Trop. Anim. Prod. Invest. 16 (1): 01-06 (2013)
ISSN: 1115-2540
http://animalsci.agric.ui.edu.ng/TAPI/index.php/tapijournal/index

ORIGINAL RESEARCH ARTICLE

Dry matter digestibility and gut histomorphology of broiler chickens fed groundnut cake based–diets supplemented with crystalline L-lysine and DL-methionine

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ABSTRACT

Effects of supplementing Groundnut Cake (GNC) based-diets with crystalline L-lysine and DL-methionine on dry matter digestibility and broiler chickens gut histomorphology were investigated in an experiment using 168 one-day old Arbor acre broilers. In a completely randomized design, chicks were randomly allotted to seven dietary treatments each replicated thrice with eight birds per replicate. The treatments were GNC diet without supplementation (T1); GNC diet + 0.2% lysine (T2); GNC diet + 0.4% lysine (T3); GNC diet + 0.2% methionine (T4); GNC diet + 0.4% methionine (T5); GNC diet + 0.2% lysine + 0.2% methionine (T6) and (GNC diet + 0.4% lysine + 0.4% methionine (T7). At day 56, two birds from each replicate were selected and sacrificed; the digestive tract was carefully excised for gut histomorphology using standard procedure. Two birds were also randomly chosen from each replicate and kept in metabolic cage for dry matter digestibility determination. . There were no significant variations (P>0.05 in the height and width of villi of birds across the diets. However, there were significant (P<0.05) differences in the crypt depth and ileal length of birds with those on T1 having the highest crypt depth (159.77µm) while those on T7 the least value (84.08µm). Dry matter digestibility was highest (91.63%) for birds on T2 while those on T4 (57.08%) was lowest. In conclusion, strategic formulation of GNC based diets for chicken with supplemental amino acids improved productivity of birds on T2, T5 and T6. Therefore, metabolism of groundnut cake based diets could be enhanced with appropriate amino acids supplementation.

Keywords: Supplemental amino acids, Crypt depth, Ileo-caeco-colonic junction and Broiler digestibility

INTRODUCTION

Groundnut is available in the tropics, being favoured by the edaphic factors, especially in Nigeria. Groundnut is consumed by humans and when its oil is extracted, it is used as groundnut cake for livestock and poultry production. The cake is high in crude protein but had been reported to be limiting in lysine and methionine (FAO, 1983). L-Lysine is a necessary building block for all proteins in the body. L-Lysine plays a major role in calcium absorption; building muscle protein; recovery from surgery or sports injuries; and the body's production of hormones, enzymes, and antibodies (Cheeke and Derenfeld, 2010; Bhattacharya, 2010; Vansudevan *et al.*, 2011; Ovie and Eze, 2012). Lysine deficiency causes immunodeficiency in chicken (Chen *et al*., 2003). Methionine is an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidylcholine, and other phospholipids. Improper conversion of methionine can lead to atherosclerosis in animals (Refsum *et al*., 1998). Early reports for broilers (Olomu and Offiong, 1980), pullet chickens (Chhillar *et al*., 1971; Wethli *et al*., 1975) and cockerel starters (Nwokoro and Tewe, 1992; Nwokoro, 1992) demonstrated that groundnut-based-diets were deficient in methionine and lysine. A similar study (Okosun, 1987) with cotton seed cake and palm kernel cake to replace GNC in cockerel starter diets revealed deficiencies of proteins except when supplemented with fish meal or lysine and methionine. Thus, the present study was designed to investigate the gut histomorphology and dry matter digestibility of broiler chicken fed groundnut cake based-diets supplemented with crystalline L-lysine and DL-methionine.

MATERIALS AND METHODS

Arbor acre broiler, one-day old chicks (n=168) purchased from CHI Farms Limited were randomly allotted to seven dietary treatments. Each treatment was replicated three times with eight birds per replicate. Experimental diets and clean water were given *ad libitum* to birds. Routine vaccines and antibiotics were administered. Starter and finishers diets were formulated and were offered to birds from day 1 to 21 and day 42 to 56 respectively as shown in Tables 1 and 2. Using completely randomized design, seven experimental diets were formulated as follows: Treatment 1, Sole GNC without any amino acid supplementation;

Table 1: Gross composition (g/100gDM) of experimental starter diets

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
Limestone	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
Table salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mycofix^{R}	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
ME (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 Phosphorous (%)	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

ME - Metabolizable energy

*Broiler Premix = 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

Treatment 2, GNC + 0.2% lysine; Treatment 3, GNC + 0.4% lysine; Treatment 4, GNC + 0.2% methionine; Treatment 5, GNC + 0.4% methionine; Treatment 6, GNC + 0.2% lysine + 0.2% methionine; Treatment 7, GNC + 0.4% lysine + 0.4% methionine. Details of the formulation of experimental starter and finishers diets are shown in Tables 1 and 2 respectively. At day 56, two birds from each replicate were selected and conditioned in metabolic cage for dry matter digestibility determination. For proper adaptation, the birds were fed for a period of five days after which the excreta was subsequently collected from day 6–10. At the end of each day of collection period, droppings from individual birds were first weighed and 10% of the bulk kept. From the portions, two samples were taken, one larger portion for drying and the other for analysis in fresh condition, mainly for dry matter estimation. Apparent dry matter digestibility was calculated using the equation of NAS (1963).

At week 8, two birds from each replicate were selected and weighed. The birds were slaughtered and the digestive tract was carefully excised. Digesta samples from terminal two thirds of the section between Meckel diverticulum and 2cm anterior to the ileo-caeco-colonic junction were flushed out as described (Agboola, 2011). Approximately, 5cm of ileum (5cm after Meckel's diverticulum) were removed and histological examinations were undertaken (Iji *et al.*, 2001). Intestinal samples from each section were immersed in formaldehyde prior to fixation in Bouin's solution and paraffin embedding. The samples were transferred into 70% ethanol after 24 hours. Paraffin sections at 6µm thickness made from each sample were stained with haematoxylin and eosin, and examined by light microscopy. Villus height, crypt depth and the ratio of crypt depth to villus height were measured from each preparation. The length of the intestinal villi and the depth of the intestinal crypt were measured with linear scaled graticule. The length of the ileum was determined using a measuring tape. The dry matter of excreta samples was determined (AOAC, 2000).

RESULTS

The dry matter digestibility of birds on experimental diets is shown in Table 3. There were significant variations ($P<0.05$) across the dietary treatments. However, the dry matter digestibility (%) of birds on 0.2% lysine supplementation was highest (91.63) while lowest value (57.08) was recorded in birds on 0.2% methionine. The ileal morphological indices obtained for birds fed experimental diets are shown in Table 4. There were no significant differences observed in the villus height, dry matter digestibility and gut width

Table 2: Gross composition (g/100gDM) of experimental broiler finisher diets

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
Soya bean meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00
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
Limestone	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
Table salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mycofix^{R}	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
ME (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 Phosphorous (%)	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

ME= Metabolizable energy

*Broiler premix = 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

of birds across the diets. However, the villus height of birds on control diets recorded the highest value (1079.3µm) while the least value (890.5µm) was recorded in birds on supplemental 0.2% lysine and 0.2% methionine.

There were significant (P<0.05) differences in crypt depth and ileal length of birds fed groundnut based diets supplemented with L-lysine and DL-methionine. Birds fed sole groundnut cake without supplementation had the highest crypt depth (159.77µm) value while least value (84.08µm) was recorded in those on 0.4% lysine and 0.4% methionine supplementation. The longest ileal length (82.33cm) was recorded in birds on GNC supplemented with 0.2% lysine while birds on diets supplemented with 0.2% methionine had the shortest ileal length (66.63cm). El-Tahawy and Ismaeil (2013) reported that concentrate supplemented with methionine increased the coefficient of dry matter, organic matter; crude protein, ether extract and nitrogen free extract but had no significant effect on crude fibre digestibility in lambs when fed these diets. He further posited that the increased digestibility coefficient was a good indicator of promicrobial activity of methionine. In the present research, dry matter digestibility in birds on 0.2% methionine supplemented diets and GNC supplemented with 0.4% methionine were of the same trend as reported (El-Tahawy and Ismaeil. 2013). This observation could also be a direct fallout of amino acid imbalance in that only methionine was supplemented without corresponding addition of lysine as earlier opined (Furlan, 2006). Digestion is a complex process involving enzyme and fluid secretions, motility, absorption and ultimately evacuation. Therefore, a healthy digestive system is crucial for optimal animal performance (The Fish Site, 2008). Awad *et al*. (2009) reported that increasing villus height was suggestive of an increased surface area for absorption of available nutrients while deeper crypt depth was implicated in a greater production of enterokinase which is the

Table 3: Digestibility of nutrients by broiler chickens fed experimental finisher diets

Digestibility (%)	**Treatments**							
Parameter	1	2	3	4	5	6	7	SEM
DM	90.31^{a}	91.6^{a}	75.93^{b}	57.08^{c}	71.96^{b}	62.32^{c}	86.62^{a}	2.73

Means with the same superscripts are not significantly different (P>0.05)

SEM-Standard Error of Mean

Table 4: Ileal histomorphology indices of broilers on experimental finisher diets

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Villus Height (µm)	1079.3	1010.50	1017.9	928.50	970.70	890.5	925.60	124.66
Crypt Depth (µm)	159.77^{a}	118.60^{ab}	115.83^{ab}	104.82^{ab}	106.82^{ab}	88.45^{b}	84.08^{b}	27.64
Villus Width (µm)	118.34	137.83	143.32	116.87	131.21	124.53	120.48	16.08
Ileal Length (cm)	71.20^{ab}	82.33^{a}	72.13^{ab}	66.63^{b}	75.68^{ab}	79.63^{ab}	78.73^{ab}	6.47

Means with the same superscript are not significantly different (P>0.05)
SEM-Standard Error of Mean
Treatment 1: Sole groundnut cake [GNC] without supplementation; Treatment 2: GNC + 0.2% lysine; Treatment 3: GNC + 0.4% lysine; Treatment 4: GNC + 0.2% methionine; Treatment 5: GNC + 0.4% methionine; Treatment 6: GNC + 0.2% lysine + 0.2% methionine; Treatment 7: GNC + 0.4% lysine + 0.4% methionine

precursor for the production of trypsin. Trypsin is needed for digestion of protein which culminates into increased availability of amino acids which is vital for improved birds' performance. The villus height of birds on other diets compared favourably with those on sole groundnut cake without supplementation. It was reported (Poultry CRC, 2006; 2013) that efficiency of absorption is influenced by the surface area available for the nutrients to move through, and the more the villi the better the absorption. Least villi height reported on 0.2% lysine and 0.2% methionine supplemented diets could be as a result of imbalance in amino acids in these diets (NRC, 1994). Birds on sole groundnut cake without supplementation had the highest villi height. This contrasted with the result for broiler chicken (Teixeira *et al.*. 2004), especially on the performance of birds on sole groundnut cake without supplementation. Furlan *et al.* (2001) opined that a thorough understanding of the macroscopic development of the intestine is imperative in that growth and maintenance of the digestive tract are factors that contribute to increased efficiency of digestive processes in birds. The rapid weight gain of chicken and feed utilization are directly related to their nutrition and the morpho functional integrity of the digestive system (Smith *et al*., 1990) especially in the small intestine where part of the digestive processes and absorption of nutrients occurs in enterocytes (Furlan *et al.*, 2001).

The crypt depth values followed the same trend as those of villi height. However, the least values observed in birds on 0.4% lysine and 0.4% methionine supplemental diets could be as a result of amino acid toxicity (Ueda *et al.*, 1981; Edmonds and Baker, 1987 and NRC, 1994). Also, imbalance of amino acids in some of the diets could have resulted to wear, tear (mucosa cell wastages) and lowered production of some enzymes responsible for protein digestion along the gastrointestinal tracts. This may lead to a decreased proliferation of enterocytes with resultant effect on the crypt depth. This invariably affects the ability of birds to digest and absorb nutrients necessary for their maintenance and production (Furlan, 2006).

CONCLUSION

The study showed that groundnut cake supplemented with crystalline L-lysine and DL-methionine was a valuable replacement for soybean meal or fishmeal in broiler diets without adverse effects. Dry matter digestibility and the gut histomorphology revealed no severe effects on health status of the experimental birds and appropriate supplementation of GNC based diets with both L-lysine and DL-methionine resulted in better performance of broiler chicken.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exist concerning this manuscript.

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