ISSN 0189-0514
J. Anim. Prod. Res. (2017) 29(1):240-248

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CALCIUM AND PHOSPHORUS RETENTION BY BROILER CHICKENS FED GROUNDNUT CAKE BASED-DIET SUPPLEMENTED WITH L-LYSINE AND DL-METHIONINE

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ABSTRACT

Effect of supplementing groundnut cake (GNC) based-diets with L-lysine and DL-methionine on calcium and phosphorus retention in broiler chickens was evaluated in this study. In a completely randomized design, one-day-old Arbor Acre chicks (n=168) were randomly allotted to seven treatments each in triplicate of eight birds per replicate. Control birds (T_1) were fed GNC based-diets without any supplementation while those on T_2 and T3 had 0.2 and 0.4 % L-lysine, respectively. Birds on T4 and T5 had 0.2 and 0.4 % DL-methionine while diets of birds on T6 and T7 were supplemented with 0.2% L-lysine and 0.2% DL-methionine and 0.4% L-lysine and 0.4% DL-methionine, respectively. Faecal calcium of birds on treatment 7 (2.37%) was significantly higher ($P<0.05$) compared with those on T5 (2.16%). Birds on T_7 also had higher ($P<0.05$) phosphorus (1.67%) than those on treatment 1 (1.46%). Tibia phosphorous of birds on control diet was similar ($P>0.05$) to those on treatments 2, 3, 5, 6 and 7 while the birds on T4 recorded significantly lower ($P<0.05$) tibia phosphorus. At the starter phase, birds on sole amino acid supplementation (T_2, T_3, T_4 and T_5) had lower but similar ($P>0.05$) sera calcium. At the finisher phase sera Ca and P of birds were similar ($P>0.05$). Correlation of feed and faecal calcium and phosphorus was positive and highly significant ($p<0.01$) at the starter phase which were conversely not observed among calcium and phosphorus levels in blood and tibia bone as well as in the serum alkaline phosphatase at the finisher phase. Supplementation of GNC based diets with both L-lysine and DL-methionine at 0.2% and 0.4%, respectively elevated especially the starter chicks sera as well as faecal calcium and phosphorus thereby also implying on bone strength.

Keywords: Amino acids, Broiler, Bone, Calcium, Chickens, Faecal, Phosphorus, Serum.

INTRODUCTION

Macro minerals like calcium, phosphorous, magnesium, potassium, sodium, chloride and sulphur are very important to poultry. Feeds that will supply all these nutrients in sufficient quantities to meet animals' requirement are usually expensive (Onu and Mdubuike, 2006). This is attributable to the high cost of feedstuff which rose by about 400% within the last 20 years (Udedibie, 2003).

For optimum poultry production, adequate level of protein and energy must be provided through feed and this requires the inclusion of protein-rich feed ingredients like fish meal, soybean and groundnut cake which are relatively expensive. Protein requirement is one of the most important criteria upon which any feed formulation is based and groundnut cake happens to be one of the feed ingredients that are very rich in protein.

Protein from GNC is known to be deficient in L-lysine and DL-methionine and also has a limited amount of tryptophan and threonine but amino acid quality improves in artificial diets when reinforced with lysine, methionine and tryptophan (Eyo and Olatunde, 1998). According to

Mukhtar *et al*. (2007), ideal poultry diet should satisfy the requirement of the animals for amino acid to achieve maximum economic return. However, in Nigeria, the main problem facing poultry farmers is not just high cost of protein feed resources but supplying the right quantity and quality of protein (amino acid) in the diets (Okpaniezeali *et al*., 2001). In addition, the proportion of lysine and methionine to all other essential amino acids (EAA) is of great concern for optimal performance of broilers. May and Vardaman (1972) stated that the most essential amino acids that are likely to be deficient in poultry diets are lysine, methionine and cystine. Unfortunately, protein from a single feed ingredient is not always adequate to satisfy these essential amino acid requirements. This problem has tended to reduce the rate of growth and performance of animals and has added to the low level of animal protein intake of Nigerians. A possible way of increasing the productivity of our animals and maximizing efficiency of protein use is by improving the dietary levels of essential amino acids.

Bawa *et al*. (2008) reported that dietary supplementation with L- lysine and DL-methionine resulted in significant weight increase in broiler chicken. Similar study (Ogunwole *et al*., 2013) revealed enhanced dry matter digestibility and gut histomorphology of broiler chickens when fed GNC based-diets supplemented with varying inclusion levels of L-lysine and DL-methionine. Also, Ogunwole *et al*. (2016) reported the effect of L-Lysine and DL-methionine supplementation in groundnut cake-based diets on meat and bone characteristics of broiler chickens. The present endeavour was aimed at further documenting the effects of supplementing GNC-based diets with L-lysine and DL-methionine on calcium and phosphorus utilisation in broiler chickens.

MATERIALS AND METHOD

Location of Experiment

The study was undertaken at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The site is located on latitude 7^0 20'N and 30^0 50'E, 200m above the sea level.

Experimental Design and Diets

One-day old Arbor Acre broiler plus chicks (n=168) were randomly allocated to seven treatments and three replicates of eight birds each. The birds were placed on dietary treatments for a period of eight weeks. Broilers on T_1 (Control) were fed GNC-based diets without amino acids supplementation while those on Treatments 2 and 3 had their GNC based diets supplemented with 0.2 and 0.4% lysine, respectively. Birds on Treatments 4 and 5 were fed GNC based diets supplemented with 0.2 and 0.4% DL-methionine, respectively while those on Treatments 6 and 7 were fed GNC based diets supplemented with both 0.2% L-lysine and 0.2% DL-methionine; 0.4% L-lysine and 0.4% DL-methionine, respectively. Details of the composition of experimental starter and finisher diets have been published (Ogunwole *et al*., 2013; Ogunwole *et al.,* 2016) and are also shown in Tables 1 and 2, respectively. Birds were given feed and water *ad-libitum* for the duration of the experiment. The experimental design was a completely randomised design.

Blood collection and analysis

At weeks 3 (starter phase) and 6 (finisher phase), blood (5 mL) was sampled from the jugular vein of two birds per replicate with the use of syringe and needle into bottles without anticoagulants for the collection of serum. Alkaline phosphatase (ALP) was determined using a photoelectric colorimeter as described (Duncan *et al.,* 1994). Serum calcium was determined by precipitation methods (Pybius *et al*., 1972) and serum phosphorus by colorimetric method (Fiske and Sub-Barow, 1925)

Faecal Analysis

Birds were placed in metabolic cages for a total of ten days, they were allowed to adjust in the first 5 days and thereafter, total voided faeces were collected from two birds per replicate for five days. Faecal materials were oven dried and samples taken for calcium and phosphorus determination (AOAC, 1990).

Bone Collection

Two birds with weight closest to mean group weight of each replicate were selected, sacrificed and right tibia leg of the birds collected. Flesh, cartilages and fat were removed after boiling and momentary soaking in concentrated hydrochloric acid. The bones were thereafter weighed, air dried, crushed then oven dried at 105^0C for 24 hours. Samples were ashed at 600^0C for 6 hours. The calcium and phosphorus content were thereafter determined (AOAC, 2009).

Statistical Analysis

Data were subjected to Pearson correlation and analysis of variance (ANOVA) using SAS (1999) package. Means were separated using Duncan's multiple range test option of the same software.

Table 1: Composition (g/100gDM) of experimental broiler chicken starter diet

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
Lysine	0.00	0.20	0.40	0.00	0.00	0.20	0.40
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)	3054.63	3050.89	3047.15	3050.89	3047.15	3047.15	3039.67
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

** Premix composition: Each 2.5Kg pack contained vitamin A - 10,000,000IU; vitamin D_3 - 2,000,000I;, vitamin E - 40,000mg; vitamin K_3 - 2,000mg; vitamin B_1 - 1500mg; vitamin B_2 - 5000mg; vitamin B_6 - 4000mg; vitamin B_{12} - 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*

T_1 = (Control) GNC-based diets without amino acids supplementation, T_2 = GNC based-diets supplemented with 0.2% lysine, T_3 = GNC based-diets supplemented with 0.4% lysine T_4 = GNC based-diets supplemented with 0.2% DL-methionine, T_5 GNC based-diets supplemented with 0.4% DL-methionine, Treatments T_6 = GNC based-diets supplemented with 0.2% lysine and 0.2% DL-methionine and T_7 = GNC based-diets supplemented with 0.4% lysine and 0.4% DL-methionine

Table 2: Composition (g/100gDM) of experimental finisher diets 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
Lysine	0.00	0.20	0.40	0.00	0.00	0.20	0.40
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)	3155.44	3151.70	3147.76	3150.68	3147.25	3147.25	3139.67
Calcium (%)	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Available P (%)	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

** Premix composition: Each 2.5Kg pack contained vitamin A - 10,000,000IU; vitamin D_3 - 2,000,000I;, vitamin E - 40,000mg; vitamin K_3 - 2,000mg; vitamin B_1 - 1500mg; vitamin B_2 - 5000mg; vitamin B_6 - 4000mg; vitamin B_{12} - 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*

T_1 = (Control) GNC-based diets without amino acids supplementation, T_2 = GNC based-diets supplemented with 0.2% lysine, T_3 = GNC based-diets supplemented with 0.4% lysine T_4 = GNC based-diets supplemented with 0.2% DL-methionine, T_5 GNC based-diets supplemented with 0.4% DL-methionine, Treatments T_6 = GNC based-diets supplemented with 0.2% lysine and 0.2% DL-methionine and T_7 = GNC based-diets supplemented with 0.4% lysine and 0.4% DL-methionine

Available P = Available Phosphorus

RESULTS AND DISCUSSION

Table 3 shows the calcium and phosphorus contents of tibia bone and excreta of broilers fed supplemental amino acid. Significant differences (P<0.05) were observed in the measured faecal and tibia parameters. Faecal calcium (%) were 2.22, 2.19, 2.28, 2.34, 2.16, 2.24 and 2.37 for broiler finisher chickens on treatments 1, 2, 3, 4, 5, 6 and 7, respectively and all the values differed significantly (P<0.05). The higher (P<0.05) faecal calcium in T7 may suggest that co-

supplementation of methionine and lysine had negative influence on calcium absorption as also evident in T6 with lower level of combination of the amino acid compared with the control (T1). Sole supplementation of lysine in T2 and T3 and methionine at T4 lowered faecal calcium indicating that these amino acids improved calcium retention in the body when compared with other treatments. Tibiae calcium was however not significantly different (P>0.05) across the treatments at the finisher phase. This suggests that supplemental amino acid did not influence the utilisation of calcium by broilers in this phase of study.

Table 3: Calcium and phosphorus in tibia bone and excreta of finisher broiler chickens fed groundnut cake based diet supplemented with graded levels of L lysine and DL methionine

Parameter	T1	T2	T3	T4	T5	T6	T7	SEM
				Faeces				
Ca (%)	2.22^{e}	2.19^{f}	2.28^{c}	2.34^{b}	2.16^{g}	2.24^{d}	2.37^{a}	0.005
P (%)	1.46^{e}	1.54^{c}	1.59^{b}	1.61^{b}	1.49^{de}	1.51^{d}	1.67^{a}	0.008
Tibia Bone								
Ca (%)	28.56	29.81	30.07	28.63	28.89	28.94	28.83	0.634
P (%)	16.99^{a}	16.99^{a}	16.70^{ab}	16.10^{b}	16.70^{ab}	17.07^{a}	17.02^{a}	0.206

a,b=Means across the rows with different superscripts are significantly different (p<0.05)

T_1 = (Control) GNC-based diets without amino acids supplementation, T_2 = GNC based-diets supplemented with 0.2% lysine, T_3 = GNC based-diets supplemented with 0.4% lysine T_4 = GNC based-diets supplemented with 0.2% DL-methionine, T_5 GNC based-diets supplemented with 0.4% DL-methionine, Treatments T_6 = GNC based-diets supplemented with 0.2% lysine and 0.2% DL-methionine and T_7 = GNC based-diets supplemented with 0.4% lysine and 0.4% DL-methionine

Dietary supplementations of amino acids in broiler chickens have been implicated in the up keep of good skeletal health. Kerstetter *et al.* (1998; 1999; 2003) reported elevated faecal calcium levels as a result of inadequate amino acids supplementation which resulted in decreased intestinal calcium absorption. Similarly, Civitelli *et al*. (1992) demonstrated that dietary supplements of certain amino acids, particularly L-lysine could increase calcium absorption in the gut. This observation was similar to results in this study as supplemental lysine at 0.2% (T2) lowered faecal calcium compared with the control. Further supplementation of the diets with lysine (T3) increased faecal calcium thus implying that inclusion beyond T2 would reduce calcium retention. Also, supplementation at 0.2% methionine (T4) increased faecal calcium but further supplementation in (T5), significantly improved (P<0.05) calcium absorption compared to chicks on other treatments. Conversely, co-supplementation of these amino acids at lower and higher levels did not improve the retention of calcium as evident in higher faecal calcium of chicks on T6 and T7 compared with control. The observed elevated calcium levels suggested that combination of methionine and lysine may have antagonised one another in intestinal absorption of calcium. Remer (2003) had earlier posited that excessive intake of dietary amino acids caused urinary Ca loss, negative Ca^{2+} balance and bone loss. The observations were however attributed to high acid load from metabolism of animal protein. Similarly, foods or supplements with excess sulphur containing amino acids have been reported to increase endogenous sulphuric acid production and therefore have the potential to increase calcium excretion and alter bone metabolism (Zwant, 2009).

Addition of amino acids to diets of broiler chicks also significantly influenced the excretion of P (%) by birds on various treatments. Broiler chickens on treatments 3, 4 and 5 had lowered tibiae P compared with other treatments. Earlier reports (Kerstetter *et al.*, 1998; Roughead *et al*., 2003) have

implicated of amino acid roles in bone development of broiler chicken. Skinner *et al.* (1991) had earlier observed a relationship between amino acid and calcium levels on tibia weight of broiler chickens. In this study however, calcium retention was not significantly affected by amino acid supplementation at all levels of supplementation (Table 3). Faecal P however, was significantly reduced at 0.4% methionine supplementation.

The serum composition of calcium and phosphorus as influenced by dietary amino acid supplementation are shown in Table 4. The values obtained in this study conformed to the reported reference values for chicken (Mitruka and Rawnsley, 1977). Owen and Amakin (2012) highlighted the importance of ions in the maintenance of proper osmotic and electrolyte balance in birds. Sera calcium of birds on T5 (8.23), T6 (8.30) and T7 (8.83) at the starter phase were not significantly different ($p>0.05$) from the control (9.14); although values for T2 (7.49), T3 (7.35) and T4 (7.68) were significantly different ($p<0.05$) from those on T1, indicating that supplemental amino acid at these levels reduced serum calcium at the starter phase. Serum P levels of birds on other treatments were not different from those on control diet while ALP of birds on T3 was not different from those on T2 but differed significantly ($p<0.05$) from those on other treatments. At the finisher phase however, Ca and P concentration in the serum were not different ($P>0.05$) across treatment. Elevated ALP levels have been reportedly associated with liver, gall bladder or bile dysfunction (Kaslow, 2011). The ALP of chicks on T1 was similar to those on treatments 2, 3, 4, 6 and 7 but was significantly different from that obtained for birds on 0.4% methionine supplementation (T4). Values reported were however within the reference range of 24.50-44.40 iu/L for serum alkaline phosphatase in chickens (Mitruka and Rawnsley, 1977).

Table 4: Serum indices of broilers fed diets containing groundnut cake supplemented with graded levels of L-lysine and DL-methionine at the starter and finisher phases

Parameter	Ca (mg/dI	P (mg/dL	ALP (ui/I	Ca (mg/dI	P (mg/dL	ALP (ui/I
		Starter			*Finisher*	
T1	9.14^{a}	6.30^{ab}	37.58^{a}	8.57	7.76	30.11^{b}
T2	7.49^{b}	5.20^{ab}	36.77^{ab}	7.14	6.76	36.22^{ab}
T3	7.35^{b}	7.12^{ab}	35.65^{b}	7.99	9.19	41.31^{ab}
T4	7.68^{b}	4.82^{b}	37.49^{a}	8.57	5.22	44.67^{ab}
T5	8.23^{ab}	5.23^{ab}	37.56^{a}	10.78	8.63	47.10^{a}
T6	8.30^{ab}	4.80^{b}	37.42^{a}	7.33	6.29	42.39^{ab}
T7	8.83^{a}	9.03^{a}	37.39^{a}	11.36	7.17	42.62^{ab}
SEM	0.30	1.18	4.62	1.96	1.40	3.61

*a, b, Treatments means within columns followed by different superscripts are significantly different ($p<0.05$) *Ca – Calcium *P- Phosphorus *ALP- Alkaline Phosphatase*

T_1 = (Control) GNC-based diets without amino acids supplementation, T_2 = GNC based-diets supplemented with 0.2% lysine, T_3 = GNC based-diets supplemented with 0.4% lysine T_4 = GNC based-diets supplemented with 0.2% DL-methionine, T_5 GNC based-diets supplemented with 0.4% DL-methionine, Treatments T_6 = GNC based-diets supplemented with 0.2% lysine and 0.2% DL-methionine and T_7 = GNC based-diets supplemented with 0.4% lysine and 0.4% DL-methionine

Correlation of serum calcium, phosphorus and alkaline phosphatase levels to calcium and phosphorus levels in the feed are shown in Tables 5 and 6. At the starter phase, feed calcium and phosphorus were positively correlated while faecal calcium and phosphorus were positively

correlated at the finisher phase in Table 6. This suggested that there was a balance in the P and Ca of feeds administered to the chickens. Also, there was no observable significant correlation among other parameters indicating that there was no strong association among these factors due to dietary amino acid supplementation. This however, was contrary to report of Bosante *et al*. (2013) on relationships of amino acid and serum calcium and phosphorus in infants.

Table 5: Correlation of dietary calcium and phosphorus with serum indices of broilers fed diet containing groundnut cake supplemented with graded levels of L-lysine and DL-methionine at the starter phase

Parameters	Feed Calcium	Feed Phosphorus	Blood Calcium	Blood Phosphorus	ALP
Feed Ca	1.00				
Feed Phosp	0.84**	1.00			
Blood Ca	0.18	0.16	1.00		
Blood Phosp	-0.12	0.03	0.18	1.00	
ALP	0.25	0.29	0.39	-0.17	1.00

*** - r value is significant at 0.01% significance level*

T_1 (Control) were fed GNC-based diets without amino acids supplementation while those on Treatments 2 and 3 had their GNC based diets supplemented with 0.2% and 0.4% lysine respectively. Birds on Treatments 4 and 5 were fed GNC based diets supplemented with 0.2% and 0.4% DL-methionine respectively while those on Treatments 6 and 7 were fed GNC based diets supplemented with both 0.2% L-lysine and 0.2% DL-methionine; 0.4% L-lysine and 0.4% DL-methionine,

Table 6: Correlation of dietary calcium and phosphorus with serum, bone and faecal calcium and phosphorus of broilers fed diet containing groundnut cake supplemented with graded levels of L-lysine and DL-methionine at the finisher phase

Parameter	**Feed Ca**	**Feed P**	**Blood Ca**	**Blood P**	**ALP**	**Faecal Ca**	**Faecal P**	**Bone Ca**	**Bone P**
Feed Ca	1.00								
Feed P	-0.23	1.00							
Blood Ca	-0.11	0.12	1.00						
Blood P	0.32	0.25	0.31	1.00					
ALP	0.25	-0.44	0.46	0.08	1.00				
Faecal Ca	0.22	-0.17	0.16	-0.24	0.15	1.00			
Faecal P	0.38	-0.39	0.1	- 0.17	0.29	0.86**	1.00		
Bone Ca	0.46	-0.21	-0.65	-0.02	-0.29	-0.11	0.16	1.00	
Bone P	-0.18	-0.06	0.04	-0.13	-0.26	-0.27	-0.24	0.06	1.00

** - r value is significant at 0.01% significance level

T_1 (Control) were fed GNC-based diets without amino acids supplementation while those on Treatments 2 and 3 had their GNC based diets supplemented with 0.2% and 0.4% lysine respectively. Birds on Treatments 4 and 5 were fed GNC based diets supplemented with 0.2% and 0.4% DL-methionine respectively while those on Treatments 6 and 7 were fed GNC based diets supplemented with both 0.2% L-lysine and 0.2% DL-methionine; 0.4% L-lysine and 0.4% DL-methionine,

CONCLUSION AND RECOMMENDATIONS

This study revealed that groundnut cake-based diet supplemented with lysine and methionine had no negative effect on tibia bone. Also, sole supplementation with lysine and methionine at 0.2 and 0.4%, respectively resulted in lower faecal calcium and phosphorus and improved bone strength. It is recommended that sole supplementation of groundnut cake-based diets with 0.2% lysine and 0.4% methionine should be used in broiler nutrition as they gave the best results in terms of skeletal health balance. Further research should be conducted using 0.4% methionine and 0.2% lysine combination as this may elicit better response. It is also recommended that this trial be conducted for birds in cages as birds on deep litter are exposed to nutrients in their excreta. This is to enable a more definite conclusion on the effect of supplemental first two limiting amino acids in broiler production.

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