Effect of feed grade acidifiers on total tract mineral retention, intestinal pH and phytate utilization by broiler finisher chickens

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Mineral chelators such as organic acids have been reported to compete favourably with phytate by forming soluble complexes with feed minerals. Effect of feed grade acidifiers on total tract mineral retention and intestinal pH of broiler finisher chickens was investigated. A total of 300-broiler finisher chickens were assigned to four treatment groups. Each treatment was replicated thrice with twenty chickens per replicate in a completely randomized design. The control treatment (T₁) was fed the control diet adequate in available phosphorus at 0.5% while other treatments (T₂-T₄) were fed sub-optimal available phosphorus diets at 0.4%. Three blends of acidifiers; Fysal[®], Orgacid[®] and Acidomix[®] were supplemented in the diets of chickens in treatments 2, 3 and 4 at 0.1% (1kg per ton/feed). Chickens fed diet supplemented with Fysal[®] showed the best (P<0.05) results in total tract mineral retention 56.5+/-5.75 compared to the control and other treatments. The poor mineral retention i.e. 35 and 46% for calcium and phosphorus respectively by chickens fed the control diet was attributed to the insoluble phytate-mineral complex that limits mineral bioavailability. Intestinal segments of chickens fed acidified diets showed significant reduction (P<0.05) in pH in the range of 5.26-7.16 across duodenum to ileum compared to the control group 6.83-8.76. It is concluded that feed acidification at 0.1% improves mineral utilization in broiler chickens as it competes favourably with phytate in the lumen of the gut leading to better nutrient utilization.

Keywords: Acidifier, supplementation, phytate, minerals, intestinal pH

Introduction

Phytate is the primary storage form of phosphate in seeds and by its structure attracts feed nutrients in a hexaphosphate ring on its inositol molecule, making nutrients less available to poultry chickens (Woyengo and Nyachoti, 2013). Phytate has been reported by Catalá-Gregori *et al.* (2006) to form insoluble complex with protein, starch, minerals and vitamins thereby affecting weight gain and other productive parameters. Some 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 basic in pH. Phytate in a basic pH strongly attracts divalent and trivalent cations in feed stuffs thereby becoming stable and unavailable to monogastric animals. Unfortunately, feed minerals and particularly phosphorus are held bound by phytate leading to increased faecal losses due to inability of simple stomach animals to hydrolyze and utilize the nutrients them (Rath *et al.*, 2000). As a result, leg problems resulting from poor mineralization may become a concern in production of meat-type chickens.

Mineral chelators such as organic acids have been reported to compete favourably with phytate by forming soluble complexes with feed minerals and other nutrients in the lumen of the gut. This soluble complex is

readily absorbed by the body of chickens thereby enhancing nutrient uptake. Boling *et al.* (2000a) showed that the supplementation of citric acid in broiler diet increased phosphorus utilization and reduced dietary available phosphorus requirement by 0.10%. Similarly, Park *et al.* (2009) asserted that organic acid supplementation in corn/soybean based diets fed to broiler 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) reported 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. Organic acids are powerful mineral chelators and as such they attract divalent and trivalent cations in feedstuffs by decreasing phytate's ability to chelate feed minerals. This makes phytate unstable and more susceptible to hydrolysis by endogenous or exogenous enzymes as reported by Boling *et al.* (2000b). It was reported by Julian (2005) that the pH reducing property of organic acids reduces alkalinity in the small intestine to a weakly acid state, thereby reducing phytate's ability to strongly attract feed cations in an unstable complex. Several studies on the use of organic acids to improve phytate hydrolysis in broiler chickens were carried out using feed grade acidifiers that exist in single forms. However, not much has been done on the supplementation of blends of acidifiers in broiler diet. The focus of this study is 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.

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° 9' 45" N and longitude 7° 38' 8" E, at an altitude of 610m above sea level. The temperature ranges between 26-40°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 is between May to October with annual rainfall of about 1500mm (Institute for Agricultural Research Meteorological Unit, 2012).

Source of experimental chickens

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

Source of organic acids

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

Experimental diets

Four maize/soybean meal based broiler finisher diets were formulated as presented in Table 1. The control diet (T₁) was formulated to meet NRC (1994) requirement for available phosphorus while treatments 2-4 (T₂-T₄) had sub-optimal level at 0.40%. Treatment one was the basal diet without supplemental organic acids while this 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 diets were formulated as follows;

T₁ (basal diet, 0.5%AP), T₂ (basal diet, 0.40%AP, 0.1% Fysal[®]), T₃ (basal diet, 0.40%AP, 0.1% Orgacid[®]) and T₄ (basal diet, 0.40%AP, 0.1% Acidomix[®]).

Table 1: Composition of experimental broiler finisher diets supplemented with blends of feed grade acidifiers

Ingredients (%)	Control	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
Calculated analysis	100.00	100.00	100.00	100.00
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 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

Experimental design and management of birds

Three hundred (300) finishing broiler chickens weighing averagely 510.23g were allotted to four experimental treatments each replicated three times with twenty (20) chickens per replicate in a completely randomized design. They were raised on deep litter with feed and water provided *ad libitum*. All routine and management practices were strictly adhered to. The chickens were raised for a period of 28days after which the finisher phase was terminated.

Evaluation of some selected parameters post the finisher phase

Determination of minerals and total tract retention

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 replicate 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, Mg, K and Na) and micro (Mn, Zn and Cu) minerals using atomic absorption

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spectrophotometer (AAS). Results obtained from mineral determination were used to calculate total tract retention of macro and trace minerals using the formula below.

$$\text{Mineral retention} = \frac{(\text{Feed intake} \times \text{Mineral}_{\text{Diet}}) - (\text{Excreta output} \times \text{Mineral}_{\text{Excreta}})}{100 \text{Feed intake} \times \text{Mineral}_{\text{Diet}}}$$

Determination of pH of intestinal segments

The chickens were then bled, eviscerated and intestinal contents in the duodenum, jejunum, ileum and caeca were collected in clean sample bottles after which a pH probe was inserted to determine the pH of each segment.

Data analyses

Data obtained from determination of mineral retention and intestinal pH were subjected to statistical analysis using the General Linear Model (GLM) procedure of SAS (2001) software for analysis of variance. Duncan's (1955) multiple range test was used to determine differences between treatment means.

Results

Total tract retention of selected minerals by broiler chickens fed diets supplemented with blends of organic acids

Table 2 shows the total tract retention of selected minerals by broiler chickens fed diets supplemented with blends of organic

acids. Chickens fed diets supplemented with Fysal[®] showed the best (P<0.05) result in total tract retention of calcium and phosphorus at 69 and 66% respectively. Chickens fed diets supplemented with Orgacid[®] and Acidomix[®] showed significantly (P<0.05) better retention of calcium at 59 and 52% respectively compared to the control. Phosphorus retention did not differ between the control and treatments supplemented with Orgacid[®] and Acidomix[®].

Total tract retention of magnesium was significantly (P<0.05) better for chickens fed diet supplemented with Fysal[®] while other treatments showed comparable (P>0.05) results with each other. Chickens fed diets supplemented with Fysal[®], Orgacid[®] and Acidomix[®] were significantly (P<0.05) better than the control which showed the poorest result in retention of potassium, manganese and zinc retention. Chickens fed diets supplemented with Fysal[®] showed the best (P<0.05) retention of copper while this was followed by treatments supplemented with Orgacid[®] and Acidomix[®]. Chickens fed the control diet showed the poorest (P<0.05) result in retention of copper.

Intestinal pH of broiler chickens fed diets supplemented with blends of organic acids

Table 3 shows the intestinal pH of chickens fed diets supplemented with blends of organic acids. Duodenum, jejunum, ileum

Table 2: Total tract retention of selected minerals by broiler chickens fed diets supplemented with blends of acidifiers

Minerals	(%) Control	Fysal [®]	Orgacid [®]	Acidomix [®]	SEM
Calcium	46.0 ^d	69.0 ^a	59.0 ^b	52.0 ^c	0.18
Phosphorus	35.0 ^b	66.0 ^a	43.0 ^b	39.0 ^b	2.25
Magnesium	54.0 ^b	64.0 ^a	46.0 ^c	57.0 ^b	3.21
Potassium	42.0 ^c	60.0 ^a	66.0 ^a	59.0 ^b	3.00
Manganese	36.0 ^b	61.0 ^a	65.0 ^a	58.0 ^a	3.50
Zinc	49.0 ^b	62.0 ^a	61.0 ^a	62.0 ^a	2.03
Copper	50.0 ^c	65.0 ^a	55.0 ^b	55.0 ^b	2.10

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

and caeca were significantly ($P<0.05$) affected by feed acidification. Chickens fed the control diet had significantly ($P<0.05$) higher duodenal pH compared to chickens fed the acid supplemented diets. Compared to the acid not supplemented

group, duodenal acidity increased across other treatment groups. Similarly, pH in the jejunum, ileum and caeca followed similar trend as the duodenum and showed significant ($P<0.05$) reduction with feed acidification.

Table 3: Intestinal pH of broiler chickens fed diets supplemented with blends of acidifiers

Gut Segments	Control	Fysal®	Orgacid®	Acidomix®	SEM
Duodenum	6.83 ^d	5.26 ^a	5.47 ^b	5.55 ^c	0.02
Jejunum	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
Caeca	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

Discussion

Total tract retention of selected minerals by broiler chickens fed diets supplemented with blends of organic acids

Total tract mineral retention is an index that indicates feed digestibility vis a vis the nutrient retention by an animal. The control treatment showed the poorest results in retention of calcium, potassium, manganese, zinc and copper because phytate is known to limit the bioavailability of dietary mineral components. Julian (2005) reported that the negative charges on phytate molecule increases as intestinal pH rises or becomes alkaline. This in turn increases phytate's ability to react and strongly attract positively charged multivalent cations such as magnesium, calcium, zinc and iron thereby becoming more stable and rendering minerals and other feed nutrients biologically unavailable to chickens. Modification of intestine pH from alkalinity to a weak acidic state by use of acidifiers reduces the negative charges on phytate molecule thereby limiting its ability to attract positively charged feed minerals.

The increased mineral retention across treatment groups fed acidified diets may be attributed to improved phytate degradation and better utilization of nutrient

components in the feed. Organic acids have been reported to chelate multivalent cations such as calcium, magnesium, iron and zinc by reducing the amount of cations that bind to phytate making phytate less stable Maenz *et al.* (1999). Similarly, Omogbenigun *et al.* (2003) observed results that are consistent with this study as they reported better mineral retention when pigs were fed corn-soybean based diets supplemented with feed grade acidifiers.

Youn *et al.* (2005) reported that organic acids tend to lower gastric pH and accelerate the conversion of pepsinogen to pepsin which improves absorption of protein and minerals. The best results in retention of calcium, phosphorus and magnesium which are the main agents for bone mineralization was observed for chickens fed diets supplemented with Fysal®. Fysal® is the only acidifier used in this study that contains citric acid as a constituent blend. Citric acid has been reported to have greater affinity compared to other organic acids to displace phytate in the lumen of the gut by forming soluble complex with divalent and trivalent cations, thus limiting phytate's ability to form insoluble complex with feed minerals Boling *et al.* (2001a). Atapattu and Nelligaswatta (2005) also stressed that

organic acids containing citric acid better increase mineral retention in poultry chickens.

Afsharmanesh and Pourreza (2005) reported that feed acidification with citric and ascorbic acid increased the solubility of wheat phytate and reduced faecal excretion of minerals. Another reason for the improved mineral retention in the acid supplemented treatments stemmed from the fact that organic acids inhibit the proliferation of harmful bacteria species that colonizes intestinal segments of farm animals. This may support the development of intestinal morphometry such as increased villus height for better absorption of digested nutrients.

Hedayati *et al.* (2013) reported that the proliferation of unfavourable micro-organisms in the intestinal lumen of chickens results in competition for digested nutrients as well as reduction in intestinal absorptive capacity. This assertion supports the values obtained in the present study as total tract mineral retention was better for chickens fed acidified diets compared to the acid non supplemented group.

Intestinal pH of broiler chickens fed diets supplemented with blends of organic acids

All intestinal segments of chickens fed diets supplemented with organic acids showed that feed acidification resulted in significant reduction in gut pH compared to the control group. It is well documented that organic acid supplementation in broiler diets has pH lowering property (Canibe *et al.* 2001; Abdel Fattah *et al.* 2008). The lowered pH is conducive for growth of favourable bacteria as it hampers the growth of pathogenic bacteria which are known to grow at relatively higher pH levels (Langhout, 2000; Canibe *et al.* 2001). It was reported by Guinotte *et al.* (1995) that the pH reducing property of organic acids increases gut acidity and this stimulates

pepsin activity, improves nitrogen retention and increases the solubility of various mineral fractions in feedstuffs. This is in consonance with the result obtained in this study as mineral utilization improved with dietary feed acidification as compared to the control group.

Afsharmanesh and Pourreza (2005) reported that feed acidification with citric and ascorbic acid reduced pH of intestinal segments and increased the solubility of wheat phytate. Feed acidification has been reported to induce an inhibitory effect on growth and proliferation of harmful bacteria in gastrointestinal tract of chickens thereby preventing pathological diseases and reducing competition for digested nutrients.

Conclusion

Phytate reduces the bioavailability of minerals and other nutrients in cereal grains and oil seed based diets fed to chickens. Supplementation of 0.1% blends of acidifiers reduced phytate's affinity to bind feed nutrients in an unstable complex. This improved total tract mineral retention and reduced faecal wastage of feed minerals. Supplementation of 0.1% blends of acidifiers can increase the utilization of minerals in feedstuffs and reduce available phosphorus requirement in broiler diets by about 0.1%. Feed grade acidifiers reduced intestinal pH, thereby improving the solubility of feed nutrients and intestinal health.

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