Response in Growth Performance, Physico-chemical Properties, and Fatty acid Composition of Broiler Meat to Different Levels of *Mucuna Pruriens* Seed Meal

Makiwa Simeon Mthana
University of Fort Hare Faculty of Science and Agriculture
Christian Sabelo Gajana (SGajana@ufh.ac.za)
University of Fort Hare
Bethwell Moyo
Fort Cox College of Agriculture and forestry
Arno Hugo
University of the Free State
Nasiphi Makhamba
University of Fort Hare Faculty of Science and Agriculture
Fortune Thabethe
University of Kwazulu-Natal

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Abstract

The study was conducted to determine the response in growth performance, physicochemical properties and fatty acid composition of broiler meat fed incremental levels of *M. pruriens* seed meal. A total of 112 Cobb 500, seven days old, unsexed broiler chicks with body weight of 150 ± 5.47 g/bird (mean ±SD) were used. Chicks were allotted to pens in a completely randomized design hence assigned to four experimental diets containing 0, 10, 15, and 20% of MPSM, respectively. Each diet was offered *ad libitum* with clean water to 28 broilers in each treatment. There was a linear decrease in average daily feed intake (ADFI), average daily gain (ADG) and final body weight (FBW) with incremental levels of MPSM ($P < 0.05$). Feed conversion ratio (FCR) increased linearly with increasing MPSM levels while Final Body Wight (FBW) decreased linearly with increasing levels of MPSM ($P < 0.05$). Meat pH_{24} and colour parameters showed no relationship with increasing levels of MPSM ($P > 0.05$). Thawing loss, carcass weight and cooking loss showed a linear increase with increasing levels of MPSM ($P < 0.05$). However, shear force showed a linear decrease with increasing levels of MPSM ($P > 0.05$). Muscle fat, fat free dry matter (FFDM) and moisture, mono-unsaturated fatty acids and margaric acid showed no relationship with increasing levels of MPSM ($P > 0.05$), however, a linear decrease ($P < 0.05$) on myristic acid and palmitic acid with incremental levels of MPSM was observed. Arachidic acid, and α-Linolenic acid increased linearly with inclusion level of MPSM ($P < 0.05$). Other poly-unsaturated fatty acids, fatty acid ratios and health lipid indices indicators showed no relationship with increasing levels of MPSM ($P > 0.05$). Increasing inclusion levels of MPSM impairs growth performance of broilers and may also improve broiler quality and fatty acid some fatty acids.

Introduction

The increase in human population has led to high demand in poultry meat hence forcing broiler farmers to double or triple production. According to Department of Agriculture, Forestry and Fisheries (DAFF), (2013) and National Department of Agriculture (NDA), (2018), from 2012 to 2017 broiler production in South Africa increased by 16.2 to 17% and it contributed about 1.7% of the total worth of agricultural products. Poultry meat is one of the cheapest meats (R 22.89/kg) when compared to pork (R 44.98/kg) and beef (R 25.13/kg) (SAPA, 2019). This makes it to be an easily accessible source of protein for people from under poverty-stricken communities or countries. Besides being a protein source, chicken meat has also been reported to have many nutritional traits such as low lipid content and relatively high concentrations of polyunsaturated fatty acids (Maltin et al., 2003). The health benefits of broiler meat have led to a growing demand by health-conscious consumers for broiler meat which has an attractive colour, and is tender, with low saturated fats (Al-Hassan et al., 2014). Secondary compounds such as antioxidants which are naturally present in indigenous plants can be a source of non-synthetic antioxidants when included in broiler diets, and they can be an alternative source of feed additives for broiler under organic production (Botsoglou et al., 2002).

Factors such as climate change, increasing human population and changes in price of the currently available feed ingredients for broilers has motivated animal nutritionists to explore other potential feed

ingredients from indigenous seeds such as *Mucuna pruriens*. *Mucuna pruriens* plants can adapt to undesirable environmental conditions, unlike other conventional feed sources which are not drought tolerant, such as soybean meal (Mureithi et al., 2003). Raw *Mucuna pruriens* seed contains high levels of anti-nutritional factors such as tannins, phytic acid, oxalate phenolic, and a toxic amino acid called levodopa (L-Dopa) (Mugendi et al., 2010). However, it has been shown that seed treatment can reduce anti-nutrition factors to safe consumption levels and without affecting the nutritional value of the seed (Carew et al., 2003). Treatment techniques include heating, fermentation, autoclaving and boiling of which heat treatment has been considered the most effective among the others (Mugendi et al., 2010). Heat processed *Mucuna pruriens* seed meal (MPSM) contains 32.48% crude protein, 7.41% crude fibre, 8.50% ether extract, 4.10% ash, and 47.51% nitrogen free extract (Fathima et al., 2010). Although its crude protein is not comparable to commonly used protein source [soybean (47%)], it however contains high levels of lysine (6.1 – 6.7 g/16 gN) which is comparable to that of soybean meal (6.2 g/16gN) (Banaszkiewicz, 2011; Tuleun et al., 2009). Furthermore, the seeds can be produced at a low cost under rain fed conditions by resource limited smallholder famers in many countries (Mureithi et al., 2003; Jiri and Mafongoya, 2018).

To reduce the quantity of soybean meal used when formulating poultry diets, *M. pruriens* seeds can be used as a protein source and as a source of lysine which is one of the limiting amino acid that is usually supplemented in broiler diets (Jayaweera et al., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010). However, its effect on meat quality has not been investigated, as it has been found that dietary ingredients affect meat quality (Mir et al., 2017). The inclusion of high protein-rich feed ingredients in non-ruminant diets improves growth performance and meat quality but some bioactive compounds found in seeds may compromise it (Ahmed et al., 2017; Altmann et al., 2018). Some studies have shown that bioactive compounds in seeds such as flavonoids, condensed tannins, oxalate, and phytic acid are potent antioxidants which scavenge free radicals and reduce the harmful effects caused by oxidation and they contribute to oxidative stability hence improving meat quality (Seeram et al., 2005; Reddy et al., 2007; Fayemi et al., 2019).

Other researchers have explored the use of MPSM as a protein source in broiler diets in some parts of Asia and East Africa (Del Carmen et al., 1999; Iyayi and Taiwo, 2003; Nyirenda et al., 2003; Jayaweera et al., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010; Mangwiro et al., 2013). The focus has mainly been on its potential benefits as an alternative source of protein on the growth performance of birds, little has been reported on its effect on the quality fatty acid composition of broiler meat. To the best of our knowledge, only one study reported the effect of feeding MPSM on meat quality attributes of Guinea fowl (Dahouda et al., 2009), thus there is a dearth of evidence on its effect on broiler meat quality. The objective of this study was to fill this research gap by determining the response in growth performance, physicochemical properties and fatty acid composition of broiler meat to increasing levels of *M. pruriens* seed meal. It was hypothesised that incremental levels of *M. pruriens* seed meal does not alter broiler growth performance, meat quality and fatty acid composition of broiler meat.

Materials And Methods

Experimental study site

The feeding trial was conducted at Fort Cox Agriculture and Forestry Training Institute in South Africa for a period of 35 days. The institution is under the following geographical coordinates: 32°43'48"S and 27°1'32"E and is at an altitude of 508 m above sea level. The study was conducted in autumn (March to April) when the environmental temperature ranges from 15°C to 30°C. The annual rainfall is about 480 mm, of which most of it is received in summer to autumn season (Mukumbo, 2013).

M. pruriens sourcing and determination of chemical composition

Raw black variety of *M. pruriens* seeds were purchased from McDonald Seed Company in Pietermaritzburg, Kwa-Zulu Natal, South Africa. At arrival the seeds were heat processed at 130°C for 30 min (Carew et al., 2003) using a laboratory oven, to reduce the harmful effects of condensed tannins, total free phenolic, phytic acid and oxalate to enable safe consumption. They were then ground to produce MPSM using a laboratory mill with the allowance to pass through a 2 mm sieve and were stored in sealed plastic bags at room temperature. The milled samples were later analyzed for proximate composition using the Association of Official Analytical Chemists (AOAC, 2005) methods for dry matter (method no. 930.15), crude protein (method no. 984.13), crude fiber (method no.978.10) and ether extract (method no. 920.39). For the analysis of minerals, the powdered seed sample was burnt at 550°C overnight using muffle furnace (Abdou et al., 2011). Macro (Calcium, Phosphorus, Potassium, and Magnesium) and micro (Iron, Copper, Zinc, and Manganese) minerals were then analyzed using an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 4110ZL, London, UK) as described by Okalebo et al. (2002). Nitrogen Free Extract (NFE) was calculated as NFE = DM - (Crude protein + Crude fiber + Ether extract + Ash) using AOAC (2005) method: 942.05. The metabolisable energy (ME) in kcal/kg was calculated using the following method: ME = (35 × CP %) + (81.8 × EE %) + (35.5 × NFE %) described by Pauzenga (1985). The total phenolic and condensed tannin content of the seed was analysed as described by Idris et al. (2017) using distilled water extracts. The oxalate and phytic acid content of the seed was determined as described by Unuofin et al. (2017) using distilled water extracts (Table 2). The neutral detergent fibre and acid detergent fibre contents of the experimental diets were analysed using an ANKOM200/220 fibre analyser (ANKOM Technology, Fairport, NY, USA) by the methods described by Van Soest et al. (1991) (Table 3).

Diets formulation

Diets were formulated using the FORMAT New Century Single-mix 250 version Formulation Software to be iso-caloric and iso-nitrogenous to meet the broiler nutrient requirements as recommended by National Research Council (NRC, 1994) at two (starter and finisher) different stages of growth as shown in Table 1.

The diets (T_1, T_2, T_3 and T_4) were formulated such that soybean meal was replaced by MPSM in terms of crude protein, at 0% MPSM, 10% MPSM, 15% MSM, and 20% MPSM, respectively.

Table 1
Feed composition of starter and finisher diets for broilers fed increasing levels of *M. pruriens* seed meal.

Ingredients (%)	Starter				Finisher			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Yellow maize	61.00	60	53.66	53.68	70	65	58.39	52.53
Soya Oil-cake 47	29.09	23.89	21.83	13.86	20.63	15.80	11.73	12.53
Full Fat Soya	4.07	4.63	3.00	6.14	2.99	4.1	9.82	9.90
M. prurien seed meal	0	10	15	20	0	10	15	20
Canola Oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone Fine	1.91	0.13	0.00	1.50	1.38	1.43	1.34	1.32
Mono calcium phosphate	0.59	0.00	0.84	0.85	0.65	0.47	0.50	0.51
Sodium Bicarbonate	0.09	0.14	0.31	0.25	0.61	0.06	0.09	0.08
Salt fine	0.38	0.34	0.22	0.27	0.02	0.35	0.33	0.34
Lysine	0.20	0.30	0.41	0.52	1.20	0.17	0.17	0.16
Methionine	0.18	0.15	0.18	0.20	0.08	0.14	0.15	0.15
Threonine	0.00	0.00	0.00	0.22	0.00	0.03	0.03	0.03
Tryptophan	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
Axtra Phy broiler enzyme blend 600 px	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Aviax plus	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Surmax	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Min-Vit Premix	0.34	0.33	0.33	0.33	0.29	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100

T_1, T_2, T_3 and T_4 contain incrementing levels of *M. pruriens* seed meal at 0, 10, 15 and 20% respectively.

Table 2
Chemical composition of oven heated (130°C) *M. pruriens* seed meal.

Chemical nutrients	Composition
Proximate Composition (g/kg DM)	
Dry matter	925
Crude protein	334
Crude fibre	78
Ether extract	84
Ash	33
Nitrogen free extract	396
Metabolizable energy (Kcal/Kg)	3261.92
Minerals (g/kg DM)	
Calcium	0.7
Phosphorus	7
Potassium	1.9
Magnesium	0.9
Iron	0.126
Copper	0.013
Zinc	0.017
Manganese	0.04
Anti-nutritional factors	
Condensed Tannins (mg CE/g)	61.95
Total free phenolic (mg GAE/g)	1912.54
Phytic acid (%)	19.96
Oxalate (mg)	3.81

mg CE/g- milligram of catechin equivalent per grams, mg GAE/g- milligram in gallic acid equivalent per grams, %- percentage, mg- milligram.

Table 3
Nutrient composition of incremental levels of *M. pruriens* seed meal diets.

Nutrients	Starter				Finisher			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Dry matter (g/kg)	908	914	905	901	896	892	898	899
Crude protein (g/kg)	211	218	206	213	177	175	185	186
Ether extracts (g/kg)	27	30	28	33	29	27	34	38
Ash (g/kg)	46	42	51	48	45	44	50	43
Nitrogen free extract (g/kg)	603	595	585	585	607	615	597	604
Calcium (g/kg)	9	11	14	6	12	7	7	9
Phosphorus (g/kg)	4	6	4	8	4	6	5	3
ADF (%)	3.6	4.9	3.7	4.1	2.7	3.7	3.3	3.3
NDF (%)	19.9	25.9	20.1	21.3	33.7	42.8	20.6	29.3
ME (Kcal/Kg)	3.100	3120	3026	3092	3011	3016	3044	3106
ADF = Acid detergent fibre, NDF = Neutral detergent fibre, ME = Metabolizable energy.								

Experimental design

A total of 112 Cobb 500 seven-day-old, unsexed chicks with an average weight of 150 ± 5.469 g/bird were purchased from a reliable commercial supplier (Buffalo chicks) in Berlin, East London, South Africa. The chicks were fed commercially supplied pre-starter diet before sourced. The dietary treatments used were four diets with incremental levels of MPSM. T_1 was a control diet with 0% MPSM, and continuing to increase from T_2 (10%), T_3 (15%), and T_4 (20%) of MPSM. The broiler chicks were randomly allocated to four replicates per treatment group where each replicate pens (1.5 m long × 1.5 m × wide × 0.8 m high) had seven chicks.

Broiler management and growth performance measurements

The experiment was carried out under a deep litter system with 5-8 cm depth saw dust in the poultry house. A two phased feeding program was used, which include starter (1 to 21 days) and finisher (22 to 35 days) diets. Fresh experimental diets and water were offered *ad libitum* throughout the feeding trial. Broilers were housed in a low-cost housing unit, where ventilation, humidity and temperatures were not artificially controlled. The ventilation was manually controlled by opening the curtains every morning. Initial live body weights were measured at arrival and subsequently measured weekly by weighing all the

broilers in each pen until day 35 to determine the weekly body weight gain and divided by seven to obtain the average daily gain (ADG). Average daily feed intake (ADFI) was measured by subtracting the weight of the feed refusals from that of the feed offered every day from day 2 to day 35. The feed conversion ratio (FCR) was determined by dividing the ADFI with ADG.

Slaughter procedure

Birds were slaughtered at day 35 of the experiment. Eighty birds were randomly selected, 20 birds per treatment were fasted for seven hours with water offered *ad libitum*. Prior slaughter, all selected birds were weighed to obtain the live/final body weight and thereafter slaughtered humanely. Slaughtering was done following usual commercial procedures at Fort Cox poultry abattoir, whereby birds were individually stunned on the head using electrical stunning method with 70 volts, and frequency of 50 Hz. While unconscious, a single cut to the base of the throat of birds was performed by a trained worker using a sharp knife. All slaughtered birds were allowed to bleed for 5 min. They were then placed in hot water (60°C) and de-feathered using chicken feather plucker machine. Immediately, evisceration was done where internal organs, head and feet were removed to obtain the carcass weight.

Meat pH and colour determination

The meat pH measurement was performed 24 hours after slaughter on 80 breast muscles that were chilled and stored at 4°C using a pH meter (Crison pH 25, Crison instruments, S.A., Alella, Spain). The pH meter was calibrated with pH 4 and pH 7 standard solutions. Colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was also determined 24 hours after slaughter using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, at 10º observation angle. Colour components were determined during blooming after removal of the fillets from the packaging, and exposure to air for 30 minutes to allow the oxygenation of myoglobin as described by Tapp et al. (2011). Three readings were taken by rotating the Colour Guide 90º between each measurement, to obtain a representative average value of the colour as previously reported by Fayemi and Muchenje (2018). Hue angle (H) and chroma (C) values were calculated following the procedure described by American Meat Science Association (AMSA, 2012).

Thawing loss, cooking loss and Warner-Bratzler Shear Force (WBSF) determination

The 80 breast meat samples that were used for thawing and cooking loss determination were frozen at -20°C for seven days. A day before preparation, all samples were weighed using digital scale (RADWAG, WLC 10/A2, Torunska, Radom, Poland) and thawed over 24 hours at 4°C and weighed again after thawing as described by Sekali et al. (2016). The breast meat was packed in airtight plastic bags and cooked using a water bath at 85ºC for 45 minutes (Ding et al., 2010). Raw and cooked weights were recorded. The tenderness of 80 breast meat samples was determined using Instron- Warner-Bratzler Shear Force (WBSF). Following cooking, three sub-samples of 10mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner

Bratzler (WB) shear device mounted on an Instron, model 3344, Universal Testing. The mean maximum load in Newton's (N) was recorded for the batch.

Fatty acid profile analysis

A total of 40 breast muscle portions were randomly selected from all treatments (10 breast muscles per treatment) and analysed for fatty acid composition. Total lipid from each muscle sample was quantitatively extracted, according to the method of Folch et al. (1957), using chloroform and methanol in a ratio of 2:1. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as moisture adsorbent. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as % fat (w/w) per 100 g tissue. Approximately 25 mg of extracted lipid muscle was transferred into a Teflon-lined screw-top test tube. Fatty acid methyl esters (FAME) were prepared for gas chromatography by methylation of the extracted fat, using methanol–BF3 (Slover and Lanza, 1979). FAMEs from muscle were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Analysis was performed using an initial isothermic period (40°C for 2 min). Thereafter, temperature was increased at a rate of 4°C / min to 230°C. Finally, an isothermic period of 230°C for 10 min followed. FAMEs n-hexane (1µl) was injected into the column using a Varian 8200 CX Autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. Fatty acid data were used to calculate the following ratios of FAs: total SFAs; total MUFAs; total PUFAs; PUFA/SFA; Δ^9 desaturase index (C18:1c9 / C18:0); total omega-6; total omega-3; the ratio of omega-6 to omega-3 (n-6)/(n-3) FAs. Atherogenicity index (AI) was calculated as: AI = (C12:0 + 4 x C14:0 + C16:0) / (MUFA + PUFA) (Chilliard et al., 2003).

Statistical analysis

The PROC MEANS (SAS, 2009) procedure was used to determine the mean ± SE (standard error) between inclusion level of MPSM and growth performance characteristics (ADFI, ADG, FCR and final body weight), meat quality characteristics and fatty acid composition of broiler meat. Thereafter, the polynomial regression procedure of SAS, (2009) was used to determine the linear and quadratic effects of feeding incremental levels of MPSM and growth performance (ADFI, ADG and FCR, final body weight), meat quality characteristics and fatty acid composition of broiler meat. The following regression model was used:

$$Y = \beta_0 + \beta_1 T + \beta_2 T^2 + E$$

Where: Y = is the response variables (growth performance, meat quality attributes and fatty acid composition of broiler meat).

$\beta 0 + \beta_1 + \beta_2$ = are the regression coefficients.

T = is the inclusion level of MPSM.

E = is the residual error.

Results

Influence of MPSM inclusions on growth performance

The effects of incremental levels of MPSM on growth performance parameters are shown in Table 4. There was a linear decrease on ADFI [Y = -0.06(0.14)x + 7.97(8.60)], ADG [Y = -0.85(9.32)x + 1.12(11.58)], and final body weight [Y = - 0.49(1.34)x + 47.28(99.53)] with incremental levels of MPSM, however, FCR [Y = 0.04(0.06)x – 0.15(5.80)] increased linearly with incremental levels of MPSM ($P < 0.05$).

Influence of MPSM inclusions on meat quality characteristics of broilers

Table 5 shows the effect of increasing levels of *M. pruriens* seed meal on physico-chemical characteristics of broiler breast muscle. There was a linear increase ($P < 0.05$) in carcass weight [Y = 0.01(0.03)x – 3.89(23.06)], shear force [Y = -0.23(0.22)x + 3.12(1.46)], thawing loss [Y = 0.40(0.47)x +0.02(2.01)] and cooking loss [Y = 0.30(0.50)x +2.51(4.91)] with incremental levels of MPSM ($P < 0.05$). However, incremental levels of MPSM had no effect on dressing percentage, meat pH24, colour parameters (L*, a*. b*), hue angle and Chroma ($P > 0.05$).

Influence of MPSM inclusions on fatty acid composition

Table 6 and 7 shows the effect of MPSM inclusion on fatty acids of broiler meat. Incremental levels of MPSM had no effect on broiler breast muscle fat, free fat dry matter (FFDM) and moisture of broiler meat and mono-unsaturated fatty acids and margaric acid (Table 6) ($P > 0.05$). Myristic acid [Y = -72.98(69.08)x + 15.96(10.89] and Palmitic acid [Y = -3.42(14.05)x +46.06(17.16)] decreased linearly with increasing levels of MPSM, however Arachidic acid [Y = 33.64(38.18)x +1.38(1.08)]) and α-Linolenic acid [Y = 8.37(22.53)x+2.74(0.27)] was observed to increase. There was no dietary influence ($P > 0.05$) on other poly-unsaturated fatty acids, fatty acid ratios and health indices fatty acids with response to MPSM inclusion as shown in Table 7.

Table 4
Effect of increasing levels of *M. pruriens* seed meal on broiler growth performance.

Parameters	Inclusion levels of *M. pruriens* seed meal				SEM	*P* – values	
	0%	10%	15%	20%		Linear	Quadratic
ADFI (g/bird/day)	133.80	100.08	103.95	101.75	1.028	0.0308	0.8369
ADG (g/bird/day)	65.22	50.40	42.90	42.55	89.79	0.0131	0.2700
FCR	2.05	1.99	2.42	2.39	1.231	0.0023	0.9607
Final Weight (g/bird)	2438.80	1915.15	1963.10	1953.03	0.979	0.0175	0.5023

ADFI: Average daily feed intake, ADG: Average daily gain, FCR: feed conversion ratio, SEM: Standard error of the means. *P*-value of linear and quadratic effect of less than 0.05 ($P < 0.05$) was considered statistically significant.

Table 5
Effect of increasing levels of *M. pruriens* seed meal on physic-chemical characteristics of broiler breast muscle.

Parameters	Inclusion levels of *M. pruriens* seed meal				SEM	*P* – values	
	0%	10%	15%	20%		Linear	Quadratic
CW (g/bird)	1787.55	1488.15	1416.83	1452.28	38.60	0.0013	0.5447
DP (%)	73.85	77.98	72.65	74.40	1.166	0.7963	0.2213
pH_{24}	5.75	5.83	5.76	5.79	1.134	0.6250	0.4475
Lightness - L*	47.45	46.19	46.13	45.75	1.103	0.1020	0.1163
Redness - a*	5.90	6.20	7.12	6.17	1.127	0.3405	0.3591
Yellowness - b*	11.29	10.86	11.43	11.71	1.115	0.3536	0.1137
Hue Angle - H*	61.61	60.46	58.42	61.87	1.128	0.8826	0.2053
Chroma - C	12.99	12.68	13.62	13.86	1.119	0.1052	0.7252
Shear force (N)	11.73	12.52	13.04	14.20	1.062	0.0039	0.1008
Thawing Loss (%)	7.46	8.82	8.01	9.66	1.074	0.0028	0.6571
Cooking Loss (%)	21.26	20.22	18.72	18.33	0.948	<.0001	0.2443

CW: Carcass weight, DP: Dressing Percentage, pH_{24}: Meat pH 24 h post slaughter, SEM: Standard error of the means. *P*-value of linear and quadratic effect of less than 0.05 ($P < 0.05$) was considered statistically significant.

Table 6
Effect of increasing levels of *M. pruriens* seed meal on proximate composition of broiler breast muscle.

Proximate analysis	Inclusion levels of M. pruriens seed meal				SEM	*P* – values	
	0%	10%	15%	20%		Linear	Quadratic
Fat (%)	1.81	2.05	2.00	2.01	0.49	0.5623	0.6680
FFDM (%)	23.70	23.29	23.61	23.87	0.66	0.5413	0.2683
Moisture (%)	74.47	74.66	74.71	74.11	0.78	0.5159	0.1500

FFDM: Fat free dry matter, SEM: Standard error of means. *P*-value of linear and quadratic effect of less than 0.05 ($P < 0.05$) was considered statistically significant.

Table 7
Effect of *M. pruriens* seed meal on fatty acid composition of broiler breast muscle.

Fatty acids (%)	Inclusion levels of *M. pruriens* seed meal					*P* – values	
	0%	10%	15%	20%		Linear	Quadratic
SFA							
C14:0	0.34	0.32	0.33	0.36	0.04	0.0317	0.3959
C16:0	24.42	24.56	24.80	23.94	0.98	0.0026	0.8195
C17:0	0.04	0.04	0.03	0.03	0.03	0.7187	0.9656
C18:0	9.95	8.46	9.17	9.47	1.60	0.7420	0.3874
C20:0	0.03	0.01	0.03	0.05	0.02	0.0176	0.4989
Total SFA	40.45	44.50	41.83	40.70	1.84	0.8256	0.6469
MUFA							
C14:1c9	0.03	0.06	0.05	0.02	0.03	0.5616	0.6240
C16:1c9	4.04	4.66	4.35	3.86	0.88	0.6526	0.8345
C18:1c9	30.08	34.07	31.57	30.86	0.24	0.2617	0.8249
C18:1c7	5.22	4.89	5.01	4.99	0.20	0.6545	0.7442
C22:1c13	1.09	0.83	0.85	0.97	0.05	0.0548	0.9721
Total MUFA	24.77	22.03	22.80	25.55	1.23	0.5151	0.8422
PUFA							
C18:2n6t	0.45	0.23	0.40	0.46	1.14	0.3549	0.2827
C18:2n6c	17.06	16.40	16.76	17.66	0.02	0.4601	0.2179
C18:3n6	0.04	0.08	0.08	0.06	0.14	0.1671	0.2195
C18:3n3	0.40	0.27	0.33	0.35	0.13	0.0425	0.3557
C20:2n6	0.40	0.27	0.33	0.30	0.34	0.6394	0.9390
C20:3n3	0.08	0.00	0.02	0.00	1.40	0.4364	0.1104
C20:4n6	3.93	3.18	3.83	4.41	0.18	0.8063	0.2326
C22:5n3	0.55	0.38	0.48	0.55	0.24	0.5759	0.6602

SFA: Saturated fatty acids; MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, FAIR: Fatty Acids Ratios and Indices, AI: Atherogenicity Index, DI: Desaturase Index, SEM: Standard error of means. *P*-value of linear and quadratic effect of less than 0.05 ($P < 0.05$) was considered statistically significant.

Fatty acids (%)	Inclusion levels of *M. pruriens* seed meal					*P* – values	
	0%	10%	15%	20%		Linear	Quadratic
C22:6n3	0.45	0.16	0.44	0.46	1.99	0.6253	0.8080
Total PUFA	0.62	0.50	0.58	0.64	0.49	0.3926	0.9585
FAIR							
Omega-6 fatty acid	21.89	20.15	21.40	22.89	0.56	0.8061	0.7854
Omega-3 fatty acid	2.88	2.05	2.41	2.65	0.07	0.8466	0.6195
Omega-6/Omega-3	7.79	9.88	9.23	8.78	0.02	0.4784	0.8519
PUFA:SFA	0.71	0.66	0.69	0.76	0.12	0.6023	0.4504
PUFA/MUFA	0.62	0.50	0.58	0.64	1.10	0.3821	0.4265
AI	0.40	0.38	0.40	0.38	0.94	0.9679	0.7038
DI	3.19	4.11	3.51	3.42	0.84	0.4816	0.8483

SFA: Saturated fatty acids; MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, FAIR: Fatty Acids Ratios and Indices, AI: Atherogenicity Index, DI: Desaturase Index, SEM: Standard error of means. *P*-value of linear and quadratic effect of less than 0.05 ($P < 0.05$) was considered statistically significant.

Discussion

The potential protein sources such as leaf meals, oil cakes and seed or nut meals from indigenous plants are valued for their nutritional characteristics which can benefit the livestock feed industry if they are considered. Exploring other potential protein sources for non-ruminants is imperative to sustain the ever-increasing poultry enterprises as well as human population in Sub-Saharan Africa. To date, Soybean production has been criticized due its low production outputs in South Africa, thus majority (72%) is imported from other countries (Grain SA, 2016). This consequently leads to high purchasing value, hence disadvantaging many resource limited poultry farmers, therefore it of paramount importance that other local feed ingredients of local origin such MPSM be considered when formulating poultry diets.

Average daily feed intake showed a linear decrease with increasing inclusion levels of MPSM. Our results are in agreement with (Carew et al., 2003; Jayaweera et al., 2007) who also reported a decline in average daily feed intake of broilers. Generally, heat processing results in detoxification of the anti-nutritional factors (ANFs), hence, enhancing protein absorption, bioavailability of the amino acids in the gut and higher palatability (Emenalom et al., 2005; Tuleun and Igba, 2008; Gurumoorthi and Uma, 2011). However, the observed decrease in feed intake suggests that the secondary compounds of the seed meal such as

condensed tannins, total free phenolic, phytic acid and oxalate present in *Mucuna pruriens* seeds might not be heat-labile and also increase with the inclusion level of the seed meal. When consumed, they bind dietary protein to complexes that are not readily digestible and inactivate digestible enzymes (Alhassan et al., 2019). On other hand, phytic acid, phenolic, and oxalate interfere with essential minerals absorption such as calcium, phosphorus, magnesium, and zinc (Natesh et al., 2017). Condensed tannins are known to as one of the major groups of ANFs that inhibit feed consumption in non-ruminants. Broilers were selective when feeding hence avoiding some of the feed due to unpleasant aroma of the anti-nutritional factors (Manyelo, 2018).

It is with no surprise that average daily gain decreases with increasing levels of MPSM as expected. Similar observations were reported by (Iyayi et al. 2006; Tuleun and Igba, 2008; Gurumoorthi and Uma, 2011). Poor nutrient intake mostly protein and absorption lead to the unavailability of essential amino acids from the gastrointestinal tract resulting in broilers utilising body reserves to sustain their nutrient needs subsequently leading to decreased body weight (Mabusela et al., 2018). Furthermore, anti-nutrients inhibit the activity of hydrolytic enzymes such as trypsin, chymotrypsin, lipase, and amylase which leads to high by-pass nutrients (Lampariello et al., 2012). Due to evident decrease in body weight gain, the observed increase in FCR with increasing levels of MPSM in diets was expected. The higher FCR indicates that the feed was not efficiently converted to muscle (meat). Our results corroborate with Miya et al. (2019) who also reported a linear increase in FCR on broiler chicken fed increasing levels of *Vachellia* leaf meal. The decrease in final body weight of broilers to increasing levels of MPSM might be attributed to the linear decrease in ADFI and ADG which indicates that nutrients absorption was constrained at higher levels of the seed meal.

In this study, the carcass weight decreased linearly with increasing levels of MPSM in diets. This decreasing carcass weight was also observed by Emenalom and Udedibie (2005); Iyayi et al., (2006). The carcass weight results trend obtained across treatments in the present study was expected due to broiler final body weight trend. The meat pH_{24} and colour was not influenced by diets. The pH_{24} of meat fell within the ideal range in all treatments (5.75 to 5.83) (Hambrecht et al., 2004). Generally, over 24 hours after slaughter the pH of poultry meat decreases from approximately from 7.0-7.2 down to a range of 5.5-5.8 for acceptable meat where there is no case of pale soft and exudative (PSE) meat (Hambrecht et al., 2004). Increasing levels of MPSM in the diets increased the meat shear force. These results may have been driven by the presence of condensed tannins that are not heat-labile in *Mucuna pruriens* seeds which triggers oxidation in meat (Seeram et al., 2005; Reddy et al., 2007). Increasing meat shear force implies that, as the level of MPSM is increased the tougher the meat becomes. The toughening of meat is related to the oxidation of myofibrillar proteins due to tanniferous diets, which promotes accumulation of muscle fibres thus the meat becomes less tender (Harris et al., 2001; Morán et al., 2012). Currently, there is no traceable evidence on the meat quality analysis of broiler meat fed increasing levels of MPSM. However, the determination of tenderness through sensory evaluation was done by Adzitey et al. (2010). In this study it was observed that the inclusion of MPSM seemed not to influence meat tenderness. While in other related legume seeds fed to broilers, Milczarek et al. (2016) observed that the inclusion of faba

beans to broiler diet resulted in more tender of meat. In this case, the vast difference in meat tenderness between broilers fed *Mucuna pruriens* and faba bean seeds may have been influenced by factors such as the age at slaughter, sarcomere shortening during rigor mortis, the amount and solubility of connective tissues, and post-mortem proteolysis of myofibrillar proteins (Warner et al., 2010). Furthermore, the *M. pruriens* seeds contain a toxic amino acid called L-Dopa which is a precursor of dopamine that increases the skeletal muscles resulting in tougher meat (Reichart et al., 2011).

Thawing loss increased linearly with increasing levels of MPSM in diets. During thawing process, small muscle fibres attained from smaller carcass weight have high muscle water loss due to myofibrillar proteins denaturation caused by freezing temperatures (Waritthitham et al., 2010). However, breast meat thawing loss on Guinea fowls fed diet containing high level of MPSM was found to be lower (Dahouda et al., 2009). Since Guinea Fowls might be comparable to indigenous chicken, therefore the ability of their muscle fibres to retain intrinsic water becomes poor because of age at slaughter and the amount of connective tissues (Listrat et al., 2016; Soglia et al., 2016). This suggests that higher MPSM in a diet lowers the amount of substance lost during cooking as observed in the current study where cooking loss decreased linearly with increasing levels of MPSM. These findings are in line with a study by Dahouda et al. (2009), where lower cooking loss of breast meat from Guinea fowls fed 20% cooked, and toasted MPSM were observed. Cooking loss is known as the amount of water lost during cooking (Tornberg, 2005). These losses may include volatile substances from the volatile aromatic substances and the decomposition of fat (Thu, 2006). Moreover, they include nitrogenous and non-nitrogenous extractives and salts, which are beneficial to meat consumers (Adzitey et al., 2010).

According to Wolmarans (2009), animal tissue is known to contain high levels of saturated fatty acid composition when comparing to plant materials. Myristic (C14:0) and palmitic (C16:0) were observed to decrease linearly in response to increasing levels of MPSM, which is a positive observation. These fatty acids are recognised as saturated fatty acids and has also been identified as a potential risk factor on human health. Specifically, palmitic acid is known to increase blood cholesterol (Peña et al., 2009), while myristic is known to accumulate fat in the body (Verruck et al., 2019). Unfortunately, one of the saturated fatty acid [arachidic acid (C20:0)] was observed to increase with increasing levels of MPSM in diets. These saturated fatty acids have the capacity to increase triglycerides in animal blood which increases risk of cardiovascular disease and other chronic diseases in meat consumers (Verbeke et al., 1999; Pottel et al., 2014; Navidshad et al., 2015). This indicates that MPSM exhibit a wide variety of pharmacological properties such as anti-diabetic and antioxidants (Lampariello et al., 2012), which in this case may have resulted to decrease some saturated fatty acids on broiler meat. These medicinal properties may have played a significant role in increasing the composition of α-Linolenic acid (C18:3n3) as observed in this study. Furthermore, the finding observed by Ndukwe et al. (2011) showed an increase in white blood cell count, as well as in bilirubin concentrations and alkaline phosphatase and a decrease in alanine aminotransferase and aspartate aminotransferase in rats fed diets containing *M. pruriens* seed extracts. Which also confirms its medicinal properties (Lampariello et al., 2012).

In conclusion, incremental levels of MPSM in diets reduce broiler performance, does not alter meat pH_{24} and colour. However, shear force increases with increasing levels of MPSM in diets. The diets with MPSM lowered the composition of some unsaturated fatty acid (Myristic, palmitic and arachidic acid) but improved the composition of α-Linolenic acid in meat. This suggests that incorporating *Mucuna pruriens* seed in broiler diets may therefore have a low-density lipoprotein (LDL)-Cholesterol lowering effect, of which is a positive implication on consumers' health. However, further confirmation needs to be done to provide a more convincing evidence.

Declarations

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Conflict of interest:

Authors would like to declare that there is no conflict of interest.

Ethics approval:

Broiler handling practices and welfare were considered as recommended by National Society of the Prevention of Cruelty to Animals (NSPCA) in South Africa. Ethical clearance to carry out the study was approved by the University of Fort Hare's Research ethics committee (UREC) (Clearance Number: GAJ041AMTH01).

Informed consent:

Not applicable.

Consent for publication:

Not applicable.

Data availability:

All data analyzed in the study are available on request from the corresponding author.

Code availability:

Not applicable

Authorship contribution:

Conceptualization: M.S.M., C.S.G., B.M.; data curation: M.S.M., A.H. and N.M.; investigation: M.S.M., C.S.G., B.M. and N.M.; methodology: M.S.M. and C.S.G.; validation: M.S.M., C.S.G. and B.M.; writing - original draft: M.S.M.; formal Analysis: C.S.G., B.M. and F.T.; project administration: C.S.G., supervision: C.S.G. and B.M., resources: B.M., A.H. and F.T., writing - review & editing: C.S.G., B.M., A.H. and F.T.

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