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Effects of *Brachiaria decumbens* leaf meal supplementation on the health, blood biomarkers, and relative telomere length of broiler chickens raised in tropical environments

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Abstract

The main goal of this investigation was to determine the effects of *Brachiaria decumbens* leaf meal on the health performance, blood biomarkers, and relative telomere length of broiler chickens raised in tropical conditions. A total of 300, day-old, male, Ross 308 broilers were divided into six groups and given various feeding regimens based on a completely randomized design model. T1 commercial feed was devoid of antibiotics (negative control) whereas T2 commercial feed was supplemented with 100 mg/kg oxytetracycline (positive control). Commercial diets supplemented with 25, 50, 75, and 100 mg/kg of powdered *B. decumbens* leaf meal was given to the birds in treatments T3, T4, T5, and T6, respectively. Throughout the 42-day study, there were a few occurrences of leg problems, diarrhoea, and fatalities, but no statistical association was detected. The growth performance, leucocyte profile, immunoglobulin and cytokine concentrations, and relative telomere length, however, differed substantially among treatments.T3 broilers fed with diets supplemented with 25 mg/kg of *B. decumbens* leaf meal exhibited the best growth performance (highest final body weight and body weight gain; lowest cumulative feed conversion ratio), leucocyte profiling (increased total white blood cell count, monocytes, and basophil count; decreased heterophil to lymphocyte ratio), immunoglobulins (up-regulated IgG, IgA, and IgM), cytokines (increased IL4 and IL7), and telomere length. In conclusion, *B. decumbens* leaf meal can be used instead of antibiotics to enhance the growth and health of broilers raised in hot, humid conditions.

Keywords: *Brachiaria decumbens*, growth, leucocyte, immunoglobulins, cytokines, telomere length, Ross 308
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Introduction

As the human population is expanding exponentially, there is a sharp rise in the demand for broiler meat on a global scale. Unfortunately, the chicken industry is having a difficult time maximizing productivity to meet demand because of inadequate management, high feed production costs, disease outbreaks, unclear government policy, and pressure from technological innovation (Alghirani *et al*., 2021a). Furthermore, broilers may experience thermal stress due to climate change, high temperatures, and heavy humidity in tropical areas, which could negatively affect their growth and health (Entezari *et al*. 2021; Kpomasse *et al*., 2021). Thus, in-feed antibiotics have been widely utilized in the chicken industry to increase meat yield, health, and feed efficiency (Engberg *et al*., 2000; Ziaie *et al*., 2011). The ingestion of antibiotics by food-producing livestock is one of the two major sources of antibiotic transmission that has led to the emergence of resistant bacteria in hospitals and human communities,

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according to a recent study. This is true even though antibiotics may be effective in controlling infectious pathologies and successfully preventing the negative effects of various avian diseases (Abadi *et al*., 2019). This has sparked a global movement to forbid the use of antibiotics in the human food animal industry (Ahmad & Khan, 2019).

Attributable to their origin in herbs and natural bases, phytobiotics have recently gained international attention (Dhama *et al*., 2015). Due to the high concentration of pharmacologically-active chemicals found in phytobiotics, which may also serve as a natural growth booster for broiler production, they are a possible replacement for antibiotics (Grashorn, 2010). One phytobiotic sub-derivative with pharmacological qualities, including anti-inflammatory, immunostimulant, hypocholesterolaemic, antifungal, cytotoxic activity, antimicrobial, anticancer, and antioxidant capabilities, is saponins (Francis *et al*., 2002; Guclu-Ustundag & Mazza, 2007). According to a review by Francis *et al*. (2002), supplementing with saponins has an important impact on livestock growth performance, antioxidant activity, anti-inflammatory response, antifungal activity, immune response, nervous system stimulation, cholesterol metabolism, and protein digestibility.

In regard to this, *B. decumbens*, commonly known as signal grass, is widely planted in more than 80% of the grassland in the tropics and has significant quantities of steroidal saponins (Riet-Correa *et al*. 2011; Chung *et al*., 2018). With the climatic circumstances in the area, *B. decumbens* has the agronomic potential to be an outstanding pasture species (Assumaidaee & Mustapha, 2012). As a result, *B. decumbens* grass is more prevalent than the other *Brachiaria* species (Chung *et al*., 2018). The high saponin content found in *B. decumbens* is assumed to be advantageous to the growth and health performance of broilers due to the pharmacologically advantageous effects of saponins. However, there is a lack of information on the effect and mechanisms of growth and health of broiler chickens supplemented with *B. decumbens*, especially in tropical climates. With the ban on antibiotics despite their value as a growth promoter, there is increasing pressure on the livestock sector to seek alternatives for antimicrobials to prevent antimicrobial resistance and antibiotic residue in the poultry industry without compromising the performance of the broilers. This study was therefore carried out to investigate the effects of *B. decumbens* grass meal, as an alternative feed additive to replace antibiotics in broiler diets, on the health performance, leucocyte profiling, immunoglobulins, and cytokine concentrations, as well as the expression of telomere length.

Material and methods

The Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) granted approval for this experiment in accordance with the Animal Utilization Protocol (approval number: UPM/IACUC/AUP-R005/2020).

Based on an analysis of variance (ANOVA) with a power of 90% and a significance level of 5%, a total of 300, day-old, male, Ross 308 broilers were weighed individually and randomly assigned to six dietary treatments, with five replicates consisting of ten broilers each, according to the completely randomized design model. The broilers were kept in multi-level stainless steel wired flooring battery cages (Henan Silver Star Poultry Equipment Co., Ltd, China) measuring 88 cm in length by 45 cm in height by 118 cm in width in an open-sided house. The average temperature and relative humidity throughout the course of the 42 d were 29 °C and 79%, respectively, with 23 h of continuous light (LED lighting system). Anti-stress (Vet Pharma 1000) was added to the water supply for the first three days. On day 7, all broilers received an eye vaccine for infectious bronchitis (IB) and Newcastle disease (ND), and on day 14, they received an oral vaccine for infectious bursal disease (IBD) (Chung *et al*., 2021).

B. decumbens grass was cultivated at Farm 15, Field Laboratory, Department of Animal Science, Faculty of Agriculture, UPM, and was harvested at five-weeks-old. The grass was then dried for a further 72 h at 60 °C until maintaining a constant weight. The grass was then chopped into small pieces and ground into a powder (1 mm), which was then kept at room temperature for later use. Dry matter, crude protein, crude fibre, ether extract, and ash of the leaf meal were measured according to the methods of the AOAC (2006). The *B. decumbens* leaf meal underwent phytochemical screening according to the methods of Osuntokun *et al*. (2016) to determine the presence of secondary metabolites, which revealed the presence of saponins, tannins, flavonoids, and alkaloids. The total saponins were then calculated in accordance with Makkar *et al.* (2007). Table 1 lists the nutritional composition of *B. decumbens* leaf meal.

Table 1 Nutritional composition of five-week-old *Brachiaria decumbens* leaf meal

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

Note: All values are expressed as mean ± standard error

From days 1 to 21, all broilers received starter diet in mash form, and from days 22 to 42, they received a commercial finisher diet in crumble form. Primary ingredients of the commercial diets were soybean meal and maize. The T1 commercial feed was devoid of antibiotics (negative control) whereas T2 commercial feed was supplemented with 100 mg/kg oxytetracycline (positive control). Commercial diets supplemented with 25, 50, 75, and 100 mg/kg of powdered *B. decumbens* leaf meal were given to the birds in treatments T3, T4, T5, and T6, respectively. Tables 2 and 3 display the nutritional composition of starter and finisher diets supplemented with different dosages of powdered *B. decumbens* leaf meal. The broilers received unlimited access to fresh water and feed for the duration of the 42-day experiment. To determine the growth performance, the total body weight (BWG), feed intake (FI), and feed conversion ratio (FCR) of broilers were monitored weekly. A digital weighing scale (Mettler Toledo Industrial Scale, BBA211 series, Greifensee, Switzerland) was used to measure weight with a two-decimal point accuracy. Daily records were kept of the mortality rates and clinical signs (including coughing, ruffled feathers, diarrhoea, and problems with the legs; Chakma, 2015). After the finisher phase, 10 broilers from each treatment were randomly selected and sacrificed for leucocyte profiling, immunoglobulins, cytokines, and telomere length analysis.

Using peripheral blood smears stained with Wright Stain, the estimated total white blood cell (TWBC) and differential white blood cell (WBC) counts were manually determined. To see the leucocytes, the blood smears were examined with a Novex microscope and a CMEX CMOS camera connected to Image Fokus v3.0 software. The leucocyte subtypes were identified as heterophils, lymphocytes, monocytes, basophils, and eosinophils. By counting the WBC in 10 separate fields of the slide at a 40× objective, the TWBC was counted, and the average of the number of WBC was computed. To calculate an estimated TWBC count per microliter, the average value was multiplied by 2000 according to the formula of Carisch *et al*. (2019):

$$\text{Estimated TWBC/µl} = (\text{Mean of 10 views}) \times 2000 \quad (1)$$

In addition, 200 WBC were manually counted and identified at a magnification of 100× using emulsion oil to provide a sharper view of the white blood cells for the differential WBC counts, and the data were transformed into percentages (Lilliehook *et al*., 2008). The formula: Total heterophils/Total lymphocytes was used to calculate the heterophil and lymphocyte (H/L) ratio.

Using commercial enzyme-linked immunosorbent assay (ELISA) kits from QAYEE-BIO (China), the concentrations of immunoglobulins (IgM, IgA, and IgG) and cytokines (IL4 and IL7) in each blood serum sample were determined. The manufacturer's recommendations for the ELISA methods were followed. A microplate reader was used to measure the optical density (OD) at 450 nm (Bio-Rad Microplate Reader, USA). Using MyAssays software, the sample concentration was determined in accordance with the standard curve linear regression equation based on the concentrations of the standards and the related OD values.

Table 2 Composition and nutrient content of the broiler starter diet supplemented with different concentrations of powdered *Brachiaria decumbens* leaf meal

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredients (%)						
Corn (%)	41.27	41.27	41.27	41.27	41.27	41.27
Soybean meal (%)	40.60	40.60	40.60	40.60	40.60	40.60
Palm oil (%)	6.00	6.00	6.00	6.00	6.00	6.00
Wheat pollard (%)	6.88	6.88	6.88	6.88	6.88	6.88
Dicalcium phosphate (%)	2.28	2.28	2.28	2.28	2.28	2.28
Calcium carbonate (%)	1.75	1.75	1.75	1.75	1.75	1.75
Salt (%)	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine (%)	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix (%)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix (%)	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant (%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (%)	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder (%)	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analysis						
Metabolizable Energy (MJ/kg)	13.01 ± 0.03	12.88 ± 0.10	12.90 ± 0.04	13.10 ± 0.08	12.90 ± 0.28	13.18 ± 0.04
Dry matter (%)	89.77 ± 0.23	90.77 ± 0.53	90.33 ± 0.52	91.33 ± 0.20	90.57 ± 0.72	90.43 ± 0.87
Crude protein (%)	23.10 ± 0.46	23.57 ± 0.15	23.17 ± 0.52	22.90 ± 0.30	23.07 ± 0.27	23.13 ± 0.32
Crude fibre (%)	3.40 ± 0.15	3.40 ± 0.06	3.47 ± 0.09	3.13 ± 0.09	3.23 ± 0.37	2.97 ± 0.27
Ether extract (%)	6.85 ± 0.49	7.20 ± 0.29	7.35 ± 0.20	7.15 ± 0.09	7.20 ± 0.29	7.00 ± 0.00
Ash (%)	5.90 ± 0.10	5.67 ± 0.20	5.77 ± 0.23	5.80 ± 0.10	6.00 ± 0.17	5.80 ± 0.10

Note: All values are expressed as mean ± standard error. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

Table 3 Composition and nutrient content of the broiler finisher diet supplemented with different concentrations of powdered *Brachiaria decumbens* leaf meal

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredients (%)						
Corn (%)	49.50	49.50	49.50	49.50	49.50	49.50
Soybean meal (%)	33.44	33.44	33.44	33.44	33.44	33.44
Palm oil (%)	6.00	6.00	6.00	6.00	6.00	6.00
Wheat pollard (%)	6.35	6.35	6.35	6.35	6.35	6.35
Dicalcium phosphate (%)	1.61	1.61	1.61	1.61	1.61	1.61
Calcium carbonate (%)	1.83	1.83	1.83	1.83	1.83	1.83
Salt (%)	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine (%)	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix (%)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix (%)	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant (%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (%)	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder (%)	0.15	0.15	0.15	0.15	0.15	0.15
Calculated analysis						
Metabolizable Energy (MJ/kg)	13.41 ± 0.07	13.67 ± 0.06	13.60 ± 0.06	13.76 ± 0.01	13.61 ± 0.13	13.62 ± 0.19
Dry matter (%)	90.30 ± 0.00	90.20 ± 0.59	89.57 ± 0.43	90.43 ± 0.59	89.97 ± 0.67	90.43 ± 0.30
Crude protein (%)	19.33 ± 0.26	19.77 ± 0.32	19.37 ± 0.19	19.87 ± 0.29	19.00 ± 0.38	19.30 ± 0.32
Crude fibre (%)	3.95 ± 0.20	4.53 ± 0.19	3.70 ± 0.12	3.40 ± 0.38	4.50 ± 1.55	3.40 ± 0.17
Ether extract (%)	4.67 ± 0.20	4.70 ± 0.00	4.57 ± 0.13	4.70 ± 0.00	4.67 ± 0.33	5.00 ± 0.17
Ash (%)	5.43 ± 0.13	5.67 ± 0.20	5.57 ± 0.13	5.57 ± 0.13	5.43 ± 0.13	5.80 ± 0.10

Note: All values are expressed as mean ± standard error. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

DNA was extracted from whole blood samples using the innuPREP Blood DNA Micro Kit (Analytik Jena, China) according to the manufacturer's recommendations. The yield and purity of the DNA that was extracted were evaluated from the 260 nm/280 nm ratio using the Multiskan Go (Thermo Scientific). The qPCR was used to calculate the relative telomere length using the 2-ΔΔCt technique (Livak & Schmittgen, 2001). SensiFAST™ SYBR No-ROX Kit was used as the master mix solution; samples were run in triplicate. The qPCR cycling conditions and primer sequences of O'Callaghan and Fenech (2011) were used.

The data were subjected to one-way ANOVA based on a completely randomized design model for growth performance, leucocyte profiling, immunoglobulins, and cytokine concentrations, as well as telomere length using Statistical Analysis System (SAS, 2012). The significance of the differences among the treatment groups was determined using Tukey's Post-Hoc Test. The Chi-squared test was used to examine the clinical signs and mortality rates. All data were deemed significant at P <0.05. The mathematical model for each analysis is produced below:

One-way Analysis of Variance: $F = \frac{MS_{group}}{MS_{error}}$ (2)

Tukey's Post-Hoc Test: $HSD = q * \sqrt{\frac{MS_{within}}{n}}$ (3)

Chi-squared Test: $\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$ (4)

where MS_{group} = Mean squares of group
MS_{error} = Mean squares of error
MS_{within} = Mean squares error within group
q = Standardized range statistic
O_i = Observed value
E_i = Expected value
n = Sample size

Results

The growth performance of broiler chickens supplemented with *B. decumbens* leaf meal is shown in Table 4. There were differences in the final body weight (P <0.001), body weight gain (P <0.001), total feed intake (P <0.001), and cumulative FCR (P <0.004) among the treatments. T3 broiler chickens supplemented with 25 mg/kg *B. decumbens* leaf meal had the highest final body weight and body weight gain and the lowest cumulative FCR, indicating better growth performance. A higher (P< 0.001) feed intake was observed in chickens from the negative control compared to the other treatments.

Table 4 Effect of *Brachiaria decumbens* leaf meal supplementation on the growth performance of broilers

	Treatments						
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	***P* value**
Final body weight (kg)	2.23 ± 0.01^{b}	2.23 ± 0.02^{b}	2.30 ± 0.01^{a}	2.22 ± 0.02^{b}	2.22 ± 0.02^{b}	2.23 ± 0.02^{b}	<0.0001
Body weight gain (kg)	2.19 ± 0.01^{b}	2.19 ± 0.02^{b}	2.25 ± 0.01^{a}	2.17 ± 0.02^{b}	2.17 ± 0.02^{b}	2.19 ± 0.02^{b}	<0.0001
Feed intake (kg)	4.24 ± 0.02^{a}	4.12 ± 0.02^{c}	4.18 ± 0.01^{b}	4.15 ± 0.01^{bc}	4.15 ± 0.01^{bc}	4.13 ± 0.01^{c}	<0.0001
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 are expressed as mean ± standard error; $^{a, b, c}$ values with different superscripts within a row are different at P <0.05
FCR: Feed conversion ratio. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

Table 5 reports the clinical signs and mortality rates of broiler chickens supplemented with *B. decumbens* leaf meal. Throughout the 42-day study, only one case of leg problems was observed in T3 and T4. In addition, only one case of diarrhoea was observed in T2 and T5. Correspondingly, there was a total of 18 mortalities recorded from all the treatments. However, the mortality rates were similar between treatments (P = 0.714). This indicates that supplementing broiler chickens with *B. decumbens* leaf meal had no detrimental effect on the birds.

Table 5 Effect of *Brachiaria decumbens* leaf meal supplementation on the clinical observations of broilers

Clinical Observation	Treatments						*P* value
	T1	T2	T3	T4	T5	T6	
Mortality (birds)	3	3	2	3	5	2	0.714
Leg problem (birds)	0	0	1	1	0	0	-
Diarrhoea (birds)	0	1	0	0	1	0	-

Note: Different at $P < 0.05$ using a Chi-Square test
T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

The leucocyte profile of broiler chickens fed with *B. decumbens* leaf meal is presented in Table 6. There were differences in the total WBC counts ($P = 0.01$), heterophils ($P = 0.02$), lymphocytes ($P = 0.01$), and H/L ratio ($P = 0.01$) between treatments. Comparatively, T2 and T3 broilers exhibited the highest total WBC and lymphocyte count and the lowest heterophil and H/L ratio. This result demonstrated that 25 mg/kg of *B. decumbens* leaf meal had similar responses to the oxytetracycline antibiotic by enhancing leucocyte production, while reducing stress in the birds.

Table 6 Effect of *Brachiaria decumbens* leaf meal supplementation on the leucocyte profile of broilers

White Blood Cells	Treatments						*P* value
	T1	T2	T3	T4	T5	T6	
Total WBC count	199.40 ± 13.52^{c}	294.80 ± 17.23^{a}	294.40 ± 12.99^{a}	211.60 ± 7.96^{b}	208.80 ± 8.25^{b}	267.60 ± 8.84^{ab}	0.01
Heterophil (%)	30.40 ± 1.60^{a}	20.00 ± 0.63^{b}	20.00 ± 1.64^{b}	28.20 ± 2.31^{a}	24.20 ± 1.82^{ab}	21.40 ± 1.02^{b}	0.02
Lymphocyte (%)	66.20 ± 1.74^{b}	76.40 ± 0.870^{a}	74.60 ± 2.15^{a}	66.00 ± 2.38^{b}	69.80 ± 0.86^{b}	73.40 ± 1.20^{ab}	0.01
H/L Ratio	0.46 ± 0.03^{a}	0.26 ± 0.01^{b}	0.27 ± 0.02^{b}	0.43 ± 0.05^{a}	0.35 ± 0.02^{ab}	0.29 ± 0.01^{b}	0.01
Monocytes (%)	1.80 ± 0.37	1.60 ± 0.24	3.00 ± 0.77	2.40 ± 0.67	1.60 ± 0.67	2.20 ± 0.77	0.72
Eosinophil (%)	0.20 ± 0.20	0.60 ± 0.24	0.60 ± 0.24	0.80 ± 0.37	0.50 ± 0.6	0.80 ± 0.37	0.57
Basophil (%)	1.40 ± 0.24	3.20 ± 0.66	1.80 ± 0.37	2.20 ± 0.37	3.20 ± 0.66	3.00 ± 0.63	0.24

Note: All values are expressed as mean ± standard error; $^{a, b, c}$ values with different superscripts within a row are different at $P < 0.05$
H/L: heterophil and lymphocyte ratio. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

Table 7 illustrates the blood biomarkers of broiler chickens supplemented with *B. decumbens* leaf meal. All immunoglobulins and cytokine concentrations demonstrated differences ($P < 0.05$) among treatments. T3 broilers showed higher concentrations of IgG ($P = 0.008$), IgA ($P = 0.0452$), IgM ($P = 0.0203$), and IL4 ($P < 0.001$) than the other treatments. T2 expressed a higher concentration of IL7 ($P < 0.05$) than T3. The lowest expression of immunoglobulins and interleukins was observed in T1 (negative control group). Hence, this study revealed that an optimum supplementation of 25 mg/kg of *B. decumbens* leaf meal was capable of up-regulating the immune status of broilers.

Table 7 Effect of *Brachiaria decumbens* leaf meal supplementation on the blood biomarkers of broilers

Parameter	Treatments						*P* value
	T1	T2	T3	T4	T5	T6	
IgG (ng/ml)	1153 ± 0.48^{c}	1296 ± 1.20^{bc}	1569 ± 0.90^{a}	1231 ± 0.79^{c}	1251 ± 0.64^{bc}	1510 ± 1.12^{ab}	0.008
IgA (ng/ml)	799 ± 0.72^{bc}	970 ± 0.18^{ab}	1004 ± 0.54^{a}	762 ± 0.86^{c}	898 ± 0.40^{abc}	914 ± 0.38^{abc}	0.0452
IgM (mg/ml)	242 ± 0.08^{bc}	287 ± 0.23^{ab}	366 ± 0.37^{a}	265 ± 0.09^{abc}	342 ± 0.41^{c}	326 ± 0.06^{abc}	0.0203
IL4 (pg/ml)	2938 ± 1.85^{d}	4242 ± 1.11^{c}	4961 ± 1.04^{a}	458 ± 0.78^{bc}	4481 ± 0.59^{c}	4876 ± 1.30^{ab}	<0.0001
IL7 (pg/ml)	646 ± 0.68^{d}	1614 ± 2.41^{ab}	1304 ± 2.88^{bc}	822 ± 1.06^{cd}	749 ± 0.41^{cd}	2092 ± 0.63^{a}	<0.0001

Note: All values are expressed as mean ± standard error; $^{a, b, c}$ values with different superscripts within row are different at *P* <0.05
Ig: immunoglobulin; IL: interleukin. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

The relative telomere length of broiler chickens fed with *B. decumbens* leaf meal is reported in Figure 1. There was a difference (*P* = 0.0016) between treatments; T3 had a longer telomere length than the control, followed by the other treatments. Supporting the previous findings of the lowest heterophil and H/L ratio, a longer telomere length is also an indication that *B. decumbens* supplementation can alleviate stress effects in broiler chickens reared under tropical conditions.

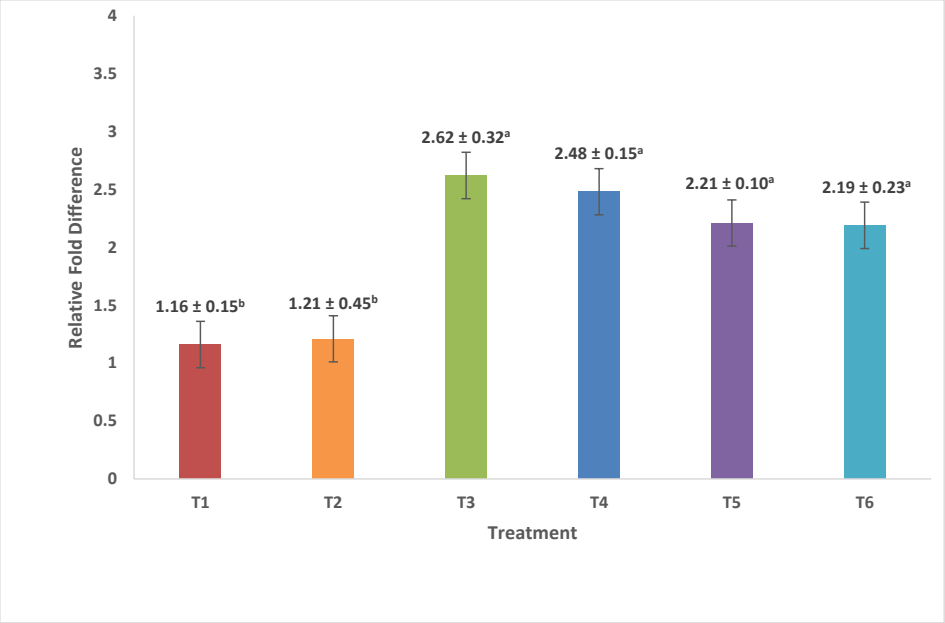

Figure 1 Effect of *B. decumbens* leaf meal supplementation on the relative telomere length of broilers
All values are expressed as mean ± standard error; $^{a, b}$ values with different superscripts are different at *P* <0.05
Rel. Fold Dif.: Relative fold difference. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *B. decumbens*; T4: 50 mg/kg *B. decumbens*; T5: 75 mg/kg *B. decumbens*; T6: 100 mg/kg *B. decumbens*

Discussion

According to earlier studies, phytobiotics contain antimicrobial properties that support growth and have antibacterial, antioxidant, and immunostimulant properties. Phytobiotics, according to Windisch *et al*. (2009), alter the microbial population to a more advantageous state, which promotes the release of digestive enzymes and leads to better gut functioning. Additionally, phytobiotics have been found to improve the palatability of broiler feed, promote growth, reduce mortality rate, improve gut functioning and nutrient digestibility, reduce gut microflora-related disease, improve carcass quality, improve meat quality and safety by reducing microbial load, and possibly improve sensory sensitivity (Grashorn, 2010). In the current study, it was shown that feeding T3 broilers 25 mg/kg of *B. decumbens*

leaf meal greatly improved broiler growth performance. Because *B. decumbens* leaf meal has a high concentration of saponins, as well as other phytocompounds, including alkaloids, tannins, and flavonoids, the large growth effect was demonstrated even with a small supply of *B. decumbens*. To support this, it has been shown that adding dried herbs and spices to chicken feed increases feed intake, average daily weight gain, and FCR (Alghirani *et al*., 2021b). Thus, just a small quantity is needed to enhance growth performance since the phytocompounds found in *B. decumbens* may help with gut health and enzyme production.

Phytobiotics have been shown by Montzouris *et al*. (2009) to promote growth, increase nutrient digestibility, and boost immunological response due to the enhanced enzyme secretion, leading to better digestion and absorption. Broiler chickens are dependent on enzyme-based systems, where feed is ground in the gizzard and is then digested further in the small intestine. Intestinal fluids and digestive enzymes, such as aminopeptidase, amylase, maltase, and invertase, are then used to break down the meal before it is absorbed (Clavijo & Florez, 2018). Nevertheless, plant-based supplementation must be given in the right quantities, notwithstanding their potential as feed additives, as they may influence growth performance and increase mortality owing to anti-nutritional elements or toxins found in the plant (Naidoo *et al*., 2008). For instance, the characteristics of saponins make ruminant consumption more problematic, especially in sheep and goats, since they can hinder digestion and absorption, which can result in bloat and death (Chung *et al*., 2018; Muniandy *et al*., 2020). In contrast, the current study shows that due to their toxicological adaptability, monogastric animals, in particular broiler chickens, do not present any issues since the death rate was minimal in all treatments. As broilers do not have rumen bacteria, the soapy properties of the saponins will not have a negative impact. The clinical symptoms of diarrhoea and leg problems seen in the current study might be related to other issues, including the genetic tendency of broilers for rapid growth or the housing conditions (Mack *et al*., 2013).

As lymphocytes are crucial for humoral immunity and function in cell-mediated immunity, the leucocyte profile is a general indicator of the health state of broilers. Several secondary metabolites from plant sources, like as alkaloids, tannins, and flavonoids, have comparable antibacterial, anti-inflammatory, and antioxidant effects to saponins and can benefit broiler health (Alagbe *et al*., 2020). Additionally, it has been reported that these phytochemicals enhance lipid metabolism and meat quality by enhancing the expression of glutamate-cysteine ligase catalytic (GCLC) and Nrf-2 antioxidants (Alghirani *et al*., 2021a). For instance, due to their anti-oxidation, anti-inflammation, immunomodulation, and gut protection properties, flavonoids are frequently associated with animal health defence against diseases (Kamboh *et al*. 2015). Moreover, flavonoids may alter endocrine, circulatory, mucosal, and cellular immunity in broiler chickens, as well as other health indices (Kamboh *et al*., 2018). In light of their antibacterial, antitumor, anthelminthic, analgesic, anti-inflammatory, and immunostimulant properties, plant-based feed additive supplementation with a high concentration of secondary metabolites presents a therapeutic remedy for a variety of diseases. The immunity and health of broilers will subsequently increase when these ingredients are included in meals for them at an ideal concentration. Similar findings were made in the current investigation, where broilers that received 25 mg/kg of *B. decumbens* leaf meal as a supplement showed improved leucocyte responses. With regard to the haematological parameters, broilers treated with *Persicaria odorata* leaf meal showed substantial (P <0.05) increases in the leukocyte count, RBC count, haemoglobin, and packed cell volume (PCV) (Basit *et al*., 2020).

A crucial part of systemic humoral immunity is played by the protein, immunoglobulin, which is generated by plasma cells. While they generate secretion proteins and signalling proteins for the biological functioning of leucocytes and immunoglobulins, cytokines are principally responsible for the operation of the immune system (Chung *et al*., 2021). According to Kamboh *et al*. (2018), dietary phytobiotic supplementation can assist the immune system and overall health of the broiler by controlling their cellular and mucosal immunity, as well as their endocrine and vascular indicators. Due to their properties of anti-oxidation, anti-inflammation, immunomodulation, and gut protection, secondary phytocompounds can also improve health performance (Kamboh *et al*., 2015). According to the results of the current study, *B. decumbens* leaf meal showed beneficial effects by boosting immunity in broiler chickens; just a modest amount of supplementation was needed. The greatest immunoglobulin and cytokine concentrations were seen in T3 broilers treated with 25 mg/kg of *B. decumbens* leaf meal. This can be explained by the fact that *B. decumbens* leaf meal contains secondary metabolites such saponins, alkaloids, and flavonoids. This is supported by research showing that broilers supplemented with plant-derived alkaloids have improved growth and intestinal health (Arora *et al*., 2012).

Telomeres are functional nucleoprotein structures that protect and cap the eukaryotic chromosomal end to preserve the genomic integrity against recombination, exonuclease destruction, and end-to-end fusion. They are found at the ends of chromosomes (Sohn & Subramani, 2014; Badmus *et al*., 2021). Non-genetic variables, such as environmental stress caused by housing systems, temperature and humidity, disease, oxidative stress, or stochastic influences, may have an impact on the natural factor telomere shortening (Factor-Litvak *et al*., 2017). Lee *et al*. (2008) reported that chickens supplemented with phytobiotics were observed have a lower rate of telomere shortening as a result of increased antioxidant and anti-inflammatory activity. Hence, plant material rich in various kinds of secondary metabolites can aid in maintaining the length of telomeres, therefore enhancing the health and wellbeing of broiler chickens. In the current study, the use of *B. decumbens* leaf meal is a component of oxidative release that aids in preserving the telomere length. The combination of phytocompounds, including saponins, alkaloids, tannins, and flavonoids, which enhance the preservation of telomere length, is likely to be responsible for the beneficial impact of the low concentration of *B. decumbens* leaf meal. According to Lee *et al*. (2008), Hyline brown commercial laying hens treated with Siberian ginseng leaf and *Eucommia* leaf plants with significant antioxidant properties at 0.5% and 1% exhibited longer telomeres between the ages of 20–30 and 60–70 weeks. Moreover, consuming foods high in antioxidants and anti-inflammatory properties, such as fruits, vegetables, and nuts, has been shown to maintain and control the biological telomere length, which may have a positive influence on an organism's health and lifespan (Hou *et al*., 2009; Lian *et al*., 2015).

Conclusion

Supplementing *B. decumbens* leaf meal at a modest dosage of 25 mg/kg was sufficient to promote growth performance and health benefits. These were proven in T3 broilers, which demonstrated the best growth performance with no marked clinical symptoms or mortality. Moreover, the leucocytes, immunoglobulins, and cytokines were up-regulated. In comparison to the other treatments, the relative telomere length was also maintained. In conclusion, this study confirms that 25 mg/kg of *B. decumbens* leaf meal is a promising feed additive to replace antibiotics in promoting the growth and health performance of broilers in tropical environments.

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Authors' contributions

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

Conflict of interest declaration

All authors declare no competing interests.

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