High dietary marula (Sclerocarya birrea subsp. caffra) seed (nut) cake induces detrimental effects on performance, carcass characteristics and immuno- physiology of broiler chickens

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Abstract

Background

The objective of this study was to investigate effects of dietary incremental levels of marula seed cake (MSC), partially replacing soya bean meal (SBM) on growth performance, carcass characteristics, and haemato-biochemistry of broiler chickens from starter to finisher phases. In a completely randomized design, 400 day-old Ross 308 broilers were randomly allotted to 5 diets with 0, 5, 10, 15 and 20% MSC, each with 8 replicates of 10. Weekly feed intake, body weight gain and feed conversion efficiency were calculated whilst haemato-biochemistry was measured at d42.

Results

Overall, feed intake was quadratically decreased ($P < 0.01$) by MSC, of which the optimum inclusion was 15%. Body weight gain and feed conversion efficiency was linearly decreased ($P < 0.001$ and $P < 0.01$, respectively) by dietary inclusion of MSC. Also, MSC linearly decreased slaughter weight ($P < 0.001$), hot carcass weight ($P < 0.001$) and cold carcass weight ($P < 0.001$). Similarly, it linearly decreased white blood cells ($P < 0.01$) and lymphocytes ($P < 0.05$) and symmetric dimethylarginine ($P < 0.001$), as it linearly increased ($P < 0.001$) serum cholesterol.

Conclusion

In conclusion, up to 15% MSC can be incorporated into broiler diets in replacement of SBM without adverse effects.

1. Introduction

Expected to reach 9.4 to 10.1 billion people by 2050 with additional 0 to 2.7 billion by 2100 [1], the rapidly growing world human population, together with concomitant improvements in living standards and incomes, has increased the global demand for food (proteins) especially in low- and middle-income (developing) countries particularly in Africa [2]. To meet this demand, global food production needs to increase by 70% by 2050 [3]. Whilst most consumed protein is derived from plants, global trends indicate animal-derived proteins to gradually become predominant sources of food in these countries, similarly to food consumption trends in the developed world [2, 4]. Indeed, white meat consumption is on an upward trajectory in all regions of the world including Africa [4], with broiler chickens topping global annual production forecasts at 105.26 million metric tonnes in 2023 and a predicted high growth rate of 1.73% in 2019–2023 [5]. Consequently, there has been increased need for feed for production of broilers [6]. Among other ingredients, high quality (amino acid-rich) protein sources are important to incorporate in the diets of broilers to ensure efficient production [7]. Intensive genetic selection for rapid growth and massive meat yields over the past decades have heightened the requirements for amino acids in broilers [8, 9].

Due to its high crude protein (CP) content (400–550 g/kg DM) and well-balanced profile of highly digestible amino acids, especially the essential ones, in comparison with other oilseed grains, SBM is the preferred protein source for broiler diets in sub-Saharan Africa (SSA) and elsewhere [10]. Notwithstanding, the use of SBM in animal feeding is both economically and environmentally unsustainable. Large-scale soya bean cultivation incurs high variable costs, and results to deforestation, biodiversity loss, climate change, pollution, coastal and riverine eutrophication, and acidification, and worsens the feed/food competition [11].

Against this background, there is an urgent need for investigation of alternative protein-rich feed resources that are not only easily accessible and abundantly available particularly to resource-limited smallholder farmers but also the production of which is non-destructive to the environment such as MSC. Marula (*Sclerocarya birrea* subsp. *caffra*) seed (nut) cake (MSC) is an industrial by-product (residue) that remains after oil extraction from the seed kernels [12] of fruits fallen from marula trees that are indigenous and abundantly available throughout most of SSA from Niger to South Africa [13–15]. In light of predicted future increases in climate change associated frequency and severity of droughts in SSA [16, 17], MSC is an ideal alternative dietary protein source for broiler and other animal diets instead of SBM as it is produced from marula tree plants that are moderately resistant to drought and wide-ranging environmental temperatures (27 to 37°C), that promote marula seed germination [18, 19], as well as rainfall (400 to 1 000 mm per annum) [20, 21]. This feed resource has recently aroused great research interest mainly in Southern Africa due to its high CP (470 g/kg DM) and essential amino acid content similarly to SBM, except for lysine, as well as residual oil rich in the n-9 monounsaturated fatty acid (MUFA) oleic acid (72–85%) [22–24]. Previously, MSC has been successfully employed as alternative protein source in the diets of cattle (beef: [12] and dairy: [24]), goats [25], Japanese quails [26], and pigs [27, 28]. Whilst it has been used also in broiler diets [23, 29], no studies, however, have investigated dietary effects of the marula oil extraction by-product on the full repertoire of haemato-biochemical parameters including immuno-physiological biomarkers of the meat-producing birds. Also, no previous studies involved feeding of MSC-containing diets over the full production cycle (d1 to 42) of broiler chickens. Therefore, the objective of this study was to investigate effects of dietary incremental levels (0 to 20%) of MSC on growth performance, internal organs, carcass characteristics, and haemato-biochemical parameters of broiler chickens during the starter (d1 to 14), grower (d15 to 28) and finisher (d29 to 42) phases.

2. Materials and Methods

2.1. Study site description and ethical approval

All methods were performed in accordance with North-West University (NWU) guidelines and regulations for the care and welfare of animals in research and the rearing and slaughtering of broilers used in this study was approved by the NWU Animal Production Sciences Research Ethics Committee (Ethical clearance number: NWU-00806 -22-A5). The study was conducted at NWU Molelwane Experimental Farm during the summer season (October – November

2022). The farm is located (coordinates: 25°40.459'S, 26°10.563'E) outside Mahikeng City in the Mahikeng Local Municipality in Ngaka Modiri Molema District, North-West Province of South Africa.

2.2. Sourcing of MSC and chemical analysis

The MSC was obtained from The Marula Company in Phalaborwa, Limpopo Province, South Africa. On arrival, 100g of MSC was milled using a laboratory mill (screen size: 1 mm) and stored in sealed labelled polyethylene bags at room temperature for chemical analysis. It was then analyzed for dry matter (DM) (method 930.15), ash (method 942.05), ether extract (EE) (method 920.39) and CP (method 954.01) following the guidelines of the AOAC [30] whilst neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analyzed following procedures of Van Soest et al. [31]. The DM was determined by putting 1 g of sample into pre-weighed crucibles and oven-drying it at 105°C for 12hrs after which it was placed in a desiccator for cooling and then weighed. The DM was then calculated as the difference between the initial sample weight and moisture weight. The ash content was determined by calcinating a dry sample (1 g) in the muffle furnace (Nabertherm GmbH, Germany) for 6hrs at 550 ℃. Then the OM content was calculated by subtracting the % ash from 100%. The EE was determined by extracting crude fat with ether using an automated Soxhlet Fat analyzer ANKOMXT^{15} extractor following operator's manual (ANKOM Technology, Macedon, NY, USA). The CP content was determined following the Kjeldahl method by analyzing the nitrogen content of each sample and multiplying it by a factor of 6.25. The NDF and ADF contents were analyzed using an automated ANKOMDELTA fibre analyzer (ANKOM Technology, Macedon, NY, USA). The residue bags of ADF were then immersed in 72% (wt/wt) H_2SO_4 for 3hrs to determine the ADL content. Condensed tannins (CTs) were analyzed according to the method described by Makkar [32]. Briefly, CTs were extracted from 200 mg of plant material using 10 mL of aqueous acetone (70%). Then 0.5 mL of the tannin extract was mixed with 3 mL of butanol-HCl and 0.1 mL of ferric reagent in a test tube. The test tubes were vortexed and subjected to heating on a block at 100 ℃ for 60 min and allowed to cool at room temperature. The absorbance was then recorded at 550 nm and CTs in % DM as leucocyanidin equivalents were calculated using the following equation:

Condensed tannins (% DM) = (Absorbance × 78.26) / (% dry matter) (1)

2.3. Diet formulation, experimental design, and management

Five iso-caloric and iso-nitrogenous diets were formulated such that they contained incremental levels (0, 5, 10, 15, and 20%) of MSC partially replacing soya bean products to meet the nutritional requirements of broiler chickens at starter (d1–14), grower (d15–28), and finisher (d29–42) phases (Table 1) [33]. The metabolizable energy (ME) value of 15.44 MJ/kg dry matter [24] and amino acid composition [23] of MSC were used for diet formulation. In a completely randomized design, 400 day-old Ross 308 broiler chicks with average initial weight of 45.61 ± 0.67 g were randomly allocated to the dietary treatments, each with 8 replicate pens of 10 birds (pen = 1.8m high x 1.5m long x 1.5m wide; density = 4.4 broilers/m^2). Each pen had 5 males and 5 females. The experiment was carried out in a deep litter system in an environmentally controlled broiler house where the temperature was maintained within 18 ˚C and 21 ˚C by continuously recording daily temperatures using a thermometer. Also, the broiler house had semi-automatic curtains fitted that were opened during the day (08h00 to 17h00) to allow natural lighting and closed in the evenings / night until the next morning (17h00 to 08h00). However, in week 1, lighting was provided 24hrs a day using electric lights in addition to infra-red lamps (1 per pen) for brooding. Thereafter, infra-red lamps were removed, and electric lighting was provided for 24hrs a day until the end of the experiment. Each pen had 1 feeder and 1 drinker. On placement, chicks were offered StressPack which provided vitamins and electrolytes for the first 48hrs. Fresh feed and water were offered *ad libitum* throughout the feeding trial. All pens were checked daily for mortalities, and dead birds were removed and recorded.

Table 1
Ingredient and nutrient composition (g/kg on as-fed basis, unless otherwise stated) of experimental starter (d1–14), grower (d15–28), and finisher (d29–42) diets.

Ingredients (g/kg)	Starter					Grower					Finisher				
	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20
Yellow maize	587.7	604.4	545.0	472.4	409.2	594.8	646.7	605.4	528.0	480.3	604.5	642.4	661.0	595.4	532.5
Marula seed (nut) cake	0.0	50.0	100.0	150.0	200.0	0.0	50.0	100.0	150.0	200.0	0.0	50.0	100.0	150.0	200.0
Soya bean full fat	124.9	0.0	0.0	0.0	0.0	211.8	21.7	0.0	0.0	0.0	150.0	128.6	0.0	0.0	0.0
Soya bean meal (CP: 46.5%)	255.0	252.7	125.0	100.6	100.9	165.8	251.9	104.8	117.8	104.6	190.4	149.9	147.4	65.6	66.1
Sunflower oilcake (CP: 34%)	0.0	59.8	150.0	76.1	0.0	0.0	0.0	148.3	15.0	0.0	0.0	0.0	60.4	76.6	0.0
Wheat bran	0.0	0.0	44.6	163.8	217.7	0.0	0.0	08.6	155.1	104.7	0.0	0.0	0.0	78.7	125.8
Crude soya bean oil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0	0.0
Silica	0.0	0.0	0.0	0.0	35.1	0.0	0.0	0.0	0.0	76.3	0.0	0.0	0.0	0.0	40.2
L-Lysine	0.0	1.0	3.4	4.0	4.2	0.0	1.1	4.1	4.1	4.6	0.0	0.6	02.5	4.1	4.4
DL-Methionine	1.1	0.6	0.3	0.5	0.0	0.6	0.9	0.5	0.8	0.0	0.8	0.6	0.3	0.3	0.4
L-Threonine	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.3	0.7	0.0	0.0	0.0	0.0	0.3	0.7
L-Tryptophan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.3
Limestone, fine	13.9	13.7	13.4	13.8	13.9	12.7	12.7	12.3	12.8	12.5	13.1	13.1	12.9	13.0	13.0
Mono-dicalcium phosphate	9.9	10.4	10.6	10.7	11.4	7.4	8.0	8.3	8.4	9.9	7.7	8.4	9.0	9.2	9.9
Fine salt	2.8	0.3	2.3	2.1	2.1	2.8	2.8	2.1	2.1	2.1	2.8	2.8	2.7	2.1	2.1
Sodium bicarbonate	1.0	1.0	1.7	2.0	2.0	1.0	1.0	2.0	2.0	2.0	1.0	1.0	1.2	2.0	2.0
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Betaine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Quantum blue 10000G	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BSGF premix	3.0	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Chemical composition															
Dry matter	879.6	881.3	885.4	887.6	894.2	879.9	880.1	884.6	886.0	898.6	881.9	881.2	883.0	886.3	893.6
Crude protein	210.0	210.0	210.0	210.0	210.0	200.0	200.0	200.0	200.0	200.0	190.0	190.0	190.0	190.0	190.0
Ether extract	49.9	44.1	60.3	76.9	92.2	65.9	48.8	60.9	77.8	91.1	81.0	68.0	61.7	78.1	93.0
Crude fibre	28.7	35.2	52.9	52.9	46.7	30.5	26.8	49.2	42.9	36.3	28.0	28.9	35.4	45.1	38.1
Ash	42.2	43.4	45.6	46.7	46.8	38.5	38.6	41.0	41.7	41.0	37.4	37.7	38.7	40.4	40.3
ME (MJ/Kg)	11.5	11.5	11.5	11.5	11.5	12.0	12.0	12.0	12.0	12.0	12.5	12.5	12.5	12.5	12.5
Calcium	10.0	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0

Ingredients (g/kg)	Starter					Grower					Finisher				
	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20
Total phosphorus	5.9	6.5	7.5	8.1	8.4	5.2	5.6	6.6	7.2	7.2	5.1	5.5	6.2	7.0	7.3
Potassium	9.8	8.6	7.4	7.0	6.3	9.3	8.2	6.7	6.6	5.3	8.8	7.7	6.4	5.6	4.8
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chloride	2.2	2.4	2.6	2.6	2.6	2.7	2.4	2.6	2.6	2.6	2.2	2.3	2.6	2.6	2.0

ME = metabolizable energy.

2.4. Feed intake and growth performance measurements

Following measurement of initial live weights at arrival, broilers were weekly weighed between 08h00 and 10h00 throughout the feeding trial by weighing all the birds in each pen until week 6. Weekly body weights, and daily amounts of feed offered and leftovers were recorded. Then body weight gain (BWG: g/bird/week) was calculated by subtracting the previous weekly body weight (g) from the current body weight (g) divided by the number of broilers per pen. Daily feed intake (FI: g/day) was calculated by subtracting the weight (g) of the leftover feed from the weight (g) of feed offered divided by the number of birds per pen. The daily FI values were then converted into weekly averages of FI (g/bird/week) by combining pen averages over 7 days whilst feed conversion efficiency (FCE) was calculated by dividing the weekly BWG by the weekly FI.

2.5. Haemato-biochemistry analysis

A day before slaughter (d42), blood samples for haematology and serum biochemistry analysis were collected from 16 broilers per treatment (2 birds per pen) in the morning under veterinary supervision. Blood was collected from the wing vein with a 21-gauge needle and placed into purple-top EDTA-coated vacutainer tubes for haematological analysis. Red blood cells, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, red cell distribution width, reticulocytes, white blood cells, heterophils, lymphocytes, monocytes, eosinophils, basophils, platelets, and platelet distribution width were analyzed using an automated IDEXX LaserCyte Hematology Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa). The heterophils-to-lymphocytes ratio (N:L ratio) was calculated as heterophil values divided by the lymphocytes values. For serum biochemistry analysis, blood samples were collected into red top Vacuette® Serum Clot Activator tubes without EDTA (Greiner Bio-One, GmbH, Frickenhausen, Germany). Serum biochemical parameters including glucose, symmetric dimethylarginine (SDMA), urea, phosphate, calcium, total protein, albumin, globulin, albumin/globulin, alanine transaminase, alkaline phosphatase, total bilirubin, cholesterol, amylase, and lipase were analyzed using an automated IDEXX Vet Test Chemistry Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa).

2.6. Slaughter procedure and carcass characteristics measurements

In the evening of d42, 6 broilers per pen (3 males and 3 females) were fasted for 12hrs (18h00 to 06h00) whilst provided with clean drinking water *ad libitum*. Next morning after group-weighing per pen to obtain the final live (slaughter) weights, birds were transported to Rooigrond poultry abattoir, located ≈30 km from the NWU experimental site. An hour upon arrival at the abattoir, they were sacrificed humanely by cervical dislocation after electrical stunning (70 volts). The jugular vein was cut with a sharp knife at the base of the throat and allowed to bleed for 5 min. Following thorough bleeding, feathers were un-plucked and the carcasses washed. The heads, necks, and feet were removed. Visceral organs [liver, spleen, heart, gizzard, and intestine (duodenum, jejunum, ileum, colon, and caecum)] were removed by hand through an opening from the vent to the sternum and weighed individually. Hot carcass weight (HCW) was recorded immediately after slaughter at the abattoir whereas cold carcass weight (CCW) was recorded 24hrs post chilling (4 °C). Subsequently, chilling loss was calculated and expressed as a percentage using the following formula:

Chilling loss (%) = [HCW (g) - CCW (g) / (HCW (g)] × 100% (2)

Carcass cuts (breast, wing, thigh, and drumstick) were then removed by cutting from the joints of the carcass and through the shoulder area to remove the backbone from the breast. The internal organs and carcass cuts were then weighed and expressed as percentages of the HCW.

2.7. Statistical analysis

Weekly FI, BWG and FCE data were analyzed using the repeated measures option in the General Linear Model (GLM) procedure whilst overall FI, BWG and FCE as well as haemato-biochemistry, internal organs and carcass characteristics data were analyzed using the PROC GLM procedure of SAS [34]. The least square means (LSMEANS) were compared using the probability of difference (PDIFF) option in the LSMEANS statement [34] and differences among them were deemed significant at $P \leq 0.05$. Thereafter, the response surface regression (PROC RSREG) analysis [34] was performed to estimate the optimum inclusion level of MSC according to the quadratic model: $y = ax^2 + bx + c$, where y = response variable; a and b = coefficients of the quadratic equation; c = intercept; x = MSC level (%); and − b/2a = x value for optimal response.

3. Results

3.1. Proximate composition of MSC

The proximate composition of MSC is shown in Table 2. The results indicated MSC with a notably high CP (471.8 g/kg DM) and OM contents, with low levels of EE, ash, fiber (NDF, ADF and ADL) and CTs.

Table 2
Chemical composition of MSC (g/kg DM, unless otherwise stated).

Nutrient	Composition
Dry matter	938.7
Crude protein	471.8
Ether extract	168.2
Ash	75.1
Organic matter	924.9
Neutral detergent fibre	122.1
Acid detergent fibre	62.3
Acid detergent lignin	27.4
Condensed tannins (%)	0.11

3.2. Effects of MSC on growth performance

Table 3 shows the effects of incremental levels of MSC on weekly and overall FI, BWG and FCE, as well as mortality, with associated regression equations demonstrated in Table 4. Repeated measures analysis revealed a significant diet × time (week) interaction for weekly BWG ($P < 0.001$) but not for weekly FI ($P > 0.05$) and FCE ($P > 0.05$). Regarding FI, dietary MSC inclusion induced a linear decrease ($P < 0.01$) in this parameter in week 1, a quadratic decrease in weeks 2 ($P < 0.05$), 3 ($P < 0.01$), and 4 ($P < 0.01$), as well as a linear decrease in week 5 ($P < 0.01$). On the other hand, there was no effect ($P > 0.05$) of dietary MSC inclusion on FI in week 6. However, analysis of overall FI showed this parameter to have been quadratically decreased ($P < 0.01$) by dietary inclusion of incremental levels of MSC. In terms of FI, the optimum dietary inclusion level of MSC was 15% beyond which there was a sharp decrease in this parameter.

Concerning BWG, dietary inclusion of MSC induced a linear decrease in this parameter in weeks 1 ($P < 0.001$) and 3 ($P < 0.01$), a quadratic decrease in week 4 ($P < 0.001$), and a linear decrease in week 5 ($P < 0.01$). However, there was no effect ($P > 0.05$) of dietary MSC on BWG in weeks 2 and 6. Notwithstanding, analysis of overall BWG showed this parameter to have been linearly decreased ($P < 0.001$) by dietary inclusion of increasing levels of MSC. Regarding BWG, the optimum dietary inclusion level of MSC was found to be 10% beyond which there was a decrease in this parameter (Table 3).

As far as FCE is concerned, inclusion of dietary incremental levels of MSC linearly decreased this parameter in broilers in weeks 1 ($P < 0.01$), 3 ($P < 0.01$), and 5 ($P < 0.01$) whilst it quadratically decreased it in week 4 ($P < 0.01$). Otherwise, similarly to BWG, there was no effect ($P > 0.05$) of dietary MSC on FCE in weeks 2 and 6. Notwithstanding, analysis of overall performance data showed a linear decrease ($P < 0.01$) in FCE with the inclusion of increasing levels of MSC in broiler diets. Overall, our FCE data showed the optimum dietary inclusion level of MSC to be 10% beyond which there was a decrease in the efficiency of broilers to convert feed into body weight (Table 3). Interestingly, results also showed no effects ($P > 0.05$) of dietary MSC at all inclusion levels including 20% on all performance parameters (FI, BWG and FI) in broilers in week 6 of the study. Mortality was not affected ($P > 0.05$) by dietary MSC (Table 3).

Table 3
Effect of dietary inclusion of MSC on weekly and overall feed intake, body weight gain and feed conversion efficiency, as well as mortality, of broiler chickens.

Parameter	Week	Dietary inclusion of MSC (%)					SEM	*P*-value				
		0	5	10	15	20		Linear	Quadratic	Diet	Week	Diet × Week
FI (g/bird)	1	137.95^{a}	140.52^{a}	131.27^{a}	126.92^{ab}	125.50^{b}	4.406	0.008	0.851	0.001	0.001	0.207
	2	225.98^{ab}	238.29^{a}	247.26^{a}	227.50^{ab}	211.63^{b}	8.988	0.165	0.014			
	3	394.61^{ab}	425.07^{a}	435.11^{a}	395.71^{ab}	353.27^{b}	16.210	0.032	0.002			
	4	591.32^{b}	636.09^{ab}	693.48^{a}	618.69^{b}	578.63^{b}	23.008	0.564	0.001			
	5	805.49^{a}	845.47^{a}	846.03^{a}	777.47^{ab}	713.24^{b}	25.698	0.004	0.005			
	6	1005.29	996.22	989.60	983.21	928.31	30.465	0.084	0.417			
BWG (g/bird)	1	90.60^{a}	92.71^{a}	86.09^{a}	77.13^{b}	72.26^{b}	2.537	0.001	0.097	0.001	0.001	0.028
	2	137.89	139.37	154.42	131.34	124.37	7.524	0.153	0.061			
	3	279.99^{ab}	302.17^{a}	302.99^{a}	245.65^{b}	228.18^{b}	14.678	0.002	0.018			
	4	326.00^{b}	370.14^{b}	421.40^{a}	378.46^{ab}	331.69^{b}	15.742	0.695	0.001			
	5	503.77^{a}	493.42^{a}	492.50^{a}	423.24^{b}	396.74^{b}	21.536	0.002	0.216			
	6	717.97	698.18	601.77	614.61	595.86	47.733	0.335	0.529			
FCE (gain/feed)	1	0.66^{a}	0.66^{a}	0.66^{a}	0.61^{b}	0.58^{b}	0.017	0.004	0.113	0.004	0.001	0.251
	2	061	0.58	0.62	0.58	0.59	0.018	0.526	0.876			
	3	0.70^{a}	0.71^{a}	0.70^{a}	0.62^{b}	0.65^{b}	0.019	0.005	0.777			
	4	0.55^{b}	0.58^{ab}	0.61^{a}	0.61^{a}	0.58^{ab}	0.016	0.093	0.008			
	5	0.63^{a}	0.58^{a}	0.58^{a}	0.54^{b}	0.56^{b}	0.018	0.002	0.277			
	6	0.72	0.71	0.62	0.63	0.64	0.053	0.152	0.402			
Overall FI (g/bird)		3158.64^{a}	3281.67^{a}	3342.76^{a}	3129.56^{a}	2910.59^{b}	90.822	0.027	0.007			
Overall BWG (g/bird)		2056.22^{a}	2095.98^{a}	2059.18^{a}	1870.42^{b}	1749.10^{b}	59.720	0.001	0.038			
Overall FCE (gain/feed)		0.65^{a}	0.64^{a}	0.62^{ab}	0.59^{b}	0.60^{b}	0.039	0.003	0.481			
Mortality (%)		8.90	5.59	5.59	7.86	6.63	0.280	0.751	0.449			

Means in the same row with different superscripts (^{ab}) are significantly different. BWG = body weight gain, FCE = feed conversion efficiency, FI = feed intake, MSC = marula seed cake, and SEM = standard error of the mean.

Table 4
Regression equations of weekly and overall feed intake, body weight gain and feed conversion efficiency.

Parameter	Week	Regression equation	R^2	*P*-value
FI	1	y = -0.009(± 0.047)x + 139.690(± 4.112)	0.174	*P* < 0.01
	2	y = -0.243(± 0.094)x^2 + 4.073(± 1.967)x + 225.874(± 8.304)	0.146	*P* < 0.05
	3	y = -0.558(± 0.169)x^2 + 8.921(± 3.536)x + 395.262(± 14.926)	0.205	*P* < 0.01
	4	y = -0.862(± 0.248)x^2 + 16.392(± 5.179)x + 589.079(± 21.861)	0.244	*P* < 0.01
	5	y = -0.804(± 0.269)x + 806.622(± 23.703)	0.175	*P* < 0.01
BWG	1	y = -0.047(± 0.027)x + 91.881(± 2.406)	0.512	*P* < 0.001
	3	y = -0.393(± 0.159)x + 284.192(± 13.989)	0.212	*P* < 0.01
	4	y = -0.789(± 0.169)x^2 + 16.167(± 3.518)x + 322.166(± 14.848)	0.370	*P* < 0.001
	5	y = -0.288(± 0.228)x + 504.403(± 20.086)	0.315	*P* < 0.01
FCE	1	y = -0.0003(± 0.0002)x + 0.662(± 0.016)	0.279	*P* < 0.01
	3	y = -0.00006(± 0.0002)x + 0.712(± 0.020)	0.195	*P* < 0.01
	4	y = -0.0005(± 0.0002)x^2 + 0.011(± 0.003)x + 0.546(± 0.014)	0.163	*P* < 0.01
	5	y = 0.0002(± 0.0002)x + 0.625(± 0.016)	0.217	*P* < 0.01
Overall FI		y = -2.738(± 0.950)x^2 + 41.795(± 19.819)x + 3157.385(± 83.652)	0.164	*P* < 0.01
Overall BWG		y = -1.355(± 0.629)x + 2066.411(± 55.388)	0.328	*P* < 0.001
Overall FCE		y = -0.005(0.003)x + 0.656(0.013)	0.220	*P* < 0.01

BWG = body weight gain, FCE = feed conversion efficiency, FI = feed intake.

3.3. Effects of MSC on internal organs and carcass characteristics

The internal organs and carcass characteristics of broiler chickens fed diets supplemented with varying inclusion levels of MSC are shown in Tables 5 and 6, with associated regression equations shown in Table 7. There were neither linear nor quadratic effects ($P > 0.05$) of MSC on the weights and lengths of all internal organs (Table 5). Similarly, there were neither linear nor quadratic effects ($P > 0.05$) of MSC on all carcass characteristics, except for the SW, HCW and CCW (Table 6). In this regard, dietary inclusion of MSC linearly decreased the SW ($P < 0.001$), HCW ($P < 0.001$), and CCW ($P < 0.001$) of broilers in response to increasing dietary inclusion levels of MSC.

Table 5
Effect of dietary MSC inclusion on weights (% of HCW, unless stated otherwise) and lengths of internal organs of broiler chickens.

Parameters	Dietary inclusion of MSC (%)					SEM	*P*-value	
	0	5	10	15	20		Linear	Quadratic
Liver (%)	1.68	1.82	1.88	1.91	1.86	0.119	0.246	0.392
Spleen (%)	0.11	0.13	0.12	0.11	0.13	0.014	0.593	0.942
Proventriculus (%)	0.42	0.41	0.39	0.45	0.46	0.023	0.080	0.167
Gizzard (%)	1.76	1.87	1.74	1.84	2.23	0.082	0.170	0.195
Duodenum (%)	0.78	0.80	0.78	0.81	0.82	0.067	0.615	0.920
Jejunum (%)	1.39	1.42	1.23	1.31	1.46	0.049	0.988	0.147
Ileum (%)	1.25	1.14	1.17	1.22	1.24	0.099	0.827	0.429
Caecum (%)	0.52	0.62	0.78	0.57	0.68	0.118	0.478	0.452
Colon (%)	0.11	0.10	0.11	0.10	0.15	0.015	0.074	0.112
Duodenum length (cm)	28.81	30.21	30.39	29.78	30.22	1.484	0.606	0.621
Jejunum length (cm)	61.49	65.48	65.51	61.87	60.40	2.453	0.451	0.114
Ileum length (cm)	66.89	73.16	73.38	68.03	68.48	15.595	0.191	0.395
Caecum length (cm)	18.88	18.74	18.60	18.44	17.95	0.616	0.365	0.617
Colon length (cm)	4.83	4.79	5.28	4.75	5.11	0.402	0.679	0.894

MSC = marula seed cake, SEM = standard error of the mean.

Table 6
Effect of dietary MSC inclusion on carcass characteristics (% of CCW, unless stated otherwise) of broiler chickens.

Parameters	Dietary inclusion of MSC (%)					SEM	*P*-value	
	0	5	10	15	20		Linear	Quadratic
SW (g)	2101.79^{a}	2141.79^{a}	2104.99^{a}	1915.72^{b}	1794.64^{b}	59.894	0.001	0.038
HCW (g)	1478.57^{ab}	1504.47^{a}	1489.28^{a}	1361.61^{b}	1264.29^{b}	43.208	0.001	0.030
CCW (g)	1446.62^{a}	1472.38^{a}	1452.97^{a}	1326.67^{b}	1230.75^{b}	42.999	0.001	0.033
Chilling loss (%)	2.17	2.14	2.46	2.56	2.67	0.189	0.061	0.921
Dressing %	70.47	70.22	70.73	71.03	70.45	0.618	0.691	0.706
Breast (%)	18.65	15.23	18.65	17.55	15.88	0.969	0.342	0.796
Drumstick (%)	7.28	6.75	7.46	7.12	7.21	0.297	0.819	0.871
Thigh (%)	9.15	7.66	8.87	8.39	8.87	0.301	0.882	0.083
Wing (%)	5.82	5.14	6.04	5.63	5.98	0.231	0.313	0.420
Back length (cm)	20.02	18.35	30.56	18.74	18.91	4.607	0.904	0.256

Means in the same row with different superscripts (^{abc}) are significantly different. SW = slaughter weight, HCW = hot carcass weight, CCW = cold carcass weight, MSC = marula seed cake, SEM = standard error of the mean.

Table 7
Regression equations of slaughter weight, hot carcass weight, and cold carcass weight.

Parameter	Regression equation	R^2	*P*-value
SW	y = -1.356(± 0.631)x + 2112.052(± 55.551)	0.328	$P < 0.001$
HCW	y = -1.026(± 0.453)x + 1482.653(± 39.901)	0.301	$P < 0.001$
CCW	y = -1.001(± 0.451)x + 1451.337(± 39.703)	0.309	$P < 0.001$

SW = slaughter weight, HCW = hot carcass weight, CCW = cold carcass weight.

3.4. Effects of MSC on haemato-biochemistry

The haematological responses of broiler chickens to dietary graded levels of MSC are shown in Table 8, with regression equations in Table 10. The results demonstrated no effects ($P > 0.05$) of dietary MSC inclusion on all haematological parameters, except for white blood cells and lymphocytes. In this regard, MSC inclusion in broiler diets linearly decreased white blood cells ($P < 0.01$) and lymphocytes ($P < 0.05$).

Table 8
Effect of dietary inclusion of MSC on haematological parameters of broiler chickens.

Parameter	Dietary inclusion of MSC (%)					SEM	*P*-value	
	0	5	10	15	20		Linear	Quadratic
Red blood cells ($\times 10^{12}$/L)	1.39	1.39	1.08	1.18	1.29	0.153	0.385	0.282
Hematocrit (L/L)	8.11	8.35	6.57	7.29	7.69	0.486	0.073	0.599
Hemoglobin (g/dL)	9.03	10.34	7.39	7.54	13.13	0.808	0.069	0.698
MCV (fL)	42.500	55.35	42.09	46.13	49.66	2.896	0.259	0.918
MCH (pg)	35.91	46.04	36.68	38.76	43.75	2.817	0.355	0.970
White blood cells ($\times 10^{9}$/L)	9.15^{ab}	14.85^{a}	11.58^{a}	8.23^{b}	8.57^{b}	2.053	0.009	0.160
Heterophils ($\times 10^{9}$/L)	3.15	5.83	5.29	4.06	4.07	0.911	0.193	0.086
Lymphocytes ($\times 10^{9}$/L)	2.73^{b}	5.89^{a}	2.84^{b}	1.93^{b}	2.12^{b}	0.997	0.034	0.253
H:L ratio	1.72	2.47	2.33	4.38	2.31	1.176	0.410	0.437
Monocytes ($\times 10^{9}$/L)	1.55	2.43	2.81	1.78	1.85	0.698	0.316	0.334
Eosinophils ($\times 10^{9}$/L)	0.56	0.59	0.55	0.38	0.47	0.144	0.152	0.406
Basophils ($\times 10^{9}$/L)	0.06	0.08	0.09	0.09	0.05	0.018	0.158	0.201
Platelet (K/µL)	150.19	28.13	90.19	76.94	177.50	24.505	0.712	0.119
PDW (%)	18.61	14.30	12.78	11.99	13.16	1.412	0.077	0.851

Means in the same row with different superscripts (ab) are significantly different. H:L ratio = heterophils-to-lymphocytes ratio, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, PDW = platelet distribution width, MSC = marula seed cake, and SEM = standard error of the mean.

The effects of dietary incorporation of graded levels of MSC on serum biochemical parameters of broiler chickens are shown in Table 9, with regression equations in Table 10. Results showed no effect ($P > 0.05$) of dietary MSC on all serum biochemical parameters, except for SDMA and cholesterol. In this connection, dietary MSC linearly decreased ($P < 0.001$) serum SDMA concentrations as the marula oil extraction by-product level increased from 0 to 15% beyond which it increased from 15 to 20%. In contrast, serum cholesterol concentrations showed a linearly increasing ($P < 0.001$) response to increasing dietary inclusion levels of MSC.

Table 9
Effect of dietary MSC inclusion on serum biochemistry of broiler chickens.

Parameters	Dietary inclusion of MSC (%)					SEM	*P*-value	
	0	5	10	15	20		Linear	Quadratic
Glucose (mmol/L)	6.77	6.89	7.11	7.58	6.31	0.618	0.795	0.310
SDMA (µg/dL)	24.81^{a}	23^{a}	17.13^{b}	14.94^{b}	19.38^{c}	1.288	0.004	0.006
Urea (mmol/L)	0.60	0.60	0.94	0.64	1.21	0.302	0.195	0.699
Phosphate (mmol/L)	4.01	3.92	3.96	3.84	3.95	0.207	0.865	0.904
Calcium (mmol/L)	2.38	2.38	2.38	2.38	2.41	0.028	0.540	0.390
Total protein (g/L)	36.56	33.88	36.56	31.69	35.63	1.846	0.738	0.987
Albumin (g/L)	14.44	13.63	14.38	13.38	14.63	0.630	0.331	0.732
Globulin (g/L)	22.19	20.13	22.00	18.38	21.25	1.243	0.959	0.887
Albumin/globulin	0.66	0.69	0.66	0.74	0.69	0.017	0.227	0.717
ALT (U/L)	27.81	25.56	24.25	29.00	28.75	3.047	0.209	0.723
ALP (U/L)	680.25	693.06	743.88	937.50	686.38	90.729	0.516	0.341
Total bilirubin (µmol/L)	16.63	9.19	16.63	11.19	10.38	3.563	0.946	0.404
Cholesterol (mmol/L)	2.39^{b}	2.44^{b}	2.67^{ab}	2.65^{b}	3.01^{a}	0.121	0.001	0.856
Amylase (U/L)	400.25	477.13	389.06	536.06	304.06	75.174	0.741	0.136
Lipase (U/L)	208.44	208.88	187.44	405.44	232.69	83.141	0.395	0.738

Means in the same row with different superscripts (^{abc}) are significantly different. ALT = Alanine transaminase, ALP = Alkaline phosphatase, MSC = marula seed cake, SDMA = symmetric dimethylarginine, SEM = standard error of the mean.

Table 10
Regression equations of white blood cells, lymphocytes, SMDA, and cholesterol.

Parameter	Regression equation	R^2	*P*-value
White blood cells	y = -0.034(± 0.024)x + 13.230(± 2.021)	0.219	$P < 0.01$
Lymphocytes	y = -0.016(± 0.014)x + 4.635(± 1.192)	0.154	$P < 0.05$
SDMA	y = -0.043(± 0.014)x + 26.089(± 1.322)	0.263	$P < 0.001$
Cholesterol	y = 0.0002(± 0.0012)x + 2.281(± 0.107)	0.418	$P < 0.001$

SDMA = symmetric dimethylarginine.

4. Discussion

This study was undertaken to investigate MSC as an alternative to the economically and environmentally unsustainable conventional SBM protein source for broiler chicken diets. In keeping with previous studies [23, 24], our results found MSC to have a similar CP content as SBM. Considering its amino acid composition also mimicking that of SBM, except for lysine [22, 23], MSC offers great promise to replace SBM in poultry diets in Southern Africa and elsewhere. Interestingly also, local marula oil-extracting factories have improved their efficiency of oil extraction from marula kernels as evidenced by relatively low residual oil content in MSC used in this study compared to previous MSC products (289.6–343.5 g/kg DM) [23, 24]. With more improvements in oil extraction efficiency, iso-energetic MSC-containing broiler and other animal diets can henceforth be formulated with greater ease and the feed product is expected to have less problems with fungal and hence mycotoxin infestation as observed previously [23]. Of interest also is the relatively low fibre content in MSC used in this study in comparison to values observed in previous studies [27, 35]. The observed low fibre content of MSC renders the feed product even more ideal for use in diets of broiler chickens and other non-ruminants that are unable to utilize high fibre-containing diets [36, 37]. Further, our study showed MSC to be richer in ash, an indicator of the mineral content, compared to previous MSC products (48.5–54.3 g/kg DM: [23, 27, 35]). Furthermore, the concentration of CTs observed in MSC used in this study is higher than that reported by Malebana et al. [22] yet within the nor-mal range that is considered safe for the feed product to be used in broiler diets without induction of adverse effects on bird growth performance [38]. Indeed, previous studies have shown that inclusion of up to 3% tannins in broiler diets improves gut health and digestive performance [39, 40].

As part of the investigation of the nutritive value of MSC for broiler chickens, this study tested effects of incremental dietary inclusion levels of the marula by-product on broiler growth performance and mortality during the whole production cycle from d1 to 42 (i.e. starter, grower, and finisher phases). The observed increase in FI from 0 to 15%, as well as BWG and FCE from 0 to 10%, of dietary MSC inclusion level beyond which they decreased, corroborates

observations in pig studies by Hlongwana et al. [35], Mabena et al. [27] and Thabethe et al. [28] but contradicts those of Mazizi et al. [41] in Japanese quails. In a previous study, the decrease in performance parameters with increasing inclusion levels of MSC was suspected to be induced by extensive lipid peroxidation and mycotoxin infestation of MSC [23]. Indeed, these researchers found high lipid peroxidation and low concentrations of mycotoxins deoxynivalenol (DON) and T-2 toxin in MSC.

Notwithstanding, there is a possibility that the observed decrease in performance at high (15 to 20%) dietary MSC inclusion levels may also be related to the high oleic acid content in the residual oil-rich MSC. Indeed, dietary oleic acid was previously shown to decrease food intake through induction of satiety in mice [42] and to decrease food energy intakes in humans [43] through its eliciting of production of oleoylethanolamide [44], which is known to decrease food intake [45–47] through a mechanism involving the histaminergic system [48]. Oleic acid also has capacity to regulate body weight in animals as was demonstrated through a decrease in this parameter in rats fed a diet supplemented with 10% olive oil [49], a rich (70–80%) source of oleic acid. Mechanistically, this might occur through high MUFA diets exhibiting greater rates of oxidation leading to decreased body weight [50]. Otherwise, another putative mechanism might involve oleic acid inducement of a laxative (cathartic) effect as has been demonstrated in rats [51]. Hence, these effects of oleic acid may explain the decreased FI, BWG and FCE in broiler chickens fed high dietary levels of MSC in this study. These mechanisms may also explain the observed significant decrease in slaughter weight, HCW and CCW in these broilers when fed high (15 to 20%) dietary inclusion levels of MSC.

Otherwise, considering the lack of effect of dietary MSC on the weights and lengths of all internal organs, it would therefore appear that the marula by-product does not contain detrimental antinutritional factors and is thus safe to incorporate at 5 to 10% inclusion levels in broiler diets. This notion is further supported by the lack of effect of dietary MSC on mortality in this study. Generally, the feeding of alternative plant-derived feedstuffs or their extracts with high levels of antinutritional factors and fibre is associated with in-creased size and length of the digestive system [52, 53]. Further, the observation of lack of effects of dietary MSC on all performance parameters even at as high inclusion levels of the marula by-product as 20% in week 6 suggests age-dependent attainment of adaptation to consumption of the novel alternative protein source by birds. This was reflected in the significant diet x time (week) interaction for weekly BWG. Indeed, the digestive system of broiler chickens undergoes major anatomical and physiological changes as the birds grow with increases in size and length as well as ability to secrete digestive enzymes, alongside improved digestive ability, as they grow older [54]. If the MSC contained any antinutritional substances, their adverse impact appears to have decreased as the age of birds advanced, similarly to previous observations [55, 56].

The lack of significant effects of dietary MSC inclusion on most haemato-biochemical parameters of broiler chickens in this study is indicative of reasonable biosafety of the marula by-product in relation to the health of the birds. Indeed, the kernels of marula fruits are a safe and delicious source of nutrition generally indulged upon by millions of mainly rural people in numerous countries in Africa without any health perturbations. Generally, they are consumed as a snack [57], incorporated into porridge and boiled meat as flavour enhancers [58] or their extracted oil used for meat preservation [59–61]. In poultry nutrition, their dietary consumption in the form of MSC has also elicited no deleterious effects in Japanese quails [41]. Hence, their deleterious effects on broiler chicken white blood cells including lymphocytes at dietary inclusion levels beyond 10% and 5%, respectively, as observed in this study, was unexpected. Notwithstanding, many studies have reported inhibitory effects of oleic acid in its neat form [62–64] or as dietary olive oil [65, 66] or cashew kernel oil [67] on lymphocytes and their proliferation in different tissues including blood. Also, consumption of an oleic acid-rich Mediterranean diet decreased the number of leukocytes and platelets in human subjects [68]. The mechanisms underlying these deleterious effects of oleic acid on leukocytes including lymphocytes seem to involve the MUFA-induced cellular oxidative stress, mitochondrial depolarization [68–71] and apoptosis [72, 73]. This was the first study to investigate the full repertoire of haemato-biochemical parameters including immuno-physiological biomarkers in broilers. Hence, it remains to be seen in future studies whether diets supplemented with high levels of MSC would induce similar deleterious effects on leukocytes in other breeds of chicken. Also, there is a need for elucidation of molecular mechanisms underlying the observed MSC-induced perturbations in immunological parameters of broilers and other breeds of chicken.

The safety of MSC as broiler chicken feed at low (0 to 15%), and its apparent toxicity at high (20%), dietary inclusion levels was clearly embodied in SDMA responses. Correlated well with renal function, SDMA is a biomarker of acute kidney injury [74] and has previously been measured in broiler and quail studies of alternative protein sources and phytogenic feed additives [75, 76]. Considering the linearly decreasing serum SDMA concentrations in broilers fed diets with 0 to 15% MSC, it is evident that MSC was safe to use at these relatively low dietary inclusion levels but induced kidney injury in the chickens when it was included at a higher (20%) level, mirroring the observed decremental responses in performance and immunological parameters of birds fed diets with high dietary inclusion levels of the marula by-product. As mentioned above, it is argued that the high oleic acid content of MSC particularly at 20% inclusion level of the marula by-product induced cellular oxidative stress [69–71] and possibly apoptosis as well [72, 73] in the chickens. Lending support to this contention are previous observations of elevated SDMA levels in disease conditions involving oxidative stress including diabetes mellitus, atherosclerosis, inflammation, apoptosis, and compromised immune function [77]. In fact, some studies have postulated that SDMA itself may be an inducer of oxidative stress by elevating reactive oxygen species in monocytes [78] whilst enhancing NADPH-oxidase through endothelial Toll-like receptor-2 activation [79]. Further, literature evidence reporting abrogative effects of administration of antioxidants including epigal-locatechin-3-gallate, melatonin, N-acetylcysteine, vitamin E, and others on kidney injury, measured as asymmetric dimethylarginine (ADMA), a structural isomer of SDMA [77], further reinforces the contention of oleic acid having induced oxidative stress in broilers at high dietary MSC inclusion levels. Unfortunately, our data could not be compared with literature values as this was the first study to investigate SDMA responses to dietary MSC supplementation in broilers. In future studies, there is a need for investigation of biomarkers of and mechanisms underlying oxidative stress in birds fed high MSC-containing diets.

Another intriguing finding in the current study was the linear increase in broiler se-rum cholesterol responses to incremental dietary inclusion levels of MSC. Whilst our se-rum cholesterol values were about 1.8 times lower than plasma cholesterol ones previously observed in quails [80], there are currently no comparable literature serum cholesterol responses to dietary MSC supplementation in broilers. However, it is evident that the observed increase in the chicken serum cholesterol concentrations with increasing dietary MSC inclusion levels in the current study is again associated with oleic acid. Indeed, a

previous study showed consumption of oleic acid-rich olive oil to increase blood plasma and adipose tissue concentrations of high-density lipoprotein cholesterol (HDL-C) [49]. Apparently, the MUFA has unique ability to selectively increase the levels of the health-beneficial blood HDL-C whilst decreasing those of its cardiovascular disease (CVD)-associated low-density lipoprotein cholesterol (LDL-C) counterpart [49, 81, 82] resulting to its attenuation of CVD risk in hypercholesterolemic patients [83, 84]. Hence, it will be necessary to measure concentrations of both HDL-C and LDL-C in MSC-fed broilers in future studies in order to discern which exactly between the two cholesterol species is responsible for the observed elevation in serum cholesterol levels of birds fed incremental marula by-product-containing diets. Also, the observed dietary MSC-associated in-crease in broiler serum cholesterol suggests a need for investigation of underlying molecular mechanisms in terms of cholesterol biosynthesis in future studies.

5. Conclusions

In conclusion, up to 15% MSC can be incorporated into broiler diets in substitution of SBM without adverse effects on growth performance, carcass yield and immuno-physiology of birds. Notwithstanding, there is a need for strategies to resolve antinutritional effects of MSC in order to optimize its inclusion in broiler diets.

Abbreviations

ADF - Acid detergent fibre

ADL - Acid detergent lignin

ADMA - Asymmetric dimethylarginine

ALP - Alkaline phosphatase

ALT - Alanine transaminase

BWG - Body weight gain

CCW - Cold carcass weight

CP - Crude protein

CT - Condensed tannins

DON - Deoxynivalenol

FCE - Feed conversion efficiency

FI - Feed intake

GLM - General linear model

HCW - Hot carcass weight

HDLC - High-density lipoprotein cholesterol

LDLC - Lipoprotein cholesterol

MCH - Mean corpuscular haemoglobin

MCV - Mean corpuscular volume

ME - Metabolizable energy

MSC - Marula seed cake

MSC - marula seed cake

MSC - Marula seed cake

MUFA - Monounsaturated fatty acid

NDF - Neutral detergent fibre

NWU - North-West University

PDW - Platelet distribution width

SBM - Soyabean meal

SDMA - Symmetric dimethylarginine

SEM - Standard error of the mean

SW - Slaughter weight

Declarations

Author Contributions

Conceptualization, M.S. Mthana. and D.M.N. Mthiyane.; methodology, M.S. Mthana. and D.M.N. Mthiyane.; validation, M.S. Mthana.; D.M.N. Mthiyane.; D.C. Onwudiwe.; and M. Mwanza.; formal analysis, M.S. Mthana.; and D.M.N. Mthiyane.; investigation, M.S. Mthana.; and D.M.N. Mthiyane.; resources, D.M.N. Mthiyane.; data curation, M.S. Mthana.; writing—original draft preparation, M.S. Mthana.; writing—review and editing, D.M.N. Mthiyane and D.C. Onwudiwe.; supervision, D.M.N. Mthiyane.; D.C. Onwudiwe.; and M. Mwanza.; project administration, D.M.N. Mthiyane. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data used in supporting the conclusions of this article will be made available by the corresponding author, without undue reservation.

Ethics Approval

The animal study protocol was approved by the North-West University (NWU) Animal Production Sciences Research Ethics Committee (Ethical clearance number: NWU-00806 -22-A5).

Consent for publications

Not Applicable

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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