efficient, and this may be the result of an increase in the diet's flavonoid concentration. Earlier reports [17] showed that when birds suffer from challenges such as heat stress and immune suppression, their growth performance is affected. Also, Kimura et al. [18] demonstrated that supplementing broiler chicken diets with bamboo leaf flavonoids at a concentration of 1.6% mitigated the growth-inhibiting effects of heat stress and immunological suppression in broiler chickens. It could therefore be inferred that flavonoids in bamboo leaves are responsible for the observed growth improvement in this study. Livestock is more effective at converting feed into food when the FCR is lower. Under stressful environmental conditions, broiler chicken diets are often supplemented with vitamin C to improve productivity traits, including FCR. The better FCR of the birds fed the diet containing 2% BLM is comparable with that of those on a diet containing 0.2% vitamin C; this observation suggests that BLM enhanced the FCR of the experimental animals, thereby corroborating a previous report [11]. This observation implies that BLM supplementation can prevent the growth-suppressing consequences of heat stress in broiler chickens. The inclusion of BLM in the diets fed to the experimental birds in this study, regardless of the inclusion level, did not affect the mortality rates across the treatment groups, suggesting that BLM is suitable for the birds' survivability.

The hematology values for the experimental birds were within the reported normal range of values for healthy broiler chickens [19,20]. The hematology profile reflects an animal's health and physiological status; these are affected by its genetics, environment, and nutrition [21]. Hence, hematological values could indicate an animal's nutritional health. The inclusion of BLM in the feed of the broiler chickens in this study did not have a detrimental effect on their health. The serum GSH-Px, CORT, and MDA levels in the experimental birds were an index of their adaptability to heat stress. GSH-Px, found within the mitochondria, cytosol, and extracellular space, is an antioxidant that effectively catalyzes hydrogen peroxide, prevents lipid peroxidation in the cell membrane, and binds free radicals. The reduced glutathione reacts with H_2O_2 in the presence of GSH-Px, which might be dose-dependent since it varies, in the present study, with BLM levels in the diets. An earlier report [22] showed that bamboo leaf extract improved serum and liver glutathione enzyme activity in rats. The flavonoid content of the BLM in the experimental diets can stimulate antioxidant enzymes, chelate metal catalysts, transfer electrons to free radicals, and suppress oxidases. These abilities are responsible for the antioxidant effects of bamboo leaf flavonoids in animal cells [23]. Consequently, the flavonoid content of the BLM-included diets had positive impacts by improving the antioxidant enzyme activities within the animal cells, confirming the peroxide scavenging capacity of the bamboo leaf.

Heat stress conditions can activate the hypothalamic-pituitary-adrenal axis, eliciting an array of responses such as body temperature alteration, water and feed intake regulation, and endocrine changes, including an increase in serum CORT [11,24]. The CORT levels in the present study were reduced in the BLM-fed chickens; this observation reveals that BLM flavonoids modulated serum CORT levels in the experimental birds under heat-stress conditions. The MDA levels might inadvertently reflect lipid peroxidation as the level of ROS rises due to diminished antioxidant protection. The generally low concentration of serum MDA in the present experimental birds may be due to the experimental diets' capacity to offer sufficient antioxidant protection against lipid peroxidation in the broiler chickens during exposure to heat stress conditions. The phytochemicals in BLM may have enhanced the ability of the broiler chickens to adequately adapt during heat stress by increasing the oxidative stability of the birds. The improved MDA observed agrees with the observations of Cao et al. [11] and AOAC [22], indicating that bamboo leaves decreased serum MDA levels and increased the antioxidant capacity of birds.

SOD protects cells from the damaging effects of the superoxide anion and mutates the superoxide radicals to form molecular oxygen and hydrogen peroxide [25]. The levels of SOD observed in birds fed BLM in the present study reflect an improvement in the oxidative status of the chickens. Lv et al. [26] reported decreased SOD levels in the liver and kidney of cholesterol-fed rats after administering bamboo leaf extract. Cao et al. [11] demonstrated that bamboo leaves could remarkably raise the SOD enzyme levels in birds exposed to heat stress, enhancing the antioxidant potential. BLM included in the broiler diets provides a protective mechanism against heat stress by decreasing free radicals rather than increasing antioxidant enzyme activity. The bamboo leaf plays the role of an antioxidant by preventing the chain reactions of lipid peroxidation in the process of metabolism; therefore, the need for antioxidant enzymes will be less when lipid peroxidation products are fewer. It was assumed that increasing BLM levels in the experimental diets would increase their antioxidant capacity since bamboo leaf has a peroxide scavenging capacity. In addition, other studies using other plant materials have demonstrated that broilers given plant extracts high in flavonoids, polyphenols, and polysaccharides can increase their capacity to scavenge free radicals [7]. We suggest that more studies be carried out on this subject; in particular, a non-heat stressed condition should be included to investigate the mode of action of the test ingredient; also, other higher dose-response studies are needed to determine the optimum requirement and likely effect if exceeded.

Conclusion

This study concludes that the inclusion of BLM at 2% in the broiler feed could alleviate heat stress and have no damaging consequences on the birds' health. Furthermore, broiler chickens fed 2% BLM inclusion showed improvement in growth performance despite being reared under heat-stress conditions. Leaf meal from the *Bambusa vulgaris* bamboo species has an inhibitory effect on lipid peroxidation and excellent anti-oxidant characteristics.

Acknowledgment

The authors acknowledge the assistance of the Landmark University Teaching and Research Farm staff during the fieldwork. Also, we recognize the assistance of the Landmark University Animal Science laboratory staff, rendered during the laboratory studies.

List of abbreviations

CP, crude protein; CF, crude fiber; CORT, Corticosterone; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SAS, Statistical Analytical Software; SOD, superoxide dismutase; ROS, Reactive oxygen species.

Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

FAO and BMA conceived, designed, and led the research work. BMA and FAO analyzed the data, interpreted the results, and developed and prepared the manuscript. BMA, RAA, RCO and FAO carried out the experiments. OEO, OOA, SOO, EOB, AJS, and FAO revised the manuscript.

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