Supplementation value of Mucuna seed powder on performance, antioxidant enzymes, meat cholesterol and peroxidation, and serum metabolites of broiler chickens

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Abstract

This study examined the effect of Mucuna seed powder (MSP) supplementation on performance, meat, and health status of broiler chickens. A total of 300 broiler chicks were randomly allocated into 5 treatments with 6 replicates of 10 birds each, as follows: Diet 1 (control), Diet 2 (diet supplemented with 1.1% oxytetracycline, OXYT), Diet 3 (diet with 0.5% MSP), Diet 4 (diet with 1.0% MSP) and Diet 5 (diet with 1.5% MSP). The dietary MSP supplementation significantly (P<0.05) increased the daily body weight gain of the broiler chickens, compared to those fed the control diet at the starter phase and overall (1-42 days) period of the feeding trial. No significant differences were observed in the measured aspartate aminotransferase (AST), creatinine, and alanine aminotransferase (ALT) among the treatments. The broiler chicken fed diets supplemented with MSP had higher (P<0.05) total serum glutathione peroxidase and superoxide dismutase compared to those fed the control diet. The concentration of muscle cholesterol and lipid peroxidation reduced significantly (P<0.05) in the birds fed MSP supplemented diets compared to those fed the control diet. In conclusion, this study has shown that MSP can be used up to 1.5% as a potential phytogenic feed supplement in a broiler diet to enhance the growth performance, maintain the carcass traits, boost endogenous antioxidants and reduce meat cholesterol level and lipid oxidation.

Keywords: antioxidant enzymes, cholesterol, lipid peroxidation, mucuna seed meal, phytogens

Introduction

Poultry production is one of the fastest-growing agricultural subsectors that is contributing greatly to the global economy by providing livelihood, nutrition, food security, employment, and poverty alleviation for hundreds of millions of people across the world (Nabarro and Wannous, 2014). Despite this, the poultry industry is still faced with substantial challenges ranging from strategies to meet global demand for high-quality animal proteins which is anticipated to nearly double by 2050 (Flees et al., 2020) to the identification of effective alternatives /replacements for antibiotic feed additives due to its global ban to provide food that is safe and healthier (i.e free of antimicrobial residues) for consumers (Flees et al., 2020).

Interestingly, some alternatives such as prebiotics, probiotics, symbiotic and phytogens have been proposed for consideration in poultry production in recent times (Cheng et al., 2014, Reis et al., 2018; Flees et al., 2020), despite that the use antibiotic feed additives has been instrumental in the reduction of harmful microorganisms in the intestinal microbiota and enhancement of growth performance and immunity of broiler chickens (Reis et al., 2018). Presently, phytogen is rated as one of the fastest-growing sectors of animal feed additives, accounting for a total sale of about USD 586.8 million and 631.4 million in 2017 and 2018, respectively, with an expectation to reach a total amount of USD 962.5 million by 2023 (Flees et al., 2020).

These phytogenic feed additives, which are commonly referred to phytobiotics or botanicals, are usually derived from a wide variety of plant materials such as herbs (flowers, leave, seed, stem, root, etc.), spices and their extracts such as essential oils (Reis et al., 2018). These plants are rich in secondary plant metabolites with a robust capacity to elicit antioxidant, antimicrobial, antiviral, and immune-modulatory effects in a biological system (Applegate et al., 2010). Application of phytogenics feed additives has been reported to enhanced body weight and feed efficiency (Reis et al., 2018, Flees et al., 2020), egg production (Sharma et al., 2020), improve carcass yield, gut integrity and meat quality (Reis et al., 2018; Oloruntola et al., 2018), boost reproductive performance, serum enzymatic and antioxidant activity (Reis et al., 2018; Oloruntola et al., 2019), and reduced intestinal bacterial pathogenicity and medicinal cost (Reis et al., 2018). One of such phytogenic feed additives is mucuna seed (*Mucuna pruriens var. utilis*).

Mucuna seed is a widely available tropical legume that is nutritionally rich in crude protein (29.37%), crude fibre (5.53%), ether extract (5.90%), ash (4.43%), energy (3.49 Kcal/g), minerals and other nutrients (Tuleun et al., 2009). The antioxidant activity of mucuna seed has been demonstrated with a strong ability to scavenge free radicals (DPPH and ABTS radicals), reactive oxygen species, and inhibit lipid peroxidation (Dhanasekaran et al., 2008). Mucuna seed possesses phytate, tannins, oxalate, saponin (Sarmiento-Franco et al., 2019) and a high phenolic compound including levodopa (containing approximately 5%) which has been used as neuroprotective agents and treating in Parkinson's disease in an animal model (Dhanasekaran et al., 2008; Longhi et al., 2011).

Dietary inclusion of mucuna seed powder as an alternative protein source in poultry diet at a high level has been reported although with adverse outcomes. Tuleun and Igba, (2008) observed in a study that dietary inclusion of mucuna seed powder at 20% significantly decreased the performance traits of the birds (feed intake, growth rate, and feed conversion ratio) compared with control. Similarly, Vadivel and Pugalenthi (2010) reported that dietary replacement of soybean meals with mucuna seed powder above 15.7% significantly decreased body weight growth performance of broiler chickens compared to control. Besides, the study of Adzitey et al. (2010) revealed that the inclusion of mucuna seed powders at 25 % and 30 % did not influence carcass traits and internal organ weight except gizzard when compared to control.

Based on these pieces of evidence, many authors have recommended a low inclusion rate of mucuna seed powder to maintain the performance of broiler chickens (Akinmutimi and Okwu, 2006; Tuleun and Igba, 2008; Iyayi et al., 2008, Vadivel and Pugalenthi, 2010). Therefore, the objective of this study was to examine the dietary supplementation of mucuna seed powder as a phytogenic feed additive on growth performance, antioxidant enzymes, meat cholesterol and peroxidation, and serum metabolites of broiler chickens.

Materials and Methods

Phytogens, diets, animal management and experimental design

Mucuna pruriens (Mucuna) seeds were collected from the mother plants within the vicinity of The Federal Polytechnic, Ado Ekiti, Nigeria. These seeds were separated from their pods and sundried for 14. Thereafter, the Mucuna seeds were ground with a hammer mill to about 100µm to produce Mucuna seed powder (MSP). The MSP were analyzed for flavonoids (Bohm and Kocipal-Abyazan, 1994), phenol (Ignat et al., 2013), saponin (Brunner, 1984), terpenoids (Sofowora 1993), ferric-reducing antioxidant property (Pulido et al. 2002) and 2,2-diphenyl-1-picrylhydrazy hydrate (Gyamfi et al., 1999).

A basal diet each was formulated for the broilers' starter and finisher phases (NRC, 1994) (Table 1). At each of the phases, the basic diet was divided into five equal portions and named diets 1 to 5. Diets 1 and 2 had 0 and 1.1% OXYT supplementation, while the diets 3, 4 and 5 were supplemented with 0.5%, 1.0% and 1.5 % MSP, respectively (Table 1).

Table 1. Composition of experimental basal diets and Mucuna seed powder.

	Basal diets		Mucuna seed powder	
Ingredients (%)	Starter phase (1 to 21 days)	Grower phase (22 to 42 days)	Parameter	Quantity (mg/g)
Maize	39.00	51.20	Flavonoids	71.87
Wheat offal	14.3	15.70	Phenol	18.80
Soybean meal	38.00	26.7	Saponin	64.54
Fish meal	2.5	5.00	Terpenoids	109.22
Vegetable oil	2.20	2.20	FRAP	22.18
Bone meal	2.00	2.00	DPPH (%)	71.87
Limestone	1.00	0.8		
Premix	0.25	0.25		
Methionine	0.25	0.25		
Lysine	0.32	0.32		
Salt	0.18	0.18		
Chemical analysis (%)				
Crude protein	23.44	21.06		
Crude fibre	4.49	3.75		
Calculated analysis (%)				
ME (kcal/kg)	2894.21	3019.71		
Ca	1.33	1.38		
Available P	0.77	0.71		
Methionine	0.62	0.61		
Lysine	1.66	1.47		

ME: Metabolizable energy, FRAP: Ferric-reducing antioxidant property, DPPH: 2,2-diphenyl-1-picrylhydrazyl hydrate.

Three hundred 1-day old Cobb 500 broiler chicks were randomly assigned to five diets (10 birds/replicate; 60 birds/diet) using a completely randomized design. A space of 210 x 100 cm was provided per replicate, and the floor was covered with wood shaving. The experimental pen temperature was maintained at 31ºC±2 for the first 7 days and gradually reduced by 2ºC after each consecutive week until the experimental house temperature was 26ºC±2. The experimental pen was lighted 23 hours/day, and the experimental birds fed *ad libitum*. The broiler chickens were vaccinated against Newcastle disease and fowlpox during the feeding trial.

Growth performance, carcass traits, and blood analysis

The birds' growth performance indices were determined on 7 days interval and the feed conversion ratio estimated as a ratio of the total feed ingested to the total weight gain. On day 42 of the experiment, three birds per replicate were randomly selected, weighted, stunned, and sacrificed. The jugular veins in the neck region of the selected birds were cut with a sharp and clean knife. The birds' blood was allowed to flow into a plain sample bottle for serum metabolites (aspartate aminotransferase, creatinine and alanine aminotransferase) and serum antioxidant enzymes (catalase and glutathione peroxidase) determination. The serum metabolites were determined with a Reflectron ® Plus 8C79 (Roche diagnostic, GombH Mannheim, Germany). The glutathione peroxidase and superoxide dismutase were determined as described by Rotruck et al. (1973), and Aebi (1974), respectively.

The weights of the slaughtered and dressed birds were determined with a sensitive scale, and the birds' dressed percentage was estimated as a percentage of the slaughtered weight. About 100g of the bird's breast meat was cut out the determination of the meat cholesterol (Allain et al., 1974), and meat lipid peroxidation (Bostogloun et al., 1994).

Analysis of data

The model: $Dny = \mu + \alpha n + \beta ny$, was used in this experiment, where Dny = any of the response variables; n = the overall mean; αn = effect of the *nth* treatment (*D* = diets 1, 2, 3, 4 and 5); and βny = random error due to experimentation. Data were exposed to one-way ANOVA using SPSS version 20. The differences among the means were determined ($p<0.05$) by Duncan multiple range test of SPSS.

Results

Phytochemical composition and antioxidant activity of Mucuna seed powder

The phytochemical constituents and antioxidant activity of Mucuna seed powder (MSP) are presented in Table 1. The result shows that MSP possessed high flavonoid (71.89mg/g), phenol (18.80mg/g), saponin (64.54mg/g) and terpenoids (109.22mg/g) contents. Additionally, the result of the antioxidant activity revealed that MSP exhibited 71.87% 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 22.18mg/g ferric-reducing antioxidant property (FRAP).

Growth performance, carcass traits and relative internal organs of broiler chickens

Table 2 shows the effect of Mucuna seed powder supplementation on the growth performance of broiler chickens. The body weight gain of the broiler chickens at both starter and overall (1-42 days) was significantly ($P<0.05$) improved by OXYT and MSP supplementations, compared to the control.

The carcass traits and relative internal organs weights of the broiler chickens were not affected ($P>0.05$) by the OXYT and MSP supplementation (Table 3).

Table 2. Effects of Mucuna seed powder on the performance of broiler chickens

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5%MSP	Diet 4 1.0%MSP	Diet 5 1.5%MSP	SEM	P value
Starter phase (1-21day)							
IBW (g/bird)	43.09	43.34	43.34	42.84	43.93	0.15	0.26
BWG (g/bird/day)	31.47[b]	36.54[a]	37.94[a]	37.24[a]	36.29[a]	0.68	0.01
FI (g/bird/day)	45.78	50.81	51.27	49.98	54.17	1.71	0.68
FCR	1.45	1.39	1.35	1.34	1.48	0.04	0.82
Grower phase (22-42day)							
BWG (g/bird/day)	74.49	88.01	85.17	90.04	83.61	2.17	0.18
FI (g/bird/day)	135.23	139.34	136.67	133.01	128.71	2.29	0.69
FCR	1.84	1.58	1.61	1.50	1.56	0.04	0.16
Overall (1-42day)							
BWG (g/bird/day)	105.97[b]	124.54[a]	123.12[a]	127.28[a]	119.90[ab]	2.56	0.04
FI (g/bird/day)	181.02	190.15	187.95	183.00	182.88	3.30	0.92
FCR	1.31	1.13	1.13	1.07	1.09	0.03	0.09

MSP: Mucuna seed powder, 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.

Table 3. Effects of Mucuna seed powder supplementation on the carcass traits and relative weights (% slaughtered weight) of the broiler chickens

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5%MSP	Diet 4 1.0%MSP	Diet 5 1.5%MSP	SEM	P value
Slaughtered weight (g/bird)	2266.66	2616.67	2566.65	2600.00	2590.01	64.12	0.42
Dresses weight (g/bird)	1636.97	1932.65	1871.02	1936.68	1891.00	42.95	0.13
Dressed percentage (%)	72.71	73.91	73.32	74.86	73.00	1.34	0.99
Heart	0.31	0.32	0.34	0.26	0.30	0.01	0.22
Kidney	0.38	0.42	0.50	0.51	0.43	0.03	0.73
Liver	1.75	1.76	1.77	1.69	1.55	0.06	0.85
Lung	0.45	0.47	0.46	0.36	0.37	0.02	0.28
Pancreas	0.22	0.25	0.22	0.16	0.22	0.01	0.12
Gizzard	1.61	1.61	1.58	1.31	1.54	0.05	0.36
Proventriculus	0.21	0.25	0.22	0.16	0.22	0.01	0.12
Gall bladder	0.07	0.09	0.09	0.06	0.10	0.01	0.57
Spleen	0.11	0.12	0.15	0.13	0.11	0.01	0.77

MSP: Mucuna seed powder, OXYT: Oxytetracycline; SEM: Standard error of the mean.

Serum metabolites, serum antioxidant enzymes, and meat analysis

The aspartate aminotransferase (AST), creatinine, and alanine aminotransferase (ALT) were not affected (P>0.05) by the dietary treatment (Table 4). The serum glutathione peroxidase and superoxide dismutase of the birds fed MSP supplemented diets were significantly higher (P<0.05) than those fed the control and OXYT supplemented diets. The meat cholesterol level decreased significantly (P<0.05) in the birds fed the MSP supplement compared to the control and OXYT supplemented diet. In the same vein, the lipid peroxidation of meat of broiler chickens fed MSP supplemented diets were significantly (P<0.05) lower compared to those fed the control and OXYT supplemented diets.

Table 4. Effects of Mucuna seed powder supplementation on the serum metabolites, serum antioxidant enzymes, and meat analysis of the broiler chickens

Parameters	Diet 1 Control	Diet 2 OXYT	Diet 3 0.5%MSP	Diet 4 1.0%MSP	Diet 5 1.5% MSP	SEM	P value
Serum metabolites							
Aspertate amino transferase (μ/l)	104.30	63.70	70.70	92.90	70.55	5.71	0.88
Creatinine (mmol/l)	20.10	23.50	28.65	25.15	23.75	1.29	0.36
Alanine amino transferase (μ/l)	59.75	61.55	59.65	60.70	59.55	0.57	0.82
Serum antioxidant enzyme							
Glutathione peroxidase (IU/ml)	43.41^{e}	50.21^{d}	53.88^{c}	59.12^{b}	62.13^{a}	1.77	0.00
Superoxide dismutase (%)	45.29^{d}	52.01^{c}	52.52^{c}	68.40^{b}	73.92^{a}	2.90	0.00
Meat analysis							
Cholesterol (mg/dl)	71.55^{a}	69.94^{a}	38.37^{b}	37.40^{b}	33.91^{b}	5.36	0.01
Lipid peroxidation (mg/MDA)	3.75^{a}	1.72^{b}	0.95^{e}	1.29^{d}	1.47^{c}	0.26	0.00

MSP: Mucuna seed powder, OXYT: Oxytetracycline, SEM Standard error of the mean.

Discussion

In this study, the result of the phytochemical and antioxidant analysis revealed that MSP is rich in flavonoids, phenols, saponin, terpenoids and possessed a strong ability to scavenge free radical and other reactive oxygen species when applied as phytogenic feed additives. Phytogenic feed additives are often reported to possess high polyphenols, flavonoid, saponin, terpenoids, and other bioactive compounds which possess antioxidant properties and are useful in scavenging free radicals and improve poultry health and performance (Gade et al., 2017; Lillehoj et al., 2018; Sharma et al., 2020). These non-enzymatic antioxidants of plants and phytochemical origin interact directly or indirectly with reactive oxygen species by chelating metals and prevent these transition metals from participating in metal-mediated Haber-Weiss reaction. Besides, these non-enzymatic antioxidants from phytochemicals can also scavenge free radicals by donating electrons from radicals to make them stable and thereby prevent the attack of the biological targets (Kohen and Gati, 2000; Samuni et al., 1983). This result is in line with other authors who have reported mucuna seed meal to contain high phenolic compounds (Longhi et al., 2011) and free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radicals (Siddhuraju and Becker, 2003).

The similar daily body weight gain (BWG) observed in the broiler chickens fed the OXYT (feed-based antibiotic supplement) and MSP supplemented diets which were an improvement over the daily BWG recorded for those birds fed the control diet suggests the phyto-constituents of the phytogens (MSP) used in this study promote the growth of the broiler chickens (Stanley et al., 2014). For instance, the phenolic compounds inhibit microbial enzymes by reacting with sulfhydryl groups or through non-specific interaction with the protein (Masson and Wasserman, 1987; Panda and Rath, 2012). Tannins can bind to protein and metals, thus inhibit the growth of microorganisms through the substrate and metal ion deprivation (Stern et al., 1996), while it was being speculated that terpenoids are involved in membrane disruption by lipophilic compounds (Ahmed et al., 1993; Panda and Rath, 2012). Besides, phytochemicals exhibit biological activities by modulating the gut microflora, controlling the adhesion of antimicrobial pathogen, maintain the integrity of the intestinal epithelium and health status of the animals by reducing toxins and improving the nutrient availability for absorption (Costa et al., 2013, Dhama et al., 2014).

Phytochemical feed supplements could produce effects that may have direct and indirect anabolic modulating effects on animal metabolism in favour of increasing the growth of target tissues (Valenzuela-Grijalva et al., 2017; Olorunmtola et al., 2018). The phytochemicals modulate animal metabolism in a way similar to the action of β-adrenergic agonist compounds because, some compounds of plant origin such as hydroxycinnamic acid derivatives of the amino acid phenylalanine and catecholamines; the natural animal hormones (Valenzuela-Grijalva et al., 2017). The catecholamines interact with β-adrenergic receptor agonists to change animal metabolism by increasing the protein synthesis and lipolysis and by decreasing lipogenesis (Dominguez-Vara et al., 2009). Besides, there could be changes in the relative weights of the internal organs of the animals in response to some dietary constituents (Ayodele et al., 2016). Therefore, the observed non-significant difference in carcass traits and relative internal organ weights of the broiler chickens across the dietary treatments suggests the safety of the MSP dietary supplementation at these levels when applied as phytogenic feed additives in poultry diet. A similar result has been reported by Adzitey et al. (2010) who found no significant difference in carcass characteristics and relative internal organ weights except gizzards of broiler chickens fed a diet containing mucuna seed meals at 25 and 35% inclusion levels.

Most serum enzymes are tissue specific and their abnormal rise in serum concentration connotes tissue damage (Oloruntola et al., 2020). Besides, the result of the serum enzyme constituents is important in determining the health status and the physiological responses of animals to treatments. Findings from this study revealed no significant influence of MSP supplements on serum aspartate aminotransferase (AST), creatinine and alanine aminotransferase (ALT) concentration of the broiler chickens compared to other treatments. These observed similarities demonstrate the safety and nutritional adequacy and safety of MSP supplement on broiler chickens. It also showed that the diets elicit similar effects and cause no injury to the heart, liver, skeletal muscles and kidney cells of the broiler chickens (Lording and Friend, 1991; Jiwuba and Onunwa, 2018), thereby ascertaining the suitability of the MSP at these inclusion levels as phytogenic feed additives for chicken production.

The adoption of phytogens as an antioxidant for ameliorating the effects of oxidative stress is on the increase (Gupta et al., 2006). Glutathione peroxidase and superoxide dismutase are among the various antioxidants that prevent oxidation (Afolabi and Oloyede, 2014). The observed increase serum glutathione peroxidase and superoxide dismutase activities in the birds fed MSP diets further revealed the antioxidant properties of MSP and its potentials to improve the antioxidant status of the experimental birds. This result is in tandem with the report of Oloruntola et al., (2018) who reported increased antioxidant activities in rabbits fed mucuna leaf meal supplemented diets. The improved oxidative status observed in birds fed the MSP supplemented diets in this study could also be linked to the improved performance recorded in these same set of birds because oxidative stress has been reported as one of the important factors preventing tropical domestic animals from attaining their full growth potential (Jimoh, 2018).

The reduced meat cholesterol level recorded in the broiler chickens fed MSP supplemented diets in this study is of health benefits because the consumers are now conscious of the quality of the products that they are consuming. Meat low in cholesterol is suitable for health, in particular for those predisposed to heart attack and related health challenges (Oloruntola et al., 2016; Oloruntola et al., 2018). This result clearly showed the hypocholesterolemic effect of the MSP supplements. The observed hypocholesterolemic effect may be due to the activities of the phytosterols in MSP by inhibiting cholesterol absorption in the intestine due to the similarity in the structure of phytosterol and cholesterol (Plat and Mensink, 2002). This finding is in agreement with the report of Jayaweeya et al (2007) who reported a significant decrease in cholesterol content of broiler chickens fed MSP diet. Similarly, the reduction in lipid peroxidation recorded in the meat of the birds fed MSP supplemented diets, compared to control could be a result of the higher amount of the antioxidant enzymes recorded in the muscle of broiler chickens in this study. Lipid peroxidation is known as the leading non-microbial cause of quality deterioration in muscle food, producing off-flavour, and oxidized compounds that are detrimental to consumer health (Falowo et al., 2014). Another study has reported the capacity of phytogenic feed additives to reduce lipid peroxidation in muscle food due to inherent bioactive components and antioxidant activity (Oloruntola et al., 2019; Valenzuela-Grijalva et al., 2017).

Conclusion

Mucuna seed powder contains rich bioactive compounds with a strong antioxidant capacity to scavenge free radical and other reactive species. It is also observed from this study that the application of MSP at 0.5. 1.0, and 1.5% enhanced body weight gain of the broiler chickens and maintain the carcass trait and internal organ weights of broiler chickens. While the inclusion of MSP will reduce meat cholesterol level, and meat lipid peroxidation of the broiler chickens. It could, therefore, be recommended that dietary inclusion of MSP up to 1.5% to enhance the weight gain, maintain the health status, and improved the meat quality of the broiler chickens.

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