Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tjas20

Phytobiotic effects of Guinea grass supplementation on production and health performances of coloured-broilers

Yee Lyn Ong, Eric Lim Teik Chung, Nazri Nayan, Muhamad Faris Ab Aziz, Faez Firdaus Abdullah Jesse & Awis Qurni Sazili

To cite this article: Yee Lyn Ong, Eric Lim Teik Chung, Nazri Nayan, Muhamad Faris Ab Aziz, Faez Firdaus Abdullah Jesse & Awis Qurni Sazili (2025) Phytobiotic effects of Guinea grass supplementation on production and health performances of coloured-broilers, Italian Journal of Animal Science, 24:1, 149-163, DOI: 10.1080/1828051X.2024.2444457

To link to this article: https://doi.org/10.1080/1828051X.2024.2444457

Published online: 27 Dec 2024.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2025, VOL. 24, NO. 1, 149–163
https://doi.org/10.1080/1828051X.2024.2444457

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Phytobiotic effects of Guinea grass supplementation on production and health performances of coloured-broilers

Yee Lyn Ong[a], Eric Lim Teik Chung[a,b], Nazri Nayan[a,b], Muhamad Faris Ab Aziz[b,c], Faez Firdaus Abdullah Jesse[d] and Awis Qurni Sazili[b,c]

[a]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Malaysia; [b]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia; [c]Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Malaysia; [d]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia

ABSTRACT

Growing concerns about antibiotic resistance in the livestock industry have prompted researchers to explore alternative solutions. This study investigated the effects of *Megathyrsus maximus* as a phytobiotic supplement on the production performance and health of Sasso broilers raised in tropical environments. 216 Sasso mixed-sex broiler chicks were randomly assigned to six treatment groups (six replicates per treatment): Treatment 1 (T1) as a negative control (no antibiotics), Treatment 2 (T2) as a positive control (100 mg/kg oxytetracycline), and Treatments 3–6 received 1.25, 2.50, 3.75, and 5.00 g/kg of *M. maximus* grass meal on top the basal diet, respectively, without antibiotics. At day 56, broilers in T6 had the highest final body weight (1.87 kg) and superior FCR of 1.93. Nutrient digestibility was optimised at higher doses of *M. maximus*, with the highest CP (67.68%) and EE (74.50%) digestibility in the T6 group ($p < 0.001$). *Megathyrsus maximus* also improved intestinal morphology, with higher VH:CD in all segments. However, microbial counts and carcase characteristics remained largely unaffected, though meat quality was significantly improved in terms of reduced drip loss and shear force at supplementation levels of 5.00 g/kg. Blood biomarkers analysis and decreased liver enzyme activity in the *M. maximus* groups, particularly in T6, suggests lowered inflammation and enhanced liver function. This study concludes that *M. maximus* supplementation, particularly at the highest inclusion level (T6) shows potential as an effective antibiotic replacement in tropical poultry production.

HIGHLIGHTS

- Supplementing Sasso broilers with 5.00 g/kg of *Megathyrsus maximus* improved their growth and feed efficiency over antibiotic and non-antibiotic controls.
- *Megathyrsus maximus* significantly enhanced health metrics, including nutrient digestibility and gut health, while lowering stress indicators.
- 5.00 g/kg of *M. maximus* supplementation improved meat quality and lipid profile, showing promise as an antibiotic alternative in poultry feed.

ARTICLE HISTORY
Received 29 July 2024
Revised 17 November 2024
Accepted 13 December 2024

KEYWORDS
Megathyrsus maximus; phytocompounds; alkaloid; feed additive; Sasso broiler

Introduction

The poultry industry is a crucial contributor to global food production as it is a significant source of protein. However, one of the main challenges faced by this industry is maintaining a consistent production output whilst ensuring efficient and healthy growth rates in broilers. Generally, antibiotics have been widely used as a growth promoter to achieve these goals (Selaledi et al. 2020). However, growing global concerns of antibiotic resistance have driven researchers to explore alternative solutions (Frieri et al. 2017). One promising alternative is phytobiotics, known for their phytocompounds that can enhance growth performance, improve gut health, and immune function (Ni et al. 2016; Aljumaah et al. 2020; Kikusato et al. 2021). Henceforth, by leveraging on the natural bioactive compounds found in plants, phytobiotics present a potential strategy for improving the productivity of

CONTACT Eric Lim Teik Chung eric88lim@gmail.com

the poultry industry without the associated risk of antibiotic resistance (Chung et al. 2018).

Megathyrsus maximus, or known as Guinea grass and green panic grass is a forage grass that is native to tropical and subtropical parts of Africa that was introduced across Asia, Europe, North America, and South America (Soti and Thomas 2022). As a major pantropical grass, it is widely used throughout the tropics for pasture, cut-and-carry, silage, and hay due to its fast growing and leafy grass, which is palatable and nutritious to livestock (Aswanimiyuni et al. 2018). Guinea grass has an excellent reputation in the ruminant livestock industry due to its nutritional content and ease of cultivation, yet its potential in the poultry industry has yet to be explored. Henceforth, besides being a key fodder species for ruminants, phytocompounds present in the plant-typically considered as antinutritional factors at high concentrations-may offer a promising alternative to antibiotics in the broiler industry, as they have been found to enhance gut function and health when present in modest levels (Grashorn 2010; Huang et al. 2018). A study by Abu Hafsa and Hassan (2021) found that Guinea plant contains 29.6 mg/g of alkaloids, followed by 7.2 mg/g of tannins, 11.4 mg/g of saponins, 4.8 mg/g of phytates, and 3.1 mg/g of hydrogen cyanide. Another analysis by Kanife and Doherty (2012) reported alkaloids at 2.83%, saponins at 2.60%, tannins at 0.85%, and flavonoids at 0.54% in Guinea plant leaves. The differences in these findings might be due to varying management practices, such as cutting age or fertilisation, which were not detailed in either study (Onyeonagu and Ukwueze 2012; Onyeonagu et al. 2013).

On the other hand, Kanife and Doherty (2012) noted that the principal physiologically active compounds in Guinea grass, including alkaloids, tannins, saponins, and flavonoids, may have antifungal properties. Alkaloids, in particular, may contribute to antimicrobial activity against pathogens like *Klebsiella pneumoniae* and *Candida albicans*. Moreover, feeding broilers with alkaloid-rich leaf meals, such as *Sauropus androgynus* and *Polyalthia longifolia*, has been observed to promote growth, suggesting they could serve as antibiotic replacements (Sugiharto et al. 2019). Additionally, alkaloids and saponins in the aqueous leaf extract of *Azadirachta indica* have been shown to reduce *Eimeria* oocyte counts by binding to and disrupting critical macromolecules on the parasite's membrane, leading to its death (Onyiche et al. 2021). Given the beneficial phytocompounds found in Guinea grass, this study aims to investigate its impact on the growth performance, apparent ileal digestibility, gut histomorphology, caecal microbial population, carcase and meat quality, and blood indices of Sasso broilers. The findings may support its potential as a practical alternative feed additive for the poultry industry.

Materials and methods

Plant preparation

Megathyrsus maximus was grown at Field 15, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The leaves were manually harvested at four weeks old and dried in an oven at 60 °C until they reached a constant weight. They were then processed into a powder (50–300 mesh) using an electric grinder (RRH-800A) and stored at room temperature for later use. The levels of dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash content in *M. maximus* were determined using the Official Methods of Analysis of AOAC International (Horwistz 2010). The nutritional composition of *M. maximus* is summarised in Table 1.

Phytochemical and antioxidant activity analyses

The quantification of saponins, tannins, flavonoids, and alkaloids in *M. maximus* was performed using a modified version of the method by Kordali et al. (2005). Additionally, the DPPH radical scavenging activity of *M. maximus* leaf meals was evaluated according to the protocol outlined by Jack et al. (2020), with slight adjustments. Table 1 details the phytochemical content and antioxidant activity of *M. maximus*.

Table 1. Nutrient composition, quantitative analysis of phytochemical screening and antioxidant activity of *M. maximus* leaf meal.

Parameters	*M. maximus*
Nutrient composition	
Metabolizable energy, MJ/kg	18.24 ± 0.23
Dry matter, %	26.10 ± 0.15
Crude protein, %	8.61 ± 0.28
Crude fibre, %	30.00 ± 0.45
Ether extract, %	1.75 ± 0.15
Ash, %	6.42 ± 0.16
Phytochemical screening	
Saponins, %	1.06 ± 0.04
Tannin, %	1.41 ± 0.02
Alkaloid, %	4.59 ± 0.17
Flavonoid, %	1.00 ± 0.04
Antioxidant activity	
DPPH radical scavenging activity, %	12.78 ± 0.00

MJ/kg: megajoules per kilogram; DPPH: 2,2-Diphenyl-1-picrylhydrazyl.
All values were expressed as mean ± standard error.

Broilers and husbandry

All procedures involving animal care, handling, and sampling were conducted in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti of Putra Malaysia before the start of the research (Approval number: UPM/IACUC/AUP-R047/2022). In a completely randomised design (CRD) experiment, 216 day-old Sasso mixed-sex broiler chicks were sourced from a local hatchery. The chicks were weighed and randomly assigned to six treatment groups, each comprising six replications and six broilers per replicate. They were raised in an open-sided house for 56 days, housed in stainless-steel tiered cages measuring 113 × 82 × 45 cm, with a stocking density of six chickens per cage (0.154 m^2 per chicken). Throughout the study, the average temperature and relative humidity were 30.9 °C and 71.24%, respectively. Lighting program was implemented based on guidance from the Sasso Management Guide Rural Poultry by Hendrix Genetics (2022) and were maintained with natural day light, 40-W fluorescent lamps, and a 60-W bulb placed centrally in each cage cell, above the heads of the birds. After two weeks, the 60-W bulb was turned off and lighting was sustained with natural day light and florescent lamps. Light hours was provided constantly for 24 h from the day of chick arrival until the seventh day, then lowered to 20 h each day from the eighth to the fourteenth, followed by maintaining 12 h of natural daylight until the day of slaughter. On day 7, chicks were immunised intraocularly against infectious bronchitis (IB) and Newcastle disease (ND), and on day 14, they were immunised against infectious bursal disease (IBD) *via* eye drop (Chung et al. 2021). Feed and freshwater were provided ad libitum throughout the study.

Diets

The broilers were fed commercial diets based on maize and soybean meal, provided in crumble form. The starter diet was given from day 0 to day 28, and the finisher diet from day 29 to day 56. Broilers in Treatment 1 (negative control) received commercial diets without antibiotics, while those in Treatment 2 (positive control) broilers were fed commercial feeds containing 100 mg/kg oxytetracycline antibiotic. Treatments 3, 4, 5, and 6 were given the same commercial diets, supplemented with 1.25, 2.50, 3.75, and 5.00 g/kg of *M. maximus* grass meal, on top of the base diet, respectively, without antibiotics. The dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash content were analysed using the respective methods specified by AOAC International, following protocols AOAC 934.01 for DM, AOAC 978.10 for CF, AOAC 990.03 for CP, AOAC 920.39 for EE, and AOAC 942.05 for ash content (Horwistz, 2010). Table 2 provides a breakdown of the basal diets' composition, while Table 3 presents the nutritional composition of the treatment groups for both the starter and finisher phases.

Growth performance

Throughout the eight-week study, body weight (BW) and feed intake (FI) were recorded weekly for each replicate. Accurate measurements were obtained using a digital weighing scale with a precision of two decimal points (Mettler Toledo Industrial Scale, BBA211 series, Greifensee, Switzerland). These records were used to calculate body weight gain (BWG) and feed intake. The feed conversion ratio (FCR) was then calculated and recorded. On the 56th day of the study, six chickens (three males and three females) were randomly selected from each treatment group for slaughter. This was conducted to evaluate nutritional digestibility, gut histomorphology, caecal microbiota population, carcase characteristics, and meat quality. The slaughtering process adhered to Halal standards and was carried out at the slaughterhouse of the Department of Animal Science, Faculty of Agriculture, UPM. Blood samples were collected to assess concentrations of acute phase proteins, corticosterone, and

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	52.10
Wheat bran	4.50	6.00
Soybean meal	40.00	32.00
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[a]	0.15	0.15
Vitamin premix[b]	0.15	0.15
Toxin binder	0.10	0.10
Total	100	100

[a]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.

[b]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 3. Nutrient composition of Sasso broilers diets added with different levels of *M. maximus* grass meal.

	Treatments					
Parameters	T1	T2	T3	T4	T5	T6
Starter diet (1–28 days)						
Metabolisable energy, MJ/kg	13.00 ± 0.84	12.45 ± 0.51	12.15 ± 0.50	12.17 ± 0.79	12.13 ± 0.66	13.02 ± 0.69
Dry matter, %	90.67 ± 0.33	91.33 ± 0.71	90.83 ± 0.60	91.17 ± 0.31	90.50 ± 0.34	90.67 ± 0.21
Crude protein, %	21.96 ± 1.70	22.13 ± 0.08	22.56 ± 0.09	22.65 ± 0.40	22.93 ± 1.42	22.02 ± 0.95
Crude fibre, %	3.50 ± 0.50	3.40 ± 0.68	3.80 ± 0.20	3.60 ± 0.40	3.25 ± 0.63	3.83 ± 0.17
Ether extract, %	4.93 ± 0.12	4.70 ± 0.27	4.86 ± 0.31	4.57 ± 0.87	4.53 ± 0.19	4.60 ± 0.33
Ash, %	5.70 ± 0.34	5.94 ± 0.24	6.43 ± 0.35	7.13 ± 0.46	6.99 ± 0.36	6.07 ± 0.25
Finisher (29–56 days)						
Metabolisable energy, MJ/kg	13.04 ± 0.13	12.00 ± 0.15	12.71 ± 0.36	12.00 ± 0.11	12.76 ± 0.25	12.09 ± 0.25
Dry matter, %	89.85 ± 0.43	88.04 ± 0.34	89.00 ± 0.17	88.55 ± 0.20	89.34 ± 0.44	89.20 ± 0.23
Crude protein, %	19.78 ± 0.43	19.36 ± 0.17	19.92 ± 0.25	20.16 ± 0.33	19.63 ± 1.34	19.63 ± 0.82
Crude fibre, %	3.29 ± 0.11	3.69 ± 0.14	4.03 ± 0.36	3.76 ± 0.32	3.68 ± 0.46	3.46 ± 0.21
Ether extract, %	5.24 ± 0.17	5.78 ± 0.42	5.13 ± 0.15	6.11 ± 0.41	5.52 ± 0.33	5.09 ± 0.62
Ash, %	13.00 ± 0.84	12.45 ± 0.51	12.15 ± 0.50	12.17 ± 0.79	12.13 ± 0.66	18.02 ± 0.69

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
All values were expressed as mean ± *SE*.

heat shock proteins, as well as other biochemical markers including liver function and serum lipid profile.

Apparent ileal digestibility

Three days before slaughter, 300 mg/kg titanium dioxide (TiO_2) was added to the finisher diets as an indigestible marker. Following slaughter, ileal material was collected and stored at −20 °C for subsequent nutritional analysis. Samples were ashed at 580 °C for 13 h, treated with sulphuric acid, and dissolved by boiling before filtration. A working TiO_2 standard solution (0.3 mg/ml) was prepared and used to create a standard curve for spectrophotometric analysis at 410 nm (Alghirani, Chung, Sabri, et al. 2021). The apparent ileal digestibility (AID) of dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), and ash was then determined by calculating the titanium marker ratios in the diet and ileal content using the provided equations, as outlined by Hartinger et al. (2022):

$$\text{Apparent ileal digestibility (AID)} = 100\% - \left[\left(\frac{\%\ TiO_2\ \text{in feed}}{TiO_2\ \text{in ileal content}}\right) \times \left(\frac{\%\ \text{of nutrient in ileal content}}{\%\ \text{nutrient in feed}}\right) \times 100\right]$$

Gut histomorphology

The procedure for gut histomorphology followed standard protocols at the Histopathology Lab, Faculty of Veterinary Medicine, UPM (Alghirani, Chung, Jesse, et al. 2021). Initially, 5 cm segments from the duodenum, jejunum, and ileum were excised, rinsed with neutral buffered saline, and preserved in 10% formalin solution overnight. Subsequently, all samples were dehydrated in a tissue processing machine and embedded in paraffin wax before sectioning. The intestinal samples were then cut into 4 μm slices, mounted on glass slides, stained with haematoxylin and eosin using a hot plate at 60 °C, and prepared for observation under a Nikon DS-U2/L2 light microscope. NIS-Elements D software was used to examine, capture, and measure villi height and crypt depth. Villi height was determined from the tip to the crypt transition, while crypt depth was measured at the invagination between two villi.

Caecal microbial population

After slaughtering on day 56, caecal samples were collected from each treatment group and promptly transported to the Microbiology Lab, Faculty of Veterinary Medicine, UPM. The samples were placed in an ice box with ice to maintain their temperature during transportation. In the laboratory, the samples were subjected to microorganism isolation and identification, salmonella identification, standard plate count, and coliform count, following the protocol outlined by Park and Kim (2020). Specifically, microorganism isolation and identification were carried out to determine the microbial composition of the caecal contents, with a focus on beneficial and pathogenic bacteria. Salmonella species were identified using selective media and biochemical tests to assess the presence of pathogenic microorganisms. The standard plate count method was employed to quantify the total viable aerobic bacteria present in the caecal content, providing a measure of overall microbial load. Additionally,

coliform counts were performed using selective media to evaluate the abundance of coliform bacteria, which serve as indicators of gut health and hygiene. The viable bacteria colonies were then enumerated and expressed as the logarithm of colony-forming units (cfu)g^{-1} per gram of caecal content.

Carcase and meat quality

After slaughtering, all carcase parameters were manually dissected, and the following measurements were recorded: final live weight, de-feathered weight, dressing percentage, the weight of the breast muscle (both left and right pectoralis major and minor), drumsticks, wings, head, shank, gastrointestinal tract, heart, liver, full and empty gizzard (Chung et al. 2020). Subsequently, the right pectoralis major (breast muscle) and right soleus muscle (drumstick) were extracted from each replicate for all meat quality assessments. Protocols outlined by Chung et al. (2021) were adhered to for measuring pH, colour, drip loss, cooking loss, and tenderness.

Blood biomarkers

Blood samples for plasma were collected into blood collection tubes containing anticoagulant EDTA (BD vacutainer®, New Jersey, USA) and kept on ice. Blood samples were centrifuged at 3000 g for 10 min at 4 °C. For further analysis, the plasma was decanted into 1.5 mL microcentrifuge tubes and stored at −80 °C. Acute phase proteins (serum amyloid A, alpha-1–acid glycoprotein, and ceruloplasmin), corticosterone, and heat shock protein (heat shock protein 70) responses were all measured from the blood samples using commercial enzyme-linked immunoabsorbent one-step process assay (ELISA) kit purchased from QAYEE-BIO for Life Science (China) and was used according to manufacturer's instructions. Results were then computed and calculated using MyAssays software.

Blood biochemistry

Alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST) parameters were determined in the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Putra Malaysia using a BA400 biochemical and turbidimetry analyser from Spain (Manual code TEUS00048-07–EN). Besides, the plasma total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured using an automatic analyser (Automatic analyser 902, Hitachi, Germany). The Friedewald Equations were used to estimate LDL and VLDL:

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{VLDL}$$

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

Statistical analysis

All data collected was uploaded in RStudio version 4.1.3 where the data was analysed using one-way analysis (ANOVA) based on the completely randomised design model. Tukey's HSD *post-hoc* test was used to determine the significant difference among treatment groups if any. Results were considered significant at $p < 0.05$. For mortality, Chi-square test was used as a non-parametric statistical test.

Results

Growth performance

Table 4 shows the effect of *M. maximus* leaf meal supplementation on the growth performance of Sasso broilers on days 28 and 56. Treatments showed significant differences ($p < 0.05$) in all growth performance indicators throughout the starter and finisher phases. Despite having a higher feed consumption throughout both stages, broiler chicks treated with *M. maximus* leaf meal exhibited a greater body weight increase (+10.65%) and final body weight (+10.9%) in comparison to the negative control. Thus, higher levels of *M. maximus* supplementation also resulted in lower FCR. There was no significant different in the FCR between T6 and T2 broilers indicating the supplementation of 5.00 g/kg of *M. maximus* leaf meal could enhance the growth performance of broilers which is comparable to the usage of antibiotics.

Apparent ileal digestibility

According to Table 5, the addition of *M. maximus* leaves considerably ($p < 0.001$) impacted the apparent ileal nutritional digestibility of broilers on day 56. Across all of the treatments, all of the parameters revealed results that were significantly different ($p < 0.05$). Comparing T6 to the other treatments, it was more digestible for CP, CF, and EE, but less so for DM and ash. This shows how the inclusion level of 5.00 g/kg of *M. maximus* leaf meal enabled better

Table 4. Effect of *M. maximus* leaf meal supplementation on the growth performance of Sasso broilers on days 28 and 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
28-day-old (starter phase)							
Initial body weight, kg	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.835
Final body weight, kg	0.65 ± 0.02^{ab}	0.62 ± 0.02^{b}	0.60 ± 0.01^{b}	0.65 ± 0.02^{ab}	0.65 ± 0.02^{ab}	0.71 ± 0.02^{a}	0.011
Body weight gain, kg	0.61 ± 0.02^{ab}	0.57 ± 0.02^{b}	0.56 ± 0.01^{b}	0.60 ± 0.02^{ab}	0.61 ± 0.02^{ab}	0.67 ± 0.02^{a}	0.011
Feed intake, kg	1.00 ± 0.04^{a}	0.78 ± 0.03^{c}	0.88 ± 0.02^{bc}	0.89 ± 0.02^{ab}	0.86 ± 0.01^{bc}	0.91 ± 0.02^{ab}	<0.001
Cumulative FCR	1.63 ± 0.00^{a}	1.35 ± 0.01^{c}	1.42 ± 0.01^{a}	1.48 ± 0.02^{b}	1.42 ± 0.04^{bc}	1.37 ± 0.02^{c}	<0.001
Mortality bird	0	1	0	1	0	1	0.849
56-day-old (finisher phase)							
Final body weight, kg	1.69 ± 0.04^{ab}	1.62 ± 0.05^{b}	1.63 ± 0.05^{b}	1.70 ± 0.04^{ab}	1.80 ± 0.05^{ab}	1.87 ± 0.04^{a}	0.002
Body weight gain, kg	1.65 ± 0.04^{ab}	1.58 ± 0.05^{b}	1.59 ± 0.05^{b}	1.67 ± 0.04^{ab}	1.76 ± 0.05^{ab}	1.83 ± 0.04^{a}	0.002
Feed intake, kg	3.39 ± 0.09^{ab}	3.03 ± 0.1^{b}	3.22 ± 0.11^{ab}	3.36 ± 0.09^{ab}	3.38 ± 0.05^{ab}	3.54 ± 0.07^{a}	0.006
Cumulative FCR	2.06 ± 0.01^{a}	1.92 ± 0.01^{b}	2.02 ± 0.01^{a}	2.02 ± 0.02^{a}	1.93 ± 0.03^{b}	1.93 ± 0.01^{b}	<0.001
Mortality bird	1	1	1	0	0	4	0.098

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal; FCR: feed conversion ratio; FCR: Feed Conversion Ratio.
All values were expressed as mean ± *SE*; superscripts [a, b, and c] values within the row are significantly different at $p < 0.05$.

Table 5. Effect of *M. maximus* leaf meal supplementation on the apparent ileal nutrient digestibility of Sasso broilers on day 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
Dry matter, %	70.23 ± 0.05^{a}	71.49 ± 0.10^{a}	67.95 ± 0.19^{b}	66.25 ± 0.79^{b}	62.58 ± 0.43^{c}	60.60 ± 0.27^{d}	<0.001
Crude protein, %	55.75 ± 0.20^{e}	64.44 ± 0.16^{b}	58.24 ± 0.06^{d}	60.54 ± 0.3^{c}	66.24 ± 0.35^{b}	67.68 ± 0.34^{a}	<0.001
Crude fibre, %	26.63 ± 0.35^{e}	27.71 ± 0.29^{d}	28.39 ± 0.03^{cd}	29.23 ± 0.14^{bc}	29.45 ± 0.23^{b}	30.50 ± 0.10^{a}	<0.001
Ether extract, %	65.63 ± 0.14^{e}	72.53 ± 0.12^{b}	69.40 ± 0.19^{d}	70.30 ± 0.03^{c}	72.68 ± 0.28^{b}	74.50 ± 0.04^{a}	<0.001
Ash, %	37.64 ± 0.33^{a}	38.27 ± 0.24^{a}	37.40 ± 0.21^{a}	37.07 ± 0.14^{a}	35.05 ± 0.37^{b}	34.48 ± 0.13^{b}	<0.001

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
All values were expressed as mean ± *SE*; superscripts [a, b, c, d, and e] values within the row are significantly different at $p < 0.05$.

Table 6. Effect of *M. maximus* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
Villi height, μm							
Duodenum	481.95 ± 152.41^{b}	626.25 ± 198.04^{a}	587.13 ± 185.67^{a}	584.82 ± 184.94^{a}	598.08 ± 189.13^{a}	619.03 ± 195.76^{a}	<0.001
Jejunum	572.01 ± 180.88^{b}	593.43 ± 187.63^{ab}	501.21 ± 158.50^{c}	510.16 ± 161.33^{c}	588.66 ± 186.15^{b}	628.19 ± 198.65^{a}	<0.001
Ileum	313.19 ± 99.07^{c}	362.20 ± 114.54^{b}	330.49 ± 104.51^{bc}	357.79 ± 113.14^{b}	459.02 ± 145.16^{a}	487.71 ± 154.23^{a}	<0.001
Crypt depth, μm							
Duodenum	118.48 ± 37.47^{a}	117.95 ± 37.30^{a}	112.50 ± 35.58^{ab}	109.96 ± 34.77^{ab}	111.75 ± 35.34^{ab}	101.64 ± 32.14^{b}	0.001
Jejunum	126.98 ± 40.16^{a}	78.81 ± 24.92^{b}	77.24 ± 24.42^{b}	70.33 ± 22.24^{c}	67.01 ± 21.19^{c}	65.76 ± 20.80^{c}	<0.001
Ileum	130.25 ± 41.19^{a}	73.40 ± 23.21^{bc}	80.01 ± 25.30^{b}	73.23 ± 23.16^{c}	65.82 ± 20.82^{d}	57.18 ± 18.08^{e}	<0.001
Villi height: crypt depth							
Duodenum	4.07 ± 1.29^{c}	5.32 ± 1.68^{b}	5.22 ± 1.65^{b}	5.35 ± 1.69^{b}	5.36 ± 1.69^{b}	6.09 ± 1.93^{a}	<0.001
Jejunum	4.51 ± 1.43^{e}	7.56 ± 2.39^{c}	6.50 ± 2.05^{d}	7.26 ± 2.30^{c}	8.80 ± 2.78^{b}	9.59 ± 3.03^{a}	<0.001
Ileum	2.41 ± 0.76^{e}	4.96 ± 1.57^{c}	4.14 ± 1.31^{d}	4.91 ± 1.55^{c}	6.99 ± 2.21^{b}	8.55 ± 2.70^{a}	<0.001

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
All values were expressed as mean ± *SE*; superscripts [a, b, c, d, and e] values within the row are significantly different at $p < 0.05$.

nutritional absorption in the ileum as CP, CF, and EE's digestibility were improved by 21.40, 14.53, and 13.53% respectively in contrast to T1.

Gut histomorphology

Table 6 illustrates the effect of *M. maximus* leaf meal supplementation on the small intestine histomorphology of Sasso broilers on day 56. In the duodenum, T3 to T6 broilers showed no significant differences ($p > 0.05$) when compared to the positive control group (T2). Whereas in the jejunum, only T6 showed comparable ($p > 0.05$) in comparison to T2. On the other hand, T5 (+26.73%) and T6 (+34.65%) broilers exhibited better ($p < 0.05$) villi height in the ileum when compared to T2. Additionally, T6 showed the lowest ($p < 0.05$) crypt depth in the duodenum, jejunum, and ileum. Therefore, T6 had the best ($p < 0.05$) small intestine histomorphology results as T6 showed the highest villi height: crypt depth ratio in

Table 7. Effect of *M. maximus* supplementation on the caecal microorganisms of Sasso broilers on day 56.

	Treatment					
Parameters	T1	T2	T3	T4	T5	T6
Isolation and identification	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (4+)	*E. coli* (3+) NFL E. coli (2+)	*E. coli* (4+) NFL *E. coli* (2)	*E. coli* (4+) NFL *E. coli* (2+)	*E. coli* (3+)
Salmonella identification	Negative	Negative	Negative	Negative	Negative	Negative
Coliform count, cfu/g	8.3×10^6	2.5×10^7	1.6×10^7	3.2×10^7	7.6×10^7	1.6×10^8
Standard plate count, cfu/g	4.3×10^6	1.2×10^8	2.1×10^7	5.1×10^7	3.6×10^7	1.4×10^9

cfu: colony-forming unit; NLF *E. coli*: non-lactose fermenting *E. coli*; T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.

Table 8. Effect of *M. maximus* supplementation on the carcase characteristics of Sasso broilers on day 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
Final live weight, kg	1.67 ± 38.51	1.69 ± 8.48	1.72 ± 49.69	1.67 ± 9.39	1.76 ± 89.24	1.79 ± 25.93	0.487
Dressing, %	78.47 ± 1.09	79.75 ± 0.59	77.53 ± 1.17	78.85 ± 1.62	79.12 ± 1.26	81.37 ± 1.13	0.329
Breast, %	16.91 ± 0.42	17.70 ± 0.87	16.69 ± 0.19	17.90 ± 0.16	17.53 ± 0.57	17.44 ± 0.91	0.693
Drumstick, %	10.92 ± 0.59	10.52 ± 0.47	10.32 ± 0.32	10.61 ± 0.25	11.04 ± 0.34	11.13 ± 0.48	0.723
Wing, %	9.00 ± 0.46	9.55 ± 0.17	9.35 ± 0.40	9.38 ± 0.16	9.28 ± 0.47	9.68 ± 0.31	0.805
Full gizzard, %	1.58 ± 0.09	1.85 ± 0.15	2.05 ± 0.20	2.03 ± 0.14	1.88 ± 0.20	1.94 ± 0.14	0.349
Empty gizzard, %	1.27 ± 0.05	1.47 ± 0.06	1.47 ± 0.07	1.54 ± 0.09	1.51 ± 0.09	1.55 ± 0.11	0.190
GIT, %	6.70 ± 0.24	7.06 ± 0.34	7.00 ± 0.31	7.34 ± 0.17	6.90 ± 0.25	7.76 ± 0.55	0.294
Heart, %	0.58 ± 0.02	0.51 ± 0.02	0.52 ± 0.01	0.55 ± 0.04	0.56 ± 0.03	0.60 ± 0.04	0.267
Liver, %	2.34 ± 0.10^{ab}	1.68 ± 0.03^{c}	2.67 ± 0.27^{a}	2.09 ± 0.12^{bc}	1.93 ± 0.08^{bc}	2.09 ± 0.08^{bc}	<0.001

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal; GIT: Gastrointestinal tract.
All values were expressed as mean ± *SE*; superscripts [a, b, and c] values within the row are significantly different at $p < 0.05$.

relation to T1 across the duodenum (+49.63%), jejunum (+112.64%), and ileum (+254.77%) representing a better gut histomorphology.

Caecal microbial population

On day 56, the caecal microorganism population of broilers treated with *M. maximus* is shown in Table 7. *Escherichia coli* was found in every treatment group. Non-lactose fermenting *E. coli* was not found in T2 and T6 broilers. Throughout all treatments, no *Bacillus* sp. or *Salmonella* were found. The coliform and standard plate counts were almost identical, while T6 demonstrated the highest coliform and standard plate numbers in which could signified a better gut health.

Carcase quality

The effect of *M. maximus* supplementation on the carcase traits of Sasso broilers on day 56 is shown in Table 8. Significant differences ($p < 0.001$) were discovered in liver weight. For the liver parameter, T2 (1.68%) showed the lowest percentage and T3 (2.67%) showed the greatest, although T4 (2.09%), T5 (1.93%), and T6 (2.09%) did not vary significantly ($p > 0.05$) from T2 (1.68%) in this regard. Other parameters, such as final live weight, dressing percentage, breast weight, and other organs did not show significant differences among treatments. Thus, the overall data suggests that increasing levels of *M. maximus* in the diet had a positive impact on liver weight. This effect is further supported by accompanying data on reduced liver enzyme production, highlighting potential benefits in these areas.

Meat quality

Table 9 illustrates the effect of *M. maximus* leaf meal supplementation on Sasso broiler meat quality on day 56. There were significant differences ($p < 0.05$) between treatment groups in drip loss percentages at 24 and 48 h, L^*, and shear force of breast muscle, whereas drumstick revealed significant differences ($p < 0.05$) in cooking loss percentage, drip loss percentage at 48 h, and b^*. Group T2 had the lowest drip loss percentage in both the 24 and 48 h evaluations, but their results were not substantially different ($p > 0.05$) from the other groups supplemented with *M. maximus* leaf meal. Shear force was lowest in T6 breast meat, indicating that T6 possessed greater softness. While T2 showed the lowest drip loss at 48h, it did not significantly differ ($p > 0.05$) to T3–T6. Besides, drumstick meat analysis showed that T6 had the best results as it showed the lowest cooking loss and the highest yellowness (b^*) value which are desired. Overall, T6 supplemented with 5.00 g/kg of *M.*

Table 9. Effect of *M. maximus* leaf meal supplementation on the meat quality of Sasso broilers on day 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
Breast							
Cooking loss, %	21.40 ± 0.69	22.24 ± 0.97	22.65 ± 1.01	22.85 ± 0.53	21.44 ± 0.41	20.16 ± 0.67	0.146
Muscle pH	6.08 ± 0.09	5.92 ± 0.05	6.04 ± 0.02	6.02 ± 0.09	6.08 ± 0.04	6.11 ± 0.07	0.418
Drip loss at 24 h, %	5.90 ± 0.39^{a}	1.94 ± 0.31^{c}	5.04 ± 1.16^{ab}	2.98 ± 0.58^{abc}	2.40 ± 0.36^{bc}	2.34 ± 0.28^{c}	0.001
Drip loss at 48 h, %	6.94 ± 0.57^{a}	3.32 ± 0.36^{b}	5.71 ± 0.92^{ab}	4.36 ± 0.41^{ab}	3.85 ± 0.61^{ab}	3.55 ± 0.60^{b}	0.007
Colour *L** (lightness)	61.03 ± 0.78^{a}	56.03 ± 0.88^{b}	59.62 ± 0.49^{a}	58.06 ± 1.09^{ab}	56.55 ± 0.80^{b}	56.44 ± 0.49^{b}	<0.001
Colour *a** (redness)	9.48 ± 0.15	9.30 ± 0.22	9.71 ± 0.11	9.24 ± 0.68	9.26 ± 0.00	9.12 ± 0.24	0.430
Colour *b** (yellowness)	21.41 ± 0.23	21.34 ± 0.38	22.53 ± 0.40	20.91 ± 0.68	21.17 ± 0.45	21.24 ± 0.40	0.179
Shear force	504.27 ± 18.37^{a}	455.42 ± 13.68^{a}	544.41 ± 50.61^{a}	483.44 ± 10.70^{a}	449.73 ± 10.15^{ab}	349.15 ± 1.37^{b}	<0.001
Drumstick							
Cooking loss, %	23.04 ± 1.01^{bc}	22.94 ± 0.82^{bc}	26.81 ± 0.64^{a}	24.74 ± 0.8^{ab}	24.61 ± 0.53^{ab}	20.93 ± 0.96^{c}	<0.001
Muscle pH	6.53 ± 0.09	5.60 ± 0.14	6.53 ± 0.09	6.62 ± 0.06	6.56 ± 0.12	6.71 ± 0.09	0.828
Drip loss at 24 h, %	1.33 ± 0.27	0.55 ± 0.12	1.07 ± 0.24	1.04 ± 0.19	0.95 ± 0.36	0.76 ± 0.17	0.577
Drip loss at 48 h, %	2.26 ± 0.41^{a}	1.03 ± 0.17^{b}	1.90 ± 0.22^{ab}	1.72 ± 0.26^{ab}	1.70 ± 0.07^{ab}	1.06 ± 0.11^{ab}	0.023
Colour *L** (lightness)	52.97 ± 0.73	51.91 ± 0.96	53.32 ± 0.95	53.64 ± 1.14	52.33 ± 0.52	52.54 ± 0.80	0.797
Colour *a** (redness)	11.10 ± 0.43	12.10 ± 0.54	11.71 ± 0.3	11.32 ± 0.27	11.46 ± 0.37	11.34 ± 0.36	0.594
Colour *b** (yellowness)	16.41 ± 0.37^{b}	17.22 ± 0.26^{ab}	17.36 ± 0.56^{ab}	17.49 ± 0.41^{ab}	17.50 ± 0.23^{ab}	17.92 ± 0.18^{a}	0.028
Shear force	531.71 ± 24.71	573.84 ± 26.21	642.79 ± 13.78	558.39 ± 21.08	475.06 ± 13.61	415.00 ± 11.35	0.207

T1: negative control (basal diet only); T2: positive control (basal diet + 100mg/kg of oxytetracycline); T3: basal diet + 1.25/kg of *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
All values were expressed as mean ± *SE*; superscripts $^{a, b, \text{and } c}$ values within the row are significantly different at $p < 0.05$.

Table 10. Effect of *M. maximus* leaf meal supplementation on the blood biomarkers of Sasso broilers on day 56.

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	*p*-Value
SAA, ng/mL	8.66 ± 0.47^{a}	7.46 ± 0.24^{ab}	8.05 ± 0.28^{ab}	7.93 ± 0.23^{ab}	7.08 ± 0.12^{b}	6.90 ± 0.24^{b}	0.003
AGP, µl/mL	35.81 ± 0.77^{a}	29.09 ± 1.22^{b}	29.27 ± 1.11^{b}	28.91 ± 0.79^{b}	26.46 ± 0.47^{b}	25.74 ± 0.51^{b}	<0.001
CP, µl/mL	10.44 ± 0.21^{a}	9.12 ± 0.25^{bc}	10.32 ± 0.46^{ab}	9.18 ± 0.21^{abc}	8.22 ± 0.29^{c}	7.99 ± 0.23^{c}	<0.001
Corticosterone, pg/mL	195.73 ± 5.70^{a}	193.45 ± 6.84^{a}	190.57 ± 6.95^{a}	168.93 ± 5.28^{a}	168.33 ± 10.28^{a}	135.15 ± 5.10^{b}	<0.001
HSP 70, pg/mL	191.13 ± 4.28^{a}	151.03 ± 4.50^{b}	157.00 ± 8.26^{b}	151.00 ± 3.88^{b}	145.03 ± 6.59^{b}	134.03 ± 7.24^{b}	<0.001

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 *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
Note: All values were expressed as mean ± *SE*; $^{a, b, \text{and } c}$ values with superscript within the row are significantly different at $p < 0.05$.

maximus leaf meal presented a better breast and drumstick meat quality as compared to the other treatments.

Blood biomarkers

Table 10 shows the effect of *M. maximus* leaf meal supplementation on blood biomarkers in Sasso broilers on day 56. There were significant differences in all parameters examined ($p < 0.05$) across treatment groups, with the T6 group having the lowest values for all blood biomarker markers assessed. However, there were no statistically significant variations ($p > 0.05$) between T2 and T6 for SAA, AGP, and HSP70. Furthermore, there were no significant differences ($p > 0.05$) in CP between T2 and T3 to T6, while T4 to T6 differed considerably ($p < 0.05$) from T3. Corticosterone levels were the lowest in T6 (135.13 pg/ml) and substantially different from T1 to T5 (168.33–195.73 pg/ml) broilers. T6 reduced SAA, AGP, CP, corticosterone, and HSP 70 levels by 20.32, 28.12, 23.47, 30.96, and 29.87%, respectively, as compared to T1. Therefore, it can be concluded that T6 broilers were the less stressful in which performed better than the other treatment groups.

Blood biochemistry

The impact of *M. maximus* leaf meal supplementation on the blood biochemistry of Sasso broilers on day 56 is shown in Table 11. There were significant changes ($p < 0.05$) in all liver functions examined, including ALP, AST, GGT, and ALT. T6 had the lowest levels of ALP, AST, GGT, and ALT, with values of 129.89, 162.21, 8.86, and 1.24 U/L, respectively. However, AST levels between T6 (162.21 U/L) and T2 (172.65 U/L) did not differ substantially ($p > 0.05$). When the lipid profile was examined, significant variations ($p < 0.05$) were detected across all treatments. Despite T2 having the lowest cholesterol levels among all treatments, the cholesterol levels of T6 (4.01 mmol/L) and T2 (3.94 mmol/L) did not significantly different ($p > 0.05$)

Table 11. Effect of *M. maximus* leaf meal supplementation on the blood biochemistry of Sasso broilers on day 56.

		Treatments						
Parameters	Normal range	T1	T2	T3	T4	T5	T6	*p*-Value
Liver functions								
ALP, U/L	55–2345	174.19 ± 3.20^{b}	187.87 ± 5.27^{ab}	204.29 ± 5.63^{a}	174.05 ± 2.55^{b}	140.85 ± 4.53^{c}	129.89 ± 1.14^{c}	<0.001
AST, U/L	98–294	282.66 ± 13.19^{a}	172.65 ± 5.15^{cd}	223.93 ± 3.28^{b}	215.54 ± 5.92^{b}	201.38 ± 4.41^{bc}	162.21 ± 3.21^{d}	<0.001
GGT, U/L	8–48	14.61 ± 0.32^{bc}	$12.88 \pm 0.35\ c^{d}$	22.7 ± 0.59^{a}	14.90 ± 0.15^{b}	12.33 ± 0.53^{d}	8.86 ± 0.27^{e}	<0.001
ALT, U/L	<11	3.47 ± 0.07^{b}	2.50 ± 0.09^{c}	3.95 ± 0.04^{a}	3.67 ± 0.09^{b}	1.60 ± 0.06^{d}	1.24 ± 0.05^{e}	<0.001
Lipid profile								
Cholesterol, mmol/L	1.09–5.65	4.56 ± 0.15^{a}	3.94 ± 0.09^{b}	4.13 ± 0.09^{ab}	4.04 ± 0.07^{ab}	4.03 ± 0.11^{ab}	4.01 ± 0.16^{b}	0.017
TG, mmol/L	N/A	0.51 ± 0.02^{bc}	0.46 ± 0.01^{c}	0.59 ± 0.02^{a}	0.53 ± 0.02^{ab}	0.51 ± 0.01^{bc}	0.50 ± 0.02^{bc}	0.001
LDL, mmol/L	N/A	0.55 ± 0.02^{b}	0.37 ± 0.01^{d}	0.68 ± 0.02^{a}	0.57 ± 0.01^{b}	0.51 ± 0.01^{bc}	0.48 ± 0.02^{c}	<0.001
HDL, mmol/L	N/A	3.00 ± 0.16^{b}	3.13 ± 0.05^{ab}	3.50 ± 0.10^{ab}	3.59 ± 0.06^{ab}	3.61 ± 0.22^{a}	3.64 ± 0.12^{a}	0.008
VLDL, mmol/L	N/A	0.10 ± 0.00^{bc}	0.09 ± 0.00^{c}	0.12 ± 0.00^{a}	0.11 ± 0.00^{ab}	0.10 ± 0.00^{bc}	0.10 ± 0.00^{bc}	0.001

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 *M. maximus* grass meal; T4: basal diet + 2.5g/kg of *M. maximus* grass meal; T5: basal diet + 3.75g/kg of *M. maximus* grass meal; T6: basal diet + 5.00g/kg of *M. maximus* grass meal.
Note: All values were expressed as mean ± *SE*; $^{a, b, c, d, \text{and } e}$ values with superscript within the row are significantly different at $p < 0.05$.

from each other. Similarly, triglyceride levels in T6 and T2 were 0.46 and 0.50 mmol/L, respectively with no significant differences ($p > 0.05$). HDL levels were the highest in T6 (3.64 mmol/L), though not significantly different ($p > 0.05$) from T2 (3.13 mmol/L). LDL and VLDL were lowest in T2 at 0.37 and 0.09 mmol/L, respectively, but VLDL did not significantly differ ($p > 0.05$) from T6. Overall, this indicates that the supplementation of *M. maximus* at 5.00 g/kg of feed had similar effects to antibiotic treated group.

Discussion

Growth performance

T6 broilers supplemented with 5.00 g/kg of *M. maximus* displayed a better growth performance overall possibly due to the presence of phytocompounds in the grass meal, particularly alkaloids. Quantitative analysis of *M. maximus* used in this experiment confirmed that alkaloids were the most prevalent phytocompound present in Guinea grass as per reported by previous studies (Kanife and Doherty 2012; Abu Hafsa and Hassan 2021). The results presented show that increasing inclusion levels of the grass meal increased feed intake of broilers possibly due to improved feed status, mainly flavour and palatability (Jamroz et al. 2003; Windisch et al. 2008; Valenzuela-Grijalva et al. 2017; Alghirani et al. 2022a). Similar results were found in an experiment where dried guava leaf meals increased final body weight and average daily BWG correspondingly to those fed with higher levels of leaf meals. Rahimi et al. (2011) supports that notion by theorising that the addition of phyhtocompounds enhanced FI and FCR by stimulating appetite and increasing digestive secretion, thus improving nutrient digestion and easing absorption, as evidenced by improved BWG and final body weight in T6. Another possible method *M. maximus* might enhance broilers' growth performance is through gut fermentation modulation *via* antimicrobial effects (Valenzuela-Grijalva et al. 2017). As the presence of various physiologically active phytocompounds in *M. maximus*, such as alkaloids, tannins, saponins, and flavonoids may contribute to antifungal and antimicrobial actions against harmful pathogens in the gut which promote the growth status of broiler chickens in this experiment (Kanife and Doherty 2012). Additionally, the improved broilers' growth performance of broilers could also be credited to the anti-inflammatory and antimicrobial qualities of *M. maximus* grass meal akin to dried guava leaf meal due to the presence of several phytocompounds (Abang et al. 2023). Sugiharto et al. (2019) even proposed that alkaloid-rich leaf meals might be used as antibiotic substitutes, which is reinforced by the findings of the current experiment, which revealed no significant differences in cumulative FCR between the positive control and T6 broilers.

Apparent ileal digestibility

The current experiment revealed a decrease in DM and ash digestibility as grass inclusion amount increased. This might be related to the inclusion of insoluble fibres in broiler diets, which results in a shorter transit time of the digesta in the small intestine (Amerah et al. 2009). Backing up this theory is a review by Hetland et al. in 2004. These authors presented that insoluble fibre alters gut function and modulates nutrient digestion in the intestine. Thus, digestion of starch is higher and digesta passage rate is accelerated when a moderate amount of insoluble fibre is present in the diet (Hetland and Svihus 2001). Due to this accelerated intestinal transit, there is less risk for colonisation of pathogenic bacteria in the gut. Aside from that, the present

experiment also showed the highest digestibility for CP, CF, and EE. Dietary fibre has been known to improve nutritional digestibility by increasing pancreatic enzymatic activity and reversing peristalsis (Hetland et al. 2003; Amerah et al. 2009; Mateos et al. 2012). This enables bile salts to be transported to the gizzard by reverse peristalsis and combined with gastric secretions. Thus, fat emulsification is improved by lowering the ability of fat droplets to coat nutrients, allowing nutrients to be more easily digested and absorbed (Hetland et al. 2004). Several other studies backed these findings as feed intake and digestibility have been shown to improve with the addition of grass or leaf meals as it increases the endogenous secretion production in the small intestine, liver, and pancreas (Hetland et al. 2003; Amerah et al. 2009; Mateos et al. 2012). Results from the present study supports this statement as feed intake increases with the addition of *M. maximus*. Alkaloids which are abundantly present in *M. maximus* may also be responsible for the improvements in digestibility as they have antifungal and antimicrobial actions against pathogenic bacteria in the gut (Kanife and Doherty 2012). Supporting that claim, *Sauropus androgynus* and *Polyalthia longifolia* are other examples of alkaloid-rich leaf meals may be utilised as antibiotic replacements due to their favourable effects to broilers' growth (Sugiharto et al. 2019). Therefore, the mode of mechanism for improvements in digestibility with the addition of *M. maximus* may be attributed to the antimicrobial effects that alter gut fermentation functions, as supported by enhanced growth performance.

Gut histomorphology

T6 in the current research showed the highest VH:CD throughout the small intestine. Previous studies utilising various leaf meals of differing inclusion levels have also shown overall positive effects on the VH:CD ratio (Basit et al. 2020; Abdelatty et al. 2021; Ogwuegbu et al. 2021; Saharan et al. 2022). On the other hand, there were some other studies which found insignificant or detrimental effects on the gut with the inclusion of plant-based meals in broiler diets (Saadatmand et al. 2019; Sugiharto et al. 2020). Nonetheless, due to the various plants utilised in each experiment, it is important to determine the fibre content and type as well as the appropriate inclusion levels to fully gain its benefits. The action of several phytocompounds present in *M. maximus*, notably alkaloids (which are the most prevalent) may be related to the increased villus height and decreased crypt depth. Phytocompounds, such as alkaloids, tannins, saponins, and flavonoids have antimicrobial effects that are beneficial to the gut of broilers. Thus, making alkaloid-rich plants a suitable candidate for antibiotic replacements (Kanife and Doherty 2012; Sugiharto et al. 2019). Earlier experiments utilising extracts from *A. indica* which contain alkaloids and saponins have also been shown to reduce the oocyte counts of *Eimeria*, a common enteric parasite that causes coccidiosis in broilers (Onyiche et al. 2021). A reduction of pathogenic bacteria in the intestine reduces the risk of intestine-related diseases and, as a result, foster a healthier gut (Chrubasik et al. 2005; Pohl et al. 2012). Furthermore, less pathogenic microorganisms mean less intestine damage and the need of energy for lumen restoration (Hidayati et al. 2022). Supplementation of *M. maximus* leaf meal significantly enhanced VH:CD in Sasso broilers, particularly in the ileum, with improvements of 255% when comparing T1 and T6. This translates to greater nutrient absorption and, as a result, better growth performance, as proven by this experiment.

Caecal microbial population

The present experiment did not identify any *Salmonella* in the caecal microbiota. Alkaloids are naturally present and found in plants as water-soluble salts of organic acids. Because of the presence of one or more nitrogen atoms in their chemical structure, they have alkaline characteristics. Alkaloids can be further classified based on their chemical structures, biological activity, biosynthesis pathway, and complexity into heterocyclic and non-heterocyclic alkaloids. Beneficial effects on the growth of broilers have also been observed when broiler chickens are fed with alkaloid-rich leaf meals, such as *Sauropus androgynus* and *Polyalthia longifolia*, which may be employed as antibiotic replacements (Sugiharto et al. 2019). The antibacterial action of alkaloids is known to inhibit topoisomerase and disrupt DNA synthesis, lowering the quantity of harmful bacteria in the gut (Suresh et al. 2018). Additionally, chelerythrine a class of alkaloids, inhibits protein kinase C, which is implicated in the Toll-like receptor 2 pathway, which recognises microorganisms and controls immune responses. Therefore, various subclasses of isoquinoline alkaloids have been shown to enhance the intestinal environment, subsequently improving nutrition utilisation through various pathways (Rundle et al. 2023). By reducing the number of pathogenic microbes in the gut, intestinal absorptive cells are able to grow, promoting bird development. T6 which had the best growth performance in the current experiment also

had no NFL *E. coli* detected, similar to T2 (antibiotic-treated group). Hence, it can be inferred that the impact of *M. maximus* grass meal on the caecal microbial population is likely linked to the elevated alkaloid content found in the grass.

Carcase quality

The application of *M. maximus* in broiler feed did not show much effects on the carcase traits of Sasso broilers. Significant differences were only found in the liver weight percentage, with T3 displaying the highest liver relative weight. In a previous experiment using Arbour Acres broilers and Mucuna leaf meals, Oloruntola et al. (2022) found that increasing the dose of Mucuna leaf meal supplementation resulted in a greater dressing percentage. Mucuna leaf meals are reported to be high in alkaloids, flavonoids, saponins, tannins, terpenoids, and other phytochemicals. Additionally, Faria et al. (2011) also found better carcase yield with the addition of cassava leaf meal. Phytocompounds can have anabolic effects *via* modifying animal metabolism, mimicking β-adrenergic agonist chemicals, or boosting plasma norepinephrine levels by inhibiting catechol-o-methyltransferase. In summary, these two approaches alter the animal's metabolism by increasing protein synthesis and lipolysis while lowering lipogenesis, hence promoting muscle tissue growth (Valenzuela-Grijalva et al. 2017; Oloruntola et al. 2022). This could explain the significantly lower relative liver weight in T4-T6 in comparison to the negative control as reduced fatty acid storage in the liver may lower liver weight to some extent, though not definite (Zaefarian et al. 2019). Nevertheless, the liver weight percentage in this study was still within the normal weight range as an extreme increase in liver weight would indicate a surge of detoxification activity in the hepatocytes (Prihambodo et al. 2021). On the other hand, several other studies found opposing effects, in which the addition of various leaf meals lowered carcase quality, which reflected a decline in production performances (Donkoh et al. 2002; Kagya-Agyemang et al. 2007; Zaefarian et al. 2019). In short, the data indicates that the inclusion of *M. maximus* into broiler diets does not adversely affect broiler development.

Meat quality

Based on the results from this experiment it was demonstrated that alkaloids are responsible for the observed reduction in drip loss and cooking loss, as incremental levels of *M. maximus* leaf supplementation produced better results. Oxidative damage to skeletal muscle tissues diminishes meat quality. Rapid post-mortem metabolism which affects pH and protein denaturation in the muscle results in pale, soft, and exudative meat (PSE). This relates to acidosis of the chicken meat which occurs when anaerobic glycolysis in the muscle is activated, causing more $H+$ and lactic acid to build, resulting in a decrease of muscle pH. A reduction in muscle pH causes limited water holding capacity, which leads to PSE (Van Laack et al. 2000; Strasburg and Chiang 2009). A study on isoquinoline alkaloids demonstrated their beneficial effects on oxidative damage and protein catabolism, showing improvements in muscle conditions (Kikusato et al. 2021). Muscle degradation may occur as a metabolic reaction to recruit amino acids from the skeletal muscle to supply energy substrates or to synthesise peptides and proteins, such as acute phase proteins. In response to physiological and pathological stimuli, corticosterone is released, inducing proteolysis *via* the intracellular signalling system. Findings from this study showed that corticosterone secretions were suppressed and plasma total protein content were restored in broilers supplemented with isoquinoline alkaloids under stressful conditions (Kikusato et al. 2021). Additionally, plasma concentration of uric acid, an endogenous antioxidant, was increased with the administration of corticosterone (Liu et al. 2014). Furthermore, the addition of grass meal could have contributed to the slightly darker and more yellow meat due to the presence of xanthophyll and chlorophyll, enhancing the overall appearance and making the meat a more desirable en-product (Hu et al. 2012; Manyelo et al. 2022). The study discovered that isoquinoline alkaloids may prevent oxidative damage since plasma uric acid levels were lowered after administration. Thus, the improved meat quality in *M. maximus* supplemented chickens could be attributed to high levels of alkaloids present in the grass.

Blood biomarkers

Various studies have reported that alkaloid derivatives and/or plants high in alkaloids have been utilised as an AGP substitute in poultry and livestock production due to their positive impacts on production performances (Vacek et al. 2010; Kantas et al. 2015). Liu et al. (2022) suggested that supplementation of *Macleaya cordata* extract which are high in isoquinoline alkaloids reduced inflammation marked by decreased levels of ALT, interleukin-1β, and interleukin-6. This

shows hepatoprotective effects of alkaloids through inhibition of TLR4, NF-κB, myeloid differentiation factor 88 (MyD88), Nrf-2, and other related inflammatory signalling pathways (Hu et al. 2020). Heat-stressed broilers treated with isoquinoline alkaloids also showed improved growth performance, which was linked to reduced oxidative damage, protein catabolism, intestinal barrier function, and inflammation (Kikusato et al. 2021). These findings align with the results from the current experiment, wherein Sasso broilers supplemented with *M. maximus* exhibited blood biomarker levels that were either similar to or significantly lower than those of the group treated with antibiotics.

Earlier models have suggested a significant role for elevated HSP70 levels in the chaperone machinery, influencing the selection of proteins affected by oxidative or other toxic damage. Consequently, reduced levels of HSP70 imply a lesser extent of damage (Surai and Kochish 2017; Alghirani et al. 2022b). As observed, T6 broilers from this study revealed the lowest values for the measured blood biomarkers, indicating that the observed positive effects could be attributed to elevated levels of alkaloids.

Blood biochemistry

The present study demonstrated that supplementing *M. maximus* leaf meal at the highest level of 5.00 g/kg of feed improved liver function by reducing liver enzyme levels in the bloodstream. The outcomes of this investigation could be linked to the elevated concentration of alkaloids found in *M. maximus* leaves, aligning with prior studies that have indicated positive effects with the use of alkaloid-rich supplementation in broilers. For example, Liu et al. (2022) observed a decrease in serum ALT with dietary *Macleaya cordata* extract, indicating reduced hepatocyte damage; similar to the results shown in the present study. However, in contrast to the current experiment, *M. cordata* extract notably increased serum levels of TG, HDL, and LDL. The authors attributed this to heightened metabolism during the growth and development of broiler chickens, contributing to enhanced growth performance (Khadem et al. 2014). Nevertheless, results from the present investigation were in line with Tokofai et al. (2020) which showed lowered total cholesterol and LDL levels with the supplementation of *Vernonia amygdalina* leaf meal. A plausible explanation for the mechanism of phytobiotics with hypocholesterolaemic effects lies in the volatile oils present in plants. These oils may hinder the function of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), a liver enzyme that regulates cholesterol production, consequently leading to a reduction in blood cholesterol levels (Fujioka et al. 2003). Thus, the mixed results of lipid profile effects from this study to prior experiments could be due to varying levels and interactions of bioavailable compounds of volatile oil in each plant source.

Conclusions

The present experiment revealed that the highest supplementation level of 5.00 g/kg of *M. maximus* showcased the most favourable overall growth performance. Despite exhibiting higher feed intake, the improved final body weight and body weight gain translated into a lower cumulative FCR in both starter and finisher phases, indicative of efficient growth performance. Furthermore, digestibility in this experiment was improved. These outcomes may be attributed to the addition of *M. maximus*, which augments endogenous secretion production. Additionally, T6 exhibited the most favourable villi height to crypt depth ratio throughout the small intestine, suggesting enhanced gut health and facilitating more efficient digestion and absorption. The abundance of alkaloids in *M. maximus* may contribute to this, given their antifungal and antimicrobial properties. This hypothesis is supported by the caecal microbial population results, wherein T6 exhibited comparable outcomes to the antibiotic group, with *Salmonella* and other pathogenic bacteria not identified besides *E. coli*. Correspondingly, T6 displayed improved carcase characteristics, with significant lower liver weight values. The reduced liver weight could stem from diminished production of liver enzymes and APP, as suggested by the blood biomarkers and biochemistry results. Furthermore, T6 exhibited superior meat quality, characterised by lower cooking loss, drip loss, lightness, and shear force values. T6's meat also displayed the highest yellowness, a desirable trait in coloured-broiler meat. Lastly, T6 enhanced the lipid profile of broilers by lowering cholesterol, TG, and LDL levels while increasing HDL levels. These findings suggest that *M. maximus* holds promise as a feed supplement due to its high alkaloid content, which enhances animal performance and product quality. Therefore, *M. maximus* could potentially be used as a feed additive in broiler production to replace antibiotics.

Acknowledgements

The authors would like to thank the staff of the Department of Animal Science, Faculty of Agriculture, UPM for their technical assistant.

Ethical approval

All procedures involving animal care, handling, and sampling were conducted in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC) of Universitiy of Putra Malaysia prior to the start of the research (Approval number: UPM/IACUC/AUP-R047/2022).

Author contributions

Yee Lyn Ong: investigation, writing–original draft. Eric Lim Teik Chung: funding acquisition, resources, supervision, conceptualisation, methodology, investigation, writing–review and editing. Nazri Nayan: investigation, writing–review and editing. Muhamad Faris Ab Aziz, Faez Firdaus Abdullah Jesse, and Awis Qurni Sazili: writing–review and editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The project was funded by the Putra Grant-Impactful Putra Grant (GP-GPB), Universiti Putra Malaysia (Grant no: 9713700).

Data availability statement

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

References

Abang FBP, Echeonwu IE, Amu MU. 2023. Effect of graded levels of guava (*Psidium guajava* L.) leaf meal on productive performance and meat organoleptic properties of chicken. Online J Anim Feed Res. 13(1):73–78. doi: 10.51227/ojafr.2023.12.

Abdelatty AM, Mandouh MI, Mohamed SA, Busato S, Badr OAM, Bionaz M, Elolimy AA, Moustafa MMA, Farid OAA, Al-Mokaddem AK. 2021. Azolla leaf meal at 5% of the diet improves growth performance, intestinal morphology and P70S6K1 activation, and affects cecal microbiota in broiler chicken. Animal. 15(10):100362. doi: 10.1016/j.animal.2021.100362.

Abu Hafsa S, Hassan A. 2021. Partial berseem hay replacement by *Panicum maximum* grass on caecal fermentation activity, performance, and carcass characteristics of growing rabbits. Livest Sci. 250:104593. doi: 10.1016/j.livsci.2021.104593.

Alghirani MM, Chung ELT, Jesse FFA, Sazili AQ, Loh TC. 2021. Could phytobiotics replace antibiotics as feed additives to stimulate production performance and health status in poultry? An overview. J Adv Vet Res. 11(4):254–265. https://advetresearch.com/index.php/AVR/article/view/810.

Alghirani MM, Chung ELT, Kassim NA, Ong YL, Jesse FFA, Sazili AQ, Loh TC. 2022a. Blood biochemistry and stress biomarkers of broiler chickens supplemented with different levels of *Yucca schidigera* saponins reared under tropical conditions. Vet Integr Sci. 21(1):1–15. doi: 10.12982/VIS.2023.001.

Alghirani MM, Chung ELT, Kassim NA, Ong YL, Jesse FFA, Sazili AQ, Loh TC. 2022b. Effect of *Brachiaria decumbens* as a novel supplementation on the production performance of broiler chickens. Trop Anim Health Prod. 54(6):386. doi: 10.1007/s11250-022-03384-4.

Alghirani MM, Chung ELT, Sabri DSM, Tahir MNJM, Kassim NA, Kamalludin MH, Nayan N, Jesse FFA, Sazili AQ, Loh TC. 2021. Can *Yucca schidigera* be used to enhance the growth performance, nutrient digestibility, gut histomorphology, cecal microflora, carcass characteristic, and meat quality of commercial broilers raised under tropical conditions? Animals. 11(8):2276. doi: 10.3390/ani11082276.

Aljumaah MR, Alkhulaifi MM, Aljumaah RS, Abudabos AM, Abdullatif AA, Suliman GM, Al-Ghadi MQ, Stanley D. 2020. Influence of sanguinarine-based phytobiotic supplementation on post necrotic enteritis challenge recovery. Heliyon. 6(11):e05361. doi: 10.1016/j.heliyon.2020.e05361.

Amerah AM, Ravindran V, Lentle RG. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br Poult Sci. 50(3):366–375. doi: 10.1080/00071660902865901.

Aswanimiyuni A, Noor M, Haryani H, Norfadzrin F, Nurzillah M. 2018. A comparison of feed intake and growth performances of goats fed Guinea grass and Napier grass. Malays J Vet Res. 9(2):13–18. https://api.semanticscholar.org/CorpusID:235176201.

Basit MA, Kadir AA, Loh TC, Abdul Aziz S, Salleh A, Zakaria ZA, Banke Idris S. 2020. Comparative efficacy of selected phytobiotics with halquinol and tetracycline on gut morphology, ileal digestibility, cecal microbiota composition and growth performance in broiler chickens. Animals. 10(11):2150. doi: 10.3390/Ani10112150.

Chrubasik S, Pittler MH, Roufogalis BD. 2005. *Zingiberis rhizoma*: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 12(9):684–701. doi: 10.1016/j.phymed.2004.07.009.

Chung ELT, Alghirani MM, Kamalludin MH, Nayan N, Jesse FFA, Wei OTA, Ma Stephen FMH, Reduan MFH, Loh TC. 2021. Do different vaccination regimes affect the growth performance, immune status, carcass characteristics and meat quality of broilers? Br Poult Sci. 62(1):32–37. doi: 10.1080/00071668.2020.181732.

Chung ELT, Nayan N, Kamalludin MH, Alghirani MM, Jesse FFA, Kassim NA, Azizi A, Reduan MFH, Loh TC. 2020. The effects of alkaline water and rainwater on the production and health performance of commercial broilers under tropical conditions. Thai J Vet Med. 50(1):65–73. doi: 10.56808/2985-1130.3076.

Chung ELT, Predith M, Nobilly F, Samsudin AA, Jesse FFA, Loh TC. 2018. Can treatment of *Brachiaria decumbens* (signal grass) improve its utilisation in the diet in small ruminants?—A review. Trop Anim Health Prod. 50(8):1727–1732. doi: 10.1007/s11250-018-1641-4.

Donkoh A, Atuahene CC, Anang DM, Badu-Botah EK, Boakye KT. 2002. Response of broiler chickens to the dietary inclusion of *Chromolaena odorata* leaf meal. J Anim Feed Sci. 11(2):309–320. doi: 10.22358/jafs/67815/2002.

Faria PB, Vieira JO, Silva JN, Rodrigues AQ, Souza XR, Santos FR, Pereira AA. 2011. Performance and carcass characteristics of free-range broiler chickens fed diets containing alternative feedstuffs. Rev Bras Cienc Avic. 13(3):211–216. doi: 10.1590/S1516-635X2011000300009.

Frieri M, Kumar K, Boutin A. 2017. Antibiotic resistance. J Infect Public Health. 10(4):369–378. doi: 10.1016/j.jiph.2016.08.007.

Fujioka T, Kondou T, Fukuhara A, Tounou S, Mine M, Mataki N, Hanada K, Ozaka M, Mitani K, Nakaya T, et al. 2003. Efficacy of glycyrrhizin suppository for treatment of chronic hepatitis C: a pilot study. Hepatol Res. 26(1):10–14. doi: 10.1016/S1386-6346(02)00332-7.

Genetics H. 2022. Sasso management guide rural poultry 2022.

Grashorn M. 2010. Use of phytobiotics in broiler nutrition–an alternative to infeed antibiotics? J Anim Feed Sci. 19(3):338–347. doi: 10.22358/jafs/66297/2010.

Hartinger K, Fröschl K, Ebbing M, Bruschek-Pfleger B, Schedle K, Schwarz C, Gierus M. 2022. Suitability of *Hermetia illucens* larvae meal and fat in broiler diets: effects on animal performance, apparent ileal digestibility, gut histology, and microbial metabolites. J Anim Sci Biotech. 30(50):1–16. doi: 10.1186/s40104-022-00701-7.

Hetland H, Choct M, Svihus B. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poult Sci J. 60(4):415–422. doi: 10.1079/WPS200325.

Hetland H, Svihus B, Krogdahl Å. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br Poult Sci. 44(2):275–282. doi: 10.1080/0007166031000124595.

Hetland H, Svihus B. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br Poult Sci. 42(3):354–361. doi: 10.1080/00071660120055331.

Hidayati NA, Zuprizal Z, Kustantinah K, Indarto RE, Suryanto E, Dono ND. 2022. Intestinal health in broiler chickens treated with nanoencapsulation of *Terminalia catappa* leaf extract as an antibacterial agent. Trop Anim Sci J. 45(4):443–450. doi: 10.5398/tasj.2022.45.4.443.

Horwistz W. 2010. Official methods of analysis. 18th ed. Rockville (MD): AOAC International.

Hu CH, Wang DG, Pan HY, Zheng WB, Zuo AY, Liu JX. 2012. Effects of broccoli stem and leaf meal on broiler performance, skin pigmentation, antioxidant function, and meat quality. Poult Sci. 91(9):2229–2234. doi: 10.3382/ps.2012-02142.

Hu N, Guo C, Dai X, Wang C, Gong L, Yu L, Peng C, Li Y. 2020. Forsythiae fructuse water extract attenuates liver fibrosis via TLR4/MyD88/NF-κB and TGF-β/smads signaling pathways. J Ethnopharmacol. 262:113275. doi: 10.1016/j.jep.2020.113275.

Huang Q, Liu X, Zhao G, Hu T, Wang Y. 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim Nutr. 4(2):137–150. doi: 10.1016/j.aninu.2017.09.004.

Jack I, Clark P, Ndukwe G. 2020. Evaluation of phytochemical, antimicrobial and antioxidants of *Pennisetum purpureum* (Schumach) extracts. Chem Sci Int J. 29(4):1–14. doi: 10.9734/CSJI/2020/v29i430170.

Jamroz D, Orda J, Kamel C, Wiliczkiewicz A, Wertelecki T, Skorupińska J. 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. J Anim Feed Sci. 12(3):583–596. doi: 10.22358/jafs/67752/2003.

Kagya-Agyemang JK, Takyi-Boampong G, Adjei M, Karikari-Bonsu FR. 2007. A note on the effect of *Gliricidia sepium* leaf meal on the growth performance and carcass characteristics of broiler chickens. J Anim Feed Sci. 16:104–108. doi: 10.22358/jafs/66731/2007.

Kanife U, Doherty F. 2012. Phytochemical composition and antifungal properties of leaf, stem, and florets of *Panicum maximum* Jacq, (Poaceae). Int J Biol. 4(2):64–69. doi: 10.5539/ijb.v4n2P64.

Kantas D, Papatsiros VG, Tassis PD, Athanasiou LV, Tzika ED. 2015. Effect of a natural feed additive (*Macleaya cordata*), containing sanguinarine, on the performance and health status of weaning pigs. Anim Sci J. 86(1):92–98. doi: 10.1111/asj.12240.

Khadem A, Soler L, Everaert N, Niewold TA. 2014. Growth promotion in broilers by both oxytetracycline and *Macleaya cordata* extract is based on their anti-inflammatory properties. Br J Nutr. 112(7):1110–1118. doi: 10.1017/S0007114514001871.

Kikusato M, Xue G, Pastor A, Niewold T, Toyomizu M. 2021. Effects of plant-derived isoquinoline alkaloids on growth performance and intestinal function of broiler chickens under heat stress. Poult Sci. 100(2):957–963. doi: 10.1016/j.psj.2020.11.050.

Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. 2005. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. J Agric Food Chem. 53(5):1408–1416. doi: 10.1021/jf048429n.

Liu LL, He JH, Xie HB, Yang YS, Li JC, Zou Y. 2014. Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens. Poult Sci. 93(1):54–62. doi: 10.3382/ps.2013-03423.

Liu Y, Li Y, Niu J, Liu H, Jiao N, Huang L, Jiang S, Yan L, Yang W. 2022. Effects of dietary *Macleaya cordata* extract containing isoquinoline alkaloids supplementation as an alternative to antibiotics in the diets on growth performance and liver health of broiler chickens. Front Vet Sci. 9:950174. doi: 10.3389/fvets.2022.950174.

Manyelo TG, Sebola NA, Ng'ambi JW, Weeks W, Mabelebele M. 2022. The influence of different amaranth leaf meal inclusion levels on performance, blood profiles, and gut organ characteristics of Ross 308 broiler chickens. Front Vet Sci. 9:869149. doi: 10.3389/fvets.2022.869149.

Mateos GG, Jiménez-Moreno E, Serrano MP, Lázaro RP. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J Appl Poult Res. 21(1):156–174. doi: 10.3390/ani11020461.

Ni H, Martínez Y, Guan G, Rodríguez R, Más D, Peng H, Valdivié Navarro M, Liu G. 2016. Analysis of the impact of isoquinoline alkaloids, derived from *Macleaya cordata* extract, on the development and innate immune response in swine and poultry. Biomed Res Int. 2016:1352146–1352147. doi: 10.1155/2016/1352146.

Ogwuegbu MC, Ani AO, Oyeagu CE, Osita CO, Oyeagu U, Ugwuoke WI, Lewu FB. 2021. Sodium butyrate and rosemary leaf meal inclusion in broiler diet: effects on gut micro-floral, growth performance, ileum, jejunum and

duodenal histological traits. Adv Anim Vet Sci. 9(7):1095–1112. doi: 10.17582/journal.aavs/2021/9.7.1095.1112.

Oloruntola OD, Ayodele SO, Omoniyi IS, Adeyeye SA, Adegbeye MJ. 2022. The effect of dietary supplementation of Mucuna leaf meal on the growth performance, blood parameters, and carcass quality of broiler. Acta Sci Anim Sci. 44:e55362. doi: 10.4025/actascianimsci.v44i1.55362.

Onyeonagu C, Obute P, Eze S. 2013. Seasonal variation in the anti-nutrient and mineral components of some forage legumes and grasses. Afr J Biotechnol. 12(2):142–149. doi: 10.5897/AJB11.1359.

Onyeonagu C, Ukwueze C. 2012. Anti-nutrient components of guinea grass (*Panicum maximum*) under different nitrogen fertilizer application rates and cutting management. Afr J Biotechnol. 11(9):2236–2240. doi: 10.5897/AJB11.748.

Onyiche T, Gotep J, Tanko J, Ochigbo G, Ozoani H, Viyoff VZ, Dogonyaro BB, Makoshi MS, Kinjir H, Thekisoe O, et al. 2021. *Azadirachta indica* aqueous leaf extracts ameliorates coccidiosis in broiler chickens experimentally infected with *Eimeria* oocysts. Sci Afr. 13: e 00851. doi: 10.1016/j.sciaf.2021.e00851.

Park J, Kim I. 2020. Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. Poult Sci. 99(1):463–470. doi: 10.3382/ps/pez533.

Pohl SA, Caldwell DJ, Lee JT, Coppedge JR, Dunn-Horrocks SL, Stringfellow KD, Jessen K, Farnell MB. 2012. Effects of dietary calcium formate inclusion on broiler performance, skeletal development, and gut maturation. J Appl Poult Res. 21(2):311–317. doi: 10.3382/japr.2011-00400.

Prihambodo TR, Sholikin MM, Qomariyah N, Jayanegara A, Batubara I, Utomo DB, Nahrowi N. 2021. Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. Anim Biosci. 34(3):434–442. doi: 10.5713/ajas.20.0379.

Rahimi S, Zadeh Z, Karimi Torshizi M, Omidbaigi R, Rokni H. 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. J Agri Sci Tech. 13(4):527–539. doi: 20.1001.1.16807073.2011.13.4.6.5.

Rundle CM, Artuso-Ponte V, Stein HH. 2023. Effects of isoquinoline alkaloids on apparent ileal digestibility of amino acids, crude protein, starch, and acid hydrolyzed ether extract and apparent total tract digestibility of energy and crude protein by growing and finishing pigs fed corn-soybean meal diets. Anim Feed Sci Tech. 299:115623. doi: 10.1016/j.anifeedsci.2023.115623.

Saadatmand N, Toghyani M, Gheisari A. 2019. Effects of dietary fiber and threonine on performance, intestinal morphology and immune responses in broiler chickens. Anim Nutr. 5(3):248–255. doi: 10.1016/J.Aninu.2019.06.001.

Saharan V, Pathak AK, Sharma RK, Sharma N, Sarma K, Kumar H. 2022. Influence of *Psidium guajava* leaf meal supplementation on growth performance, nutrient utilization, intestinal micrometry, caecal fermentative metabolites and microbiota of broiler chickens. Anim Nutri Feed Tech. 22(2):367–382. doi: 10.51227/ojafr.2023.12.

Selaledi LA, Hassan ZM, Manyelo TG, Mabelebele M. 2020. The current status of the alternative use to antibiotics in poultry production: an African perspective. Antibiotics. 9(9):594. doi: 10.3390/antibiotics9090594.

Soti P, Thomas V. 2022. Review of the invasive forage grass, guinea grass (*Megathyrsus maximus*): ecology and potential impacts in arid and semi-arid regions. Weed Res. 62(1):68–74. doi: 10.1111/wre.12512.

Strasburg GM, Chiang W. 2009. Pale, soft, exudative turkey—the role of ryanodine receptor variation in meat quality. Poult Sci. 88(7):1497–1505. doi: 10.3382/ps.2009-00181.

Sugiharto S, Widiastuti E, Isroli I, Yudiarti T, Sartono TA, Wahyuni HI. 2020. Effect of feeding fermented mixture of cassava pulp and *Moringa oleifera* leaf meal on immune responses, antioxidative status, biochemistry indices, and intestinal ecology of broilers. Vet World. 13(2):392–399. doi: 10.14202/vetworld.2020.392-399.

Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Wahyuni H, Sartono T. 2019. Recent advances in the incorporation of leaf meals in broiler diets. Livest Res Rural Dev. 31(7):109. http://www.lrrd.org/lrrd31/7/sgu_u31109.html.

Surai PF, Kochish II. 2017. Antioxidant systems and vitagenes in poultry biology: heat shock proteins. Heat Shock Prot Vet Med Sci. 12:123–177. doi: 10.1007/978-3-319-73377-7_5.

Suresh G, Das RK, Kaur Brar S, Rouissi T, Avalos Ramirez A, Chorfi Y, Godbout S. 2018. Alternatives to antibiotics in poultry feed: molecular perspectives. Crit Rev Microbiol. 44(3):318–335. doi: 10.1080/1040841x.2017.137306.

Tokofai MB, Idoh K, Agbonon A. 2020. Growth performance, haematological and biochemical parameters in broilers fed diets with varying levels of *Vernonia amygdalina* leaf meal. Eur Poult Sci. 84:2020. doi: 10.1399/eps.2020.321.

Vacek J, Walterová D, Vrublová E, Šimánek V. 2010. The chemical and biological properties of protopine and allocryptopine. Heterocycle. 81(8):1773–1789. doi: 10.3987/REV-10-673.

Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Domínguez-Díaz D, González-Ríos H. 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol. 59(1):1–17. doi: 10.1186/s40781-017-0133-9.

Van Laack RLJM, Liu CH, Smith MO, Loveday HD. 2000. Characteristics of pale, soft, exudative broiler breast meat. Poult Sci. 79(7):1057–1061. doi: 10.1093/ps/79.7.1057.

Windisch W, Schedle K, Plitzner C, Kroismayr A. 2008. Use of phytogenic products as feed additives for swine and poultry. J Anim Sci. 86(14 Suppl):E140–E148. doi: 10.2527/jas.2007-0459.

Zaefarian F, Abdollahi MR, Cowieson A, Ravindran V. 2019. Avian liver: the forgotten organ. Animals. 9(2):63. doi: 10.3390/ani9020063.