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## Novel *Brachiaria decumbens* saponins extract as a feed additive improves growth, gut health and meat quality in broiler chickens

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### ABSTRACT

With restrictions on antibiotic use in poultry, plant-derived metabolites such as commercial saponins have emerged as alternatives for growth promotion. This study examined the effects of novel saponins extract from *Brachiaria decumbens* on broiler performance under tropical conditions. A total of 300-d-old female Ross 308 broilers were randomly assigned to six dietary groups, each with five replicates of ten birds. The control group received a standard diet without additives, while the positive control group was supplemented with 100 mg/kg of oxytetracycline. The remaining four groups were fed the same basal diet enriched with 25, 50, 75 or 100 mg/kg of *B. decumbens* saponins extract. The extract was obtained through maceration from five-week-old plants. Over the 42-day feeding period, weekly feed intake and body weight were recorded to assess weight gain and feed conversion ratio (FCR). Ten birds per group were slaughtered at the end of the starter and finisher phases for analysis of nutrient digestibility, intestinal morphology, caecal microflora, carcass yield and meat quality. Broilers supplemented with 100 mg/kg extract showed 6.1% higher body weight gain and 9.2% better FCR than controls ( $p < 0.05$ ). Ileal protein digestibility rose by 22% and duodenal villus height by 10%. Dressing percentage increased by 2%, while breast muscle drip loss dropped by 19% ( $p < 0.05$ ). Supplemented groups also had balanced caecal microflora with no *Salmonella* or *E. faecalis* detected. These indicate that *B. decumbens* saponins extract is a promising plant-based alternative to antibiotics for enhancing poultry production in tropical climates.

### HIGHLIGHTS

1. Novel use of *B. decumbens* saponins extract as a phytochemical feed additive improved broiler growth, carcass traits and meat quality.
2. Supplementation at 100 mg/kg enhanced ileal nutrient digestibility and gut histomorphology in broilers raised in tropical climates.
3. Caecal microflora balance was positively influenced without the presence of pathogenic *Salmonella* or *E. faecalis* in supplemented groups.

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*Brachiaria decumbens*; saponins; antibiotic replacement; production performances; Ross 308

## Introduction

The poultry sector is one of the fastest-growing areas of the agricultural economy, driven by global population growth and rising demand for animal-based protein (Alghirani et al. 2021a). This expansion brings challenges such as maintaining biosecurity, controlling disease outbreaks and managing rising feed costs. Greater awareness of food safety and animal welfare has also led to stricter industry regulations and

expectations. In response, many developed countries have banned the use of antibiotics in animal feed (Gheisar et al. 2015). This shift highlights the need for effective alternatives to antibiotics for disease prevention and growth promotion in poultry production (Yadav et al. 2016).

Plant-based compounds, known as phytobiotics, have been incorporated into poultry diets as alternatives to antibiotics. They help reduce antibiotic

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use without compromising production performance (Gadde et al. 2017). Among these, saponins have attracted particular attention for their potential to improve animal health and productivity, making them a promising natural substitute for antibiotic growth promoters (Alghirani et al. 2021a). Saponins are plant-derived glycosides with diverse biological activities, including antimicrobial, antioxidant, immunomodulatory and antiparasitic effects (Aboutaleb and Monfared 2015; Chaudhary et al. 2018; Alghirani et al. 2022a, 2022b). In poultry, commercial extracts of *Yucca schidigera* and *Quillaja saponaria* have been shown to improve growth, nutrient digestibility, gut morphology, microbial balance and meat quality, making them promising alternatives to antibiotics (Sahoo et al. 2015; Su et al. 2016; Alghirani, Chung, et al. 2021). These effects are linked to their ability to interact with intestinal membranes, stimulate digestive enzymes and regulate microbial balance (Aminullah et al. 2025).

*Brachiaria decumbens*, commonly known as signal grass, is a fast-growing tropical forage widely used in ruminant nutrition. It is one of the most common *Brachiaria* species, making up over 80% of improved pastures in tropical agriculture (Chung et al. 2018). Despite its productivity, this species contains steroidal saponins, primarily dichotomin and dioscin/protodioscin, which have been reported to cause hepatotoxicity and nephrotoxicity in small ruminants, particularly sheep. These compounds have been linked to sporadic cases of photosensitisation, reduced health performance, and mortality (Muniandy et al. 2020). Although *B. decumbens* saponins extract is rich in steroidal saponins, its application in broiler chicken nutrition remains unexplored, unlike the more widely studied *Y. schidigera* and *Q. saponaria*. Previous studies reported that broilers supplemented with 25 mg/kg of *B. decumbens* leaf meal containing 54.60% saponins showed improvements in growth performance, nutrient digestibility, gastrointestinal health, carcass yield and meat quality compared with other treatment groups (Alghirani et al. 2022a, 2022b). Similarly, Ong et al. (2024) observed enhanced performance in Sasso broiler chickens fed diets containing 5 g/kg of *B. decumbens* leaf meal. These benefits may also come from other bioactive compounds in *B. decumbens*, such as tannins, flavonoids, and alkaloids, which also support health and productivity (Seng et al. 2025).

Despite evidence from leaf meal studies, few reports have isolated the effects of purified steroidal saponins from *B. decumbens* in broilers. This knowledge gap raises uncertainty about whether saponins alone is

responsible for the observed improvements. Addressing this gap is important for understanding their potential role as phyto-genic feed additives. Therefore, this study aimed to evaluate the effects of purified steroidal saponins extracted from *B. decumbens* on growth performance, nutrient digestibility, gut morphology, microbial balance, carcass traits and meat quality in broiler chickens raised under tropical conditions. It is hypothesised that dietary supplementation with purified steroidal saponins extracted from *B. decumbens* dose-dependently enhances growth performance, nutrient digestibility, gut health, carcass traits and meat quality in broiler chickens raised under tropical conditions. By focusing on a purified extract under controlled feeding conditions, this work addresses the lack of clarity on the direct role of *B. decumbens* saponins as phyto-genic feed additives in poultry nutrition (Aminullah et al. 2025; Ibeagha-Awemu et al. 2025).

## Materials and methods

### Plant material preparation

*B. decumbens* was cultivated at Farm 15 of the Field Laboratory, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The grass was manually harvested at five weeks of age and cleaned, then oven-dried at 60 °C for 72 h until a constant weight was achieved. After drying, the material was finely chopped, milled to a 1 mm particle size, and stored at ambient temperature for subsequent saponin extraction (Ong et al. 2024; Seng et al. 2025).

### Saponins extraction

Saponins were extracted using the maceration technique described by Ashraf et al. (2013). Briefly, 1.5 kg of *B. decumbens* leaf powder was mixed with 7.5 L of distilled hexane (1 g: 5 mL) and stirred continuously with a magnetic stirrer for 24 h to remove lipophilic compounds. The mixture was then filtered through Whatman No. 1 filter paper, and the defatted residue was dried overnight at 45 °C to eliminate residual hexane. Subsequently, 1.2 kg of the defatted material was immersed in 12 L of 50% aqueous methanol at room temperature and left to macerate overnight. After centrifugation at 3000 × *g* for 10 min, the supernatant (approximately 6 L) was collected, and methanol was removed under reduced pressure at 40 °C using a rotary evaporator. The concentrated extract was subjected to liquid–liquid partitioning with *n*-butanol (1:1) in a separating funnel to isolate the saponins from the aqueous phase. The butanol fraction was retained, and

the solvent was evaporated under vacuum at a maximum of 45 °C. The resulting concentrated extract was dried overnight in an oven at 45 °C to ensure complete solvent removal. Quantification of total steroidal saponins, following the method of Li et al. (2010), indicated a saponin content of at least 78.01%.

### Birds and husbandry

All animal procedures, including care, handling and sample collection, were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (Approval No. UPM/IACUC/AUP-R005/2020). A completely randomised design (CRD) was used to assign 300 female day-old Ross 308 broiler chicks into six dietary groups, each consisting of five replicates of 10 birds. The broilers were reared for 42 d in an open-sided housing system on deep litter bedding made of wood shavings, with a stocking density of 0.07 m<sup>2</sup> per bird. Environmental conditions were maintained at an average temperature of 29 °C and 79% relative humidity, with 23 h of continuous lighting per day. Birds received intraocular vaccinations against infectious bronchitis (IB) and Newcastle disease (ND) on day 7, followed by vaccination against infectious bursal disease (IBD) on day 14 (Chung et al. 2021). Feed and clean water were provided ad libitum throughout the experimental period.

### Diets

Throughout the 42-d feeding trial, broilers were provided with commercial starter feed from day 1–21 and finisher feed from day 22–42. Both feeds were supplied in crumble form and were primarily composed of corn and soybean meal. In the negative control group (Treatment 1), birds received the basal diet without additives. The positive control group (Treatment 2) received the same diet supplemented with 100 mg/kg of oxytetracycline. Treatments 3–6 consisted of the same basal diet enriched with 25, 50, 75 or 100 mg/kg of *B. decumbens* saponins extract, respectively. The supplementation levels of 25, 50, 75 and 100 mg/kg were selected to evaluate a graded dose–response. Lower levels were based on prior studies using *B. decumbens* leaf meal (Alghirani et al. 2022a, 2022b; Ong et al. 2024), while 100 mg/kg aligned with reports of optimal responses to commercial saponins such as *Y. schidigera* and *Q. saponaria* (Sahoo et al. 2015; Alghirani, Chung, et al. 2021). The purified *B. decumbens* saponins extract was thoroughly blended into the basal diet as a premix to ensure uniform distribution before feeding. The detailed nutrient compositions of the starter and finisher diets for each treatment group are presented in Tables 1 and 2.

### Growth performance

Feed intake and body weight were measured weekly for each replicate using a Mettler Toledo Industrial

**Table 1.** Composition and nutrient content of broiler starter diet supplemented with different concentrations of *Brachiaria decumbens* saponins extract.

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
<b>Ingredient, (% in feed)</b>						
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
D,L-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
<b>Calculated analysis</b>						
Metabolizable energy, MJ/kg	13.34 ± 0.03	13.34 ± 0.03	13.14 ± 0.03	13.27 ± 0.03	13.20 ± 0.03	13.40 ± 0.03
Dry matter, %	89.33 ± 0.07	90.00 ± 0.07	89.33 ± 0.07	90.44 ± 0.07	89.44 ± 0.07	89.44 ± 0.07
Crude protein, %	24.77 ± 0.12	25.45 ± 0.12	25.05 ± 0.12	25.34 ± 0.12	25.38 ± 0.12	25.86 ± 0.12
Crude fibre, %	3.86 ± 0.12	4.71 ± 0.12	4.28 ± 0.12	4.04 ± 0.12	4.11 ± 0.12	4.22 ± 0.12
Ether extract, %	7.14 ± 0.11	7.03 ± 0.11	7.00 ± 0.11	6.94 ± 0.11	7.36 ± 0.11	7.53 ± 0.11
Ash, %	5.84 ± 0.17	5.55 ± 0.17	6.59 ± 0.17	7.00 ± 0.17	6.95 ± 0.17	5.71 ± 0.17

All values were expressed as mean ± SEM. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract. \*mineral mix comprises Fe (100 mg), Zn (100 mg), I (2 mg), Mn (110 mg), Cu (20 mg), Se (0.2 mg), and Co (0.6 mg).

<sup>†</sup>Vitamin premix contains 2 mg of retinol, 0.03 mg of cholecalciferol, 0.02 mg of  $\alpha$ -tocopherol, 0.83 mg of thiamine, 2 mg of riboflavin, 0.33 mg of folic acid, 1.33 mg of menadione, 0.03 mg of cobalamin, 0.03 mg of biotin, 3.75 mg of pantothenic acid, 23.3 mg of niacin, and 1.33 mg of pyridoxine.

**Table 2.** Composition and nutrient content of broiler finisher diet supplemented with different concentrations of *Bracharia decumbens* saponins extract.

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredient, (% in feed)						
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	12.58 ± 0.03	12.41 ± 0.03	12.47 ± 0.03	12.75 ± 0.03	12.71 ± 0.03	12.70 ± 0.03
Dry matter %	87.55 ± 0.20	87.00 ± 0.20	87.88 ± 0.20	88.00 ± 0.20	88.33 ± 0.20	88.33 ± 0.20
Crude protein %	16.22 ± 0.08	16.08 ± 0.08	16.59 ± 0.08	16.47 ± 0.08	16.91 ± 0.08	17.13 ± 0.08
Crude fibre %	4.02 ± 0.09	4.11 ± 0.09	4.08 ± 0.09	3.73 ± 0.09	3.82 ± 0.09	4.00 ± 0.09
Ether extract %	4.84 ± 0.09	4.47 ± 0.09	4.71 ± 0.09	4.85 ± 0.09	4.82 ± 0.09	5.04 ± 0.09
Ash %	5.71 ± 0.11	5.74 ± 0.11	6.57 ± 0.11	5.30 ± 0.11	6.03 ± 0.11	6.28 ± 0.11

All values were expressed as mean ± SEM. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract. \*mineral mix comprises Fe (100 mg), Zn (100 mg), I (2 mg), Mn (110 mg), Cu (20 mg), Se (0.2 mg), and Co (0.6 mg).

<sup>3</sup>Vitamin premix contains 2 mg of retinol, 0.03 mg of cholecalciferol, 0.02 mg of  $\alpha$ -tocopherol, 0.83 mg of thiamine, 2 mg of riboflavin, 0.33 mg of folic acid, 1.33 mg of menadione, 0.03 mg of cobalamin, 0.03 mg of biotin, 3.75 mg of pantothenic acid, 23.3 mg of niacin, and 1.33 mg of pyridoxine.

Scale (BBA211 series, Switzerland) with a precision of two decimal places. These measurements were used to calculate body weight gain and feed conversion ratio (FCR). On days 21 and 42, two birds per replicate (ten birds per treatment group) were randomly selected, weighed and slaughtered for subsequent analyses. The collected samples were then analysed for ileal nutrient digestibility, intestinal histomorphology, caecal microbial populations, carcass traits and meat quality (Alghirani, Chung, et al. 2021).

### Nutrient digestibility

To evaluate nutrient digestibility, titanium dioxide (TiO<sub>2</sub>) was incorporated into the starter and finisher diets at a concentration of 300 mg/kg, three days before slaughter, as an indigestible marker. After euthanasia, ileal digesta were collected and stored at -20 °C for subsequent analysis (Ong et al. 2024). Samples were digested with 7.4 M sulphuric acid and oxidised with 30% hydrogen peroxide. The concentration of TiO<sub>2</sub> in both feed and digesta was determined using spectrophotometry at an absorbance of 410 nm. Apparent ileal digestibility (AID) of dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE) and ash was calculated following the method of Ong et al. (2020), using the formula: AID (%) = 100 × [(TiO<sub>2</sub> in feed/TiO<sub>2</sub> in digesta) × (nutrient in digesta/nutrient in feed)].

### Gut histomorphology

To assess intestinal histomorphology, 5 cm segments of the duodenum, jejunum, and ileum were excised from each bird, rinsed in 10% neutral-buffered formalin, and fixed overnight. The fixed tissues were processed using a tissue processor, embedded in paraffin wax, and sectioned for microscopic evaluation. Sections were cut at a thickness of 4  $\mu$ m, mounted on glass slides, and stained with haematoxylin and eosin. Microscopic examination was carried out using a Nikon DS-U2/L2 light microscope, and measurements were recorded with NIS-Elements D imaging software. Villus height was measured from the villus tip to the crypt-villus junction, while crypt depth was measured at the point of invagination between adjacent villi (Chung et al. 2019).

### Caecal microflora population

Caecal contents from each treatment group were immediately transported to the microbiology laboratory for analysis, where samples were pooled to obtain a composite sample that was subsequently used to determine microbial populations. The procedures included standard plate counts, coliform enumeration, microbial isolation and identification, and screening for *Salmonella* species to evaluate the composition of the caecal microflora. Bacterial populations were

quantified by calculating the number of colony-forming units (CFUs) per gram of sample and expressed as  $\log_{10}$  values to indicate viable bacterial counts (Alghirani, Chung, et al. 2021).

### **Carcase characteristic**

Carcase characteristics were evaluated post-slaughter by measuring final live weight, kill-out weight, de-feathered weight, carcass weight and dressing percentage. Individual components, including the breast muscle, wings, drumsticks, head, neck, shank, digestive tract, full and empty gizzard, liver and heart, were carefully dissected, weighed and recorded following the method of Chung et al. (2020). Carcass component values are expressed as percentages relative to carcass weight.

### **Meat quality**

Meat quality was assessed using the right pectoralis major (breast) and soleus (drumstick) muscles, focusing on pH, colour, drip loss, cooking loss and shear force, in accordance with the protocols of Chung et al. (2020). For pH determination, 15 g of each muscle sample was snap-frozen in liquid nitrogen ( $-195^{\circ}\text{C}$ ) and stored at  $-80^{\circ}\text{C}$  until analysis. After 24 h, the frozen samples were pulverised with a mortar and pestle, homogenised in distilled water and the pH was measured using a portable pH metre.

To evaluate colour attributes, 15 g of muscle tissue from each bird was analysed using a ColorFlex spectrophotometer. Colour parameters were recorded as  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) based on the surface appearance of the meat.

Drip loss was measured using 20 g samples of breast and drumstick muscles from each treatment group. The samples were vacuum-sealed and stored at  $4^{\circ}\text{C}$ . Initial weights ( $W_1$ ) were recorded, and final weights ( $W_2$ ) were taken after 24 and 48 h. Drip loss (%) was calculated using the formula:  $\text{Drip loss} = [(W_1 - W_2)/W_1] \times 100$ .

For cooking loss, 15 g of each muscle sample was weighed ( $W_1$ ), sealed in vacuum bags, and cooked in a water bath at  $80^{\circ}\text{C}$  for 20 min. After cooling, the final weight ( $W_2$ ) was recorded, and cooking loss (%) was calculated using the formula:  $\text{Cooking loss} = [(W_1 - W_2)/W_1] \times 100$ .

Shear force was then assessed by cutting cooked meat samples into strips ( $1 \times 1 \times 2 \text{ cm}^3$ ), aligned with the muscle fibre direction, and subjecting them to shear force analysis using a TA.XTplus Texture Analyser equipped with a Volodkovich blade (Stable

Micro Systems, Surrey, UK). The force (kg) required to shear the muscle fibres was recorded.

### **Statistical analysis**

The Statistical Analysis System was used to perform a one-way analysis of variance (ANOVA) on all data obtained under the completely randomised design model. Prior to analysis, data normality was confirmed using the Shapiro–Wilk test. Group means for growth performance, ileal nutrient digestibility, gut histomorphology, carcass traits and meat quality were evaluated. Significant differences among treatment groups were determined using Tukey's *post hoc* test, with significance set at  $p < 0.05$ .

## **Results and discussion**

### **Growth performance**

Table 3 presents the growth performance of broilers fed diets supplemented with *B. decumbens* saponins extract at days 21 and 42. Significant differences ( $p < 0.05$ ) were observed in final body weight and weight gain during the starter phase. Broilers in Treatment 6 (T6), which received 100 mg/kg of the extract, recorded the highest final body weight and weight gain. During the finisher phase, significant differences were also observed among treatments for body weight ( $p < 0.0001$ ), weight gain ( $p < 0.0001$ ), feed intake ( $p = 0.0170$ ), and overall feed conversion ratio (FCR) ( $p = 0.0005$ ). T6 broilers achieved the greatest weight gain and final body weight, along with the most efficient FCR, indicating enhanced growth performance at the highest supplementation level.

Growth rate is a key indicator of broiler performance and is influenced by multiple factors, including diet and environmental conditions (Chung et al. 2021). In tropical regions, elevated temperatures can induce oxidative stress and damage the intestinal brush border membrane. This reduces feed intake, growth rate and feed efficiency (Santos et al. 2015). Phytochemical additives such as saponins have been shown to enhance poultry productivity by improving gut morphology, digestion and nutrient assimilation (Tavangar et al. 2021). This study is the first to evaluate the effects of *B. decumbens* saponins extract on broilers. The T6 group (100 mg/kg supplementation) showed the greatest improvements in body weight, weight gain and FCR. These findings align with previous reports demonstrating that diets supplemented with 100 mg/kg of *Y. schidigera* extract, which contains 40–60% saponins, enhanced broiler growth

**Table 3.** Effect of *Brachiaria decumbens* saponins extract supplementation on the growth performance of broilers on day 21 and 42.

Parameters	Treatment						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 0-21 (Starter phase)								
Initial body weight, kg	0.04	0.04	0.04	0.04	0.04	0.04	0.0000	0.1864
Final body weight, kg	0.90 <sup>c</sup>	0.91 <sup>b,c</sup>	0.92 <sup>b</sup>	0.91 <sup>c</sup>	0.92 <sup>b</sup>	0.95 <sup>a</sup>	0.0050	<0.0001
Body weight gain, kg	0.85 <sup>c</sup>	0.86 <sup>b,c</sup>	0.88 <sup>b</sup>	0.87 <sup>c</sup>	0.88 <sup>b</sup>	0.91 <sup>a</sup>	0.0083	<0.0001
Feed intake, kg	1.18	1.20	1.18	1.15	1.18	1.18	0.0217	0.1987
Cumulative FCR	1.37	1.39	1.34	1.32	1.33	1.30	0.0233	0.3794
Day 22-42 (Finisher phase)								
Final body weight, kg	2.18 <sup>b</sup>	2.18 <sup>b</sup>	2.09 <sup>c</sup>	2.18 <sup>b</sup>	2.16 <sup>b</sup>	2.31 <sup>a</sup>	0.0117	<0.0001
Body weight gain, kg	2.13 <sup>b</sup>	2.15 <sup>b</sup>	2.04 <sup>c</sup>	2.13 <sup>b</sup>	2.11 <sup>b</sup>	2.26 <sup>b</sup>	0.0117	<0.0001
Feed intake, kg	4.17 <sup>a</sup>	4.17 <sup>a</sup>	3.93 <sup>b</sup>	4.05 <sup>a,b</sup>	3.98 <sup>b</sup>	4.03 <sup>a,b</sup>	0.0533	0.0170
Cumulative FCR	1.96 <sup>a</sup>	1.94 <sup>ab</sup>	1.92 <sup>a,b</sup>	1.90 <sup>a,b</sup>	1.88 <sup>b</sup>	1.78 <sup>c</sup>	0.0152	0.0005

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c</sup> values with superscript within row are significantly different at  $p < 0.05$ . FCR: feed conversion ratio. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract. Each dietary group consisting of five replicates of 10 birds.

**Table 4.** Effect of *Brachiaria decumbens* saponins extract supplementation on the apparent ileal nutrient digestibility of broilers on day 21 and 42.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 0-21 (Starter phase)								
Dry matter (%)	68.43 <sup>a</sup>	67.28 <sup>a</sup>	63.98 <sup>a</sup>	64.30 <sup>a</sup>	56.80 <sup>b</sup>	54.15 <sup>b</sup>	1.7300	0.0019
Crude protein (%)	52.03 <sup>b,c</sup>	51.53 <sup>c</sup>	55.42 <sup>b,c</sup>	56.09 <sup>b</sup>	60.50 <sup>a</sup>	62.28 <sup>a</sup>	1.0567	0.0003
Crude fibre (%)	30.63 <sup>b</sup>	31.64 <sup>b</sup>	33.19 <sup>a,b</sup>	32.58 <sup>a,b</sup>	32.32 <sup>b</sup>	35.78 <sup>a</sup>	1.0567	0.0451
Ether extract (%)	72.50 <sup>b</sup>	76.22 <sup>a</sup>	73.42 <sup>ab</sup>	71.70 <sup>b</sup>	76.55 <sup>a</sup>	76.73 <sup>a</sup>	1.0033	0.0280
Day 22-42 (Finisher phase)								
Dry matter (%)	64.59 <sup>a</sup>	63.76 <sup>a,b</sup>	62.87 <sup>a,b</sup>	60.36 <sup>b</sup>	56.24 <sup>c</sup>	53.35 <sup>c</sup>	1.1200	0.0003
Crude protein (%)	50.05 <sup>c</sup>	51.83 <sup>c,b</sup>	52.44 <sup>c,b</sup>	54.92 <sup>b</sup>	58.77 <sup>a</sup>	61.08 <sup>a</sup>	1.0000	0.0003
Crude fibre (%)	27.01 <sup>b</sup>	27.08 <sup>b</sup>	29.03 <sup>b</sup>	29.26 <sup>b</sup>	30.35 <sup>a,b</sup>	33.62 <sup>a</sup>	1.1217	0.0427
Ether extract (%)	72.96	75.91	76.65	76.60	74.83	78.25	0.9567	0.1014

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract. Two birds per replicate (ten birds per treatment group) were randomly selected for sample collection.

performance (Su et al. 2016; Alghirani, Chung, et al. 2021). The observed benefits may be attributed to the high steroidal saponin content. These compounds support digestive function by stimulating enzyme activity and enhancing nutrient absorption through increased villus height (Aminullah et al. 2025). Additionally, saponins may act as natural emulsifiers, improving membrane permeability and nutrient transport (Reyer et al. 2017), or promote growth through antimicrobial, immunostimulatory, antioxidant, anti-inflammatory and free radical-scavenging activities (Chaudhary et al. 2018; Talbott et al. 2018; Ferdous et al. 2019). Therefore, the superior growth performance in broilers supplemented with 100 mg/kg of *B. decumbens* extract suggests this dosage provides an optimal concentration of bioactive compounds to support metabolic and physiological functions.

### Ileal nutrient digestibility

Table 4 presents the ileal nutrient digestibility of broilers on days 21 and 42. During the starter phase, significant differences ( $p < 0.05$ ) were observed in the digestibility

of DM, CP, CF and EE. Broilers in the T6 group, supplemented with 100 mg/kg of *B. decumbens* saponins extract, showed the highest digestibility values for CP, CF and EE, but the lowest for DM. In the finisher phase, digestibility of DM ( $p = 0.0003$ ), CP ( $p = 0.0003$ ), and CF ( $p = 0.0427$ ) also differed significantly among treatments. Notably, T6 broilers maintained higher CP and CF digestibility but lower DM digestibility, indicating that supplementation at 100 mg/kg may improve ileal nutrient absorption efficiency.

Phytobiotics have been shown to enhance gastrointestinal enzyme activity in poultry through bioactive compounds that positively affect physiological and metabolic processes. This, in turn, improves nutrient digestibility (Ibeagha-Awemu et al. 2025). Saponins, in particular, have been reported to modify membrane permeability and facilitate the transcellular transport of macromolecules within the intestinal tract (Reyer et al. 2017). In this study, broilers in the T6 group, supplemented with 100 mg/kg of *B. decumbens* saponins extract, achieved the highest ileal nutrient digestibility across all phases. Similar findings were reported in broilers supplemented with 125 mg/kg of *Y. schidigera*

**Table 5.** Effect of *Brachiaria decumbens* saponins extract supplementation on the small intestinal villus height and crypt depth of broilers on day 21 and 42.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 0-21 (Starter phase)								
Villi height (µm)								
Duodenum	881.20 <sup>e</sup>	899.44 <sup>d,e</sup>	909.27 <sup>c,d</sup>	923.46 <sup>b,c</sup>	931.60 <sup>b</sup>	956.45 <sup>a</sup>	7.5733	<0.0001
Jejunum	486.08 <sup>c</sup>	492.80 <sup>c</sup>	482.98 <sup>c</sup>	489.48 <sup>c</sup>	515.89 <sup>b</sup>	554.80 <sup>a</sup>	5.4533	<0.0001
Ileum	374.02 <sup>c</sup>	374.58 <sup>c</sup>	378.31 <sup>c</sup>	379.69 <sup>c</sup>	395.98 <sup>b</sup>	409.42 <sup>a</sup>	4.1500	<0.0001
Crypt depth (µm)								
Duodenum	101.04 <sup>a</sup>	106.87 <sup>a</sup>	98.41 <sup>a,b</sup>	91.15 <sup>b,c</sup>	90.11 <sup>b,c</sup>	87.05 <sup>c</sup>	3.9033	<0.0001
Jejunum	92.73 <sup>a</sup>	92.96 <sup>a</sup>	89.85 <sup>a</sup>	67.86 <sup>b</sup>	67.76 <sup>b</sup>	60.30 <sup>b</sup>	2.7333	<0.0001
Ileum	84.74 <sup>a</sup>	72.39 <sup>b</sup>	69.83 <sup>b</sup>	66.41 <sup>b</sup>	54.56 <sup>c</sup>	53.84 <sup>c</sup>	2.8033	<0.0001
Day 22-42 (Finisher phase)								
Villi height (µm)								
Duodenum	1146.09 <sup>c</sup>	1189.78 <sup>b</sup>	1185.17 <sup>b</sup>	1193.15 <sup>b</sup>	1207.08 <sup>b</sup>	1260.21 <sup>a</sup>	8.5367	<0.0001
Jejunum	659.63 <sup>d</sup>	688.89 <sup>c</sup>	690.30 <sup>c</sup>	705.69 <sup>c</sup>	724.84 <sup>b</sup>	778.23 <sup>a</sup>	5.9767	<0.0001
Ileum	569.72 <sup>d</sup>	571.65 <sup>d</sup>	568.77 <sup>c</sup>	582.19 <sup>c</sup>	602.74 <sup>b</sup>	666.25 <sup>a</sup>	4.9033	<0.0001
Crypt depth (µm)								
Duodenum	145.45 <sup>a</sup>	145.87 <sup>a</sup>	145.58 <sup>a</sup>	131.37 <sup>b</sup>	128.72 <sup>b</sup>	114.62 <sup>c</sup>	4.1583	<0.0001
Jejunum	123.38 <sup>a</sup>	98.60 <sup>b</sup>	97.16 <sup>b</sup>	86.33 <sup>c</sup>	79.16 <sup>cd</sup>	74.83 <sup>d</sup>	3.0233	<0.0001
Ileum	97.29 <sup>a</sup>	80.69 <sup>a</sup>	84.87 <sup>b</sup>	77.71 <sup>c</sup>	66.25 <sup>d</sup>	58.59 <sup>e</sup>	2.5017	<0.0001

All values were expressed as mean ± SEM; <sup>a,b,c,d</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

extract, which significantly improved protein and energy utilisation (Sahoo et al. 2015). Likewise, Alghirani, Chung, et al. (2021) observed enhanced nutrient absorption with 100 mg/kg of *Y. schidigera* supplementation. These improvements are likely due to the stimulatory effect of phytobiotics on pancreatic enzyme secretion. This promotes nutrient breakdown and assimilation. Furthermore, Reyer et al. (2017) demonstrated that phytogetic additives modulate the expression of genes encoding peptide and amino acid transporters, thereby enhancing protein uptake in the small intestine. The emulsifying properties of saponins, which stabilise oil-in-water emulsions and improve monoglyceride solubility, may also explain the increased EE digestibility. Together, the expanded absorptive surface area and enzymatic activation induced by saponins likely contributed to the improved digestibility observed in broilers given 100 mg/kg of *B. decumbens* extract.

### Gut histomorphology

Table 5 summarizes villus height and crypt depth in the duodenum, jejunum, and ileum of broilers on days 21 and 42. Significant differences ( $p < 0.05$ ) were observed among treatment groups during both the starter and finisher phases. Supplementation with *B. decumbens* saponins extract resulted in a progressive increase in villus height across all three intestinal segments ( $p < 0.0001$ ), with the highest values recorded in T6 broilers receiving 100 mg/kg of the extract. In contrast, increasing levels of the extract were associated with reduced crypt depth ( $p < 0.0001$ ), with T6

broilers showing the shallowest crypts in all intestinal regions. The combination of elongated villi and reduced crypt depth in the T6 group indicates enhanced intestinal integrity, which likely supported improved nutrient digestion and absorption.

Villus height and crypt depth are widely recognised as key indicators of gut health and functional efficiency in broilers. Increased villus height expands the mucosal surface area, improving nutrient absorption and supporting better growth performance. In contrast, deeper crypts are often linked to impaired nutrient uptake and higher mucosal turnover, which can compromise health (Heak et al. 2017). Madkour et al. (2024) reported that the inclusion of plant extracts in broiler diets modified intestinal morphology and stimulated the secretion of digestive enzymes such as amylase and protease, thereby enhancing growth outcomes. In this study, dietary supplementation with increasing concentrations of *B. decumbens* saponins extract produced a linear increase in villus height across the duodenum, jejunum and ileum during both the starter and finisher phases. These results are consistent with previous studies showing that broilers receiving saponin-enriched diets exhibited not only improved body weight but also greater villus height in the gastrointestinal tract (Gurbuz et al. 2011; Alghirani, Chung, et al. 2021). This enhancement may be linked to the antioxidant properties of saponins, which improve the intestinal environment and cellular architecture (Adriani et al. 2019). As villus height increases, the absorptive area expands. This facilitates more efficient nutrient uptake and feed utilisation, which is particularly important during early growth stages when

feed efficiency strongly impacts performance (Tavangar et al. 2021). Conversely, the shallowest crypt depths were observed in broilers from the T6 group supplemented with 100 mg/kg of *B. decumbens* saponins extract. Reduced crypt depth may indicate lower epithelial turnover or reduced energy requirements for tissue maintenance due to more differentiated enterocytes (Chung et al. 2019). This reduction suggests that less energy was diverted towards tissue regeneration, allowing more allocation to growth. This effect was reflected in improved body weight and FCRs. Overall, the concurrent increase in villus height and decrease in crypt depth observed in this study likely resulted from the modulatory effects of saponins on intestinal physiology, including acid secretion, pH regulation, gastrin production, epithelial cell proliferation and nutrient metabolism.

### Caecal microorganisms' population

Table 6 presents the caecal microbial populations of broilers on days 21 and 42. On day 21, *Escherichia coli* was detected in all treatment groups, while *Enterococcus faecalis* was identified only in the T1 group. Broilers in the T6 group, supplemented with 100 mg/kg of *B. decumbens* saponins extract, recorded the highest standard plate counts and coliform counts. By day 42, the overall microbial composition remained similar, with both *E. coli* and *E. faecalis* detected across all groups. However, T6 broilers continued to exhibit elevated coliform and plate count levels compared with the other treatments. Importantly, *Salmonella* species were absent in all groups at both time points.

The gastrointestinal tract of broilers harbours a complex microbial community that plays a crucial role in modulating immune responses, metabolic processes and nutrient utilisation (Sohail et al. 2012). The

composition and balance of this microflora can be influenced by dietary inclusion of plant-derived compounds. Such compounds may enhance digestive efficiency, nutrient absorption and growth performance (Talbot et al. 2018; Ferdous et al. 2019). In this study, analysis of caecal contents during the starter phase revealed variations in microbial populations, including coliform and total bacterial counts. Notably, *E. faecalis* was isolated only from the T1 group, which received no dietary supplementation. Although *E. faecalis* is part of the normal avian gut flora, it is also an opportunistic pathogen capable of causing severe disease, particularly in embryos and young chicks (Fertner et al. 2011). The absence of *E. faecalis* in supplemented groups suggests that *B. decumbens* saponins extract may exert antimicrobial effects. This observation is consistent with the findings of Low (2015), who reported that the saponin-rich content of *B. decumbens* possesses antimicrobial activity against a range of pathogens, including bacteria, fungi and insects. These effects may result from saponins interacting with microbial cell membranes. They disrupt membrane integrity and ionic transport, particularly of hydrogen and potassium ions. This disruption leads to cell lysis, interference with ATP synthesis, and reduced pathogenic load (Gilani et al. 2021). During the finisher phase, no notable differences were observed in microbial species across treatment groups, which may reflect microbial adaptation to continuous saponin exposure over time. Nevertheless, phytochemical additives such as saponins may still support beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. They do so by suppressing pathogenic proliferation and improving nutrient bioavailability (Park and Kim 2020). Supporting this, Alghirani et al. (2021a) reported that dietary plant extracts lowered intestinal pH and promoted lactic acid bacteria growth in the

**Table 6.** Effect of *Brachiaria decumbens* saponins extract supplementation on the caecal microorganisms of broilers on day 21 and 42.

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Day 0-21 (Starter phase)						
Isolation & Identification	<i>E. faecalis</i> (2+) <i>E. coli</i> (3+) NLF <i>E. coli</i> (3+)	<i>E. coli</i> (3+)	<i>E. coli</i> (3+)	<i>E. coli</i> (2+)	<i>E. coli</i> (2+) NLF <i>E. coli</i> (2+)	<i>E. coli</i> (2+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Standard plate count (cfu/mL)	$1.6 \times 10^7$	$6.2 \times 10^5$	$3.1 \times 10^6$	$5.4 \times 10^6$	$7.9 \times 10^6$	$6.3 \times 10^7$
Coliform count (cfu/mL)	$8.5 \times 10^6$	$4.2 \times 10^6$	$4.9 \times 10^5$	$3.2 \times 10^6$	$4.6 \times 10^6$	$9.6 \times 10^6$
Day 22-42 (Finisher phase)						
Isolation & Identification	<i>E. coli</i> (1+) <i>E. faecalis</i> (2+) <i>Bacillus</i> sp. (1+)	<i>E. coli</i> (1+) NLF <i>E. coli</i> (1+) <i>E. faecalis</i> (3+)	<i>E. coli</i> (1+) <i>Bacillus</i> sp. (1+) <i>E. faecalis</i> (3+)	<i>E. coli</i> (1+) NLF <i>E. coli</i> (1+) <i>E. faecalis</i> (2+)	<i>E. coli</i> (1+) NLF <i>E. coli</i> (3+) <i>E. faecalis</i> (2+)	<i>E. coli</i> (1+) <i>E. faecalis</i> (2+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Standard plate count (cfu/mL)	$2.9 \times 10^4$	$2.1 \times 10^5$	$1.9 \times 10^6$	$9.6 \times 10^4$	$1.9 \times 10^6$	$2.2 \times 10^6$
Coliform count (cfu/mL)	$1 \times 10^4$	$9.4 \times 10^4$	$2.7 \times 10^4$	$3.5 \times 10^4$	$8.2 \times 10^4$	$6.1 \times 10^5$

cfu: colony-forming unit; NLF *E. coli*: non-lactose fermenting *E. coli*. T1: no additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

**Table 7.** Effect of *Brachiaria decumbens* saponins extract supplementation on the carcass characteristics of broilers on day 21.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 0-21 (Starter phase)								
Final live weight (g)	949.60 <sup>c</sup>	964.40 <sup>c</sup>	981.60 <sup>a,b</sup>	961.40 <sup>b</sup>	992.20 <sup>a,b</sup>	1027.50 <sup>a</sup>	12.6233	0.0051
Kill-out weight (g)	915.60 <sup>b</sup>	925.00 <sup>b</sup>	933.20 <sup>a,b</sup>	910.20 <sup>b</sup>	923.60 <sup>b</sup>	974.75 <sup>a</sup>	7.3883	0.0001
De-feathered weight (g)	864.00 <sup>b</sup>	874.80 <sup>b</sup>	881.00 <sup>b</sup>	873.20 <sup>b</sup>	888.20 <sup>b</sup>	917.25 <sup>a</sup>	8.8050	0.0091
Carcass weight (g)	661.00 <sup>c</sup>	659.60 <sup>c</sup>	669.60 <sup>c</sup>	653.00 <sup>c</sup>	712.80 <sup>b</sup>	740.20 <sup>a</sup>	6.3617	<0.0001
Dressing percentage (%)	69.65 <sup>b</sup>	68.42 <sup>b</sup>	68.23 <sup>b</sup>	67.94 <sup>98b</sup>	71.84 <sup>a</sup>	72.03 <sup>a</sup>	0.6300	0.0007
Breast (%)	37.61	37.69	37.82	39.11	36.66	36.69	0.9017	0.1395
Drumstick (%)	12.28 <sup>c</sup>	12.75 <sup>b,c</sup>	12.97 <sup>a,b</sup>	13.29 <sup>a,b</sup>	11.63 <sup>b,c</sup>	12.20 <sup>a</sup>	0.2217	0.0060
Wings (%)	12.01 <sup>e</sup>	12.17 <sup>e</sup>	13.30 <sup>c</sup>	13.08 <sup>d</sup>	12.95 <sup>b</sup>	12.78 <sup>a</sup>	0.0900	<0.0001
Head (%)	4.84	5.10	5.02	5.02	4.78	4.76	0.0950	0.1756
Neck (%)	2.87	3.03	2.84	2.85	2.72	2.73	0.0767	0.2846
Shanks (%)	5.51 <sup>c</sup>	5.80 <sup>b</sup>	5.92 <sup>b</sup>	5.97 <sup>b</sup>	5.56 <sup>b</sup>	5.64 <sup>a</sup>	0.0800	<0.0001
Full gizzard (%)	4.15 <sup>c</sup>	4.16 <sup>c</sup>	4.25 <sup>b,c</sup>	4.62 <sup>a,b</sup>	4.16 <sup>a,b</sup>	4.26 <sup>a</sup>	0.0917	0.0010
Empty gizzard (%)	2.39	2.55	2.57	2.60	2.25	2.33	0.0617	0.1098
GIT (%)	16.37	16.45	16.56	17.52	16.40	14.49	0.6000	0.2142
Heart (%)	0.85	0.82	0.84	0.95	0.79	0.84	0.0533	0.5167
Liver (%)	3.96 <sup>c</sup>	4.10 <sup>b,c</sup>	4.36 <sup>a</sup>	4.47 <sup>a</sup>	3.99 <sup>a,b</sup>	3.97 <sup>a</sup>	0.0933	0.0069

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c,d,e</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: no additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

**Table 8.** Effect of *Brachiaria decumbens* saponins extract supplementation on the carcass characteristics of broilers on day 42.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 22-42 (Finisher phase)								
Final live weight (g)	2288.00 <sup>e</sup>	2390.60 <sup>d</sup>	2430.60 <sup>c</sup>	2451.00 <sup>b,c</sup>	2466.00 <sup>b</sup>	2500.40 <sup>a</sup>	9.6750	<0.0001
Kill-out weight (g)	2190.20 <sup>d</sup>	2256.60 <sup>c</sup>	2271.40 <sup>c</sup>	2344.80 <sup>b</sup>	2357.80 <sup>b</sup>	2422.75 <sup>a</sup>	7.4467	<0.0001
De-feathered weight (g)	2149.40 <sup>d</sup>	2159.60 <sup>d</sup>	2181.00 <sup>c</sup>	2262.80 <sup>b</sup>	2263.40 <sup>b</sup>	2302.25 <sup>a</sup>	6.4167	<0.0001
Carcass weight (g)	1634.60 <sup>d</sup>	1631.80 <sup>d</sup>	1705.00 <sup>c</sup>	1763.00 <sup>b</sup>	1775.40 <sup>b</sup>	1815.75 <sup>a</sup>	6.8400	<0.0001
Dressing percentage (%)	71.44 <sup>a</sup>	68.26 <sup>c</sup>	70.15 <sup>b</sup>	71.93 <sup>a</sup>	72.00 <sup>a</sup>	72.61 <sup>a</sup>	0.3533	<0.0001
Breast (%)	38.43 <sup>c</sup>	38.65 <sup>c</sup>	36.95 <sup>c</sup>	36.50 <sup>c</sup>	37.31 <sup>b</sup>	37.85 <sup>a</sup>	0.3483	<0.0001
Drumstick (%)	13.81 <sup>b</sup>	14.15 <sup>b</sup>	13.68 <sup>b</sup>	13.08 <sup>b</sup>	12.77 <sup>b</sup>	15.80 <sup>a</sup>	0.4683	0.0165
Wings (%)	12.24 <sup>d</sup>	12.29 <sup>d</sup>	12.15 <sup>c,d</sup>	12.33 <sup>b</sup>	11.93 <sup>b,c</sup>	12.52 <sup>a</sup>	0.1467	<0.0001
Head (%)	3.70 <sup>d</sup>	3.81 <sup>c</sup>	3.81 <sup>b</sup>	3.78 <sup>a,b</sup>	3.68 <sup>a,b</sup>	3.69 <sup>a</sup>	0.0333	<0.0001
Neck (%)	2.57 <sup>b,c</sup>	2.69 <sup>b</sup>	2.25 <sup>d</sup>	2.13 <sup>d</sup>	2.31 <sup>c</sup>	2.98 <sup>a</sup>	0.0417	<0.0001
Shanks (%)	5.94 <sup>b</sup>	6.13 <sup>b</sup>	6.15 <sup>a</sup>	5.46 <sup>b</sup>	5.51 <sup>b</sup>	5.87 <sup>a</sup>	0.0417	0.0001
Full gizzard (%)	2.41 <sup>d</sup>	2.91 <sup>a,b</sup>	2.64 <sup>c</sup>	2.56 <sup>c</sup>	2.59 <sup>b,c</sup>	2.67 <sup>a</sup>	0.0383	<0.0001
Empty gizzard (%)	1.84 <sup>c</sup>	1.90 <sup>c</sup>	1.92 <sup>b</sup>	1.88 <sup>b</sup>	1.88 <sup>b</sup>	1.93 <sup>a</sup>	0.0233	<0.0001
GIT (%)	12.32 <sup>b,c</sup>	12.77 <sup>a,b</sup>	11.41 <sup>b,c</sup>	10.91 <sup>c</sup>	11.15 <sup>b,c</sup>	11.90 <sup>a</sup>	0.2683	0.0196
Heart (%)	0.65 <sup>b,c</sup>	0.59 <sup>c</sup>	0.67 <sup>a,b</sup>	0.65 <sup>a,b</sup>	0.63 <sup>a,b</sup>	0.66 <sup>a</sup>	0.0167	0.0009
Liver (%)	2.94 <sup>c</sup>	3.09 <sup>b</sup>	3.13 <sup>a</sup>	3.05 <sup>a</sup>	3.09 <sup>a</sup>	3.06 <sup>a</sup>	0.0383	<0.0001

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c,d,e</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

ileum and caecum, thereby reducing populations of *Clostridium perfringens* and *E. coli*. In this study, broilers supplemented with 100 mg/kg of *B. decumbens* saponins extract consistently exhibited the highest standard plate and coliform counts. This may reflect a shift in microbial ecology towards enhanced fermentation processes and potentially beneficial bacterial activity.

### Carcass characteristics

Carcass trait data for broilers on days 21 and 42 are presented in Tables 7 and 8, respectively. On day 21, significant differences were observed among treatment groups for final live weight ( $p = 0.0051$ ), kill-out weight ( $p = 0.0001$ ), de-feathered weight ( $p = 0.0091$ ), carcass weight ( $p < 0.001$ ), dressing percentage ( $p = 0.0007$ ),

and the percentages of the drumstick ( $p = 0.0060$ ), wing ( $p < 0.0001$ ), shank wing ( $p < 0.0001$ ), full gizzard ( $p = 0.0010$ ), and liver ( $p = 0.0069$ ). By day 42, all evaluated carcass parameters showed statistically significant variation across treatments ( $p < 0.05$ ). Broilers in the T6 group, supplemented with the highest level of *B. decumbens* saponins extract (100 mg/kg), consistently recorded the highest values at both time points, indicating superior carcass development and yield.

Carcass weight and dressing percentage are key economic indicators in broiler production, as they directly determine market value and profitability. Improving these traits is essential for increasing processing yield within the poultry industry. Plant-based additives with antioxidant and antimicrobial properties have shown potential to enhance carcass characteristics and overall meat quality (Adriani et al. 2019).

Puvača and Stanačev (2011) reported that incorporating plant extracts into broiler diets increased breast muscle yield by 1.2% relative to the eviscerated carcass. In this study, broilers supplemented with 100 mg/kg of *B. decumbens* saponins extract exhibited the most favourable carcass traits, including higher carcass weight and dressing percentage. These findings agree with earlier reports showing that saponins from various plant sources improved carcass traits such as breast and thigh muscle yield (Abdulkarimi et al. 2011; Alghirani, Chung, et al. 2021). Since body weight is closely linked to dressing percentage, enhanced nutrient absorption and gastrointestinal function likely contributed to the improved carcass metrics observed in this study (Alghirani, Chung, et al. 2021). These compounds support gut health and nutrient uptake by modulating intestinal morphology and microbial balance (Tavangar et al. 2021). Such physiological improvements translate into superior growth performance and carcass development. Overall, supplementation with *B. decumbens* saponins extract appears to enhance carcass quality through combined effects on digestion, microbial ecology, intestinal structure and antioxidant defence. This, in turn, improves broiler productivity and economic returns.

### Meat quality

Tables 9 and 10 show the effects of different levels of *B. decumbens* saponin extract on the quality attributes of broiler breast and drumstick muscles. On day 21, significant treatment effects were observed for muscle pH ( $p < 0.0001$ ), cooking loss ( $p < 0.0001$ ), and drip loss ( $p <$

0.0001) at both 24 and 48 hours in both muscle types. Likewise, significant differences ( $p < 0.05$ ) were found for muscle pH, cooking loss, and drip loss at both 24 and 48 hours in both muscles. For breast muscle color, a significant difference in  $L^*$  (lightness) ( $p = 0.0341$ ) was observed only on day 42. Broilers in the T6 group, supplemented with 100 mg/kg of the extract, recorded the highest muscle pH and the lowest cooking loss, drip loss, and  $L^*$  values, indicating superior overall meat quality compared with the other groups.

Muscle pH is a critical post-mortem determinant of meat quality, as it influences colour, water-holding capacity (WHC), drip loss, cooking loss and shear force (Ong et al. 2024). The inclusion of phytobiotic compounds in broiler diets, particularly under heat stress conditions, has been shown to preserve or enhance meat quality due to their antioxidant properties (Zdanowska-Świątek et al. 2019). In this study, broilers supplemented with 100 mg/kg of *B. decumbens* saponins extract (T6) consistently recorded the highest muscle pH, along with the lowest drip loss, cooking loss and  $L^*$  (lightness) values across both phases. These results agree with previous reports. Saponin supplementation improves meat quality by increasing pH and WHC while reducing serum triglycerides, cholesterol and low-density lipoproteins (Tippel et al. 2017; Zdanowska-Świątek et al. 2019; Alghirani, Chung, et al. 2021). Oxidation of lipids and proteins in meat reduces nutritional value and compromises physicochemical stability. This creates both food safety concerns and economic risks for the poultry industry (Adeyemi et al. 2016). The improvement in meat quality observed in this study is likely due to the antioxidant activity of *B.*

**Table 9.** Effect of *Brachiaria decumbens* saponins extract supplementation on the meat quality of broilers on day 21.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 0-21 (Starter phase)								
Breast								
Cooking loss (%)	18.70 <sup>a</sup>	17.44 <sup>b</sup>	17.51 <sup>b</sup>	16.72 <sup>c</sup>	15.52 <sup>d</sup>	15.32 <sup>d</sup>	0.1267	<0.0001
Muscle pH	5.89 <sup>d</sup>	5.91 <sup>d</sup>	5.94 <sup>c</sup>	5.96 <sup>c</sup>	6.05 <sup>b</sup>	6.10 <sup>a</sup>	0.0100	<0.0001
Drip loss at 24 h (%)	3.37 <sup>a</sup>	3.87 <sup>b</sup>	3.81 <sup>bc</sup>	3.63 <sup>c</sup>	3.26 <sup>d</sup>	3.15 <sup>d</sup>	0.0533	<0.0001
Drip loss at 48 h (%)	6.29 <sup>a</sup>	6.26 <sup>a</sup>	6.21 <sup>a</sup>	5.79 <sup>b</sup>	5.53 <sup>c</sup>	5.29 <sup>d</sup>	0.0467	<0.0001
Colour $L^*$ (lightness)	50.64	51.26	50.02	50.00	50.75	50.07	0.7617	0.8501
Colour $a^*$ (redness)	6.90	7.16	7.42	7.29	7.61	7.70	0.3083	0.6298
Colour $b^*$ (yellowness)	18.45	18.64	18.81	18.34	18.17	19.33	0.2867	0.1427
Shear force (kg)	750.49	735.14	761.84	787.20	768.16	764.12	10.9317	0.0570
Drumstick								
Cooking loss (%)	20.00 <sup>a</sup>	19.82 <sup>a</sup>	19.03 <sup>b</sup>	18.60 <sup>c,b</sup>	18.07 <sup>c,d</sup>	17.45 <sup>d</sup>	0.1833	<0.0001
Muscle pH	6.41 <sup>d</sup>	6.43 <sup>c,d</sup>	6.38 <sup>e</sup>	6.44 <sup>c,b</sup>	6.46 <sup>b</sup>	6.57 <sup>a</sup>	0.0090	<0.0001
Drip loss at 24 h (%)	2.30 <sup>a</sup>	2.12 <sup>b</sup>	2.32 <sup>a</sup>	2.09 <sup>b</sup>	1.96 <sup>c</sup>	1.80 <sup>d</sup>	0.0317	<0.0001
Drip loss at 48 h (%)	3.20 <sup>a</sup>	3.03 <sup>a,b</sup>	3.09 <sup>a</sup>	3.11 <sup>a</sup>	3.04 <sup>a,b</sup>	2.85 <sup>b</sup>	0.0483	0.0246
Colour $L^*$ (lightness)	50.04	49.44	49.63	50.19	51.11	50.61	0.8300	0.7763
Colour $a^*$ (redness)	7.45	7.68	7.44	7.66	7.53	8.50	0.4367	0.6200
Colour $b^*$ (yellowness)	13.58	13.85	14.29	14.60	14.64	15.65	0.7850	0.5665
Shear force (kg)	331.05	317.63	326.40	320.86	319.50	306.54	6.9683	0.2904

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c,d,e</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

**Table 10.** Effect of *Brachiaria decumbens* saponins extract supplementation on the meat quality of broilers on day 42.

Parameters	Treatments						SEM	p Value
	T1	T2	T3	T4	T5	T6		
Day 22-42 (Finisher phase)								
Breast								
Cooking loss (%)	24.34 <sup>a</sup>	23.60 <sup>a</sup>	23.75 <sup>a</sup>	21.50 <sup>b</sup>	21.67 <sup>b</sup>	20.45 <sup>b</sup>	0.3600	<0.0001
Muscle pH	5.84 <sup>d</sup>	5.85 <sup>d</sup>	5.90 <sup>d,c</sup>	5.93 <sup>bc</sup>	5.98 <sup>ab</sup>	6.01 <sup>a</sup>	0.0167	0.0002
Drip loss at 24 h (%)	3.24 <sup>a</sup>	3.05 <sup>b</sup>	2.98 <sup>b,c</sup>	2.89 <sup>c</sup>	2.75 <sup>d</sup>	2.62 <sup>d</sup>	0.0350	<0.0001
Drip loss at 48 h (%)	4.35 <sup>a</sup>	4.30 <sup>a</sup>	3.88 <sup>b</sup>	3.96 <sup>b</sup>	3.67 <sup>bc</sup>	3.41 <sup>c</sup>	0.0900	<0.0001
Colour L* (lightness)	56.14 <sup>a</sup>	55.94 <sup>a</sup>	56.40 <sup>a</sup>	55.82 <sup>a</sup>	55.39 <sup>ab</sup>	54.36 <sup>b</sup>	0.4300	0.0341
Colour a* (redness)	6.21	6.44	6.65	6.62	6.57	7.05	0.3683	0.7728
Colour b* (yellowness)	15.75	16.59	16.39	16.26	17.44	17.47	0.5400	0.2431
Shear force (kg)	920.96	923.14	931.62	930.42	917.48	899.98	12.5717	0.6159
Drumstick								
Cooking loss (%)	23.45 <sup>a</sup>	23.49 <sup>a</sup>	23.64 <sup>a</sup>	23.60 <sup>a</sup>	20.67 <sup>b</sup>	20.10 <sup>b</sup>	0.2383	<0.0001
Muscle pH	6.20 <sup>d</sup>	6.24 <sup>c</sup>	6.24 <sup>c</sup>	6.23 <sup>c</sup>	6.26 <sup>b</sup>	6.32 <sup>a</sup>	0.0100	<0.0001
Drip loss at 24 h (%)	3.08 <sup>a</sup>	3.00 <sup>a</sup>	2.90 <sup>a</sup>	2.93 <sup>a</sup>	2.91 <sup>a</sup>	2.70 <sup>b</sup>	0.0467	0.0033
Drip loss at 48 h (%)	4.42 <sup>a</sup>	4.28 <sup>ab</sup>	4.12 <sup>b,c</sup>	4.22 <sup>ab,c</sup>	4.00 <sup>c,d</sup>	3.83 <sup>d</sup>	0.0633	0.0005
Colour L* (lightness)	55.99	56.06	56.13	56.11	55.94	55.23	0.7167	0.9593
Colour a* (redness)	6.20	6.77	6.36	6.55	6.73	6.85	0.3000	0.6539
Colour b* (yellowness)	14.42	14.50	14.72	15.13	15.77	16.02	0.6633	0.4618
Shear force (kg)	374.28	356.56	360.89	365.18	353.39	349.40	10.6250	0.6509

All values were expressed as mean  $\pm$  SEM; <sup>a,b,c,d</sup> values with superscript within row are significantly different at  $p < 0.05$ . T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg *B. decumbens* saponins extract.

*decumbens* saponins extract. Supporting this, several studies have demonstrated the strong antioxidative effects of saponins. They stabilise muscle pigments, maintain pH, and inhibit oxidative degradation of proteins and lipids. These mechanisms reduce post-mortem glycolysis and minimise cooking loss (Tippel et al. 2017; Wang et al. 2017; Bera et al. 2019). Consequently, broilers in the T6 group supplemented with 100 mg/kg of saponins extract exhibited the most favourable breast and drumstick meat quality attributes among all treatments.

### Viability of *Brachiaria decumbens* saponins extract supplementation

Overall, supplementation with *B. decumbens* saponins extract, particularly at 100 mg/kg, produced consistent and interrelated benefits across all measured parameters. Enhanced growth performance was associated with higher ileal digestibility. This was supported by improved villus height and reduced crypt depth. These morphological changes likely facilitated greater nutrient absorption, contributing to superior feed efficiency. In parallel, shifts in caecal microbial balance, including suppression of opportunistic pathogens, may have supported gut health and reduced metabolic stress. These combined effects translated into higher carcass yield and improved meat quality attributes such as pH stability, WHC and reduced drip loss. The correlations across performance, digestive physiology, microbial ecology and meat characteristics indicate that *B. decumbens* saponins act through multiple complementary pathways to enhance broiler productivity under tropical conditions.

While the present findings highlight the benefits of *B. decumbens* saponins extract at 100 mg/kg in improving broiler performance, nutrient digestibility, gut morphology and meat quality, the potential toxicity of steroidal saponins must be addressed. In ruminants, excessive intake of *B. decumbens* has been associated with hepatotoxicity, nephrotoxicity and photosensitisation. These effects are largely attributed to steroidal saponins such as protodioscin (Low 2015; Muniandy et al. 2020). Although no adverse effects were observed in the current trial, high concentrations of saponins could theoretically disrupt intestinal epithelial integrity, impair erythrocyte stability or impose subclinical stress on hepatic and renal functions (Chaudhary et al. 2018). These risks underline the importance of dose optimisation and toxicological validation. Future studies should include serum biochemistry and histopathology to confirm safety margins across different poultry strains and management systems.

Beyond biological safety, the economic feasibility of supplementing *B. decumbens* saponins in broiler diets is a critical factor for practical adoption. Cost-benefit analyses suggest that phytochemical additives from tropical grasses can improve profitability by enhancing growth efficiency while reducing reliance on costly antibiotics. Seng et al. (2025) demonstrated that signal grass supplementation in Sasso broilers not only improved physicochemical meat quality but also yielded favourable cost-to-benefit ratios under tropical production systems. Similarly, Zheng et al. (2025) reported that phytochemical supplementation with *Pennisetum purpureum* contributed dual benefits of improved performance and economic returns in antibiotic-free production models. Extrapolating these

findings, the integration of *B. decumbens* saponins extract into broiler diets has the potential to increase net margins. This improvement would come through better feed conversion efficiency, carcass yield, and meat quality, while also reducing economic risks linked to disease outbreaks and consumer resistance to antibiotics.

Therefore, while *B. decumbens* saponins extract shows promise as a safe phytogetic alternative under controlled supplementation, its commercial viability hinges on both ensuring toxicological safety and demonstrating consistent economic benefits across diverse production contexts.

## Conclusions

In summary, *B. decumbens* harvested at 5 weeks of age contained a high concentration of saponins that can be effectively extracted and supplemented in broiler diets at a dosage of 100 mg/kg. Broilers receiving this level of supplementation demonstrated improved growth performance, enhanced ileal nutrient digestibility, healthier gut morphology, superior carcass yield and better meat quality. An increase in beneficial caecal microbial populations was also observed. While these findings support the potential of *B. decumbens* saponins extract as a phytogetic alternative to antibiotic growth promoters, caution is required in defining it as universally safe or optimal. The present trial was conducted under specific conditions, including a single broiler strain, controlled housing and a tropical climate. Other variables such as genetic background, management practices, and long-term health effects were not assessed. Therefore, further studies under diverse production systems and environmental contexts are needed to confirm the reproducibility, safety and economic feasibility of *B. decumbens* saponins supplementation before its widespread application in poultry production.

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## Author contributions

Eric Lim Teik Chung: Funding acquisition, Resources, Supervision, Conceptualisation, Methodology, Investigation, Writing–review & editing. Mohamed M. Alghirani: Investigation, Writing–original draft. Alvin Lim Teik Zheng: Investigation, Writing–review & editing. Faez Firdaus

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## Data availability statement

Data of this study are available from the authors on request.

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