ISSN 0189-0514
J. Anim. Prod. Res. (2018) 30(2):22-31

National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journals.napri.gov.ng

EFFECT OF ACIDIFIER (FYSAL®) ON PHYTATE UTILIZATION, MINERAL DIGESTIBILITY AND BONE DENSITY OF BROILER FINISHER CHICKENS

[1]Idachaba, C. U., [2]Duru, S., [2]Omage, J. J., [2]Onimisi, P. A. and [3]Abdullahi, I.
[1]Department of Animal Production, University of Jos, Nigeria
[2]Department of Animal Science, Ahmadu Bello University Zaria, Nigeria
[3]National Animal Production Research Institute, Shika Zaria, Nigeria
Corresponding Author: philiousgee@gmail.com

ABSTRACT:

The effect of acidifier (Fysal®) on phytate utilization, mineral digestibility and bone density of broiler finisher chickens was investigated. Three hundred broiler finisher chickens were assigned to five treatment groups each replicated thrice with twenty chickens per replicate in a completely randomized design. Chickens in the control treatment were fed basal diet while those in other treatments were fed diets supplemented with 0.1, 0.2, 0.3 and 0.4% levels of acidifier (Fysal®). At the end of the 56-day feeding period, nine chickens per treatment were selected for mineral digestibility trial and later processed to remove the left tibia bone for determination of bone density. Supplementation of 0.3% organic acid was found to liberate minerals bound by phytate and significantly (P<0.05) increased apparent digestibility of calcium, phosphorus and magnesium by 28.57, 27.77, 13.89% respectively. It also significantly (P<0.05) increased bone density, tibia ash, calcium and phosphorus by 11.69, 11.58, 14.44 and 14.89% respectively. The study conclude that the use of acidifiers at 3kg per ton/feed is beneficial in broiler production for bone mineralization and efficient utilization of feed nutrients.

Key words: Acidifier, Organic acid, Phytate, Mineral

INTRODUCTION

Minerals are required for normal life processes and the dietary requirement of farm animals is well documented (NRC, 1994). Minerals required for growth and development in poultry chickens are derived primarily from feedstuffs. It was reported by Thomas and Ravindran (2010), that the bioavailability of minerals in feedstuffs is manifested by the efficiency to which the body utilizes and retains them. Unfortunately, feed minerals and particularly phosphorus is bound by phytate leading to increased faecal losses due to inability of simple stomach animals to hydrolyze and utilize them (Rath *et al*., 2000). As a result, bone problems resulting from poor mineralization has become a concern in the production of meat-type chickens (Idachaba, 2017). Phytate has also been reported by Abdoulaye *et al.* (2011) to form insoluble complex with protein, starch, minerals and vitamins thereby affecting weight gain and other productive parameters. Conventional vegetable feed sources have low (30-40%) available phosphorus because most of it is bound by phytate (NRC, 1994). Mineral chelators such as acidifiers compete favourably with phytate by forming soluble complexes with minerals and other nutrients in the lumen of the gut (Boling *et al*., 2000a). The soluble complex that results from this interaction is readily absorbed by the body and therefore enhances nutrient utilization. Boling *et al*. (2000a) further showed that citric acid supplementation in diet of broiler chicks increased phosphorus utilization and reduced dietary available phosphorus requirement by 0.10%. Similarly, Park *et al.* (2009) reported that organic acid supplementation in corn/soybean meal based diets fed to poultry chickens increased mineral utilization only in

phosphorus deficient diets (0.30%AP). The authors reported that organic acid supplementation did not improve mineral utilization in diets adequate in available phosphorus. Snow *et al.* (2004) asserted that supplementing acidifiers in diets with 0.39% available phosphorus as against 0.50% recommended by NRC (1994) increased weight gain and mineral utilization in broiler chicks. It was speculated that organic acids such as citric acid, being a strong chelator of calcium, may complex with calcium and decrease its ability to bind phytate, thereby making phytate less stable and more susceptible to endogenous enzymes (Boiling *et al*., 2000b). According to Rafacz-Livingston *et al.* (2005) organic acids improve apparent digestibility of feeds in broiler chickens by lowering pH of digesta in the small intestine thereby inhibiting the ability of phytate to form insoluble complexes that are resistant to hydrolysis by endogenous enzymes. Although improving the genetic potentials of meat-type chickens may enhance utilization of feed ingredients, the feed consumed by the animal needs to be buffered to ensure better digestion and reduction of nutrient losses as faecal wastage. Some nutritionists have tailored their research towards finding alternative feed ingredients that are cheap and affordable (Abeke, 2005; Idachaba, 2012; Abdullahi, 2012). However, there should be a focus geared towards improving the bioavailability of already existing feed ingredients, since anti-nutrients such as phytate can affect nutrient utilization despite the adequacy of ingredients in formulated rations. This informed the decision to investigate the effect of acidifier (Fysal®) on phytate utilization, mineral digestibility and bone density of broiler finisher chickens

Justification of the study

Broiler chickens are characterized by rapid growth rate which should be associated with bone mineralization to support the increase in body weight. The binding effect of phytate on feed minerals can result in leg problems as ossification may not be sufficient to support the rapid increase in body weight. Phytate is the primary storage form of phosphate in seeds and by its structure, it attracts feed nutrients particularly minerals in a hexaphosphate ring on its inositol molecule, making them less available to chickens (Woyengo and Nyachoti, 2013). Feed nutrients such as protein, minerals and vitamins are attracted to the negative charges on phytate which are the binding site used to complex feed nutrients as reported by Julian (2005). It was reported by Abdoulaye *et al.* (2011) that the negative charges on phytate increases as feed moves from the acidic region of crop, proventriculus and gizzard to the small intestine which is of basic pH. This further increases phytate's ability to strongly attract divalent and trivalent cations (minerals) in feedstuffs. This interaction results to formation of phytate-mineral complex that limits mineral utilization.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the poultry unit of the Teaching and Research Farm, Department of Animal Science, Ahmadu Bello University Samaru, Zaria. The Departmental farm is located on latitude 11^0 $9^{'}$ $45^{''}$ N and longitude 7^0 $38^{'}$ $8^{''}$ E, at an altitude of 610m above sea level. The temperature ranges between 26-40^{o}C depending on the season while the relative humidity during the dry and wet seasons are 21 and 72% respectively. The wet periods in Zaria are between May to October with annual rainfall of about 1500mm (IAR, 2012).

Breed and source of experimental chickens

Arbor Acre breed of broiler chickens were purchased from Vertex farms in South Western Nigeria and used for the trial.

Source of organic acids

Commercial feed grade acidifier (Fysal®) containing blends of organic acids was

purchased from commercial feed outlets in Zaria, Kaduna state, Nigeria.

Experimental diets

The organic acid (Fysal®) was supplemented in the diets at 0.1, 0.2, 0.3 and 0.4% for treatments two to five respectively while the control treatment was fed the basal diet without supplemental organic acid. The control diet (T_1) was formulated to meet NRC (1994) requirement for available phosphorus at 0.50% while treatments two to five (T_2-T_5) had sub-optimal available phosphorus at 0.40%. The diets were formulated as follows: T_1 (Basal diet adequate in available phosphorus), T_2 (Basal diet, 0.40% available phosphorus, 0.1% Acidifier), T_3 (Basal diet, 0.40% available phosphorus, 0.2% Acidifier), T_4 (Basal diet, 0.40% available phosphorus, 0.3% Acidifier) and T_5 (Basal diet, 0.40% available phosphorus, 0.4% Acidifier). The experimental diets are presented on Table 1.

Experimental design and management of birds

Three hundred (300) finishing broiler chickens weighing averagely 510.23g were allotted to five experimental treatments each replicated three times with twenty (20) birds per replicate in a completely randomized design. The chickens were raised on deep litter with feed and water provided *ad libitum*. All management practices were strictly adhered to.

Determination of mineral digestibility

At the end of the trial, three chickens were selected from each replicate and housed in cages for faecal collection which lasted for seven days. Faecal samples of a treatment were pooled, dried and milled to pass through 1.0mm sieve and taken to the laboratory, Department of Soil Science, A.B.U. Zaria, for determination of macro (Ca, P, Mg, K and Na) and micro minerals (Fe, Mn, Zn and Cu) using the Atomic Absorption Spectrophotometer (AAS). Results obtained from mineral determination were used to calculate mineral digestibility of macro and trace minerals using the formula.

Mineral retention coefficient =

$$\frac{(\text{Feed intake} \times \text{Mineral}_{\text{Diet}}) - (\text{Excreta output} \times \text{Mineral}_{\text{Excreta}})}{\text{Feed intake} \times \text{Mineral}_{\text{Diet}}}$$

Table 1: Ingredients and percentage composition of broiler finisher diets supplemented with graded levels of acidifier (Fysal®)

	Fysal® (%)				
Ingredients	**0.00**	**0.10**	**0.20**	**0.30**	**0.40**
Maize	59.50	57.70	57.70	57.70	57.70
Soya cake	20.00	24.00	24.00	24.00	24.00
Groundnut cake	16.00	14.00	14.00	14.00	14.00
Limestone	0.60	1.20	1.20	1.20	1.20
Bone meal	2.90	2.10	2.10	2.10	2.10
Common salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix*	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
ME (Kcal/kg)	2915.00	2901.00	2901.00	2901.00	2901.00
Crude protein (%)	21.38	21.39	21.39	21.39	21.39
Ether extract (%)	4.56	4.57	4.57	4.57	4.57
Crude fibre (%)	3.93	3.93	3.93	3.93	3.93
Calcium (%)	1.05	1.06	1.06	1.06	1.06
Av. Phosphorus(%)	0.50	0.40	0.40	0.40	0.40
Lysine (%)	1.16	1.24	1.24	1.24	1.24
Methionine (%)	0.61	0.60	0.60	0.60	0.60
Cost/kg diet (₦)	79.42	82.15	82.10	82.25	84.15

*Biomix chick premix provide per kg of diet vit A, 10,000 I.U; vit D3, 2000 I.U; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg; cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.
ME= Metabolizable energy
Av. Phosphorus= Available phosphorus

Determination of bone density and mineralization

The same chickens selected and housed in cages were then sacrificed, after which the left tibia bones were removed. The tibia bones were labelled and immersed in boiling water (100 °C) for 10 minutes and then solvent extracted to remove any traces of fat. Tibia length was measured using a meter rule and measured as the distance from cranial to distal extremities. Bone density (Robusticity index) was calculated

using the following formula as described by Reisenfeld (1972).

$$\text{Bone density} = \frac{\text{Bone length}}{\text{Cube root of bone weight.}}$$

To determine bone ash, bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to procedures described by AOAC (1994). Tibia ash was expressed as percentage of the dry weight of the tibia bone.

Statistical analysis

Data obtained from the experiment was subjected to analysis of variance using the general linear model procedure of SAS (1993). Significant treatment means were separated using Duncan's Multiple Range Test.

RESULTS

Mineral Digestibility of Broiler Chickens Fed Diets Supplemented with Graded Levels of Acidifier (Fysal®)

Table 2 shows the mineral digestibility of broiler chickens fed diets supplemented with graded levels of acidifier (Fysal®). Chickens fed diet supplemented with 0.3% acidifier showed the highest (P<0.05) digestibility of calcium and phosphorus. This was followed by treatment supplemented with 0.2% acidifier. Calcium digestibility did not differ significantly (P>0.05) between the control, 0.1 and 0.4% levels of acidifier supplementation. No significant difference (P>0.05) was observed in phosphorus digestibility diets having 0.1 and 0.4% levels of acidifier supplementation, while the control treatment diet showed the poorest (P<0.05) result.

The best (P<0.05) result in terms of magnesium digestibility was observed in diet containing 0.3% acidified diet. This was followed by treatments supplemented with 0.2 and 0.4% levels of feed acidification which showed significantly (P<0.05) better results compared to the control and 0.1% level of organic acid supplementation. Significant (P<0.05) differences were observed in potassium digestibility as diets supplemented with acidifiers showed better results compared to the control group.

Manganese digestibility was similar (P>0.05) at 0.1, 0.3 and 0.4% levels of organic acid supplementation as they were significantly (P<0.05) better than other treatments. This was followed by the control diets while treatment diet supplemented with 0.2% acidifier showed the poorest (P<0.05) result. Diets containing 0.2 and 0.3% acidified showed significantly (P<0.05) better results in zinc digestibility compared to other treatment groups. The control and treatment supplemented with 0.1 and 0.4% levels of organic acid did not differ significantly (P>0.05) in zinc digestibility. The best (P<0.05) result in copper digestibility was observed for chickens fed 0.4% acidified diet while 0.2 and 0.3% levels of acidifier supplementation showed comparable (P>0.05) result with each other. The control and treatment diets supplemented with 0.1% level of feed acidication showed the poorest (P<0.05) results for copper digestibility.

Bone Density and Mineralization of Broiler Finisher Chickens Fed Diets Supplemented with Graded Levels of Acidifier (Fysal®)

Table 3 shows the bone density and mineralization of broiler finisher chickens fed diets supplemented with graded levels of acidifier. All parameters measured showed significant (P<0.05) differences across the treatments. Bone density of chickens fed 0.3%acidified diet was significantly (P<0.05) better than other treatment groups. This was followed by treatments supplemented with 0.1, 0.2 and 0.4% acidifier as they showed comparable values (P>0.05) and were significantly better than the control.

No significant difference (P>0.05) was observed in percentage tibia ash of chickens fed 0.2 and 0.3% acidifier as they showed significantly (P<0.05) higher values than other treatment groups. This was followed by treatment

supplemented with 0.1% acidifier while the poorest result was observed for chickens fed the control and diet supplemented with 0.4% organic acid.

Tibia calcium was significantly (P<0.05) higher in treatment supplemented with 0.3% acidifier while other treatments were similar (P>0.05) to each other with exception of treatment supplemented with 0.1% acidifier which was the poorest. No significant difference (P>0.05) was observed in tibia phosphorus between chickens fed diets supplemented with 0.2 and 0.3% organic acid as they showed the best (P<0.05) results. This was followed by treatment supplemented with 0.1% acidifier while the poorest (P<0.05) results were observed for chickens fed the control and diet supplemented with 0.4% organic acid. No leg deformity was observed in chickens in all treatment groups.

Table 2: Mineral digestibility of broiler chickens fed diets supplemented with graded levels of acidifier (Fysal®)

Minerals (%)	Level of Fysal® (%)					SEM	LOS
	0.0	0.1	0.2	0.3	0.4		
Calcium	57.0^{c}	59.0^{c}	67.0^{b}	77.0^{a}	55.0^{c}	3.02	*
Phosphorus	39.0^{d}	44.0^{c}	47.0^{b}	54.0^{a}	43.0^{c}	1.00	*
Magnesium	62.0^{c}	63.0^{c}	67.0^{b}	72.0^{a}	66.0^{b}	0.50	*
Potassium	73.0^{c}	75.0^{a}	74.0^{b}	74.0^{b}	74.0^{b}	0.03	*
Manganese	24.0^{b}	27.0^{a}	22.0^{c}	29.0^{a}	29.0^{a}	0.51	*
Zinc	30.0^{b}	27.0^{b}	33.0^{a}	35.0^{a}	29.0^{b}	1.00	*
Copper	46.0^{c}	47.0^{c}	50.0^{b}	50.0^{b}	58.0^{a}	1.15	*

a, b, c, d=Means with different superscript on the same row differ significantly (P<0.05)
SEM= Standard error of mean, LOS= Level of significance

Table 3: Bone density and mineralization inbroiler finisher chickens fed diets supplemented with graded levels of acidifier (Fysal®)

Parameters	Level of Fysal® (%)					SEM	LOS
	0.0	0.1	0.2	0.3	0.4		
Tibia length (cm)	10.22^{c}	10.68^{bc}	11.06^{b}	11.97^{a}	10.74^{b}	0.23	*
Tibia weight (g)	9.00^{b}	8.00^{c}	9.00^{b}	10.00^{a}	8.00^{c}	0.21	*
Bone density	4.91^{c}	5.34^{b}	5.32^{b}	5.56^{a}	5.37^{b}	0.09	*
Tibia Ash (%)	41.02^{c}	43.48^{b}	45.85^{a}	46.39^{a}	41.97^{c}	0.57	*
Tibia Calcium (%)	26.39^{b}	25.41^{c}	26.80^{b}	30.12^{a}	25.77^{b}	0.60	*
Tibia Phosphorus (%)	14.73^{c}	16.12^{b}	16.77^{a}	17.12^{a}	14.57^{c}	0.23	*
Leg Deformity (%)	0.00	0.00	0.00	0.00	0.00	0.00	NS

a, b, c=Means with different superscript on the same row differ significantly (P<0.05) SEM= Standard error of mean
LOS= Level of significance, NS= Non-significant

DISCUSSION

Mineral Digestibility of Broiler Finisher Chickens Fed Diets Supplemented with Graded Levels of Acidifier (Fysal®)

Although treatments supplemented with acidifiers had sub-optimal level of available phosphorus (0.40%), yet they showed better mineral digestibility at higher levels (0.2 and 0.3%) of supplementation compared to the control and other groups. This is because acidifiers are powerful chelators that distort phytate's affinity to bind feed minerals in an unstable complex. This contributed to increased calcium and phosphorus digestibility as feed acidity increased up to 0.3%. This is in concordance with the report of Sheikh *et al.* (2010) who reported better feed utilization and mineral digestibility at 0.3% level of organic acid supplementation. Maenz *et al.* (1999) reported that organic acids chelated multivalent cations such as calcium, magnesium, zinc and iron thereby reducing the amount of the cations that bind to phytate making it less stable.

The marked decline in calcium and phosphorus retention at the highest level of acidifier supplementation (0.4%) was because the level of acidity was high and it resulted in depressed feed digestion and nutrient retention. It was reported by Salgado-Transito *et al*. (2011) that supplementation of acidifiers at high levels may affect productive parameters in broiler chickens.

The control treatment though adequate in phosphorus, was poor in calcium and phosphorus digestibility because phytate was known to strongly bind feed minerals limiting their digestibility and bioavailability. Magnesium, manganese, zinc and copper digestibility also increased from the control group across the levels of acid supplementation, thus giving an indication that acidifiers improved feed utilization and reduced faecal losses of dietary minerals. Youn *et al.* (2005) reported that acidifiers lower gastric pH and accelerate the conversion of pepsinogen to pepsin which improved absorption of protein

and minerals. Rafacz-Livingston *et al.* (2005) also reported that acidifiers are effective in phytate degradation because they displace phytate in the lumen of the gut by preventing the formation of insoluble phytate complexes that are excreted as faecal losses.

Bone Density and Mineralization in Broiler Finisher Chickens Fed Diets Supplemented with Graded Levels of Acidifier (Fysal®)

Bone density (robusticity index) was better in the acid supplemented treatment groups compared to the control because acidifiers improve phytate hydrolysis by increasing the availability of minerals for skeletal development. The control treatment showed poor result in bone density because phytate increases its stability by binding phosphorus in a hexaphosphate ring thereby strongly attracting to its inositol structure some cations such as calcium and magnesium that would have aided skeletal mineralization Abdoulaye *et al.* (2011). This can result to mineral losses in faeces thereby leading to increased feed consumption to compensate for nutrient requirement.

Chickens fed 0.3% acidified diet showed the best results in tibia weight, length and density thereby implying that 0.3% was the ideal level at which supplemental acidifiers were able to hydrolyze phytate and liberate feed minerals. Increasing the level of feed acidification beyond 0.3 to 0.4% elicited a decline in tibia bone mineralization and density showing that the optimum level at which the acid could be tolerated may have been exceeded.

Tibia ash has been used by several authors as a viable indicator of bone mineralization as an increase in percentage ash was related to better mineral deposition in tibia bone (Ahmad *et al.* 2000; Leeson *et al.,* 2000). Tibia ash, calcium and phosphorus improved with increasing supplemental levels of the acidifiers up to 0.3%, thus showing that there was better digestion and skeletal development with feed acidification. It was observed that 0.3% was the most ideal level at which the acidifier effectively hydrolyzed phytate as shown by tibia ash, calcium and phosphorus percentages. Liem *at al*. (2007) found that increasing the levels of feed acidifiers beyond 0.1% resulted to increase in tibia ash percentage and reduced incidence of phosphorus deficiency rickets in broiler chickens fed low available phosphorus diets. Orban *et al*. (1993) reported that addition of organic acids to broiler diet facilitated skeletal bone development and increased femur breaking strength.

There was no incidence of leg deformity in all acid supplemented treatments even though they had sub-optimal phosphorus diets. This may have occurred because the acidifiers helped to improve nutrient uptake from the feed to support bone development. Similarly, chickens in the control treatment had no incidence of leg deformity because their diet was adequate in available phosphorus and minerals that may have been bound by phytate were compensated for by increased feed intake.

CONCLUSION

Graded levels of acidifier (Fysal®) in the diets, significantly increased mineral digestibility and bone density.

Both parameters were better among chickens fed diet supplemented with 0.3% acidifier. Acidifiers are able to liberate at least 0.1% phosphorus and other minerals bound by phytate since sub-optimal available phosphorus at 0.3% was used in the acid supplemented treatments and leg deformity did not occur.

The study concluded that the use of acidifiers at 3kg per ton/feed is beneficial in broiler production for bone mineralization and efficient utilization of feed nutrients.

REFERENCES

Abdoulaye, C., Brou, K. and Jie, C. (2011). Phytic acid in cereal grains: Structure, Healthy or Harmful ways to reduce phytic acid in cereal grains and their effect on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, 1:1-22.

Abdullahi, I. (2012). Nutritive value of differently processed mango seed kernel (Local Variety (Kanbiri) and mixed Samples) meal in broiler diets. M.Sc. thesis, Department of Animal Science, A.B.U. Zaria.

Abeke, F.O. (2005). Evaluation of the nutritive value of *lablab purpureus* beans in replacing groundnut cake in poultry diets. Ph.D. thesis, Department of Animal Science, ABU, Zaria.

Ahmad, T., Rasool, S., Sarwar, M., Haq, A. and Zia-ul,H. (2000). Effect of microbial phytase from a fungus*Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. *Animal Feed Science and Technology*, 83: 103-114.

Association of Official Analytical Chemists (1994). Official Methods of Analysis.16th ed. Association of Analytical Chem. Washington, DC.

Boling, S. D., Douglas, M. W., Snow, J. L., Parsons, C. M. and Baker, D. H. (2000a). Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. *Poultry Science*, 79:1335–1337.

Boling, S. D., Webel, D. M., Mavromichalis, I., Parsons, C. M. and Baker, D. H. (2000b). The effects of citric acid on phytate phosphorus utilization in young chicks and pigs. *Journal of Animal Science*, 78:682–689.

IAR (2012). Institute for Agricultural Research, A.B.U. Zaria, Meteorological Station Data.

Idachaba, C. U. (2012). Effect of graded levels of stone grit with or without enzyme supplementation on utilization of rice offal based diets by broiler chickens. M.Sc. thesis, Department of Animal Science, A.B.U. Zaria

Idachaba, C. U. (2017). Effect of Different Organic Acids with and without Phytase Supplementation on Performance and Phytate Utilization by Broiler Chickens. Ph.D Thesis, Department of Animal Science, Ahmadu Bello University, Zaria.

Julian, R. J. (2005). Production and growth related disorders and other metabolic diseases of poultry. A review. *Veterinary Journal*, 169: 350–369.

Leeson, S., Namkung, H., Cottrill, M. and Forsberg,C.W. (2000). Efficacy of new bacterial phytase in poultry diets. *Canadian Journal of Animal Science*, 80: 527-528.

Liem, G. M., Pesti and Edwards, H. M. Jr. (2007). The Effect of Several Organic Acids on Phytate Phosphorus Hydrolysis in Broiler Chicks. *Poultry science*, 87(4): 689-693.

Maenz, D. D., Engele-Schaan, C. M., Newkirk, R. W. and Classen, H. L. (1999). The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. *Animal Feed Scienceand Technology*, 81:177–192.

NRC (1994). Nutrient Requirements of Poultry (9th Ed.).National Academy Press, Washington, DC, USA.

Orban, J. I., Roland, D. A., Bryant, M. M. and Williams, J. C. (1993). Factors influencing bone mineral content, density, breaking strength, and ash as response criteria for assessing bone qualityin chickens. *Poultry Science*, 72:437–446.

Park, K. W., Rhee, A. R., Um, J. S. and Paik, I. K. (2009). Effect of dietary available phosphorus and organic acids on the

performance and egg quality of laying hens. *Journal of Applied Poultry Research*, 18:598–604.

Rafacz-Livingston, K.A., Martinez-Amezcua, C., Parsons, C.M., Baker, D.H. and Snow, J. (2005). Citric Acid Improves Phytate Phosphorus Utilization in Crossbred and Commercial Broiler Chicks. *Poultry Science,* 84:1370–1375.

Rath, N., Huff, G., Huff, W. and Balog, J. (2000). Factors regulating bone maturity and strength in poultry. *Poultry Science,*79: 1024-1032.

Reisenfeld, A. (1972). Metatarsal robusticity in bi-pedal rats. *American Journal of Physiology and Anthropol*ogy, 40: 229–234.

Salgado-Tránsito, L., Del Río-García, J. C., Arjona-Román, J. L., Moreno-Martínez, E. and Méndez-Albores, A. (2011). Effect of citric acid supplemented diets on aflatoxin degradation, growth performance and serum parameters in broiler chickens. *Archivos De Medicina Veterinaria*, 43:215-222.

SAS (1993). Statistical Analysis System, Computer Software, Version 9: Statistics, SAS Institute Inc. Cary, NC27513, NC27513, USA.

Sheikh, A., Tufail, B., Gulam, A. B., Masood, S. M. and Manzoor, R. (2010). Effect of Dietary Supplementation of Organic Acids on Performance, Intestinal Histomorphology, and Serum Biochemistry of Broiler Chicken. *Veterinary Medicine International*, 10:1-7.

Snow, J.L., Baker, D.H. and Parsons, C.M. (2004). Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poultry Science*, 83:1187–1192.

Thomas, D.V. and Ravindran, V. (2010). Mineral Retention in Young Broiler Chicks Fed Diets Based on Wheat, Sorghum or Maize. *Asian-Australian Journal Animal Science*, 23(1): 68 – 73.

Woyengo, T.A. and Nyachoti, C.M.(2013). Review: Supplementation of phytase and carbohydrases to diets for poultry. *Canadian Journal of Animal Science,*91:177–192.

Youn, B.S., Nam, K.T., Chang, K.M., Hwang, S.G. and Choe, I. S. (2005). Effects of wood vinegar addition for meat quality improvement of old layer. *Korean Journal of Poultry Science*, 32:101–106.