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ORIGINAL RESEARCH ARTICLE

Cholesterol profile and gut microbial population of laying birds fed L-Dopa supplemented diets

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Abstract

In a 42-day feeding trial, effect of L-Dopa on performance, serum cholesterol and intestinal microbial load in laying birds was investigated. One hundred and twenty 34-weeks-old layers were allotted to 5 dietary treatments of 8 replicates and three birds per replicate. The birds were fed diets supplemented with L-Dopa at graded inclusion levels (0.0, 0.1, 0.2, 0.3 and 0.4% of the diet) in a completely randomized design. Performance parameters were monitored. At day 42, egg, meat and 2.5 mL of blood were sampled to determine total cholesterol and lipoproteins using standard procedures. The ilea digesta was collected for microbial load analysis. Data were analysed at α0.05.

The L-Dopa inclusion had no effect on the performance parameters and egg cholesterol profile. Serum cholesterol levels of birds fed the control diet and those on 0.1% and 0.2% L-Dopa supplemented diets were similar but significantly higher than those fed 0.3% and 0.4% L-Dopa. In meat, the cholesterol and low density lipoprotein of birds fed control diet were significantly higher than 0.1% and 0.3% L-Dopa but similar in birds fed other diets. Also, Escherichia coli population was highest (7.25 x10^{4}cfu/mL) in birds on control diet and least (1.35 x10^{4}cfu/mL) in birds fed 0.3% L-Dopa. Lactobacillus counts of birds on L-Dopa supplemented diets were significantly higher than those on the control diet.

In conclusion, L-Dopa, at 0.3% inclusion, significantly improved the cholesterol profile in the blood and meat, reduced the population of Escherichia coli and effectively increased the population of Lactobacillus in laying birds.

***Keywords**: Levodopa, Performance, lipid profile, microbial load, Layers.*

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Introduction

Among the anti-nutritional factors in raw velvet beans, L-Dopa (3,4-dihydroxyphenyl alanine) have been proved to have a profound effect on the growth response of some poultry species (Prasad *et al.,* 2007; Omidiwura *et al*., 2015a). Naturally, L-Dopa is secreted in birds through the biosynthesis of L-tyrosine in the presence of tyrosine hydroxylase (Nakashima *et al.,* 2009). It is a direct precursor to dopamine and its supplementation in feed increases dopamine levels in the body. It 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 L-Dopa is known as Levodopa, one of the components used clinically in the treatment of Parkinson's disease, building of muscle and reduction of cholesterol level (González-Maldonado, 2018). Dopamine also reduces the cholesterol content in eggs and the blood stream of laying birds (Prasad *et al.,* 2007). The retardation in reproductive activity in aging rat correlated with hypothalamic deficiency of catecholamine has been corrected with the administration of L-Dopa (Quadri *et al.,* 1973).

Feeding considerable levels of L-Dopa has also been reported to cause significant increase in brain dopamine (Wilson and Meier, 1989) which affects neuro endocrine gonadal axis resulting in changes in body growth and reproductive conditions. In addition, the injection of L-Dopa into the blood stream of poultry has the effect of reducing cholesterol content in their eggs and blood stream. The injection of L-Dopa in Japanese quail was found to stimulate testicular and ovarian development and the production of follicle stimulating hormone and lutenizing hormone (Chaturvedi and Meier, 1988; Prasad *et al.,* 2007). Outside the nervous system, dopamine functions in several parts of the body as a local messenger. Earlier studies indicated that L-Dopa reduces gastro intestinal motility and also the activity of lymphocytes (Shultz, 2007). As a pharmacologically active ingredient, ingesting large amounts of L-Dopa could be toxic (Pieris *et al*., 1980). However, Omidiwura *et al*. (2015b) 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. It is worthy to note also that each region of the gastrointestinal tract develops its special microbial profile, and this community becomes more complex as chickens age (Gong *et al.,* 2002). Omidiwura *et al*. (2015a) worked on the influence of L-Dopa on gut mucosa integrity of broiler chicken and reported that L-Dopa improved the villus to crypt depth ratio. As a result of the earlier and recent studies indicating the effectiveness of L-Dopa in growth and reproductive stimulation in some poultry species, the motive behind this study is to investigate the effect of L-Dopa on the performance, intestinal microbial population, and egg, blood and meat cholesterol profile of laying birds.

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Materials and Methods

One hundred and twenty 34-weeks old laying birds were obtained from the Poultry Unit of the Teaching and Research Farm, University of Ibadan. The birds were tagged, weighed and randomly allotted to five dietary treatments (24 layers per treatment) with three replicates using completely randomised design. Experimental diets and fresh water were supplied *ad libitum* for six weeks. Recommended vaccines and other medications were also administered at the appropriate time while other routine management was strictly carried out.

The experimental layer's diets used (16% Crude protein and 2755kcal/kg Metabolisable energy) were prepared at the Feed mill unit of the University. Treatment 1 was the control, a basal diet without L-Dopa while treatments 2, 3, 4, 5 contained the basal diets and L-Dopa at inclusion levels of 0.1, 0.2, 0.3, and 0.4% respectively. The L-Dopa used in the experiment was a pure extract from *Mucuna pruriens* seed which was purchased from a reliable source. The proximate composition of the diets is shown in Table 1.

Table 1: Proximate composition (%) of layers' diet supplemented with L-Dopa

Parameter (%)	Control (0% L-Dopa)	0.1% L-Dopa	0.2% L-Dopa	0.3% L-Dopa	0.4% L-Dopa
Dry matter	93.14	93.11	93.05	93.20	93.21
Crude protein	16.94	15.12	16.40	16.49	17.83
Crude fibre	3.20	3.30	3.10	3.20	3.10
Ether extract	7.30	8.20	7.50	7.50	7.50
Ash	15.00	11.00	14.00	17.00	18.00
Nitrogen free extract	50.70	55.49	52.05	49.01	46.78

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Data collection

Weight Gain (WG): The initial weight was measured on the first day of the experiment and subsequently on weekly basis. It was computed as final weight minus theinitial weight.

Feed Intake (FI): Amount of feed consumed minus the amount of feed remaining.

Feed Conversion Ratio (FCR): This was computed as weight gain per unit feed intake in gram.

Egg yolk cholesterol quantification: The eggs used for this analysis were prepared for cholesterol quantification according to the procedure described by Elkin and Rogler (1990). Total Cholesterol (TC), High Density Lipoprotein (HDL), and Total Triglyceride (TG) concentration in the egg yolk were determined using the respective cholesterol assay kit.

Low Density Lipoprotein (LDL) was calculated as expressed by Friedewald *et al.* (1972).

$$LDL = TC-HDL-(TG/5)$$

Very Low Density Lipoprotein (VLDL) was calculated as expressed by Friedewald *et al.* (1972).

$$VLDL = TG/5$$

Serum cholesterol: The cholesterol level in the serum was determined using serum cholesterol kit (Cell Biolabs' HDL and LDL/VLDL Cholesterol Assay Kit) and by the procedure of Siedel *et al.* (1981)

Meat cholesterol analysis: At the end of the experiment, 2 birds per replicate were sacrificed and breast meat samples collected for further analysis. About 2g of sample were saponified according to a modified version of the method described by Stewart *et al,* (1992)

Intestinal microbial load evaluation: The ilea digesta of the 2 birds sacrificed was collected for intestinal microbial load evaluation. Microbial count was done using methods described by Barrow and Feltharn (1993).

Statistical analysis: Data collected were analysed using descriptive statistics and ANOVA $\alpha_{0.05}$. Means were separated using Duncan Multiple Range Test (DMRT).

Results and Discussion

Effect of L-Dopa supplementation on the performance of layers

The result of L-Dopa supplemented diets on the performance of layers is shown in Table 2. There were no significant (P>0.05) differences observed in the feed conversion ratio, feed intake (hen-day production) and egg weight across all the treatments. 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 but contradicts the report of Vadivel and Pugalenthi (2010). The supplementation of L-Dopa at graded levels had no significant (P>0.05) effect on Hen-day production of layers. This was not in line with the reports of Wilson and Meier (1989) who reported that feeding considerable levels of L-Dopa could cause significant increase in brain dopamine which affects neuroendocrine gonadal axis resulting in changes in body growth and reproductive conditions. Earlier studies by Bhatt and Chaturvedi (1993) also revealed that the dopaminergic activity may influence neuro-endocrine-gonadal axis which could induce and maintain reproductive conditions.

Table 2: Effect of L-Dopa supplemented diet on the performance of laying birds

	L-Dopa inclusion level (%)						
Parameter	0.0	0.1	0.2	0.3	0.4	SEM	P-value
Hen-day (%)	83.93	82.44	79.76	84.62	81.15	3.26	0.83
Feed Intake (g/hen/day)	101.87	98.65	103.18	102.52	100.48	1.75	0.39
FCR (g/feed/g/egg)	0.91	0.92	1.18	0.85	1.16	0.15	0.35
Egg weight (g)	58.26	57.49	60.65	60.73	61.76	1.92	0.48

SEM= Standard Error of Mean. FCR: Feed Conversion Ratio

Effect of L-Dopa supplementation on blood cholesterol of laying birds

The result of L-Dopa supplemented diets on blood cholesterol of laying birds is shown in Table 3. The L-Dopa inclusion had no effect on high density lipoprotein, low density lipoprotein and very low density lipoprotein of birds on the experimental diets. There were significant (P<0.05) differences observed in triglycerides and total cholesterol. As the level of L-Dopa increased in the diet the level of

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triglycerides and cholesterol tended to decrease. Although, birds fed 0.2%, 0.3% and 0.4% L-Dopa supplemented diets had similar concentration of triglycerides. Cholesterol levels of birds fed the control diet and those on 0.1% and 0.2% L-Dopa supplemented diets were similar but significantly higher ($P<0.05$) than those fed 0.3% but birds on 0.2% was also similar to 0.4% L-Dopa supplemented diets. Similar reports by Jayaweera *et al*. (2007) suggested that mucuna contains a strong cholesterol lowering effect in broilers. The reduction in blood cholesterol also agrees with the findings of Carew *et al*. (2003) who reported that this effect may be due to adsorption of intestinal cholesterol by dietary fiber and rapid excretion of some other components in mucuna.

Very low density lipoprotein was not ($P>0.05$) significantly affected by L-Dopa supplementation but there were reductions in their values across the treatments. This is in consonance with the findings of Iauk *et al*. (1993) who also observed reduction in blood cholesterol when birds were fed raw mucuna diets.

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Table 3: Effect of L-Dopa on blood cholesterol in laying birds

	L-Dopa inclusion level (%)						
Parameter (mg/dL)	0.0	0.1	0.2	0.3	0.4	SEM	P-value
Triglyceride	337.06[a]	331.63[a]	286.30[b]	255.21[b]	254.02[b]	15.30	0.0003
HDL	10.23	7.67	12.97	8.031	6.15	2.52	0.3687
Cholesterol	107.51[a]	102.32[a]	83.74[ab]	48.09[c]	64.65[bc]	10.09	0.0007
LDL	20.24	26.33	20.01	9.31	15.08	4.85	0.1647
VLDL	67.41	66.33	57.26	51.04	50.80	3.06	0.0003

*Means on the same row with different superscripts are significantly ($P< 0.05$) different; SEM= Standard Error of Mean.; HDL - High Density Lipoprotein; LDL - Low Density Lipoprotein; VLDL- Very Low Density Lipoprotein

Effect of L-Dopa supplementation on egg cholesterol of laying birds.

The effect of L-Dopa diet supplementation on egg cholesterol of laying birds is shown in Table 4. There were no significant ($P>0.05$) differences in the cholesterol, triglycerides high density lipoprotein, low density lipoprotein, and very low density lipoprotein of birds on L-Dopa supplemented diets. Egg which is considered as a whole food for human consumption due to the presence of numerous vitamins, minerals and essential amino acids has been reported by ASN (2010) to be one of the largest sources of phosphatidycholine in human diet. In the present study, diet had no effect on the cholesterol profile of eggs. This was contrary to the reports of Prasad *et al*. (2007) who reported that incorporation of L-Dopa in the feed of poultry has the effect of reducing the cholesterol content in the eggs and also the ratio of saturated to unsaturated fatty acids in eggs produced by such poultry species.

Table 4: Effect of L-Dopa on cholesterol profile of chicken eggs

	L-Dopa inclusion level (%)						
Parameter (mg/dL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**SEM**	**P-value**
Cholesterol	88.77	63.00	100.30	94.37	72.23	10.33	0.1084
Triglycerides	429.06	378.67	417.58	435.05	400.34	39.66	0.8509
High Density Lipoprotein	5.58	5.79	11.38	9.37	6.92	2.68	0.5038
Low Density Lipoprotein	4.64	0.00	11.05	15.22	3.89	6.38	0.4829
Very Low Density Lipoprotein	85.81	75.73	82.77	87.01	80.07	8.11	0.8638

*Means on the same row with different superscripts are significantly (P< 0.05) different; SEM= standard Error of Mean.

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Effect of L-Dopa supplemented diet on meat cholesterol

The result on the effect of L-Dopa supplemented diet on meat cholesterol is shown in Table 5. It was observed that there were no significant (P>0.05) differences in triglyceride concentration. High Density Lipoprotein of birds fed 0.1% L-Dopa was significantly higher than those fed other diets which were statistically similar.. The Cholesterol and Low Density Lipoprotein concentration in birds fed the control diet was significantly (P<0.05) higher than 0.1% L-Dopa and 0.3% L-Dopa but similar to those fed 0.2% and 0.4% L-Dopa supplementation. The inclusion of L-Dopa in the feed helped to reduce the level of cholesterol and Low Density Lipoprotein in the meat especially at 0.3% L-Dopa inclusion. The result agrees with the reports by Jayaweera *et al*. (2007) who suggested that mucuna contains a strong cholesterol lowering effect in broilers.

Table 5: Effect of L-Dopa Supplemented Diet on Meat Cholesterol

	L-Dopa inclusion level (%)						
Parameter (mg/dL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**SEM**	**P-value**
Triglyceride	31.38	33.89	43.05	32.22	41.56	5.58	0.4569
Cholesterol	177.33[a]	87.12[b]	205.52[a]	60.31[b]	171.81[a]	17.1	<0.0001
High Density Lipoprotein	5.35[b]	17.50[a]	6.18[b]	5.28[b]	5.29[b]	1.07	<0.0001
Low Density Lipoprotein	165.71[a]	57.84[b]	190.74[a]	48.58[b]	158.21[a]	16.53	<0.0001

Means on the same row with different superscripts are significantly (p < 0.05) different. SEM: Standard Error of mean; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Effect of L-Dopa supplementation on intestinal microbial population of layers.

The result of L-Dopa supplemented diets on the intestinal microbial population of layers is shown in Table 6. Significant differences ($P<0.05$) were observed in the microbial population of *Escherichia coli* and Lactobacillus. The *Escherichia coli* population ($P<0.05$) was highest (7.25 x10^4cfu/mL) in birds on control diet and least (1.35 x10^4cfu/mL) on birds fed 0.3% L-Dopa diet. Lactobacillus counts of birds on L-Dopa supplemented diets were ($P<0.05$) significantly improved compared with those on the control diet. According to the results, the population of *Escherichia coli* in the control diet was higher than those in the L-Dopa supplemented diets. The inclusion of L-Dopa in the diets had greatly reduced the population of *Escherichia coli* in the laying birds. More so, L-Dopa inclusion at graded levels had a significant effect on the population of Lactobacillus in the layers. Lactobacillus population in L-Dopa supplemented diet of 0.1%, 0.2% 0.3% and 0.4% L-Dopa were found to be higher than the Lactobacillus population in diets supplemented with 0.0% L-Dopa (the control diet). This implies that for an effective increase in the population of Lactobacillus and reduction of that of *Escherichia coli* to confer improved gut integrity for better nutrient utilisation, laying birds' diet needs L-Dopa supplementation.

Table 6: Effect of L-Dopa supplementation on Intestinal Microbial Population of Layers

Parameter ($x10^4$ cfu/ml)	L-Dopa inclusion level (%) 0	0.1	0.2	0.3	0.4	SEM	P-value
E-coli	7.25^a	3.38^b	3.08^b	1.35^c	2.75^b	1.97	<0.0001
Total Coliform	4.75	3	2.6	3.18	5.25	5.09	0.039
Lactobacillus	2.05^b	6.98^a	7.00^a	5.50^a	4.88^a	3.74	<0.0001

*Means on the same row with different superscript are significantly ($P<0.05$) different: SEM= Standard Error of Mean.

Conclusions and Recommendation

The result of this present study showed that the dietary supplementation of L-Dopa, especially at 0.3% inclusion, significantly improved the cholesterol profile in the blood and meat, reduced the population of *E coli* and effectively increased the population of Lactobacillus in laying birds. It could, therefore, be concluded that L-Dopa diet supplementation in the diets of layers at 0.1 to 0.4% did not elicit any deleterious effect on the overall performance of layers.

It is therefore recommended that further studies should be carried out to investigate the influence of L-Dopa in other species of poultry.

Conflict of Interest

The authors of this study declare that no conflict of interest including financial, personal or other relationships with people and organisations within two to three years of performing the experiment that could inappropriately influence, or be perceived to influence the work.

References

ASN, 2010. Egg consumption and risk of type 2 diabetes in older adults".*American Society for Nutrition* 2010.Retrieved 12 December, 2010.

Barrow G. H. and Feltham. R. K. A. 1993. Cowan and Steel's Manual for Identification of Medical Bacteria. Third edition. Cambridge University Press, Cambridge. pp. 331.

Bhatt, R. and Chaturvedi, 1993. L-DOPA treatment induces scotosensitivity in Japanese quail. Reproduction. *Biol. Comp. Endocrinol*.,5:75-83.

Carew, L.B., Hardy, D., Weis, J., Alster, F.A., Mischler, S.A., Gernat, A.G. and Zakrzewska, E.I. 2003. Heating Raw Velvet Beans *(Mucuna pruriens)* Revers Some Antinutritional Effects on Organ Growth, Blood Chemistry, and Organ Histology in Growing Chickens. *Trop. Subtrop. Agroecosys*. 1: 267 – 275.

Chaturvedi, C.M. and Meier, A.H. 1988. Neurotransmitter affecting drugs reset relative photorefractoriness in Japanese quail. In Fourth International Symposium, Avian Endocrinol. Japan, pp:122

Elkin, R.G. and Rogler, J.C. 1990. Reduction of the cholesterol content of eggs by theoral administration of lovastatin to laying hens. *J Agric. Food Chem*, 38:1635-1641.

Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultra-centrifuge. *Clinical Chemistry*.18(6):499-502.

Gong, G., Entekhabi, D, and Cohen, J. 2002. A large-ensemble model study of 298 the wintertime AO/NAO and the role of interannual snow perturbations, 299 *J. Clim*., 15, 3488 – 3499

González-Maldonado R. 2018. Mucuna and Parkinson's Disease: Treatment with Natural Levodopa. IntechOpen (The world's leading publisher of Open Access books Built by scientists, for scientists). 2018 edition Chapter 6: 95-116

Iauk, L., Galati, E.M., Kirjavainem, S., Forestieri, A.M. and Trovato, A. 1993. Analgesic and antipyretic effects of *Mucuna pruriens*. *Int. J. Phcog*. 31: 213-216.

Jayaweera, T.S.P., Cyril, H.W., Samarasinghe, K., Ruwandeepika, H.A.D., Wickramanayake, D.D. and Thotawaththe, T. S. J. 2007. Effect of feeding velvet beans (*Mucuna pruriens*) on the lipid profile of broiler chickens. *Tropical Agricultural Research* 19: 273 - 282

Meites, J., Simpkins, J., Bruni, J. and Advis, J. 1977. Role of biogenic amines in control of anterior pituitary hormones. *IRCS J. Med. Sci.* 5: 1-7.

Nakashima A, Hayashi N, Kaneko YS, Mori K, Sabban EL, Nagatsu T and Ota A. 2009. Role of N-terminus of tyrosine hydroxylase in the biosynthesis of catecholamines. *J Neural Transm (Vienna).* ;116(11):1355-62.

Omidiwura, B. R. O., Agboola, A. F., Adeoye, M. K. and Iyayi, E. A.

GALLEY PROOF YET UNPUBLISHED

2015a. Influence of L-dopa of *Mucuna pruriens* on growth response and gut mucosa integrity of broiler chickens**.** British Society of Animal Science Annual Conference 2015 - Science with Impact. University of Chester, Parkgate Road, Chester, CH1 4AR 14-15 April 2015.

Omidiwura, B. R. O, Agboola, A. F and Iyayi E. A. 2015b. Effect of L-Dopa in replacing dl-methionine on the performance of broiler chickens. In: Odoi, F. N. A., Hagan, J., Adam, I., Ayorinde, L. K., Awuma, K. S., Teye, M. and Anna-Prah, A. A. (Eds). Proceedings of the 19th Biennial Conference of the Ghana Society for Animal Production (GSAP) - Climate change and livestock development held on August 5-8, 2015 at the University of Cape Coast, Ghana, Pp 122-129.

Omidiwura, B. R. O., Odu, O., Agboola, A. F., Akinbola, D. D. and Iyayi, E. A. 2016. Crude protein and energy requirements of Japanese quail (*Coturnix coturnix japonica*) during rearing period. *World Poultry Research* 6(2):99-104.

Pieris, N., Jansz, E. R. and Dharmadasa, H. M. 1980. Studies on mucuna species of Sri Lanka 1 - The L-Dopa content of seeds. *Journal of Natural Science* 8: 35-40.

Prasad, S. K., Qureshi, T. N., Sexana, S., Qureshi, S., Mehar, M. and Thakur, S. K., 2007. L-Dopa feeding Induces Body Growth and Reproductive Conditions in Japanese Quail, *Coturnix coturnix Japonica*. *International Journal of Poultry Science* 6 (8): 560-566.

Quadri, S. K., Kledzik, G. S. and Meites, J. 1973. Reinitiation of estrous cycles in old constant estrous rats by central acting drugs. *Neuroendocrinol.* 11: 248–255.

Shultz, 2007. Multiple dopamine function at different time course. *Annu.Rev.Neurosci 30:259-88.*

Siedel, J., Schlumberger, H., Klose, S., Ziegenhorn, J., Wahlefeld, A. W. 1981. Improved reagent for the enzymatic determination of serum cholesterol.. *J. Clin. Chem. Clin. Biochem*. 19: 838-847.

Stewart, G., Gosselin, C. and Pandian, S. 1992. Selected ion monitoring of tert-butyldimethylsilyl cholesterol ethers for determination of total cholesterol content in foods. *Food Chem.* 44:.377-380.

Vadivel, V. and Pugalenthi, M. 2010. Evaluation of growth performance of broiler birds fed with diet containing different levels of effectively processed velvet bean seeds collected from South India. *Livestock Science* 127: 76 – 83.

Wilson, J. M. and Meier, A. H. 1989. Tryptophan feeding induced sensitivity to short daylength in photorefractory Hamsters. *Neuro endocrinol*., 36: 59-63

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