



# The effect of dietary supplementation of mucuna leaf meal on the growth performance, blood parameters, and carcass quality of broiler

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**ABSTRACT.** Three hundred 1-day old broiler chickens were used to assess the effects of Mucuna leaf meal (MLM) dietary supplementation on the performance, haemato-biochemical indices, oxidative status and meat of broiler chickens. Five experimental supplemented diets were formulated: diets: 1 (0% supplement), 2 (1.1 % OXYT), 3 (0.5% MLM), 4 (1.0 % MLM) and 5 (1.5% MLM). The final weight gain of the birds fed diets 2 and 5 was higher ( $p < 0.05$ ) than those birds fed the control and other diets. The relative weights of the lung were affected ( $p < 0.05$ ) by dietary supplementation. Serum cholesterol concentration reduces ( $p < 0.05$ ) with increased dietary MLM supplementation levels from 1.0% to 1.5%. Superoxide dismutase and glutathione peroxidase levels increased ( $p < 0.05$ ) in the broiler chickens fed a 1.5% MLM supplemented diet, compared to those fed the control and other diets. Meat cholesterol of the chickens fed 1.0%, and 1.5% MLM supplemented diets were lower ( $p < 0.05$ ) than the experimental birds fed the rest diets. In conclusion, the 1.5 % MLM dietary supplementation improves body weight gain, reduces the serum cholesterol concentration, increases the serum superoxide dismutase and glutathione peroxidase activities of the chickens and reduced the meat cholesterol.

**Keywords:** Phytochemicals; supplements; chickens; performance; antioxidative status; meat.

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## Introduction

Globally, poultry production is one of the stable means of meeting the animal protein requirement of man. The world poultry meat utilization in 2019 was 133.4 million tonnes and was forecasted to rise by 2.6% to 135 million tonnes in 2020. This is due to the foreseen increased production due to customers' current efforts to substitute bovine and pig meats with alternatives (Lee, Kwak, Joo, Kang, & Lee, 2018). In many countries such as Brazil, China, the United States of America, South Africa and Mexico, the poultry meat output was increasing in the year 2020, though at a relatively slower rate, compared to the previous three years, reflecting rises in the production cost in the middle of soaring animal feed ingredients such soybean meal and maize prices and cost needed to execute the COVID-19 protocols in butchery (Kearney, 2010). The use of phytochemicals as a dietary supplement as a replacement for antibiotic growth promoters (AGPs) and health status enhancers has been in practice for poultry (Oloruntola, Agbede, Ayodele, & Oloruntola, 2018; Oloruntola, Ayodele, Adeyeye, & Agbede, 2018).

Among the various under-utilized wild legumes is Mucuna (*Mucuna pruriens*), otherwise known as velvet bean is native to Africa and tropical Asia, but has been introduced to several countries and widely naturalized and cultivated as a soil improver (Holo, Achigan-Dako, & Sinsin, 2013; Oloruntola, Ayodele, Adeyeye, & Agbede, 2018). *Mucuna pruriens* (Fabaceae) is an orthodox therapeutic annual plant (Lampariello, Cortelazzo, Guerranti, Sticozzi, & Valacchi, 2012). Every single part of *M. pruriens* has treasurable medicinal properties. It has been explored in various contexts, including for its anti-microbial, anti-neoplastic, anti-diabetic, and aphrodisiac activities (Lampariello, Cortelazzo, Guerranti, Sticozzi, & Valacchi, 2012). *Mucuna* has antioxidant, anti-venom, immunomodulatory, neuroprotective, and growth performance-enhancing properties (Rai, Chaturvedi, Singh, Singh, & Singh, 2020; Oloruntola, Ayodele, Adeyeye, & Agbede, 2018). Mucuna leaf meal also has nutritive value

with 26.54% crude fibre, 26.09% crude protein, 4.91% ether extract, 10.25% ash and 21.18 % nitrogen-free extract (Adejoh, Okwori, & Orayaga, 2019). The effects of Mucuna leaf meal dietary supplementation on performance, health status, and chickens' meat have not been intensively evaluated. This experiment was conducted to unveil the effects of Mucuna leaf powder/meal dietary supplementation on the performance, erythrogram, serum biochemical indices, antioxidant status, and meat cholesterol of broiler chickens.

## Material and methods

### Leaf collection, processing, and analysis

Fresh mucuna leaves harvested from mother plants were chopped into smaller pieces with stainless knives and spread lightly on a clean tarpaulin to air-dry for two weeks, milled to mucuna leaf meal (MLM) having an average size of about 100 $\mu$ m and analyzed for alkaloids (Jan, Faridullah, Sherani, & Jahan, 2017), cardiac glycoside (Sofowora, 1993), and flavonoid (Zhao et al., 2018). The saponin and tannin (Senguttuvan, Paulsamy, & Karthika, 2014), steroid (Panchal, & Charuben, 2021), and terpenoid (Sofowora, 1993) were also determined. The ferric reducing antioxidant property (Iqbal, Salim, & Lim, 2015) and 2-diphenyl-1-picrylhydrazylhydrate (Zhao et al., 2018) were determined.

### Experimental diets

The broiler starter (0-21 days) and finisher (22-42 days) diets were formulated and prepared for feeding the broiler chickens (Table 1). The experimental diets were divided into five equal portions in the starter and finisher phases: diet 1 (0% MLM), and diet 2(1.1% OXYT). In contrast, diets 3, 4 and 5 were supplemented with 0.5 %, 1.0 % and 1.5% MLM, respectively. After that, all the diets were analyzed for their crude protein and crude fibre composition (Association of Official Analytical Chemistry [AOAC], 2016).

### Birds, housing, and experimental design

Three hundred (300) a-day old Arbor acres breed of broiler chickens were distributed at random to the five experimental diets (6 replicates per dietary treatment, 10 birds per replicate, and 60 birds per experimental diet). Each replicate (10 birds each) was housed in 200x100cm pens. The experimental house's average temperature was maintained within 30°C $\pm$ 2 for the first seven days and gradually reduced by 2°C after each consecutive seven days until the house temperature was 26°C $\pm$ 2. Twenty-three hours of light/day was provided, while feed was provided as often as necessary (*ad libitum*) during the experimental period.

### Growth performance, blood collection, slaughtering, and carcass analysis

The body weight (BW), the body weight gain/increase (BWG), and feed intake (FI) were estimated at a seven-day interval. The feed conversion ratio (FCR) was estimated as the birds' ratio of feed consumed to their body weight gain. On day 42 of the feeding trial, three birds/replicate were randomly selected, tagged, weighed, stunned, and sacrificed by cutting the two jugular veins in their neck region. The slaughtered chickens' blood was collected in plain bottles for serum biochemical indices (aspartate aminotransferase, cholesterol, and creatinine) and serum antioxidant enzyme (superoxide dismutase and glutathione peroxidase) analysis; and into EDTA bottles for the haematological studies (Sastry, 1983).

The blood samples collected in the plain bottles were centrifuged, and their serum was separated into another set of plain bottles and frozen at -20°C before analysis. The serum enzymes were determined with a Reflectron® Plus 8C79 (Roche Diagnostic, GonbH Mannheim, Germany), using kits. The serum superoxide dismutase and serum glutathione peroxidase (Wang, et al., 2018) were also determined. The slaughtered birds were dressed and weighted to determine the dressed percentage. After that, the birds' internal organs were weighed and expressed as the percentage of slaughtered weight. The breast meat cholesterol (Grasso, Harrison, Monahan, & Brunton, 2019) was also determined.

### Data analysis

The Completely Randomized Design with the following model:  $Y_{dij} = \mu + a_d + e_{dij}$  was used in this experiment. Where  $Y_{dij}$  = any of the response variables;  $\mu$  = the overall mean;  $a_d$  = effect of the  $i$ th treatment ( $d$  = diets 1, 2, 3, 4 and 5) and  $e_{dij}$  = random error due to experimentation. The variance among the data collected within the treatments was analyzed with SPSS version 20 software. Duncan's test of SPSS separated the treatment means.

## Results and discussion

### Composition of the *Mucuna* leaf meal (MLM)

The result of phytochemicals determination shows that MLM has some phytochemicals (alkaloids: 11.92 g kg<sup>-1</sup>, cardiac glycoside: 9.88 g kg<sup>-1</sup>, flavonoid: 90.33 g kg<sup>-1</sup>, saponin: 32.04 g kg<sup>-1</sup>, steroid: 9.58 g kg<sup>-1</sup>, tannin: 3.04 g kg<sup>-1</sup> and terpenoid: 12.66 g kg<sup>-1</sup>) of health benefits (Table 1). Phytochemicals are rich sources of chemical compounds of biological importance which exert protective effects against degenerative diseases (Nwanna, Adebayo, Ademosun, & Oboh, 2019; Oloruntola et al., 2020). The protective roles (e.g. antibacterial, anticancer, anti-hypertensive, etc.) of alkaloids were reported (Kuate, 2014). Cardiac glycosides exert a beneficial stimulation on the cardiac muscle, thus causing increased heart contraction without concomitant increased oxygen consumption but the efficiency of the myocardium in pumping and meeting the demands of the circulatory system (Atalay & Durmaz, 2018). The flavonoids and terpenoids (isoprenoids) cytotoxicity roles against tumour cells and their preventive roles against cancer were reported in addition to their antioxidant, antibacterial and antiviral properties (Kozłowska & Szostak-Wegierek, 2014). Tannins are anti-microbial and antioxidative (Sung et al., 2012), while saponins were reported to lower cancer risks, decrease blood lipids, and lower blood glucose (Shi et al., 2004). This study's antioxidant property determination shows that MLM has 37.98 mg g<sup>-1</sup> ferric reducing antioxidant property and 22.56% 2-diphenyl-1-picrylhydrazylhydrate. The revealed the antioxidant potential of MLM was revealed in this study and supported by the earlier report of Oloruntola, Ayodele, Adeyeye, and Agbede (2018).

**Table 1.** Composition of experimental basal diets and *Mucuna puriens* leaf meal.

Ingredients (g kg <sup>-1</sup> )	Experimental diets		Phytogen	
	Starter (1-21 days)	Grower (22-42 days)	Parameters (g kg <sup>-1</sup> )	MLM
Maize	426.60	485.60	Alkaloid	11.92
Wheat offal	121.00	101.00	C Gly	9.88
Soybean meal	386.90	347.90	Flavonoid	90.33
Vegetable oil	22.00	23.00	Saponin	32.04
Di-calcium phosphate	18.00	17.00	Steroid	9.58
Limestone	14.00	14.00	Tannin	3.04
Premix	3.00	3.00	Terpenoid	12.66
Methionine	3.00	3.00	FRAP (mg/g)	37.98
Lysine	2.50	2.50	DPPH (%)	22.56
Salt	3.00	3.00		
Chemical analysis (g kg <sup>-1</sup> DM)				
Crude protein	220.00	205.60		
Crude fibre	43.30	45.80		
Calculated analysis (g kg <sup>-1</sup> DM)				
ME (kcal kg <sup>-1</sup> )	2955.88	3 000.24		
Ca	10.20	9.30		
Available P	6.00	5.50		
Methionine	6.30	3.80		
Lysine	11.50	10.30		

MLM: *Mucuna pruriens* leaf meal; C gly: Cardiac glycoside; ME: Metabolizable energy; FRAP: Ferric reducing antioxidant property; DPPH: 2-diphenyl-1-picrylhydrazyl hydrate; \*Composition of vitamin premix: \*Composition of vitamin premix: Vitamin A (10,000 iu) D (2,000,000 iu), E (35, 000 iu); K (1,900 mg); B12 (19 mg); Riboflavin (7,000 mg). Nicotinic acid (45,000 mg) Folic acid (1,400 mg); Pyridoxine (3800 mg); Thiamine (2,200 mg); Pantothenic acid (11,000 mg); Biotin (113 mg) and trace element such as Cu (8,00 mg), Mn (64,000 mg); Zn(40,000 mg), Fe(32,000 mg), Se(160 mg), I(800 mg); and other items as Ca (400 mg); Chlorine (475,000 mg) Methionine (50, 000 mg); BHT (5,000 mg) and Spiramycin (5,000 mg) in 2.5 kg of premix.

### The performance, carcass traits and internal organs of broiler chicken

Table 2 shows the effects of MLM supplements on performance, carcass traits, and broiler chickens' relative internal organs. The final weight recorded in the birds fed diets 2, 4 and 5 in this study were higher ( $p < 0.05$ ) than those fed the control (diet 1) and diet 3. Almost similarly, the body weight gain of the birds fed diets 2 and 5 was higher ( $p < 0.05$ ) than those fed the control and the rest diets. The performance parameters such as body weight gain, feed conversion ratio, and broiler chickens' livability are affected by nutrition (Martins et al., 2016). The observed better growth performance recorded in birds fed diet 5 may be due to activities of the bioactive components of MLM which supports better growth performance by improving the food status (Ahmad et al., 2018), by producing anti-microbial, antioxidant and flavour enhancer effects (Valenzuela-Grijalva, Pinelli-Saavedra, Muhlia-Almazan, Domínguez-Díaz, & González-Ríos, 2017).

Therefore, there is a likelihood that the phenolic compounds, present in MLM have demonstrated free radical scavenging and antioxidant activities, which supports the maintenance of the intestinal mucosa integrity (Oloruntola, Ayodele, Adeyeye, & Agbede 2018), and increased absorption surface of the duodenum and the ileum of broiler chicken (Cardoso, Lima, Lima, Dorneles, & Danelli, 2012). This study's experimental treatment tends to affect the feed intake ( $p = 0.06$ ) and feed conversion ratio ( $p = 0.08$ ) in this study.

**Table 2.** Effects of OXYT and MLM supplementation on the performance, carcass traits and internal organs of broiler chickens.

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5% MLM	Diet 4 1.0% MLM	Diet 5 1.5% MLM	SEM	P-value
Performance (1-42 days)							
IBW (g bird <sup>-1</sup> )	33.93	33.65	33.41	33.72	33.27	0.31	0.98
FWG (g bird <sup>-1</sup> )	2656.53 <sup>b</sup>	2966.03 <sup>a</sup>	2608.80 <sup>b</sup>	2864.29 <sup>a</sup>	2949.01 <sup>a</sup>	46.07	0.01
BWG (g bird <sup>-1</sup> )	2622.60 <sup>c</sup>	3032.42 <sup>a</sup>	2575.38 <sup>c</sup>	2830.56 <sup>b</sup>	3015.66 <sup>a</sup>	54.48	0.01
FI (g bird <sup>-1</sup> )	3536.20	3774.73	3549.26	3612.33	3759.53	36.34	0.06
FCR	1.35	1.24	1.37	1.27	1.24	0.02	0.08
Carcass traits and internal organ (day 42)							
Dressing %	70.35	71.08	72.30	72.63	76.43	1.05	0.45
Liver	2.20	2.58	1.95	2.17	2.30	0.09	0.18
Heart	0.39	0.46	0.47	0.45	0.45	0.01	0.14
Kidney	0.54	0.58	0.54	0.57	0.57	0.02	0.96
Gizzard	2.05	2.10	2.21	2.03	2.06	0.04	0.79

Means within a row with different superscripts are significantly different ( $p < 0.05$ ). MLM: *Mucuna pruriens* leaf meal; OXYT: Oxytetracycline; SEM Standard error of the mean. IBW: Initial body weight; FWG: Final weight gain; BWG: body weight gain; FI: Feed intake; FCR: Feed conversion ratio.

Some phytochemicals were recently proposed to exert effect directly and indirectly on animal metabolism by increasing muscle tissue. Besides, the relative weights of the animals' internal organs may deviate from the normal range in response to dietary toxins (Oloruntola et al., 2020). In this study, the MLM supplementations did not affect ( $p > 0.05$ ) the dressing percentage and relative internal organs' weights of the broiler chicken. The stability of the dressed percentage and the relative organ weights in this feeding trial indicates that this study's dietary supplement supports the healthy development of the edible parts and the internal organs (liver, heart, kidney and gizzard) relative weight of the chicken. This result/observation suggests that dietary treatment did not pose a harmful threat to the chickens' relative lung weights.

### The erythrogram and serum biochemical indices of broiler chickens

Table 3 shows that the haematological indices were not ( $p > 0.05$ ) affected by MLM supplementation. The existence of influence of nutrition on the haematological traits and the correlation existing between the haematological traits and the performance of animals (Oloruntola, Ayodele, Adeyeye, & Agbede, 2018) are among the reasons for making blood indices primary tools for assessing the physiological, nutritional and pathological status of animals (Agbede, Omotoso, Oloruntola, Ayeni, & Aletor, 2019). The stability of the erythrocytes counts in the experimental birds across the dietary treatments shows appropriate erythrocyte production and oxygen transportation to the body cells and the delivery of carbon dioxide to the lungs.

Serum biochemistry was reported as a chemical analysis used when studying the organs' physiological state in the body of an organism (Oloruntola, Agbede, Ayodele, & Oloruntola, 2018). Similar ( $p > 0.05$ ) aspartate aminotransferase and creatinine concentrations were recorded across the various treatments in this study (Table 3). Aspartate aminotransferase (AST) concentration in the serum is usually used to detect liver damage. The stability of aspartate aminotransferase concentration across the various treatments in this study agreed with the earlier report of Oloruntola, Agbede, Ayodele, and Oloruntola (2018). It signalled the non-negative impact of the supplements used in this study on the experimental birds liver's normal physiological and anatomical function (Peter & Susan, 1999). The birds' serum cholesterol concentration reduces ( $p < 0.05$ ) with increased dietary supplementation levels from 1.0% to 1.5% compared to the experimental birds fed diets 1 and 2. The reduction of the serum cholesterol concentration following 1.0 and 1.5% MLM supplementation indicates that MLM dietary supplementation at 1.0% and 1.5% produced hypo-cholesterol effects in the birds. The decreased serum cholesterol concentration recorded in MLM supplemented diets in this study is in tandem with earlier reports of Oloruntola, Agbede, Ayodele, and Oloruntola (2018), and could be the function of phytosterol (sitosterol[24-ethylcholesterol] and campesterol [24-methylcholesterol]) which naturally occur in small quantities in plants and known for decreasing cholesterol levels by impeding with the absorption of cholesterol by competing effectively with cholesterol for inclusion in mixed micelles, a necessary step for

cholesterol absorption (Poli et al., 2021). An abnormally elevated level of creatinine signals possible kidney failure. However, the indifference of the creatinine concentration among the birds distributed to the different dietary treatments in this study indicates the safety of MLM as a dietary supplement (Peter & Susan, 1999). This result is in tandem with Oloruntola, Ayodele, Agbede, and Oloruntola, 2016 and Oloruntola, Agbede, Ayodele, and Oloruntola (2018) who recorded insignificant effects of phyto-genic feed ingredient or supplement on the creatinine levels in broiler chickens and growing rabbits.

**Table 3.** Effects of OXYT and MLM on the erythrogram and serum biochemical indices of broiler chickens.

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5% MLM	Diet 4 1.0% MLM	Diet 5 1.5% MLM	SEM	P-value
<b>Erythrogram</b>							
Red blood cells ( $\times 10^{12} \text{ L}^{-1}$ )	1.70	2.10	2.20	2.25	2.20	0.08	0.23
Haemoglobin conc. (g $\text{dL}^{-1}$ )	9.83	9.66	9.83	10.16	10.17	0.10	0.46
Packed cell volume (%)	29.50	29.00	29.50	30.50	30.50	0.30	0.47
<b>Serum biochemical indices</b>							
Aspartate aminotransferase (IU $\text{L}^{-1}$ )	111.30	127.60	131.45	123.75	116.40	6.13	0.88
Cholesterol (mg $\text{dL}^{-1}$ )	95.55 <sup>a</sup>	86.84 <sup>ab</sup>	89.18 <sup>ab</sup>	77.22 <sup>bc</sup>	69.420 <sup>c</sup>	3.11	0.03
Creatinine (mg $\text{dL}^{-1}$ )	1.20	1.74	1.66	1.58	2.11	0.18	0.70

Means within a row with dissimilar letters and significantly different ( $p < 0.05$ ). MLM: *Mucuna pruriens* leaf meal; OXYT: Oxytetracycline; SEM Standard error of the mean.

### The serum antioxidant enzyme and meat cholesterol

The superoxide dismutase is a metalloenzyme that accelerates the simultaneous oxidation and reduction (dismutation) of a pair of molecules of superoxide anion to hydrogen peroxide and molecular oxygen, consequently causing the potentially harmful superoxide anion less hazardous (Ighodaro & Akinloye, 2018); while the glutathione peroxidase (GPx) breakdown hydrogen peroxides to water; and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol (Ighodaro & Akinloye, 2018). The superoxide dismutase and glutathione peroxidase levels of the chickens fed 1.5% MLM supplemented diet was significantly higher ( $P < 0.05$ ), compared to those birds fed the rest diets (Table 4). The elevation of superoxide dismutase and glutathione peroxidase levels in the experimental birds fed 1.5% MLM supplemented diet may also be linked to the activities of flavonoid, ferric reducing antioxidant properties and 2-diphenyl-1-picrylhydrazyl hydrate in MLM (Table 1). For instance, the flavonoids reaction with free radical results in the delocalization of the gained electron over the phenolic antioxidant and the stabilization by the resonance effect of the aromatic nucleus and subsequent aversion of the continuation of the free radical chain reaction (Lee, Lin, Yu, & Lee, 2017). The cholesterol in the chickens' meat fed 1.0%, and 1.5% MLM supplemented diets were lower ( $P < 0.05$ ) than those on the rest diet. This result agreed with earlier reports by Think et al. (2018) and could be associated with the reduced plasma cholesterol level recorded in the birds fed 1.5% MLM supplemented diet in this study. The factors responsible for this reduced cholesterol in the plasma and meat may be similar. Besides, tannin (one of the phytochemicals detected in MLM) produced an inhibitory effect on intestinal absorption of lipid and subsequent prevention of excessive lipid accumulation in the tissues (Think et al., 2018).

**Table 4.** Effects of OXYT and MLM on the serum antioxidant enzymes and meat cholesterol of the broiler chickens.

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5% MLM	Diet 4 1.0% MLM	Diet 5 1.5% MLM	SEM	P-value
Superoxide dismutase (%)	41.80 <sup>b</sup>	35.53 <sup>b</sup>	40.23 <sup>b</sup>	40.31 <sup>b</sup>	49.92 <sup>a</sup>	1.52	0.02
Glutathione peroxidase (mg $\text{mL}^{-1}$ )	11.48 <sup>c</sup>	13.53 <sup>b</sup>	12.81 <sup>b</sup>	10.31 <sup>d</sup>	25.04 <sup>a</sup>	1.42	0.01
Meat Cholesterol (mg $\text{dL}^{-1}$ )	140.38 <sup>a</sup>	136.45 <sup>a</sup>	135.24 <sup>a</sup>	78.76 <sup>b</sup>	92.58 <sup>b</sup>	7.11	0.01

Means within a row with dissimilar superscripts are significantly different ( $p < 0.05$ ); MLM: *Mucuna pruriens* leaf meal; OXYT: Oxytetracycline; SEM: Standard error of the mean.

### Conclusion

The 1.5 % MLM dietary supplementation improves the body weight gain, reduces the serum cholesterol concentration, increases the serum superoxide dismutase and glutathione peroxidase of the broiler chickens and reduced their meat cholesterol.

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