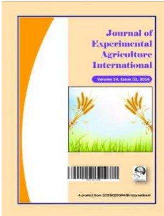

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# Effect of L-Dopa in Replacing Dl-Methionine on the Performance of Broiler Chickens

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***Author's contribution***

*The sole author designed, analysed, interpreted and prepared the manuscript.*

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## ABSTRACT

The L-Dopa found in mucuna seed, when present with other antinutritional factors, has been implicated on nutritional disorders of monogastric animals. It is also reputed to influence muscular development. However, information on its effect on methionine utilisation is scanty. Therefore, the effect of L-Dopa on growth performance and its replacement for DL-methionine in broiler chickens was investigated.

In a 42-day trial, between August and September 2014, two hundred and forty one-day old chicks were allotted to six diets which contained a diet with 0 % L-Dopa + 0 % DL-methionine and other five diets containing L-Dopa replacing DL-methionine at 0, 25, 50, 75 and 100 %. All treatments had 4 replicates with 10 birds per replicate in a completely randomized design. Feed Intake (FI), Final Weight (FW), Body Weight Gain (BWG), Feed Conversion Ratio (FCR), Serum Methionine (SM), Methionine Adenosyltransferase (MAT), Breast Meat Protein (BMP), liver enzymes, haematological parameters and serum biochemical indices were determined following standard procedure. Data were analysed using descriptive statistics and ANOVA at $P$ = .05.

The result revealed that the FI, FW and BWG of birds fed diets containing 0%L-Dopa+100% methionine (3491.2 g, 2148.8 g and 2108.5 g) and 50 % L-Dopa + 50 % methionine (3556.1 g, 2137.6 g and 2097.3 g) were similar but significantly better than other diets. The FCR (1.86) of broilers fed 0 % L-Dopa +0 % methionine diet was better than other diets. It was also observed that

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the MAT (0.45 ng/ml) of broilers fed diet containing 50 % L-Dopa + 50 % methionine was higher than that of other diets but similar to MAT of birds on 75 % L-Dopa + 25 % Methionine. Haematology, SM, BMP and liver enzymes were not significantly affected across treatments.
L-Dopa extract, at levels tested, had no detrimental effect on broilers and can replace synthetic DL-methionine up to 50 %. Therefore, 0.2 % inclusion of L-Dopa is recommended in diets of broiler chickens for improved performance and utilisation of available methionine.

*Keywords: L-Dopa; DL-methionine; broilers; performance.*

## 1. INTRODUCTION

Methionine, essential amino acid, plays a significant role in energy production, regulation of cell division, reduces reactive oxygen, transmethylation reaction and protein synthesis. Deficiency of Methionine can result into poor feathering in birds, increased atmospheric ammonia, poor feed conversion, retarded growth in broilers, kidney problems, increased heat stress, increased potassium intake, increased pododermatitis, increased coccidiosis problem and reduced egg production in layers and breeders. Methionine is required to provide the building blocks for immune cells and tissues. This includes the nonspecific mechanisms such as the skin and mucosa, and the specific mechanisms that include T and B lymphocytes. This is particularly important for newly hatched chicks that are highly susceptible to infection during the first two weeks of life as discussed by Jacob [1].

Synthetic methionine, which can be metabolised into highly toxic compounds such as methylpropionate, is listed among the prohibited synthetic substances and its usage has been questioned in organic farming practices [2]. Level of adulteration and high cost are also sensitive factors to be considered in the inclusion of synthetic methionine in modern feed formulation. Keeping these facts in mind, it is a high time to think about an alternative.

Methionine is converted to its active form which is the most important methyl donor in the body, S-Adenosyl Methionine Transferase (SAM), in presence of Methionine Adenosyl Transferase (MAT). Methylation by SAM is a critical step in the stabilisation of many proteins. According to experiments conducted by Benson et al., Zhao et al. [3,4], L-Dopa, one of the antinutritional factors in mucuna seed was observed to increase the activity of MAT and catechol-O-methyl transferase (COMT). Benson et al. [3] concluded that SAM will react with L-Dopa, producing 3-O-methyldopa, and with dopamine, producing 3-methoxytyramine and 3,4-dimethoxyphenylethylamine. Consequently, the increased production of SAM would cause the depletion of available DA and its precursor L-Dopa. The MAT activity was determined by a Western blot analysis which showed that MAT is more clearly characterised in 10 % mercaptoethanol reducing buffer, Hence, the fact that L-Dopa induces the enzymes MAT and COMT and activates them to catalyse metabolic reaction necessitated this study; to investigate the effect of L-Dopa on growth performance and its replacement for DL-methionine in broiler chickens.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The experiment was carried out at the Poultry Unit of Teaching and Research Farms, University of Ibadan, Nigeria.

### 2.2 Experimental Birds and Layout

Two hundred and forty one-day old Arbor Acre broiler chicks were randomly allotted to six dietary treatments.

| Treatment (Replacement levels - %) | Control | 0 | 25 | 50 | 75 | 100 |
|---|---|---|---|---|---|---|
| L-Dopa | 0 | 0 | 0.1 | 0.2 | 0.3 | 0.4 |
| Methionine | 0 | 0.4 | 0.3 | 0.2 | 0.1 | 0 |

### 2.3 Management of Experimental Birds

Each diet was replicated 4 times and group of 4 pens of 10 birds each to each treatment in a completely randomised design. The corn-soyabean meal- based diets, for both starter (22.3 % crude protein and 3033 Kcal/Kg Metabolisable Energy) and finisher (20.5 % crude protein and 2923 Kcal/Kg Metabolisable Energy) phases, were formulated based on the nutritional requirements recommended by NRC [5] Feed and water were supplied *ad libitum* during the experimental period that lasted for 6 weeks.

## 2.4 Data Collection and Data Analysis

Feed intake and body weight gain of birds were calculated on weekly basis Blood samples were collected from two randomly selected birds from each replicate on days 21 and 42 by venipuncture to harvest serum samples for haematology, serum biochemical indices, serum methionine and Methionine Adenosyl Transferase. These were determined using ELISA kit. Proximate analysis was determined by the procedure of AOAC [6]. Data were analysed using GLM procedure in SAS [7] and differences between treatment means were separated using Duncan Multiple Range Test.

# 3. RESULTS AND DISCUSSION

## 3.1 Performance of broilers fed L-Dopa in replacement of DL-methionine

It was observed from this study that L-Dopa supplementation did not elicit any deleterious effect on performance parameters measured in both the starter and finisher phases.

As shown in Table 1, Dry matter intake and body weight gain of experimental broilers showed that broilers fed 0 % L-Dopa + 100 % methionine and those fed 50 % L-Dopa + 50 % Methionine were similar at both phases. From this evaluation, substitution of L-Dopa for methionine especially at 50% inclusion, influenced feed intake as much as 100 % methionine inclusion thereby increases the utilisation of available methionine to meet the requirement of broiler chickens. This is similar to study conducted by Wang et al. [8], where they reported increase in weight gain with increasing level of methionine in diets of broilers.

The best response of feed conversion was found in the birds fed diet 25 % L-Dopa + 75 % methionine (1.34) at the starter phase and 0 % L-Dopa + 100 % methionine (1.78) at the finisher phase though they were similar to other diets at both phases. This result further confirms the efficiency of L-Dopa to influence the utilisation of available methionine and performance improvement observed in broiler chickens fed L-Dopa supplemented diets as concluded by Omidiwura et al. [9]. As variance with the conclusion of [10] who fed L-Dopa supplemented diet to pullets, L-Dopa significantly improved the feed intake, feed conversion ratio and body weight gain in this study. The influence of. The least performance was observed in birds fed 0 % L-Dopa + 0 % methionine and 100 % L-Dopa + 0 % methionine which may be attributed to absence of methionine supplementation in the diets and/or toxicity of L-Dopa at 100 % inclusion.

## 3.2 Serum Methionine and Meat Protein of Broilers Fed L-Dopa in Replacement of DL-methionine

The results of serum methionine, breast meat protein and Methionine Adenosyltransferase concentration of birds fed dietary treatments are shown in Table 2. The concentration of methionine in the serum and breast meat protein were not significantly ($P > 0.05$) influenced by L-Dopa in the dietary treatments. This can be attributed to the utilisation of available methionine as described by Miller et al. [11]. The utilisation is further confirmed by the significant increase in the concentration of methionine adenosyltransferase (MAT) in the serum of birds fed diet containing 50% L-Dopa+ 50% methionine (0.45 ng/ml), the enzyme that catalyses the reaction between Methionine and adenosine triphosphate to yield S-adenosylmethionine (SAMe). The result is in accordance with experiment conducted by Benson et al., Zhao et al. [3,4], where L-Dopa was observed to increase the activity of MAT and increases the transmethylation process by inducing MAT, and catechol-O-methyl transferase (COMT), the enzyme that transfers the methyl group from SAM to L-Dopa and Dopamine.

## 3.3 Haematological Parameters, Serum Biochemical Indices and Liver Enzyme of Broilers fed L-Dopa in Replacement of DL-methionine

As indicated on Table 3, it was observed that replacing methionine with L-Dopa does not have effect on the haematological parameters of broilers fed dietary treatments. All the haematological indices measured fell within the normal range as reported by Mitruka and Rawnsley [12]. This is an indication that the physiological state of the birds was not compromised. Also, as shown on Table 4, no significant ($P$ = .05) differences were observed in the liver enzymes of broilers fed dietary treatments. Hyperproteinemia in most birds is indicated by plasma total protein concentrations of greater than 4.5 g/dL [13].

The mean values for serum total protein obtained from this study were below 4.5 g/dL, indicating that dietary treatments did not affect the concentration of total serum protein and albumin.

**Table 1. Performance of broilers fed L-Dopa in replacement of DL-methionine**

| Growth period | Diet | Initial weight (g) | Final weight (g) | Body Weight Gain (g/chick) | Feed intake (g/chick) | FCR | Dry Matter intake (g/chick) |
|---|---|---|---|---|---|---|---|
| Starter 0-21d | 0 % L-D + 0 % Met | 40.2 | 551.12[c] | 510.88[c] | 859.77[b] | 1.68[a] | 775.69[b] |
| | 0 % L-D + 100 % Met | 40.23 | 750.86[a] | 710.61[a] | 993.33[a] | 1.39[cd] | 899.26[a] |
| | 25 % L-D + 75 % Met | 40.25 | 744.42[a] | 704.16[a] | 943.63[a] | 1.34[d] | 856.06[a] |
| | 50 % L-D + 50 % Met | 40.3 | 747.64[a] | 707.21[a] | 983.82[a] | 1.39[cd] | 892.72[a] |
| | 75 % L-D + 25 % Met | 40.3 | 682.78[b] | 642.48[b] | 961.28[a] | 1.51[bc] | 872.26[a] |
| | 100 % L-D + 0 % Met | 40.2 | 551.5[c] | 511.30[c] | 808.88[b] | 1.58[ab] | 734.86[b] |
| | SEM | 1.60 | 19.87 | 19.03 | 27.06 | 0.04 | 24.53 |
| | P-value | 1.0000 | <.0001 | <.0001 | 0.0006 | 0.0002 | 0.0006 |
| Finisher, 22-42d | 0 % L-D + 0 % Met | 551.12[c] | 1763.06[c] | 1211.94[b] | 2340.8 | 1.94[ab] | 2131.33[bc] |
| | 0 % L-D + 100 % Met | 750.86[a] | 2148.75[a] | 1397.89[a] | 2497.9 | 1.78[b] | 2292.80[ab] |
| | 25 % L-D + 75 % Met | 744.42[a] | 1919.58[b] | 1175.16[b] | 2419.3 | 2.07[a] | 2205.63[abc] |
| | 50 % L-D + 50 % Met | 747.64[a] | 2137.60[a] | 1389.96[a] | 2572.3 | 1.85[ab] | 2354.42[a] |
| | 75 % L-D + 25 % Met | 682.78[b] | 1885.00[bc] | 1202.23[b] | 2392.8 | 1.99[ab] | 2162.81[abc] |
| | 100 % L-D + 0 % Met | 551.5[c] | 1766.11[c] | 1218.64[b] | 2333.3 | 1.92[ab] | 2069.67[c] |
| | SEM | 19.87 | 47.40 | 42.19 | 73.46 | 0.08 | 67.27 |
| | P-value | <.0001 | <.0001 | 0.0027 | 0.2069 | 0.2482 | 0.073 |
| 0-42d | 0 % L-D + 0 % Met | 40.2 | 1763.06[c] | 1722.86[c] | 3200.6[b] | 1.86[a] | 2907.0[bc] |
| | 0 % L-D + 100 % Met | 40.23 | 2148.75[a] | 2108.53[a] | 3491.2[a] | 1.65[c] | 3192.1[a] |
| | 25 % L-D + 75 % Met | 40.25 | 1919.58[b] | 1879.33[b] | 3362.9[ab] | 1.8[ab] | 3061.7[ab] |
| | 50 % L-D + 50 % Met | 40.3 | 2137.60[a] | 2097.30[a] | 3556.1[a] | 1.69[bc] | 3247.1[a] |
| | 75 % L-D + 25 % Met | 40.3 | 1885.00[bc] | 1844.70[bc] | 3354.0[ab] | 1.82[ab] | 3035.1[abc] |
| | 100 % L-D + 0 % Met | 40.2 | 1766.11[c] | 1725.91[c] | 3142.2[b] | 1.82[ab] | 2804.5[c] |
| | SEM | 1.60 | 47.40 | 47.01 | 85.33 | 0.04 | 78.00 |
| | P-value | 1.0000 | <.0001 | <.0001 | 0.0215 | 0.0155 | 0.0071 |

**Met: Methionine. L-D: L-Dopa. Values are means of 4 replicates pens of 10 birds each, [abc] Means in column in each growth period with different superscripts are significantly different at P = .05.*

**Table 2. Serum methionine and meat protein of broilers fed L-Dopa in replacement of DL-methionine**

| Diet | Starter | Finisher | | |
|---|---|---|---|---|
| | Serum Methionine (μmol/ml) | Serum Methionine (μmol/ml) | Meat Protein (%) | MAT concentration (ng/ml) |
| 0 % L-D + 0 % Met | 4.33 | 4.78 | 79.19 | 0.26[b] |
| 0 % L-D + 100 % Met | 5.07 | 5.38 | 75.94 | 0.19[b] |
| 25 % L-D + 75 % Met | 4.33 | 4.63 | 72.29 | 0.26[b] |
| 50 % L-D + 50 % Met | 4.98 | 5.03 | 81.16 | 0.45[a] |
| 75 % L-D + 25 % Met | 5.48 | 5.05 | 77.22 | 0.30[ab] |
| 100 % L-D + 0 % Met | 6.57 | 6.23 | 72.3 | 0.21[b] |
| SEM | 0.37 | 0.65 | 1.49 | 0.06 |
| P-value | 0.3992 | 0.5808 | 0.2508 | 0.0434 |

**Met: Methionine. L-D: L-Dopa. Values are means of 4 replicates pens of 10 birds each, abc Means in row with different superscripts are significantly different at P = .05. MAT - Methionine Adenosyl Transferase, Meth: Methionine.*

**Table 3. Haematological parameters of broilers fed L-Dopa in replacement of DL-methionine**

| Diet | PCV (%) | HB (g/dL) | RBC ($10^6$UI) | WBC ($10^3$UI) | LYMP (%) | HETERO (%) | MONO (%) | Eos (%) | BASO (%) | Platelet x$10^3$/mm$^3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 % L-D + 0 % Met | 28.89 | 9.39 | 3.08 | 14.44 | 56.63 | 25.25 | 3.13 | 2.38 | 0.38 | 15.16 |
| 0 % L-D + 100 % Met | 23.25 | 7.55 | 2.56 | 15.46 | 47.13 | 23.13 | 2.63 | 1.88 | 0.13 | 14.28 |
| 25 % L-D + 75 % Met | 27.13 | 8.86 | 2.97 | 17.04 | 48.13 | 34.63 | 2.63 | 1.38 | 0.50 | 14.18 |
| 50 % L-D + 50 % Met | 25.38 | 8.21 | 2.76 | 14.68 | 50.75 | 32.75 | 2.25 | 2.50 | 0.13 | 15.48 |
| 75 % L-D + 25 % Met | 29.38 | 9.79 | 3.04 | 18.45 | 47.63 | 34.63 | 3.13 | 1.75 | 0.13 | 16.06 |
| 100 % L-D + 0 % Met | 26.75 | 8.61 | 3.02 | 17.73 | 54.88 | 26.88 | 3.50 | 2.25 | 0.13 | 15.48 |
| SEM | 4.08 | 1.32 | 0.43 | 9.56 | 8.43 | 6.37 | 0.67 | 0.58 | 0.14 | 0.87 |
| P-value | 0.901 | 0.8617 | 0.9496 | 0.4188 | 0.9451 | 0.6767 | 0.7978 | 0.7443 | 0.2772 | 0.9946 |

**Met: Methionine. L-D: L-Dopa. SEM: Standard Error of Mean; PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cell; WBC: White blood cell; Lymp: Lymphocyte; Hetero: Heterophil; Mono: Monocyte; Eos: Eosinophil*

**Table 4. Serum indices and liver enzyme of broilers fed L-Dopa in replacement of DL-methionine**

| Diet | ALB (g/dL) | TP (g/dL) | GLO (g/dL) | creatinine (mg/dL) | ALP (µ/L) | AST (µ/L) | ALT (µ/L) |
|---|---|---|---|---|---|---|---|
| 0 % L-D + 0 % Met | 1.88 | 3.08 | 1.21 | 0.76 | 577.21 | 111.51 | 3.74 |
| 0 % L-D + 100 % Met | 1.55 | 2.69 | 1.14 | 0.71 | 474.20 | 122.89 | 5.20 |
| 25 % L-D + 75 % Met | 2.55 | 3.01 | 1.63 | 0.69 | 482.60 | 119.36 | 4.60 |
| 50 % L-D + 50 % Met | 1.81 | 2.75 | 0.94 | 0.69 | 523.84 | 117.77 | 3.69 |
| 75 % L-D + 25 % Met | 1.51 | 2.76 | 1.26 | 0.74 | 400.31 | 122.96 | 5.96 |
| 100 % L-D + 0 % Met | 1.74 | 2.94 | 1.20 | 0.69 | 573.28 | 124.27 | 4.24 |
| SEM | 0.39 | 0.28 | 0.25 | 0.05 | 56.25 | 6.99 | 0.87 |
| P-value | 0.486 | 0.8965 | 0.6428 | 0.8563 | 0.2607 | 0.8003 | 0.4326 |

**Met: Methionine. L-D: L-Dopa. SEM: Standard Error of Mean; T.P: Total protein; ALB: Albumin; GLO: Globulin, ALP: Alkaline Phosphatase, ALT: Alanine Transaminase, AST: Aspartate Transaminase*

**Table 5. Cost benefit ratio of L–dopa supplementation in replacing DL – methionine in broiler chickens**

| | 0 % L-Dopa + 0 % Methionine | 0 % L-Dopa + 100% Methionine | 25 % L-Dopa + 75 % Methionine | 50 % L-Dopa + 50% Methionine | 75 % L-Dopa + 25% Methionine | 100 % L-Dopa + 0% Methionine |
|---|---|---|---|---|---|---|
| **Starter Phase** | | | | | | |
| Weight gain (kg/b) | 0.51 | 0.71 | 0.70 | 0.71 | 0.64 | 0.51 |
| Feed Intake (kg) | 0.86 | 0.99 | 0.94 | 0.98 | 0.96 | 0.81 |
| Feed Conversion Ratio | 1.68 | 1.40 | 1.34 | 1.39 | 1.51 | 1.58 |
| Feed Cost/kg (N) | 120.80 | 125.20 | 133.20 | 141.20 | 149.20 | 157.20 |
| Feed Cost/kg/b (N) | 103.85 | 124.37 | 125.70 | 138.92 | 143.43 | 127.16 |
| Feed Cost/kg weight gain (N/kg) | 203.27 | 175.02 | 178.64 | 196.67 | 224.57 | 248.87 |
| **Finisher Phase** | | | | | | |
| Weight gain (kg/b) | 1.21 | 1.40 | 1.18 | 1.39 | 1.20 | 1.22 |
| Feed Intake (kg) | 2.34 | 2.50 | 2.42 | 2.57 | 2.39 | 2.33 |
| Feed Conversion Ratio | 1.94 | 1.79 | 2.07 | 1.85 | 2.00 | 1.92 |
| Feed Cost/kg (N) | 102.90 | 107.70 | 115.70 | 123.70 | 131.70 | 139.70 |
| Feed Cost/kg/b (N) | 240.78 | 268.90 | 279.79 | 318.07 | 315.01 | 325.85 |
| Feed Cost/kg weight gain (N/kg) | 199.74 | 192.31 | 239.85 | 228.86 | 263.15 | 268.14 |

*Kg- Kilogram, kg/b- kilogram per bird, N- Naira, N/kg- Naira per kilogram, N/kg/b- Naira per kilogram per bird*

The relative means of creatinine was within the same range (0.69 – 0.76 mg/dL) in all the treatments which indicated that there was no tissue wastage. The liver enzymes were not influenced by the inclusion of L-Dopa from 0 % to 100 % replacement of methionine. These results showed that L-Dopa is not toxic, at the levels tested, on the liver, tissues, bone marrows and other organs from which these enzymes can sip into the blood.

### 3.4 Cost Benefit Ratio of L–Dopa Supplementation in Replacing DL – Methionine in Broiler Chickens

The cost analysis of L-Dopa supplementation in replacing synthetic methionine broiler diet, as shown on Table 5, revealed that, even though the body weight gain and feed conversion ratio of broilers fed 0 % L-Dopa + 100 % methionine and those fed 50 % L-Dopa + 50 % Methionine were statistically similar at both phases, the cost of L-Dopa in achieving kilogramme weight gain is higher than that of methionine. This difference can be attributed to the freight cost of a single shipment of 10 kg L-Dopa used for this study as compared to tons of methionine imported for commercial use.

## 4. CONCLUSION

It was observed that L-Dopa supplementation for synthetic methionine did not have any adverse effect on the performance and blood metabolites of broilers. Cost effectively, at 50 % replacement (50 % L-Dopa and 50 % methionine) body weight gain was improved. The results showed that methionine utilisation will be improved in the presence of L-Dopa, as it increases the availability of methionine adenosine transferase the enzyme that convert methionine to its biological active form, S-adenosyl methionine, thereby, speed up methylation and other functions of methionine. This will improve sulphur amino acids utilisation in birds.

## DISCLAIMER

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## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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