

Growth performance, protein utilization and haemato-biochemical parameters of broilers fed marama bean (*Tylosema esculentum*) based diets

Samuel Tjetjeo

North-West University

upenyu Marume

upenyu.marume@nwu.ac.za

North West University <https://orcid.org/0000-0003-1285-5797>

Research Article

Keywords: growth performance, protein utilization efficiency, haematology, serum biochemistry

Posted Date: February 20th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3905782/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

This study investigated growth performance, protein utilization and blood parameters in broiler chickens fed diets containing marama bean (MB). Three hundred day-old broiler chicks were randomly allocated to 5 dietary treatments with eight replicates. The chicks were fed commercial starter diets from 1 to 14 days. The experimental diets were offered during the grower and finisher phases. Five isonitrogenous and isoenergetic diets with the inclusion of marama bean at 0 (MB0, control), 7 (MB7), 14 (MB14), 21 (MB21) and 28% (MB28) were formulated. From the results, Diets containing marama bean had lower average daily gain (ADG) and higher feed conversion ratios (FCR) compared to the control diet. In both phases, the birds fed diet containing MB7 diet had higher ADG compared with other marama bean containing diets. The birds fed control diet also had high cumulative weight gain followed by the birds fed the MB7 diet. In both feeding phases, protein efficient ratio (PER) and specific growth rate (SGR) were higher in birds fed the control diet followed by those fed MB7 diet. With regards to serum metabolites, birds fed control diet had the highest amounts of calcium, phosphorus and total albumin followed by those fed the MB7 diet. However, total protein, total globulin and cholesterol were higher in the birds fed MB7 compared to the control. Higher values for alkaline transferase (ALT) were obtained in all diets containing marama bean compared with the control. In conclusion, inclusion of marama bean meal in diets at 7% provided the optimum performance.

1. Introduction

Global human population growth, increased income, and the demand for quality protein by people for good health and wellbeing are increasingly becoming key drivers for higher animal protein production (OECD-FAO, 2016). Annual per capita consumption of meat is projected to reach 35.5 kg and 88 to 100 kg per person in developing and developed countries respectively by 2025 (OECD-FAO, 2016). The increase in the demand for animal protein has challenged geneticists, nutritionist and meat producers to focus on producing animals within the least possible time (Raha, 2007). Consequently, poultry, especially broilers, are becoming more dominant within the meat complex. The short production cycle has enabled rapid responses to market signals by producers, whilst allowing for expeditious improvements in genetics, animal health, and feeding practices. Nevertheless, under commercial broiler production, one of the major drawbacks has been the increasing cost of feeds which can take as much as 70% of the total cost of production (Disetlhe et al., 2018). In particular, the protein and energy components of the broiler diets are becoming rather expensive and scarce due to factors that include increased pressure on land footprint, recurring droughts and climate change that have resulted in reduced production, culminating in intense competition between humans and livestock for the same feed (Mengesha, 2012). Khan et al. (2009) opined that scarcity and high price of good quality feed, especially protein and energy rich feed ingredients, are one of the most important constraints to the improvement and sustenance of broiler productivity.

The higher prices of poultry feed ingredients have provoked intense efforts by nutritionists in seeking alternative ingredients that optimize broiler production at a lesser cost (Babiker, 2012; Moreki and Gabanakgosi, 2014). As a result, Müsseler and Schönfeldt (2006) recommended the investigation of little-known but high potential plant feed sources which are native to dry areas requiring little seasonal rainfall. Among other native plant feed sources, marama bean (*Tylosema esculentum*) has been shown to have great potential as a protein source. Marama bean is a protein rich legume that thrives in arid and semi-arid regions

with feasible agronomic and economic returns. The plant has been observed to have nutrient composition similar to that of peanuts (*Arachis hypogaea*) and soybeans (*Glycine max*) (Tibe et al., 2016; Keegan and van Staden, 1981) and thus has the potential to replace conventional protein ingredients in poultry diets. Marama contain approximately 34.1% crude protein and 33.5% oil, respectively (Amarteifio and Moholo, 1998). Although the protein is low in the essential sulphur-containing amino acids; methionine and cysteine, it has adequate level of lysine. Lysine is considered the first limiting amino acids in poultry diets and hence its inclusion in diets may reduce the amounts of additional elementary lysine during diet formulation (Siqueira et al. 201; Nasr and Kheiri, 2012).

Although there are many non-conventional feeds such as marama bean with substantial nutritional value and inexpensively available in large quantities, their inclusion in formal animal feed database and feed formulation systems is being limited by the lack of adequate nutritional information (Olukosi et al., 2019; Usayran et al., 2014). The nutritional potential of marama bean as food for humans has been investigated in a number of studies (Bower et al., 1988; Müseler and Schönfeldt, 2006). However, no studies have been done on the value of this plant as feed for livestock particularly poultry. Some research exploring the use of diets containing non-conventional feed ingredients as protein sources reported positive responses on growth performance and carcass quality of broilers (Olukosi et al., 2019; Usayran et al., 2014). We therefore postulate that marama bean may be included in broiler diets without negative effects on performance. The study was hence conducted to assess the effects of inclusion of marama bean in broiler diets on growth performance, protein utilisation and physiological responses.

2. Materials and methods

2.1 Study site

The study was conducted from September to October at Rooigrond Poultry Farm in North-West province of South Africa. The geographical coordinates are 25° 55' 0" South, 25° 48' 0" East and average rainfall ranges between 300 and 700 mm annually. All the experimental procedures were approved by the NWU-ANIMPROD research ethics committee following the Guide for the Care and use of Laboratory Animals of the National Research Council (NRC, 2011) and the following number was given: NWU-00480-18-A5

2.2 Protein sources

Soybean meal and marama oil cake were used largely as protein sources. Marama beans were purchased from farmers in Kweneng Districts of Botswana. The marama bean samples were manually cracked before oil is extracted by cold press method which retains nutrients and flavour otherwise destroyed by heat that arise from conventional methods of oil extraction. The obtained marama cake was subjected to chemical analysis (Table 1).

Table 1
Chemical composition of Marama beans

Parameter	% of dry matter
Dry matter	95.05
Ash	3.03
Moisture	4.97
Protein	39.03
Neutral Detergent Fibre	21.00
Acid Detergent Fibre	31.33
Acid Detergent Lignin	5.29

2.3 Diets formulation

Five experimental diets were formulated with the inclusion of marama bean to meet the nutritional requirements for broiler chickens at grower and finisher phases as recommended by Ross Broiler: Nutrition Specifications (2019). The isonitrogenous and isocaloric experimental diets were formulated by partially replacing soybean meal with marama bean meal (MB) in broiler diets at 0% (MB0, control), 7% (MB7), 14% (MB14), 21% (MB21) and 28% (MB28). The ingredients and compositional analysis of the diets are presented in Tables 2 and 3.

Table 2
Ingredients composition of experimental diets fed to broiler chickens

¹ Dietary treatments										
	Grower					Finisher				
	MB0	MB7	MB14	MB21	MB28	MB0	MB7	MB14	MB21	MB28
Yellow Maize 8.0%	55.2	55.8	56.4	57.4	58.5	64.0	64.4	64.8	65.2	65.6
Fullfat Soya	12.0	12.0	12.0	8.4	2.9	12.0	12.0	12.0	12.0	12.0
Marama Bean Meal	0.0	1.6	3.3	4.9	6.5	0.0	1.0	2.0	3.1	4.1
Soya O/C 47%	23.2	21.8	20.4	21.7	24.5	14.6	13.7	12.8	11.9	11.1
Sunflower O/C 36%	3.5	3.5	3.5	3.5	3.5	4.0	4.0	4.0	4.0	4.0
Limestone	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1
Mono Calcium Phos	0.9	0.9	0.9	1.0	1.0	0.6	0.6	0.6	0.6	0.6
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium Bicarbonate	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
DL Methionine	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2
L-Threonine	0.1	0.1	0.1	0.1	0.1	0.002	0.01	0.01	0.01	0.02
L- Tryptophan	0.01	0.01	0.01	0.01	0.01	0.003	0.003	0.002	0.001	0.0
Lysine HCL	0.3	0.3	0.3	0.4	0.4	0.2	0.2	0.2	0.2	0.2
Crude Soya Oil mixer	2.1	1.2	0.3	0.0	0.0	2.4	1.9	1.3	0.8	0.2
Lignobond	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Grower/Finisher Premix	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2
Axtra Phy10000 Broiler	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Anti-Oxidant	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Salinomycin 12%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

¹Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean;

²ME = Metabolizable Energy.

¹ Dietary treatments										
Zinc Bacitracin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean; ² ME = Metabolizable Energy.										

Table 3
Nutrition composition of experimental diets fed to broiler chickens

¹ Dietary treatments										
	Grower					Finisher				
	MB0	MB7	MB14	MB21	MB28	MB0	MB7	MB14	MB21	MB28
Dry matter (g/kg)	888.8	889.4	890.0	891.1	892.5	885.7	886.1	886.5	886.9	887.2
² ME (MJ/kg)	12.98	12.98	12.98	12.98	12.98	13.40	13.40	13.40	13.40	13.40
Crude Protein (g/kg)	215.0	215.0	215.0	215.0	215.0	180.0	180.0	180.0	180.0	180.0
Lysine (g/kg)	12.2	12.2	12.2	12.2	12.2	9.1	9.1	9.1	9.1	9.1
Methionine (g/kg)	7.2	7.2	7.2	7.2	7.2	4.9	4.9	4.9	4.9	4.9
Threonine (g/kg)	8.1	8.1	8.1	8.1	8.1	5.9	5.9	5.9	5.9	5.9
Tryptophan (g/kg)	2.3	2.3	2.3	2.3	2.3	1.9	1.9	1.9	1.9	1.9
Crude Fat (g/kg)	67.2	65.1	63.1	60.6	57.8	73.4	72.1	70.8	69.6	68.3
Crude Fibre (g/kg)	36.6	36.8	37.0	36.5	35.7	36.5	36.6	36.7	36.8	36.9
Phosphorus (g/kg)	4.5	4.5	4.5	4.5	4.5	3.8	3.8	3.8	3.8	3.8
Calcium (g/kg)	9.0	9.0	9.0	9.0	9.0	7.6	7.6	7.6	7.6	7.6
Chloride (g/kg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium (g/kg)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Density (kg/m)	674.7	671.3	667.8	666.0	665.1	680.9	678.7	676.5	674.4	672.2
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean; ² ME = Metabolizable Energy.										

2.4 Animals and experimental design

A total of 300 unsexed day-old broiler chicks were randomly allocated to 40 pens (each pen measuring 2.0 x 1.5 x 1.7 meters) designed to meet the animal welfare standards for optimum production in broiler chickens.

The pens were also randomly divided into five (5) treatments. Each treatment had eight replicate pens with a pen holding eight chicks as the experimental unit in a completely randomized design (CRD). Upon arrival, the chicks were offered stress packs. The chicks were fed commercial starter diets from 1 to 14 days. Experimental diets were offered from grower phase (15 to 28 days) and finisher phase (29 to 37 days). Water and feed were provided ad-libitum.

2.5 Feed intake and growth performance

Feed intake was measured daily and weight gain measured weekly. All birds from the forty pens were weighed at the beginning of the trial at day 11 (initial body weight) and subsequently weighed weekly (14, 21, 28 and 35 days) using a weighing scale (TSW Adam equipment, SA). The feed offered to the birds and left overs (refusals) were weighed daily. The average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) for each feeding phase were calculated as:

$$ADFI = \frac{\text{Feed offered} - \text{feed refused}}{14 \text{ days}}$$

$$ADG = \frac{\text{Finish weight} - \text{Start weight}}{\text{Age (days)}}$$

$$FCR = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

2.6 Protein utilisation efficiency

Protein consumed (PC g/bird) was calculated by multiplying the concentration of crude protein (CP_d) in the diet (g/kg DM consumed) by daily feed intake (FI kg/bird), while the protein efficiency ratio (PER g/kg) was calculated by dividing mean body weight gain (BWG) by the mean protein consumed. Specific growth rate (SGR), which is percent growth per feeding phase and growth efficiency (GE) was calculated using the following formulas:

$$PC = FI \times CP_d$$

$$PER = \frac{BWG}{PC}$$

$$SGR = \frac{(\text{In final weight} - \text{In initial weight})}{14d} \times 100$$

$$GE = \frac{BWG}{\text{Initial weight}}$$

2.7 Haematology and serum biochemistry

From each pen, 2 broilers were randomly selected for blood collection at the 28th day of the experiment. Blood was collected from the brachial vein using 21 gauge needle and syringe into two types of tubes; the red top anti-coagulant free vacutainer tubes and the purple top ethylene diamine tetra-acetic acid (EDTA) coated vacutainer tubes. Blood collected in the red top anti-coagulant vacutainer tubes was used for serum biochemical indices analysis. Liver enzyme activity, serum metabolites and mineral content were quantified using an auto-analyser (Hitachi-704, Boehringer Mannheim Ltd, Germany). Likewise, blood collected in the purple top vacutainer tubes was used for haematological parameters analysis. The Idexx lasercyte

(Haematology analyser) was used to analyse for haematocrit, haemoglobin, erythrocyte, leucocyte, neutrophils, lymphocytes, monocytes, eosinophil and normoblasts.

2.8 Data analysis

Data on growth, protein utilisation efficiency, haematology and serum biochemistry parameters measured was analysed using GLM procedure of SAS (2010) with diet as the only fixed effect. Data on cumulative weight gain was measured on a weekly basis and analysed using mixed model procedure of SAS (2010) that take into consideration the effect of both diet and week of measurement. Proc univariate procedure of SAS (2010) was used to test for normality. The PDIFF option of SAS (2010) was used to perform pairwise comparisons of the least square means. Where significant the quadratic response surface was done to determine the optimum inclusion level of marama bean

The statistical models was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} = observation (growth parameters and blood parameter), μ = population mean constant common to all observations, T_i = effect of diet, and ε_{ij} = random error term.

$$Y_{ijk} = \mu + T_i + W_j + (T * W)_{ij} + \varepsilon_{ijk}$$

Where: Y_{ij} = observation (Cumulative weight gain parameter), μ = population mean constant common to the observation, T_i = effect of diet, $T*W_{ij}$ = effect of diet interacting with week and ε_{ij} = random error term. For all tests, the level of significance was set at ($P < 0.05$).

3. Results

3.1 Growth performance

Diets had no effect ($P > 0.05$) on ADFI in both the grower and finisher phases although a marginal decline was observed in diets containing marama bean. However, diet had an effect ($P < 0.05$) on ADG and FCR in both the grower and finisher phases (Table 4). Diets containing marama bean had lower ADG compared to the control diet. A negative linear relationship existed between marama inclusion level and ADG. In both phases, the birds fed diet containing 7% marama bean (MB7) had higher ADG compared with other marama bean containing diets. Over the experimental period, the birds fed MB0 diet had high cumulative weight gain followed by the birds fed MB7 diet. (Fig. 1). Birds fed MB28 diet consistent had the lowest cumulative weight gain. With regards to FCR, a similar trend was reflected. Birds fed the control diet has the lowest ($P < 0.05$) values for FCR in both the grower and finisher phases followed by those fed MB7. A positive linear relationship existed between marama inclusion level and FCR in both feeding phases. Birds fed the MB0 diet has the lowest ($P < 0.05$) values for FCR in both the grower and finisher phases followed by those fed MB7.

Table 4
The effect of Marama based diets on growth performance in broiler chickens

¹ Dietary treatments						
Feeding phase	MB0	MB7	MB14	MB21	MB28	SEM
<i>Grower</i>						
ADFI (g)	119.91	111.36	110.55	116.16	108.46	4.57
ADG (g)	88.32 ^a	73.23 ^b	70.21 ^b	73.02 ^b	64.07 ^b	3.97
FCR	1.36 ^a	1.57 ^b	1.60 ^b	1.60 ^b	1.72 ^b	0.08
<i>Finisher</i>						
ADFI (g)	247.17	244.47	240.38	234.84	229.79	7.93
ADG (g)	236.21 ^a	184.76 ^b	169.03 ^{bc}	167.30 ^{bc}	156.69 ^c	8.46
FCR	1.10 ^a	1.33 ^b	1.43 ^b	1.41 ^b	1.47 ^b	0.07
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean & MB28 = experimental broiler diet containing 28% marama bean. ² ADFI = Average Daily Feed Intake; ³ ADG = Average Daily Gain; ⁴ FCR = Feed Conversion Ratio. ⁵ SEM = Standard Error of Means; ^{a,b,c} In a row, dietary treatment means with common superscripts do not differ ($p > 0.05$).						

3.2 Protein utilisation efficiency

Diet had a significant effect ($P < 0.05$) on all protein utilisation efficiency parameters apart from the PC (Table 5). In both the grower and finisher phases, PER was higher in birds fed the control diet. SGR was also higher ($P < 0.05$) in birds fed the control diet followed by those fed MB7 diets in both feeding phases. However, with regards to GE, birds fed the MB21 had higher value compared to the other diets in the grower phase. In the finisher phase, the birds fed the MB0 had higher GE value followed by those fed MB21 diet. As with growth performance parameters, a linear negative relationship existed between MB inclusion level and PER and SGR. Nevertheless. The relationship between MB inclusion and GE was non-significant in both feeding phases.

Table 5
The effect of Marama based diets on protein utilization in broiler chickens

¹ Dietary treatments						
Parameters	MB0	MB7	MB14	MB21	MB28	SEM
<i>Grower</i>						
² PC (g/bird)	2398.26	2394.18	2376.91	2497.52	2331.81	96.30
³ PER (g/Kg)	0.04 ^a	0.03 ^b	0.03 ^b	0.03 ^b	0.03 ^b	0.001
⁴ SGR (%)	42.80 ^a	29.99 ^b	28.24 ^b	31.21 ^b	25.01 ^b	2.26
⁵ GE	0.44	0.32	0.33	0.50	0.31	0.08
<i>Finisher</i>						
PC (g/bird)	4449.04	4400.53	4326.78	4227.05	4136.14	142.78
PER (g/Kg)	0.05 ^a	0.04 ^b	0.04 ^b	0.04 ^b	0.04 ^b	1.26
SGR (%)	130.85 ^a	102.14 ^b	98.67 ^b	95.42 ^b	85.61 ^c	2.80
GE	1.21	0.83	0.80	1.18	0.77	0.22
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean; ² PC = Protein Consumed; ³ PER = Protein Efficiency Ratio; ⁴ SGR = Specific Growth Rate; ⁵ GE = Growth Efficiency; ⁶ SEM = Standard Error of Means; ^{a,b,c} In a row, dietary treatment means with common superscripts do not differ (p > 0.05).						

3.3 Haematology and serum biochemistry

The effect of diet on haematology is presented on Table 6 while Table 7 shows the effect on diet on serum biochemical parameters. Diet had no effect on all haematological parameters apart from the basophils. Broilers fed MB14 diets had highest ($P < 0.05$) basophil concentration while those fed the MB7 diet had the lowest. With regards to serum biochemistry, diet significantly ($P < 0.05$) affected all parameters except glucose, urea, creatinine and γ -glutamyl transferase. The birds fed control diet had the highest amounts of calcium, phosphorus and total albumin followed by those fed the MB7 diet. However, total protein and total globulin and were higher in the birds fed MB7 diet followed by those fed MB14 diet. Total bilirubin and cholesterol were also higher in the birds fed MB7 diet compared to those fed MB0 diet. With regards to liver enzymes, higher values for alkaline transferase (ALT) were obtained in all diets containing marama bean compared with the control. The positive linear relationship existed between marama inclusion level and levels of ALT.

Table 6
Haematological parameters of broiler chickens fed marama based diets

¹ Dietary treatments						
Parameters	MB0	MB7	MB14	MB21	MB28	SEM
Erythrocytes (x 10 ¹² /L)	1.85	2.14	1.72	1.78	2.09	0.31
Haematocrit (%)	11.76	13.50	10.20	10.96	13.22	2.26
Haemoglobin (g/dL)	10.04	9.95	8.66	9.33	8.83	0.48
Neutrophils (x 10 ⁹ /L)	37.45	32.86	33.14	39.60	39.92	6.86
Lymphocytes (x10 ⁹ /L)	40.99	48.64	46.36	42.48	42.58	8.72
Monocytes (10 ⁹ /L)	16.93	13.01	13.05	12.19	14.16	2.28
Eosinophils (x 10 ⁹ /L)	4.05	4.95	6.48	5.13	2.81	1.74
White blood cells (x 10 ⁹ /L)	27.44	30.13	23.50	28.49	25.60	4.41
Basophils (x 10 ⁹ /L)	0.59 ^{ab}	0.50 ^b	1.00 ^a	0.63 ^{ab}	0.53 ^b	0.15
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean; ² SEM = Standard Error of Means; a,b,c In a row, dietary treatment means with common superscripts do not differ (p > 0.05).						

Table 7
Serum biochemical parameters of broiler chickens fed marama based diets

¹ Dietary treatments						
Parameters	MBO	MB7	MB14	MB21	MB28	SEM
Glucose (mg/dL)	5.38	6.61	6.07	7.19	7.81	1.05
Urea (mmol/L)	0.68	0.66	0.66	0.62	0.68	0.26
Creatine (μ/L)	9.00	9.00	9.20	9.00	9.00	0.09
Calcium (mmol/L)	2.45 ^a	1.55 ^b	1.43 ^b	1.21 ^b	1.57 ^b	0.14
Phosphorus (mmol/L)	2.99 ^a	2.57 ^{ab}	2.24 ^b	2.07 ^b	2.71 ^{ab}	0.23
Total Protein (g/L)	46.50 ^{ab}	53.00 ^a	51.60 ^{ab}	40.40 ^b	45.80 ^{ab}	4.09
Albumin (g/L)	15.67 ^a	13.80 ^{ab}	12.00 ^{bc}	10.40 ^c	12.40 ^{bc}	0.91
Globulin (g/L)	30.83 ^{ab}	39.40 ^{ab}	40.00 ^a	29.80 ^b	33.40 ^{ab}	3.28
Total bilirubin (μmol/L)	10.50 ^a	12.60 ^a	6.60 ^{ab}	2.80 ^b	7.80 ^{ab}	2.08
Alkaline phosphatase (IU/L)	313.00 ^a	934.25 ^b	454.8 ^a	643.50 ^{ab}	947.50 ^b	142.19
Gamma-glutamyl transferase (IU/L)	19.17	22.00	24.20	23.00	27.00	3.06
Cholesterol (mg/dL)	2.68 ^a	3.53 ^b	2.67 ^a	2.25 ^a	2.70 ^a	0.26
¹ Dietary treatments: MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean; ² SEM = Standard Error of Means; a,b,c In a row, dietary treatment means with common superscripts do not differ (p > 0.05).						

4. Discussion

The results of this study shows showed that inclusion of marama bean in diets did not depress feed intake. Ferket and Gerket (2006) postulated that maintaining maximum feed intake is the single-most important factor that determines growth and nutrient utilization efficiency. However, the maintenance of feed intake in the current study did not translate to the similarity in growth performance of all the birds across dietary treatments in both the grower and finisher phases. Contrary to observation by Olukosi et al. (2019) and Usayran et al. (2014), replacement of soya bean with graded levels of marama bean resulted in a linear decline in ADG and increase in FCR. The declining ADG and inclining FCR with mamara bean inclusion levels could be a manifestation of the effect of some intrinsic characteristics of marama bean which could have reduced feed utilisation efficiency. The intrinsic characteristics, particularly the high levels of trypsin inhibitors could have the effect of depressing digestion of dietary protein and utilization of nutrients in diets resulting in poor growth rates as observed in other studies (Manyeula et al., 2013; Palliyeguru et al., 2011; Pacheco et al., 2014). Marama bean is reported to have trypsin inhibitors which makes about 5–10% of total protein (Nadaraja et al.,

2010) and dietary fibre varying between 18.7–26.8% (Holse et al., 2010). Trypsin inhibitors are reported to form complexes with pancreatic proteases reducing their activity in the small intestine (Heger et al., 2016). This subsequently affects digestion of dietary protein and utilization of nutrients in diets resulting in poor growth rates.

The probable depressant effect of trypsin inhibitor contained in marama bean was also manifested by the reduced protein utilisation efficiency. While there was some decline in PER, the SGR and GE suppression was more apparent. This strongly supports the belief that some intrinsic factors, including trypsin inhibitor, have the ability to negatively influence protein digestion dynamics through alterations of some endogenous enzymes functions (Sarikhani et al., 2010), increasing digesta viscosity which interferes with digestive enzymes activities (Günel et al., 2004) and causing perturbations in intestinal microflora plasticity (Diseth et al., 2018; Malayoğlu et al., 2010; Eits et al. 2002; Jafarnejad and Sadegh, 2011). The trypsin inhibitors might also cause an overactive and enlarged pancreas (Ivarsson and Wall, 2017). While this is an initial attempt to assess the efficacy of marama bean inclusion in broiler diet, further investigations can be done to explore treatment methods of marama bean, including heat treatment, which reduces the effects of antinutritional factors. Heat treatment has been observed to be effective in mitigating trypsin inhibitor effects in some bean varieties as trypsin inhibitors have been observed to be heat labile (Ivarsson and Wall, 2017; Rubio et al., 1990).

Hematology is a useful diagnostic tool in veterinary medicine (Merck Manual, 2012) that is commonly to assess health status of birds housed in cage systems. The tool allows for detection of various stresses that may be environmental, nutritional, and pathological in nature (Toghyani et al., 2010; Li et al., 2015). Results from the study showed lack of influence of diet on almost all the hemato parameters. All hematological indices for the birds across treatments were observed to be within the anticipated ranges for healthy broilers (Merck Manual, 2012). Nevertheless, the influence of diet on some serum biochemical parameters was quite significant. While most of the serum parameters were within the expected range (Merck Manual, 2012), reduced level of total albumin and elevated levels of total bilirubin and alkaline phosphatase may be indicative of the strain subjected to the liver in dealing with antinutritional factors toxicity in diets containing marama bean. More critically, the high levels of ALP in marama fed birds are reflective of an overworking liver as the body fight to reduce the effects of mainly trypsin inhibitors as observed in other studies (Usayran et al., 2014; Viveros et al., 2007). The increase in cholesterol with inclusion of marama bean is however consistent with finding from other studies (Usayran et al., 2014).

5. Conclusion

In conclusion, the partial replacement of soya bean meal with graded levels of marama bean meal resulted in a linear decline ADG and an incline in FCR. In addition the inclusion of marama bean meal also resulted in a decrease in protein utilisation efficiency which could be attributed to higher levels of trypsin inhibitor in marama bean meal. This was also confirmed by the elevation of some blood parameters particularly bilirubin and ALP. Overall, inclusion of marama bean meal in diets at 7% provided the optimum performance. While this is a first attempt to assess the efficacy of inclusion of marama bean meal in broiler diets, more research need to be done, in particular, on the heat treatment, enzyme inoculation and use of some organic acids that have

potential to reduce the effects of ant nutritional factors in marama bean meal and improve performance of marama bean fed broilers.

Declarations

Acknowledgements

We acknowledge NWU Department of Animal Science for providing the infrastructure and the research of animals. The assistance rendered by the NWU Animal Science Department is greatly appreciated.

Author contributions

S Tjetjoo: Conceptualization, writing - original draft, Formal analysis, Validation. **U Marume:** Conceptualization, writing - review and editing, Supervision and funding acquisition.

Data Availability

The data sets used in the current study are available from the corresponding author on request.

Declaration of Competing Interest

All authors declare that they have no conflicts of interests.

Funding

We are grateful to the NWU Department of Animal Science for provision of research funds used in the study.

Ethical approval

The experimental procedures were approved by the NWU-ANIMPROD Research Ethics Committee of North-West University following the guidelines for use and care of animals in research and the following ethics number was granted: NWU-00238-18-A5

References

1. Amarteifio, J.O., Moholo, D., 1998. The chemical composition of four legumes consumed in Botswana. *Journal of Food Composition and Analysis*, 11, 329–332. <https://doi.org/10.1006/jfca.1998.0595>.
2. Babiker, M.S., 2012. Chemical composition of some non-conventional and local feed resources for poultry in Sudan. *International Journal of Poultry Science*, 11, 283–287. <http://dx.doi.org/10.3923/ijps.2012.283.287>.
3. Bower, N., Hertel, K., Oh, J., Storey, R., 1988. Nutritional evaluation of marama bean (*Tylosema esculentum*, Fabaceae): Analysis of the seed. *Economic Botany*, 42, 533–540. <https://doi.org/10.1007/BF02862798>.
4. Disetlhe, A.R.P., Marume U., Mlambo, V., 2018. Humic acid and enzymes inclusion in canola based-diets generate different responses in growth performance, protein utilization dynamics and hemato-

- biochemical parameters in broiler chickens. *Poultry Science*, 97 (1), 2745–2753.
<https://doi.org/10.3382/ps/pey047>.
5. Eits, R.M., Kwakkel, R.P., Verstegen, M.W.A., Stoutjesdijk, P., De Greef, K.H., 2002. Protein and lipid deposition rates in male broiler chickens: separate responses to amino acids and protein-free energy. *Poultry Science*, 81, 472–480. <https://doi.org/10.1093/ps/81.4.472>.
 6. Ferket, P.R., Gernat, A.G., 2006. Factors that affect feed intake of meat birds: A review *International Journal of Poultry Science*, 5, 905–911.
 7. Günal, M., Yaşar, S., 2004. Performance and some digesta parameters of broiler chickens given low or high viscosity wheat-based diets with or without enzyme supplementation. *Turkish Journal of Veterinary and Animal Sciences*, 28, 323–327.
 8. Heger, J., Wiltafsky, M., Zelenka, J., 2016. Impact of different processing of full-fat soybeans on broiler performance. *Czech Journal of Animal Science*, 61, 57–66. <https://doi.org/10.17221/8728-CJAS>.
 9. Holse, M., Husted, S., Hansen, Å., 2010. Chemical composition of marama bean (*Tylosema esculentum*) – A wild African bean with unexploited potential. *Journal of Food Composition and Analysis*, 23, 648–657. <https://doi.org/10.1016/j.jfca.2010.03.006>.
 10. Ivarsson, E., Wall, H., 2017. Effects of toasting, inclusion levels and different enzyme supplementations of faba beans on growth performance of broiler chickens. *Journal of Applied Poultry Research*, 26(4), 467–475. <https://doi.org/10.3382/japr/pfx016>.
 11. Jafarnejad, S., Sadegh, M., 2011. The effects of different levels of dietary protein, energy and using fat on the performance of broiler chicks at the end of the third weeks. *Asian Journal of Poultry Science*, 5, 35–40. <http://dx.doi.org/10.3923/ajpsaj.2011.35.40>.
 12. Keegan, A.B., van Staden J.V., 1981. Marama bean, *Tylosema esculentum*, a plant worthy of cultivation. *South African Journal of Science*, 77, 387. https://hdl.handle.net/10520/AJA00382353_1549.
 13. Khan, M.K., Akbar, M.A., Khaleduzzana, A.B.M., Rahman, M.M., 2009. Utilization of leucaena and sesbania leaf meals as protein supplements in broiler ration. *Bangladesh Journal of Animal Science*, 38, 123–131.
 14. Lara, L.J., Rostagno, M.H., 2013. Impact of heat stress on poultry production. *Animal*, 3, 356–369. <https://dx.doi.org/10.3390.ani3020356>.
 15. Li, X.L., He, W.L., Wang, Z.B., Xu, T.S., 2015. The effects of Chinese herbal mixture on performance, egg quality and blood biochemical parameters of laying hens *Journal of Animal Physiology and Animal Nutrition*, 100(6), 1041–1049. <https://doi.org/10.1111/jpn.12473>.
 16. Malayoğlu, H.B., Yilmaz, H., Turan, N., 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers on wheat-soybean meal diets. *British Poultry Science*, 51, 66–80. <https://doi.org/10.1080/00071660903573702>.
 17. Manyeula, F., Tsopito, C., Kamau, J., Mogotsi, K.K., Nsoso, S.J., Moreki, J.C., 2013. Effect of *Imbrasia belina* (westwood), *Tylosema esculentum* (Burchell) Schreiber and *Vigna subterranean* (L) as protein sources on growth and laying performance of Tswana hens raised under intensive production system. *Scientific Journal of Animal Science*, 2, 1–8. <https://doi.org/10.14196/sjas.v2i1.472>

18. Mengesha, M., 2012. The issue of feed-food competition and chicken production for the demands of foods of animal origin. *Asian Journal of Poultry Science*, 6, 31–43.
<http://dx.doi.org/10.3923/ajpsaj.2012.31.43>.
19. Merck Manual., 2012. Merck Manual of diagnostic therapy, MSD Vet Manual 10th Ed. Kenilworth, NJ, USA.
20. Moreki, J.C., Gabanakgosi, K., 2014. Potential use of *Moringa Olifera* in poultry diets. *Global Journal of Animal Scientific Research*, 2, 109–115.
21. Mùseler, D.L., Schönfeldt H.C., 2006. The nutrient content of marama bean (*Tylosema esculentum*), an underutilized legume from Southern Africa. *Agricola*, 16, 7–13.
22. Nadaraja, D., Weintraub, S.T., Hakala, K.W., Sherman, N.E., Starcher, B., 2010. Isolation and partial sequence of a kunitz-type elastase specific inhibitor from marama bean (*Tylosema esculentum*). *Journal of Enzyme Inhibition and Medicinal Chemistry*, 25, 377–382. <https://doi.org/10.3109/14756360903179500>.
23. Nasr, J., Kheiri, F., 2012. Effects of lysine levels of diets formulated based on total or digestible amino acids on broiler carcass composition. *Brazilian Journal of Poultry Science*, 14, 249–258.
<https://doi.org/10.1590/S1516-635X2012000400004>.
24. National Research Council., 2011. Guide for the Care and use of Laboratory Animals. 8th Edition. The National Academies Press, Washington, DC, 20001
25. OECD/FAO., 2016. OECD-FAO Agricultural Outlook, OECD Agriculture statistics (database),
<http://dx.doi.org/10.1787/agr-data-en>.
26. Olukosi, O.A., Walker, R.L., Houdijk, J.G.M., 2019. Evaluation of the nutritive value of legume alternatives to soybean meal for broiler chickens. *Poultry Science*, 98(11), 5778–5788.
<https://doi.org/10.3382/ps/pez374>.
27. Pacheco, W.J., Stark, C.R., Ferket, P.R., Brake, J., 2014. Effects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreas. *Poultry Science*, 93, 2245–2252. <https://doi.org/10.3382/ps.2014-03986>.
28. Palliyeguru, M.W.C.D., Rose, S.P., Mackenzie, A.M., 2011. Effect of trypsin inhibitor activity in soybean on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks. *British Poultry Science*, 52, 359–367. doi: 10.1080/00071668.2011.577054.
29. Raha, S.K., 2007. Poultry industry in Bangladesh: Is it growing? *Bangladesh Journal of Agricultural Economics*, 2, 93–101.
30. Ross. 2019., Broiler: Nutrition Specifications. Aviagen, Meyerton, South Africa.
31. Rubio, L. A., Brenes, A., Castano, M., 1990. The ~utilization of raw and autoclaved faba beans (*Vicia faba* L., var. minor) and faba bean fractions in diets for growing broiler chickens. *British Journal of Nutrition*, 63, 419–430. <https://doi.org/10.1079/bjn19900130>.
32. Sarikhan, M., Shahryar, H.A., Gholizadeh, B., Hosseinzadeh, M.H., Beheshti, B., Mahmoodnejad, A., 2010. Effects of insoluble fibre on growth performance carcass traits and ileum morphological parameters on broiler chick males. *International Journal of Agriculture and Biology*, 12, 531–536.
33. SAS. 2010. Users Guide; Statistical Analysis System Institute Inc.: Cary, NC, USA.

34. Sgavioli, S., Santos, E.T., Domingues, C.H.F., Quedros, T.C.O., Castiblanco, D.M.C., Andrade-Garcia, G.M., Amoroso, L., Nààs, I.A., Garcia, R.G., Baraidi-Artoni, S.M., 2016. Effect of high incubation temperature on the blood parameters of chicks. *Revista Brasileira de Ciencia Avicola*, 18, 41–47. <http://dx.org/10.1590/1806-9061-2015-0095>.
35. Siqueira, J.C., Sakomura, N.K., Dourado, L.R.B., Ezequiel, J.M.B., Barbosa, N.A.A., Fernandes, J.B.K., 2013. Diet formulation techniques and lysine requirements of 1 - to – 22-day old broilers. *Brazilian Journal of Poultry Science*, 15, 123– 134. <https://doi.org/10.1590/S1516-635X2013000200008>.
36. Tibe, O., Legwatagwata, B., Motlogelwa, K., 2016. The effect of processing on the anti- nutrients in selected legumes in Botswana. *Asian Journal of Agricultural and Food Sciences*, 4, 265– 269.
37. Toghyani, M., Toghyani, M., Gheisari, A., Ghalamkari, G., Mohammadrezaei, M., 2010. Growth performance, serum biochemistry and blood haematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). *Livestock Science*, 129, 173– 178. <https://doi.org/10.1016/j.livsci.2010.01.021>.
38. Usayran, N., Sha'ar, H., Barbour, G.W., Yau, S.K., Maalouf, F., Farran, M.T., 2014. Nutritional value, performance, carcass quality, visceral organ size, and blood clinical chemistry of broiler chicks fed 30% tannin-free fava bean diets. *Poultry Science*, 93(8): 2018–2027. <https://doi.org/10.3382/ps.2014-03872>.
39. Viveros, A., Centeno, C., Arija, I., Brenes. A., 2007. Cholesterol lowering effects of dietary lupin (*Lupinus albus* var. Multolupa) in chicken diets. *Poultry Science*, 86, 2631–2638. <https://doi.org/10.3382/ps.2007-00128>.

Figures

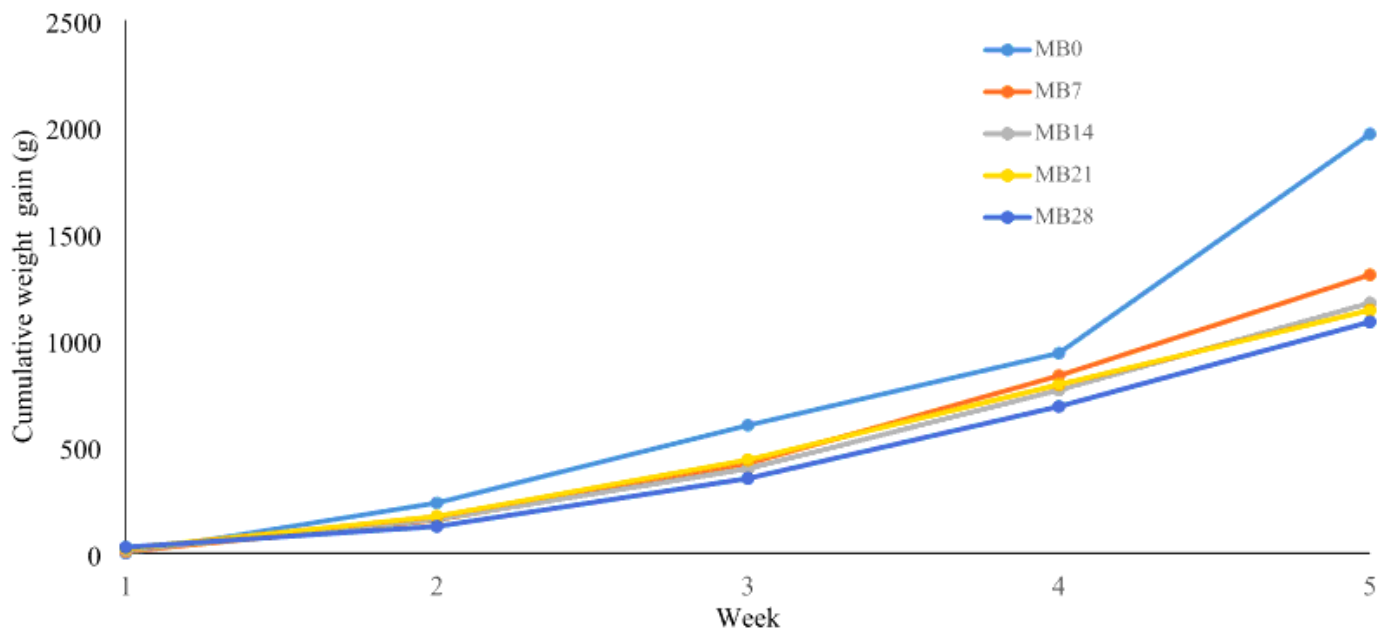


Figure 1

Cumulative weight gains of broiler chickens fed marama based diets

MB0 = commercial broiler diet (Control), MB7 = experimental broiler diet containing 7% marama bean, MB14 = experimental broiler diet containing 14% marama bean, MB21 = experimental broiler diet containing 21% marama bean and MB28 = experimental broiler diet containing 28% marama bean