Dual Role of *Pennisetum purpureum* as a Phytogenic Feed Additive: Enhancing Growth and Profitability in Antibiotic-Free Broiler Production

Alvin Lim Teik Zheng,[a] Kelly Wong Kai Seng,[b] Yee Lyn Ong,[c] Jacqueline Lease,[d] Yoshito Andou,[d] Faez Firdaus Abdullah Jesse,[e] and Eric Lim Teik Chung,[c,*]

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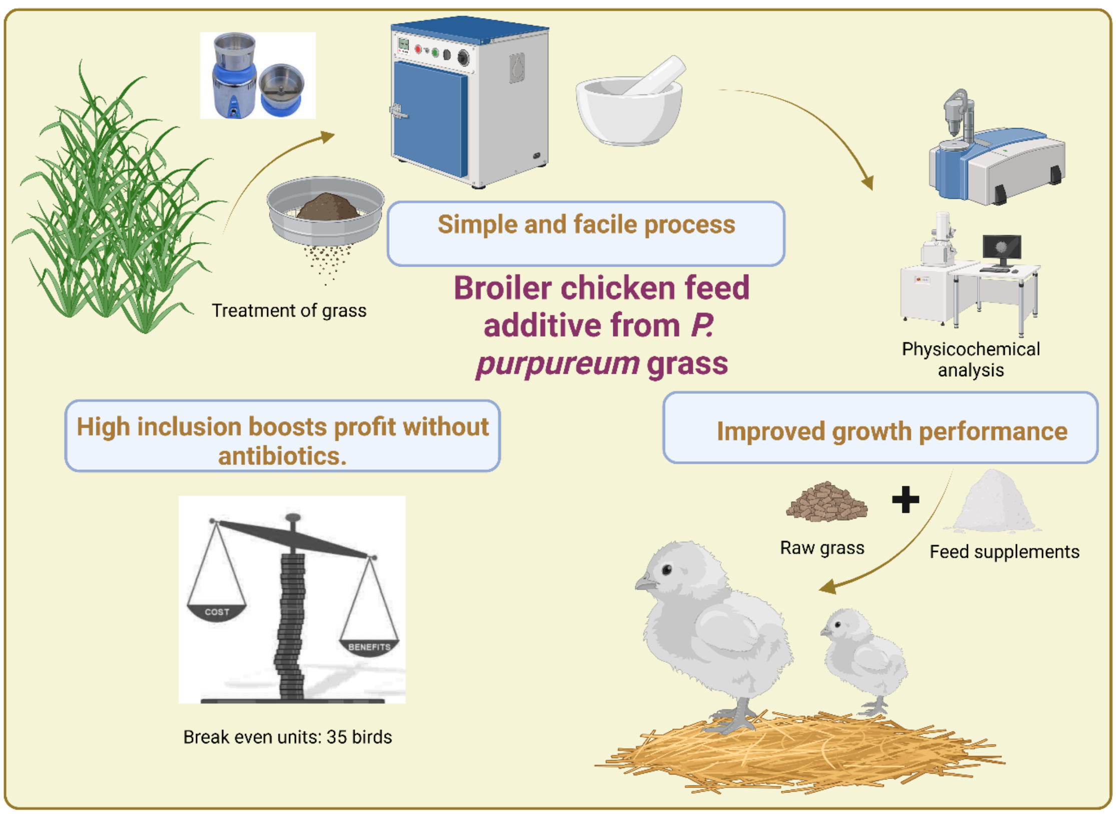
GRAPHICAL ABSTRACT



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Amid growing concerns about antibiotic resistance in livestock systems, there is a global shift toward identifying plant-based alternatives to conventional synthetic feed additives. This study explored the physicochemical characteristics of raw Napier grass (*Pennisetum purpureum*) and assessed its viability as a low-cost, functional feed additive for colored broiler chicken diets. Comprehensive characterization was conducted on the raw grass, revealing structural features and functional groups characteristic of bioactive, fibrous biomass. A subsequent feeding trial was conducted with 216 Sasso broiler chicks divided among six dietary regimens: a negative control (basal diet), a positive control (100 mg/kg oxytetracycline), and four supplementation levels of *P. purpureum* grass meal (1.25 to 5.00 g/kg). The highest supplementation level (5.00 g/kg) significantly enhanced growth performance (p < 0.05), reducing feed conversion ratio (FCR) while maintaining low feed intake. Economic analyses demonstrated that this treatment yielded the greatest profitability, exhibiting superior net profit margin, break-even efficiency, and margin of safety. These findings indicated *P. purpureum* as a promising phytogenic feed additive with dual benefits of enhancing production efficiency and promoting sustainable poultry farming.

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Keywords: Pennisetum purpureum; Phytogenic feed additive; Broiler performance; Physicochemical characterization; Antibiotic alternative; Cost-benefit analysis

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INTRODUCTION

The intensive use of antibiotics in animal agriculture particularly in poultry has long been a subject of concern due to its contribution to the global surge in antimicrobial resistance. Antibiotics are traditionally administered not only for therapeutic purposes but also as prophylactic agents and growth promoters. However, this non-therapeutic application, especially in sub-therapeutic doses, has contributed to the emergence of antibiotic-resistant pathogens, posing a serious threat to both animal and public health

(Selaledi *et al.* 2020). In response, there has been a growing research emphasis on phytogenic feed additives or natural bioactive compounds derived from plants that offer antimicrobial, antioxidant, and growth-promoting properties. Despite considerable exploration into herbal extracts and essential oils, the potential of whole plant-based feed materials, particularly leaf and grass meals, remains under-investigated (Alghirani *et al.* 2021, 2022).

Napier grass (*Pennisetum purpureum*), a fast-growing tropical forage crop, is widely cultivated in tropical and subtropical regions. Traditionally used as ruminant fodder, it is characterized by high biomass yield, adaptability to marginal soils, and notable agronomic value (Negawo *et al.* 2017; Islam *et al.* 2023). Nutritionally, *P. purpureum* is rich in crude protein, fiber, and an array of secondary metabolites including flavonoids, saponins, tannins, and alkaloids compounds known for their functional roles in modulating gut health, immunity, and metabolic efficiency in livestock (Cao *et al.* 2012; Ng *et al.* 2020). Additionally, flavonoids have demonstrated estrogenic and antioxidant activities that may positively influence lipid metabolism and disease resistance in poultry (Seomoon and Jang 2022; Tan *et al.* 2022).

Although *P. purpureum* is extensively recognized as a high-yield forage grass with diverse applications in ruminant nutrition, its potential use in poultry feed formulations remains largely unexplored. Unlike ruminants, poultry possess a relatively short and simple digestive tract, which limits their ability to ferment and extract nutrients from high-fiber forages. Consequently, the inclusion of grass-based materials in poultry diets must be carefully controlled, as excessive crude fiber (>5%) can impair nutrient digestibility and growth performance (Jha and Mishra 2021). However, at low inclusion levels, grass-derived bioactives and functional fibers may provide prebiotic and gut-modulating effects without compromising digestibility, supporting their potential as phytogenic additives in broiler nutrition (Alghirani *et al.* 2022). Recent studies have indicated that the young leaves and tender shoots of *P. purpureum* are abundant in dietary protein, essential minerals, vitamins, and antioxidant compounds. Notably, its extracts have demonstrated antifungal activity, which has been primarily attributed to the presence of phenolic compounds (Ng *et al.* 2020). A phytochemical analysis indicated that sun-dried young shoots have comparatively high concentrations of flavonoids (0.021%), followed by glycosides (0.008%) and saponins (0.002%) (Jack *et al.* 2020). Flavonoids, in particular, are known to exert estrogenic and antioxidant effects in animals, with reported benefits including improved lipid metabolism (Negasa 2024). When incorporated into broiler diets, these bioactive compounds have been associated with enhanced immune responses, increased immune organ indices, and elevated humoral immunity against infectious agents such as Newcastle disease and Avian influenza virus (Sugiharto *et al.* 2019).

The application of *P. purpureum* in poultry nutrition has not been systematically evaluated, and there has been a lack of studies characterizing its raw physicochemical profile in the context of monogastric animal diets. The current study addresses this gap by (i) providing a comprehensive physicochemical characterization of untreated *P. purpureum* using FTIR, XRD, TGA, and SEM techniques; and (ii) investigating the impact of graded supplementation levels of *P. purpureum* grass meal on the growth performance and economic viability of colored-broiler (Sasso) chicken production. It is hypothesized that the bioactive and fibrous components of *P. purpureum* can serve as a functional and sustainable feed additive that enhances broiler performance while improving production economics. The results from this investigation aim to contribute to the growing body of evidence supporting phytogenic alternatives in antibiotic-free poultry systems.

EXPERIMENTAL

Materials

Unless otherwise specified, all chemicals and reagents used in this study were of analytical grade and applied without further purification.

Planting and Harvesting of *P. purpureum* Grass

P. purpureum grass was grown at Farm 15, Department of Animal Science, Universiti Putra Malaysia. At four weeks of growth, the plants were harvested, weighed, and dried in a hot-air oven at 60 °C for 72 h or until a constant weight was attained. The dried material was then milled using a mechanical grinder and stored in airtight containers to preserve its integrity for further analyses.

Nutritional Composition, Phytochemical and Antioxidant Activity Analyses

The proximate composition of *P. purpureum* grass meal, including dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), and ash content, was determined in accordance with the Official Methods of Analysis by AOAC International as shown in Table 1. Quantification of key phytochemicals such as saponins, tannins, flavonoids, and alkaloids was carried out using a modified protocol (Osuntokun 2014). Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay following the method described previously with appropriate modifications (Jack *et al.* 2020).

Table 1. Proximate Composition, Phytochemical constituents, and Antioxidant capacity of *P. purpureum* Grass Meal

Parameters	Values
Nutrient Content	
Metabolizable energy (MJ/kg)	17.01±0.56
Crude protein (%)	13.63±0.40
Dry matter (%)	8.00±0.26
Ether extract (%)	2.02±0.05
Crude fiber (%)	29.5±0.67
Ash (%)	7.50±0.09
Phytochemical Analysis	
Flavonoid (%)	1.96±0.03
Saponins (%)	1.16±0.23
Alkaloid (%)	1.45±0.14
Tannin (%)	1.50±0.07
Antioxidant activity	
DPPH radical scavenging activity (%)	67.73±0.01
All data are presented as mean ± standard error.	

Physicochemical Characterizations

To determine the structural and thermal characteristics of *P. purpureum*, the following analytical techniques were employed. Surface morphology was examined using a JEOL JSM-6000 microscope operated at 15 kV. Prior to imaging, samples were sputter-coated with carbon to improve conductivity and resolution.

Crystallinity was analyzed using a Rigaku diffractometer with Cu K-α radiation (λ = 1.5406 Å), operated at 40 kV and 15 mA, with a scanning rate of 10°/min across a 2θ range of 5° to 70°. Spectral FTIR analysis was performed using a Nicolet iS5 spectrometer to identify functional groups characteristic of plant biomass. Thermal stability was assessed using an EXSTAR TG/DTA7000 system under a nitrogen atmosphere, with a heating rate of 10 °C/min from 30 to 600 °C.

Broiler Chickens Feeding Trial

The study was evaluated and endorsed by the Universiti Putra Malaysia Institutional Animal Care and Use Committee (IACUC; Approval ID: UPM/IACUC/AUP-R047/2022). Two hundred and sixteen one-day-old Sasso broiler chicks were sourced from a licensed hatchery, individually weighed, and then randomly assigned to six dietary treatment groups (36 birds per treatment), with each treatment subdivided into six replicates of six birds. Birds were housed in stainless steel tiered cages (113 cm × 82 cm × 45 cm) in an open-sided facility under standard management conditions, with *ad libitum* access to feed and water (Chung *et al.* 2020, 2021). All birds received standard vaccinations: intraocular vaccination against Infectious Bronchitis (IB) and Newcastle Disease (ND) on day 7, and eye-drop vaccination for Infectious Bursal Disease (IBD) on day 14. Environmental parameters were recorded with a mean temperature of 30.9 °C and relative humidity of 71.24%. All broilers received standard vaccinations *via* intraocular route on day 7, and IBD by eye drop on day 14 under trial conditions of 30.9 °C and 71.2% humidity. The broiler chickens were fed with commercial starter and finisher diets formulated mainly from crumbled soybean meal and maize, administered from day 0 to 28 and day 29 to 56, respectively. For the negative control group (Treatment 1), antibiotic-free commercial feed was provided. In contrast, Treatment 2 (positive control) received commercial feed supplemented with 100 mg/kg of oxytetracycline (Alhendi *et al.* 2000; Alghirani *et al.* 2022). Treatments 3 through 6 were offered the same basal diet without antibiotics but incorporated with *P. purpureum* grass meal at incremental inclusion levels of 1.25, 2.50, 3.75, and 5.00 g/kg, respectively. It is important to note that *P. purpureum* grass meal was supplemented into the basal diet for treatments T3 to T6, rather than replacing any portion of the existing ingredients such as corn or soybean meal. This approach ensured that the basal nutritional composition (energy and protein content) remained constant across treatments, thereby isolating the effects of *P. purpureum* supplementation on growth performance and economic outcomes. Table 2a outlines the composition of the basal starter and finisher feeds, while Table 2b summarizes the nutrient profiles of each treatment group. Body weight (BW) and feed intake (FI) were recorded per replicate to calculate BW gain (BWG), total feed intake (TFI), and feed conversion ratio (FCR = TFI / BWG).

Table 2a. 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	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.

Table 2b. Nutrient Profiles of Broiler Diets Formulated with Graded Supplementation 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 fibre (%)	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 fibre (%)	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 data are presented as mean ± standard error. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetraxycline); 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.

Statistical Analysis

All data were analyzed using RStudio version 4.1.3. A one-way analysis of variance (ANOVA) was performed based on a completely randomized design. Tukey's Honest Significant Difference (HSD) test was used for post-hoc comparisons, with significance set at $p < 0.05$. Mortality rates were analyzed using the Chi-square test.

Cost and Benefit Analysis

A comprehensive economic evaluation was conducted to assess the cost-effectiveness and financial viability of *P. purpureum* grass meal supplementation in broiler production. The analysis considered both fixed and variable costs, as well as profitability metrics across all treatment groups. Fixed costs, including labor and electricity, were standardized at RM 558.97 for each treatment, as they remained constant regardless of feed formulation or output. Variable costs, which included expenditures for chicks, feed, water, antibiotics (for T2 only), and *P. purpureum* grass meal (for T3–T6), varied based on the supplementation level and treatment type. The following financial indicators were computed including total cost (RM), total revenue (RM), total profit (RM), average cost per bird (RM), average revenue per bird (RM), average profit per bird (RM), net profit margin (%), break-even point (birds), margin of safety (birds) and margin of safety (%), as previously reported (Seng *et al.* 2025).

$$\text{Total Cost (RM)} = \text{Fixed Cost} + \text{Variable Cost} \tag{1}$$

$$\text{Total Revenue (RM)} = \text{Number of live birds} \times \text{Final body weight per bird (kg)} \times \text{Market price (RM 18/kg)} \tag{2}$$

$$\text{Total Profit (RM)} = \text{Total Revenue} - \text{Total Cost} \tag{3}$$

$$\text{Average Cost per Bird (RM)} = \text{Total Cost} / \text{Number of birds sold} \tag{4}$$

$$\text{Average Revenue per Bird (RM)} = \text{Total Revenue} / \text{Number of birds sold} \tag{5}$$

$$\text{Average Profit per Bird (RM)} = \text{Average Revenue} - \text{Average Cost} \tag{6}$$

$$\text{Net Profit Margin (\%)} = (\text{Total Profit} / \text{Total Revenue}) \times 100 \tag{7}$$

$$\text{Break-even Point (birds)} = \text{Total Fixed Cost} / (\text{Selling Price per Bird} - \text{Variable Cost per Bird}) \tag{8}$$

$$\text{Margin of Safety (birds)} = \text{Actual birds sold} - \text{Break-even birds} \tag{9}$$

$$\text{Margin of Safety (\%)} = (\text{Margin of Safety} / \text{Actual birds sold}) \times 100 \tag{10}$$

These metrics were applied to each treatment group to determine relative cost efficiency and financial resilience. A lower break-even threshold and higher margin of safety reflect improved cost control and the ability to withstand market or operational fluctuations. Treatments that resulted in positive profit margins with lower average costs and higher per-bird returns were considered economically superior. All economic data were processed using Microsoft Excel and validated through manual cross-checking for accuracy. All data were analyzed using RStudio version 4.1.3. A one-way analysis of variance (ANOVA) was performed based on a completely randomized design. Tukey's Honest Significant Difference (HSD) test was used for post-hoc comparisons, with significance set at $p < 0.05$. Mortality rates were analyzed using the Chi-square test.

RESULTS AND DISCUSSION

Physicochemical Characterization

The surface morphology of ground *P. purpureum* was examined using SEM to assess its structural features. Prior to imaging, samples were carbon-coated using a vacuum sputter coater to improve electrical conductivity and image resolution. At low magnification (100×), the SEM micrographs shown in Fig. 1(a) revealed irregularly shaped agglomerates distributed across the surface.

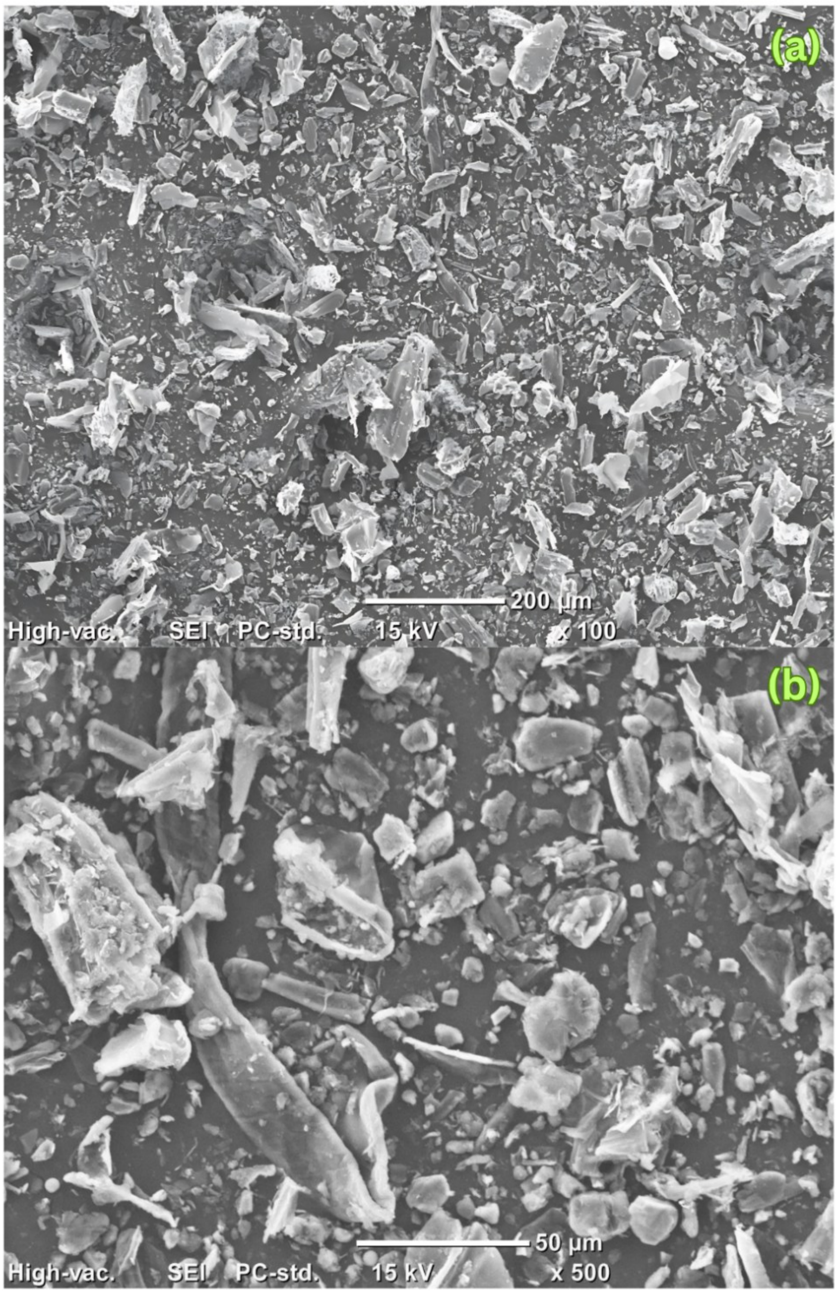
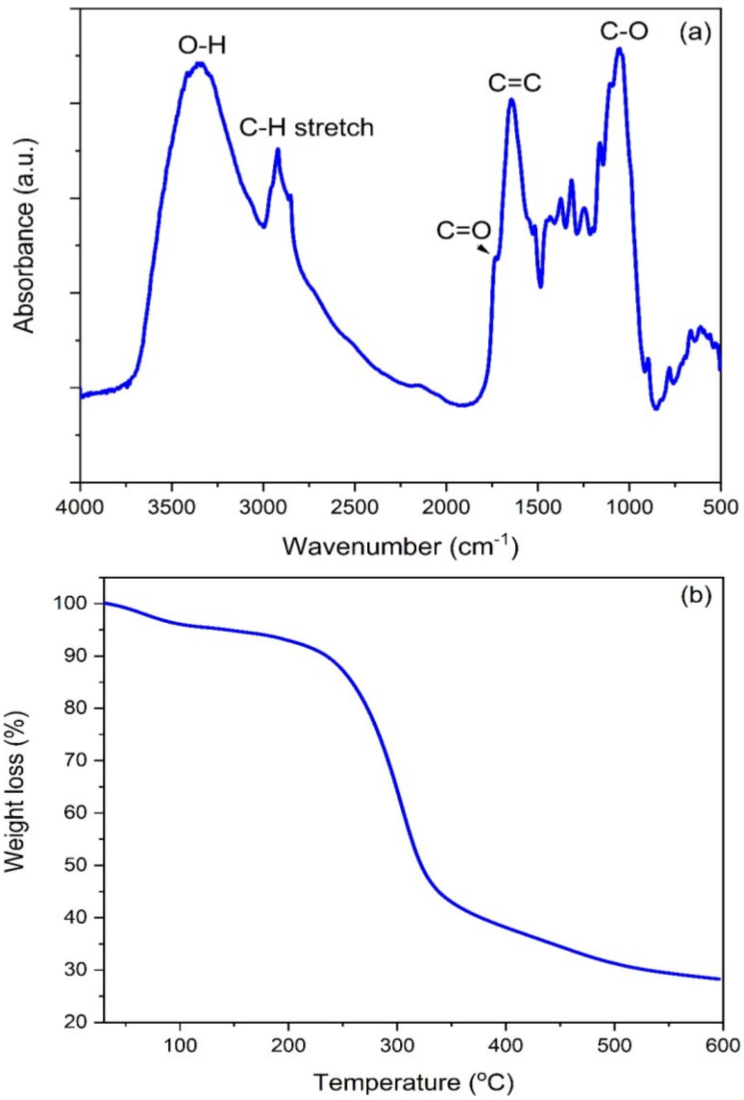


Fig. 1. SEM micrographs of the *P. purpureum* grass under (a) 100x magnification and (b) 500x magnification

This was indicative of a heterogeneous and porous matrix (Wang *et al.* 2008). At higher magnification (500×) shown in Fig. 1(b), fibrous-like particles and aggregated structures were more pronounced, suggesting a complex network of cellulose, hemicellulose, and lignin commonly found in lignocellulosic biomass (Bajpai 2016, 2022). The average particle size was estimated at 28 µm, ranging from 40 to 110 µm, confirming that the grinding process yielded sufficiently small particles for feed incorporation without requiring chemical pretreatment.

FTIR spectroscopy was carried out to identify the functional groups present in *P. purpureum*. The resulting FTIR spectrum depicted in Fig. 2(a) exhibited characteristic absorbance bands typical of plant biomass.



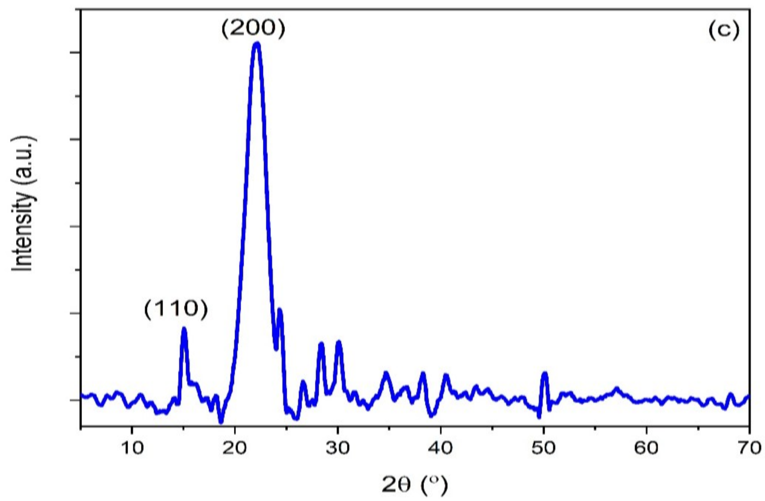


Fig. 2. (a) XRD spectra, (b) TGA thermogram, (c) and XRD spectra of *P. purpureum* grass

A broad peak centered at 3372 cm^{-1} was attributed to O–H stretching vibrations, primarily arising from hydroxyl groups in cellulose, hemicellulose, and lignin, as well as from adsorbed water molecules (Soliman *et al.* 2016; Cichosz and Masek 2020; Baker and Oguntoye 2023; Talik *et al.* 2023). Distinct symmetric and asymmetric C–H stretching vibrations appeared at 2850 cm^{-1} and 2916 cm^{-1}, indicating the presence of aliphatic hydrocarbons. Peaks at 1055 cm^{-1}, 1161 cm^{-1}, and 1311 cm^{-1} were assigned to C–O stretching and C–H bending modes within the cellulose structure (Rana *et al.* 2018). A shoulder at 1739 cm^{-1} corresponded to C=O stretching in ester linkages, likely originating from lipid membranes and cell wall pectins (Wu *et al.* 2020). A notable absorption peak at 1641 cm^{-1} corresponded to C=C stretching vibrations of aromatic rings, indicative of phenolic compounds within the plant matrix.

Thermogravimetric analysis (TGA) was used to assess the thermal decomposition profile of *P. purpureum* under a nitrogen atmosphere depicted in Fig. 2(b). An initial weight loss below 100 °C was attributed to moisture evaporation and the volatilization of light compounds (Zheng *et al.* 2021; Jeffrey *et al.* 2024). The major degradation phase occurred between 250 and 321 °C, corresponding to the breakdown of hemicellulose and cellulose, and accounted for approximately 45% of the total weight loss (Gu *et al.* 2021). A continued decline in weight between 322 and 600 °C was linked to the decomposition of lignin and more thermally stable macromolecules. These results indicate that *P. purpureum* contains approximately 70% volatile matter, highlighting its potential for thermochemical conversion into bio-crude and other energy-dense products, as supported by previous findings (Toor *et al.* 2022).

XRD analysis was performed to assess the crystallographic properties and degree of structural order in the *P. purpureum* grass meal as shown in Fig. 2(c). The resulting diffractogram exhibited distinct peaks centered at approximately 16° and 22° (2θ), which are indicative of the cellulose I polymorph specifically representing the (110) and (200) crystallographic planes, respectively (French 2022; Salem *et al.* 2023). These peaks confirm the presence of semi-crystalline cellulose, a major component of lignocellulosic biomass. The sharpness and intensity of these peaks reflect the partial ordering of the cellulose chains within the plant matrix, which plays a critical role in determining mechanical strength, enzymatic accessibility, and digestibility in animal feed applications. In addition to the characteristic cellulose reflections, several minor yet welldefined peaks were detected between 28° and 60° (2θ). These are likely attributable to crystalline impurities or trace amounts of inorganic constituents such as metal oxides or mineral residues, which may be naturally incorporated into the biomass from the soil during plant growth (Alves *et al.* 2022). These smaller peaks can also be attributed to additional crystalline regions of cellulose (Sofla *et al*. 2016; Soltani *et al*. 2024). Their presence could influence the mineral profile and bioavailability of micronutrients when the biomass is used as a feed ingredient. Overall, the XRD results affirm that *P. purpureum* possesses a heterogeneous fibrous structure with moderate crystallinity, which may influence both its physicochemical behavior and nutritional utility in poultry feed formulations. These findings align with crystallographic data reported for *P. purpureum* grass reported previously (Luengnaruemitchai and Anupapwisetkul 2020).

Growth Performance

Table 3 illustrates the impact of *P. purpureum* grass meal supplementation on the growth performance metrics of Sasso broiler chickens.

Table 3. Impact of *P. purpureum* Grass Meal Supplementation on Growth Performance Parameters in Sasso Broiler Chickens

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	p-value
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}	<0.000
Mortality bird	0	0	2	2	1	1	0.416

All values were expressed as mean ± SE; superscripts $^{a, b, c, \text{ and } d}$ 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 oxytetraxycline); 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 4. Total Production Costs, Income, & Net Returns for Sasso Broilers Fed Varying Levels of *P. purpureum* Grass Meal

Treatments	T1	T2	T3	T4	T5	T6
VARIABLE COST (RM)						
Grass	0.00	0.00	4.02	8.04	12.07	16.09
Antibiotic	0.00	0.72	0.00	0.00	0.00	0.00
Chick	132.74	132.74	125.36	125.36	129.05	129.05
Starter feed	111.74	105.98	107.14	102.53	101.38	101.38
Finisher feed	278.77	263.61	278.70	254.47	263.09	252.88
Water	0.69	0.65	0.71	0.66	0.66	0.64
Total Variable Cost (RM)	523.94	503.70	515.94	491.06	506.24	500.04
FIXED COST (RM)						
Electricity	58.97	58.97	58.97	58.97	58.97	58.97
Labour	500.00	500.00	500.00	500.00	500.00	500.00
Total Fixed Cost (RM)	558.97	558.97	558.97	558.97	558.97	558.97
TOTAL COST (RM)	1,082.91	1,062.67	1,074.90	1,050.03	1,065.21	1,059.00
REVENUE						
No. of live birds sold	36	36	34	34	35	35
Final weight (kg)	1.61	1.69	1.67	1.64	1.68	1.75
TOTAL REVENUE (RM)*	1,043.28	1,095.12	1,022.04	1,003.68	1,058.4	1,102.5
TOTAL PROFIT (RM)	-39.63	32.45	-52.86	-46.35	-6.81	43.50

* Total revenue was computed using a fixed farm gate price of RM 18 per kg of live bird weight. T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100 mg/kg of oxytetraxycline); 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.

Key performance indicators including final body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were used to assess treatment efficacy. While final BW and BWG did not differ significantly among the treatment groups ($p > 0.05$), notable differences were observed in FI ($p = 0.037$) and FCR ($p < 0.001$), suggesting that dietary modifications influenced feed efficiency more than overall weight gain. Among the groups, Treatment 6 (T6), which included 5.00 g/kg of *P. purpureum* grass meal, yielded the most favorable FCR (1.82 ± 0.00), significantly outperforming both the negative control (T1) and lower supplementation treatments (T3 to T5). Remarkably, this improved efficiency occurred despite T6 having the lowest total feed intake, indicating that birds on this diet utilized their feed more effectively to support growth. This observation suggests that *P. purpureum* supplementation, particularly at higher supplementation levels, may confer functional benefits beyond basic nutrition. It is important to note that the FCR values observed in this study (1.82 to 2.14) were higher than typical values for fast-growing commercial broilers (1.5 to 1.7), reflecting differences in genotype (slow-growing Sasso birds), housing (open-sided tropical facility), and production conditions. These contextual factors should be considered when comparing our results to intensive commercial systems.

Several phytochemical constituents identified in *P. purpureum*, particularly flavonoids, saponins, and tannins, are known to exert bioactive effects that could contribute to enhanced gut health, nutrient digestibility, and metabolic regulation. Flavonoids, for example, have been shown to stimulate digestive enzyme secretion, reduce oxidative stress, and modulate gut microbiota composition, thereby improving nutrient absorption and energy utilization (Rahimi *et al.* 2011; Kuralkar and Kuralkar 2021). The reduction in feed intake observed in T6 could be partially attributed to increased nutrient density or satiety signaling induced by the presence of these compounds, while the improved FCR indicated the physiological efficiency with which nutrients were converted into body mass.

Although *P. purpureum* contains a relatively high crude fiber content, its supplementation at up to 5 g/kg did not raise the total dietary fiber beyond the tolerable limit for broilers (≤5% CF). The improved feed conversion ratio and body weight gain observed at this inclusion level may be partly explained by the action of bioactive compounds, such as flavonoids and saponins, which are known to enhance gut morphology, stimulate digestive enzyme secretion, and improve nutrient absorption. However, it is acknowledged that digestibility was not directly evaluated in this trial, which limits mechanistic interpretation. Future work should incorporate ileal digestibility and gut histological assessments to validate these findings.

Moreover, the enhanced feed efficiency observed in the absence of antibiotic growth promoters (as in T6) supports the potential of *P. purpureum* as a viable phytogenic alternative. Notably, while the positive control group (T2) also showed good FCR (1.93 ± 0.01), it was still inferior to T6, reinforcing the comparative efficacy of the grass meal supplement. These results align with existing literature on the use of phytobiotic additives in poultry diets, which report similar improvements in feed efficiency and health status under antibiotic-free conditions (Ong *et al.* 2024). Mortality rates were low across all treatments and did not differ significantly ($p = 0.416$), indicating that the supplementation of *P. purpureum* did not compromise animal welfare or survivability. Collectively, the findings from this trial suggest that 5.00 g/kg of *P. purpureum* grass meal is an optimal supplementation level that enhances feed efficiency without adversely affecting feed intake or survival, offering a promising natural alternative to in-feed antibiotics in sustainable poultry production systems.

Cost and Benefit Analysis

The cost of production was calculated based on the summation of the variable cost and fixed cost (Table 4). The fixed cost (RM 558.97) remained constant across all six treatments, indicating that the cost for electricity and labor used across all treatments will stay constant regardless of the poultry production levels. Among the six treatments, the proportion of fixed costs to total costs was around 53%, indicating the importance of fixed costs in firm production, particularly for firms with low output. Furthermore, variable costs among treatments ranged from RM 491.06 to RM 523.94, according to Table 4. T4 had the lowest variable costs and T1 had the highest variable costs. In terms of variable costs, T1 had the highest total cost of production (RM 1,082.91), while T4 had the lowest total cost of production (RM 1,050.03). Producing output with a lower cost per unit implies that the rational producer has an economy of scale to produce more output with the least amount of waste in the production chain. Table 5 shows that T2 was the most cost-effective of the five treatments, with the lowest average variable cost, average fixed cost, and average total cost. T3 and T4, on the other hand, had the highest average cost (cost inefficiency), at RM 31.61 and RM 30.88 per bird, respectively. T5 and T6, which produced a chicken at an average cost of RM 30.43 and RM30.26 per bird, respectively, were moderately cost-effective.

It is important to note that while the unit price of chicks, feed, and water remained constant across treatments, the total cost per treatment varied due to differences in mortality-adjusted live bird counts (affecting chick costs), as well as actual recorded consumption of feed and water in each group. This approach ensures that the economic analysis reflects real-world production dynamics rather than theoretical uniform costs. Electricity and labor were treated as fixed costs, as they were independent of consumption patterns.

Comparing the overall cost performance among the six treatments, T2 with antibiotics had the lowest average cost relative to other treatments, but the cost difference was not significant. According to Azabo *et al.* (2022), the use of antibiotics will help to reduce the cost of production in broiler production. However, reducing antibiotic use in broiler farming can lead to higher production costs due to increased mortality rates and additional management expenses (Azabo *et al.* 2022). This supports the idea that the average cost of production for T2 is lower than other treatments. However, the average cost of Treatment 6 was almost the same as T2 if it substituted the conventional feed without antibiotics with 5 grams of grass treatment. Furthermore, compared to the final weight gained by each chicken, T6 (1.75 kg per bird) was even higher than T2 (1.69 kg per bird).

The revenue ranged from RM1,003.68 to RM 1,102.5, with T6 asserting its dominance by yielding the most revenue and T4 yielding the least (Table 4). The four treatments with the loss were T1, T3, T4, and T5. On the other hand, this indicates that only T2 and T6 generated a positive profit and that the total revenue exceeded the total cost. T6, for example, generated a higher revenue and profit of RM 1,102.50 and RM 43.50, respectively. This substantial return demonstrates that T6 had effective cost management and a healthy return on investment (higher profit margin of 4.96%). T6 can generate the highest returns of about RM 31.50 per bird, or a net profit of about RM 1.24 per bird. When it comes to profitability, treatments ranged from a loss of RM 52.86 (T3) to a substantial profit of RM 43.50 (T6). This disparity highlights the critical role of revenue generation and cost management strategies in determining profitability. Furthermore, profit margins range from -5.17% (T3) to 3.95% (T6), emphasizing T6's continued superiority in terms of profitability and efficiency.

As a rational investor with good decision-making, the main objective must aim for higher profit with minimum costs of production (Dahan and Srinivasan 2011). As a result, comparing revenue and profit (financial accounting information) across treatments is critical for providing additional evidence to support the best treatment investment decision (Ball *et al.* 2003; Al-Sehali and Spear 2004). T6 continued to produce the highest revenue per bird among treatments based on the estimated average revenue. This substantial return demonstrates that T6 exhibited effective cost management (efficient) and a healthy return on investment (profitability).

Table 5 displays the estimated break-even points for the six treatments, which ranged from 33 to 39 birds, as well as the margins of safety, which ranged from -10.45% to 7.22%. Treatments with lower break-even points, such as T6 with 33 birds, were more efficient in covering costs and achieving profitability, allowing for a greater margin of safety against losses. At the same time, T6 had a significantly positive margin of safety, *i.e.*, 7.22%, indicating that it could cover costs and withstand market fluctuations better than other treatments.

Break-even analysis is an important cost-and-benefit analysis indicator that identifies the point at which total revenue equals total costs, thereby explaining the minimum number of sales required to avoid losses. Treatments with lower break-even points are more efficient in terms of covering costs and generating profits, *i.e.*, T6. Furthermore, margins of safety are important too in determining the amount of output or sales that can fall before a business reaches its break-even point or becomes unprofitable. Higher positive margins of safety imply a larger buffer against losses and greater resilience to market fluctuations (Fatmawatie 2021). Overall, the current T6 produces more birds (35 birds) than the break-even units (33 birds) as well as shows the highest percentage of margins of safety among other treatments. This indicates that T6 was more profitable and more cost-efficient than other treatments.

Table 5. Summary of Economic Performance Indicators for Sasso Broilers Fed Diets with Incremental Levels of *P. purpureum* Grass Meal Supplementation

Treatments	T1	T2	T3	T4	T5	T6
Average Variable Cost (RM per bird)	14.55	13.99	15.17	14.44	14.46	14.29
Average Fixed Cost (RM per bird)	15.53	15.53	16.44	16.44	15.97	15.97
Average Cost (RM per bird)	30.08	29.52	31.61	30.88	30.43	30.26
Average Revenue (RM per bird)	28.98	30.42	30.06	29.52	30.24	31.50
Average Profit (RM per bird)	-1.10	0.90	-1.55	-1.36	-0.19	1.24
Net Profit Margin (%)	-3.80%	2.96%	-5.17%	-4.62%	-0.64%	3.95%
Break-even (birds)	38.75	34.02	37.55	37.07	35.43	32.47
Margin of safety (birds)	-2.75	1.98	-3.55	-3.07	-0.43	2.53
Margin of safety (%)	-7.63%	5.49%	-10.45%	-9.04%	-1.23%	7.22%

T1: Negative control (Basal diet only); T2: Positive control (Basal diet+100mg/kg of oxytetraxycline); 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.

Future research should elucidate the mechanisms by which *P. purpureum* bioactives interact with the gut microbiome, immune pathways, and nutrient transport systems in monogastric animals, and evaluate performance under commercial-scale production.

CONCLUSIONS

1. Physicochemical characterization (SEM, FTIR, TGA, XRD) confirmed that *P. purpureum* grass is a heterogeneous lignocellulosic material composed of semi-crystalline cellulose, amorphous hemicellulose and lignin, and rich in bioactive functional groups (hydroxyl, carbonyl, aliphatic chains).
2. The biochemical complexity of *P. purpureum* suggests inherent health-promoting properties when incorporated into animal feed.
3. Broiler supplementation at 5.00 g/kg significantly improved feed conversion efficiency, reduced total feed intake, and enhanced body weight gain without the use of synthetic antibiotics.
4. Unchanged mortality rates across treatments confirm the safety of *P. purpureum* supplementation at the tested inclusion level.
5. Economic analysis identified Treatment 6 (5 g/kg) as the most profitable strategy, delivering the highest revenue, net profit margin, and margin of safety with a low break-even threshold.
6. While this study demonstrated growth and economic benefits, a key limitation is the absence of direct assessments of digestibility, immune response, antioxidant status, and gut microbiota, which should be prioritized in future research; additionally, exploring enzymatic (*e.g.*, peroxidase, laccase) or fungal pretreatment of *P. purpureum* could improve its digestibility and enhance the bioavailability of its phytogenic compounds for poultry.

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