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Original Article

Napier grass (*Pennisetum purpureum*) meal modulates growth efficiency, gut morphology, and systemic health in broiler chickens

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Abstract

Grass and leaf meals, which include vital phytocompounds, have the potential to be a natural and safe alternative to antibiotics in the poultry industry. Therefore, this study aims to assess *Pennisetum purpureum* leaf meal and its phytocompounds on the health performances of colored-broilers. A total of 216 unsexed Sasso chicks were assigned to six diet groups. The negative control (T1) received a commercial basal diet without antibiotics, while the positive control (T2) received the same diet with oxytetracycline (100 mg/kg). Groups T3 to T6 received no antibiotics and included *P. purpureum* meal at 1.25, 2.50, 3.75, or 5.00 grams per kilogram of feed, respectively. The feeding trial was conducted for 8 weeks. Feed intake and body weight were recorded weekly to calculate the feed conversion ratio (FCR). Other measures included intestinal morphology, cecal microbiota, and serum biochemical profiles. The 5.00 g/kg diet (T6) delivered the most consistent gains, showing significant improvements in growth performance, intestinal structure, and cecal microbial composition ($P < 0.05$). Several blood biochemical markers improved with *P. purpureum*, with the strongest effects at 5.00 g/kg. Overall, *P. purpureum* meal at 5.00 g/kg appears to be a practical plant-based alternative to antibiotic growth promoters, supporting better health performance and selected health-related outcomes in tropical broiler systems.

Keywords: flavonoids, health biomarkers, *Pennisetum purpureum*, phytogenic alternative, Sasso broiler

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Introduction

In the poultry sector, in addition to vaccines, antimicrobials are used as growth promoters and for the treatment and prevention of infectious diseases in livestock (Chung *et al.*, 2020; Alghirani *et al.*, 2021). As a result, sub-therapeutic use in animal feeds has been associated with the increased rates of antimicrobial resistance among pathogens isolated from animals, as evidence showed that antibiotic resistance genes can be and are transmitted from animal to human microbiota. Alternatively, common natural feed additives that could be used as an antibiotic alternative are phytogenic groups, which include essential oils, enzymes, prebiotics and probiotics, herbal extract, and others (Ong *et al.*, 2024). These phytogenics possess a plethora of bioactive chemicals or phytocompounds that account for their antioxidant and antimicrobial properties. Therefore, they could potentially be utilized to enhance the diet of broilers in order to establish a viable and sustainable production cycle.

Even though *Pennisetum purpureum* is widely grown and used as forage, its nutritional value in poultry diets remains underexplored. Young leaves and tender shoots contain useful amounts of protein, vitamins, minerals, and antioxidant compounds (Ng *et al.*, 2021). Studies using extracts also report antifungal effects, often linked to phenolic fractions (Ojo *et al.*, 2022). Phytochemical screening of sun-dried young shoots found flavonoids (0.021%), glycosides (0.008%), and saponins (0.002%) (Jack *et al.*, 2020). Aqueous stem extracts were also reported to contain high levels of tannins (67.89%) and alkaloids, with lunamarine measured at 26.21% (Akuru *et al.*, 2015). Other identified compounds include catechin (2.49%), rutin (2.10%), kaempferol (0.09%), and anthocyanin (0.06%), along with total phenols (1.16%) (Akuru *et al.*, 2015). Together, these profiles point to both nutritional and functional value.

Plant-based flavonoids have drawn interest as functional compounds that may help regulate metabolism and immune function in animals. Feeding studies link certain flavonoids to estrogen-like effects and better lipid metabolism, often tied to their antioxidant activity and their influence on redox balance (Cao *et al.*, 2012). In broilers, flavonoid-rich ingredients have also been linked to immune benefits, such as better development of immune organs and stronger humoral responses. Some studies report improved antibody-based protection against Newcastle disease virus (ND) and avian influenza virus (Sugiharto *et al.*, 2019). These findings highlight the need to set inclusion levels that deliver benefits without reducing growth performance. This study, therefore, tests graded levels of *P. purpureum* leaf meal as a phytobiotic feed additive and evaluates effects on key health-related indicators in colored-broiler chickens raised under tropical conditions.

Materials and Methods

Plant preparation: Fresh *P. purpureum* leaves were hand-harvested at four weeks of growth. The leaves were oven-dried at 60 °C until they reached a constant weight to standardize moisture content. The dried leaves were then milled into a fine powder using an electric mill (RRH-800A, China), producing particles in the 50 to 300 mesh range. The powder was stored in airtight containers at room temperature until feed formulation. The leaf powder was analyzed for dry matter (DM), crude fiber (CF), crude protein (CP), ether extract (EE), and ash using standard AOAC methods (Horwitz, 2010). Table 1 summarizes the nutritional composition.

Phytochemical and antioxidant activity analyses: Analysis of the *P. purpureum* leaf powder measured key phytochemicals, including flavonoids, tannins, saponins, and alkaloids, using methods adapted from Barbouchi *et al.* (2024) to suit the facilities available in the local laboratory. Antioxidant potential was evaluated using the DPPH free radical scavenging assay described by Jack *et al.* (2020). Table 1 presents the full phytochemical profile and the antioxidant activity results.

Broilers and husbandry: The International Animal Care and Use Committee at Universiti Putra Malaysia approved the study (UPM/IACUC/AUP-R003/2023). The trial used 216 day-old mixed-sex Sasso broiler chicks sourced from a regional hatchery and set up in a completely randomized design. Mixed-sex chicks were used to reflect common commercial rearing conditions for colored broilers. To minimize sex-related bias, chicks were randomly distributed across treatments, and post-slaughter sampling was balanced by sex. On arrival, each chick was weighed, then randomly assigned to six treatment groups. Each group had six replicates, with six birds per replicate. Birds were housed for 56 days in stainless-steel multi-tier cages (113 cm × 82 cm × 45 cm) inside an open-sided poultry house. Average temperature and relative humidity during the study were 30.9 °C and 71.24%, respectively. The health program included intraocular vaccination for Newcastle disease and infectious bronchitis on day 7, followed by ocular vaccination for infectious bursal disease on day 14. Feed and water were available at all times throughout the trial.

Diets: A two-phase feeding program was used with commercially prepared maize and soy-bean meal-based crumble diets. Birds received the starter diet from hatch to day 28, then switched to the finisher diet from day 29 to day 56. Six dietary treatments were tested. Treatment 1 was the negative control and received the basal diet with no antimicrobial additives. Treatment 2 was the positive control and received the basal diet plus oxytetracycline at 100 mg/kg. Treatments 3 to 6 received the same antibiotic-free basal diet, with *P. purpureum* leaf meal added at 1.25, 2.50, 3.75, or 5.00 grams per kilogram of feed, respectively. All diets were analyzed using AOAC methods (AOAC, 2006) for dry DM, CF, CP, EE, and ash. Table 2 lists the ingredients in the basal starter and finisher diets, while Table 3 summarizes the nutrient composition of all treatment diets.

Table 1 Nutrient composition and quantitative analysis of the phytochemical screening of *P. purpureum* leaf meal.

Parameters	*P. purpureum*
Nutrient composition	
Metabolizable energy (MJ/kg)	17.01±0.56
Dry matter (%)	8.00±0.26
Crude protein (%)	13.63±0.40
Crude fiber (%)	29.5±0.67
Ether extract (%)	2.02±0.05
Ash (%)	7.50±0.09
Phytochemical screening	
Saponins (%)	1.16±0.23
Tannin (%)	1.50±0.07
Alkaloid (%)	1.45±0.14
Flavonoid (%)	1.96±0.03
Antioxidant activity	
DPPH radical scavenging activity (%)	67.73±0.01

All values were expressed as mean ± standard error.

Table 2 Composition of the basal diets for starter and finisher phases from day 1 to day 28 and day 29 to day 56, respectively.

Ingredients (%)	Starter	Finisher
Corn	46.00	52.1
Wheat bran	4.50	6.0
Soybean meal	40.00	32.0
L-Lysine	1.32	1.05
DL-Methionine	0.55	0.45
Choline chloride	0.18	0.20
Calcium carbonate	0.80	0.80
Palm oil	3.35	5.10
Dicalcium phosphate	2.60	1.60
Salt	0.30	0.30
Mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.15	0.15
Toxin binder[3]	0.10	0.10
Total	100	100

[1]Mineral mix (provided per kg of the product): Selenium 0.30 g; iron 80.0 g; manganese 100.0 mg; zinc 80.0 g; copper 16.0 g; potassium 6.0 g; sodium 1.80 g; iodine 1.25 g and cobalt 0.25 g; [2]Vitamin premix (provided per kg of the product): Vitamin D3 9.0 MIU; vitamin A 35.0 MIU; vitamin K3 6.0 g; vitamin E 90.0 g; vitamin b2 22.0 g; vitamin B1 7.0 g; vitamin B12 0.070 g; vitamin B6 12.0 g; nicotinic acid 120.0 g; pantothenic acid 35.0 g; folic acid 3.0 g; cobalamin 0.05 mg; biotin 300.000 mg; phytase 25,000.0 FTU; folic acid 0.56 mg; thiamine 1.43 mg; riboflavin 3.44 mg; pantothenic acid 6.46 mg; biotin 0.05 mg; niacin 40.17 mg, and pyridoxine 2.29 mg; [3]Toxin binder is comprised of naturally hydrated sodium, calcium, and aluminum silicate.

Table 3 Nutrient composition of broiler diets added with different levels of *P. purpureum* grass meal.

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Starter diet (1-28 days)						
Metabolizable energy (MJ/kg)	12.67±0.22	12.45±0.19	12.67±0.22	12.45±0.19	12.67±0.22	12.45±0.19
Dry matter (%)	90.97±0.33	91.33±0.71	91.33±0.33	90.83±0.70	91.17±0.31	91.17±0.48
Crude protein (%)	22.46±0.20	22.13±0.07	22.42±0.09	22.42±0.18	22.60±0.51	23.20±0.19
Crude fiber (%)	3.50±0.50	3.50±0.56	3.50±0.50	4.33±0.42	3.67±0.33	4.00±0.52
Ether extract (%)	2.10±0.12	1.70±0.27	2.10±0.12	1.72±0.22	2.10±0.12	1.72±0.22
Ash (%)	5.53±0.28	5.95±0.19	5.95±0.26	6.45±0.30	5.83±0.35	5.45±0.16
Finisher diet (29-56 days)						
Metabolizable energy (MJ/kg)	19.05±0.13	19.01±0.15	19.05±0.03	19.09±0.19	18.79±0.19	19.01±0.12
Dry matter (%)	89.18±0.21	88.38±0.29	88.10±0.19	89.10±0.26	89.38±0.34	88.46±0.09
Crude protein (%)	19.78±0.43	19.53±0.02	19.55±0.11	19.97±0.21	19.86±0.11	19.79±0.38
Crude fiber (%)	3.29±0.11	3.69±0.14	3.61±0.36	3.23±0.25	3.55±0.29	3.75±0.04
Ether extract (%)	5.24±0.17	5.58±0.42	5.39±0.41	5.09±0.26	5.01±0.48	5.09±0.82
Ash (%)	4.76±0.19	5.59±0.06	4.68±0.45	4.75±0.08	5.38±0.41	5.71±0.17

All values were expressed as mean ± SE. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Growth performance: Body weight gain (BWG) and feed intake (FI) were recorded on a replicate basis for the overall 8-week feeding period. Feed conversion ratio (FCR) was calculated from cumulative feed intake and body weight gain over the 56-day trial. All weights were measured using a high-precision digital scale (Mettler Toledo Industrial Scale, BBA211 series, Greifensee, Switzerland) calibrated to 0.01-unit accuracy. FCR was calculated using the standard equation:

$$FCR = FI/BWG$$

Growth performance over the 8-week period was used to compare treatments. At day 56, six birds per treatment were selected through stratified random sampling, balanced by sex (3 males, 3 females), for post-slaughter evaluation. Assessments covered small intestinal morphology and cecal microbiota. Whole blood samples were collected for biochemical analysis, including liver enzyme markers and serum lipid profiles. Additional measures included acute-phase proteins, corticosterone, and heat shock protein expression to evaluate homeostasis and stress-related responses. Slaughter followed Halal standards, and sample collection was carried out at the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). Birds were humanely euthanized by severing the neck following Halal slaughter procedures. Blood samples were collected immediately during exsanguination from the jugular vein, and approximately 3 to 5 mL of blood was transferred into EDTA tubes (BD Vacutainer®, New Jersey, USA).

Gut histomorphology: To assess small intestinal structure, histomorphometric analysis was carried out at the Histopathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), using a modified method based on Alghirani *et al.* (2021). Samples were taken as 5 cm sections from the duodenum, jejunum, and ileum. Each section was rinsed with neutral buffered saline and fixed overnight in 10% neutral buffered formalin. Tissues were processed using standard paraffin-embedding methods. They were dehydrated through increasing ethanol concentrations, cleared in xylene, and infiltrated with molten paraffin using an automated tissue processor. Sections were cut at 4 µm with a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin. Slides were then heated at 60 °C before microscopy using a Nikon DS-U2/L2 brightfield system. Morphometric measurements were performed with NIS-Elements D software. Villus height was defined as the perpendicular distance from the villus tip to the villus-crypt junction. Crypt depth was defined as the distance from the base of the crypt to the point where adjacent villi meet.

Cecal microbial population: Cecal digesta were collected at slaughter on day 56 from birds in each dietary group and transported on ice to the Microbiology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). Microbiological analysis included three components: (i) quantitative counts of coliforms and total aerobic bacteria, (ii) screening for Salmonella, and (iii) isolation and taxonomic identification of other cecal microbes, following Park and Kim (2020). Bacterial counts were reported as $\log_{10}$ colony-forming units (cfu) per gram of cecal digesta.

Blood biomarkers: Samples were centrifuged at 3000 × g for 10 min at 4 °C to separate plasma. The plasma was then aliquoted into 1.5 mL microcentrifuge tubes and stored at −80 °C until analysis. Commercial ELISA kits (QAYEE-BIO for Life Science, China) were used to measure acute-phase proteins, corticosterone, heat shock proteins, and selected biochemical markers, following the manufacturer's procedures. Concentrations were calculated using the MyAssays platform with four-parameter curve fitting against the assay standards.

Blood biochemistry: Clinical biochemistry tests were run at the Hematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). Liver-related enzyme activities, including aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and alanine transaminase (ALT), were measured using a BA400 automated biochemistry and turbidimetric analyzer (TEUS00048-07-EN, Spain). Serum lipids, including total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL), were measured using an Automatic Analyzer 902 (Hitachi, Germany). Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated using the Friedewald formula:

$$LDL = \text{Total cholesterol} - HDL - VLDL$$
$$VLDL = (\text{Triglycerides})/5$$

Statistical analysis: All statistical analyses were run in RStudio (version 4.1.3) using a completely randomized design (RStudio Team, 2020). Treatment effects were tested with one-way ANOVA. When the overall test was significant ($P < 0.05$), Tukey's HSD was used for pairwise comparisons among treatment means. Mortality data, treated as categorical outcomes, were analyzed using a Chi-square test of independence. On the other hand, the isolation and identification findings were descriptive microbiological observations and were not analyzed statistically.

Result

Growth performance: Table 4 summarizes how *P. purpureum* leaf meal affected Sasso broiler growth performance on day 56. The feed intake ($P = 0.037$) and cumulative FCR ($P < 0.001$) differed among treatments. Across both phases, Treatment 6 (T6, 5.00 g/kg *P. purpureum*) produced the lowest FCR. T6 also had the lowest feed intake (3.11 kg), but it differed significantly only from T3 (3.46 kg) and was similar to the other treatments ($P > 0.05$). Overall, the lower FCR with largely comparable feed intake points to better feed efficiency in T6 over the 56-day period.

Gut histomorphology: Table 5 summarizes small intestinal histomorphology at day 56 after graded inclusion of *P. purpureum* leaf meal. Supplementation increased villus height in the duodenum, jejunum, and ileum compared with both controls ($P < 0.05$). Villi height was determined from the tip to the crypt transition, while crypt depth was assessed at the invagination between two villi (Fig. 1). The strongest response occurred at 5.00 g/kg (T6), which produced the greatest villus height in all segments. *P. purpureum* also reduced crypt depth across segments relative to

the negative control ($P < 0.05$), except in the duodenum for T3. In the duodenum and jejunum, crypt depth in T4 to T6 was lower than the positive control ($P < 0.05$). In contrast, T3 to T6 did not differ from the positive control in the ileum ($P > 0.05$). Overall, T6 showed the most favorable architecture, with the highest villus height-to-crypt depth ratio in the duodenum and ileum. The highest jejunal ratio was observed in T2, but it did not differ from T6 ($P > 0.05$).

Table 4 Effect of *P. purpureum* leaf meal supplementation on the growth performance of Sasso broilers on day 56.

Parameters	Treatments						*P*-value
	T1	T2	T3	T4	T5	T6	
Final body weight (kg)	1.61±0.04	1.69±0.02	1.67±0.04	1.64±0.05	1.68±0.03	1.75±0.06	0.284
Body weight gain (kg)	1.57±0.04	1.65±0.02	1.63±0.04	1.59±0.05	1.64±0.03	1.71±0.06	0.284
Feed intake (kg)	3.36±0.08^{ab}	3.18±0.03^{ab}	3.46±0.08^{a}	3.20±0.10^{ab}	3.20±0.06^{ab}	3.11±0.10^{b}	0.037
Cumulative FCR	2.14±0.02^{a}	1.93±0.01^{c}	2.12±0.02^{a}	2.01±0.00^{b}	1.96±0.01^{bc}	1.82±0.00^{d}	<0.001
Mortality bird	0	0	1	2	1	1	0.639

All values were expressed as mean ± SE; superscripts $^{a, b, c, and d}$ indicate values within the row are significantly different at $P < 0.05$. FCR: feed conversion ratio. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Table 5 Effect of *P. purpureum* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56.

Parameters	Treatments						*P*-value
	T1	T2	T3	T4	T5	T6	
Villi height (µm)							
Duodenum	481.95±152.41^{e}	626.25±198.04^{b}	560.70±177.31^{c}	502.07±158.77^{de}	535.69±169.40^{cd}	675.19±213.51^{a}	<0.001
Jejunum	572.01±180.88^{a}	593.34±187.63^{a}	471.42±149.08^{b}	446.94±141.34^{b}	452.05±140.95^{b}	463.86±146.69^{b}	<0.001
Ileum	313.19±99.07^{c}	362.12±114.54^{b}	382.52±120.96^{ab}	409.42±129.47^{a}	416.79±131.80^{a}	423.50±133.92^{a}	<0.001
Crypt depth (µm)							
Duodenum	118.48±37.47^{b}	117.95±37.30^{b}	131.57±41.61^{a}	99.42±31.44^{c}	82.44±26.07^{d}	78.15±24.71^{d}	<0.001
Jejunum	126.98±40.16^{a}	78.81±24.92^{b}	80.22±25.37^{b}	70.64±22.34^{c}	68.75±21.74^{c}	67.58±21.37^{c}	<0.001
Ileum	130.25±41.19^{a}	73.40±23.21^{b}	73.03±23.09^{b}	68.60±21.69^{b}	68.49±21.57^{b}	68.47±21.65^{b}	<0.001
Villi height: Crypt depth ratio							
Duodenum	4.07±1.29^{d}	5.32±1.68^{c}	4.27±1.35^{d}	5.06±1.60^{c}	6.53±2.06^{b}	8.66±2.74^{a}	<0.001
Jejunum	4.51±1.43^{d}	7.56±2.39^{a}	5.92±1.87^{c}	6.35±2.01^{bc}	6.59±2.08^{bc}	6.90±2.18^{ab}	<0.001
Ileum	2.41±0.76^{d}	4.96±1.57^{c}	5.32±1.6^{bc}	5.97±1.89^{ab}	6.13±1.94^{a}	6.21±1.96^{a}	<0.001

All values were expressed as mean ± SE; superscripts $^{a, b, c, d, and e}$ indicate values within the row are significantly different at $P < 0.05$. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Cecal microbial population: Table 6 summarizes cecal microbiota at day 56 in broilers given graded *P. purpureum* leaf meal. *Escherichia coli* was found in all treatments, while non-lactose-fermenting *E. coli* appeared only in the control groups (T1 and T2). *Bacillus cereus* was detected only in T1, whereas other *Bacillus* spp. were isolated in T3 to T6, suggesting a shift in the cecal microbial profile with *P. purpureum* inclusion. Salmonella was not detected in any group. Coliform and total aerobic counts were numerically highest in T6, although differences among treatments were small.

Blood biomarkers: Table 7 summarizes stress- and inflammation-related blood biomarkers at day 56 after adding *P. purpureum* leaf meal. All biomarkers differed among treatments ($P < 0.05$). Overall, T6 (5.00 g/kg) showed the lowest levels of corticosterone, HSP70, alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and serum amyloid A (SAA). Some markers showed overlap among the higher-inclusion diets. SAA did not differ across T4, T5, and T6 ($P > 0.05$). AGP in T4 to T6 was similar to T2 ($P > 0.05$). CP did not differ between T5 and T6 ($P > 0.05$), and HSP70 in T6 was comparable to T2 and T5 ($P > 0.05$). Compared with the negative control (T1), T6 reduced SAA by 24.88%, AGP by 23.36%, CP by 35.71%, corticosterone by 43.96%, and HSP70 by 24.64%. Taken together, the T6 profile suggests lower systemic stress and reduced acute-phase activation, consistent with better physiological stability under tropical rearing conditions.

Blood biochemistry: Table 8 summarizes plasma biochemistry on day 56 with graded inclusion of *P. purpureum* leaf meal. All liver-related enzymes differed among treatments, including ALT, GGT, AST, and ALP ($P < 0.05$). T6 recorded the lowest activities, with ALP at 131.32 U/L, AST at 99.46 U/L, GGT at 10.02 U/L, and ALT at 2.17 U/L. For GGT, T6 did not differ from T5 (10.37 U/L) ($P > 0.05$). Lipid-related indices also differed among treatments. Total cholesterol, TG, LDL, and VLDL decreased with supplementation ($P < 0.05$), while HDL did not change ($P > 0.05$). Overall, T6 produced the most favorable lipid profile, with the lowest total cholesterol, TG, LDL, and VLDL, alongside lower liver enzyme activity.

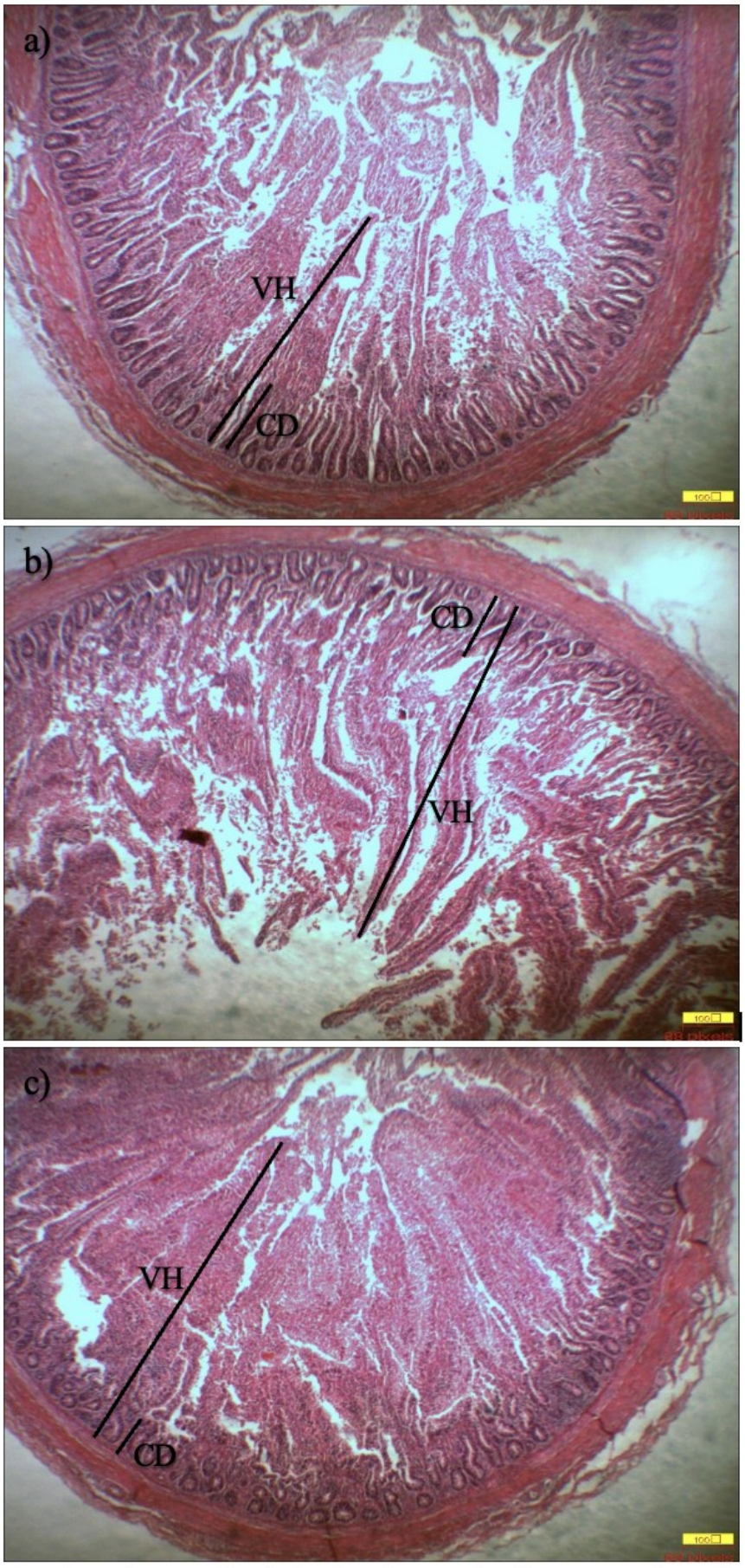

Figure 1 Villi height (VH) and crypt depth (CD) of Sasso broilers at 56 days of age. (a) Duodenum (b) Jejunum (c) Ileum.

Table 6 Effect of *P. purpureum* supplementation on the cecal microorganisms of Sasso broilers on day 56.

Parameters	Treatment					
	T1	T2	T3	T4	T5	T6
Isolation and Identification	*E. coli* (3+) NFL *E. coli* *Bacillus cereus* (3+)	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (3+) *Bacillus* sp. (3+)	*E. coli* (4+) *Bacillus* sp. (3+)	*E. coli* (3+) *Bacillus* sp. (3+)	*E. coli* (3+) *Bacillus* sp. (3+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Coliform count (cfu/g)	3.5×10^6	2.5×10^7	1.0×10^6	2.3×10^6	6.6×10^6	1.6×10^8
Standard plate count (cfu/g)	2.2×10^7	1.2×10^8	2.5×10^7	2.7×10^7	1.1×10^8	1.4×10^9

Note: cfu: Colony-forming unit. NLF *E. coli*: Non-lactose fermenting *E. coli*. Values in parentheses (3+ & 4+) indicate semi-quantitative colony growth intensity. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Table 7 Effect of *P. purpureum* leaf meal supplementation on the blood biomarkers of Sasso broilers on day 56.

Parameters	Treatments						*P*-value
	T1	T2	T3	T4	T5	T6	
SAA (ng/ml)	8.28±0.40^{a}	8.22±0.13^{ab}	8.11±0.43^{ab}	7.53±0.39^{abc}	6.82±0.20^{bc}	6.22±0.22^{c}	0.007
AGP ((µl/ml)	29.71±0.84^{a}	24.92±0.93^{bc}	27.60±0.81^{ab}	25.62±0.39^{bc}	25.01±0.20^{bc}	22.77±0.22^{c}	0.003
CP (µl/ml)	9.24±0.15^{a}	8.60±0.17^{ab}	9.40±0.22^{a}	7.98±0.31^{b}	6.28±0.32^{c}	5.94±0.21^{c}	<0.001
Corticosterone (pg/ml)	235.79±6.86^{a}	227.72±7.25^{a}	228.80±7.65^{a}	161.78±2.85^{b}	160.51±3.40^{b}	132.14±3.45^{c}	<0.001
HSP 70 (pg/ml)	156.72±4.80^{a}	126.48±5.48^{bc}	150.08±4.67^{a}	132.08±2.17^{b}	122.58±3.16^{bc}	118.10±4.39^{c}	<0.001

Note: All values were expressed as mean ± SE; $^{a, b, \text{and } c}$ values with superscript within row are significantly different at $P < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Table 8 Effect of *P. purpureum* leaf meal supplementation on the blood biomarkers of Sasso broilers on day 56.

Parameters	Normal range	Treatments						*P*-value
		T1	T2	T3	T4	T5	T6	
Liver functions								
ALP (U/L)	55-2345	174.19±3.20^{b}	187.87±5.27^{a}	179.94±3.51^{ab}	140.05±1.09^{c}	136.47±1.35^{c}	131.32±0.64^{c}	<0.001
AST (U/L)	98-294	282.66±13.19^{a}	172.65±5.15^{b}	166.19±0.52^{b}	157.74±6.08^{b}	145.17±2.44^{b}	99.46±0.97^{c}	<0.001
GGT (U/L)	8-48	14.61±0.32^{ab}	12.88±0.35^{c}	14.61±0.43^{ab}	13.92±0.28^{bc}	10.37±0.24^{d}	10.02±0.52^{d}	<0.001
ALT (U/L)	<11	3.47±0.07^{a}	2.50±0.09^{bc}	2.72±0.05^{bc}	2.76±0.06^{bc}	2.47±0.05^{c}	2.17±0.06^{d}	<0.001
Lipid profile								
Cholesterol (mmol/L)	1.09-5.65	4.01±0.16^{ab}	3.94±0.09^{b}	4.50±0.11^{a}	3.90±0.15^{b}	3.87±0.07^{b}	3.06±0.12^{c}	<0.001
TG (mmol/L)	N/A	0.51±0.02^{ab}	0.46±0.01^{bc}	0.57±0.02^{a}	0.55±0.02^{a}	0.53±0.01^{a}	0.44±0.01^{c}	<0.001
LDL (mmol/L)	N/A	0.55±0.02^{b}	0.37±0.01^{c}	0.69±0.01^{a}	0.51±0.02^{b}	0.39±0.02^{c}	0.27±0.01^{d}	<0.001
HDL (mmol/L)	N/A	3.00±0.16	3.13±0.05	3.11±0.08	3.12±0.14	3.29±0.05	3.46±0.15	0.115
VLDL (mmol/L)	N/A	0.11±0.00^{ab}	0.09±0.00^{bc}	0.11±0.00^{a}	0.11±0.00^{a}	0.11±0.00^{a}	0.09±0.00^{c}	<0.001

Note: All values were expressed as mean ± SE; $^{a, b, c, \text{and } d}$ values with superscript within row are significantly different at $P < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase; N/A: Not Available. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetracycline); T3: Basal diet+1.25/kg of grass meal; T4: Basal diet+2.5g/kg of grass meal; T5: Basal diet+3.75g/kg of grass meal; T6: Basal diet+5.0g/kg of grass meal.

Discussion

Growth performance: Adding *P. purpureum* at 5.00 g/kg (T6) reduced cumulative FCR in both the starter and finisher phases, and feed intake also dropped in the finisher period. This leads to better feed efficiency driven by improved nutrient use, not higher feed consumption. A plausible explanation is phytogenic support of gut function, including stronger digestive enzyme secretion, which can improve digestion and absorption (Rahimi *et al.*, 2011). Phytochemical screening in this trial confirmed tannins, saponins, flavonoids, and phenolic compounds in *P. purpureum*. These compound classes link to functional effects relevant to growth and gut health in poultry (Kuralkar and Kuralkar, 2021). Phenolics, especially flavonoids, may improve glucose use by modulating intestinal glucose transport, supporting metabolic pathways such as AMPK, and reducing oxidative stress. They may also influence gut microbial activity in ways that improve nutrient use and help explain better feed efficiency in broilers (Zheng *et al.*, 2025). This aligns with the lower FCR despite reduced finisher intake (Mbikay, 2012). Evidence on *P. purpureum* as a poultry feed additive remains limited, and few studies focus on broiler performance. The results, therefore, fit best alongside findings from other flavonoid-rich leaf ingredients. Kudzu leaf extract improved feed efficiency, alongside higher flavonoid exposure and a more favorable microbial balance (Xue *et al.*, 2021).

Similar effects have been reported for guava leaf meal, where antimicrobial and antioxidant actions were linked to intestinal integrity and nutrient use (Abang *et al.*, 2023). Earlier work also suggests phytogenic growth responses often reflect combined effects on microbial load and digestive secretions, not a single pathway (Jang *et al.*, 2004; Czech *et al.*, 2009). Given the flavonoid content in *P. purpureum*, these compounds likely contributed to the efficiency gains through linked effects on intestinal function, microbial regulation, and nutrient absorption.

Gut histomorphology: Phytobiotics often link to positive changes in gut structure, and these shifts often track with better feed efficiency and growth (Gilani *et al.*, 2021). In this trial, *P. purpureum* at 5.00 g/kg gave the most consistent structural benefits. Birds in T6 showed the highest villus height to crypt depth (VH: CD) ratios in the duodenum and ileum, which suggests a larger absorptive surface and slower epithelial turnover. In the jejunum, the VH: CD ratio in T6 was not the highest across all treatments, but it was similar to the antibiotic control (T2). This suggests the response extended across segments, even if the pattern was not identical in each region. These changes align with pathways reported for flavonoid-rich materials. Flavonoids and related phenolics support antioxidant defense, which helps protect the mucosa from oxidative stress and supports epithelial maturation. When the mucosa remains stable, villi tend to be taller, and crypt activity is more controlled. This improves nutrient contact time, brush-border enzyme function, and overall absorption (Olukosi and Dono, 2014). Similar results were reported in broilers fed 5.00 g/kg rosemary leaf powder, where the authors linked the effect to combined antioxidant and antimicrobial actions that supported microbial balance and mucosal integrity (Ogwuegbu *et al.*, 2021). Not all phytogenic additives produce clear histological changes. For example, cinnamon essential oil, alone or combined with bamboo leaf flavonoids, did not change intestinal morphology in another study (Yang *et al.*, 2019). Differences across studies likely stem from variation in phytochemical profiles, bioavailability, and dose, as well as delivery form, such as whole leaf meals versus extracts or essential oils. These factors shape how much of each bioactive reaches the intestinal surface and whether structural adaptation becomes measurable.

Cecal microbial population: Cecal cultures at day 56 detected *E. coli* in all treatments, while Salmonella was not found in any sample. *Bacillus cereus* appeared only in the negative control (T1). Other *Bacillus* spp. were isolated from the grass meal groups, with no *Bacillus* recovered from the antibiotic control (T2) or the highest *P. purpureum* group (T6). This pattern suggests a diet-related shift that reduced *B. cereus* recovery with *P. purpureum*, and it resembled the antibiotic group. Bacillus is an aerobic, Gram-positive, rod-shaped genus that includes both harmless and pathogenic members (Henriques and Moran Jr., 2000). The non-cereus *Bacillus* isolates in the grass meal groups were not identified to species level, so the data do not support strain-level conclusions. Still, the wider context matters. Many *Bacillus* species in poultry, including probiotic strains, are linked to better gut function and performance. In this trial, T6 delivered the strongest growth response even though coliform and total aerobic counts were numerically higher. This suggests that culture totals did not reflect functionally important shifts in community composition, and non-pathogenic taxa may have contributed to performance. Other studies support the idea that leaf meals and plant extracts can shift cecal microbiota away from pathogen dominance. Several leaf-derived additives have reduced harmful cecal bacteria in broilers (Mandal *et al.*, 2014; Abdelatty *et al.*, 2021; Hidayati *et al.*, 2022). For example, *Terminalia catappa* leaf extract, which contains flavonoids, tannins, saponins, and alkaloids, inhibited *S. aureus*, *E. coli*, *S. typhi*, and *B. cereus* (Hidayati *et al.*, 2022). A similar antimicrobial range is biologically plausible for *P. purpureum* because the material analyzed here also contained flavonoids and other phenolics. Flavonoids show antibacterial activity through mechanisms such as protein binding and disruption of bacterial membranes, which can reduce viability in sensitive species and reshape competition in the gut (Shamsudin *et al.*, 2022). Overall, the cecal culture results support a microbiota-modulating effect of *P. purpureum*, marked by the absence of *B. cereus* recovery and a cecal environment compatible with normal growth. Follow-up work with species-level identification and broader community profiling is needed to confirm which taxa drove these shifts and whether the response depends on dose (Vidanarachchi *et al.*, 2006).

Blood Biomarkers: Higher inclusion of *P. purpureum* leaf meal was linked to lower acute-phase proteins (APP) and lower HSP70, a pattern that fits with reduced systemic inflammation and a less activated stress response. In this dataset, T4 to T6 reduced serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and corticosterone compared with the control diets, with the lowest values consistently in T6 (5.00 g/kg). This biomarker pattern supports better physiological stability under the rearing conditions and suggests a lower inflammatory load. Studies on grass or leaf meals and APP changes in broilers remain limited, so these results add to the phytogenic literature beyond standard performance and gut measures. Evidence on HSP70 responses to plant-based additives is also limited and mixed, which likely reflects differences in the stress context and when sampling occurs. Flavonoids have been linked to immune regulation in poultry, including effects on pro-inflammatory pathways such as NF-κB, which can reduce tissue stress linked to prolonged immune activation (Esmaeil *et al.*, 2017). Along similar lines, *Artemisia annua* reduced HSP70 gene expression in broilers, and the effect was linked to antioxidant phenolics and flavonoids that lower oxidative load (Song *et al.*, 2017). In contrast, *Rosmarinus officinalis* extract increased HSP70 expression before and during induced heat stress, and this rise was interpreted as cardioprotective during an acute challenge (Tang *et al.*, 2018). These differences suggest HSP70 direction depends on the setting. An additive may trigger a protective HSP70 rise during acute stress or reduce baseline HSP70 when birds face sustained

environmental pressure. HSP70 supports cellular protection during stress, but persistent elevation often signals ongoing strain. Tropical rearing brings prolonged heat exposure, so lower HSP70 in the higher *P. purpureum* groups fits better with reduced chronic activation than with weaker stress defense. Similar interpretations appear in thermal conditioning studies, where improved adaptation links to lower stress biomarkers over time (Balakrishnan *et al.*, 2023). Mechanistically, flavonoid-rich diets can reduce reactive oxygen species, support mitochondrial stability, and weaken upstream signaling that activates heat shock transcription factors. As oxidative and inflammatory pressure falls, HSP70 expression can also drop. Taken together, the concurrent reductions in corticosterone, APP markers, and HSP70 with *P. purpureum* supplementation point to lower systemic stress and reduced acute-phase activation, consistent with effects from the plant's phenolic and flavonoid content.

Blood Biochemistry: Liver enzyme activity declined as *P. purpureum* leaf meal increased, with the lowest values at 5.00 g/kg (T6). This dose-related trend points to better liver stability under the trial conditions and suggests a hepatoprotective effect. A similar pattern has been reported with other leaf meals. For example, *Persicaria odorata* supplementation lowered AST, ALT, and ALP in broilers, which was taken to indicate reduced enzyme leakage into plasma (Basit *et al.*, 2020). These enzymes are mainly intracellular, so higher plasma activity often signals hepatocellular membrane disruption or metabolic strain (Tavangar *et al.*, 2021). The reductions seen with *P. purpureum,* therefore, align with less cellular injury or stronger membrane integrity. Its phytochemical profile, especially flavonoids and other phenolics, offers a plausible explanation. Polyphenolic antioxidants can reduce oxidative stress in liver tissue, limiting lipid and protein oxidation and lowering the chance of enzymes leaking into circulation (Vitaglione *et al.*, 2004). The highest inclusion level also produced the most favorable lipid profile, with lower total cholesterol, triglycerides, and LDL. Similar lipid shifts have been reported with *P. odorata* leaf meal and were linked to bioactive secondary metabolites, including flavonoids (Basit *et al.*, 2020c). The weaker lipid response at lower *P. purpureum* levels suggests a dose threshold, where a minimum bioactive load is needed to shift systemic lipid markers. Flavonoids have been linked to changes in lipid handling and fatty acid balance, which can reduce plasma cholesterol and triglycerides (Kamboh and Zhu, 2013). Added benefit may come from limiting oxidative reactions that affect LDL formation and stability, given the role of lipid peroxidation in lipoprotein modification (Habibian *et al.*, 2019).

In conclusion, dietary supplementation with *P. purpureum* leaf meal improved several health and performance indicators in colored broilers, with responses generally becoming more pronounced as the inclusion level increased. Although 5.00 g/kg produced the most consistent overall benefits across feed efficiency, intestinal morphology, blood biomarkers, and lipid profile, the lower inclusion levels also showed positive effects in selected parameters. No clear adverse response was detected at 5.00 g/kg under the present conditions, but the study did not test higher levels, so this value should be interpreted as the most effective level within the tested range rather than a definitive optimum. Overall, the findings support the potential of *P. purpureum* leaf meal as a phytogenic feed additive for broilers reared under tropical conditions.

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