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Original Article

Impact of Brachiaria decumbens Leaf Meal Supplementation on Broiler Chickens Raised in Tropical Environments in Terms of Growth Performance, Blood Biochemistry, and Stress Biomarkers

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■Keywords

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ABSTRACT

This study's main objective was to determine the novel impact of supplementing *Brachiaria decumbens* leaf meal on the growth performance, blood biochemistry, and stress biomarkers of commercial broilers raised in hot and humid environments. A total of 300 male Ross 308 day-old broilers were divided into six different treatment groups at random. In Treatment 1, commercial diets without any additional additives were given to the broilers. In Treatment 2, broilers were fed commercial diets containing 100 mg/kg of the antibiotic oxytetracycline. Similar commercial diets supplemented with 25, 50, 75, and 100 mg/kg of *B. decumbens* leaf meal were respectively given to Treatments 3, 4, 5, and 6 without the use of antibiotics. Growth performance, serum lipid profiles, acute phase proteins, hormones, and heat shock proteins all differed significantly ($p<0.05$) among the treatments. The greatest final body weight, body weight gain, and lowest feed conversion ratio were generally seen in T3, for broilers supplemented with 25 mg/kg of *B. decumbens* leaf meal. Moreover, T3 broilers supplemented with 25 mg/kg of *B. decumbens* leaf meal showed the lowest AGP, corticosterone, and HSP 70 concentrations, while T6 broilers treated with 100 mg/kg of *B. decumbens* leaf meal had the lowest LDL and highest HDL values. The results of this study clearly demonstrate that supplementing broilers with *B. decumbens* leaf meal only requires a small amount to improve growth and reduce the effects of stress, but that a higher concentration is necessary to improve lipid profiles.

INTRODUCTION

Heat stress is one of the most difficult environmental conditions to manage in many parts of the world, especially on hot summer days (Attia *et al.,* 2009). Since the recommended ambient temperature for poultry is between 16 and 25 °C, broiler chicks raised in low-tech systems that are constantly exposed to high temperatures during the finisher stage suffer from chronic heat stress (Sahin *et al.,* 2009; Entezari *et al.*, 2021). According to Hoan *et al.* (2021), heat stress has a negative impact on growth, feed digestibility, and the immune system, reducing yield and meat quality as well as increasing fat storage, which lowers farmers' economic efficiency. Broiler chicks under heat stress also experience disturbances in the acid-base balance, which causes panting and respiratory alkalosis (Chung *et al.,* 2020). Moreover, blood metabolites and hormones are also altered by heat stress (Attia *et al.,* 2009). Broilers exposed to prolonged heat stress showed endocrinological changes that increased lipogenesis, decreased lipolysis, and increased amino acid catabolism, which together stimulated lipid accumulation (Lara & Rostagno, 2013). The production of heat shock proteins, elevated lipid peroxidation, and higher cholesterol levels in chicken plasma are additional signs of the increased oxidative stress brought on by heat exposure (Attia *et al.,* 2009; Sahin *et al.,* 2009).

Phytobiotics may be used in stressful situations, such as during heat stress, and can affect the blood indices of poultry through their antioxidant and other advantageous properties. According to Greene *et al.* (2021), a wide range of plants and their associated phytocompounds may be used under heat stress conditions for their antioxidant activity, which can affect chicken serum biochemistry and blood biomarkers. For instance, the lipid profile, liver profile, and stress biomarkers were all reportedly reduced by using garlic powder, papaya seeds, turmeric, and green tea (Khan *et al.,* 2014; Muazu & Aliyu-Paiko, 2020; Khodadadi *et al.,* 2021). However, the concentration and relationship of these substances, as well as their addition or supplementation in broilers' diets, determine the use of secondary plant metabolites. Due to their astringent, anti-oxidant, antimicrobial, and inflammatory properties, plant products rich in secondary metabolites, such as *B. decumbens*, used in small concentrations, may therefore have a positive impact on broilers' responses. These properties may affect the physiological and chemical functions of the digestive tract (Alghirani *et al.,* 2021a). In addition to tannins, flavonoids, and alkaloids, *B. decumbens* also has steroidal saponins concentrations that range from high to extremely high (5.9-24.1mg/g), being 7 to 30 times higher than the necessary level for photosensitization (Muniandy *et al.,* 2020). Dichotomin and dioscin/protodioscin have been found to be the two main steroidal saponins in *B. decumbens* (Chung *et al.,* 2018). By triggering digestive enzymes and increasing nutrient absorption in the intestinal tract by increasing villi height, these saponins benefit broiler chickens by assisting in the absorption of substances that are not typically utilized in the gastrointestinal system (Alghirani *et al.,* 2022).

Even though *B. decumbens* has a high nutritional value and a high concentration of saponins, only a small number of plants, including *Quillaja saponaria* and *Yucca schidigera*, have been studied to determine their effects as feed additives in the poultry industry. There is still a knowledge gap regarding the potential use and influence of phytocompounds on the blood biochemistry and stress biomarkers of broilers in the tropics, despite the widespread use of phytobiotics as growth promoters instead of antibiotics. Therefore, the goal of this study was to examine the novel effects of supplementing commercial broilers raised in hot and humid environments with various concentrations of *B. decumbens* leaf meal on their growth performance, lipid profiles, liver functions, acute phase proteins, hormone levels, and production of heat shock proteins.

MATERIALS AND METHODS

Broilers management

All experimental procedures were carried out in accordance with University Putra Malaysia's (UPM) Institutional Animal Care and Use Committee (IACUC) Research Policy (approval number: UPM/IACUC/AUP-R005/2020). A total of 300 male Ross 308 day-old broilers were purchased, weighed, and randomly assigned to one of six treatments, with five repeats of ten broilers each, in a completely randomized design (CRD). The broilers were raised in battery cages with wired floors for 42 days, in an open-sided building. For the first three days, the drinking water contained anti-stress (VP1000). Throughout the rearing period, the average temperature and relative humidity were respectively 29°C and 79%. All broilers received intraocular vaccinations against Infectious bronchitis and Newcastle disease on day 7, and against Infectious bursal disease on day 14 (Chung *et al.,* 2021).

Brachiaria decumbens preparation

At Farm 15, Field Lab, Department of Animal Science, Faculty of Agriculture, UPM, *B. decumbens* grass was sown and harvested after five weeks. The grass was then dried for a further 72 hours at 60°C, ensuring that the weight remained constant. The grass was then chopped into small pieces and ground into a fine powder (1 mm), which was then kept at room temperature for later use. The leaf meal's dry matter, crude protein, crude fiber, ether extract, and ash were measured according to AOAC (2012) standards. Following Osuntokun *et al.* (2016), secondary metabolites from the phytochemical screening of *B. decumbens* leaf meal revealed the presence of saponins, tannins, flavonoids, and alkaloids. After that, the total saponins were calculated as described by Makkar *et al.* (2007). Table 1 shows the nutritional composition of the *B. decumbens* leaf meal.

Table 1 – Nutritional composition of five-week-old *B. decumbens* leaf meal.

Parameters	
Metabolizable Energy (MJ/kg)	9.23±0.23
Dry matter (%)	31.04±1.75
Crude protein (%)	10.87±0.16
Crude fiber (%)	27.85±1.55
Ether extract (%)	2.33±0.00
Ash (%)	6.95±0.16
Saponins concentration (%)	54.60±0.47

Note: All values were expressed as mean ± standard error.

Experimental design

A commercial starter diet in crumble form with a corn and soybean meal basal composition was given to the broilers from day 1 to day 21; while from day 22 to day 42 they were given a finisher diet. T1 commercial feed was devoid of antibiotics (negative control), whereas T2 commercial feed was supplemented with 100 mg/kg oxytetracycline (positive control). T3, T4, T5, and T6 were given commercial feed that was supplemented with different amounts of *B. decumbens* leaf meal (respectively 25, 50, 75, and 100 mg/kg), without any antibiotics. The nutritional breakdown of starter and finisher meals containing various amounts of *B. decumbens* leaf meal is displayed in Table 2. The broilers were given unrestricted access to food and clean water for the duration of the 42-day feeding period. To measure the body weight gain and feed conversion ratio (FCR), the total feed intake and body weight for each replicate were recorded weekly. Six broilers from each treatment were then randomly selected and slaughtered on day 42. Blood samples were taken at the time of slaughter and examined for the lipid profile, liver function, acute phase proteins, corticosterone, and heat shock protein concentrations.

Blood biochemistry

Total serum cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured using an automatic analyzer (Automatic analyzer 902, Hitachi, Germany), and very low-density lipoprotein cholesterol (VLDL) and low-density lipoprotein cholesterol (LDL) were estimated using the Friedewald Equations: LDL = Total cholesterol - HDL- VLDL, where VLDL = Triglycerides/5.

The following parameters for liver function were determined using a BA400 biochemical and turbidimetry analyzer from Spain (Manual code TEUS00048-07-EN): alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST).

Stress biomarkers

Using commercial enzyme-linked immunosorbent assay (ELISA) kits (QAYEE-BIO, China), the concentrations of serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and heat shock protein 70 (HSP 70) in each blood serum sample were measured. Although the working phases of all the ELISA kits are similar, each kit uses a unique set of standards with known concentrations. A microplate reader (Bio-Rad Microplate Reader, USA) was used to determine optical density (OD) at 450 nm. The standard curve linear regression equation was established using the concentration of the standards and the corresponding OD values, and the sample concentration was computed using this same equation.

Statistical analysis

Using the Statistical Analysis System (SAS, 2012), the collected data were subjected to a one-way analysis of variance (ANOVA) based on the completely random design model. The Tukey Post-Hoc Test was used to determine whether there was a significant difference between the treatment groups. A significance level of $p<0.05$ was applied to all data.

Table 2 – Nutrient content of broiler diets supplemented with *B. decumbens* leaf meal at different concentrations.

	Treatments					
Parameters	T1	T2	T3	T4	T5	T6
Starter diet (1-21 d)						
Metabolizable Energy (MJ/kg)	13.39±0.02	13.37±0.03	13.18±0.06	13.24±0.06	13.52±0.10	13.46±0.01
Dry matter (%)	89.33± 0.00	90.00± 0.00	89.33± 0.00	90.22± 0.22	89.55± 0.11	89.55± 0.11
Crude protein (%)	25.13±0.25	25.51±0.12	25.78±0.06	25.52±0.27	25.26±0.13	25.27±0.18
Crude fiber (%)	3.65±0.30	4.65±0.10	4.45±0.10	4.25±0.05	3.85±0.00	3.16±0.16
Ether extract (%)	7.22±0.11	7.44±0.11	7.11±0.22	7.00±0.19	7.77±0.40	7.77±0.11
Ash (%)	5.84±0.12	5.92±0.00	6.34±0.00	6.87±0.10	5.71±0.25	6.58±0.13
Finisher diet (22-42 d)						
Metabolizable Energy (MJ/kg)	12.47±0.14	12.47±0.06	12.46±0.01	12.47±0.03	12.44±0.02	12.49±0.14
Dry matter (%)	87.66±0.00	87.55±0.11	88.00±0.19	88.00±0.00	88.22±0.29	88.44±0.29
Crude protein (%)	16.32±0.27	16.87±0.17	16.80±0.07	16.35±0.06	16.74±0.15	16.49±0.14
Crude fiber (%)	4.15±0.05	4.05±0.05	3.93±0.03	3.68±0.16	3.61±0.03	3.55±0.10
Ether extract (%)	4.55±0.72	4.33±0.19	4.00±0.00	4.00±0.00	3.88±0.11	3.88±0.11
Ash (%)	6.09±0.00	5.75±0.15	6.57±0.10	6.31±0.25	6.53±0.22	6.41±0.02

Note: All values were expressed as mean ± standard error. Note: T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

RESULTS

Growth performance

Table 3 shows the impact of feeding broilers *B. decumbens* leaf meal on their growth performance on days 21 and 42. Final body weight, body weight gain, feed intake, and cumulative FCR varied significantly ($p<0.05$) throughout the study. During the starter phase, broilers fed with antibiotics and different concentrations of *B. decumbens* leaf meal had heavier final body weights and body weight gains as compared to the control broilers. Nonetheless, only T3 and T4 broilers had lower cumulative FCR comparable to the positive control broilers. On the other hand, T3 broilers fed with 25 mg/kg of *B. decumbens* leaf meal showed the highest final body weight, body weight increase, and lowest FCR during the finisher phase, indicating a better growth performance.

Table 3 – Effect of *B. decumbens* leaf meal supplementation on the growth performance of broilers on days 21 and 42.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p* value
21 days old (Starter phase)							
Initial body weight (kg)	0.044±0.00^{a}	0.045±0.00^{a}	0.044±0.00^{a}	0.044±0.00^{a}	0.045±0.00^{a}	0.044±0.00^{a}	0.8789
Final body weight (kg)	0.898±0.00^{d}	0.907±0.00^{cd}	0.946±0.00^{a}	0.934±0.00^{ab}	0.919±0.00^{bc}	0.907±0.00^{cd}	<.0001
Body weight gain (kg)	0.853±0.00^{d}	0.861±0.00^{cd}	0.901±0.00^{a}	0.889±0.00^{ab}	0.874±0.00^{bc}	0.862±0.01^{cd}	<.0001
Feed intake (kg)	1.200±0.02	1.202±0.02	1.237±0.01	1.232±0.01	1.244±0.01	1.243±0.01	0.0806
Cumulative FCR	1.40±0.03^{ab}	1.39±0.02^{ab}	1.37±0.00^{ab}	1.38±0.01^{ab}	1.42±0.01^{ab}	1.44±0.00^{a}	0.0416
42 days old (Finisher phase)							
Final body weight (kg)	2.230±0.01^{b}	2.230±0.02^{b}	2.298±0.01^{a}	2.219±0.02^{b}	2.219±0.02^{b}	2.230±0.02^{b}	<.0001
Body weight gain (kg)	2.185±0.01^{b}	2.185±0.02^{b}	2.253±0.01^{a}	2.174±0.02^{b}	2.173±0.02^{b}	2.185±0.02^{b}	<.0001
Feed intake (kg)	4.235±0.02^{a}	4.119±0.02^{c}	4.175±0.01^{b}	4.153±0.01^{bc}	4.145±0.01^{bc}	4.133±0.01^{c}	<.0001
Cumulative FCR	1.93±0.01^{ab}	1.89±0.01^{bc}	1.85±0.01^{c}	1.91±0.01^{b}	1.90±0.01^{b}	1.88±0.01^{bc}	0.0004

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within row are significantly different at $p<0.05$. FCR: Feed conversion ratio. T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

Blood biochemistry

Table 4 displays the impact of supplementing *B. decumbens* leaf meal on the lipid profiles and liver function of broilers on day 42. Only the LDL and HDL values significantly ($p<0.05$) differed between the treatments. Measures of liver function and other lipid profiles did not change significantly ($p>0.05$). The lowest LDL and highest HDL values were found in T6 broilers treated with 100 mg/kg of *B. decumbens* leaf meal, as compared to the other treatment groups, suggesting that improved lipid profiles can improve broiler meat quality with no toxic effects.

Table 4 – Effect of *B. decumbens* leaf meal supplementation on the blood biochemistry of broilers on day 42.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p* value
Lipid profile							
Cholesterol (mmol/L)	3.58±0.02	3.73±0.07	3.18±0.08	3.43±0.28	3.38±0.20	3.37±0.10	0.3052
TG (mmol/L)	0.29±0.01	0.37±0.01	0.36±0.02	0.37±0.02	0.34±0.01	0.34±0.02	0.0930
LDL (mmol/L)	0.47±0.01^{a}	0.30±0.00^{cd}	0.38±0.01^{bc}	0.41±0.01^{ab}	0.27±0.01^{d}	0.27±0.05^{d}	0.0052
HDL (mmol/L)	2.42±0.14^{ab}	2.54±0.06^{a}	2.13±0.12^{b}	2.38±0.07^{ab}	2.47±0.08^{ab}	2.52±0.10^{a}	0.0179
Liver function							
ALP (U/L)	4616.52±104.98	4447.10±288.03	4006.86±17.55	4125.52±62.74	4126.38±200.65	4168.89±141.97	0.1708
AST (U/L)	407.87±30.21	300.74±19.01	330.75±16.60	361.18±16.00	343.34±28.53	299.23±13.85	0.0644
GGT (U/L)	21.50±2.50	25.00±3.00	21.00±1.00	27.50±2.50	20.50±0.50	23.50±1.50	0.2534
ALT (U/L)	18.00±2.00	18.50±0.50	11.50±1.50	18.00±2.00	12.00±2.00	13.50±0.50	0.0511

Note: All values were expressed as mean ± SE; $^{a, b, c, d}$ values with superscript within row are significantly different at $p<0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase. T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

Stress biomarkers

Table 5 shows the effects of *B. decumbens* leaf meal supplementation on indicators of broiler stress at day 42. Significant variations were seen in the serum concentrations of AGP, corticosterone, and HSP 70 ($p<0.05$). Among treatment groups, there were no significant differences in CP and SAA concentrations ($p>0.05$). The lowest AGP, corticosterone, and HSP 70 concentrations were observed in T3 broilers supplemented with 25 mg/kg of *B. decumbens* leaf

Table 5 – Effect of *B. decumbens* leaf meal supplementation on the stress biomarkers of broilers on day 42.

	Treatment						
Parameters	T1	T2	T3	T4	T5	T6	*p* value
SAA (ng/ml)	109.85±1.47	96.97±1.39	95.85±5.11	108.29±3.40	100.40±3.91	110.65±8.99	0.0747
AGP (ng/ml)	680.84±11.94^{ab}	643.75±9.78^{b}	643.38±6.84^{b}	659.91±13.34^{ab}	682.71±13.56^{a}	693.16±16.63^{a}	0.0263
CP (µg/ml)	260.38±13.81	261.96±20.21	251.70±13.69	270.65±14.33	248.21±12.45	266.30±15.45	0.0540
Corticosterone (ng/ml)	36.75±2.68^{ab}	31.46±7.52^{ab}	19.40±2.11^{b}	25.28±6.26^{ab}	28.24±4.12^{ab}	43.60±11.38^{a}	0.0132
HSP 70 (pg/ml)	5791.75±19.59^{c}	5274.67±23.24^{d}	5250.00±32.76^{d}	5767.42±24.22^{c}	5876.67±17.96^{b}	6108.00±13.65^{a}	<.0001

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within row are significantly different at $p<0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70. T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

meal, comparable to T2 broilers fed with 100 mg/kg oxytetracycline. Although there were no statistically significant differences between T3 and other concentrations of *B. decumbens* leaf meal, T3 had the lowest stress biomarker values when compared to the other treatments, signifying less stress and inflammatory reactions in those broilers.

DISCUSSION

Broiler development, productivity, and health can all be enhanced by using phytobiotics, or compounds derived from plants. The current study discovered that T3 broilers fed the least amount of *B. decumbens* displayed the best growth performance in both starter and finisher phases. The growth-promoting properties of *B. decumbens* may be due to its high concentration of steroidal saponins. By triggering digestive enzymes and increasing nutrient absorption in the intestinal tract through increases in villi height, these saponins support the absorption of substances that are not typically utilized in the gastrointestinal system, leading to positive effects (Alghirani *et al.,* 2023). Additionally, because *B. decumbens* contains additional phytochemical components like tannins, flavonoids, and alkaloids, the production efficiency of T3 broilers can also increase as a result. These elements have antimicrobial action and promote immunological enhancement, which maintain microflora balance, reduce pathogenic bacterial load, and stabilize intestinal health, which involves changing digestive organ function, nutrition absorption, and broiler weight gain (Chaudhary *et al.,* 2018). However, supplementation of *B. decumbens* at higher levels could lower body weight gain, which could be caused by the bitter taste of steroidal saponins and the anti-nutritional effect of both saponins and tannins (Alghirani *et al.,* 2021a). This was observed in the current study, where T5 and T6 broilers fed with 75 and 100 mg/kg of *B. decumbens* had lower growth performances throughout the whole study.

Additionally, adding phytobiotic supplements to the diet of broilers can help raise HDL levels while lowering serum levels of cholesterol, triglycerides, and LDL in both starter and finisher broilers (Gilani *et al.,* 2018). In comparison to the other treatment groups, T6 broilers supplemented with 100 mg/kg of *B. decumbens* leaf meal displayed the lowest and highest values of LDL and HDL, respectively. This may have been influenced by the plant's high concentrations of saponins and other secondary metabolites. Previous research has demonstrated that adding leaf powders containing various phytocompounds (such as Chinese bayberry leaves, green tea leaves, peppermint, roots of *Glycyrrhiza glabra*, and *Ginkgo biloba* leaves) to broilers' diets also lowers serum cholesterol, TG, and LDL levels, while raising HDL levels (Zhang *et al.,* 2017; Gilani *et al.,* 2018; Niu *et al.,* 2019). Similar to what was previously mentioned, Suganya *et al.* (2016) found that higher intakes of onion and garlic prevent LDL oxidation and consequently lower serum LDL levels. In line with this, Khan *et al.* (2014) discovered that adding 2 and 3 g/kg of garlic powder to broiler meals significantly lowers blood TG, cholesterol, and LDL, while increasing HDL. These could be attributed to numerous phytochemical substances, including saponins, tannins, flavonoids, and alkaloids, which were sufficient to cause interactions in the gut and trigger the formation of large mixed micelles. These encourage cholesterol excretion, resulting in a decrease of serum cholesterol levels by deferring the intestinal absorption of nutritional fat via inhibition of pancreatic lipase activity (Alghirani *et al.,* 2021b). Therefore, a greater concentration of *B. decumbens* leaf meal in the current study also contributed to improved LDL reduction and an increase in HDL.

One of the largest and most important organs in all living things, the liver, is essential for the metabolism, detoxification, and elimination of both endogenous and foreign substances (Paul *et al.,* 2016). Plant supplementations have been shown to improve liver function regulation and serum biochemical characteristics (Basit *et al.,* 2020). Despite adding *B. decumbens* leaf meal at different concentrations, no

noticeable changes in liver parameters were seen in the current study. Increased AST and ALT levels are sensitive indicators of hepatocellular disease, and are used to identify liver disorders and determine hepatotoxicity (Gilani *et al.,* 2018). Therefore, every pathological symptom or toxin that raises AST and ALT activity levels is considered a specific indicator of liver damage or dysfunction. The current study has demonstrated that broilers did not suffer any negative effects from the various levels of *B. decumbens* leaf meal supplementation. In support of this, broiler chickens' liver parameters like AST, ALT, and GGT were unaffected by the addition of red ginseng root powder containing high saponins content at 75, 150, or 225 mg/kg (Yener *et al.,* 2021). Similarly, broiler chickens supplemented with 25, 50, 75, and 100 mg/kg of *Y. schidigera* powder containing 60.60% saponins showed no significant differences between treatments for liver parameters like ALP, AST, ALT, and GGT (Alghirani *et al.,* 2023).

On the other hand, stress biomarkers like APP, hormones, and HSP are crucial for determining how stress affects broilers' welfare and growth efficiency (Imik *et al.,* 2012). Due to their antioxidant activity, which can impact poultry blood biomarkers and serum biochemistry, adding phytobiotics to broilers' feed could improve growth performance under heat stress conditions (Salah *et al.,* 2019). The lowest levels of AGP, corticosterone, and HSP 70 were observed in T3 broilers supplemented with 25 mg/kg of *B. decumbens* leaf meal, indicating lower inflammatory and stress responses. AGP is used to evaluate the health of chickens and plays a significant role in maintaining homeostasis by minimizing tissue damage brought on by inflammatory response in extrahepatic cells (Zulkifli *et al.,* 2018). This reduction in AGP levels may be attributed to the anti-inflammatory properties of the various phytobiotics found in *B. decumbens* leaf meal.

Meanwhile, corticosterone, which is primarily linked to stress in avian species, has been used to track physiological reactions to stressors (Najafi *et al.,* 2015). There is a link between serum cholesterol and serum corticosterone, and feeding saponins to broilers has been shown to have hypocholesterolemic effects (Rokade *et al.,* 2016). Since serum cholesterol is thought to be a precursor to serum corticosterone, a decline in serum corticosterone levels may be associated with a decline in serum cholesterol. Therefore, the decrease in serum corticosterone level seen in the current study may be caused by the decrease in serum cholesterol that occurred after supplementation with saponins.

Stress protein biomarkers, such as HSP, are a group of proteins present in every cell of every living thing, which are produced in large amounts in response to high or low temperatures or other stressors. The ability of saponins and other phytochemical components to scavenge radicals, chelate metals, and collaborate with other antioxidants may be the cause of the decrease in HSP 70 levels (Hajati *et al.,* 2015). For instance, dietary phytocompounds in broilers can influence thermal stress biomarkers, like HSP 70 mRNA, in a positive manner, improving the growth performance of chickens under heat stress (Abdel-Moneim *et al.,* 2021). Furthermore, by controlling the production of reactive oxygen species (ROS) and the breakdown of proteins, activating antioxidant enzymes, and boosting the expression of avian uncoupling protein (avUCP), the antioxidant properties of other phytocompounds in *B. decumbens* leaf meal may be able to lessen oxidative damage and enhance heat stress tolerance (Toyomizu *et al.,* 2019).

CONCLUSIONS

In conclusion, T3 broilers treated with 25 mg/kg of *B. decumbens* leaf meal had superior final body weight, body weight gain, and the lowest FCR. Meanwhile, T6 broilers fed with 100 mg/kg of *B. decumbens* leaf meal had better LDL and HDL levels, but T3 broilers supplied with 25 mg/kg of *B. decumbens* leaf meal had lower AGP, corticosterone, and HSP 70 levels. According to the results, a higher concentration of *B. decumbens* leaf meal supplementation is necessary to improve lipid profiles in broilers, whereas a small amount of *B. decumbens* leaf meal supplementation is sufficient to improve growth performance and reduce the effects of stress. The effects of phytocompounds like saponins, tannins, flavonoids, and alkaloids present in *B. decumbens* leaf meal may influence all of these factors. Furthermore, no significant changes in liver parameters were found, proving the safety and lack of adverse effects on hepatic functions of *B. decumbens* leaf meal.

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AUTHORS' CONTRIBUTIONS

ELTC: Supervision, conceptualization, writing and editing. MMA, NAK, and YLO: Data collection, analysis and writing. FFAJ, AQS, and TCL: Review and editing. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST DECLARATION

The authors declare that they do not have any competing interests.

DATA AVAILABILITY STATMENT

Data of the current study is available from the authors upon request.

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