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(Cont'd)

Response of Broiler Chickens Exposed to Heat Stress, and fed with Dietary Inclusion of *Bambusa Vulgaris* Leaf Meal

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ABSTRACT
A fifty-six (56) days study was conducted to determine the effect of varying levels of *Bambusa vulgaris* leaf meal (BLM) inclusion in the diets of broiler chickens exposed to heat stress. A total of one hundred and fifty Arbor Acres broilers were allotted randomly into five (5) dietary treatments (T1-T5) of thirty per treatment and ten per replicate. T1, T2, T3, and T4 were fed 0%, 1%, 1.5%, and 2% BLM included diets respectively, while T5wase fed 200g/mg of Vitamin C included diet. The experimental birds were exposed to a temperature of 50±1°C between the hours of 12-4pm daily for four weeks. Data collected on growth performance, and stress indicators were subjected to one way analysis of variance. The result showed that birds on T4 had the highest ($p<0.05$) feed intake (267g), body weight gain (1504g), and the best FCR (3.64). The serum corticosterone (3.4865U/ml) and glutathione peroxidase (230.3U/ml) levels decreased with an increase in BLM inclusion in the diet with birds on T4 having the lowest ($p<0.05$) values. However, malondialdehyde and superoxide dismutase values increased with an increase in BLM inclusion in the diets with birds on T4 having the highest values (1.7618nmol/ml and 1865.7U/ml respectively). In conclusion, *Bambusa vulgaris* leaf meal inclusion up to 2% in the broiler diet could ameliorate heat stress conditions and improved broiler chicken performance.
Keywords: Heat stress, Broiler chicken, Bamboo leaf, Stress indicators, Serum

INTRODUCTION
Among poultry meats, broiler chickens have assumed great global recognition (Seifi *et al*., 2018) because the production cycle is short and the meat is universally acceptable with little or no restrictions. Over the decades, genetic tools have been used to develop broiler chickens to meet demands (Athrey, 2020) however, heat stress especially in the tropics has constrained the effectiveness and efficiency of broiler chicken production (Sugiharto *et al*., 2017a). Heat stress is a physiological state whereby an animal is subjected to temperatures that are higher than 30 ^{0}C (Seifi *et al*., 2018) resulting in concurrent physiological difficulties, poor growth, the spread of diseases, and death (Sugiharto *et al*., 2017b). Authors have used plant materials like *Moringa oleifera* (Mona *et al.* 2017), bamboo, pawpaw, and

neem leaf (Oloruntola *et al.* 2018) as a dietary supplement, antioxidant or as a feed additive in broiler chicken production.
The bamboo plant found in many parts of the world, and used culinary and medicinal purposes for long, contains antioxidants and free radical scavenging properties (Shen *et al.*, 2019). Research has shown that *bamboo* leaf contains polysaccharides, polyphenols, and flavonoids, these active ingredients contain anti-inflammatory and antioxidant properties which could lower lipids, thereby reducing heat stress both in animals and in humans (Yu *et al.,* 2019). Little research has been carried out on the use of bamboo leaf meals to alleviate heat stress in broiler chickens, therefore, this study investigated the use of bamboo leaf meal as a feed additive in combating heat stress in broiler chickens.

MATERIALS AND METHOD

The experiment was carried out at the poultry unit of the Teaching and Research Farm of Landmark University, Omu-Aran Kwara state, Nigeria. A total of one hundred and fifty (150) day old unsexed broiler chickens were distributed, using a completely randomized design (CRD), into five treatments of 3 replicates each. The birds were kept in an electrical heated pen house on deep litter with wood shavings as litter material, they were given standard vaccinations and medications. The birds were fed with broiler starter diets for three weeks after which they were fed the experimental broiler finisher diets from week four to week eight. Birds were exposed to excess heat between 12pm-4pm at 50 ± 1^0C on days 29-56. Thermometers were provided in each pen to measure the heat generated. All experimental birds were placed under the same environmental condition. Bamboo leaf meal (BLM) was included into the broiler finisher diets at 0%, 1.0%, 1.5% and 2.0% inclusion levels for birds in treatments 1, 2, 3 and 4, respectively, while the broiler finisher diet for birds in treatment 5 contained 0.2% (200mg/kg) vitamin C and fed to the birds **as** shown in Table 1.
The proximate analysis of broiler diets was conducted using the AOAC procedure (AOAC, 2005), photochemical screening: alkaloids, phytate, flavonoid, saponin, and tannin were carried out by standard procedure of Kokate, (1994). All chemical analyses were done in triplicates. On day 56, blood sampling was performed by randomly selecting two birds per replicate. The collection of blood samples was achieved through the brachial veins of the birds. Superoxide dismutase (SOD), serum glutathione peroxidase (GPx), corticosterone (CORT), and malondialdehyde (MDA) were determined using the method described by Akhavan-Salamat *et al.,* (2015).
The means of the samples were compared using analysis of variance (ANOVA), with the level of significant difference set at (p-0.05). The Duncan multiple range test was used to separate the means and the SAS program was used to conduct this analysis (version 9.4)

Table 1: COMPOSITION OF EXPERIMENTAL DIETS (DM%)

Ingredients (%)	0.00% BLM (T1)	1.00% BLM (T2)	1.50% BLM (T3)	2.00% BLM (T4)	0.20% Vit.C (T5)
Maize	64.70	63.70	63.70	63.70	64.50
Soya bean meal	30.00	30.00	29.50	29.00	30.00
Fish meal	1.50	1.50	1.50	1.50	1.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Bambusa vulgaris	0.00	1.00	1.50	2.00	-
Vitamic C	-	-	-	-	0.20
[1]Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100

[1]Contain the following: vit A 10,000IU, vit D_3 1500 IU; vit E 10,000mg, vit k_3, 1500mg, vit B_1 1600mg, vit B_2 4000mg, pentothenic acid 10mg; nicotinic acid 2.5mg chlorine 3.5mg; folic acid 1mg; magnesium 56mg; lysine 1mg; iron 20mg; zinc 50mg; cobalt 1.25mg, biotin 7.5mg copper 30mg, manganese 40mg, selenium 2mg antioxidant 1250mg.

RESULTS

The proximate composition and phytochemicals of the experimental diets is shown in Table 2. The result indicates that the CP, CF and ash values of Diets T4 (187.21g, 375.50, and 110.01g respectively) were higher (p<0.05) than CP, CF and ash values for the other diet while the lowest (p<0.05) CP (135.01g), CF (261.55g) and ash (105.20g) values were recorded for diet T1, T5 and T5 respectively. The CP, CF and ash levels were noted to increase with increase in the BLM inclusion level in the diet. The phytochemical results showed the phytate, flavonoids, tannin, and saponin values increased with the increased addition of BLM in the diets. Flavonoids contents were similar and higher (p>0.05) for diets T4 and T5, whereas it is low (p>0.05) for T1 compared with the other diets.

The growth performance of broiler chickens fed BLM at different inclusion levels in the diet is shown in Table 3. The feed intake and weight gains of the birds increased as BLM increased in the diet, however, it was observed birds fed diet 1 had the lowest (p<0.05) feed intake and weight gain compared to birds fed the other diets while birds fed diet 4 had the highest (p<0.05) values for these parameters. The FCR values were comparable (p>0.05) for birds fed diets1, 4, and 5 with the best FCR from birds on diet 4.

Table 4 indicated that there was no significant difference (P>0.05) between glutathione peroxidases (GPx), corticosterone (CORT), and malondialdehyde (MDA) in all the diets. However, the range of glutathione peroxidase and malondialdehyde reported in this study is high (226.5-419.6U/ml and 1.2854-1.7618nmol/ml respectively). The superoxide dismutase is higher in diet 1 with 0% BLM (4477.6 U/ml) and diet 5 with 0.20% vitamin C (8955.2 U/ml) compared with other diets with BLM inclusion (1492.5, 1726.4, and 1865.7 U/ml), but much higher in diet 5.

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

Proximate	0.00% BLM (T1)	1.00% BLM (T2)	1.50% BLM (T3)	2.00% BLM (T4)	0.20% Vit.C (T5)	P value	SEM
Crude protein	135.01^{c}	137.80^{c}	157.20^{b}	187.21^{a}	160.02^{a}	0.0122	5.72
Crude fibre	350.04^{b}	355.00^{b}	360.01^{a}	375.50^{a}	261.55^{c}	0.0220	4.95
Ether extract	79.82^{a}	79.80^{b}	80.70^{a}	82.80^{a}	91.05^{a}	0.0170	1.88
Ash	105.20^{c}	107.57^{b}	109.57^{a}	110.01^{a}	107.24^{b}	0.0203	1.76
Phytochemical							
Phytate	39.93^{c}	40.22^{b}	42.99^{a}	43.02^{a}	40.11^{b}	0.0081	4.00
Flavonoids	50.99^{c}	70.64^{b}	72.85^{b}	74.90^{a}	75.10^{a}	0.0254	2.32
Saponin	20.90^{c}	21.82^{c}	38.95^{a}	39.31^{a}	36.05^{b}	0.0040	3.92
Tannin	0.15^{c}	1.20^{b}	4.30^{a}	4.32^{a}	0.99^{b}	0.0045	0.17

Table 3: Growth Performance of broiler chickens fed with experimental diets

Parameters	0.00% BLM (T1)	1.00% BLM (T2)	1.50% BLM (T3)	2.00% BLM (T4)	0.20% Vit.C (T5)	p-value	SEM
Initial weight (g)	630.00	623.33	633.33	622.22	620.00	0.7136	1.22
Final weight (g)	1714.17^{c}	1882.04^{bc}	1999.26^{ab}	2125.23^{a}	2087.04^{a}	0.0040	3.39
D/weight gain (g)	51.943^{c}	59.463^{bc}	65.203^{b}	71.573^{a}	69.857^{a}	0.0027	1.58
T/weight gain (g)	1074.17^{c}	1248.71^{bc}	1355.93^{ab}	1503.01^{a}	1457.04^{a}	0.0027	3.32
Av. feed intake (g)	235.02^{b}	255.99^{a}	259.25^{a}	266.66^{a}	260.93^{a}	0.0007	0.82
FCR (%)	3.95^{b}	4.54^{a}	4.50^{a}	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

Means with different superscripts (a, b and c) are significantly different (p< 0.05) across the rows, SEM= Standard error of mean. Dwt gain = Daily weight gain, FCR= Feed conversion ratio.

Table 4: Stress indicators levels of broiler chickens fed with experimental diets

Parameters	0.00% BLM (T1)	1.00% BLM (T2)	1.50% BLM (T3)	2.00% BLM (T4)	0.20% Vit.C (T5)	p-value	SEM

GPx (U/ml)	419.6	301.7	226.5	230.3	244.0	0.6609	4.1
MDA (nmol/ml)	1.2854	1.5314	1.7416	1.7618	1.7467	0.6818	0.21
SOD (U/ml)	4477.6^{b}	1492.5^{c}	1726.4^{b}	1865.7^{c}	8955.2^{a}	0.0019	9.81
CORT (U/ml)	1.5250	1.7317	1.7173	3.4865	2.5827	0.0548	0.25

Means with different superscripts (a, b and c) are significantly different ($p < 0.05$) , SEM= Standard error of mean. GPx = glutathione peroxidases, MDA = malondialdehyde, SOD = superoxide dismutase, CORT = corticosterone

DISCUSSION

The high phytochemical contents in diet 4 (2.0% BLM) appears better to alleviate heat stress and this is contrary to the report of Abubakar *et* al., (2021) and El-Wardany *et al.,* (2015). The increase in feed intake and weight gain with increasing BLM in the diet may be of positive value since inclusion of BLM at 2% level seems to improve the appetite of the broiler chickens with a resulting increase in weight gain comparable with and better than results from broiler chickens fed diet 5 (0.2% Vit. C). This observation is corroborated by the report of Li *et al*. (2017) who stated that the inclusion of *bamboo* leaves in broiler chicken diets improved body weight gain by 17.6% because of the presence of flavonoids. it has been hypothesized that flavonoids might play the role of growth hormones in animals (Havsteen, 2002), since flavonoids hydroxyl groups of aglycone are positioned in space in the same manner as estrogen's hydroxyl groups. This seems to indicate that the inclusion of BLM at of 2% level is the most efficient, and this dose-dependent impact may be the result of increase in concentrations of flavonoids in the diet. Earlier reports have shown that when birds suffer from challenges such as heat stress and immune suppression, growth performance would be affected (Yang *et al*., 2010). Authors have demonstrated that supplementing broiler chicken diets with bamboo leaf flavonoids at a concentration of 1.6% could mitigate the growth-inhibiting effects of heat stress and immunological suppression in broilers (Qi *et al*., 2014). It could be inferred that flavonoids in bamboo leaves may have been responsible for the observed growth improvement observed as seen in this study. The high feed conversion ratio of the diet contained 2% bamboo leaf that comparable with the diet containing vitamin C suggesting that the bamboo leaves enhanced the feed conversion ratio of the birds with little or no stress and is in line with the report of El-wardany *et al*. (2015) that bamboo leaf can be fed to reduce stress. The inclusion of the bamboo leaf in the diets fed in this study regardless of the level did not affect the percent mortality across the treatment groups and suggesting that it is non-detrimental to the health of the bird, though this is contrary to the report of Krzyszt *et al*. (2011).

The non-significant difference among glutathione peroxidases (GPx), corticosterone (CORT), and malondialdehyde (MDA) in all the diets may indicate less-observable stress within the birds during the study**.** Earlier authors have demonstrated that exposure to the bamboo leaf can remarkably raise the levels of the enzyme superoxide dismutase and glutathione peroxidase, and corticosterone hormone in birds that have been subjected to heat stress, and can enhance the birds' antioxidant potential (Liu *et al*., 2014). Research shows that the ability of flavonoids to stimulate antioxidant enzymes, chelate metal catalysts, transfer electrons to free radicals, and suppress oxidases seems to be responsible for the antioxidant effects that flavonoids have in living systems (Wan *et al*., 2016). We postulated that with the increasing the BLM in the diet, increased the antioxidant capacity of the diets since bamboo leaf has a peroxide scavenging capacity (Ni *et al*., 2011). Inhibition of the synthesis of thiobarbituric acid-reactive compounds can be attributed, in part, to flavonoids and polyphenols (Niu *et al*., 2016). In addition, research has demonstrated that broilers given plant extracts high in flavonoids, polyphenols, and polysaccharides are able to increase their capacity to scavenge free radicals (Yu *et al*., 2019).

CONCLUSION

This study concludes that bamboo leaf meal inclusion at 2% in the broiler feed was able to improve performance, alleviated heat stress and had no adverse effect on health of broiler chickens. Furthermore, broiler chickens fed 2% BLM inclusion showed improvement in terms of growth performance despite being reared under heat stress. This improvement in performance may not be unconnected to the induction of the excessive release of glutathione peroxidase (GPx) and superoxide dismutase (SOD), and reduced levels of corticosterone (CORT) and malondialdehyde (MDA) which positively influenced performance.

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