Trop. Anim. Prod. Invest. 28 (02):6-11 (2025)
ISSN: 1115-2540
https://journals.ui.edu.ng/index.php/tapi

ORIGINAL RESEARCH ARTICLE

Influence of L-Dopa on the performance and lipid profile of pullet chicks

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ABSTRACT

The effect of L-Dopa on growth performance, cholesterol profile and blood metabolites was investigated in a 56-day experiment using 300 one-day old Nera Black pullet chicks that 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 monitored using standard procedures. Blood (5 mL) 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 Initial weight, final weight, body weight gain and feed conversion ratio did not differ significantly (P>0.05) in the birds fed diets containing graded levels of L-Dopa. It was observed, however, that the feed intake of pullets fed 0.3% L-Dopa was significantly (P>0.05) higher than that of birds fed 0.0% L-Dopa but statistically similar to other diets. Monocyte counts of birds fed 0.1% and 0.2% L-Dopa were significantly (P<0.05) higher than that of birds fed 0.0% L-Dopa and 0.4% L-Dopa but all were similar to that of birds fed 0.1% L-Dopa diet. No significant difference was observed in the cholesterol profile and haematological parameters of the birds fed pullet starter diets with L-Dopa inclusions except the Basophils (10^3µL) where pullets fed 0.1% L-Dopa inclusion was significantly higher than those fed 0.4% L-Dopa but similar to other diets. Although, L-Dopa up to 0.4% inclusion in its pure state did not significantly affect performance, haematology and cholesterol because of the reproduction stage but had no detrimental effects on pullet chicks.
Keywords: growth, haematology, levodopa, lipid profile, pullet chicks

INTRODUCTION

The raw seed of Mucuna contains anti-nutritional factors such as trypsin inhibitors, tannins, and Cyanogenic glucosides, L-Dopa (3, 4 Dihydroxy-L-phenylalanine, a potential neurotoxic agent). L-Dopa, (L-3-4-dihyroxyphenyalanine) also known as Levodopa is a non-protein phenolic amino acid with molecular formular of $C_9H_{11}NO_4$, has a negative effect on the performance of broilers fed raw velvet beans (Harms *et al.,* 1961; Ferriera *et al.,* 2003, Emiola, 2004). Miller *et al*. (2009) reported that the L-Dopa (levodopa) is found in various kinds of food and herbs (e.g., Mucuna pruriens, or velvet bean) as a naturally occurring dietary supplement and psychoactive drug form.

L-Dopa is the precursor to the neurotransmitter dopamine, nor-epinephrine (noradrenaline), and epinephrine (adrenaline) collectively known as catecholamines. Aside from its natural and essential biological role, L-Dopa is also used in the clinical treatment of Parkinson's disease and other neurological disorders (Raju *et al.,* 1993). L-Dopa has been recognized to increase testosterone level, leading to deposition of protein in the muscle and increase muscle mass and strength. These then suggest that L-Dopa may not be responsible for the negative effects observed when mucuna was fed.

This study therefore was undertaken to investigate the effect of dietary inclusion of L-Dopa on performance, haematological parameters and cholesterol of pullet chicks.

MATERIALS AND METHODS

The L-Dopa used in this experiment was purchased from a reliable commercial source. Three hundred one-day 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 in a completely randomised design. Experimental diets and fresh water were supplied *ad libitum* during the 8 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.

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; 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.

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

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).

Packed Cell Volume**:** According to wintrobesmicrohaematological 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 scaled 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:
RBC Total count = RBC counts x 10 x dilution factor (200) = RBC x 10,000

Table 1: Gross composition of pullet chick starter diets containing different inclusion levels of L-Dopa

		L-DOPA			
Feed Ingredients (g)	Control	0.10%	0.20%	0.30%	0.40%
Maize	560	559	558	557	556
Soya Bean meal	330	330	330	330	330
Fish meal	30	30	30	30	30
Soya oil	30	30	30	30	30
Dicalcium Phosphate	20	20	20	20	20
Limestone	20	20	20	20	20
Salt	3	3	3	3	3
Methionine	2	2	2	2	2
Lysine	2	2	2	2	2
*Vit/Min premix	3	3	3	3	3
L-Dopa	0	1	2	3	4
Total	1000	1000	1000	1000	1000
		Calculated analysis			
ME (kcal/kg)	2948.5	2948.5	2948.5	2948.5	2948.5
CP (g/kg)	210.8	210.7	210.6	210.6	210.5
CF(g/kg)	35.63	35.63	35.63	35.63	35.63
P (%)	7.96	7.96	7.95	7.95	7.95
Ca:Total P	1.81	1.81	1.81	1.81	1.81
Fat (j/kg)	35.30	35.30	35.30	35.30	35.30

ME: Metabolisable energy; P: total phosphorus; Ca: calcium, CP: crude protein, CF: crude fibre
*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.

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).

Statistical Analysis: Experimental data collected were subjected to One-way Analysis of Variance procedure - PROC ANOVA of Statistical Analysis System (SAS, 2011) and where significant differences were observed between treatments; the means were compared using the Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Production performance

The performances of the birds fed on the experimental diets are presented in Tables 2. The Initial weight, final weight, body 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. This is confirmed by 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.

It was observed, however, that the feed intake of pullets fed 0.3% L-Dopa was significantly (P>0.05) higher than that of birds fed 0.0% L-Dopa but statistically similar to other diets. This observation clarifies the conclusions of Flores *et al.,* (2000) and Del Carmine *et al*. (2002) who reported that L-Dopa is not a factor involved in the low feed take of Mucuna diets, but the presence of other anti-nutritional factors that can be reduced through processing thereby reducing the anti-trypsic factors in it. This is in contrary to the study by Carew *et al*. (2002) that reported that there is a marked reduction in feed intake, growth rate, body weight gain when chicks were fed 20% raw velvet beans (VB). The result obtained in this study is an indication that L-Dopa extract from *Mucuna pruriens* is not responsible for the intoxication reported by Afolabi *et al.* (1985) and Ravindran and Ravindran (1988) who reported that L-Dopa intoxication associated with the consumption of mucuna beans is related to their L-Dopa content. There is possibility, therefore, that growth performance was not improved due to the development of the reproductive system of the birds because of the reproduction stage.

Haematology parameter and white blood cell counts

The haematological parameters records of the pullets fed Starter diet with L-Dopa inclusions showed that there are no significant differences. This is in consonance with the report of Omidiwura *et al*. (2015) that observed that replacing methionine with L-Dopa does not have effect on the haematology parameters of broilers fed dietary treatments. In this study, the packed cell volume values ranged between 26.80 and 30.00% which is in accordance with the reported value of 26-45.2% for apparently healthy birds and also the haemoglobin values (8.66 - 9.82g/dL) obtained are in line with the haemoglobin values of 7.50-13.1g/dL reported by Mitruka and Rawnsley (1981). In Red blood cell count, White blood cell count and platelet, no significant differences was observed between the control and all other experimental diets containing different levels of L-Dopa inclusion. This is the due to the fact that L-Dopa inclusion in the diets did not have an effect on the circulatory system of the birds.

Table 2: Performance of layer pullets (g/bird) fed starter diet with L-Dopa inclusion

	Parameters				
Diets	Initial BW	Final BW	BW gain	Feed Intake	FCR
0%L-Dopa	37.95	484.20	446.25	1631.72^{b}	3.67
0.1% L-Dopa	37.77	514.27	476.50	1665.30^{ab}	3.53
0.2% L-Dopa	37.51	493.47	455.96	1652.58^{ab}	3.65
0.3% L-Dopa	38.08	532.97	494.88	1685.45^{a}	3.41
0.4% L-Dopa	38.22	508.02	469.80	1655.92^{ab}	3.53
SEM	0.38	16.38	16.32	11.61	0.13
P-Value	0.709	0.292	0.294	0.051	0.603

^{a,b,c} Means in column in each growth period with different superscripts are significantly different at P<0.05, SEM: Standard error mean, BW: Body weight, FCR: feed conversion ratio

Table 3: Haematology and white blood cell counts of layer pullets fed starter diet with L-Dopa inclusion

	L-Dopa						
Parameters	**0%**	**0.1%**	**0.2%**	**0.3%**	**0.4%**	**SEM**	**P-value**
Pack cell volume (%)	27.40	29.20	26.80	27.40	30.00	1.74	0.657
Haemoglobin (g/dL)	8.86	9.74	8.66	9.24	9.82	0.63	0.626
Red blood cell (10^6µL)	2.90	3.15	2.78	3.28	2.98	1.45	0.793
White blood cell (10^3µL)	20.68	16.54	17.61	19.24	20.33	0.31	0.240
Platelet (x10^9/L)	293.2	232.5	226.9	197.8	243.0	350.7	0.439
Lymphocyte (×10^3µL)	66.80	62.60	62.00	66.80	70.20	2.67	0.211
Heterophils (×10^3µL)	27.40	29.20	30.80	26.40	23.60	2.85	0.467
Monocyte (×10^3µL)	3.20^b	4.80^a	4.40^a	3.80^{ab}	2.80^b	0.55	0.010
Eosinophils (×10^3µL)	3.20	3.00	2.60	2.80	3.40	0.79	0.956
Basophils (×10^3µL)	0.20^b	0.80^a	0.20^b	0.20^b	0.00^b	0.18	0.050

[a,b] Values are means of 5 replicates pens of 12 birds each
SEM: Standard error mean

There were no significant (P>0.05) difference in the lymphocytes, eosinophils, and heterophils counts. The result of the lymphocytes recorded on pullets fed all levels L-Dopa inclusion in this study is a confirmation that the pullets were not stressed which was envisaged at high inclusion level as concluded by Li *et al.* (2007) that increase in the value of lymphocytes might be as a result of response to stress resulting from amino acids imbalance. Monocyte counts of birds fed 0.1% L-Dopa and 0.2% L-Dopa were significantly (P<0.05) higher than that of birds fed 0.0% L-Dopa and 0.4% L-Dopa but all were similar to the monocyte count of birds fed 0.1% L-Dopa diet. It was also observed that the Basophil count of birds fed 0.1% L-Dopa diet was significantly (P<0.05) higher than that of birds fed other diets. In this study, monocyte and eosinophils counts recorded were within the normal range physiological range. Basophils have limited phagocytic and bactericidal capacities. Dvorak *et al* (1980) and Deldar (1994) reported that basophils play a major role in allergic and inflammatory reactions, lipid metabolism and blood coagulation. With the role of Basophil, birds fed L-Dopa supplemented diet may have better lipid profile. Circulatory monocytes are macrophages found in the tissue, known as mononuclear phagocyte system, play an important role in the engulfing and destruction of intracellular organisms such as fungi, protozoa and viruses and transformed cells (Deldar, 1994). Base on this conclusion, there is possibility that birds fed L-Dopa supplemented diet will be able to withstand intracellular organisms.

Cholesterol level

The result of serum cholesterol profile of pullets fed with starter diets with L-Dopa inclusion is presented on Table 4. It was observed that the serum total cholesterol, low density lipoproteins (LDL) and high density lipoproteins (HDL) were not influenced by the addition of L-Dopa to the diets. The findings of the current study are in agreement with that of Tuleun *et al.* (2009) and Sese *et al.* (2013) who reported that no influence was observed on serum cholesterol, low density lipoproteins LDL and HDL by *Mucuna utilis*. 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. It is 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.

Table 4: Serum cholesterol profile of Layer pullets fed with starter diets with L-Dopa inclusion

Diets	Chol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
0% L-Dopa	109.20	85.80	33.75	21.84
0.1% L-Dopa	109.86	80.35	37.20	21.97
0.2% L-Dopa	114.64	78.42	45.61	22.93
0.3% L-Dopa	110.06	73.33	52.11	22.01
0.4% L-Dopa	113.37	68.23	46.61	22.68
SEM	15.71	8.81	13.81	3.14
P-value	0.999	0.678	0.880	0.999

Values are means of 5 replicates pens of 12 birds each and each are significantly different at P<0.05. SEM: Standard Error of Mean; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; CHOL: Cholesterol.

In isolation, L-Dopa may not be able to drive this process. There were no observable significant differences in all the parameters measured probably because of the starting and growing stage of the experimental birds used for this study.

CONCLUSION

The result from this study showed that L-Dopa supplementation did not cause any variation in the parameters measured. It can therefore be concluded that L-Dopa supplementation in the diets of pullet chicks at 0.1% to 0.4% inclusion levels did not cause any deleterious effect on the overall performance of laying birds at the starter stage. 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.

ACKNOWLEDGMENT

The author, hereby, acknowledge the financial support given by Tertiary Education Trust Fund (TETFund), Nigeria to carry out this study.

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