



Dietary supplementation of low levels of unprocessed *Mucuna pruriens utilis* seed meal induces mild antinutritional entero-physio-metabolic perturbations without compromising performance and meat quality in finisher broilers

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Abstract

The rapidly growing world human population accentuates the need for improved production especially of protein-rich food. Broiler meat production offers opportunity to ensure security of this food. However, the production of modern broilers is not only limited by high feed costs due to dietary use of expensive energy and protein sources but also their meat possesses undesirable quality attributes. This study thus examined the effect of dietary *Mucuna pruriens utilis* seed meal (MSM) on growth performance, blood profile, carcass traits, and meat quality in finisher broiler chickens. In a completely randomised design (CRD), 320 21-day-old chicks were randomly allocated to 32 pens in which they were allotted 4 dietary treatments with 0, 2.5, 5, and 10% MSM, each with 8 replicate pens of 10 birds, for 28 days. Growth performance, carcass characteristics, internal organs, haemato-biochemistry, and meat quality were measured. Results showed that dietary MSM did not affect ($P > 0.05$) broiler performance, weights, and lengths of carcass cuts and internal organs, haematology, and meat quality. The only exception was MSM-induced increase in duodenal weight (linear, $P < 0.05$) and serum phosphorus (quadratic, $P = 0.05$) in contrast to a decrease in procalcitonin (quadratic, $P < 0.01$) and serum levels of total protein (linear, $P < 0.05$; and quadratic, $P < 0.01$), albumin (quadratic, $P < 0.05$), and bilirubin (quadratic, $P = 0.001$). Therefore, MSM could be supplemented up to 10% without compromising performance, carcass traits, internal organs, haemato-biochemistry, and meat quality in finisher broiler diets.

Keywords Legume · Alternative protein source · Phytogetic feed additive · Meat production and quality

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Introduction

The world human population continues to grow rapidly and is envisaged to reach between 9.4 and 10.1 billion by 2050 (United Nations, 2019). This concomitantly increases the demand for food and thus accentuates the need for strategies to improve production especially of protein-rich food to eradicate both food insecurity and malnutrition particularly in low- and middle-income countries of Africa (Ayodele et al., 2021; OECD/FAO, 2021). Considering its fastest global growth among all agricultural subsectors (Research and Market, 2019), its perception as being relatively nutritionally valuable with a preponderance of health-desirable unsaturated fatty acids (Barroeta, 2007; Cavani et al., 2009), as well as its affordability to most consumers (Valceschini, 2006), broiler meat is the most strategic type of protein-rich food for which development interventions are required to enhance its capacity and efficiency of production.

Notwithstanding, the production of broiler chickens is limited by high feed costs. These represent about 65–75% of total variable costs (Panda et al., 2014), of which about 95% are ascribable to energy and protein (plant and animal derived) sources (Mallick et al., 2020). To decrease these costs, replacement of expensive energy and protein ingredients with cheaper and locally abundant alternative feedstuffs, among other options, has been the most prudent strategy to sustain broiler production (El-Deek et al., 2020). Particularly, the focus of replacement has been directed at maize and soya bean meal (SBM), the major dietary plant-derived protein sources commonly used in broiler diets (Khalil et al., 2023). Aside exorbitant feed costs, modern fast-growing broiler strains produce meat with undesirable quality attributes imputable to intensive genetic selection and breeding programmes over the past decades. They deposit excessive abdominal fat (Tumova et al., 2021), and produce meat with high fat content and low pH (Kocer et al., 2018). The pH of meat is an important quality characteristic, the decline of which is associated with paler and softer meat with higher drip loss. Drip loss is visually unattractive to consumers and can result to excessive cooking losses and cooked meat dryness (Marchewka et al., 2023). Therefore, there is a need for strategies to improve not only production but also the quality of broiler meat to ultimately improve its appeal among consumers. One of the alternative feedstuffs with nutritional attributes that qualify it for partial replacement of maize and soya bean meal in broiler diets, as well as bioactive compounds with great potential to enhance desirable quality properties of the modern birds' meat, is MSM.

The seeds of *Mucuna pruriens* (L.) DC var. *utilis* (Wall. ex Wight) Baker ex Burck (a.k.a velvet bean), a tropical/sub-tropical

legume, are rich in proteins (25–30 g/100g) and starch (39–41 g/100g) (Ezeagu et al., 2003) as well as essential amino acids lysine, methionine, and others (Kouakou et al., 2022). They are also endowed with a plethora of bioactive phytochemical compounds primary among which is a high content ($\leq 9\%$) of 3,4-dihydroxy-L-phenylalanine (L-DOPA) (Pulikkalpara et al., 2015; Daffodil et al., 2016; Konishi et al., 2022). L-DOPA is a precursor for dopamine and other catecholamines (epinephrine and norepinephrine) (Molinoff and Axelrod, 2022; Weiner, 1979) that physiologically behave as β -agonists due to their enhancement of muscle accretion (Navegantes et al., 2000). Hence, consumption of MSM induces anabolic effects and increases muscle mass mediated through its ability to increase growth hormone (Alleman et al., 2011), testosterone (Suresh and Prakash, 2012), and dopamine (Lieu et al., 2012). Also, consumption of MSM has been demonstrated in poultry and rodent studies to induce hypoglycemic, lipid, and low-density lipoprotein cholesterol lowering effects (Jayaweera et al., 2007; Dharmarajan et al., 2012; Ngatchic et al., 2016). Further, MSM incorporation into beef diets has been shown to decrease meat cooking losses (Yantika et al., 2016).

Notwithstanding, due to its endowment with L-DOPA, dietary intake of MSM induces weight loss and decreases feed intake and feed conversion efficiency in non-ruminants including broilers (Del Carmen et al., 2002; Flores et al., 2002) especially when included at high levels ($\geq 15\%$) (Vadivel and Pugalenth, 2010a). For this reason, it may be prudent to include MSM at low levels ($\leq 10\%$) in broiler diets as it has recently been recommended (Ayodele et al., 2021) as a strategy to impart its anabolic effects on broiler meat as it was shown in mice (Imbs and Schwartz, 2011) whilst minimising the likelihood of its toxicity. Therefore, the objective of this study was to investigate the growth performance, carcass characteristics, internal organs, haemato-biochemistry, and meat quality in finisher broilers fed low-graded levels of unprocessed MSM as a partial replacement for maize and SBM as well as a phyto-genic feed additive.

Materials and methods

Study site and ethical consideration

The protocols used in this study were approved by the North-West University (NWU) Animal Production Sciences Research Ethics Committee (NWU-00814-31-AS). The study was conducted using broiler facilities at the NWU Experimental (Molelwane) farm (coordinates: 25° 40.459' S, 26° 10.563' E) situated in the Mahikeng Local Municipality, North-West Province, South Africa.

Sourcing of *M. pruriens utilis* seeds and other ingredients, diet formulation, and chemical analysis

Mucuna seeds were purchased from TWK (Piet Retief, RSA), whilst all other dietary ingredients were procured from Optifeeds (PTY) LTD., Lichtenburg, RSA. The experimental diets were formulated at SimpleGrow Agric Services (Pty) Ltd. (Irene, Gauteng, South Africa) to produce 5 iso-caloric and iso-nitrogenous (18% CP) diets with maize, SBM, and full fat soya bean being partially substituted with incremental inclusion levels of MSM (0, 2.5, 5, and 10%) to meet nutritional requirements of finisher broilers (d22–49) (Table 1) (NRC, 2001). Then, 100 g samples of MSM and experimental diets were milled (1 mm screen) and analysed for DM (930.15), CP (954.01), EE (920.39), ash (942.05), and CF (AOAC, 2005). Their DM was determined by oven-drying (105 °C) 1 g of sample in pre-weighed crucibles for 12 h followed by cooling in a desiccator and weighing. It was then calculated as a difference between initial sample weight and moisture weight. CP was determined following the Kjeldahl method and multiplying nitrogen values by 6.25. EE was determined by ether extraction of crude fat using an automated Soxhlet Fat analyser ANKOMXT15 extractor following the operator's manual (ANKOM Technology, Macedon, NY, USA). Ash was determined by calcinating a dry 1 g sample in the muffle furnace (550 °C) (Nabertherm GmbH, Germany) for 6 h. CF was analysed using the ANKOM2000 Fibre Analyzer with 0.255 N crude fibre acid solution and 0.313 N crude fibre base solution. Also, NDF, ADF, and ADL were analysed following protocols of Van Soest et al. (1991). Both NDF and ADF were analysed using the automated ANKOMDELTA fibre analyser (ANKOM Technology, Macedon, NY, USA), whilst ADL was determined by immersing ADF residue bags for 3 h in 72% (wt/wt) sulphuric acid.

Study design and broiler management

Day-old Cobb 500 male broiler chicks purchased from Eagle Pride Hatchery were initially ad libitum fed a commercial starter diet from d1 to 21, after which they were introduced to experimental finisher diets from d22 to 49. They were supplied with StressPack with electrolytes and vitamins for 48 h. In a CRD, 320 21-day-old chicks were randomly allocated to 32 pens (1.8 m high × 1.5 m long × 1.5 m wide each) in which they were allotted 4 dietary treatments (0, 2.5, 5, and 10% MSM), each with 8 replicate pens of 10 birds, for 28 days. The pen was the experimental unit. Fresh feed and water were ad libitum supplied throughout the duration of the trial. The feeding trial was carried out in a deep-litter system in a broiler house wherein temperature and ventilation were controlled by opening curtains during the day (0800 h to 1700 h). Daily house temperature was

Table 1 Ingredient, chemical, and amino acid composition (%) of experimental finisher diets

Ingredient	Dietary MSM inclusion level				
	0%	2.5%	5%	10%	MSM
Yellow maize	64.3	63.5	62.8	61.2	-
Soya bean oil cake (47%)	18.0	17.6	17.3	16.7	-
Full fat soya bean	14.0	12.6	11.2	8.3	-
<i>Mucuna</i> seed meal	0.00	2.50	5.00	10.0	-
Mono-calcium phosphate	1.35	1.36	1.37	1.40	-
Limestone	1.28	1.24	1.20	1.13	-
Broiler finisher premix	0.25	0.25	0.25	0.25	-
Sodium bicarbonate	0.18	0.19	0.20	0.21	-
DL-Methionine (98%)	0.20	0.21	0.22	0.25	-
Lysine (78%)	0.10	0.14	0.17	0.23	-
Threonine (98%)	0.03	0.04	0.05	0.08	-
Salt	0.31	0.30	0.30	0.28	-
<i>Chemical composition</i>					
Dry matter	93.4	84.5	88.7	88.6	90.6
Crude protein	18.0	18.0	18.0	18.0	31.4
Ether extract	7.60	6.60	7.20	7.20	7.4
Ash	6.40	6.20	6.10	6.40	4.7
Crude fibre	3.40	3.50	3.60	3.70	-
Neutral detergent fibre	38.2	29.9	39.1	35.8	33.6
Acid detergent fibre	22.1	28.4	29.9	25.7	11.1
Acid detergent lignin	6.60	9.30	10.5	14.0	8.16
<i>Amino acid composition</i>					
Lysine	10.3	10.3	10.2	10.2	-
Methionine	4.70	4.80	4.80	4.90	-
Cysteine	3.00	2.90	2.90	2.70	-
Methionine + cysteine	7.80	7.70	7.70	7.60	-
Threonine	7.10	7.10	7.00	7.00	-
Tryptophan	2.00	2.00	1.90	1.80	-
Isoleucine	7.60	7.30	7.00	6.40	-
Arginine	12.1	11.6	11.2	10.4	-
Valine	8.60	8.30	8.10	7.70	-
Glycine + serine	19.7	19.6	19.4	19.1	-

MSM, mucuna seed meal

maintained in the range of 18 to 21 °C and humidity at 40 to 60%, with a multi-metre device used to monitor them.

Growth performance

Average feed intake (AFI) was calculated by subtracting the weight of leftover feed from the weight of feed offered and dividing the difference by the total number of birds per pen. Body weight was initially measured on d21 and thereafter weekly by weighing all the birds in each pen using a weighing balance. Body weights were used to calculate average body weight gain (ABWG) according to the equation:

$$ABWG(t0, T) = W(T) - W(t0)$$

where $t0$ = initial time (days); T = final time, $W(T)$ = final body weight (g), and $W(t0)$ = initial body weight (g). Weekly feed conversion efficiency (FCE) was calculated as ABWG divided by AFI.

Carcass characteristics and internal organs

Following 12 h of feed withdrawal, 4 birds were randomly selected from each replicate pen on d50, electrically stunned, bled, and de-feathered. Hot carcass weight (HCW) was measured by weighing eviscerated carcasses within 45 min after slaughter, whilst cold carcass weight (CCW) was measured after chilling the carcasses at 4 °C for 24 h. The weight and length of various carcass cuts (breast, wing, drumstick and thigh) and internal organs (gizzard, proventriculus, spleen, liver, duodenum, jejunum, ileum, caecum and colon) were measured using a digital scale and tape.

Meat quality

Breast muscle pH and temperature were measured 45 min post-slaughter and 24 h after chilling (4 °C) using a Corning Model 4 pH-temperature metre (Corning Glass Works, Medfield, MA), whilst meat colour (lightness L^* , redness a^* , and yellowness b^*) was measured within 45 min and 24 h after euthanasia using a colorimeter (Minolta, Tokyo, Japan). Shear force was determined using a Meullent-Owens razor shear blade whilst drip loss, water holding capacity, and cooking loss were measured according to Cheng et al. (2019).

Haemato-biochemistry

On d 50, blood was collected from 2 birds per pen (16 per treatment) from the wing vein using a 21-gauge needle. It was placed into purple-top EDTA-coated vacutainer tubes and analysed for haematology using an automated IDEXX LaserCyte Hematology Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa), whilst blood collected into red top Vacuette® Serum Clot Activator tubes without EDTA (Greiner Bio-One, GmbH, Frickenhausen, Germany) was analysed for biochemistry using an automated IDEXX Vet Test Chemistry Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa).

Statistical analysis

All data were tested for normality using Levene test (Levene, 1960) and homogeneity of variance using Shapiro Wilk test (Shapiro and Wilk, 1965). Then, normally distributed data including weekly ADWG, FI, FCE, weights, and lengths of carcass cuts and internal organs, haemato-biochemistry,

and meat quality were analysed for linear and quadratic effects by employing polynomial contrasts. The optimum dietary inclusion level of MSM was estimated using response surface regression analysis (SAS, 2002–2012 n.d.) 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 = MSM level (%), and $-b/2a = x$ value for optimal response. Also, weekly ADWG, FI, and FCE data were analysed as repeated measures to determine the diet by week interaction effect, whilst the rest of data (overall ADWG, FI, and FCE; weights and lengths of carcass cuts and internal organs; haemato-biochemistry; and meat quality) were analysed using the general linear models procedure of SAS (2002–2012) in a CRD, with diet being the only main factor. Least square means were compared using the probability of difference option and differences among them deemed significant at $P \leq 0.05$.

Results and discussion

Our results showed that dietary MSM did not affect ($P > 0.05$) growth performance indices (Table 2), weights and lengths of carcass cuts and internal organs (Table 3), haemato-biochemistry (Tables 4 and 5), and meat quality (Tables 6 and 7) measurements. The only exception was MSM-induced linear increase in the weight of the duodenum ($y = 0.08 (\pm 0.661)x + 15.78 (\pm 1.302)$; $R^2 = 0.141$; $P < 0.05$) (Table 3) and quadratic increase in serum phosphorus ($y = 0.01 (0.006)x^2 - 0.10 (0.065)x + 2.79 (0.126)$; $R^2 = 0.153$; $P = 0.05$) (Table 5) in contrast to a quadratic decrease in procalcitonin ($y = 0.01 (\pm 0.003)x^2 - 0.08 (\pm 0.029)x + 0.49 (\pm 0.054)$; $R^2 = 0.2622$; $P < 0.01$) (Table 4), linear and quadratic decrease in serum total protein [$y = -2.17 (\pm 0.639)x + 41.54 (\pm 1.252)$, $R^2 = 0.131$, $P < 0.05$; and $y = 0.18 (\pm 0.061)x^2 - 2.17 (\pm 0.639)x + 41.54 (\pm 1.252)$, $R^2 = 0.237$, $P < 0.01$], quadratic decrease in serum albumin ($y = 0.09 (\pm 0.043)x^2 - 0.74 (\pm 0.456)x + 14.67 (\pm 0.893)$; $R^2 = 0.1654$; $P < 0.05$), and a quadratic decrease in serum bilirubin ($y = 0.14 (\pm 0.035)x^2 - 1.47 (\pm 0.364)x + 7.11 (\pm 0.712)$; $R^2 = 0.4107$; $P = 0.001$) (Table 5).

These results suggest that MSM could be supplemented up to 10% in broiler finisher diets without compromising bird performance, carcass cuts and internal organs, haematology, and meat quality. The results are not unexpected considering the low dietary inclusion levels of MSM employed in the current study. In contrast, the literature mostly reports deleterious effects of dietary MSM on broiler growth performance, carcass characteristics, internal organs, and meat quality when the legume seed meal is included at high levels (≥ 10 to 30%) as replacement for SBM (Carmen et al., 1999; Jayaweera et al., 2007; Mthana et al., 2022). Even a dietary level as low as 5% has previously elicited detrimental effects

Table 2 The effect of dietary MSM inclusion on growth performance parameters of Cobb 500 broilers

		Dietary MSM inclusion level				SEM	P value	
		0%	2.5%	5%	10%		Linear	Quadratic
IBW (g)		885	879	904	916	183.0	0.145	0.851
ADWG								
	Week 4	78.6	69.0	69.4	71.9	6.32	0.524	0.356
	Week 5	54.2	65.4	68.5	73.9	10.38	0.239	0.611
	Week 6	79.9	96.8	70.8	116	33.51	0.950	0.516
ADFI								
	Week 4	125.9	130.7	135.6	133.6	4.81	0.224	0.373
	Week 5	168.6	160.6	173.3	178.8	8.81	0.219	0.618
	Week 6	184.4	195.4	179	205.8	17.40	0.495	0.633
FCE								
	Week 4	0.62	0.53	0.52	0.54	0.04	0.272	0.223
	Week 5	0.32	0.41	0.39	0.43	0.06	0.346	0.599
	Week 6	0.50	0.50	0.60	0.60	0.28	0.760	0.259

ADWG, average daily body weight gain; ADFI, average daily feed intake; FCE, feed conversion efficiency; MSM, mucuna seed meal; SEM, standard error of the mean based on a pooled estimate of variation ($n = 80$)

Table 3 The effect of dietary MSM inclusion on weights and lengths of carcass cuts and internal organs of Cobb 500 broilers

Parameters	Dietary MSM inclusion level				SEM	P value	
	0%	2.5%	5%	10%		Linear	Quadratic
HCW (g)	2231	2198	3056	2705	513.72	0.399	0.524
CCW (g)	2140	2052	2956	2111	394.39	0.864	0.213
Wing length (cm)	21.1	21.0	21.6	20.9	0.26	0.829	0.154
Wing weight (g)	113	113	98.8	118	7.73	0.727	0.174
Drumstick length (cm)	12.0	12.0	12.2	12.0	0.18	0.913	0.322
Drumstick weight (g)	132	133	116	133	9.22	0.939	0.302
Thigh length (cm)	8.70	9.20	8.60	8.90	0.39	0.959	0.954
Thigh weight (g)	169	152	149	141	12.11	0.132	0.525
Breast length (cm)	24.5	18.3	18.7	24.6	4.15	0.779	0.165
Breast weight (g)	401	409	407	409	12.72	0.749	0.805
Spleen (g)	3.00	3.19	3.04	2.85	0.22	0.466	0.527
Liver (g)	48.5	46.7	53.1	47.6	1.88	0.915	0.178
Gizzard (g)	38.9	38.3	40.5	39.8	1.47	0.511	0.737
Proventriculus (g)	9.20	8.59	9.62	9.86	0.41	0.864	0.210
Duodenum length (cm)	71.9	66.5	78.4	76.2	3.73	0.191	0.851
Duodenum weight (g)	15.6 ^b	18.1 ^{ab}	18.3 ^{ab}	20.0 ^a	1.37	0.036	0.578
Jejunum length (cm)	68.44	68.1	71.3	75.2	4.12	0.186	0.799
Jejunum weight (g)	28.1	28.6	29.3	30.5	1.23	0.133	0.997
Ileum length (cm)	20.9	21.9	22.3	22.2	1.62	0.599	0.673
Ileum weight (g)	22.0	31.1	25.0	23.9	3.41	0.868	0.251
Caecum length (cm)	4.55	4.62	5.50	5.36	0.45	0.141	0.503
Caecum weight (g)	13.2	20.4	12.3	12.4	3.67	0.505	0.578
Colon length (cm)	4.60	4.60	5.50	5.40	0.20	0.667	0.428
Colon weight (g)	1.60	2.20	1.80	1.90	0.26	0.787	0.408

CCW, cold carcass weight; HCW, hot carcass weight; MSM, mucuna seed meal; SEM, standard error of the mean based on a pooled estimate of variation ($n = 80$)

Table 4 The effect of dietary MSM inclusion on haematology of Cobb 500 broilers

Parameters	Dietary MSM inclusion level					P value	
	0%	2.5%	5%	10%	SEM	Linear	Quadratic
Red blood cells ($\times 10^{12}/L$)	2.60	2.30	2.40	2.10	0.31	0.309	0.899
Haematocrit (L/L)	14.5	14.4	13.1	13.4	1.71	0.606	0.779
Haemoglobin (g/dL)	9.30	9.80	9.60	9.50	0.44	0.841	0.390
MCV (fL)	57.9	56.4	56.5	59.4	2.60	0.426	0.094
MCH (pg)	43.4	47.8	51.6	49.0	5.98	0.503	0.679
RCDW (%)	28.6	28.0	28.4	27.1	1.04	0.322	0.689
Reticulocytes (%)	129	218	155	177	36.1	0.472	0.461
WBC ($\times 10^9/L$)	67.3	129	137	117	2.72	0.289	0.250
Heterophils ($\times 10^9/L$)	3.70	4.00	4.40	4.70	0.87	0.266	0.487
Lymphocytes ($\times 10^9/L$)	72.42	112	131	103	0.29	0.518	0.467
Monocytes ($\times 10^9/L$)	4.90	4.60	4.30	4.50	1.17	0.907	0.951
Eosinophils ($\times 10^9/L$)	6.89	10.6	11.2	10.2	0.15	0.169	0.138
Basophils ($\times 10^9/L$)	1.28	1.23	1.31	1.75	0.28	0.264	0.356
Platelet (K/ μ L)	1755	1740	1598	2195	308.29	0.296	0.1450
MPV (fL)	3.20	3.89	4.16	2.42	0.79	0.413	0.064
PDW (%)	15.0	16.8	17.9	15.1	1.33	0.907	0.334
Procalcitonin (%)	0.49 ^a	0.40 ^a	0.37 ^b	0.59 ^a	0.06	0.142	0.005

MCH, mean corpuscular haemoglobin; *MCV*, mean corpuscular volume; *MSM*, mucuna seed meal; *MPV*, mean platelet volume; *PDW*, platelet distribution width; *RCDW*, red cell distribution width; *WBC*, white blood cells; *SEM*, standard error of the mean based on a pooled estimate of variation ($n = 80$)

Table 5 The effect of dietary MSM inclusion on serum biochemistry of Cobb 500 broilers

Parameters	Dietary MSM inclusion level					P value	
	0%	2.5%	5%	10%	SEM	Linear	Quadratic
Symmetric dimethylarginine (μ g/dL)	31.7	43.6	34.7	34.4	1.30	0.764	0.155
Blood urea nitrogen (mmol/L)	0.62	0.60	0.60	0.61	0.01	0.999	0.083
Phosphorus (mmol/L)	2.70	2.77	2.45	3.00	0.04	0.174	0.050
Calcium (mmol/L)	6.51	2.42	2.39	2.39	0.04	0.259	0.317
Total protein (g/L)	41.5 ^a	38.0 ^b	35.8 ^b	37.6 ^b	0.50	0.044	0.009
Albumin (g/L)	14.7 ^a	13.8 ^a	13.4 ^b	15.9 ^a	0.40	0.133	0.039
Globulin (g/L)	26.5	25.3	22.8	23.7	0.50	0.083	0.378
Alkaline phosphatase (U/L)	232	234	242	259	8.60	0.265	0.469
Gamma-glutamyl transferase (U/L)	12.8	4.70	6.79	31.6	8.55	0.142	0.120
Bilirubin (μ mol/L)	6.80 ^a	5.30 ^a	2.60 ^b	6.40 ^a	0.30	0.427	0.001

MSM, mucuna seed meal; *SEM*, standard error of the mean based on a pooled estimate of variation ($n = 80$)

in finisher broilers (Osei and Dei, 1998). It is only when processed that high (16 to 25%) MSM inclusion levels induced no untoward effects in broilers (Vadivel and Pugalenth, 2010b; Sarmiento-Franco et al., 2019).

Interestingly, our results also showed increased serum phosphorus in broilers fed MSM-containing diets. This corroborates a report of mucuna seeds having the highest content of phosphorus (244.6 mg/100g) of all other minerals (Bhat et al., 2007) with a calcium to phosphorus ratio of 2.28, indicating that the seeds would be a suitable source of the macro-mineral for bone development in broilers.

Notwithstanding, a previous study showed decreased blood phosphorus and alkaline phosphatase (hypophosphataemia) in rats following consumption of unprocessed MSM-containing (10%) diets (Huisden et al., 2019). The reason for the differences in animal responses to dietary MSM between the current study and that of Huisden et al. (2019) is unknown. Hence, it would be of interest to further investigate blood (serum) phosphorus and other minerals in broilers fed MSM-supplemented diets in future.

Notwithstanding, our data also demonstrated deleterious effects of dietary MSM on entero-physio-metabolic

Table 6 The effect of dietary MSM inclusion on meat quality parameters of Cobb 500 broilers

Parameters	Dietary MSM inclusion level				SEM	<i>P</i> value	
	0%	2.5%	5%	10%		Linear	Quadratic
<i>45 min</i>							
pH	6.00	6.00	6.00	6.00	0.7	0.673	0.970
Temperature (°C)	27.7	25.6	27.4	27.7	0.78	0.532	0.318
<i>L</i> *	63.2	63.5	63.3	63.1	1.14	0.863	0.862
<i>a</i> *	2.80	2.70	3.10	3.10	0.38	0.454	0.868
<i>b</i> *	12.4	11.2	12.4	12.1	0.63	0.952	0.728
<i>24 h</i>							
pH	5.90	5.90	8.90	5.90	0.02	0.367	0.662
Temperature (°C)	14.4	14.5	14.5	14.3	0.37	0.757	0.729
<i>L</i> *	53.0	53.5	53.7	51.0	1.78	0.384	0.427
<i>a</i> *	2.10	2.50	1.90	1.70	0.30	0.207	0.685
<i>b</i> *	10.9	9.80	9.80	9.50	0.56	0.143	0.387

24 h refers to meat quality parameters 24 h after slaughter (i.e., on the cold carcass); 45 min refers to meat quality parameters 45 min after slaughter; *MSM*, mucuna seed meal; *SEM*, standard error of the mean based on a pooled estimate of variation ($n = 80$)

Table 7 The effect of dietary MSM inclusion on meat quality parameters of Cobb 500 broilers

Parameter	Dietary MSM inclusion level				SEM	P value	
	0%	2.5%	5%	10%		Linear	Quadratic
Drip loss	0.40	0.40	0.50	0.70	0.19	0.364	0.864
Cooking loss	30.0	31.2	31.6	29.9	0.70	0.750	0.055
Shear force (N)	5.40	5.90	5.70	6.20	0.35	0.157	0.931
WHC	84.1	85.2	79.2	84.1	3.16	0.984	0.340

MSM, mucuna seed meal; *WHC*, water holding capacity; *SEM*, standard error of the mean based on a pooled estimate of variation ($n = 80$)

processes particularly when included at 5 and 10% levels, with most of these effects elicited in blood serum components. In this regard, the observed increase in the weight of the duodenum with incremental dietary levels of MSM (Table 3) points to the presence of toxic phytochemicals most likely L-DOPA in the legume seed meal, if not the high dietary fibre content (Agyekum et al., 2012) (Table 1). Osei and Dei (1998) observed similar hypertrophic effects on the intestine in broilers fed high (15%) MSM-containing diets. Also, other studies observed increased lengths of the duodenum and other intestinal segments due to consumption of diets supplemented with MSM (Oloruntola et al., 2018) and tannin-rich leguminous leaf meals (Miya et al., 2020), in addition to dietary fibre stimulation (Wu et al., 2004) in broilers. However, it is interesting that the relative change in duodenal weight did not influence carcass weights, implying that the observed enteric weight change was merely an adaptation mechanism.

The observation of quadratic decreases in procalcitonin and serum total protein, albumin, and bilirubin appears to be congruent with the observed increase in the weight of the duodenum and suggests MSM-induced enteric and liver toxicity

particularly at 10% inclusion level. Produced by liver neuroendocrine cells and macrophages (Matzaraki et al., 2007), increased blood procalcitonin is indicative of liver injury (Rule et al., 2015), and this appears to have been more intense at 10% MSM inclusion level. Also, serum total protein (composed mainly of albumin, globulins, and other proteins) is ordinarily measured as a biomarker of the body's nutritional status and liver function, with its levels usually decreased in liver dysfunction-associated conditions such as ageing and malnutrition (Furruqh et al., 2004; Sabatino et al., 2017). Constituting 65% of serum total proteins, albumin is a biomarker for malnutrition and is responsible for the transportation, among other substances, of unconjugated bilirubin (Tian et al., 2014). Decreased serum bilirubin levels, on the other hand, occur in physiological conditions associated with oxidative stress and inflammation (Salomone et al., 2013; Tian et al., 2018). It would therefore appear that dietary consumption of high (10%) L-DOPA-infested MSM induced hepatotoxicity that led to protein undernutrition alongside compromised production of albumin, hence poor transportation and decreased serum levels, of bilirubin in broilers. Indeed, there is a positive correlation between procalcitonin and total bilirubin level (Qu et al., 2016) as well as serum total protein and bilirubin

(Jonathan et al., 2021). Otherwise, the observed concentrations of blood procalcitonin are similar to those (0.40 to 0.49%) of Matshogo et al. (2021) whilst serum total protein values are within normal ranges (25.0 to 45.0 g/L) for broiler chickens (Harr et al. 2002) and serum bilirubin values are slightly lower but comparable with those in the literature (3.2 to 5.0 µmol/L) (Egbu et al., 2022).

Conclusion

Our results showed that dietary MSM feeding did not affect growth performance, carcass characteristics, internal organs, as well as haemato-biochemical and meat quality characteristics. Dietary MSM increased duodenum weight and serum phosphorus and decreased procalcitonin and serum levels of total protein, albumin, and bilirubin. The MSM could therefore be supplemented up to 10% without compromising growth performance, carcass traits, internal organs, haemato-biochemistry, and meat quality in finisher broiler diets.

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Author contribution PNZ and DMNM conceptualised and designed the study; PNZ collected the data and wrote the original draft; PNZ, DMNM, and MCO analysed and interpreted the data; PNZ drafted the manuscript; MCO reviewed the manuscript; DMNM and SEM supervised the study, reviewed the manuscript, and approved the final draft. All authors read and approved the manuscript.

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Data availability The datasets generated during the current study are not publicly available due to cooperating producer privacy and confidentiality but are available on request from the corresponding author.

Declarations

Ethical approval The study followed standards required by the North-West University (NWU) Animal Production Sciences Research Ethics Committee (Ethical clearance number: NWU-00814-31-AS).

Conflict of interest The authors declare no competing of interests.

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References

- Agyekum, A.K., Slominski, B.A., Nyachoti, C.M., 2012. Organ weight, intestinal morphology, and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multienzyme supplement. *J Anim Sci*, 90 (11): 3032 – 3040. <https://doi.org/10.2527/jas.2011-4380>.
- Alleman Jr, R.J., Canale, R.E., McCarthy, C.G., Bloomer, R.J., 2011. A blend of *Chlorophytum borivilianum* and velvet bean increases serum growth hormone in exercise-trained men. *Nutrition and Metabolic Insights* 4, 55 – 63.
- Association of Official Analytical Chemists (AOAC), 2005. Official Methods of Analysis. 18th Edn, Association of Official Analytical Chemists (AOAC), Washington, DC, USA.
- Ayodele, S.O., Oloruntola, O.D., Adeyeye, S.A., Jimoh, O. A., Falowo, A.B., Omoniye, I.S., 2021. Supplementation value of *Mucuna* seed powder on performance, antioxidant enzymes, meat cholesterol and peroxidation, and serum metabolites of broiler chickens. *Malaysian Journal of Animal Science*, 24, 11–22. <https://doi.org/10.26480/mahj.02.2021.43.48>.
- Barroeta, A.C., 2007. Nutritive value of poultry meat: relationship between vitamin E and PUFA. *Worlds Poultry Science Journal*, 63 (2), 277–284. <https://doi.org/10.1017/S0043933907001468>.
- Bhat, R., Sridhar, K.R., Seena, S., 2007. Nutritional quality evaluation of velvet bean seeds (*Mucuna pruriens*) exposed to gamma irradiation. *Int J Food Sci Nutr*, 59 (4), 261–278. <https://doi.org/10.1080/09637480701456747>.
- Carme, J.D., Gernat, A.G., Myhrman, R., Carew, L.B., 1999. Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broiler. *Poult Sci*, 78, 866–872.
- Cavani, C., Petracci, M., Trocino, A., Xiccato, G., 2009. Advances in research on poultry and rabbit meat quality. *Italian Journal of Animal Science* 8 (2), 741–750. <https://doi.org/10.4081/ijas.2009.s2.741>.
- Cheng, Y., Chen, Y., Li, J., Qu, H., Zhao, Y., Wen, C., 2019. Dietary beta-sitosterol improves growth performance, meat quality, antioxidant status, and mitochondrial biogenesis of breast muscle in broilers. *Animals (Basel)*, 9, 71. <https://doi.org/10.3390/ani9030071>.
- Daffodil, E., Tresina, P., Mohan, V., 2016. Nutritional and antinutritional assessment of *Mucuna pruriens* (L.) DC var. *utilis* (Wall ex. Wight) Bak. Ex Burck and *Mucuna deeringiana* (Bort) Merrill: An underutilized tribal pulse. *Int Food Res J*, 23 (4), 1501 – 1513.
- Del Carmen, J., Gernat, A.G., Myhrman, R., Carew, L.B., 2002. Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broilers. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), *Food and Feed from Mucuna: Current Uses and the Way Forward*. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO), Tegucigalpa, Honduras, pp. 258–271. <https://doi.org/10.1093/ps/78.6.866>.
- Dharmarajan, S.K., Arumugam, K.M., 2012. Comparative evaluation of flavone from *Mucuna pruriens* and coumarin from *Ionidium suffruticosum* for hypolipidemic activity in rats fed with high fat diet. *Lipids Health Dis*, 11, 126. <http://www.lipidworld.com/content/11/1/126>.
- Egbu, C.F., Motsei, L.E., Yusuf, A.O., Mnisi, C.M., 2022. Evaluating the efficacy of *Moringa oleifera* seed extract on nutrient digestibility and physiological parameters of broiler chickens. *Agriculture*, 12, 1102. <https://doi.org/10.3390/agriculture12081102>.

- El-Deek, A.A., Abdel-Wareth, A.A.A., Osman, M., El-Shafey, M., Khalifah, A.M., Elkomy, A.E., Lohakare, J., 2020. Alternative feed ingredients in the finisher diets for sustainable broiler production. *Sci Rep* 10: 17743. <https://doi.org/10.1038/s41598-020-74950-9>.
- Ezeagu, I.E., Maziya-Dixon, B., Tarawali, G., 2003. Seed characteristics and nutrient and anti-nutrient composition of 12 *Mucuna* accessions from Nigeria. In Eilittä, M., Mureithi, J., Muinga, R., Sandoval, C., & Szabo, N. (Eds.) Increasing *Mucuna*'s Potential as a Food and Feed Crop, Proceedings of an International Workshop held on September 23–26, 2002, Mombasa, Kenya. *Tropical and Subtropical Agroecosystems* 1, 129–140. <https://doi.org/10.4314/wajae.v6i1.45607>.
- Flores, L., Esnaola, M.A., Myhrman, R., 2002. Growth of pigs fed diets with *Mucuna* bean flour (*Mucuna pruriens*) compared to soybean meal. In: B. M. Flores, M. Eilittä, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), *Food and Feed from Mucuna: Current Uses and the Way Forward*. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO), Tegucigalpa, Honduras, pp. 288–305.
- Furruqh, S., Anitha, D., Venkatesh, T., 2004. Estimation of reference values in liver function test in health plan individuals of an urban South Indian population. *Indian Journal of Clinical Biochemistry*, 19, 72 – 79. <https://doi.org/10.1007/BF02894260>.
- Harr, K.E., 2002. Clinical chemistry of companion avian species: a review. *Vet Clin Pathol*, 31(3), 140 – 151.
- Huisden, C. M., Butterweck, V., Szabo, N. J., Gaskin, J. M., Arriola, K. G., Raji, A., Adesogan A. T., 2019. Effects of detoxification of *Mucuna pruriens* on the feed intake, behavior, organ weights, blood cell counts and metabolites of rats. *Tropical and Subtropical Agroecosystems* 22, 379–389.
- Imbs, J. L., Schwartz, J., 2011. *Advances in the Biosciences – Peripheral Dopaminergic Receptors*. Pergamon Press 20, 95–99.
- Jayaweera, T.S.P.I., Cyril, H.W., Samarasinghe, K., Ruwandeepika, H.A.D., Wickramanayake, D.D., Thotawatthe, T.S.J., 2007. Effect of feeding velvet beans (*Mucuna pruriens*) on the lipid profile of broiler chickens. *Sabaragamuwa University Journal*, 7 (1), 78 – 85.
- Jonathan, O., Mnisi, C.M., Kumanda, C., Mlambo, V., 2021. Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels. *PLoS ONE* 16(11), e0259630. <https://doi.org/10.1371/journal.10.1371/journal>.
- Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., Ravindran, V., 2023. Broiler age influences the apparent metabolizable energy of soybean meal and canola meal. *Animals*, 13, 219. <https://doi.org/10.3390/ani13020219>.
- Kocer, B., Bozkurt, M., Ege, G., Tüzün, A.E.T., Konak, R., Olgun, O., 2018. Effects of a meal feeding regimen and the availability of fresh alfalfa on growth performance and meat and bone quality of broiler genotypes. *Br Poultry Sci*, 59, 318 – 329.
- Konishi, F., Furusho, T., Soeda, Y., Yamauchi, J., Kobayashi, S., Ito, M., Araki, T., Kogure, S., Takashima, A. Takekoshi, S., 2022. Administration of mucuna beans (*Mucuna pruriens* (L.) DC. var. *utilis*) improves cognition and neuropathology of 3x Tg-AD mice. *Sci Rep*, 12, 1–12. <https://doi.org/10.1038/s41598-022-04777-z>.
- Kouakou, A.S.A., Konan, H.K., Kané, F., Kanga, K.A., Kouadio, E.J.P. and Kouamé, L.P., 2022. Valorization of some minor plants of Côte d'Ivoire: Biochemical parameters and nutritional composition of the legume *Mucuna pruriens* seeds according to their maturity stage. *GSC Biological and Pharmaceutical Sciences*, 20 (2), 037 – 045. <https://doi.org/10.30574/gscbps.2022.20.2.0269>.
- Levene, H., 1960. Robust tests for the equality of variance. In: *Contributions to Probability and Statistics: Essays in honor of Harold Hotelling*. Ed. Stanford, (Palo Alto, CA: University Press), 278–292.
- Lieu, C.A., Venkiteswaran, K., Gilmour, T.P., Rao, A.N., Petticoffer, A.C., Gilbert, E.V., Deogaonkar, M., Manyam, B.V., Subramanian, T., 2012. The anti-parkinsonian and anti-dyskinetic mechanisms of *Mucuna pruriens* in the MPTP-treated nonhuman primate. *Evid Based Complement Alternat Med*, 2012, 840247.
- Mallick, P., Muduli, K., Biswal, J.N., Pumwa, J., 2020. Broiler poultry feed cost optimization using Linear Programming technique. *Journal of Operations and Strategic Planning*, 3 (1), 31–57. <https://doi.org/10.1177/2516600X19896910>.
- Marchewka, J., Sztandarski, P., Solka, M., Louton, H., Rath K, Vogt, L, Rauch, E, Ruijter, D, de Jong, I.C., Horbanczuk, J.O., 2023. Linking key husbandry factors to the intrinsic quality of broiler meat. *Poult Sci*, 102, 102384. <https://doi.org/10.1016/j.psj.2022.102384>.
- Matshogo, T.B., Mlambo, V., Mnisi, C.M., Manyeula, F., 2021. Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens. *Livest Sci*, 251, 104652. <https://doi.org/10.1016/j.livsci.2021.104652>.
- Matzaraki, V., Alexandraki, K.I., Venetsanou, K., Piperi, C., Myrianthefs, P., Malamos, N., Giannakakis, T., Karatzas, S., Diamanti-Kandarakis, E., Baltopoulos, G., 2007. Evaluation of serum procalcitonin and interleukin-6 levels as markers of liver metastasis. *Clin Biochem*, 40, 336 – 342.
- Miya, A., Sithole, A.N., Mthethwa, N., Khanyile, M., Chimonyo M., 2020. Response in carcass yield, organ weights, and gut morphology of broiler chickens to incremental levels of *Vachellia tortilis* leaf meal. *Canadian Journal of Animal Science*, 100, 282–291. <https://doi.org/10.1139/cjas-2019-0041>.
- Molinoff, P.B., Axelrod, J., 2022. Biochemistry of catecholamines. *Annu Rev Biochem*, 40, 465–500. <https://doi.org/10.1146/annurevi.40.070171.002341>.
- Mthana, M.S., Gajana, C.S., Hugo, A., Makhamba, N., 2022. Response in growth performance, physico-chemical properties, and fatty acid composition of broiler meat to different levels of *Mucuna pruriens* seed meal. *Research Square Preprint*, 1–23. <https://doi.org/10.21203/rs.3.rs-1127078/v1>.
- National Research Council, 2001. *Nutrient Requirements of Poultry*. 7th Edn., National Research Council, Washington, DC, USA.
- Navegantes, L.C.C., Resano, N.M.Z., Migliorini, R.H., Kettelhut, I.C., 2000. Role of adrenoceptors and cAMP on the catecholamine-induced inhibition of proteolysis in rat skeletal muscle. *Am J Physiol Endocrinol Metab*, 279, E663 – E668. <https://doi.org/10.1152/ajpendo.2000.279.3.e663>.
- Ngatchic, J.T.M., Njintang, N.Y., Bernard, C., Oben, J., Mbofung, C.M., 2016. Lipid-lowering properties of protein-rich mucuna product. *Nutrire*, 41 (2). <https://doi.org/10.1186/s41110-016-0003-0>.
- OECD/FAO, 2021. *OECD-FAO Agricultural Outlook 2021 – 2030*. OECD Paris, France. p. 163 – 177.
- Oloruntola, O.D., Agbede, J.O., Ayodele, S.O., Adeyeye, S.A., Agbede, J.O., 2018. Performance, haemato-biochemical indices and antioxidant status of growing rabbits fed on diets supplemented with *Mucuna pruriens* leaf meal. *World Rabbit Science*, 26, 277 – 285. <https://doi.org/10.4995/wrs.2018.10182>.
- Ose, S.A., Dei, H.K., 1998. The nutritive value of raw *Mucuna pruriens* (var. *utilis*) for broiler finishers. *Ghana Journal of Agricultural Science*, 31, 55 – 59.
- Panda, A.K., Zaidi, P.H., Rama Rao, S.V., Raju, M.V.L.N., 2014. Efficacy of quality protein maize in meeting energy and essential amino acid requirements in broiler chicken production. *Journal of Applied Animal Research*, 42, 133 – 139.
- Pulikallapura, H., Kurup, R., Mathew, P. J., Baby, S., 2015. Levodopa in *Mucuna pruriens* and its degradation. *Sci Rep*, 5, 1 – 9. <https://doi.org/10.1038/srep11078>.
- Qu, J., Feng, P., Luo, Y., Lü, X., 2016. Impact of hepatic function on serum procalcitonin for the diagnosis of bacterial infections in

- patients with chronic liver disease: a retrospective analysis of 324 cases. *Medicine (Baltimore)* 95 (30), e4270. <https://doi.org/10.1097/MD.0000000000004270>.
- Research and Market, 2019. Global poultry (broiler) market with focus on US, Brazil and Mexico: insights, trends and forecast (2019 – 2023), Research and Market, Dublin, Ireland.
- Rule, J.A., Hynan, L.S., Attar, N., Sanders, C., Korzun, W.J., Lee, W.M., et al., 2015. Procalcitonin identifies cell injury, not bacterial infection, in acute liver failure. *PloS One*, 10 (9), e0138566. <https://doi.org/10.1371/journal.pone.0138566>.
- Sabatino, A., Regolisti, G., Karupaiah, T., Sahathevan, S., Singh, B.K.S., Khor, B.H., Salhab, N., Karavetian, M., Cupisti, A., Fiaccadori, E., 2017. Protein-energy wasting and nutritional supplementation in patients with end-stage renal disease on hemodialysis. *Clin Nutr*, 36, 663 – 671.
- Salomone, F., Li Volti, G., Rosso, C., Grosso, G., 2013. Unconjugated bilirubin, a potent endogenous antioxidant, is decreased in patients with non-alcoholic steatohepatitis and advanced fibrosis. *J Gastroenterol Hepatol*, 28 (7), 1202 – 1208.
- Sarmiento-Franco, F., López-Sántiz, F., Santos-Ricalde, R., Sandoval-Castro, C., 2019. *Mucuna pruriens* seeds given in broiler diets on growth performance and carcass yield. *Ecosistemas y Recursos Agropecuarios*, 6 (16), 121 – 127.
- SAS, 2002 – 2012. SAS/STAT User's Guide. Statistics. Cary, New York, USA SAS Institute Inc.
- Shapiro, S. S., Wilk, M. B., 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52, 591 – 611. <https://doi.org/10.2307/2333709>.
- Suresh, S., Prakash, S., 2012. Effect of *Mucuna pruriens* (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. *J Sex Med* 9, 3066 – 3078.
- Tian, C.R., Qian, L., Shen, X.Z., Li, J.J., Wen, J.T., 2014. Distribution of serum total protein in elderly Chinese. *PloS One*, 9, e101242.
- Tian, S., Li, J., Li, R., Liu, Z., Dong, W. 2018. Decreased serum bilirubin levels and increased uric acid levels are associated with ulcerative colitis. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, 24, 6298 – 6304.
- Tumova, E., Chodova, D., Skrivanova, E., Lalouckova, K., Subrtova-Salmonova, H., Ketta, M., Machander, V., Cotozzolo E., 2021. The effects of genotype, sex, and feeding regime on performance, carcasses characteristic, and microbiota in chickens. *Poult Sci* 100, 760–764.
- United Nations, 2019. World Population Prospects. The 2019 Revision. Department of Economic and Social Affairs, Population Division, United Nations. <https://population.un.org/wpp/Download/Standard/Population/>. (Accessed 13 March 2023).
- Vadivel, V., Pugalenth, M., 2010a. Evaluation of growth performance of broiler birds fed with diet containing different levels of velvet bean meal as an alternative protein ingredient. *Livest Sci*, 127, 76 – 83. <https://doi.org/10.1016/j.livsci.2009.09.002>.
- Vadivel, V., Pugalenth, M., 2010b. Studies on the incorporation of velvet bean (*Mucuna pruriens* var. *utilis*) as an alternative protein source in poultry feed and its effect on growth performance of broiler chickens. *Tropical Animal Health Production*, 42, 1367 – 1376. <https://doi.org/10.1007/s11250-010-9594-2>.
- Valceschini, E. 2006. Poultry Meat Trends and Consumer Attitudes. https://www.researchgate.net/publication/228586389_Poultry_meat_trends_and_consumer_attitudes [Accessed: 29 March 2023].
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74 (10), 3583 – 3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Weiner, N., 1979. Tyrosine-3-monooxygenase (tyrosine hydroxylase). In: Youdim, M.B.H., editor. *Aromatic Amino Acid Hydroxylases and Mental Disease*. John Wiley & Sons, Ltd; New York. pp. 141 – 190.
- Wu, Y.B., Ravindran, V., Thomas, D.G., Birtles, M.J., and Hendriks, W.H., 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Br Poultry Sci*, 45 (1), 76 – 84. <https://doi.org/10.1080/00071660410001668897>.
- Yantika, S. M., Evvyernie, D., Diapari, D., & Winaga, K., 2016. Performance, carcass production, and meat quality of Sumba Ongolebulls fed ration supplemented velvet bean (*Mucuna pruriens*). *Media Peternakan* 39, (1), 20 – 26. <https://doi.org/10.5398/medpet.2016.39.1.20>.

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