



Meat and Bone Characteristics of Broiler Chickens Fed Groundnut Cake-Based Diets as Affected by Graded Dietary Supplements of Crystalline L-Lysine and DL-Methionine

Ogunwole, O. A., Majekodunmi, B. C., Faboyede, R. A. and D. Ogunsiyi
Animal Nutrition Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

1.0 ABSTRACT

Effects of supplemental L-lysine and DL-methionine in a Groundnut Cake (GNC) based diets on meat and bone characteristics of broiler chickens were investigated. In a completely randomized design, a total of 168 one - day – old Arbor acre broiler chicks were randomly allocated to seven dietary treatments each in triplicate of eight birds per replicate. The seven starter and finishers' diets were: GNC based diets without any amino acid (L-lysine or DL-methionine) supplementation (T1); GNC diet + 0.2% L-lysine (T2); GNC diet + 0.4% L-lysine (T3); GNC diet + 0.2% DL-methionine (T4); GNC diet + 0.4% DL-methionine (T5); GNC diet + 0.2 L-lysine and 0.2% DL-methionine (T6) and GNC diet + 0.4% L-lysine and 0.4% DL-methionine (T7). Experimental diets and water were offered to birds *ad libitum* in an experiment lasting six-week. At day 42, two birds per replicate were slaughtered, meat and bone characteristics determined. There were significant variations ($P<0.05$) in the crude protein (%) and ether extract (%), pH₁ and pH₂ of meat. Thiobarbituric acid reactive substances composition of meat at days 0, 5, and 10 were similar ($P<0.05$) and were not affected by dietary amino acid supplementation. Tibiotarsal index (mg/mm) of bone (22.10, 27.25, 33.35, 31.40, 28.70, 31.45 and 29.75 for broilers on T1, T2, T3, T4, T5, T6 and T7, respectively) was increased significantly ($P<0.05$) by amino acid supplementation. Significant differences ($P<0.05$) were observed in the calcium, phosphorus and potassium (%) contents of broilers' bone across treatments. Supplemental L-lysine as well as both L-lysine and DL-methionine improved meat quality and bone development of broiler chickens in this study.

1.1 Keywords:

Supplemental amino acids; Meat proximate composition; Bone tibiotarsal index; Bone minerals

Academic Discipline and Sub Disciplines

Animal science ; Animal production ; Animal nutrition

SUBJECT CLASSIFICATION

Animal nutrition ; Nutritional additive ; Animal production

TYPE (METHOD/APPROACH)

Research article

2.0 INTRODUCTION

The deficit of animal protein in the diets of most Nigerians and developing countries is now a matter of urgent concern and measures to save people from imminent protein malnutrition are imperative. Even more disturbing is the fact of a recent decline in poultry production. Esonu *et al.* (2001) stated that more than 50% of Nigeria's poultry farmers have closed down and another 30% were forced to reduce their production capacity due to feed shortage.

According to Longe and Adetola (1983), feed represents a major proportion of the overall production cost in the poultry and livestock industry in Nigeria. Feed costs accounts for up to 60- 80% of the total cost of production. Protein components of poultry diets are expensive and to a large the important determinant of poultry sustainability and profitability (Adeyemo and Longe, 2008).

Protein is one of the most important nutrients upon which any feed formulation is based and groundnut cake happens to be one of the feed ingredients rich in protein. Groundnut cake is a known source of vitamin E, K, thiamine and niacin (FAO, 2000) and have been utilized as protein supplying feedstuff in poultry production. Most earlier studies based on the use of GNC in poultry have emphasized the need for supplemental limiting amino acids especially lysine and methionine for optimum performance of birds (Olomu and Offiong, 1980; Nwokoro and Tewe, 1992; Nwokoro, 1993) Also, in these earlier studies, there have been reported improvements in performance (Hickling *et al.*, 1990; Odufuwa, 2014), nutrients utilization (Ogunwole *et al.*, 2013; Odufuwa, 2014), immunity and health status of broilers (Chen *et al.*, 2003) with virtually no emphasis on broilers meat and bones characteristics. The present study was therefore aimed at evaluating the effect of supplemental DL-methionine and L-lysine in GNC – based diets on broiler chickens meat and bone characteristics.

3.0 MATERIALS AND METHODS

The experiment was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The farm location is about 200 – 300m above sea level and it is located on the latitude 7, 23°N and longitude 3, 53°E. The average day time temperature is 25°C – 28°C and relative humidity is 75 – 80%. A total of 168, one - day- old Arbor acre broiler chicks were used for the experiment. The birds were randomly allocated to seven dietary treatments in triplicates of eight birds per replicate in a completely randomized design. The birds were weighed prior to their allotment to various dietary



treatments. Routine medication, vaccination and husbandry practices were administered on the birds as outlined (Oluyemi and Robert, 2000).

Seven GNC based diets were formulated at the starter and finishers' phases. Treatment 1 (T1) was the control GNC based diet with 0% supplemental L-lysine and DL-methionine; treatment 2 (T2) had 0.2% supplemental L-lysine only; treatment 3 (T3) had 0.4% supplemental L-lysine; treatment 4 (T4) was supplemented with 0.2% DL-methionine; treatment 5 (T5) had 0.4% supplemental DL-methionine; treatment 6 (T6) was supplemented with both L-lysine and DL-methionine each at 0.2% while treatment 7 (T7) was also GNC based diet supplemented with both L-lysine and DL-methionine each at 0.4%. The starter diets had crude protein of 23.21 – 23.72% and metabolizable energy of 3039.67 – 3063.85 kcal/kg while the finishers' diets had crude protein of 20.12 – 20.25% and metabolizable energy of 3139.67 – 3163.65 kcal/kg. Details of the formulation of the experimental starter and finishers diets have been documented (Ogunwole *et al.*, 2013) and are presented in Tables 1 and 2. The diets and water were offered to the birds *ad libitum* in an experiment which lasted six weeks.

At day 42, all experimental birds were sacrificed by severing the carotid arteries with subsequent exsanguination. The pH of meat samples was measured at 30 minutes interval, using a pH- meter (MP230, Mettler, Switzerland) which was calibrated daily with standard pH buffers of 4.0 and 7.0 at 25°C. Lipid oxidation of meat samples was estimated using cuts (2 x 5g) from breast and thigh for thiobarbituric acid reactive substances (TBARS) by aqueous acid extraction method of Raharjo and Sofos (1993).

The left and right tibia of each bird with the drumsticks were removed with the flesh intact. The drumsticks were labeled and immersed in boiling water (100 °C) for 10 min. After cooling to room temperature, the drumsticks were defleshed by hand. They were then air-dried for 24 h at room temperature. The tibiotarsal length and bone weight were determined. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al.*, 1991). The tibiotarsal and the robusticity indexes were determined using the formular: robusticity index = bone length / cube root of bone weight (Reisenfeld, 1972). The bone ash content was determined according to the procedure of AOAC (1995) and percentage ash related to dry weight of tibia.

4.0 STATISTICAL ANALYSIS

All data were subjected to analysis of variance of completely randomized design using the SAS (1999) package and the means separated ($\alpha_{0.05}$) using Duncan multiple range test of the same software.

Table 1: Composition (g/100g DM) of experimental starter diet fed to broilers

Ingredients	T1	T2	T3	T4	T5	T6	T7
Maize	48.80	48.80	48.80	48.80	48.80	48.80	48.80
Groundnut cake	39.00	39.00	39.00	39.00	39.00	39.00	39.00
Wheat offal	5.20	5.00	4.80	5.00	4.80	4.80	4.40
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Calcium carbonate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.250.
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mycofix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.00	0.20	0.40	0.00	0.00	0.20	0.40
DL-Methionine	0.00	0.00	0.00	0.20	0.40	0.20	0.40
Total	100	100	100	100	100	100	100
Calculated Nutrient							
Crude Protein (%)	23.31	23.28	23.25	23.28	23.25	23.25	23.72
Metabolizable energy (kcal/kg)	3054.6	3050.9	3047.2	3050.9	3047.2	3047.2	3039.7
Calcium (%)	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Available Phosphorus (%)	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Lysine (%)	0.79	0.95	1.11	0.79	0.79	0.95	1.11
Methionine (%)	0.29	0.29	0.29	0.45	0.61	0.45	0.61



*Broiler Premix = vitamin A - 10,000,000IU; vitamin D₃ - 2,000,000IU; vitamin E - 40,000mg; vitamin K₃ - 2,000mg; vitamin B₁ - 1500mg; vitamin B₂ - 5000mg; vitamin B₆ - 4000mg; vitamin B₁₂ - 20mg; niacin - 40,000mg; calpan - 10,000mg; folic acid - 1000mg; biotin - 100mg; antioxidant - 100,000mg; choline chloride - 300,000mg; manganese - 80,000mg, iron - 40,000mg; zinc - 60,000mg, copper - 80,000mg; iodine - 800mg; cobalt - 300mg; selenium - 200mg

Table 2: Composition (g/100gDM) of experimental finishers' diet fed to broilers

Ingredients	T1	T2	T3	T4	T5	T6	T7
Maize	58.80	58.80	58.80	58.80	58.80	58.80	58.80
Groundnut cake	29.00	29.00	29.00	29.00	29.00	29.00	29.00
Wheat offal	5.20	5.00	4.80	5.00	4.80	4.80	4.40
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Calcium carbonate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.250.
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mycofix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.00	0.20	0.40	0.00	0.00	0.20	0.40
DL-Methionine	0.00	0.00	0.00	0.20	0.40	0.20	0.40
Total	100	100	100	100	100	100	100
Calculated Nutrient							
Crude Protein (%)	20.22	20.19	20.15	20.19	20.15	20.15	20.12
Metabolizable Energy (kcal/kg)	3155.4	3151.7	3147.8	3150.7	3147.3	3147.3	3139.7
Calcium (%)	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Available Phosphorus (%)	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Lysine (%)	0.67	0.83	0.99	0.67	0.67	0.83	0.99
Methionine (%)	0.24	0.24	0.24	0.40	0.56	0.40	0.56

*Broiler premix = vitamin A - 10,000,000IU; vitamin D₃ - 2,000,000IU; vitamin E - 40,000mg; vitamin K₃ - 2,000mg; vitamin B₁ - 1500mg; vitamin B₂ - 5000mg; vitamin B₆ - 4000mg; vitamin B₁₂ - 20mg; niacin - 40,000mg; calpan - 10,000mg; folic acid - 1000mg; biotin - 100mg; antioxidant - 100,000mg; choline chloride - 300,000mg; manganese - 80,000mg, iron - 40,000mg; zinc - 60,000mg, copper - 80,000mg; iodine - 800mg; cobalt - 300mg; selenium - 200mg

5.0 RESULTS

The proximate composition of meat from broiler birds fed groundnut cake-based diet supplemented with L-lysine and DL-methionine are shown in Table 3. The values of ash, crude fibre and moisture content were similar ($p > 0.05$) across treatments. However, percentage crude protein of meat of birds on Treatments 4 (22.82%) and 5 (22.03%) were significantly ($p < 0.05$) higher than those on treatment 2 (18.41%). Similarly, ether extract value of meat of birds on Treatment 7 (5.73) was significantly ($p < 0.05$) higher than those from birds on treatment 6 (4.65).

Table 3: Proximate composition of broilers' meat fed groundnut cake-based diets supplemented with DL-Methionine and L-Lysine

Parameter(%)	T1	T2	T3	T4	T5	T6	T7	SEM
Crude Protein	21.49 ^{ab}	18.41 ^b	21.10 ^{ab}	22.82 ^a	22.03 ^a	19.96 ^{ab}	20.51 ^{ab}	0.970
Ash	0.905	0.935	0.820	0.815	0.845	0.835	0.800	0.048
Ether Extract	5.30 ^{ab}	5.30 ^{ab}	4.99 ^{ab}	5.63 ^{ab}	4.75 ^{ab}	4.65 ^b	5.73 ^a	0.273
Crude Fibre	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.000
Moisture Content	52.51	50.72	55.13	56.12	56.00	59.06	54.06	3.003



a, b, c, d means along the same row with dissimilar superscripts differ significantly ($p < 0.05$). T1- sole groundnut cake (0% L-Lysine + 0% DL-Methionine); T2- groundnut cake + 0.2% L-Lysine; T3- groundnut cake + 0.4% L-Lysine, T4- groundnut cake + 0.2% DL-Methionine; T5- groundnut cake + 0.4% methionine; T6- groundnut cake + 0.2% L-Lysine + 0.2 DL-Methionine; T7- groundnut cake + 0.4% L-Lysine + 0.4% DL-Methionine; SEM – Standard Error of Mean

The pH of broilers meat fed groundnut cake - based diet supplemented with graded levels of L-Lysine and methionine presented in Table 4 varied significantly ($p < 0.05$) among treatments. The values ranged from 5.92 - 6.39 and 5.72 - 6.15 for pH₁ and pH₂ respectively with meat from treatment 1 having the lowest value.

Table 4: The pH of broilers meat fed groundnut cake based diets supplemented with graded levels of L-Lysine and DL-Methionine

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
pH	5.92 ^b	6.13 ^{ab}	6.25 ^a	6.22 ^{ab}	6.27 ^a	6.23 ^{ab}	6.39 ^a	0.0099
pH 2	5.72 ^c	5.97 ^{ab}	6.15 ^a	5.92 ^{abc}	6.11 ^{ab}	5.88 ^{bc}	6.12 ^a	0.008

a, b, c, d means along the same row with dissimilar superscripts differ significantly ($p < 0.05$). T1- sole groundnut cake (0% L-Lysine + 0% DL-Methionine); T2- groundnut cake + 0.2% L-Lysine; T3- groundnut cake + 0.4% L-Lysine, T4- groundnut cake + 0.2% DL-Methionine; T5- groundnut cake + 0.4% DL-Methionine; T6- groundnut cake + 0.2% L-Lysine + 0.2 DL-Methionine; T7- groundnut cake + 0.4% L-Lysine + 0.4% DL-Methionine; SEM – Standard Error of Mean

The TBARS values which ranged from 0.030- 0.695 showed no variation among treatments (Table 5). Values obtained for bone length and robusticity index (Table 6) were similar ($p > 0.05$) across treatments. However, tibiotarsal index values of birds on treatments 3 (33.35), 4 (31.40) and 5 (31.45) were significantly ($p < 0.05$) higher than those on treatment 1 (22.10).

Table 5: Thiobarbituric acid reactive substance of broilers fed groundnut cake based diets supplemented with L lysine and DL methionine

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Day 0	0.040	0.035	0.050	0.040	0.035	0.030	0.045	0.060
Day 5	0.390	0.350	0.355	0.360	0.345	0.350	0.350	0.017
Day 10	0.565	0.575	0.530	0.695	0.605	0.680	0.550	0.065

T1- sole groundnut cake (0% both L-Lysine + 0% DL-methionine); T2- groundnut cake + 0.2% both L-Lysine; T3- groundnut cake + 0.4% both L-Lysine, T4- groundnut cake + 0.2% DL-methionine; T5- groundnut cake + 0.4% DL-methionine; T6- groundnut cake + 0.2% both L-Lysine + 0.2 DL-methionine; T7- groundnut cake + 0.4% both L-Lysine + 0.4% DL-methionine; SEM – Standard Error of Mean

Mineral composition in bone of the experimental birds is shown in Table 7. The tibia ash and magnesium across treatments were similar ($p > 0.05$). However, values of calcium and the phosphorous in bones of birds on treatments 1 (16.99), 2 (16.99), 6 (17.07) and 7 (17.02) were significantly ($p < 0.05$) higher than those on treatment 4 (16.10).

Table 6: Physical characteristics of broilers bone fed groundnut cake based diets supplemented, with varying levels of DL-methionine and both L-Lysine

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
BL (cm)	0.155	0.205	0.115	0.185	0.160	0.175	0.145	0.024
RI	41.25	38.00	43.95	40.70	41.45	42.10	42.45	2.232
TI (mg/mm)	22.10 ^b	27.25 ^{ab}	33.35 ^a	31.40 ^a	28.70 ^{ab}	31.45 ^a	29.75 ^{ab}	2.525

a, b means along the same row with dissimilar superscripts differ significantly ($p < 0.05$). T1- sole groundnut cake (0% both L-Lysine + 0% DL-methionine); T2- groundnut cake + 0.2% both L-Lysine; T3- groundnut cake + 0.4% both L-Lysine, T4- groundnut cake + 0.2% DL-methionine; T5- groundnut cake + 0.4% DL-methionine; T6- groundnut cake + 0.2% both L-Lysine + 0.2 DL-methionine; T7- groundnut cake + 0.4% both L-Lysine + 0.4% DL-methionine, BL- Bone length/ bone weight index ; RI- Robusticity Index; TI- Tibiotarsal Index; SEM – Standard Error of Mean

**Table 7: Bone minerals of broilers fed groundnut cake based diet supplemented with graded levels of both L-Lysine and DL-methionine**

Parameters (%)	T1	T2	T3	T4	T5	T6	T7	SEM
Tibia Ash	30.32	31.00	30.06	30.97	30.42	30.15	30.97	0.432
Calcium	28.56 ^c	29.81 ^a	30.07 ^a	28.63 ^{bc}	28.89 ^b	28.94 ^b	28.83 ^{bc}	0.634
Phosphorus	16.99 ^a	16.99 ^a	16.70 ^{ab}	16.10 ^b	16.70 ^{ab}	17.07 ^a	17.02 ^a	0.206
sPotassium	0.059 ^{abc}	0.054 ^c	0.054 ^{bc}	0.064 ^a	0.059 ^{abc}	0.063 ^{ab}	0.061 ^{abc}	0.003
Magnesium	0.045	0.045	0.041	0.050	0.043	0.041	0.044	0.003

a, b,c means along the same row with dissimilar superscripts differ significantly ($p < 0.05$). T1- sole groundnut cake (0% both L-Lysine + 0% DL-methionine); T2- groundnut cake + 0.2% both L-Lysine; T3- groundnut cake + 0.4% both L-Lysine, T4- groundnut cake + 0.2% DL-methionine; T5- groundnut cake + 0.4% DL-methionine; T6- groundnut cake + 0.2% lysine + 0.2 DL-methionine; T7- groundnut cake + 0.4% both L-Lysine + 0.4% DL-methionine; SEM- Standard Error of Means

6.0 DISCUSSION

Effects of dietary treatments on crude protein and ether extract of broilers' meat may be due to the role played by methionine in protein synthesis in broilers (NRC, 1984) and high protein content of groundnut cake which is the main test ingredient as reported by Rostagno and Barbosa, (1995). Groundnut cake was reported to contain high level of edible oil (Atasi *et al.*, 2009) and this might be responsible for significant dietary effect of treatments on ether extract of broilers' meat. Test diets had no effect on proximate ash, crude fibre and moisture composition of broilers' meat.

The pH is a measure of acidity, which ranges from 0 (very acidic) to 14 (very alkaline). Meat pH is an indication of meat sample durability and shelf life. The pH of the meat also affects its colour, tenderness, and the eating quality (Fletcher, 1999). Results indicated that broiler meat on experimental diets had varying meat colour, tenderness and juiciness. And this agreed with pH < 5.7 for pale, soft and exudative (PSE) meat, 5.7 – 6.1 for standard quality meat and > 6.1 for dark, firm and dry meat. reports of Fletcher (2006).

Lipid oxidation is a factor that reduces meat quality and malondialdehyde is a soluble degraded products of lipids and an indicator widely used to reflect the extent of lipid oxidation in meat (Raharjo and Sofos, 1993). Result obtained in this study showed that treatments had no significant effect on broilers' meat TBARS.

The bone weight/bone length index is a simple indication of bone density. The higher the index, the denser is the bone (Monteagudo *et al.*, 1997). Conversely, low robusticity index indicates a strong bone structure (Reisenfeld, 1972). Also the high value of the tibiotarsal index shows the high mineralization level of the bone (Von Hartung and Van Hasselt, 1988). Result indicated that experimental diets had little or no effects on bone length/bone weight index and robusticity index of broilers. While tibiotarsal index of experimental birds showed increased degree of bone mineralization and development. The significant effects observed in diet supplemented with 0.4% L-Lysine might be due to the role L-Lysine played in calcium absorption (Toride, 2011). Although, Mutus *et al.* (2006), showed that there was no significant difference in the tibiotarsal weight, length and weight/length index and robusticity index of broiler fed dietary supplementation of probiotic, this study however revealed a contrary result with respect to the tibiotarsal index which could be due to the supplemental amino acids. Also, Adebisi *et al.* (2009) reported a significant effect on weight/length index when cockerels were fed varying levels of fossil flour-supplemented diets contrary to observations for broilers in this study.

The roles played by calcium and phosphorus in the formation of the skeletal system and body metabolism can not be overemphasized. Calcium affects bone structure, blood coagulation, adhesion of molecules, neural transmission, muscle contraction, cellular motility, differentiation and proliferation, hormonal secretion, and apoptosis (Vasudevan, 2011). Also, phosphorus is a constituent of bone, nucleic acids, high-energy compounds, and phospholipids found in membranes. Deficiency of calcium or phosphorus will adversely affect these important processes that are all a part of growth. Increased bone ash also improve bone mineralization (Onyango *et al.*, 2003). Results indicated that supplemental L-Lysine in a GNC – based diets improved calcium absorption which invariably increased bone development. Also, supplemental L-Lysine and methionine improved phosphorus absorption while supplemental DL-methionine improved potassium absorption.

7.0 CONCLUSION AND RECOMMENDATION

Proximate composition, meat pH, TBARS, bone minerals composition and physical attributes of broilers in this study revealed that test diets did not precipitate any severe effects on meat quality and bone development in the experimental birds. Dietary supplementation of 0.2% of both L-Lysine and DL-methionine improved utilisation of groundnut cake based diets by broiler chickens.



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