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Phytate Utilization Using Feed Grade Acidifiers to Improve Tibia Composition, Gut Microbiology and Intestinal pH of Broiler Chickens

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Abstract

Phytate is an anti-nutrient in feedstuffs that inhibits nutrient utilization by chelating multivalent cations and other nutrients. Hence, the need to buffer feeds to improve digestibility. Two hundred and eighty eight (288) broiler finisher chickens were used for the study. They were allotted to four treatments each replicated thrice with twenty four (24) broiler chickens per replicate in a completely randomized design. The control diet had adequate available phosphorus at 0.5% while others were sub-optimal at 0.4%. Three acidifiers; Fysal®, Acidomix® and Orgacid® were supplemented in the diets of broiler chickens in treatments two to four at 0.1%. Investigations carried out were determination of intestinal pH, tibia bone compositions and gut microbiology. Broiler chickens fed with acidified diets showed significantly (P<0.05) lower pH levels compared to those on the control diet. Tibia weight, calcium and robusticity index were significantly (P<0.05) better in broiler chickens fed with diets containing acidifier supplementation. Those broiler chickens fed diets supplemented with Fysal® showed the best results in all tibia compositions. Organic acids significantly (P<0.05) reduced Escherichia coli, Salmonella sp. and Staphylococcus sp. in the intestinal segments of broiler chickens fed with acidified diets. It was concluded that supplementation of organic acid in broiler chickens' diets at 0.1% can reduce phytate's chelation on fed minerals, improving bone mineralization and enhancing gut health for better performance.

Keywords: Phytate, Acidifier, Broiler Chickens, Supplementation, Utilization

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INTRODUCTION

Phytate also known as inositol hexaphosphate (IP4) or phytic acid is synthesized from myo-inositol via a series of phosphorylation steps; thus it consists of an inositol ring having six phosphate ester bonds (Woyengo and Nyachoti, 2013). Most oil seeds and cereal grains used in feed formulation contain phytate which is largely the storage form of phosphorus in vegetative sources (Park *et al*., 2009). Conventional vegetable feed sources have low (30-40%) available phosphorus because most of it is bound by phytate (National Research Council, 1994). Hence, the ability of monogastric animals such as poultry and pigs to utilize phytate phosphorus is poor due to insufficient intestinal phytase secretion (NRC, 1994). Consequently, large amount of phosphorus is excreted in faeces causing environmental hazard as reported by Paik (2003). Phytate by its structure attracts feed nutrients to its inositol hexaphosphate ring making them less available to poultry (Woyengo and Nyachoti, 2013).

Phytate has 12 replaceable reactive sites, carrying strong negative charges in the pH range of the digestive tract of farm animals (Kumar *et al*., 2012). Hence, phytate is able to bind multivalent cations (minerals) present in feeds, forming very stable complexes thereby decreasing their bioavailability (Idachaba *et al*., 2017). Phytate is also able to strongly attract other feed nutrients such as protein, carbohydrate and vitamins to the phytate-mineral complex (Idachaba *et al*., 2017). 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 basic in pH. The net result of this interaction is formation of stable salts which precipitate out of solution and become unavailable to simple stomach animals (Abdoulaye *et al.,* 2011). Since the small intestine is the principal site for nutrient absorption, it implies that bioavailability of feed minerals can be affected by the presence and level of phytate in feedstuff.

Leg weakness, lameness and other bone abnormalities are encountered by rapidly growing meat-type chickens which result to poor mineral utilization from feedstuff according to Kommera *et al.* (2006). Mineral chelators such as organic acids have been reported to compete favourably with phytate thereby forming organic acid-mineral complex (Boling *et al*., 2000). Organic acids are negatively charged, thus, they have the tendency to chelate multivalent cations such as calcium, thereby reducing the amount of the cations that bind to phytate making phytate less stable and more susceptible to hydrolysis as reported by Maenz *et al.* (1999). Furthermore, Sonet *et al.* (2002) reported that organic acid supplementation in broiler diets tends to slow down passage rate of feed through the digestive tract leading to increased retention time and better utilization of feed nutrients. Kommera *et al.* (2006) reported that reducing the emptying rate of the gastrointestinal tract could be a mechanism towards improving utilization of feed nutrients. Sheikh *et al.* (2010) reported that organic acid supplementation in broiler diets may increase villus height and other histological changes in the small intestines of broiler chickens, thus improving better nutrient absorption.

It is well documented that organic acid supplementation in broiler diets have pH lowering property of intestinal segments (Canibe *et al.,* 2001; Abdel Fattah *et al.,* 2008). The pH lowering property of organic acids imposes a weakly acidic pH in the intestines of chickens, making it conducive for growth of beneficial bacteria which may have more acid tolerance unlike pathogenic bacteria which grow at relatively higher pH levels (Langhout, 2000; Canibe *et al.,* 2001). Organic acids are known to penetrate bacteria cell wall, disrupting normal cellular functions including synthesis and replication of protein by bacteria (Denyer and Stewart, 1998; Davidson, 2001). Idachaba (2017) found that *Escherichia coli sp., Salmonella sp., Staphylococcus sp.* and coliform bacteria were significantly reduced in duodenum, jejenum and ileum of chickens on diets with acidifier supplementation. The author further reported that acidifier supplementation in broiler diets improved intestinal absorptive capacity of the chickens. It was reported by Hedayati *et al.* (2013) that the proliferation of unfavourable micro-organisms in the intestinal lumen of broiler chickens resulted in competition for digested nutrients as well as reduction in intestinal absorptive capacity. Conventionally, the liberation of nutrients bound by phytate requires supplementation of exogenous phytase. However, studies have shown the potency of organic acids in phytate utilization, gut modification and inhibition of proliferating pathogenic bacteria in the intestinal segments of chickens. This study focused on supplementation of feed grade acidifiers in broiler chickens diets to determine the effect on intestinal pH, tibia composition and gut microbiology.

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 610 m 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 1500 mm (Institute for Agricultural Research, 2012).

Source of Experimental Chicks

Arbor Acre breed of broiler chicks were purchased from Vertex farms located in South Western Nigeria and used for the trials.

Source of organic acids

Three commercial acidifiers; Fysal®, Acidomix® AFG and Orgacid® containing different blends of organic acids were purchased from commercial feed milling outlets in Kaduna state.

Experimental Design and Management of Birds

Two hundred and eighty eight (288) broiler finisher chickens weighing 510.23 g were used for the study. They were weighed and allotted to four experimental treatments each replicated thrice with twenty four (24) chickens per replicate in a completely randomized design. The chickens were raised for four weeks in the finisher phase. Deep litter system was used to house the birds with feed and water provided *ad libitum*. All management practices ideal for broiler chickens were strictly practiced.

Experimental Diets

Four maize/soybean meal based broiler finisher diets were formulated. The control diet (T_1) was formulated to meet NRC (1994) requirement for available phosphorus at 0.5% while treatments two to four (T_2-T_4) had sub-optimal available phosphorus at 0.40%. Treatment one was the control diet without supplemental organic acids while it was supplemented in treatments two to four at the manufacturers recommended level of 0.1%. Fysal®, Acidomix® AFG and Orgacid® were used as the blends of organic acids. Orgacid® contains formic, malic, tartaric, lactic and ortho-phosphoric acid. Acidomix® AFG contains formic, lactic, fumaric and ammonium formate. Fysal® contains sorbic, lactic, propionic, ascorbic and citric acid. The experimental diets were formulated as follows; T_1 (control diet adequate in available phosphorus), T_2 (control diet, 0.40% available phosphorus, 0.1% Fysal®), T_3 (control diet, 0.40%

available phosphorus, 0.1% Acidomix®) and T_4 (control diet, 0.40% available phosphorus, 0.1% Orgacid®). The experimental diet is presented in Table 1.

Table 1: Composition of Broiler Finisher Diets Supplemented with Different Organic Acid

Ingredients (%)	Control 0.00%	Fysal® 0.10 %	Orgacid® 0.10%	Acidomix® 0.10%
Maize	59.50	57.70	57.70	57.70
Soya cake	20.00	24.00	24.00	24.00
Groundnut cake	16.00	14.00	14.00	14.00
Limestone	0.60	1.20	1.20	1.20
Bone meal	2.90	2.10	2.10	2.10
Common salt	0.30	0.30	0.30	0.30
Vitamin premix*	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME (Kcal/kg)	2915.00	2901.00	2901.00	2901.00
Crude protein (%)	21.38	21.39	21.39	21.39
Ether extract (%)	4.56	4.57	4.57	4.57
Crude fibre (%)	3.93	3.93	3.93	3.93
Calcium (%)	1.05	1.06	1.06	1.06
Available phosphorus (%)	0.50	0.40	0.40	0.40
Lysine (%)	1.16	1.24	1.24	1.24
Methionine (%)	0.60	0.60	0.60	0.60
Cost/kg diet (₦)	79.42	82.15	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 23 mg; vit K, 2 mg; calcium pantothenate, 7.5mg; B12, 0.015 mg; folic acid, 0.75 mg; choline chloride, 300 mg; vit B1, 1.8 mg; vit B2, 5 mg; vit B6, 3 mg; manganese, 40 mg; iron, 20 mg; zinc, 53.34 mg; copper, 3 mg; iodine, 1 mg; cobalt, 0.2 mg; selenium, 0.2 mg; zinc, 30mg.

Determination of Tibia Composition of Boiler Chickens fed Diets Supplemented with Different Acidifiers

Four chickens were randomly selected from each replicate and bled after which the left tibia bone of each chickens was removed. Tibia bones were labeled and immersed in boiling water (100 °C) for 10 minutes to enable fat removal. Bones were weighed using a sensitive digital scale while the length was measured using a meter rule. The distance from cranial to distal extremities of each bone was taken as the tibia length. Robusticity index was determined using the formula described by Reisenfeld (1972).

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

Bone ash was also determined by oven-drying the bones at 100 °C for 24 hours. They were then ashed in a muffle furnace at 600 °C for 6 hours according to procedures described by the Association of Analytical Chemists (1994). The percentage ash was determined relative to dry weight of the tibia bone. Chickens that showed leg deformities in each treatment such as twisted hock, curled shank and splayed leg were recorded and expressed as percentage of the number of chickens per treatment.

Determination of Intestinal Microbial Count and pH of Broiler Chickens Fed Diets Supplemented with Different Acidifiers

Four chickens were selected from each replicate for this determination. They were bled and eviscerated to collect contents of the duodenum, jejenum, ileum and caecum in clean sample bottles after which a pH probe was inserted to determine the pH of each segment. The samples were taken to the Microbiology laboratory of the Faculty of Veterinary Medicine, ABU Zaria to culture for viable bacterial counts according to procedures described by Al-Natour and Alshwabkeh (2005) and Quinn *et al*. (1992). The number of bacteria in colony forming unit (CFU) per gram of sample was obtained by dividing the number of colonies by the dilution factor. The colony count obtained in CFU was expressed in logarithmic transformations and analyzed statistically.

Data Analysis

Data obtained from the experiment was subjected to analysis of variance using the General Linear Model Procedure of SAS (2001). Significant differences among treatment means was separated using Duncan's Multiple Range Test, Duncan (1955).

RESULTS

Tibia Compositions of Broiler Chickens Fed Diets Supplemented with Different Organic Acids

Table 2 shows the tibia compositions of broiler chickens fed diets supplemented with different organic acids. Chickens fed with diet containing Fysal® showed the best ($P<0.05$) results. The poorest ($P<0.05$) robusticity index was observed in chickens fed with the control diet. Tibia ash was significantly ($P<0.05$) higher in chickens fed diets supplemented with Fysal® while others did not differ significantly ($P>0.05$). Chickens fed with diet supplemented with Fysal® showed significantly ($P<0.05$) better result in tibia calcium which was followed by those on diets supplemented with Orgacid® and Acidomix®. Chickens fed with the control diet showed the poorest ($P<0.05$) result in bone calcium.

It was observed that tibia phosphorus was significantly ($P<0.05$) higher in chickens fed with diets supplemented with Fysal®. This was followed by treatment supplemented with Orgacid® while the control and treatment supplemented with Acidomix® gave the poorest ($P<0.05$) results. Chickens on control diet and diet supplemented with Fysal® did not have incidences of leg deformity. Treatments supplemented with Orgacid® and Acidomix® had few chickens that presented leg deformity.

Table 2: Tibia Compositions and Leg Deformity of Broiler Chickens Fed with Diets Supplemented with Different Organic Acids

Tibia Compositions	Control	Acidifiers			SEM
		Fysal®	Orgacid®	Acidomix®	
Tibia length (cm)	10.17^{b}	11.97^{a}	10.27^{b}	10.17^{b}	0.13
Tibia weight (g)	6.00^{c}	8.00^{a}	7.00^{b}	7.00^{b}	0.15
Robusticity index	5.11^{c}	5.99^{a}	5.37^{b}	5.32^{b}	0.10
Tibia Ash (%)	41.21^{b}	48.95^{a}	42.65^{b}	43.42^{b}	0.84
Tibia Calcium (%)	24.54^{c}	32.75^{a}	28.48^{b}	28.42^{b}	1.23
Tibia Phosphorus (%)	11.08^{c}	18.46^{a}	14.48^{b}	12.96^{c}	0.53
Leg Deformity (%)	4.54	0.00	4.54	4.54	0.00

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

Intestinal pH of Broiler Chickens Fed with **Diets Supplemented with Different Organic Acids**

Table 3 shows the intestinal pH of broiler chickens fed with diets supplemented with different organic acids. Duodenum, jejenum, ileum and caecum were significantly (P<0.05) affected by feed acidification. Chickens fed with the control diet showed significantly (P<0.05) higher duodenal pH compared to those fed with the acid supplemented diets. Similarly pH in the jejenum, ileum and caecum followed a similar trend as the duodenum and showed significant (P<0.05) reduction with feed acidification.

Table 3: Intestinal pH of Broiler Chickens Fed with Diets Supplemented with Different Organic Acids

Gut Segments	Control	Acidifiers			SEM
		Fysal®	Orgacid®	Acidomix®	
Duodenum	6.83^{d}	5.26^{a}	5.47^{b}	5.55^{c}	0.02
Jejenum	7.82^{b}	6.38^{a}	6.22^{a}	6.19^{a}	0.18
Ileum	8.76^{c}	7.06^{a}	7.58^{b}	7.16^{a}	0.11
Caecum	9.47^{b}	8.78^{a}	8.86^{a}	8.68^{a}	0.20

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

Bacterial Colony Count in Duodenum of Broiler Chickens Fed Diets Supplemented with Different Organic Acids

Table 4 shows the bacterial colony count in intestinal segments of broiler chickens fed diets supplemented with different organic acids. Chickens in all groups fed acidified diets showed significantly (P<0.05) lower counts of *Escherichia coli*, *Salmonella sp.*, *Staphylococcus sp.* and coliform bacteria compared to those in the control treatment. No significant (P>0.05) difference was observed in *staphylococcal* count in duodenum and jejenum of chickens in all treatment groups. However, *Staphylococcal* count in the ileum was significantly (P<0.05) higher in chickens fed the control diet compared to others.

Table 4: Bacterial Colony Count in Intestinal Segments of Broiler Chickens Fed Diets Supplemented with Different Organic Acids

Bacteria species (Log_{10} CFU/g)	Control	Acidifiers			SEM
		Fysal®	Orgacid®	Acidomix®	
Duodenum					
Escherichia coli	5.20^{b}	4.65^{a}	4.80^{a}	4.88^{a}	0.12
Salmonella sp.	4.99^{c}	4.76^{b}	4.55^{a}	4.61^{a}	0.10
Staph. sp.	4.10	4.08	4.00	4.03	0.06
Coliforms	5.26^{c}	5.06^{b}	4.67^{a}	4.80^{a}	0.07
Jejenum					
Escherichia coli	5.19^{c}	5.09^{b}	4.97^{a}	5.00^{a}	0.04
Salmonella sp.	4.81^{b}	4.58^{a}	4.63^{a}	4.57^{a}	0.03
Staph. sp.	4.60	4.56	4.51	4.57	0.05
Coliforms	5.31^{b}	5.17^{a}	5.10^{a}	5.13^{a}	0.04
Ileum					
Escherichia coli	5.01^{b}	4.56^{a}	4.90^{b}	4.99^{b}	0.10
Salmonella sp.	4.98^{b}	4.20^{a}	4.27^{a}	4.19^{a}	0.08
Staph. sp.	4.96^{b}	4.68^{a}	4.58^{a}	4.59^{a}	0.05
Coliforms	5.22^{b}	5.13^{a}	5.08^{a}	5.06^{a}	0.04

a, b, c=Means with different superscript on the same row differ significantly (P<0.05) SEM= Standard error of mean
Staph. = Staphylococcus, sp. = species

DISCUSSION

Tibia Compositions and Leg Deformity of Broiler Chickens Fed with Diets Supplemented with Different Organic Acids

Tibia bone compositions is often used as an index to determine skeletal mineralization because it is referred to as the fastest growing bone parameter in livestock animals (Osiak-Wicha *et al.*, 2023). Despite the sub-optimal dietary available phosphorus (0.4%) in diets for chickens with acidifier supplementation, they showed better tibia weights and robusticity index. This was because the acidifiers might have helped to improve the breakdown of phytate and was able to liberate calcium, phosphorus and other feed minerals held bound. According to Maenz *et al.* (1999) Acidifiers were powerful mineral chelators that distort phytate's affinity to bind feed minerals in an unstable complex. Maenz *et al.* (1999) also reported that organic acids chelate multivalent cations such as calcium, magnesium, zinc and iron thereby reducing the amount of the cations that bind to phytate, making it less stable. Rafacz-Livingston *et al.* (2005) also reported that organic acids 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. Chickens on the control diet showed poor robusticity index because phytate might have increased its stability by binding phosphorus in its hexaphosphate ring and strongly attracting to its inositol structure other cations such as calcium and magnesium that would have aided bone mineralization.

Robusticity index, tibia ash, calcium and phosphorus were better in chickens fed with diets supplemented with Fysal®. It was observed that Fysal® is the only organic acid used in this study that contained citric acid. Liem *et al.* (2007) showed that tibia ash, phosphorus and calcium were best in treatments where citric acid was among the various acidifiers supplemented. They further stated that organic acids containing citric acid reduced incidence of phosphorus deficiency rickets in broiler chicks and increased femur breaking strength. Centeno *et al*. (2007) evaluated the effect of organic acids containing citric acid on phosphorus availability and reported that citric acid being a more potent mineral chelator could compete favourably with phytate with concomitant increase in phytic acid hydrolysis.

Tibia ash has been stated to be a viable indicator of bone mineral deposition and a determinant of the utilization of feed minerals (Ahmad *et al.*, 2000; Leeson *et al.*, 2000). Calcium and phosphorus deposition in tibia bone of chickens on diets supplemented with Fysal® was found to improve tibia ash compared to chickens in other groups. Results obtained in tibia ash, calcium and phosphorus also tally with the absence of leg deformity in chickens fed diet supplemented with Fysal®. Similar to chickens in the control group, those fed with diets supplemented with acidifiers apart from Fysal® had few chickens with leg deformity. It is probable to have occurred because they were fed with diets having sub-optimal available phosphorus, hence the expected phytate hydrolysis for efficient mineral uptake may have been insufficient compared to those supplemented with Fysal®.

Intestinal pH of Broiler Chickens Fed with Diets Supplemented with Different Organic Acids

All intestinal segments of chickens fed with diets supplemented with organic acids showed that feed acidification resulted in reduction in pH levels compared to the control group. It was well documented that organic acid supplementation in broiler diets have pH lowering property of intestinal segments (Canibe *et al.* 2001; Abdel Fattah *et al.* 2008). This lowered pH property of acidifiers is apparently conducive for growth of favourable bacteria, as it tends to inhibit the growth of pathogenic bacterial species which grow at relatively higher pH levels (Langhout, 2000; Canibe *et al.* 2001). It was also reported by Guinotte *et al*. (1995) that the pH reducing property of organic acids increases gut acidity which helps to stimulate pepsin activity. This according to the authors results in increased intestinal absorption of dietary nutrients. This assertion is consistent with our earlier reports in the growth phase of this study as productive parameters of weight gain, feed intake and feed to gain ratio were better in chickens fed diets supplemented with organic acids (Idachaba *et al*., 2016).

Feed acidification has been reported to induce an inhibitory effect on growth and proliferation of harmful bacteria in the gastrointestinal tract of chickens, thereby preventing pathological diseases and reducing competition for digested nutrients (Idachaba *et al*., 2016). Hedayati *et al.* (2013) reported that the proliferation of unfavourable micro-organisms in the intestinal lumen of broiler chickens resulted in competition for digested nutrients as well as reduction in intestinal absorptive capacity.

Bacteria Colony Count in Intestinal Segments of Broiler Chickens Fed with Diets Supplemented with Different Organic Acids

The reduction in *Escherichia coli, Salmonella sp.* and coliform bacterial counts in duodenum, jejenum and ileum of chickens fed with diets supplemented with organic acids showed that the pH lowering property of feed acidifiers limited the proliferation of acid intolerant pathogenic bacteria. Organic acid was not supplemented in diet for chickens in the control group and this might have attributed to higher bacterial counts compared to others. Gunal *et al.* (2006) reported that organic acid supplementation in broiler diet reduced pathogenic bacterial counts and decreased competition with the host animal for digested nutrients. It was also reported by Sheikh *et al.* (2010) that organic acid supplementation increased villus height and other histological changes in the small intestines of broiler chickens. Similarly, Tatiane *et al.* (2011) observed increased villus height in the small intestine of chickens that had access to acidified diets suggesting that reduction in intestinal contamination by non-desirable microorganisms supported development of villus morphometry. This according to the authors increased intestinal surface area and facilitated better nutrient absorption. The small intestine is the nutrient absorptive region in farm animals and a reduction in colonization of pathogenic organisms is expected to promote gut health and enhance nutrient absorption (Wickramasuriya *et al.,* 2022). This was evident as weight gain and other productive parameters improved with feed acidification compared to the control group as shown in our previous report Idachaba *et al*., (2017).

CONCLUSION

It was concluded that supplementation of organic acid in broiler chickens' diets at 0.1% can reduce phytate's chelation on fed minerals, improving bone mineralization and enhancing gut health for better performance.

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