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Morula (*Sclerocarya birrea)* kernel cake as a partial soybean meal replacer in Ross 308 broiler diets: Effects on feed utilisation, growth performance, and selected blood parameters

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Abstract

The effects of replacing soybean meal (SBM) with graded levels of morula kernel cake (MKC) on growth performance and haemo-biochemical parameters in broiler chickens were studied. Four iso-nitrogenous and iso-caloric diets were formulated by replacing the SBM component at 0, 40, 80 and 120 g/kg with MKC as protein source in grower and finisher diets. One hundred and sixty 2-week-old chicks were randomly allocated to the treatments, which were replicated five times (eight birds/pen). Growth performance and blood parameters were measured. Results showed that neither linear nor quadratic trends were observed for weekly feed intake (WFI), weekly weight gain (WWG), weekly feed conversion ratio (WFCR), and growth performance with MKC levels. However, packed cell volume (PCV) quadratically decreased ($Y=37.4\pm1.2+1.4\pm0.5+0.13\pm0.03X^2$; $R^2=0.39$; $p=0.005$) with MKC levels, heterophils increased ($y=50.9\pm1.8+1.18\pm0.73x$; $R^2=0.35$, $p=0.009$) while lymphocytes decreased linearly ($y=40.7\pm1.67-1.42\pm0.67x$; $R^2=0.35$, $p=0.02$) with MKC levels. There was a linear decrease ($Y=0.81\pm0.02-0.01\pm0.01x$; $R^2=0.2895$; $p=0.02$) in magnesium while phosphorus increased quadratically with MKC levels. Quadratic trends ($Y=17.2\pm0.6+0.7\pm0.2+0.04\pm0.02X^2$; $R^2=0.47$; $p=0.05$) were also observed in iron as MKC levels increased. All the parameters of blood biochemistry fell within the normal range for birds. The present results indicate that inclusion of MKC up to 120 g/kg does not result in adverse effects on diet utilisation, growth performance, and health status of Ross 308 broiler chickens, indicating that MKC has the potential to be used as an alternative protein source to SBM.

Keywords: broilers, growth, haematology, plant proteins, serum biochemistry
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Introduction

Broilers are meat-type chickens bred for economically important traits such as body weight, growth rate, and feed efficiency (Kareem-Ibrahim *et al*., 2021). In most commercial broiler operations, broiler chickens reach slaughter weight between four and seven weeks of age (Bessei, 2006), whereas slower growing breeds reach slaughter weight at approximately 14 weeks of age. Protein is one of the

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most important nutrients in poultry diets and is critical in maintaining and repairing tissues in organisms to enable proper growth and development (Bondari *et al*., 1981). By virtue of its amino acid constituents, protein plays a significant role not only in growth, but also in egg production, immunity, adaptation to the environment, and in many other biological functions. Chicken meat is a cheap animal protein in Botswana compared to beef, mutton, and chevon. Broilers yield good protein in their muscles when fed diets of high-quality protein but will never make a good source of animal protein for human consumption if fed poor protein sources.

In Botswana, the most common protein source for broilers in the commercial ration is SBM. The SBM is exotic to Botswana, hence it is expensive for farmers. Therefore, it is imperative to seek an alternative source of protein that would match SBM's nutritional composition and utilisation. One of the possible substitutes of SBM is morula kernel cake (MKC), which is a by-product of oil extraction from the dry seeds of the ripe morula fruit (Nyoka *et al*., 2015). Morula (*Sclerocarya birrea*) is an indigenous fruit tree distributed throughout most of sub-Saharan Africa (SSA). The fruits, which ripen between December and March, have a light-yellow skin (exocarp), with white flesh (mesocarp). The morula fruit is climacteric; they fall to the ground when unripe (i.e., when fruits are green), and ripen to a yellow colour on the ground after a few days. The fruit is a drupe with a single seed encased within the endocarp; morula fruit can have up to four seeds. They are succulent and tart with a strong and distinctive flavour (Mukaro, 2014). Inside is a walnut-sized, thick-walled stone (endocarp). The MKC is a novel, locally available, low-cost alternative protein supplement for livestock and poultry.

The rapidly increasing human population, particularly in the developing world, has increased the demand for animal protein, especially for pork and poultry products for human nutrition (World Bank Group, 2015). Proteins play an important role in the immune system and physiological growth or muscular development of animals. There are different sources of plant proteins that are used in formulating diets for broilers. The SBM is an expensive and widely used feed supplement with a high energy content and an amino acid profile close to MKC. Morula seed cake has not been commonly used in livestock feeding, especially for chickens; hence there is limited information on its effects on animal blood parameters or serum biochemistry. Low-protein diets have a transitory effect on muscle fibre size rather than any long-term effect on the numbers of such fibres (Timson *et al*., 1983). The plant-derived proteins (e.g., SBM, decorticated sunflower, and cottonseed cakes) and animal-derived proteins (e.g., fish meal) proteins are used in intensive non-ruminant animal (broiler, pullet, piggery, and aquaculture) production in SSA (Chivandi, 2012).

The MKC is a novel, locally available, potential alternative dietary protein supplement for beef cattle (Mlambo *et al*., 2011a), dairy cattle (Mdziniso *et al*., 2016), and goats (Mlambo *et al*., 2011b). It has a remarkably high crude protein content (470 g/kg DM) and an exceptionally high amino acid composition comparable to that of SBM, except for lysine (Mthiyane & Mhlanga (2017). Thus, MKC inclusion in broiler diets would supply much needed protein, energy, and other nutrients and this justifies this research. Therefore, this study was designed to determine nutrient digestibility, protein utilisation efficiency, growth performance, and haemo-biochemical parameters of Ross 308 broiler chickens fed MKC as a partial replacement for SBM in broiler diets. It was hypothesized that partial replacement of the SBM with the MKC in broiler diets would not reduce growth performance and health of chickens.

Material and methods

The procedures used to rear, handle, and collect blood were reviewed and approved (Ethics Number BUAN-2020-08) by the Animal Research Ethics Committee (AEC) of the Botswana University of Agriculture and Natural Resources (BUAN).

The study was carried out at the BUAN Content Farm, Sebele, Gaborone (25.94°S, 24.58°E; at an altitude of 991 metres) from April to July, 2020. Two weeks before the arrival of chicks, the poultry house was cleaned and the equipment was washed with water and Biogel (detergent) purchased from Neat Solution Company (Gaborone, South Eastern Districts, Botswana). Lastly, a mixture of formalin and salt was applied to the floor, wall-junctions, and around base posts.

The MKC was obtained from DLG Natural Pty Ltd, Gabane, Botswana, and the Optimix from Optifeeds (Pty) Ltd, Gaborone, Botswana, whilst crushed yellow maize was obtained from Botswana Agricultural Marketing Board, Gaborone, Botswana. Both SBM and MKC were pressed oil cakes (Manyeula *et al*., 2021). Broiler chickens were randomly allocated to 20 pens each measuring 1.0 × 1.0 × 1.0 m^3 to which the four experimental diets were randomly allocated. Each dietary treatment had five replicate pens with each pen holding eight broilers. The study was arranged in a completely randomized design (CRD) with the pen as the experimental unit. For the first three days of rearing, chicks were exposed to 24 h of light and thereafter 23 h of light until slaughter (i.e., 6 weeks). The experimental diets were formulated to meet the nutritional requirements for broiler chicken grower and finisher phases according to Opti Feeds (Pty) Ltd recommendations. Four diets were formulated by replacing SBM in commercial grower and finisher broiler diets with graded levels of MKC (Table 1) on a crude protein basis. The isonitrogenous and iso-caloric experimental diets were formulated by replacing the SBM component with MKC as follows: MKC0 = Broiler grower and finisher diet with no MKC inclusion, MKC40 = 40 g/kg MKC/kg SBM, MKC80 = 80 g MKC/kg SBM and MKC120= 120 g MKC/kg SBM (Tables 2 and 3).

Table 1 Chemical composition (g/kg) of morula kernel cake (MKC)

Nutrients	Amount
Dry matter	998
Crude protein	366
Crude fibre	150
Crude fat	378
Ash	59
Calcium	03
Phosphorus	05
Magnesium	06

Source: Manyeula *et al*., 2021

A total of 160, one-day-old broiler chicks were purchased from the Ross Breeder hatchery, Gaborone, Botswana and were placed and brooded in pens measuring 1.0 x 1.0 x 1.0 m^3 in a broiler house. Brooding temperature was kept between 32.5 and 33 ^oC for the first three days and was reduced by 1 ^oC every three days until room temperature was attained. Vitamins and electrolytes supplements (*Virbac* Animal Health, Phenix Stresspac, South Africa) were provided to the chicks for the first three days. Birds were phase-fed starting with the provision of starter ration from days 1 to 14. Experimental diets were only offered in the grower (14–28 d) and finisher (29–42 d) phases. Diets were formulated according to the commercial feed formulation standards to meet the nutrient requirements for the grower and finisher phases (Tables 2 and 3). Water fonts were cleaned and filled with clean, fresh water on a daily basis.

Table 2 Ingredients (g/kg) of experimental diets (as-fed basis)

	Experimental diets							
	Grower diets				Finisher diets			
	MKC0	MKC40	MKC80	MKC120	MKC0	MKC40	MKC80	MKC120
Ingredients								
Opti-mix	252.6	252.6	252.6	238.3	124.2	124.2	124.2	124.2
MKC	0	40	80	120	0	40	80	120
SBM	104.7	64.7	24.7	0	145.6	105.6	65.6	25.6
Maize	642.7	642.7	642.7	641.7	730.2	730.2	730.2	730.2
Total	1000	1000	1000	1000	1000	1000	1000	1000

[1]Diets, MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean was replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean was replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean was replaced with MKC

Table 3 Nutrient composition of experimental diets (as-fed basis)

	Experimental diets							
	Grower				Finisher			
	MKC0	MKC40	MKC80	MKC120	MKC0	MKC40	MKC80	MKC120
Proximate composition (g/kg)								
Moisture	84	80	80	91	70	70	81	113
Crude protein	172.1	177	177	162	155	154	15.7	155
Crude fat	51	53	44	48	40	5	59	56
Crude fibre	32	33	30	33	38	4	42	36
Ash	49	48	44	59	66	53	59	91
ME(MJ/kg)	123	119	115	121	124	119	119	116
Mineral Composition (g/kg, otherwise stated)								
Calcium	5	7	66	7	7	5	7	6
Phosphorus	4	4	4	4	4	4	4	4
Potassium	7	8	7	6	9	7	7	7
Magnesium	2	2	2	2	2	2	2	2
Sodium	4	3	3	2	2	3	3	2
Copper (ppm)	27.3	12	6	3.3	3.6	166	74	26
Iron (ppm)	243.9	127.5	139.4	119.4	167.3	110.5	113.8	101.7
Manganese (ppm)	244.4	184.5	193.0	181.9	121.3	178.3	188.8	200.9
Zinc (ppm)	288.1	110.1	90.6	96.7	75.1	85.3	93.5	108.7

[1]Diets; MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean was replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean was replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean was replaced with MKC

After week two, all the birds in each pen were tagged and initial individual bird weight was obtained using an electronic scale sensitive to 0.01 g (Adam Equipment S.A. (Pty) Ltd, Milton Keynes, UK) to determine average initial weight (930.75 ± 65.50 g), and subsequently on a weekly basis until the end of the experiment, i.e., at 6 weeks. Feed was weighed and offered in the feeders each with a capacity of 10 kg. Feed leftovers were weighed back and changed daily until the end of the experiment. Average weekly feed intake (AWFI), weight gain (WWG), feed conversion ratio (FCR), protein consumed (PC), protein efficiency ratio (PER), growth efficiency (GE), and specific growth ratio (SGR) were calculated using the formulae in Table 4.

Table 4 Formulae used to calculate growth performance parameters in the present study

Parameters	Formula	References
AWFI (g/week)	$Feed\ offered(g) - Feed\ refusals(g)/7\ days$	a, b,c
WWG (g/week)	$Finish\ weight\ (g) - Start\ weight(g)/7\ days$	a, b,c
Feed conversion ratio	$Feed\ consumed\ (g)/Weight\ (g)$	a, b,c
Protein consumed (g)	$Feed\ consumed\ (g) \times Crude\ protein\ of\ the\ diet$	b,c
Protein efficiency ratio	$Body\ weight\ gain\ (g)/Protein\ consumed\ (g)$	b,c
SGR (% d^{-1})	$Final\ weight - Initial\ weight/7\ days\ \times 100$	c
Growth efficiency	$Body\ weight(g)/Initial\ weight(g)$	c

AWFI: Average weekly feed intake; WWG: Weekly weight gain; SGR: Specific growth ratio; d: day; a: Matshogo *et al*. (2021); b: Manyeula *et al*. (2019); c: Disetlhe *et al*. (2018)

At the end of experimental period (6 w), one bird in each replicate/pen was randomly selected and blood aspirated (~2 ml for haematology and 4 ml for serum metabolites) from the wing using twenty-one-gauge needles into vacutainer tubes. For haematological indices (packed cell volumes (PCV), heterophils, lymphocytes, eosinophils, and basophils) ethylene diamine tetra-acetic acid (EDTA)-coated vacutainer tubes (purple top) were used, whereas for serum metabolite parameters (magnesium (Mg), phosphorus (P), potassium (K), total iron (Fe), total protein (TP), calcium (Ca), and albumen (AL)), anti-coagulant-free vacutainer tubes (red top) were used. Thereafter, the samples were taken to the Diagnofirm Medical Pathology laboratory in Gaborone, Botswana, for haematological and serum biochemistry analyses.

The AWFI, AWWG, and FCR data were analysed using the repeated measures analysis option in the general linear model procedure of Statistical Analysis System (SAS) using Model 1:

$$Y_{ijk} = \mu + d_i + T_j + (d * T)_{ij} + \varepsilon_{ijk}$$ Model 1

where: Y_{ijk} = response variable (feed intake, weight gain, growth rate, FCR, haematology, and serum biochemistry); μ = general mean; d_i = diet effect; T_j = the effects of time (in weeks); $(d \times T)_{ij}$ = the interaction effects between time and diets; ε_{ijk} = random error associated with observation ijk, assumed to be normally and independently distributed.

The PC, PER, GE, SGR, and blood chemistry data were analysed using one-way analysis of variance (ANOVA) using Statistical Analysis System (SAS) (SAS, 2010) using Model 2:

$$Y_{Ij} = \mu + d_i + \varepsilon_{ij}$$ Model 2

where: Y_{Ij} = response variable; μ = general mean; d_i = diet effect; ε_{ijk} = random error associated with observation ijk, assumed to be normally and independently distributed.

Average feed intake, AWG, FCR, and blood chemistry data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (SAS, 2010) was applied to describe the responses of broiler chickens to inclusion levels of MKC using the quadratic model of: $Y = ax^2 + bx + c$

where: Y = response variable; a *and* b are the coefficients of the quadratic equation; c = intercept; and x is dietary MKC level. All tested parameters were declared statistically significant at *P* <0.05 and the least mean squares were compared using the probability of difference option in SAS.

Results

Repeated measures analyses showed significant week × diet interaction effects on AWG but not on AFI and FCR. There were neither linear nor quadratic effects observed in feed intake, AWG, and FCR in all weeks. Diets substantially affected AWG in weeks 5 and 6 but not weeks 3 and 4 (Table 5). In week 5, broilers reared on diet MKC80 had the lowest (*P* <0.05) AWG compared to those on diets MKC0 and MKC40, which were statistically similar. However, no significant differences were observed between broilers reared on diets MKC0, MKC40, and MKC120 on AWG. In week 6, broilers reared on diet MKC80 had the highest AWG compared to those reared on diets MKC0, MKC40, and MKC120, which were similar (*P* <0.05).

Table 5 Average feed intake, weight gain, and feed conversion ratio of broilers fed diets containing morula kernel cake

	Diets[1]					Significance	
Item	MKC0	MKC40	MKC80	MKC120	SEM	Linear	Quadratic
Feed intake (g/birds)							
Week 3	720.8	739.9	681.1	690.9	47.3	NS	NS
Week 4	964.9	1032.9	1116.8	1208.4	246.0	NS	NS
Week 5	1015.4	1075.0	1093.1	1187.1	240.4	NS	NS
Week 6	994.2	1024.6	1097.1	1198.7	250.0	NS	NS
Weight gain(g)							
Week 3	336.9	339.0	335.4	337.6	3.6	NS	NS
Week 4	551.3	551.5	548.3	549.2	3.1	NS	NS
Week 5	656.1[a]	656.6[a]	649.5[b]	653.6[ab]	1.8	NS	NS
Week 6	791.1[b]	791.9[b]	800.2[a]	796.7[b]	3.0	NS	NS
Feed Conversion Ratio							
Week 3	2.1	2.2	2.0	2.1	0.1	NS	NS
Week 4	1.6	1.9	2.0	2.2	0.4	NS	NS
Week 5	1.6	1.6	1.7	1.8	0.4	NS	NS
Week 6	1.3	1.3	1.4	1.5	0.3	NS	NS

NS = not significant; [1]Diets: MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean was replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean was replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean was replaced with MKC; [a,b]Within a row, different superscripts denote significant differences (*P* <0.05) between dietary treatments; SEM = standard error of the mean

Table 6 shows that dietary treatment had no influence (*P* >0.05) on growth efficiency, specific growth rate, protein consumed, and protein efficiency in both the grower and finisher phases.

Table 6 Growth performance of broilers fed diets containing morula kernel cake

	Diets[1]					Significance	
Parameters[1]	MKC0	MKC40	MKC80	MKC120	SEM	Linear	Quadratic
Grower							
Growth efficiency	0.17	0.17	0.17	0.17	0.01	NS	NS
SGR (% d^{-1})	6.70	7.13	6.93	6.69	0.20	NS	NS
Protein consumed (g)	13.17	13.85	14.04	14.83	2.12	NS	NS
Protein efficiency ratio	3.73	3.46	3.46	3.41	0.57	NS	NS
Finisher							
Growth efficiency	0.11	0.11	0.11	0.11	.002	NS	NS
SGR (% d^{-1})	6.54	5.93	5.68	5.80	1.37	NS	NS
Protein consumed (g)	13.90	14.44	15.35	16.51	3.39	NS	NS
Protein efficiency ratio	6.54	5.93	5.68	5.80	1.37	NS	NS

NS = not significant; [1]Diets: MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean was replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean was replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean was replaced with MKC; SGR = Specific growth rate; SEM = standard error of the mean

There were no significant linear and quadratic trends of haematological parameters (Table 7), except for heterophils and lymphocytes, in response to dietary levels of MKC. Heterophils linearly increased (y = 50.9 ± 1.8 + 1.18 ± 0.73x; R^2 = 0.35, *P* = 0.009) while lymphocytes decreased linearly (y = 40.7 ± 1.67 - 1.42 ± 0.67x; R^2 = 0.35, *P* = 0.02) with MKC levels.

Table 7 Selected haematological parameters (%) in 6-week-old broilers fed graded levels of morula kernel cake as a partial replacement of soybean meal

	[1]Diets					Significance	
Parameters	MKC0	MKC40	MKC80	MKC120	SEM	Linear	Quadratic
Packed cell volume	37.2[b]	41.8[a]	40.2[ab]	36.8[b]	1.25	NS	*
Heterophils	50	57.6	54.6	59.2	1.62	*	NS
Lymphocytes	41.4	34.2	36.2	33.8	1.59	*	NS
Eosinophils	6	6.2	7.6	5.6	0.99	NS	NS
Basophils	2.6	2.0	1.6	1.4	0.47	NS	NS

NS = not significant; * = p<0.05; [1]Diets: MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean ingredients were replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean ingredients were replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean ingredients were replaced with MKC; SEM = standard error of the mean.

There were no significant (*P* >0.05) linear and quadratic trends in all serum biochemical parameters except for PCV, Mg, P, and total Fe (Table 8). Packed cell volume decreased quadratically (Y = 37.4 ± 1.2) +1.4 ± 0.5x + 0.13 ± 0.03) X^2; R^2 = 0.39; *P* = 0.005) while P increased quadratically (Y = 2.2 ± 0.1 + 0.1 ± 0.03x +0.01 ± 0.003X^2; R^2 = 0.38; *P* = 0.01) with incremental levels of MKC. There was a linear decrease (Y = 0.81 ± 0.02 - 0.01 ± 0.01X; R^2 = 0.2895; *P* = 0.02) in Mg with MKC levels. However, quadratic trends (Y = 17.2 ± 0.6 + 0.7 ± 0.2x + 0.04 ± 0.02X^2; R^2 = 0.47; *P* = 0.05) were observed in total Fe in response to inclusion levels of MKC.

Table 8 Serum parameters (Mmol/l, unless stated otherwise) in 6-week-old broilers fed graded levels of morula kernel cake as a partial replacement for soybean meal

	Diets					Significance	
Parameters	MKC0	MKC40	MKC80	MKC120	SEM	Linear	Quadratic
Magnesium	0.9[a]	0.7[ab]	0.8[ab]	0.7[b]	0.2	*	NS
Phosphorus	2.3[ab]	2.0[b]	2.2[ab]	2.4[a]	0.08	NS	*
Potassium	4.8	4.5	4.7	4.8	0.11	NS	NS
Total iron	17.3[a]	14.7[b]	14.7[b]	14.6[b]	0.57	*	*
Total protein (g/l)	32.2	32.6	33	31.6	1.25	NS	NS
Calcium	3.3	3.3	3.3	3.3	0.05	NS	NS
Albumin (g/l)	4.8	4.8	4.8	4.6	0.26	NS	NS

NS = not significant * = P <0.05; [1]Diets: MKC0 = commercial broiler diet with no MKC; MKC40 = a commercial broiler diet in which 40 g/kg of soybean ingredients were replaced with MKC; MKC80 = a commercial broiler diet in which 80 g/kg of soybean ingredients were replaced with MKC; MKC120 = a commercial broiler diet in which 120 g/kg of soybean ingredients were replaced with MKC. [a,b]Within a row, different superscripts denote significant differences (P <0.05) between dietary treatments; SEM = standard error of the mean

Discussion

This study evaluated MKC as a partial replacement of SBM in commercial broiler diets. The MKC is a rich source of sulphur amino acids such as methionine (0.79%) and cysteine (1.18%) (Mthinyane & Mhlanga, 2017; Malebana *et al*., 2018) which is comparatively lower in SBM. Therefore, partially replacing SBM with MKC in the diet of broiler chickens might have balanced the nutritional composition of the diet, which culminated in improved growth performance. The MKC is very rich in protein, energy, minerals (Malebana *et al*., 2018), and other nutrients needed by broilers for optimum growth. However, in the current study, for both grower and finisher phases, graded inclusion of MKC in broiler diets effected similar feed intake and growth performance in broiler chickens across dietary treatments. This suggests that broilers were able to utilise diets containing MKC to the same extent as the control diet, implying that the concentration of anti-nutrients such as tannins, saponins, and fibre in the diets were negligible. Similar performance across treatments in terms of FI, AWG, FCR, GE, SGR, PC, and PER at different growth stages could be attributed to the diets that were isonitrogenous and iso-caloric in this study. Mazizi *et al*. (2019) also reported no treatment effects on growth parameters of broiler Japanese quail (*Cortunix cortunix japonicum)* fed diets containing MKC. In contrast, Mthiyane & Mhlanga (2017) reported depressive effects of FI, AWG, and FCR in broiler chickens fed diets containing MKC. The processing methods and the strain of birds (i.e., Ross 308 *vs.* Cobb 500) might have led to this contradiction. The palatability and functional properties of the diets can be altered by the ingredients included in the ration, which is not the case in this study. The present results suggest that MKC could be used as protein source in commercial broiler diets without causing any detrimental effects on performance.

Haematological parameters are diagnostic tools for assessing any pathophysiological changes in animals (Verheyen *et al*., 2007). To monitor the effects of inclusion of MKC in the diets on the health status of the birds, haematological parameters were evaluated and used as indicators. Interestingly, in the current study, all haematological values fell within the normal range reported for healthy chickens (Jain, 1986). The heterophils linearly increased with MKC inclusion levels but were within the normal range (55–80%) for healthy chickens, implying that the immune systems of broilers were challenged when high levels of MKC were incorporated in the diets. It is well known that tannins form an insoluble toxic complex with protein in the intestine, which causes a deficiency in protein utilisation, leading to impaired immune function (Marzo *et al*., 1990) and growth. In addition, Clark *et al*. (1988) observed increased heterophil numbers due to increased toxicants in the diets of Japanese quail. In the current study, every unit increase of MKC increased heterophils by 0.73%. Surprisingly, lymphocytes decreased linearly with MKC inclusion, suggesting that MKC contains nutraceutical properties. An additional unit of MKC decreased lymphocytes by 1.42%. Lack of significant linear and quadratic effects in eosinophil and basophil concentrations implies that the broilers were protected against parasitic infections and intracellular bacteria to the same extent as those fed the control diets. Similarly, Olorede *et al*. (1996) and Onunkwo *et al*. (2019) reported a lack of adverse effects on haematological indices when broiler chickens were fed a 32% inclusion of Roselle seed cake and 10% palm kernel cake.

Serum parameters are diagnostic tools for assessing the nutritional status of animals. In this study, it was shown that MKC provided sufficient quality dietary protein, which resulted in optimal concentrations of serum parameters; hence it can be used to replace SBM without any negative effects on the health status of chickens. The significant linear decrease observed in blood Mg concentration with MKC inclusion was not expected since formulated diets contained Mg (Table 2) above the minimum Mg required by broilers, which ranges from 0.05 to 0.06% (Pond *et al*., 1995). The MKC is rich in Mg, which ranges from 0.5 to 0.6% (Malebana *et al*., 2018 and Manyeula *et al*., 2021). Therefore, graded inclusion of MKC in poultry diets is expected to augment Mg supplies in such diets. The Mg helps in normalising metabolism of amino acids, lipids, protein, and carbohydrates (Aschner & Aschner, 2005). Contrary to the current study, Manyeula *et al*. (2019) reported a lack of difference in blood Mg in Potchefstroom koekoek (an indigenous chicken strain of South Africa) fed on graded inclusions of canola oil cake as a protein source. The serum P concentration increased quadratically with an increase in MKC inclusion levels, which could be due to high concentration of this mineral in MKC. In this study, blood Fe concentration showed a quadratic decrease (17.3–14.6 Mmol/l) with an increase in MKC inclusion levels, suggesting that dietary MKC increases lead to decreases in blood serum Fe concentration. However, in the current study, the decline in Fe levels did not result in anaemic broilers and was within the normal range (13.0–20.0 Mmol/l). Therefore, it can be concluded that MKC provided sufficient quality dietary protein, which resulted in optimal concentrations of serum parameters; however, the decline in Fe levels in the current study did not result in anaemic broilers. MKC can therefore be used to reduce SBM without any negative effect on pathophysiological and health status of chickens.

Conclusion

The results of this study suggest that feeding MKC to broiler chickens up to 120 g/kg of the diet support comparable growth, is nutritionally adequate, and has no adverse health implications.

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Author's contributions

FM and LB conceptualised this study and together with OL and KP carried out the investigation. FM, BS, OL, TK, and KP were responsible for data curation. FM, LB, SB, MM, and JCM wrote, edited, and reviewed the manuscript. FM also served as the corresponding author and, together with JCM, worked on the suggestions made by the reviewers.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

References

Aschner, J.L. & Aschner, M., 2005. Nutritional aspects of manganese homeostasis. Mol. Aspects Med. 26, 353-362. https://doi.org/10.1016/j.mam.2005.07.003.

Bessei, W., 2006. Welfare of broilers, a review. Worlds Poult. Sci. J. 62,455- 466. https://doi.org/10.1017/S0043933906001085.

Bondari, K. & Sheppard, D.C., 1981. Soldier fly larvae as feed in commercial fish production. Aquac. 24,103-109. https://doi.org/10.1016/0044-8486(81)90047-8.

Chivandi, E., Davidson, B.C. & Erlwanger, K.H., 2012. The red sour plum (*Ximenia caffra*) seed, a potential non-conventional energy and protein source for livestock feeds. Int. J. Agric. Biol. 14, 540-544. http://www.fspublishers.org.

Clark, M.W., Gildersleeve, R.P., Thaxton, J.P., Parkhurst, C.R. & McRee, D.I., 1988. Hematological effects of ethyl methanesulfonate, paraquat, and phenylhydrazine in Japanese quail. Comp. Biochem. Physiol. 89, 15-30. https://doi.org/10.1016/0742-8413(88)90140-5.

Disetlhe, A.R.P., Marume, U. & Mlambo, V., 2019. Humic and enzyme inclusion in canola-based diets generate different responses in growth performance, protein utilization dynamic, and hemato-biochemical parameters in broiler chickens. Poult. Sci. 97, 2745-2753. https://doi.org/10.3382/ps/pey047.

Jain, N.C., 1986. Schalm's Veterinary Haematology. 4th Ed. Lea and Febiger, Philadelphia, U.S.A.

Kareem-Ibrahim, K.O., Abanikannda, O.T.F. & Nwadialo, S.O., 2021. Breed differences in growth parameters of broiler chickens. Nigerian J. Anim. Sci. 23, 18-29.

Malebana, I.M.M., Nkosi, B.D., Erlwanger, K.H. & Chavandi, E., 2018. A comparison of the proximate, fibre, mineral content, amino acid and the fatty acid profile of Marula (*Sclerocarya birrea caffra*) nut and soybean (*Glycinemax*) meals. J. Sci. Food Agric. 98, 1381-1387. DOI: https://doi.org/10.1002/jsfa.8604.

Manyeula, F., Mlambo, V., Marume, U. & Sebola, N.A., 2019. Nutrient digestibility, haemo-biochemical parameters and growth performance of an indigenous chicken strain fed canola meal-containing diets. Trop. Anim. Health Prod. 51, 2343-2350. https://doi.org/10.1007/s11250-019-01949-4.

Manyeula, F., Moreki, J.C., Baleseng, L., Molapisi, M., Tabudi, L.M. & Khumoetsile, T., 2021. Effects of feeding diets containing Morula (*Sclerocarya birrea*) kernel cake on bone development in commercial broiler chickens. LRRD. Retrieved November 30, 2021, from https://www.lrrd.org/lrrd33/10/33122jmore.html.

Marzo, F., Tosar, A. & Santidrian, S., 1990. Effect of tannic acid on the immune response of growing chickens. J. Anim. Sci. 68, 3306-3312. https://doi.org/10.2527/1990.68103306x.

Matshogo, T.B., Mlambo, V., Mnisi, C.M. & Manyeula, F., 2021. Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens. Livest. Sci. 251, 1-7. DOI: https://doi.org/10.1016/j.livsci.2021.104652.

Mazizi, B.E., Erlwanger, K.H. & Chivandi, E., 2020. The effect of dietary Marula nut meal on the physical properties, proximate and fatty acid content of Japanese quail meat. Vet. Anim. Sci. 9, 1-9. https://doi.org/10.1016/j.vas.2020.100096.

Mdziniso, P.M., Dlamini, A.M., Khumalo, G.Z. & Mupangwa, J.F., 2016. Nutritional evaluation of Marula (*Sclerocarya birrea*) seed cake as a protein supplement in dairy meal. JALSI. 4, 1-11. DOI: 10.9734/JALSI/2016/23273.

Mlambo, V., Dlamini, B.J., Ngwenya, M.D., Mhazo, N., Beyene, S.T. & Sikosana, J.L.N., 2011a. *In sacco* and *in vivo* evaluation of Marula (*Sclerocarya birrea*) seed cake as a protein source in commercial cattle fattening diets, LRRD, 23. http,//www.lrrd.org/lrrd23/5/mlam23121.htm.

Mlambo, V., Dlamini, B.J., Nkambule, M.T., Mhazo, N. & Sikosana, J.L.N., 2011b. Nutritional evaluation of Marula (*Sclerocarya birrea*) seed cake as a protein supplement for goats fed grass hay. Trop. Agric. (Trinidad) 88, 35-43. http://hdl.handle.net/2139/11692.

Mthiyane, D.M.N. & Mhlanga, B.S., 2017. The nutritive value of Marula (*Sclerocarya birrea*) seed cake for broiler chickens, nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. Trop. Anim. Health Prod. 49,835-842. Doi: 10.1007/s11250-017-1269-9.

Mukaro, E., 2014. Morula Tree *Sclerocarya birrea*.https,//suntrees.co.za/sclerocarya-birrea-marula-tree/. Accessed on 22/11/2021.

Nyoka, B.I., Chanyenga, T., Mng'omba, S.A., Akinnifesi, F.K. & Sagona, W., 2015. Variation in growth and fruit yield of populations of *Sclerocarya birrea*. Hochst Agrofor. Syst. 89,397-407. DOI: 10.1007/s11250-017-1269-9.

Olorede, B.R., Onifade, A.A., Okpara, A.O. & Babatunde, G.M., 1996. Growth, nutrient retention, haematology and serum chemistry of broiler chickens fed shea butter cake or palm kernel cake in the humid tropics, J. Appl. Anim. Res. 10, 173-180. https://doi.org/10.1080/09712119.1996.9706146.

Onunkwo, D.N., Ezike, J.C., Amaduruonye, W., Nathaniel, J. & Daniel-Igwe, G., 2019. Haematological and serum biochemical indices of broiler chickens fed Roselle seed (*Hibiscus sabdariffa*) cake-based diet as replacement for soybean meal. Nig. J. Anim. Prod. 46,158-163. DOI**:** 10.51791/njap.v46i2.30.

Pond, W.G., Church, D.C. & Pond, K.R., 1995. Basic Animal Nutrition and Feeding. 4th edn. John Wiley and Sons, New York, USA.

Timson, B.F., Chi, M.S. & Bowlin, B.K., 1983. The effect of reduced dietary protein on the anterior *latissimusdorsi* muscle fibers in the single comb White Leghorn pullet. Poult. Sci. J. 62,223-234. DOI: 10.3382/ps.0622230.

Verheyen, A.J.M., Maes, D.G.D., Mateusen, B., Deprez, P., Janssens, G.P.J. & de Lange, L., 2007. Serum biochemical reference values for gestating and lactating sows. Vet. J. 174, 92-98. DOI: 10.1016/j.tvjl.2006.04.001.

World Bank Group., 2015. Ending poverty and hunger by 2030, an agenda for the global food system. 2nd edition. http://hdl.handle.net/10986/21771.