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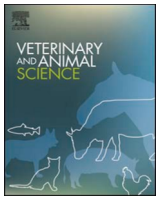

Dietary effects of *Sclerocarya birrea caffra* seed cake replacing soyabean meal on physiology, meat and bone quality of indigenous chickens

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ABSTRACT

This study investigated marula seed cake (MSC) as alternative protein source (APS) replacing soyabean meal (SBM) in indigenous chicken diets. Four hundred, 3-week-old Boschveld chicks were randomly allocated to 5 iso-energetic-nitrogenous maize and SBM-based grower diets with 0, 10, 15, 20, and 25 % MSC, each with 5 replicate pens of 16 birds, in a completely randomised design (CRD), for 9 weeks. Results showed that dietary MSC quadratically decreased bird overall feed intake (FI) ($P < 0.001$) and body weight gain (BWG) ($P < 0.01$) as it linearly decreased the weights of hot carcass (HCW; $P < 0.05$), spleen ($P < 0.05$), jejunum ($P < 0.05$), ileum ($P < 0.001$), and caecum ($P < 0.001$). In contrast, MSC increased chicken serum glucose ($P < 0.05$), cholesterol ($P = 0.001$) and phosphate ($P < 0.05$) as it decreased its amylase activity ($P < 0.01$). Also, it decreased bird meat lightness at 45 min ($P < 0.05$) and its yellowness at 45 min ($P < 0.001$) and 24 h ($P < 0.001$) whilst it increased its redness at 45 min ($P < 0.01$) and 24 h ($P < 0.05$) post-slaughter. In addition, MSC decreased chicken bone medial diaphysis ($P < 0.05$) as it induced no effects ($P > 0.05$) on overall feed conversion efficiency (FCE) and all other parameters. In conclusion, feeding of $\leq$ 15 % dietary MSC is nutritionally safe for indigenous chickens whilst detrimental on bird appetite, growth and meat yield, however without significantly affecting their physiology, at higher inclusion levels.

1. Introduction

With the rapidly increasing human population-driven demand for animal-derived protein-rich food especially in developing countries, there has been massive livestock and poultry production in the last few decades (Mottet & Tempio, 2017; Parolini et al., 2020). Due to their relatively short production cycles and affordability by most human consumers especially in these countries, chickens wield enormous potential to help in the achievement of sustainable food security and poverty alleviation (Birhanu et al., 2023). In rural areas of southern Africa in particular, indigenous chickens (*Gallus gallus domesticus*) are the most popular poultry species and serve as a source of protein-rich food (meat and eggs) and socio-economic wellbeing for rural communities (Raphulu et al., 2015; Okoro et al., 2017; Gunya et al., 2020). They are genetically highly adapted to the stressful tropical environmental conditions and endemic diseases compared to exotic birds (Alders & Pym, 2009; Okoro et al., 2017) and are excellent foragers and utilisers of poor-quality fibrous plant-based feed resources, converting them into human-edible meat and eggs (Padhi, 2016). A crossbreed of the indigenous Matebele (25 %), Ovambo (25 %) and Venda (50 %) breeds (Manyelo et al., 2020) developed in Mantsole ranch (Limpopo Province, South Africa) in 1998 (Bosch, 2011), the Boschveld chicken is a dual-purpose breed mainly bred for egg and meat production (Okoro et al., 2017).

Notwithstanding, the contribution of indigenous chickens to food and nutrition security in southern Africa is sub-optimal due to their low productivity (Okoro et al., 2017), high disease-related mortalities and predation (Guèye, 2000; Mtileni et al., 2012). One of the main contributors to their low productivity and pathogenic infections is their inadequate nutrition (Raphulu et al., 2015). To resolve this dilemma, Alabi et al. (2013) proposed that a commercial grower diet containing 178 g CP/kg DM and 14 MJ ME/kg DM could improve their growth and egg production. This could be achieved through supplementation of their diets with protein-rich feed ingredients such as soyabean meal

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(SBM) (Khobondo et al., 2015; Selaledi et al., 2020). Unfortunately, SBM prices are prohibitively too high for most smallholder farmers, which limits the economic sustainability of their chicken farming operations (Parolini et al., 2020). Hence the necessity to explore cheaper alternative protein sources (APSs) for indigenous chicken diets such as marula seed cake (MSC).

Marula (*Sclerocarya birrea caffa*) seed (kernel) cake (MSC) is the solid residue remaining after extraction of oil from the kernels dislodged from the hard nutshells of the fruits of marula trees (Mdziniso et al., 2016). The mechanical devices used to dislodge the kernels from the nutshells are unable to separate the two completely resulting to MSC containing some remnants of the nutshells that contribute to the relatively high fibre content (58.2 g/kg DM) of this by-product (Mthiyane & Mhlanga, 2017). Otherwise, MSC is a rich source of nutrients containing 470 g/kg DM CP, with a good profile of essential and non-essential amino acids similarly to SBM, except for lysine. It also contains minerals particularly Ca, P and K, as well as a high content of residual oil predominated (72 % to 85 %) by the *n*-9 monounsaturated fatty acid (MUFA) oleic acid (Mlambo et al., 2011; Mdziniso et al., 2016; Mthiyane & Mhlanga, 2017; Malebana et al., 2018). Because of the remarkable preponderance of oleic acid, the residual oil in MSC has 10 times more oxidative stability than olive oil (Mariod & Abdelwahab, 2012), which renders the oil highly desirable in terms of preservation of meat and other animal-derived products (Komane et al., 2015). In addition, marula kernels from which MSC is derived are endowed with a kaleidoscope of bioactive substances mainly the polyphenolic compounds (tannins), flavonoids, phytic acid, oxalates, and many others (Mashau et al., 2022) that can impart various beneficial and anti-nutritional effects to chickens and their edible products (Sun et al., 2019; Zhao et al., 2021).

Notwithstanding, MSC has hitherto not been investigated as a possible replacement for SBM in indigenous chicken diets. Considering its illustrious profile of crude protein and other nutrients as well as bioactive compounds, its widespread production in South Africa, Eswatini, Botswana, Namibia, Mozambique, and other southern African countries (Magaia, 2015; Mthiyane & Mhlanga, 2017; Malebana et al., 2018; Manyeula et al., 2021) and therefore its freedom from economic complications of importation over long inter-continental distances as is the case with SBM, MSC would be a perfect replacement for SBM in indigenous chicken diets in southern Africa. Thus, it was hypothesised that dietary replacement of SBM with MSC would sustain growth performance, physiology, meat and bone quality of indigenous Boschveld chickens. Therefore, this study investigated MSC incorporation at incremental levels into indigenous Boschveld chicken diets as a replacement for SBM by measuring bird responses in terms of growth performance, carcass traits, internal organs, haemato-biochemistry, meat, and bone quality.

2. Materials and methods

2.1. Study site and ingredient sources

The study was conducted between June and September 2022 at North-West University (NWU)'s Molelwane Experimental Farm (25°8600S, 25°6432E) located ~ 10 km outside Mahikeng city in Ngaka Modiri Molema district, North West Province of South Africa. Marula seed cake (MSC) was supplied by The Marula Company in Phalaborwa, Limpopo Province (South Africa) while the rest of ingredients were from Nutroteq in Centurion, Gauteng Province, South Africa.

2.2. Diet formulation

Five iso-energetic and iso-nitrogenous maize and SBM-based diets, in mash form, were formulated to meet or exceed the nutritional requirements of grower birds as recommended by the National Research Council (NRC, 1994). The experimental diets were formulated such that the graded levels of MSC (0, 10, 15, 20, and 25 %) were sequentially added in replacement of SBM (Table 1).

2.3. Chemical analyses of MSC and experimental diets

The experimental diets and MSC were milled to pass through a 2 mm-sieve (Polymix PX-MFC 90D) and subjected to proximate analysis using the Official Analytical Chemists International Methods (AOAC, 2005) and minerals using an ICP Mass Spectrometer (NexION® 2000, PerkinElmer (Pty) Ltd) as per Agri-Laboratory Association of Southern Africa (AgriLASA, 1998) (Table 1). For dry matter (DM) determination (method no.930.15), 1 g samples were added into pre-weighed crucibles and oven dried at 105 °C for 12 h followed by placing in the desiccator and then re-weighed. Thereafter, the DM was calculated as the difference between initial weight (W1) and sample weight after oven (W2) and expressed as g/kg. The ash content was determined (method no. 924.05) by burning the dried samples in a muffle furnace set at 550 °C for 6 h. Crude protein (CP) was determined following the standard Kjeldahl method (method no. 984.13) whereby the nitrogen content was determined and then multiplied by a factor of 6.25. Ether extract (EE) was determined using an automated Soxhlet Fat analyzer (ANKOM^{XT15} extractor, ANKOM Technology, Macedon, NY, USA). The amino acids were analysed using official methods of analysis (method 985.28) described by AOAC (2005).

Table 1
Ingredient and chemical composition (g/kg as fed basis, unless otherwise stated) of experimental diets with graded levels of MSC.

Ingredients		Dietary MSC (%)				
	MSC	0	10	15	20	25
Yellow maize		618.6	624.8	627.8	633.3	616.6
Full fat soyabean		48.5	31.7	30.2	0.0	10.0
Soyabean meal (46.5 % CP)		190.0	100.0	50.0	19.1	0.0
MSC		0.0	100.0	150.0	200.0	250.0
Sunflower oilcake		59.8	55.6	52.8	53.8	30.9
Soyabean oil		36.9	37.4	36.8	40.6	38.9
Pellibond		10.0	10.0	10.0	10.0	10.0
Lysine HCL 78 %		3.6	5.9	7.0	8.2	8.7
Methionine DL 98 %		2.9	2.7	2.6	2.5	2.6
Threonine 98 %		1.1	1.8	2.5	2.4	2.7
Limestone		13.0	13.5	13.8	14.0	13.6
Mono-dicalcium phosphate		8.3	8.4	8.4	8.5	8.7
Fine salt		2.2	3.4	3.0	2.6	2.3
Sodium bicarbonate		1.6	1.4	1.6	1.5	1.6
Salinomycin 12 %		0.5	0.5	0.5	0.5	0.5
Zinc bacitracin		0.3	0.3	0.3	0.3	0.3
AxtraPhy BF 1000 FTU		0.1	0.1	0.1	0.1	0.1
BSFG Premix		2.5	2.5	2.5	2.5	2.5
Total		1000.0	1000.0	1000.0	1000.0	1000.0
Analysed chemical composition						
Dry Matter	866.2	880.1	889.9	888.4	880.4	887.8
Crude Protein	465.8	193.0	192.5	192.7	192.6	192.5
Ether extract	296.3	75.7	105.7	121.0	135.9	164.5
Crude fibre	66.6	35.1	35.3	35.5	35.4	34.8
Ash	72.5	24.7	23.4	22.7	21.8	21.5
Metabolisable energy (MJ/kg)	15.7	12.9	12.9	12.9	12.9	12.9
Calcium	14.4	8.3	8.4	8.5	8.4	18.5
Phosphorus	11.3	7.2	7.5	7.5	8.1	7.8
Potassium	8.5	8.2	8.1	7.8	7.8	8.2
Lysine	5.2	10.8	10.7	10.6	10.8	10.8
Methionine	2.53	5.7	5.5	5.6	5.7	5.5
Cysteine	1.35	2.5	3.0	3.3	3.2	2.9
Threonine	3.50	6.7	6.7	6.9	7.1	6.8
Tryptophan	0.6	1.7	1.4	1.2	1.3	1.5
Valine	5.88	7.6	7.6	7.7	7.5	7.9

MSC = marula seed cake.

2.4. Experimental design and bird management

All experimental procedures used during bird rearing and slaughtering were approved by NWU Animal Production Sciences Research Ethics Committee (approval # NWU-00805-22-A5). All the protocols and procedures conformed to the guidelines for care and use of animals in research. A total of 400, 3-week-old Boschveld chicks (226.65 ± 12.455 g/bird) purchased from Boschveld Ranching PTY LTD (Bela-Bela, Limpopo, South Africa) were randomly allocated to 5 dietary treatments in a completely randomised design (CRD). Each treatment was replicated 5 times with 16 chicks (8 males and 8 females) per replicate pen (size: 1.8m long x 1.0m wide x 1.8m high) each with 1 feeder and 1 drinker. The pens had a wire mesh floor that did not require bedding; however, a polythene plastic was used to cover the floor and was changed on a regular basis. Upon arrival, the chicks were offered StressPack in drinking water for 3 consecutive days as a means to provide them with vitamins and electrolytes to relieve stress induced by transportation. Infrared lighting was used as a source of supplementary heat for 2 weeks. Clean feed and water were offered everyday *ad libitium* throughout the feeding trial. At 4 weeks of age, the birds were vaccinated against coryza. Daily mortality was monitored and recorded.

2.5. Feed intake and growth performance measurements

The growth performance data were collected as described by Ayed et al. (2015). Briefly, the daily amount of feed intake (FI) per bird was calculated by subtracting the daily amount of feed leftovers from the feed offered and dividing by the total number of birds per pen. The daily FI values were then converted into weekly averages of FI (g/bird/week) by calculating the sum of FI pen averages over 7 days. The initial weights of the birds were measured on arrival and subsequently weekly. Thereafter, the BWG was calculated by subtracting the current body weight from previous weight and dividing by the number of birds per pen. Then FCE per bird was calculated by dividing the weekly BWG by weekly FI

2.6. Blood collection and analysis

A day before slaughter, 2 birds from each pen were randomly selected for blood collection by drawing 5 mL of the fluid from the wing vein using a 21-gauge needle and placing it into tubes containing EDTA as an anticoagulant for haematological analyses whereas tubes without anticoagulant were used for serum biochemical analyses. The blood samples were analysed for haematocrit, haemoglobin, red blood cells, MCV, MCH, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets using an automated IDEXX LaserCyte Haematology analyser (IDEXX Laboratories, Inc.). In contrast, serum biochemical indices (glucose, symmetric dimethylarginine (SDMA), urea, phosphate, calcium, total protein, albumin, globulin, albumin/globulin ratio, alanine transaminase, alkaline phosphatase, total bilirubin, cholesterol, amylase, and lipase) were analysed using an automated IDEXX Catalyst one Vet Test Chemistry Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa).

2.7. Slaughter procedures, carcass traits, and internal organs measurements

At the end of the feeding trial, the chickens were subjected to a 12 h fasting period but with access to clean drinking water and final live weights were measured immediately thereafter. Chickens were then transported to Rooigrond poultry abattoir and humanely slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding stopped. The visceral organs were dissected out and the mass of each, and length of small and large intestines, measured using a measuring tape attached to a dissection board. Hot carcasses were weighed without giblets using a digital weighing scale to obtain hot carcass weight (HCW). Carcasses were then transported to NWU Animal Science laboratory for chilling at 4 °C for 24 h after which cold carcass weights (CCW) were measured. After 24 h of chilling, the breast, wing, thigh, and drumstick were removed from one side of each carcass and weighed, alongside the internal organs. Then the yields were expressed as percentages of cold carcass weight.

2.8. Meat quality measurements

Meat pH and temperature were determined on the breast muscle of each bird using a portable waterproof fibre-optic pH and temperature meter (Hanna instruments, HI98163, Romania). It was measured at intervals of 45 min and 24 h post-mortem. After every 10 measurements, the pH meter was calibrated using pH 4, pH 7 and pH 10 standard solutions meant for this purpose. For determination of meat colour, L* (lightness), a* (redness) and b* (yellowness) values of the breast were also measured at 45 min and 24 h post-mortem using a calibrated Konica Minolta colour guide [Narich (Pty) Ltd, Cape Town, RSA] following the guidelines by the Commission International De I′ Eclairage (CIE, 1978). For cooking loss measurement, the raw breast meat samples were weighed to obtain initial weight and oven cooked at 220 °C for 20 min (Honikel, 1998). Cooked samples were then allowed to cool at room temperature and then measured to obtain final weight. Cooking loss was then calculated using the following equation:

$$Cooking\ loss = \frac{Weight\ before\ cooking\ (g)\ --\ Weight\ after\ cooking\ (g)}{Weight\ before\ cooking\ (g)} \times 100\%$$

The water holding capacity (WHC) was determined using the filter paper method as described by Grau and Hamm (1957). Briefly, ~ 10 g of breast meat samples were weighed and pressed onto a Whatman 125 mm filter paper under a 60 kg mass for 5 min. After pressing, the meat samples were weighed again. The WHC was then calculated using the following equation:

$$WHC = \frac{weight\ after\ pressing\ (g)}{weight\ before\ pressing\ (g)} \times 100\%$$

The drip loss was measured using a method adapted from Honikel (1998). Approximately 2 g of breast meat samples were weighed and individually hooked inside sample bottles for 72 h followed by placing in a chiller (4 °C). The samples were hooked in a manner that they did not get in contact with the sides and surfaces of the bottles.

2.9. Bone morphometry assessment

Left tibia bones were manually removed with a knife from 100 birds (4 birds /pen). Each bone was kept in an identified plastic bag at –20 °C until analyses. The bones were individually weighed using a digital scale to obtain their weights. Geometric parameters (seedor index and robusticity index) of each bone were determined and the medial and lateral walls were measured at the midpoint mark using a digital calliper. Seedor was obtained as the ratio between the weight and the length of the bone (Seedor index = weight/length). The robusticity index of each bone was determined by dividing the bone length between the cubic of the bone weight (robustness index = length/ $\sqrt[3]{\text{weight}}$) (Evaris et al., 2021).

2.10. Statistical analysis

The data on overall FI, BWG, and FCE, as well as haemato-biochemical parameters, carcass characteristics, meat quality and bone morphometric parameters were analysed using the Generalised Linear Model (GLM) procedure (PROC GLM) of SAS (2012) using the statistical model: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where y_{ij} = response variables, μ = overall mean, α_i = effect of the diet, and + ε_{ijk} = random error. Each

replicate pen (5 per treatment) was the experimental unit, and data were presented as Least Square Means (LSMs) with respective Standard Errors of the Means (SEMs) (n = 25). The LSMs were separated using the probability of difference (PDIFF) option in the LSMEANS statement and differences among them considered significant at $P \leq 0.05$. The polynomial contrasts (PROC RSREG) were used to assess data for linear and quadratic trends. For description of linear and quadratic trends of the data in response to dietary MSC, and to determine its optimum inclusion level, response surface regression analysis in SAS (2012) was done using the non-linear model: $y = ax^2 + bx + c$, where y is the dependent variable; a and b are coefficients of the model; c is the intercept; x is dietary MSC inclusion level; and -b/2a is the x value that minimises or maximises a response parameter.

3. Results

The effect of dietary inclusion of MSC on overall FI, BWG, and FCE of Boschveld indigenous chickens is presented in Table 2. Dietary MSC quadratically decreased overall FI [y = –2.963(±0.584)x^2 + 62.635(±14.570)x + 3865.067(±74.974); $R^2 = 0.5642$; $P < 0.001$] and BWG [y = –1.126(±0.345)x^2 + 27.865(±8.618)x + 1028.584(±44.347); R^2 = 0.3972; $P < 0.01$], with optimum responses of both parameters at 15 % inclusion level, whilst it induced no effect on overall FCE ($P > 0.05$).

Table 3 shows the effect of dietary inclusion of MSC on carcass characteristics and internal organs of Boschveld indigenous chickens. Whilst there were neither linear nor quadratic effects of diet on all carcass characteristics and internal organs ($P > 0.05$), dietary MSC linearly decreased the birds' HCW [y = –0.303(±0.162)x + 894.395(±24.191); $R^2 = 0.1550$; $P < 0.05$] as well as their weights of the spleen [y = –0.00016(±0.00015)x + 0.326(±0.022); $R^2 = 0.1605$; $P < 0.05$], jejunum [y = –0.024(±0.016)x + 1.419(±0.093); $R^2 = 0.2310$; $P < 0.05$], ileum [y = –0.017(±0.010)x + 1.127(±0.059); $R^2 = 0.4533$; $P < 0.001$], and caecum [y = –0.010(±0.006)x + 0.690(±0.032); R^2 = 0.3931; $P < 0.001$].

The effects of dietary MSC inclusion on haemato-biochemistry parameters of Boschveld chickens is shown in Table 4. Whilst there were neither linear nor quadratic effects of diet on all haematological responses of birds ($P > 0.05$), dietary MSC increased serum glucose [linear: y = 0.042(±0.061)x + 11.656(±0.350); $R^2 = 0.1683$; $P < 0.05$], cholesterol [linear: y = 0.039(±0.032)x + 2.430(±0.182); $R^2 = 0.3968$; $P = 0.001$] and phosphate [quadratic: y = –0.002(±0.0006)x^2 + 0.027(±0.016)x + 2.642(±0.095); R^2 = 0.1779; $P < 0.05$] as it linearly decreased its amylase activity [y = –2.518(±4.949)x + 376.071(±28.597); $R^2 = 0.2807$; $P < 0.01$].

The effect of dietary inclusion levels of MSC on breast meat quality of Boschveld indigenous chickens is shown in Table 5. Whilst the pH and temperature at both 45 min and 24 h post-slaughter, lightness at 24 h ($L^*_{24\ h}$) post-slaughter, WHC, drip loss, cooking loss and shear force were not influenced by treatment ($P > 0.05$), dietary MSC decreased meat lightness at 45 min ($L^*_{45\ min}$) [linear: y = –0.380(±0.179)x + 46.459(±1.034); $R^2 = 0.1951$; $P < 0.05$] as well as its yellowness (b*) at both 45 min [quadratic: y = –0.009(±0.002)x^2 + 0.241(±0.056)x + 20.074(±0.323); R^2 = 0.3959; $P < 0.001$] and 24 h [quadratic: y = –0.008(±0.002)x^2 + 0.220(±0.044)x + 1.403(±0.252); $R^2 = 0.5149$; $P < 0.001$] whilst it increased its redness (a*) both at 45 min [linear: y = 0.055(±0.031)x + 0.386(±0.176); $R^2 = 0.3038$; $P < 0.01$] and 24 h [y = 0.057(±0.040)x + 0.949(±0.232); R^2 = 0.1632; $P < 0.05$] post-slaughter.

Lastly, the results showed neither linear nor quadratic effects of treatment on all bone morphometric parameters, except for the quadratic decrease in bone medial diaphysis [y = 0.0002(±0.00009)x^2 - 0.006(±0.002)x + 0.548(±0.014); $R^2 = 0.2166$; $P < 0.05$] in response to dietary incremental levels of MSC (Table 6).

4. Discussion

This study investigated MSC for complete replacement of SBM in indigenous Boschveld chicken diets by evaluating bird growth performance, carcass characteristics, internal organs, haemato-biochemistry, meat quality, and bone morphometry responses to incremental levels of the APS. Demonstrating positive effects of low dietary inclusion levels of MSC on FI, BWG and HCW, with an optimum at 15 %, our data suggested the safety of the marula oil extraction by-product at these levels against the incapacity of the indigenous birds to tolerate its ANFs at high inclusion levels (> 15 %). It is speculated that the presence of high fibre and residual lipids in MSC (Table 1) may account for the deleterious effects of the APS at high inclusion levels. Indeed, dietary inclusion of MSC has previously been shown to increase the fibre content of pig diets (Mabena et al., 2022). Notwithstanding its beneficial effects in terms of stimulating commensal and beneficial bacteria, enhancement of volatile fatty acids production, and immuno-enhancement (Singh & Kim, 2021), *inter alia*, high dietary fibre can induce antinutritional effects in chickens by decreasing metabolisable energy and nutrient digestibility whilst increasing the viscosity of their diets, resulting to compromised bird growth performance (Choct & Annison, 1992). Otherwise, the residual oil in MSC has previously been shown to undergo lipid peroxidation (Mthiyane & Mhlanga, 2017), which might have led to unpleasant odour of experimental diets with concomitantly decreased FI. Indeed, consumption of high dietary levels of peroxidised oil induced oxidative stress and decreased growth performance in birds (Oliveira et al., 2010; Zhang et al., 2021). Our results resemble those of Mabena et al. (2022), Thabethe et al. (2022) and Mthana et al. (2023) who similarly observed detrimental effects of high dietary inclusion levels (≥ 15 %) of MSC on performance parameters in broiler chickens and pigs, respectively.

The intriguingly linearly decreased weights of the spleen, jejunum, ileum, and caecum in response to dietary incremental levels of MSC would appear to be related to the high content of oleic acid in the marula oil processing by-product as well as the concomitantly decreased FI. It is speculated that this may occur through the mechanism of oleic acid dose-dependently suppressing spleen lymphocyte proliferation and natural killer cell activity (Yaqoob et al., 1994; Jeffery et al., 1996, 1997). In this connection, a corroborative study reported decreased spleen weight in broiler chickens fed a diet supplemented with brown rice (Nanto-Hara et al., 2021), the fatty acid composition of which is dominated by oleic acid (Munarko et al., 2021; Naveed et al., 2023). The oleic acid in MSC-supplemented diets might also have modified the gut microbiota thus contributing to decreased gut weight. Indeed, a high oleic acid diet was shown to alter gut microbiota diversity towards increased richness in *Prevotella* (López-García et al., 2021), the bacterial

Table 2
Effect of dietary inclusion of MSC on overall feed intake, body weight gain, and feed conversion efficiency of Boschveld indigenous chickens.

Parameter	Dietary MSC (%)					SEM	*P*-value	
	0	10	15	20	25		Linear	Quadratic
FI (g/bird)	3886.98^{c}	4004.51^{b}	4432.80^{a}	3845.93^{c}	3598.62^{d}	22.66	$P > 0.05$	$P < 0.001$
BWG (g/bird)	1037.25^{ab}	1131.22^{ab}	1177.55^{a}	1051.37^{ab}	1018.22^{b}	54.43	$P > 0.05$	$P < 0.01$
FCE (g/g)	0.27	0.28	0.27	0.27	0.28	0.01	$P > 0.05$	$P > 0.05$

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

Table 3
Effect of dietary inclusion of MSC on carcass characteristics and internal organs of Boschveld indigenous chickens.

Parameter	Dietary MSC (%)					SEM	*P*-value	
	0	10	15	20	25		Linear	Quadratic
Slaughter weight (g/bird)	1263.50	1361.22	1391.55	1268.90	1244.22	62.07	$P > 0.05$	$P > 0.05$
Hot carcass weight (g)	897.88^{a}	897.25^{a}	903.05^{a}	878.03^{ab}	815.15^{b}	25.35	$P < 0.05$	$P > 0.05$
Cold carcass weight (g)	885.64	886.03	887.10	867.50	837.22	20.48	$P > 0.05$	$P > 0.05$
Dressing %	71.12	66.04	67.10	69.47	65.68	3.62	$P > 0.05$	$P > 0.05$
Breast (%)	12.90	13.79	11.18	12.86	13.43	0.74	$P > 0.05$	$P > 0.05$
Drumstick (%)	6.82	7.36	6.16	7.54	7.49	0.46	$P > 0.05$	$P > 0.05$
Thigh (%)	8.32	8.22	6.91	8.75	8.98	0.48	$P > 0.05$	$P > 0.05$
Wing (%)	6.34	6.47	5.45	6.29	6.63	0.40	$P > 0.05$	$P > 0.05$
Liver (%)	2.03	1.87	1.89	2.08	1.91	0.139	$P > 0.05$	$P > 0.05$
Spleen (%)	0.32^{ab}	0.32^{a}	0.33^{a}	0.27^{b}	0.27^{b}	0.022	$P < 0.05$	$P > 0.05$
Proventriculus (%)	0.58	0.49	0.51	0.57	0.78	0.140	$P > 0.05$	$P > 0.05$
Gizzard (%)	2.31	2.20	2.15	2.29	2.31	0.153	$P > 0.05$	$P > 0.05$
Duodenum (%)	1.04	0.97	0.89	0.99	0.93	0.088	$P > 0.05$	$P > 0.05$
Jejunum (%)	1.41^{a}	1.26^{ab}	1.10^{ab}	1.15^{b}	1.10^{b}	0.098	$P < 0.05$	$P > 0.05$
Ileum (%)	1.12^{a}	0.99^{a}	0.92^{a}	0.81^{b}	0.82^{b}	0.062	$P < 0.001$	$P > 0.05$
Caecum (%)	0.69^{a}	0.60^{a}	0.58^{a}	0.55^{ab}	0.53^{b}	0.034	$P < 0.001$	$P > 0.05$
Colon (%)	0.17	0.19	0.17	0.10	0.10	0.042	$P > 0.05$	$P > 0.05$
Duodenum length (cm)	25.03	24.01	24.11	24.21	25.84	1.00	$P > 0.05$	$P > 0.05$
Jejunum length (cm)	52.38	50.11	48.12	48.60	48.22	2.31	$P > 0.05$	$P > 0.05$
Ileum length (cm)	53.49	51.80	48.96	49.48	47.48	2.41	$P > 0.05$	$P > 0.05$
Caecum length (cm)	16.54	18.88	22.50	14.61	14.89	2.47	$P > 0.05$	$P > 0.05$
Colon length (cm)	3.97	4.22	5.72	4.15	3.82	0.623	$P > 0.05$	$P > 0.05$

Means in the same row with different superscripts (^{abc}) are significantly different. MSC = marula seed cake, SEM = standard error of the mean.

Table 4
Effect of dietary inclusion of MSC on haemato-biochemistry of Boschveld indigenous chickens.

Parameter	Dietary MSC (%)					SEM	*P*-value	
	0	10	15	20	25		Linear	Quadratic
Haematology								
Red blood cells ($\times 10^{12}$/L)	1.28	1.16	1.15	1.17	1.17	0.10	$P > 0.05$	$P > 0.05$
Haematocrit (L/L)	8.59	8.27	9.05	7.76	8.15	0.38	$P > 0.05$	$P > 0.05$
Haemoglobin (g/dL)	8.60	9.73	9.64	9.92	10.16	0.70	$P > 0.05$	$P > 0.05$
MCV (fL)	56.35	71.56	67.13	66.29	67.45	5.64	$P > 0.05$	$P > 0.05$
MCH (pg)	64.60	81.90	72.80	81.84	78.58	4.79	$P > 0.05$	$P > 0.05$
White blood cells ($\times 10^{9}$/L)	27.60	19.89	11.84	9.86	9.34	5.13	$P > 0.05$	$P > 0.05$
Neutrophils ($\times 10^{9}$/L)	5.92	5.92	5.31	2.88	5.03	1.99	$P > 0.05$	$P > 0.05$
Lymphocytes ($\times 10^{9}$/L)	7.32	7.61	2.73	2.47	1.80	0.72	$P > 0.05$	$P > 0.05$
Monocytes ($\times 10^{9}$/L)	8.64	5.76	3.08	4.01	1.92	0.84	$P > 0.05$	$P > 0.05$
Eosinophils ($\times 10^{9}$/L)	1.97	0.46	0.66	0.41	0.53	0.07	$P > 0.05$	$P > 0.05$
Basophils ($\times 10^{9}$/L)	0.22	0.15	0.06	0.08	0.07	0.06	$P > 0.05$	$P > 0.05$
Platelet (K/μL)	25.53	38.13	194.90	124.10	131.30	83.96	$P > 0.05$	$P > 0.05$
Biochemistry								
Glucose (mmol/L)	11.57^{b}	12.39^{ab}	12.13^{ab}	12.15^{ab}	12.81^{a}	0.36	$P < 0.05$	$P > 0.05$
SDMA (μg/dL)	21.90	22.50	18.80	24.10	19.50	1.89	$P > 0.05$	$P > 0.05$
Urea (mmol/L)	0.61	0.60	0.60	0.60	0.60	0.004	$P > 0.05$	$P > 0.05$
Phosphate (mmol/L)	2.64^{a}	2.82^{a}	2.57^{ab}	2.70^{a}	2.34^{b}	0.02	$P > 0.05$	$P < 0.05$
Calcium (mmol/L)	2.44	2.47	2.46	2.50	2.51	0.03	$P > 0.05$	$P > 0.05$
Total protein (g/L)	52.30	57.10	51.40	58.40	53.20	2.50	$P > 0.05$	$P > 0.05$
Albumin (g/L)	17.80	19.30	17.70	20.80	19.00	0.77	$P > 0.05$	$P > 0.05$
Globulin (g/L)	34.80	37.50	33.80	37.60	34.30	1.73	$P > 0.05$	$P > 0.05$
Albumin/globulin	0.52	0.51	0.55	0.55	0.57	0.02	$P > 0.05$	$P > 0.05$
Alanine transaminase (U/L)	33.60	34.29	26.20	47.10	30.26	5.92	$P > 0.05$	$P > 0.05$
Alkaline phosphatase (U/L)	237.20	183.10	156.30	219.30	169.40	42.95	$P > 0.05$	$P > 0.05$
Total bilirubin (μmol/L)	24.00	32.00	22.70	38.40	26.00	5.97	$P > 0.05$	$P > 0.05$
Cholesterol (mmol/L)	2.42^{b}	2.94^{ab}	2.77^{b}	3.32^{a}	3.31^{a}	0.18	$P= 0.001$	$P > 0.05$
Amylase (U/L)	374.34^{a}	353.20^{a}	307.70^{ab}	298.85^{ab}	264.60^{b}	30.20	$P < 0.01$	$P > 0.05$
Lipase (U/L)	139.00	143.00	117.00	131.04	101.80	16.73	$P > 0.05$	$P > 0.05$

MCH = mean corpuscular haemoglobin, MCV = mean corpuscular volume, MSC = marula seed cake, SDMA = symmetric dimethylarginine, SEM = standard error of the mean.

genus associated with decreased villus height-to-crypt depth ratio (Recharla et al., 2021), and therefore decreased gut weight. Otherwise, the decreased weights of the small intestinal segments and caecum may be due to supposedly low digestive activity of the gut consequent to decreased FI in response to dietary MSC. Indeed, studies have previously demonstrated decreased weights of internal organs (Palo et al., 1995) and small intestine (Duarte et al., 2014) in feed-restricted birds.

This study also investigated the blood and its serum to assess the health and nutritional status of birds fed MSC-supplemented diets. The lack of linear and quadratic effects of dietary MSC on all haematological responses of indigenous chickens possibly indicated better adaptability of the native birds to utilising diets fraught with anti-nutritional compounds without ill effects on their health compared to broiler chickens (Manyeula et al., 2020). These data negate previous findings in broiler chickens whereby dietary incremental levels of MSC decreased white blood cells (Manyeula et al., 2021; Mthana et al., 2023) and

Table 5
Effect of dietary inclusion of MSC on breast meat quality of Boschveld indigenous chickens

Parameter	Dietary MSC (%)					SEM	*P*-value	
	0	10	15	20	25		Linear	Quadratic
$pH_{45\ min}$	5.87	5.91	5.75	5.88	5.81	0.05	$P > 0.05$	$P > 0.05$
$Temperature_{45\ min}$ (°C)	26.32	27.16	26.36	26.10	26.21	0.43	$P > 0.05$	$P > 0.05$
$L^*_{45\ min}$	46.55^{a}	42.85^{b}	44.58^{ab}	41.78^{b}	43.54^{b}	1.00	$P < 0.05$	$P > 0.05$
$a^*_{45\ min}$	0.36^{b}	0.98^{a}	0.74^{ab}	1.19^{a}	1.09^{a}	0.18	$P < 0.01$	$P > 0.05$
$b^*_{45\ min}$	0.19^{c}	1.01^{bc}	2.35^{a}	1.42^{b}	0.55^{c}	0.28	$P > 0.05$	$P < 0.001$
$pH_{24\ h}$	6.08	5.99	6.13	6.49	6.35	0.16	$P > 0.05$	$P > 0.05$
$Temperature_{24\ h}$ (°C)	12.68	12.59	12.91	12.89	13.05	0.18	$P > 0.05$	$P > 0.05$
$L^*_{24\ h}$	46.52	44.61	44.83	43.87	44.64	0.91	$P > 0.05$	$P > 0.05$
$a^*_{24\ h}$	0.91^{b}	1.57^{ab}	1.39^{ab}	1.51^{ab}	1.64^{a}	0.24	$P < 0.05$	$P > 0.05$
$b^*_{24\ h}$	1.47^{c}	2.39^{b}	3.29^{a}	2.34^{b}	1.66^{c}	0.22	$P > 0.05$	$P < 0.001$
WHC (%)	90.14	90.69	88.79	89.32	90.05	0.99	$P > 0.05$	$P > 0.05$
Drip loss (%)	16.51	16.37	19.87	17.47	13.87	1.67	$P > 0.05$	$P > 0.05$
Cooking loss (%)	24.16	25.09	27.14	25.01	26.45	1.57	$P > 0.05$	$P > 0.05$
Shear force (N)	8.14	7.51	6.79	9.85	8.32	0.85	$P > 0.05$	$P > 0.05$

Means in the same row with different superscripts (^{abc}) are significantly different. a* = redness, b* = yellowness, C* = chroma, L* = lightness, MSC = marula seed cake, SEM = standard error of the mean.

Table 6
Effect of dietary inclusion of MSC on bone morphometry of Boschveld indigenous chickens.

Parameter	Dietary MSC (%)					SEM	*P*-value	
	0	10	15	20	25		Linear	Quadratic
Bone weight (g)	9.37	8.79	8.01	9.41	9.15	0.45	$P > 0.05$	$P > 0.05$
Bone length (cm)	11.28	11.39	11.17	10.96	11.34	0.20	$P > 0.05$	$P > 0.05$
Diaphysis medial (mm)	0.55^{a}	0.50^{b}	0.52^{ab}	0.51^{ab}	0.54^{ab}	0.01	$P > 0.05$	$P < 0.05$
Diaphysis lateral (mm)	0.67	0.64	0.65	0.63	0.66	0.02	$P > 0.05$	$P > 0.05$
Proximal medial (mm)	1.02	1.17	1.29	1.32	1.32	0.16	$P > 0.05$	$P > 0.05$
Proximal lateral (mm)	1.99	1.95	1.73	1.85	1.81	0.12	$P > 0.05$	$P > 0.05$
Distal medial (mm)	0.89	0.88	0.94	0.98	0.96	0.06	$P > 0.05$	$P > 0.05$
Distal lateral (mm)	1.20	1.21	1.10	1.18	1.16	0.07	$P > 0.05$	$P > 0.05$
Seedor index (g/cm)	0.83	0.77	0.72	0.86	0.81	0.04	$P > 0.05$	$P > 0.05$
Robusticity index	5.37	5.52	5.59	5.20	5.32	0.11	$P > 0.05$	$P > 0.05$

Means in the same row with different superscripts (^{ab}) are significantly different. MSC = marula seed cake, SEM = standard error of the mean.

lymphocytes (Mthana et al., 2023). However, the observed haematology values of the birds were within the normal range for healthy chickens (Bounous et al., 2000).

The observed linear increase in serum glucose levels in response to dietary MSC is unexpected. However, this may involve oleic acid activation of the insulin receptor/PI3K/PDK1/Akt/Rac1 axis that facilitates insulin-induced uptake of glucose into adipocytes (Tsuchiya et al., 2014). Otherwise, oleic acid might have dose-dependently increased glucagon secretion by pancreatic islets mediated through NEFA receptor G-protein coupled receptor 40 (GPR40) activation and InsP3 intracellular release (Collins et al., 2007; Fujiwara et al., 2007; Hong et al., 2007). Increased pancreatic glucagon secretion would have stimulated the conversion of glycogen in the liver and possibly other storage tissues into glucose, resulting to increased serum glucose levels. In agreement with our results, Ibrahim et al. (2019) observed increased fasting blood glucose levels in mice following oleic acid administration. In contrast, dietary consumption of oleic acid-rich oils, like the residual oil of MSC, demonstrated decreased blood and plasma glucose levels in humans and rodents (Mokuda et al., 1993; Rasmussen et al., 1993). The incongruity of findings among these studies could not be explained.

The linearly increased serum cholesterol levels in response to dietary MSC seems to be related to the oleic acid-induced increased serum glucose, as discussed above. This corroborates our previous observation in broiler chickens fed MSC-supplemented diets (Mthana et al., 2023). The increased blood glucose levels have been associated with elevated systemic triglycerides and total cholesterol (Wang et al., 2020), particularly the obesity and cardiovascular disease-linked low-density lipoprotein-cholesterol (LDL-C) (Zhang et al., 2020; Wang et al., 2022). Other studies also showed dietary consumption of oleic acid-rich oil and food to elevate adipose tissue and blood levels of high-density lipoprotein cholesterol (HDL-C) (Gilmore et al., 2011; Nogoy et al., 2020) through the MUFA selectively elevating HDL-C as it abrogated LDL-C (Rudel et al., 1995; Kwon & Choi, 2015; Nogoy et al., 2020).

The quadratically increased serum phosphate content in response to dietary MSC is not unexpected considering the significantly high P content of the marula fruit by-product. In this regard, MSC from Southern Africa is reported to contain 9.27 to 11.0 g P/kg DM (Mthiyane & Mhlanga, 2017; Nkosi et al., 2019), which is 1.6 times higher than the P content of SBM (6.9 g/kg DM) (Ramachandran et al., 2007), with the marula by-product from Mozambique astonishingly containing as high as 15.2 g P/kg DM (Magaia, 2015) in contrast to much lower P contents in MSC from East Africa (Mariod & Abdelwahab, 2012). Despite the relatively high content (3.4 %) of phytic acid in marula kernels (Magaia, 2015), it is apparent from the observed MSC-induced increase in serum phosphate levels that the birds efficiently utilised dietary phytic acid-bound P. This was evidently due to the incorporated phytase enzyme in the form of AxtraPhy BF 1000 FTU (0.1 g/kg feed) in the experimental diets, though the birds fed the diet with 25 % inclusion level of MSC indicated compromised P utilisation. Cognisant of the skeletal disorders, leg diseases, and lameness globally prevalent in modern strains of broiler chickens (Liu et al., 2023), it would be of interest to investigate bone P responses of these birds to dietary MSC and to correlate them with serum phosphate levels in future studies.

Aside salivary glands, amylase is one of digestive enzymes mostly secreted by the pancreas (de-Madaria et al., 2021) for utilisation in starch digestion (Pieper-Bigelow et al., 1990). Its increased serum activity is indicative of acute pancreatitis (Regasa et al., 2022; Beiriger et al., 2023). Thus, the observed linearly decreased serum activities of this enzyme in response to dietary MSC suggested that the marula by-product incrementally supplied to the birds some bioactive

compound(s) that inhibited pancreatic amylase secretion. Indeed, marula kernels from which MSC is derived are naturally endowed with a myriad of bioactive compounds including flavonoids, phenolic acid, tannins, proanthocyanidins and others (Mashau et al., 2022). Many of these compounds particularly the flavonoids and polyphenols are well-known α-amylase inhibitors with utilities in energy metabolic regulation as anti-diabetic agents (Proença et al., 2019; Sun et al., 2019; Zhao et al., 2021). Therefore, the MSC-induced increased serum glucose levels, as discussed above, would appear to have mainly emanated from oleic acid-induced enhanced glucagon-stimulated hepatic glycogenolysis and promotion of lipolysis leading to production of glycerol, the substrate for gluconeogenesis (Hayashi, 2021), rather than from amylase enzyme-catalysed starch conversion to glucose.

The lack of effect of dietary MSC on most meat quality attributes is of great interest as it suggested that the use of the by-product as a protein source in indigenous chicken diets would not affect the marketability of the meat and purchasing decision-making processes at the level of the consumer. These findings concur with those of Mazizi et al. (2020) who also found no effect of dietary MSC on pH, colour, lightness, thawing and cooking losses, and tenderness of breast and thigh meat from Japanese quails. Notwithstanding, the observed decreased meat lightness at 45 min and yellowness at both 45 min and 24 h in contrast to increased meat redness both at 45 min and 24 h post-slaughter in response to dietary MSC suggested the marula by-product-induced heme iron-containing myoglobin protein deposition into muscle (meat). Since a higher muscle myoglobin content induces darker (redder) meat colour (Wideman et al., 2016), the high CP content, balanced amino acid composition and iron content of MSC (Phenya, 2018) and its general lack of pigments (carotenoids), suggest that its incorporation into chicken diets would be expected to decrease the lightness and yellowness whilst increasing the redness of the meat.

Lastly, the demonstrated lack of effect of dietary MSC on most tibia morphometric traits in this study indicated benign effects of MSC on the quality of bones of indigenous chickens. This suggests MSC supply of adequate amounts of minerals required for bone mineralisation and growth, as well as lack of ANFs with detrimental effects on bone quality. Notwithstanding, the observed quadratic decrease in bone medial diaphysis in response to dietary MSC suggested presence in MSC of some bioactive compound that compromised bone shaft elongation. It was speculated that this may be due to the high oleic acid content of MSC as a previous corroborative study found serum content of oleic acid to be negatively correlated with whole body bone mineral density (BMD) (Högström et al., 2007). Also, Manyeula et al. (2021) found linearly decreased tibia Ca and P contents of broiler chickens in response to incremental dietary MSC levels, suggesting negative effects of MSC on the bioavailability of these important macro-minerals in the birds. Notwithstanding, the *n*-9 MUFA has also been positively correlated with the stiffness index of bone (Paunescu et al., 2014) whilst Manyeula et al. (2021) found increased tibia length and latency-to-lie in broiler birds following consumption of incremental MSC-supplemented diets. The discrepancies among these studies suggest a need for further studies to elucidate the effects, and underlying mechanisms, of MSC on bone medial diaphysis.

5. Conclusion

Our data showed feeding of low ($\leq$ 15 %) dietary levels of MSC to be nutritionally safe for indigenous chickens whilst ANFs apparently induced detrimental effects on bird appetite, growth performance and meat yield, however without significantly affecting their physiology, at high ($>$ 15 %) inclusion levels. It is therefore recommended that MSC can be incorporated into indigenous chicken diets up to 15 % level without detrimental effects on the birds. Otherwise, our results suggest a need for a much larger future study with more replications per treatment and elucidation of underlying molecular mechanisms particularly in relation to bone quality responses of birds.

CRediT authorship contribution statement

Zibukile G. Mchunu: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Makiwa S. Mthana:** Writing – review & editing, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Doctor M.N Mthiyane:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors report no conflict of interest.

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