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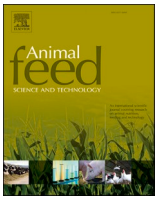

Dietary inclusion of marama bean (*Tylosema esculentum*) in broiler chicken diets compromises feed utilization, growth performance, and carcass traits

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ABSTRACT

Orphan legumes that are native to semi-arid areas of southern Africa, such as marama (*Tylosema esculentum*) bean, hold promise as sustainable nutrient sources in conventional broiler diets. Yet, their nutritive value remains largely unexplored, and the presence of antinutrients could potentially compromise their nutritional quality. Therefore, this study evaluated the effect of full-fat marama bean meal (MBM) on growth performance and physiological and meat quality parameters of growing broiler chickens. Fourteen-day-old male Ross 308 broilers (n = 385; 359.7 ± 25.48 g live-weight) were randomly assigned to five experimental diets formulated by including MBM in a standard broiler grower diet at 0 (MBM0), 16.25 (MBM16), 32.49 (MBM32), 48.74 (MBM49), and 64.98 g/kg (MBM65). Each diet was randomly allocated to 7 replicate pens (experimental units). Diets had no effect on feed intake, but body weight gain and final body weight showed negative linear responses, while feed conversion ratio showed linear and positive quadratic responses ($P < 0.05$) to increasing MBM levels. There were negative quadratic effects ($P < 0.05$) for eosinophils, lymphocytes, and alanine transaminase, whereas linear and quadratic responses ($P < 0.05$) were recorded for alkaline phosphatase. Hemoglobin, neutrophils, monocytes, and albumin:globulin ratio linearly decreased ($P < 0.05$), whereas red blood cells, symmetric dimethylarginine, cholesterol, and lipase increased linearly with MBM levels. Linear increases ($P < 0.05$) were recorded for shear force and proventriculus, gizzard, pancreas, duodenum, jejunum, and ileum sizes. In contrast, linear decreases were observed for carcass weights, breast weight, and breast meat pH. In conclusion, higher levels of MBM resulted in poor growth performance, low carcass weights, and heavier visceral organs, possibly due to anti-nutritional compounds in MBM.

Abbreviations: ALP, Alkaline phosphatase; BWG, Body weight gain; CCW, Cold carcass weight; CF, Crude fibre; DM, Dry matter; EE, Ether extract; FBW, Final body weight; FCR, Feed conversion ratio; FI, Feed intake; HCW, Hot carcass weight; MB, Marama bean; MBM, Marama bean meal; RBC, Red blood cell; SBM, Soybean meal; SDMA, Symmetric dimethylarginine.

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1. Introduction

Incorporating orphan legumes such as marama (*Tylosema esculentum*) bean (MB) as a source of nutrients into broiler chicken diets could contribute to sustainable poultry production. Marama is an indigenous legume that is currently undergoing domestication at various sites in southern Africa (Chimwamurombe, 2011). Full-fat marama beans have 300–370 g crude protein/kg DM (Amarteifio and Moholo, 1998; Amonsou et al., 2011) and about 400 g crude fat/kg DM (Omotayo and Aremu, 2021), among other important nutrients. Regarding essential amino acids, MB has arginine, cystine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine levels that are comparable to soybean (Bower et al., 1988). However, leucine is lower in MB, but tyrosine is four times higher in MB than soybean (Bower et al., 1988).

The use of MB in poultry diets could reduce reliance on imported soybeans in southern Africa, where climatic and agronomic conditions are unfavorable for soybean cultivation. However, MB is also known to contain protease inhibitors (Bower et al., 1988; Nadaraja et al., 2009), which could impede nutrient absorption and compromise animal health and growth (Liener, 1994). These inhibitors constitute about 200 g/kg of the total marama protein (Bower et al., 1988), a concentration that is about four times higher than in many other legumes (Nadaraja et al., 2009; Alabi et al., 2022). Indeed, a lower in vitro protein digestibility has been reported in MB compared to soybean (Maruatona, 2008). Given these potential drawback, it is crucial to assess the impact of incorporating MB into poultry diets on growth, hematology, serum biochemistry, size of internal organs, carcass traits, and meat quality. The outcomes of these studies will inform ongoing long-term improvement efforts for MB as well as immediate strategies to reduce levels and bioactivity of antinutritional factors.

To the best of our knowledge, there are no studies that have investigated the nutritional quality of MBM in broiler chicken diets. The objective of this study was to examine the effect of including raw full-fat MBM in a standard broiler diet on growth performance, blood parameters, carcass characteristics, meat quality, and bone breaking strength in growing Ross 308 broiler chickens. The study tested the hypothesis that the inclusion of MBM in a standard broiler diet would alter growth performance, blood parameters, carcass characteristics, meat quality, and bone breaking strength in growing Ross 308 broiler chickens.

2. Materials and methods

2.1. Ingredient sources and preparation

Whole marama seeds (hulled) were harvested in 2020 from Malwelwe village, Botswana (23.94282°S; 25.1999°E). In this area, the

Table 1
Gross ingredient composition (g/kg, as fed basis) of experimental broiler grower phase (14 – 42 day) diets.

	[a]Diets				
Ingredients	MBM0	MBM16	MBM32	MBM49	MBM65
Marama bean meal	0	16.25	32.49	48.74	64.98
Yellow maize (80 g/kg)	552.24	558.32	564.40	573.59	584.53
Full-fat soya	120.0	120.0	120.0	84.41	28.78
Soya oilcake (470 g/kg)	231.87	217.89	203.93	216.77	244.71
Sunflower oilcake (360 g/kg)	35.0	35.0	35.0	35.0	35.0
Limestone powder	12.44	12.35	12.26	12.14	12.0
Monocalcium phosphate	8.98	9.17	9.36	9.64	9.96
Salt (fine)	2.71	2.65	2.59	2.53	2.48
Sodium bicarbonate	1.27	1.37	1.46	1.55	1.63
DL-Methionine	4.14	4.22	4.30	4.36	4.42
L-Threonine	1.12	1.18	1.23	1.27	1.3
L-Tryptophan	0.09	0.08	0.06	0.06	0.05
Lysine-HCL	2.85	3.11	3.36	3.60	3.82
Crude soya oil mixer	20.95	12.07	3.20	0	0
Lignobond	2.0	2.0	2.0	2.0	2.0
Grower premix[b]	3.0	3.0	3.0	3.0	3.0
AxtraPhytase (10,000 FTU/g)	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.25	0.25	0.25	0.25	0.25
Salinomycin (120 g/kg)	0.50	0.50	0.50	0.50	0.50
Zinc bacitracin	0.50	0.50	0.50	0.50	0.50

The yellow maize, soya oilcake, crude soya oil mixer, sunflower oilcake, limestone, monocalcium phosphate, salt, sodium bicarbonate, DL-methionine, L-threonine, L-tryptophan, lysine-HCl, lignobond (high-performance lignin-based pellet binders), premix, axtraphytase, antioxidant, and salinomycin were purchased from Simplegrow Agric Services (PTY) LTD, Centurion, South Africa. The zinc bacitracin was purchased from Nutroteq (PTY) LTD, Centurion, South Africa.

[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.

[b] Premix: 0.7 mg folic acid; 30 mg niacin; 0.12 g biotin; 79 mg zinc sulphate; 8.0 mg copper sulphate; 80 mg ferrous sulphate; 100 mg magnesium sulphate; 30 mg; 0.25 mg sodium selenite; 10 mg pantothenic acid; 0.34 mg potassium iodine; 2500 IU vitamin D3; 11,000 IU vitamin A; 5.1 mg vitamin B6; 2.0 mg vitamin K3; 25 IU vitamin E; 2.5 mg vitamin B1; 4.5 mg vitamin B2.

soil type is Kalahari sands, annual rainfall ranges between 350 and 450 mm, and ambient temperatures range from 34 to 36 °C during summer, and 0 to 5 °C during winter. The seeds were mechanically cracked to remove the hard outer coat while the cotyledons were carefully handpicked to prevent contamination. The cotyledons were milled to pass through a 2-mm sieve using a cutting mill (SM100, Retsch GmbH, Haan, Germany). All other feed ingredients used in this study were purchased from a commercial feed ingredient supplier, Nutroteq (Centurion, Gauteng, South Africa).

2.2. Diet formulation

Five experimental grower diets were formulated (Table 1) to meet or exceed the nutritional specifications for Ross broiler chickens (Aviagen, 2014) by adding MBM in a standard broiler grower diet at 0 (MBM0), 16.25 (MBM16), 32.49 (MBM32), 48.74 (MBM49), and 64.98 g/kg (MBM65).

2.3. Chemical analyses

Samples of experimental diets were chemically analyzed (Table 2) using the standard methods of the Association of Official Analytical Chemists (AOAC, 2005) for dry matter (DM; method 930.15), crude protein (CP; Kjeldahl technique, N × 6.25; method 978.04), crude fibre (CF; method 978.10), ether extract (EE; method 930.09), minerals (calcium, phosphorus, chloride, and sodium; method 991.25, acid digestion followed by spectrophotometry), amino acids (method 982.30, ultra-performance liquid chromatography, Waters Corporation, Milford, MA, USA). Gross energy was determined using Gallenkamp Ballistic Bomb Calorimeter following the method described by Henken et al. (1986) and the values were used to calculate metabolizable energy (ME). In addition, MB samples were determined for DM, ash, CP, crude fibre, crude fat, lysine, and ME using the methods stated above.

2.4. Bird management and experimental design

The experimental procedures used during this study were reviewed and approved by the Research Ethics Committee for Animal Production studies at the North-West University, Mafikeng, South Africa, under protocol number NWU-02-007–20-A5. A total of 800, one-day-old, unsexed Ross 308 broiler chicks were purchased from Superior Chicks (PTY) Ltd (Gauteng, South Africa). The chicks were

Table 2
Chemical composition (g/kg, as fed) and metabolizable energy content (Kcal/kg) of marama bean meal and experimental broiler grower phase (14 – 42 days) diets.

		[a]Diets				
	[b]MBM	MBM0	MBM16	MBM32	MBM49	MBM65
Calculated values						
Dry matter		888.8	889.4	890.0	891.1	892.5
Metabolizable energy		3083	3083	3083	3083	3083
Crude protein		215.0	215.0	215.0	215.0	215.0
AP Lysine		12.20	12.20	12.20	12.20	12.2
AP Methionine		7.15	7.15	7.15	7.15	7.15
AP Threonine		8.10	8.10	8.10	8.10	8.1
AP Tryptophan		2.30	2.30	2.30	2.30	2.3
Crude fat		67.18	65.13	63.08	60.55	57.75
Crude fibre		36.63	36.81	36.98	36.53	35.72
Available phosphorus		4.50	4.50	4.50	4.50	4.50
Calcium		9.0	9.0	9.0	9.0	9.0
Chloride		2.50	2.50	2.50	2.50	2.50
Sodium		1.80	1.80	1.80	1.80	1.80
Analysed values						
Dry matter	940.3	929.6	925.3	930.7	930.2	934.4
Metabolizable energy	5489.2	3362	3370.2	3370.2	3362	3370.2
Crude protein	327.2	228.0	226.1	225.8	224.9	224.1
Crude fat	386.7	71.6	69.7	68.6	66.3	67.6
Crude fibre	34.3	40	40.3	43.1	42	4.39
Ash	28.8	68.5	67.9	60.6	66	60.6
Lysine	20.4	6.1	5.7	6.1	5.9	6.1
Methionine	-	3.8	3.8	3	3.5	3.2
Phosphorus	-	4.6	4.3	4.3	4.4	4.7
Calcium	-	8.6	8.6	9	8.7	8.7
Chloride	-	2.5	2.5	2.5	2.5	2.5
Sodium	-	2.4	2.4	2.4	2.3	2.5

[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.

[b] MBM, marama bean meal.

transported to North-West University Molelwane Research farm (25°40.459′ S and 26°10.563′ E; North West, South Africa) for rearing using a standard commercial starter diet until 10 days of age. The birds were offered vitamins and electrolytes (stress pack) via drinking water for the first three days. At 11 days of age, the birds were feather sexed and the males (n = 385) were transported to Rooigrond Commercial Farm (25°55′0″ S; 25°48′0″ E; North West, South Africa) for the feeding trial. The birds were weighed and distributed to 35 replicate pens (3.5 m length × 1.0 m width × 1.85 m height) with sunflower husk-covered floors. Each pen was equipped with a feeder and two drinkers that were cleaned daily and filled with fresh feed and water, respectively. Each of the five experimental diets was randomly allocated to 7 replicate pens, with each pen housing 11 birds. For the first 14 days, the temperature in the pens was maintained at 35 °C using infrared electric bulbs and humidity averaged 60 %. The birds had unlimited access to their respective diets and clean fresh water while natural lighting (06h00 to 18h00) was used throughout the study. Measurements, including mortality, were taken from day 14 – 42 of age. A total of 12 birds died during the study, translating to a 3 % mortality rate. Six birds died between days 14 to 35: two each in the MBM0 and MBM16, and one each in MBM32 and MBM65 diets. For the last 7 days of the trial, two birds each in MBM0 and MBM32 and one each in MBM49 and MBM65 diets died.

2.5. Growth performance

Feed intake was calculated daily as the difference between feed offered and feed refusals collected in the morning prior to feeding. Weight was recorded weekly for all the chickens in each pen using a weighing scale (ADAM® scale, readability of 0.5 to 2 g, Adam Equipment S.A. PTY, Johannesburg, South Africa) to calculate average weekly body weight gain. Feed conversion was calculated as a ratio of feed intake to weight gain. The growth performance data was corrected for all mortalities.

2.6. Blood sampling and analyses

Blood samples were collected at 40 days of age from the brachial vein of two birds, randomly selected from each pen, using 23-gauge needles and 5 mL syringes. The samples were immediately transferred into serum and whole blood tubes. An automated IDEXX® LaserCyte Hematology Analyzer (IDEXX® Laboratories Pty S.A., Midrand, South Africa) was used to determine hematological parameters; eosinophil, hematocrit, hemoglobin, lymphocyte, mean corpuscular hemoglobin, mean corpuscular volume, mean platelet volume, monocyte, neutrophil, platelet, platelet distribution width, red blood cell, red cell distribution, and reticulocyte. An automated IDEXX® Vet Test Chemistry Analyzer (IDEXX® Laboratories Pty S.A., Midrand, South Africa) was used to determine serum biochemical parameters; alanine transaminase, albumin, alkaline phosphatase, amylase, calcium, cholesterol, gamma-glutamyl transferase, globulin, glucose, lipase, phosphorus, symmetric dimethylarginine, total protein, and urea.

2.7. Carcass traits

At 42 days of age, all chickens were weighed to determine the final body weight (FBW) and, thereafter, electrically stunned and slaughtered by cutting the jugular vein using a sharp knife in a registered local abattoir. The feathers were removed using a plucking machine before manual evisceration of visceral organs. The carcasses were marked according to the experimental unit using wool fiber of different colors tied around the drumstick. Carcasses were weighed immediately after slaughter and again after being chilled (16 °C) in a cold room for 24 h to determine hot (HCW) and cold carcass weights (CCW), respectively. Carcass yield was calculated as the ratio of HCW to FBW. The weights of wing, drumstick, thigh, breast, duodenum, jejunum, colon, ceca, ileum, liver, gizzard, proventriculus, spleen and pancreas were measured using a weighing scale (ADAM® scale, readability of 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa).

2.8. Cooking loss and water holding capacity

Two breast meat samples, devoid of external fat, were randomly selected per experimental unit, pre-weighed (ADAM® scale, readability of 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa), placed in a foil plate, and cooked to reach an internal temperature of 75 °C according to the method described by Honikel (1998). The samples were removed immediately when the endpoint temperature was attained and then allowed to cool for 30 min. The meat was then taken from the foil plate, gently blotted dry, and weighed to determine the final weight. Cooking loss was expressed as the difference between the weight of raw meat sample and cooked meat in proportion to the weight of the raw meat sample. Water holding capacity was evaluated, in raw breast meat of two carcasses from each replicate, by expressing water from the meat held under pressure (60 kg pressure) using the filter-paper method as described by Grau and Hamm (1957).

2.9. Meat tenderness and bone breaking strength

Freshly cut breast meat samples from two carcasses in each replicate were sheared using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture Analyzer (TA-XT plus, Stable Micro Systems, Surrey, UK) to determine shear force (N), a measure of meat tenderness. Bone breaking strength was determined in duplicate tibia bones. Freshly cut thigh (drumstick) was placed in thin-walled plastic bags and immersed in water bath at 100 °C for 30 min. The meat was then cooled at room temperature for 15 min. Thereafter, bones were de-fleshed and cleaned of all tissues and then placed on an adjustable three-point bend/snap fixture fitted on a heavy-duty TA-XT platform of the Texture Analyzer described above and then broken with a 6-cm flat head probe attached to a 50-kg load cell

reporting the breaking force in Newtons. The breaking bone strength was recorded as the peak load before bone breakage (Disetlhe et al., 2017).

2.10. Meat color and pH

Meat pH was taken from all carcasses 24 h post-mortem on the central part of the breast muscle (from 2 birds per pen) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA, United States) fitted with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). Calibration of pH meter was performed after every ten measurements using standard solutions (pH 4, 7, and 10). This was immediately followed by determination of breast meat (from 2 birds per pen) color coordinates (lightness, L^*, redness, a^*, and yellowness, b^*) using a Minolta color-guide (BYK-Gardener GmbH, Geretsried, Germany). The color guide was set and calibrated following the guidelines by the manufacturer.

2.11. Statistical analysis

All tested variables (overall growth performance, blood characteristics, and carcass and meat quality traits) were evaluated for linear and quadratic effects using polynomial functions. The procedure of response surface regression (PROC RSREG; SAS, 2010) was used to evaluate the data using the equation: $y = ax^2 + bx + c$, where: y = response variable; a and b are the coefficients of the quadratic equation; and c is dietary MBM levels. The x value for minimum/maximum response was determined as: $\frac{-b}{2a}$.

Data from repeatedly measured variables, comprising of average weekly feed intake, body weight gain, and feed conversion ratio were subjected to repeated measures analysis in GLM procedure of SAS software package (SAS, 2010) to determine the interaction effect between dietary treatment and time (in weeks). The experimental unit used for all determined parameters was the replicate pen. Statistical significance was considered at $P < 0.05$.

3. Results

3.1. Growth performance

Repeated measures analysis revealed no diet × week (chicken age) interaction effect on average feed intake (FI, $P = 0.497$), body weight gain (BWG, $P = 0.710$), and feed conversion ratio (FCR, $P = 0.377$). There were neither linear nor quadratic responses ($P > 0.05$) for overall feed intake and overall mortality as MBM levels increased (Table 3). However, overall body weight gain linearly decreased with increasing levels of dietary MBM [$y = 2100(\pm 63.88) - 2.395(\pm 4.892)$ x, $R^2 = 0.538$, $P < 0.0001$]. There was also a negative linear effect of MBM levels on final body weight (FBW) at day 42 [$y = 2495(\pm 154.29) - 7.206(\pm 11.813)$ x, $R^2 = 0.255$; $P = 0.006$]. There were linear and quadratic responses for overall FCR [$y = 1.603(\pm 0.037) - 0.0005(\pm 0.0028)$ x + $0.0001(\pm 0.00004)$ x^2, $R^2 = 0.718$; $P < 0.05$].

3.2. Hematology and serum biochemistry

Table 4 showed that there were negative quadratic effects for eosinophils [$y = 5.35(\pm 0.850) + 0.256(\pm 0.065)$ x – $0.004(\pm 0.001)$ x^2, $R^2 = 0.397$, $P = 0.0003$] and lymphocytes [$y = 50.70(\pm 17.60) + 3.87(\pm 1.345)$ x – $0.056(\pm 0.021)$ x^2, $R^2 = 0.211$, $P = 0.012$]. Hemoglobin [$y = 9.367(\pm 0.228) - 0.005(\pm 0.017)$ x, $R^2 = 0.152$, $P = 0.039$], neutrophils, [$y = 3.77(\pm 0.301) - 0.028(\pm 0.023)$ x, $R^2 = 0.188$, $P = 0.021$] and monocytes [$y = 4.95(\pm 0.451) - 0.088(\pm 0.035)$ x, $R^2 = 0.339$, $P = 0.001$] linearly decreased with increasing levels of MBM in the diets. However, red blood cell (RBC) increased linearly [$y = 1.30(\pm 0.219) + 0.034(\pm 0.017)$ x, $R^2 = 0.157$, $P = 0.031$].

Table 3
Growth performance (g/bird, unless indicated otherwise) of Ross 308 broiler chickens offered marama bean meal-containing diets from 14 - 42 day.

	[a]Diets						Significance	
[b]Parameters	MBM0	MBM16	MBM32	MBM49	MBM65	[c]SEM	$P_{-Linear}$	$P_{-Quadratic}$
Initial BW (day 14)	362.4	361.0	370.6	355.8	348.7	9.97	0.976	0.651
Final BW (day 42)	2453.6	2405.6	2310.3	2175.6	1904.7	49.30	0.006	0.741
Overall FI	3330.8	3329.6	3163.6	3275.5	3240.2	78.40	0.074	0.884
Overall BWG	2091.1	2044.6	1862.5	1800.7	1556.0	63.50	< 0.0001	0.190
Overall FCR (g:g)	1.60	1.63	1.70	1.82	2.08	0.034	< 0.0001	0.013
Overall mortality (%)	5.32	3.92	1.30	2.60	2.60	2.143	0.317	0.605

The pen (11 birds/pen) was the experimental unit each replicated 7 times per dietary treatment.

[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.

[b] Parameters: BW, body weight; FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio.

[c] SEM, standard error of mean.

Table 4
Impact of increasing levels of marama bean meal on hematological characteristics of 40-day old Ross 308 broiler chickens.

[b]Parameters	[a]Diets					[c]SEM	Significance	
	MBM0	MBM16	MBM32	MBM49	MBM65		$P_{-Linear}$	$P_{-Quadratic}$
RBC ($\times 10^{1}$[b]/L)	1.29	1.76	2.08	1.81	1.97	0.258	0.031	0.180
Hematocrit (%)	9.65	14.3	13.71	16.11	13.21	2.455	0.162	0.300
Hemoglobin (g/dL)	9.45	8.96	9.45	8.52	8.66	0.228	0.039	0.672
Neutrophils ($\times 10^{9}$/L)	3.89	3.15	2.95	3.26	2.25	0.318	0.021	0.629
Monocytes ($\times 10^{9}$/L)	4.71	4.29	2.67	2.89	2.63	0.539	0.001	0.163
Lymphocytes ($\times 10^{9}$/L)	42.02	122.31	132.94	36.51	100.65	13.67	0.315	0.012
Eosinophils ($\times 10^{9}$/L)	5.76	7.80	10.20	6.88	3.97	0.907	0.988	0.003
MCV (fL)	50.73	63.84	58.56	61.3	61.24	5.130	0.192	0.468
MCH (pg)	50.09	50.33	47.2	39.37	50.84	6.346	0.418	0.833
RDW (%)	34.69	26.78	27.02	29.71	29.77	3.108	0.241	0.182
Reticulocytes (K/μL)	334.47	304.40	288.84	342.99	218.84	35.71	0.113	0.297
Platelets (K/μL)	2500	2500	2219	2500	2500	93.47	0.749	0.136
MPV (fL)	2.59	2.29	2.37	2.45	2.19	0.164	0.393	0.825
PDW (%)	14.64	14.56	15.02	14.38	14.42	0.314	0.840	0.233

The pen (2 birds/pen) was the experimental unit each replicated 7 times per dietary treatment.
[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.
[b] Parameters: RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red blood cells distribution width; MPV, mean platelet volume; PDW, platelet distribution width.
[c] SEM, standard error of mean.

A negative quadratic response was observed for alanine transaminase [$y = 32.34(\pm 3.031) + 0.510(\pm 0.232)\,x - 0.01(\pm 0.003)\,x^2$, $R^2 = 0.213$, $P = 0.011$] as dietary MBM levels increased (Table 5). The optimum inclusion level of MBM was calculated to be 25.5 g/kg diet for alanine transaminase. Linear and quadratic responses were recorded for alkaline phosphatase [$y = 154.3(\pm 12.69) + 3.741(\pm 0.972)\,x - 0.043(\pm 0.043)\,x^2$, $R^2 = 0.444$, $P < 0.05$]. Albumin:globulin ratio [$y = 0.453(\pm 0.020) - 0.004(\pm 0.002)\,x$, $R^2 = 0.234$, $P = 0.006$] linearly decreased, whereas symmetric dimethylarginine [$y = 28.98(\pm 2.464) + 0.229(\pm 0.189)\,x$, $R^2 = 0.456$, $P < 0.0001$], cholesterol [$y = 2.44(\pm 0.143) + 0.009(\pm 0.011)\,x$, $R^2 = 0.189$, $P = 0.021$], and lipase [$y = 193.8(\pm 20.44) + 0.554(\pm 1.566)\,x$, $R^2 = 0.551$, $P < 0.0001$] levels linearly increased in response to increasing MBM levels.

3.3. Visceral organs and carcass attributes

Table 6 shows that there were linear decreases for HCW [$y = 1903(\pm 44.25) - 1.108(\pm 3.388)\,x$, $R^2 = 0.475$, $P < 0.0001$], CCW [$y = 1891(\pm 43.43) - 1.124(\pm 3.326)$, $R^2 = 0.459$, $P < 0.0001$], and breast weight [$y = 23.50(\pm 0.769) - 0.098(\pm 0.058)\,x$, R^2

Table 5
Impact of increasing levels of marama bean meal on serum biochemical parameters of 40-day old Ross 308 broiler chickens.

[b]Parameters	[a]Diets					[c]SEM	Significance	
	MBM0	MBM16	MBM32	MBM49	MBM65		$P_{-Linear}$	$P_{-Quadratic}$
Glucose (mmol/L)	7.55	7.87	8.01	7.81	8.57	0.468	0.401	0.805
SDMA (μg/dL)	27.36	36.57	35.86	38.67	45.79	3.085	< 0.0001	0.802
Urea (mmol/L)	0.68	0.64	0.64	0.63	0.67	0.016	0.073	0.094
Phosphorus (mmol/L)	2.26	2.29	2.26	2.30	2.30	0.100	0.644	0.862
Calcium (mmol/L)	1.40	1.36	1.28	1.21	1.29	0.049	0.103	0.116
Total protein (g/L)	40.36	43.43	41.71	42.08	43.36	2.258	0.340	0.852
Albumin (g/L)	12.57	12.0	11.36	11.67	11.71	0.546	0.130	0.114
Globulin (g/L)	27.79	31.43	30.36	30.42	31.64	1.986	0.137	0.529
Albumin:Globulin ratio	0.46	0.39	0.39	0.40	0.37	0.028	0.006	0.087
ALT (U/L)	33.36	35.79	39.36	35.67	25.43	3.266	0.210	0.011
ALKP (U/L)	157.8	195.8	232.6	214.0	226.3	17.26	0.002	0.007
GGT (U/L)	15.42	16.57	20.43	18.70	19.14	1.542	0.056	0.392
Cholesterol (mmol/L)	2.37	2.79	2.54	3.03	2.98	0.147	0.021	0.964
Amylase (U/L)	466.8	391.4	464.9	601.5	542.1	55.77	0.179	0.416
Lipase (U/L)	189.6	223.0	244.9	388.1	415.6	44.12	< 0.0001	0.132

The pen (2 birds/pen) was the experimental unit each replicated 7 times per dietary treatment.
[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.
[b] Parameters: SDMA, symmetric dimethylarginine; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.
[c] SEM, standard error of mean.

Table 6
The effects of marama bean meal-containing diets on carcass traits and weights of internal organs (g /100 g hot carcass weight, unless stated otherwise) in 42-day-old Ross 308 broiler chickens.

[b]Parameters	[a]Diets					[c]SEM	Significance	
	MBM0	MBM16	MBM32	MBM49	MBM65		$P_{-Linear}$	$P_{-Quadratic}$
Carcass yield (%)	77.82	76.44	81.74	76.53	79.68	1.768	0.394	0.999
HCW (g)	1913.0	1852.1	1891.1	1660.9	1519.5	45.14	< 0.0001	0.050
CCW (g)	1904.7	1836.1	1884.6	1666.4	1518.2	44.28	< 0.0001	0.056
Wing	4.99	4.95	4.67	5.19	5.20	0.154	0.474	0.153
Drumstick	5.92	5.97	5.36	6.13	6.15	0.173	0.956	0.143
Thigh	7.09	7.03	6.27	7.15	7.08	0.210	0.493	0.1488
Breast	23.06	23.24	19.32	19.16	18.17	0.774	< 0.0001	0.824
Proventriculus	0.47	0.48	0.55	0.59	0.62	0.023	0.0001	0.342
Spleen	0.15	0.15	0.16	0.17	0.15	0.010	0.754	0.132
Liver	2.39	2.28	2.44	2.67	2.45	0.088	0.131	0.879
Gizzard	1.99	2.03	2.08	2.38	2.30	0.077	0.006	0.832
Pancreas	0.28	0.33	0.44	0.49	0.61	0.031	< 0.0001	0.869
Duodenum	0.62	0.61	0.78	0.81	0.77	0.040	0.008	0.077
Jejunum	1.59	1.54	1.82	2.01	2.01	0.070	0.0002	0.818
Ileum	1.36	1.41	1.42	1.58	1.71	0.061	0.001	0.332
Colon	0.13	0.16	0.12	0.13	0.13	0.016	0.882	0.793
Caeca	0.64	0.65	0.73	0.72	0.71	0.034	0.124	0.299

The pen (11 birds/pen) was the experimental unit each replicated 7 times per dietary treatment. The total number of birds examined was 373, as 12 birds died during the study: 4 in MBM0, 2 in MBM16, 3 in MBM32, 1 in MBM49, and 2 in MBM65 treatment groups.

[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.

[b] Parameters: HCW, hot carcass weight; CCW, cold carcass weight.

[c] SEM, standard error of mean.

$= 0.456$, $P < 0.0001$]. There was a linear increase in the weight of duodenum [$y = 0.584(\pm 0.037) + 0.008(\pm 0.003)$ x, $R^2 = 0.217$, $P = 0.008$], jejunum [$y = 1.533(\pm 0.070) + 0.008(\pm 0.005)$ x, $R^2 = 0.424$, $P = 0.0002$], gizzard [$y = 1.958(\pm 0.076) + 0.007 (\pm 0.006)$ x, $R^2 = 0.261$, $P = 0.006$], proventriculus [$y = 0.457(\pm 0.019) + 0.003(\pm 0.001)$ x, $R^2 = 0.430$, $P < 0.0001$], pancreas [$y = 0.272(\pm 0.026) + 0.004(\pm 0.002)$ x, $R^2 = 0.666$, $P < 0.0001$] and ileum [$y = 1.359(\pm 0.056) + 0.001(\pm 0.004)$ x, $R^2 = 0.333$, $P = 0.001$], as MBM inclusion level increased.

3.4. Meat quality parameters

Meat pH linearly declined [$y = 6.11(\pm 0.037) - 0.006(\pm 0.0028)x$; $R^2 = 0.188$, $P = 0.017$] while meat shear force linearly increased [$y = 3.48(\pm 0.329) + 0.097(\pm 0.024)x$; $R^2 = 0.638$, $P < 0.0001$] with increasing levels of dietary MBM (Table 7). The dietary inclusion of MBM had no effect ($P > 0.050$) on meat color, water holding capacity, cooking loss, and bone breaking strength.

Table 7
The effects of marama bean meal-containing diets on meat quality parameters and bone breaking strength of 42-day-old Ross 308 broiler chickens.

[b]Parameters	[a]Diets					[c]SEM	Significance	
	MBM0	MBM16	MBM32	MBM49	MBM65		$P_{-Linear}$	$P_{-Quadratic}$
pH	6.11	6.02	5.98	5.98	5.98	0.042	0.017	0.147
*L** (lightness)	58.77	59.74	60.04	60.51	61.21	0.807	0.307	0.241
*a** (redness)	2.03	1.47	2.06	1.83	1.25	0.264	0.431	0.712
*b** (yellowness)	9.15	9.13	9.88	10.45	9.2	0.479	0.704	0.205
Cooking loss (%)	25.17	25.20	24.84	24.22	25.98	0.620	0.675	0.166
WHC (%)	84.68	84.06	87.08	84.78	83.49	1.419	0.713	0.222
Shear force (N)	3.62	4.50	6.27	6.54	6.94	0.374	< 0.0001	0.069
BBS (N)	332.9	329.9	338.3	285.5	337.7	20.40	0.639	0.552

The pen (11 birds/pen) was the experimental unit each replicated 7 times per dietary treatment. The total number of birds examined was 373, as 12 birds died during the study: 4 in MBM0, 2 in MBM16, 3 in MBM32, 1 in MBM49, and 2 in MBM65 treatment groups.".;

[a] Diets: MBM0, a standard grower diet with no MBM; MBM16, a standard grower diet containing 16.25 g/kg MBM; MBM32, a standard grower diet containing 32.49 g/kg MBM; MBM49, a standard grower diet containing 48.74 g/kg MBM; and MBM65, a standard grower diet containing 64.98 g/kg MBM.

[b] Parameters: WHC, water holding capacity; BBS (N), bone breaking strength in Newston.

[c] SEM, standard error of mean.

4. Discussion

4.1. Growth performance and mortality

In this study, bird mortality was minimal and randomly dispersed among all five experimental diets. Consequently, there is no indication that the inclusion of MBM in broiler diets had any impact on bird mortality. Diet-induced changes in feed intake and growth performance parameters were independent of the age of the birds, as shown by the lack of a statistically significant diet × week (bird age) interaction effect on average weekly feed intake, body weight gain, and feed conversion ratio. The fact that overall feed intake was not affected by the inclusion of MBM despite the higher levels of antinutritional factors, was surprising given that a compensatory feeding response to reduced nutrient availability was expected. However, this finding is consistent with a report by Nurpaidah et al. (2021), when wringed beans were included in broiler diets at 0, 25, and 50 g/kg. Despite the similar feed intake levels, increasing MBM levels reduced final body weight and overall body weight gain while increasing feed conversion ratio (reduced feed utilization efficiency). Further, FCR was lowest (minimized) at 2.5 g/kg (calculated) suggesting that even the lowest MBM inclusion level of 16.25 g/kg used in this study was detrimental for growth performance in the chickens. This could be attributed to the adverse impact of antinutritional factors, such as trypsin inhibitors, on protein digestibility in MB. Indeed, trypsin inhibitor activity has been reported to be more than three times higher in raw MB (245.25 trypsin units inhibited (TUI) /mg) than in soybeans (66.98 TUI/mg; Coscueta et al., 2017; Alabi et al., 2022).

In a related study, growth depression in broiler chickens has been attributed to the cumulative effect of loss of essential amino acids and reduced intestinal proteolysis caused by trypsin inhibitors in raw soybeans (Chohan et al., 1993). Marama bean is comparable to soybean for most essential amino acids (g/100 g dry matter basis), including histidine (1.07 vs 0.94), isoleucine (1.54 vs 1.54), lysine (2.04 vs 2.18), phenylalanine (2.02 vs 1.77), threonine (1.22 vs 1.32) and valine (1.77 vs 1.75) (MB vs SB; Alabi et al., 2022). However, previous findings showed that MB has lower (0.46 g/100 g) levels of methionine, the first limiting amino acid in practical broiler diets, compared to soybean (0.71 g/100 g; Maruatona, 2008), and this could have contributed to poor feed utilization efficiency at higher MBM inclusion levels in the present study.

4.2. Hematological and serum biochemical parameters

The dietary inclusion of MBM quadratically affected eosinophil values indicating that MB first resulted in an increase of disease-fighting white blood cells before causing a reduction. Neutrophils account for about half of the white blood cells and are the major pathogen-fighting immune cells (Mayadas et al., 2014). Antimicrobial lipids in MB (Chingwaru et al., 2011), may have provided effective antimicrobial protection, thereby reducing the need for higher neutrophil levels in chickens. The hemoglobin concentration linearly declined due to incorporation of MBM, which could be a consequence of poor nutrient utilization. This is because protein and minerals (such as iron, calcium, and phosphorus) are essential for the formation of hemoglobin (Nworgu et al., 2007). Phytic acid in marama bean has been identified predominantly in an insoluble nutrient-phytate complex form, posing challenges to nutrient digestibility (Amonsou et al., 2011).

Linear increases were observed for symmetric dimethylarginine, alkaline phosphatase, and lipase concentrations as the level of MBM in the diets increased. Hepatic function of poultry can be assessed by serum concentrations of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and gamma-glutamyl transferase (Enemor et al., 2005). Our results on alkaline phosphatase agreed with the previous findings when laying Japanese quails were supplemented with canola seeds (Ibrahim et al., 2020). The increase in the activity of these enzymes (alkaline phosphatase, symmetric dimethylarginine, and lipase) in response to high levels of MBM in the diets may be an indication of stress on the liver, kidney, and pancreas. For instance, higher lipase concentration could be attributed to pancreatic hypertrophy and hyperplasia (Liener, 1994) induced by high levels of trypsin inhibitor activity in MB (Alabi et al., 2022). Similarly, symmetric dimethylarginine has been shown to be an accurate biomarker for renal dysfunction in animals (Hall et al., 2014).

Although the proportion of different enzymes related to metabolism and function of heart, liver, and kidney are used to determine feed toxicity, liver enzymes such as alanine transaminase or gamma-glutamyl transferase have low diagnostic value for nutritional merit due to their high variability in the blood (Enemor et al., 2005). The numerically lower calcium levels in the serum in response to higher MBM inclusion levels may be attributed to the higher levels of antinutritional factors, especially phytic acid in MB (Jackson et al., 2010). This corroborates findings reported when incremental levels (0, 50, 100, 150, 200, and 250 g/kg) of raw suckle pod seed meal were used to replace roasted soybean in broiler chicken diets (Augustine et al., 2017). We observed a positive linear response of sera cholesterol to dietary MBM levels. Generally, high lipid levels in circulation indicate enhanced de novo lipolysis, while low lipid profile in blood indicates an increased rate of amino acid transportation and improved lipid metabolism with consequent decreased fat deposition (Zhao et al., 2009). Hence, we can deduce that the increase in cholesterol concentration might have been caused by poor nutrient utilization due to antinutritional factors in MB.

4.3. Carcass yield and weight of internal organs

In the current study, the weights of jejunum, duodenum, gizzard, proventriculus, ileum, and pancreas linearly increased while breast meat yield declined as the level of MBM increased in diets. These findings agree with those of other researchers (Maidala et al., 2017; Erdaw et al., 2017; Rada et al., 2017) whose reports showed that birds fed diets containing raw soybean had heavier pancreases, small intestines, and gizzards. The reduction in weight of carcass and breast and heavier visceral organs may be attributed to declining

nutrient utilization and increasing feed conversion ratio recorded in this study. The increased weights of these internal organs may be due to cellular hypertrophy or hyperplasia. Indeed, trypsin inhibitor ingestion has been reported to increase production and secretion of trypsin and chymotrypsin enzymes leading to pancreatic hypertrophy (Rada et al., 2017).

4.4. Meat quality

The evaluation of meat quality includes physical traits such as pH, muscle color, and shear force. Although the meat pH linearly declined, it still fell within the optimum pH range of 5.35 - 6.10 expected within 24 hrs post-mortem (Janocha et al., 2022). Surprisingly, the drop in meat pH had no effect on meat color, which also contradicted the findings by Laudadio et al. (2011), who reported that a replacement of soybean meal with dehulled pea in chicken diet did not affect the breast muscle color but enhanced redness of thigh skin. In addition, Casaubon-Huguenin et al. (2004) reported that there was no effect on broiler skin redness, but skin yellowness increased upon feeding higher levels (0 %, 20 %, 40 %, 60 % and 100 %) of raw full-fat soybean. Shear force, a measure of tenderness, linearly increased in response to dietary MBM inclusion levels. The increase in meat toughness recorded in the current study may be related to the poor utilization in the MB diets. Similarly, the digestibility of nutrient, particularly protein, which influences the overall texture of meat (Samuel, 2009) might have been altered adversely by the high level of protease inhibitors in MB and consequently impacted the tenderness of the meat.

5. Conclusions

The inclusion of raw full-fat marama bean meal in growing broiler diets compromised growth performance and resulted in low carcass weights and heavier visceral organs, possibly due to high levels of protease inhibitors and phytic acid in marama bean. Marama bean improvement programs currently underway in southern Africa should also include reduction of antinutrients in this orphan legume as an important breeding objective. This will allow widespread utilization of this bean for both humans and animals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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