Effect of L-Dopa on performance and serum cholesterol of Nera black pullets

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Abstract

L-Dopa in raw or processed mucuna seed, in the presence of antinutritional factors, has been implicated to have caused nutritional disorders in monogastrics. However, the use of pure L-Dopa is established in broilers and quails to enhance growth performance, cholesterol profile, and reproductive organs without detrimental effect. Information on the effect of L-Dopa on hydrolysis of triglycerides and growth performance in laying birds is scanty. In a 56-day experiment, 300 eight-weeks old Nera Black layers were fed diets supplemented with L-Dopa at 0.0%, 0.1%, 0.2%, 0.3%, 0.4% dietary levels respectively in a completely randomized design. Feed intake, body weight gain and feed conversion ratio were determined. Blood (5mL) was sampled from two birds per replicate at the end of the feeding trial to determine serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein and blood parameters using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha 0.05$. The L-Dopa inclusion at all dietary inclusion levels did not significantly influence the feed intake, body weight gain and feed conversion ratio, serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein and blood parameters. There were no observable significant differences in all the parameters measured probably because of the growing stage of the experimental birds. It can therefore be concluded that L-Dopa supplementation in the diets of pullets at 0.1% to 0.4% inclusion levels did not cause any deleterious effect on the overall performance.

Keywords: L-Dopa, growth performance, blood metabolites, serum cholesterol, Nera black pullets

Introduction

Mucuna has been reported to contain some anti-nutritional factors like tannins, phytins, L-Dopa, cyanogenic glycosides, lectins, trypsin inhibitors, haemagglutinin (Iyayi and Egharevba 1998; Anele, 2002). Lorenzetti *et al.* (1998) also reported that the beans are known to produce the unusual non-protein amino acid, L-Dopa, a potent neurotransmitter precursor that is believed, in part, to be responsible for the toxicity of the Mucuna seeds. Among the anti-nutritional factors in raw velvet beans, L-Dopa has been reported to have a profound effect on the growth response of some

poultry species. L-Dopa is a direct precursor to dopamine and its supplementation in feed increases dopamine levels in the body. L-Dopa has also been clinically proven to be one of the few substances that could cross the blood-brain barrier where it becomes decarboxylated to dopamine which is effective in stimulating the hypothalamus and the pituitary to release and increase the level of growth hormone in the body (Meites *et al.*, 1977). The hypothalamus is considered to be of paramount importance as an integration centre for the various influences on pituitary function (Prasad *et al.*, 2007).

Effect of L-Dopa on growth and cholesterol of pullets

Despite the fact that L-Dopa is pharmacologically an active ingredient (Pieriset *et al.*, 1980), it could be potentially anti-nutritional and toxic if ingested in large amounts (Josephine and Janardhanan, 1992). L-Dopa in humans is effective for the treatment of Parkinson's disease being a precursor of dopamine; however large intake of L-Dopa causes schizophrenia; a serious disorder which affects how a person thinks, feels and acts.

Feeding considerable levels of L-Dopa has also been reported to cause a significant increase in brain dopamine which affects neuro endocrine gonadal axis resulting in changes in body growth and reproductive conditions (Wilson and Meier, 1989). Omidiwura *et al.* (2015) also reported that L-Dopa extract, at levels tested (0.1 – 0.4% L-Dopa inclusion), had no detrimental effect on broilers; rather, the serum cholesterol profile was improved and better bird performance. From earlier and recent studies (Prasad *et al.*, 2007; Omidiwura *et al.*, 2016), indicating the effectiveness of L-Dopa in growth, reproductive stimulation and cholesterol reducing effect in some poultry species, the inclusion of L-Dopa into the diet of laying birds, then, can have effect on cholesterol content in their eggs and blood stream. This study was therefore aimed at investigating the effect of L-Dopa on the growth performance, selected blood metabolites and serum cholesterol of laying birds at grower stage.

Materials and methods

The experiment was carried out at the poultry unit of the Teaching and Research Farm, University of Ibadan, Nigeria. Synthetic L-Dopa used in this study was imported from a reliable commercial source. Three hundred eight-weeks old Nera black strain of laying birds were tagged, weighed and randomly allotted to

five dietary treatments containing 60 birds each. Each treatment was replicated five times with twelve birds per replicate in a completely randomised design. Experimental diets and fresh water were supplied *ad libitum* during the 6 weeks experimental period. Recommended vaccines and other medications were administered as and when due while other routine management practices were strictly adhered to. The experimental diets as presented in Table 1, were prepared at the Feed mill unit of the Teaching and Research Farm, University of Ibadan. Treatment 1 was the control diet; basal diet with no inclusion of L-Dopa, while treatments 2, 3, 4, and 5 contained the basal diets and L-Dopa at inclusion levels of 0.1%, 0.2%, 0.3% and 0.4% respectively.

Growth performance

During the experimental period, feed intake was determined by deducting the left over from the total quantity of feed supplied to the birds. Weights of the birds were monitored weekly and feed conversion ratio calculated as follows;

Weight gain: The initial weight was measured on the first day of the experiment and subsequently on weekly basis.

Weight Gain = Final weight – Initial weight

Feed intake: This is the measurement of quantity of feed consumed on a weekly basis.

Feed Intake = Amount of feed supplied – Amount of feed remaining

Feed conversion ratio: This was computed as feed intake per weight gain in gram.

Feed Conversion Ratio = $\frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$

Blood collection

At the end of the experimental period, blood samples were collected from one randomly selected bird per replicate. The samples were taken from the jugular vein into two bottles. The bottles containing ethylene-

diamine-tetra acetate, (EDTA), anti-coagulant was used to collect 2ml of blood samples and the other bottles without EDTA were used to collect 3ml of blood samples. The blood samples with EDTA were used to determine haematological parameters, (Packed cell volume, Red blood cell, White blood cell, and Haemoglobin). While the blood samples without EDTA were used to determine serum cholesterol parameters (Total cholesterol, High density lipoprotein and Low density lipoprotein).

Determination of haematological parameters

Packed cell volume

According to wintrobemicrohaematological method (Baker and Silver, 1982), packed cell volume was estimated from the blood samples collected in bottles containing EDTA by gently mixing and drawing up in a micro haematocrit capillary tube of three quarter of its length. One end of the tube was sealed with plasticine. The capillary tube was placed in micro-haematocrit centrifuge ensuring that the plasticine end is outward. After closing it, it was centrifuged at 12,000 rotations per minute for 4 minutes. The tubes were then read in the haematocrit reader. The reading expressed the packed red blood cells as a percentage (%) of the volume of the blood.

Haemoglobin

They were determined by a cyanmethaemoglobin method (Benjamin, 1978) using Drabkin's solution as diluent.

Red blood cells

They were estimated by taking 0.02ml of the blood sample from the bottle containing EDTA and mixing with 4ml of diluting fluid (3g sodium citrate, 1ml formaldehyde in 100ml distilled water) by shaking for about 30 seconds. About a quarter of the content was expelled before filling the

haemocytometer counting chamber and allowed to settle by leaving to stand for about a minute after filling. All the red cells were then counted using the magnification of x40 for objective lens and x8 for eyepiece of the microscope, with the aid of a counter. RBC total counts were estimated using the formula below:

$$\text{RBC Total count} = \text{RBC counts} \times 10 \times \text{dilution factor (200)} = \text{RBC} \times 10,000$$

White blood cell and differential leukocytes counts

They were determined using Neubauer haemocytometer after appropriate dilution. The differential White Blood Cell counts were obtained by making a differential smear with Wright stain and the percentage counts were taken for Lymphocytes, Basophils, Monocytes, Eosinophils and Neutrophils (Wintrobe, 1956).

Serum cholesterol

The cholesterol level in the serum was determined using serum cholesterol kit (Cell Biolabs' High Density Lipoprotein and Low Density Lipoprotein/Very Low Density Lipoprotein Cholesterol Assay Kit) using the procedure of Siedel *et al.* (1981). Cholesterol esters were hydrolysed via cholesterol esterase into cholesterol, which was then oxidised by cholesterol oxidase into the ketone cholest-4-en-3-one and hydrogen peroxide. The Horseradish peroxidase catalyses the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples were compared to a known concentration of cholesterol standard within the 96-well microtiter plate format. Samples and standards were incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader. Total cholesterol (μL) =

$$\frac{\text{Sample corrected fluorescence} \times \text{Sample dilution}}{\text{Slope}}$$

$$\text{Cholesterol Ester } (\mu\text{M}) = \text{Total cholesterol} - \text{Free cholesterol}$$

Effect of L-Dopa on growth and cholesterol of pullets

Note: For the conversion of results from μM to mg/dL , divide the cholesterol concentration (μM) by 25.9.

Statistical analysis

Experimental data collected were subjected

to One-way Analysis of Variance procedure - PROC ANOVA of Statistical Analysis System (SAS, 2011). Treatment means were separated using Fisher's Least Significant Difference ($\alpha_{0.05}$) option of the same software.

Table 1: Gross composition of diets with graded levels of L-Dopa inclusion to determine the growth performance and evaluate blood metabolites and serum cholesterol fed to laying birds at grower stage

Ingredients (g/kg)	Control	Inclusion level of L-Dopa			
		0.10%	0.20%	0.30%	0.40%
Maize	53.00	52.90	52.80	52.70	52.60
Soybean Meal	18.00	18.00	18.00	18.00	18.00
Soybean Oil	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
*Vit-Min Premix	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30
Wheat offal	23.00	23.00	23.00	23.00	23.00
L-Dopa	0.00	0.10	0.20	0.30	0.40
TOTAL	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients (g/kg)					
Crude protein	16.04	16.03	16.02	16.01	16.00
Energy ME, kcal/kg	272.79	272.46	272.12	271.79	271.45
Fat	3.44	3.44	3.43	3.43	3.42
Crude fibre	4.96	4.95	4.95	4.95	4.95
Calcium (Ca)	0.54	0.54	0.54	0.54	0.54
Total Phosphorus	0.87	0.87	0.87	0.87	0.87
Non-phytate P (NPP)	0.48	0.48	0.48	0.48	0.48
Ca:NPP	1.13	1.13	1.13	1.13	1.13

*Vit-Min Premix: Vitamin A, 12,000,000 IU; Vitamin D3 2, 000, 000 IU; Vitamin E 7,000 IU; Vitamin B2 4,000 mg; Nicotinic acid 15,000 mg; Calcium d-pentothenate 8,000 mg; Biotin 40 mg; Vitamin B12 10 mg; Mn 20,000 mg; Fe 50,000 mg; Zn 100,000 mg; Cu 10,000 mg; Iodine 750 mg; Co 3000 mg.

Results and discussion

Growth performance

The growth performance characteristics of growing pullets fed graded levels of L-Dopa diets is as presented in Table 2. Total weight gain, average daily feed intake, average daily weight gain and feed conversion ratio did not differ significantly ($P>0.05$) in the birds fed diets containing graded levels of L-Dopa compared to the control birds.

Though not significant, comparable feed intake and weight gain observed in the birds fed diets with graded levels of L-Dopa inclusion compared to those on control diet

suggest that L-Dopa in isolation may not necessarily be detrimental when ingested by animals at 0.1 to 0.4% of diets. It has been documented that dopamine, for which L-Dopa is a direct precursor, influences feeding behavior in animals (TEFAN, 2004). This suggests that the growing birds fed diets with pure L-Dopa inclusion did not experience depressed feed intake owing to toxicity as the dopamine synthesized from L-Dopa could have influenced the feeding control centres of the hypothalamus to actively consume the feed given. This is in agreement with a study conducted by

Omidiwura *et al.* (2016) who found out that feed intake, weight gain and feed conversion ratio (FCR) were not significantly influenced by L-Dopa inclusion in the diets of broiler birds. There

is possibility, therefore, that growth performance was not improved due to the development of the reproductive system of the birds because they are preparing for the reproductive stage.

Table 2: Growth performance characteristics of grower laying birds fed diets with graded levels of L-Dopa inclusion

Parameters	0% L-Dopa	0.1% L-Dopa	0.2% L-Dopa	0.3% L-Dopa	0.4% L-Dopa	SEM
TWG (g)	7091	6385.2	6605	6719.8	6975.6	128.94
ADFI (g/day/bird)	68.44	64.24	61.48	64.33	67.93	1.37
ADWG (g/day/bird)	14.07	12.67	13.1	13.33	13.84	0.26
FCR	4.92	5.1	4.71	4.84	4.92	0.11

TWG: Total weight gain,

ADWG: Average daily weight gain,

ADFI: Average daily feed intake

FCR: Feed conversion ratio, SEM: Standard error of mean

Blood profile

The result of the blood profile of grower birds fed L-Dopa diets is presented in Table 3. At the end of the study, it was observed that packed cell volume of the birds on the control diet was not significantly different ($P>0.05$) from those on diets with L-Dopa inclusion. It was also observed that the haemoglobin concentration did not differ significantly among the experimental birds across the dietary treatments. White blood cells, blood lymphocytes, blood heterophils, monocytes count and Blood platelet values were also not significantly different in the birds on the control diet

compared to the birds on the L-Dopa experimental diets. L-Dopa did not significantly influence all blood parameters assessed compared to the animals on the control diet. This indicates that L-Dopa did not alter erythropoietic activities in the animals on the experimental diets. A change in the red blood parameters would have implicated L-Dopa as affecting the synthesis and activities of these crucial components of the blood. Similar values obtained for the white blood parameters of the birds on the experimental diets and those on the control diet also indicate normal health status of the growing birds.

Table 3: Blood profile of grower laying birds fed diets with L-Dopa inclusion

Parameters	0% L-Dopa	0.1% L-Dopa	0.2% L-Dopa	0.3% L-Dopa	0.4% L-Dopa	SEM
Packed Cell Volume (%)	31.20	29.80	31.60	30.20	29.20	0.51
Haemoglobin (g/dL)	10.46	9.56	10.38	9.68	9.20	0.20
Red Blood Cells ($\times 10^6$ μ L)	3.32	3.11	3.38	3.31	3.38	0.05
White Blood Cells ($\times 10^3$ μ L)	14.28	13.03	13.32	15.59	15.59	0.60
Lymphocytes (%)	59.40	66.40	64.20	60.40	58.40	1.30
Heterophils (%)	33.40	28.40	31.40	33.00	34.20	1.45
Monocytes (%)	3.20	3.00	3.00	2.80	3.80	0.20
Eosinophils (%)	3.80	1.80	2.80	3.80	3.40	0.30
Basophils (%)	0.20	0.60	0.60	0.00	0.80	0.14
Platelets ($\times 10^3$)	171.80	179.60	164.80	169.40	177.25	9.16

SEM – Standard error of mean

Effect of L-Dopa on growth and cholesterol of pullets

Serum biochemical profile

The result of the serum biochemical profile of grower laying birds fed diets with graded levels of L-Dopa inclusions is presented in Table 4. Total serum cholesterol concentration, High density lipoprotein and Low density lipoprotein did not differ significantly ($P>0.05$) in the birds fed L-Dopa containing diets compared to those on the control diet.

In the current study, serum total cholesterol, low density lipoproteins (LDL) and high density lipoproteins (HDL) were not influenced by the addition of L-Dopa to the diets of the growing hens. The findings of the current study are in agreement with that of Sese *et al.* (2013) who reported that no influence was observed on serum cholesterol, low density lipoproteins LDL and HDL by *Mucuna utilis*. It also corroborates the findings of Tuleun *et al.* (2009) who observed no influence on serum cholesterol profile, LDL and HDL by

Mucuna utilis diets in experimental subjects.

On the other hand, Carew *et al.* (2003) reported significantly lower levels in the serum cholesterol concentration of birds fed *Mucuna utilis* diets. In the said study, heated Velvet beans resulted in a more pronounced reduction in the cholesterol concentration of the experimental subjects, compared to raw Velvet beans. Heat treatment was adopted as a means of reducing the potency of anti-nutritional factors in the bean. According to Carew *et al.* (2003) reduced cholesterol may be as a result of adsorption of intestinal cholesterol by dietary fibre and then rapid excretion, or to a more specific effect of some other components of Velvet beans. It is also highly likely that certain components of the velvet beans may act as “activators” or “co-factors” in the metabolism of free fatty acids in animals. In isolation, L-Dopa may not be able to drive this process.

Table 4: Serum biochemistry profile of grower laying birds fed diets with graded levels of L-Dopa inclusion.

Parameters	0% L-Dopa	0.1% L-Dopa	0.2% L-Dopa	0.3% L-Dopa	0.4% L-Dopa	SEM
Total Cholesterol (mg/dL)	81.84	90.52	82.71	84.16	78.43	3.56
High Density Lipoprotein (mg/dL)	59.08	60.03	55.12	52.66	49.40	2.98
Low Density Lipoprotein (mg/dL)	32.08	31.99	34.13	36.40	34.66	2.07

SEM – Standard error of mean

Conclusion

This research work has demonstrated the effect of L-Dopa supplementation at different inclusion levels (0.1%, 0.2%, 0.3% and 0.4%) on growth performance, blood metabolites and serum cholesterol in layers at grower stage. The result from this study showed that L-Dopa supplementation did not cause any variation in the parameters measured probably because of the growing stage of the experimental birds used for this study. It can therefore be concluded that L-Dopa

supplementation in the diets of growers at 0.1% to 0.4% inclusion levels did not cause any deleterious effect on the overall performance of laying birds at grower stage. It is recommended that further research should be carried out at inclusion levels of L-Dopa tested in this experiment, to assess the reproductive system of layers at grower stage, to check the possibility that growth performance was not improved due to the development of the reproductive system of the birds. Research could also be carried out at inclusion levels of L-Dopa higher than

0.4% to investigate the performance of layers considering growth, blood metabolites and serum cholesterol.

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Effect of L-Dopa on growth and cholesterol of pullets

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