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Effect of Supplementary Phytase and Mineral Chelators on Chicks' Growth Performance

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ABSTRACT

Effect of supplementary phytase and mineral chelators were determined in 28-day chicks' growth assay. Six isonitrogenous, isocaloric diets, equal in minerals, sulphur amino acids and lysine were formulated; a control with no inorganic phosphorus and 5 other diets containing 0.03% phytase, 0.1% EDTA, 1.5% citric acid, 0.03% phytase + 0.1% EDTA and 0.03% phytase + 1.5% citric acid, respectively. Diets were randomly assigned to 36 individually caged chicks. Phytase, EDTA or citrate supplements resulted in increase in feed intake, body weight gain and feed efficiency improvement ($P<0.05$). Serum alkaline phosphatase and organs relative weights were insignificantly different ($P>0.05$) but a significant increase in serum phosphorus and bursa of Fabricius were observed when phytase fed combined with EDTA or citrate ($P<0.05$). Minerals in tibia, P and Mg in toes were increased by citrate or EDTA supplement ($P<0.05$). Phytase plus citrate or EDTA generates more phosphorus and enhances nutrients utilisation.

INTRODUCTION

Phytic acid can readily form divalent mineral-phytate stable-complex, which is associated with a reduction in mineral bioavailability in both poultry and pigs diets. Similarly, other dietary factors may render phytic acid less susceptible to phytase hydrolysis [1, 2, 3, 4, 5 and 6]. Theoretically, dietary supplementations with chelators have the potential to improve the efficacy of microbial phytase and subsequently, improvement in phosphorus and mineral retention in monogastric production animals. However, it is proposed that an ideal 'competitive chelator' must have a higher affinity than phytic acid for mineral binding, must be non-toxic and the minerals bound to the chelator should be available to the monogastric animal either by absorption of mineral-chelate complex or by dissociation and absorption of the minerals. Carboxylic acids, such as citric acid, have been reported to have higher affinity than phytic acid for mineral binding [1].

In humans, diets supplemented with either ascorbic or citric acid were demonstrated to enhance iron bioavailability in humans consuming vegetarian diets [7] and infant formulas [8]. However, based on the solubility of phytate in acids, the current study was undertaken to ameliorate the nutritional effects of phytic acid in growing chicks' and to examine the hypotheses that, acidification of plant-based diet with citrate or ethylene diaminetetraacetate improves phytate hydrolysis or it increase the efficacy of enzymatic hydrolysis of phytate and subsequently nutrient utilisation.

MATERIALS AND METHODS

Experimental Diets

In examining the effect of acidification of phytate, 6 plant-based diets were formulated; a control diet with phytate-P as a main source of phosphorus (where the required phosphorus level was achieved by adding a proportion of 0.03g phytate-P/kg diet) with no inorganic phosphorus source. The other five diets were identical to control diet except that they contained 0.03% phytase0.1% EDTA, 1.5% citric acid, 0.03% phytase + 0.1% EDTA or 0.03% phytase + 1.5% citric acid, respectively (Table 1).

The diets were calculated to meet the requirements for essential nutrients as in [9] and to be equal in Ca, P, Mg, Fe, Zn, Mn, Cu, sulphur containing acids and lysine. Both added citric acid C(OH)(COOH)(CH2.COOH)2.H2 and ethylene diamine tetra acetic acid (EDTA) C10H24N2O8Na2.2H2O were laboratory reagents (BDH Chemical Ltd, Poole England). The added phytase used had specific activity of 5,000U/g of the product, where; 1U is the amount of enzyme that liberates 1 µmol of inorganic phosphorus from sodium phytate per minute at pH 5.5 and 37ºC (a gift product of the Finnfeeds International Ltd). The calculated compositions of diets were based on the actual analysis of the ingredients used and as in [9].

Husbandry and Procedure

100 one-week-old male chicks of a commercial layer strain (Isabrown) were brooded for a week, then 36 chicks were selected by rejecting the lightest and the heaviest and randomly allocated into 36 individual cages and allowed 7 days to adapt to the cages. At onset of the experiment, initial chicks' body weights were determined individually then experimental diets were randomly assigned to the cages (6 chicks per diet). Feeds and water were provided *ad libitum*, *with* 24-h photoperiod was maintained throughout the four-week of the experimental period.

Feed intake, live body weight, weight gain, feed: gain ratio and incidences of leg abnormalities (determined by subjective evaluation of each bird; only chicks showing a medium or severe degree of bowing were considered to be abnormal) were recorded weekly for the individual bird mortality was recorded as it occurred.

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Table 1.Composition and Analysis of the Experimental Diets (g/kg) .

	Control	Phytase	EDTA	Citrate	Phytase + EDTA	Phytase+ Citrate
Soybean meal	313.6	313.6	313.6	313.6	313.6	313.6
Maize	505.1	505.1	505.1	505.1	505.1	505.1
Maize starch	131.2	131.2	131.2	131.2	131.2	131.2
Maize oil	2.5	2.5	2.5	2.5	2.5	2.5
Dl-methionine	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	13.7	13.7	13.7	13.7	13.7	13.7
Phytate	3.03	3.03	3.03	3.03	3.03	3.03
Ca carbonate	7.97	7.97	7.97	7.97	7.97	7.97
F. supplement[1]	5.0	5.0	5.0	5.0	5.0	5.0
Salt	2.4	2.4	2.4	2.4	2.4	2.4
Phytase	0.0	0.3	0.0	0.0	0.3	0.3
EDTA	0.0	0.0	1.0	0.0	1.0	0.0
itric acid	0.0	0.0	0.0	15.0	0.0	15.0
Filler[2]	15.3	15.0	14.3	0.3	14.0	0.00
	1000	1000	1000	1000	1000	1000
Calculated Composition						
CP	180	180	180	180	180	180
ME, MJ/kg	11.9	11.9	11.9	11.9	11.9	11.9
Lysine	10.6	10.6	10.6	10.6	10.6	10.6
Methionine	3.1	3.1	3.1	3.1	3.1	3.1
Meth. + Cystine	6.2	6.2	6.2	6.2	6.2	6.2
Calcium	9.0	9.0	9.0	9.0	9.0	9.0
Non-Phytate-P	1.4	1.4	1.4	1.4	1.4	1.4
Total P	4.0	4.0	4.0	4.0	4.0	4.0
NDF	76.2	76.2	76.2	76.2	76.2	76.2
ADF	57.3	57.3	57.3	57.3	57.3	57.3
Determined Composition						
CP	146.3	150.6	153.1	149.4	148.8	149.4
Ca	7.1	6.8	8.8	7.5	7.8	8.5
P	18	2.1	1.9	2.0	2.1	1.9
Mg	1.4	1.6	1.7	1.5	1.5	1.6
Zn	1.8	2.1	1.9	2.0	2.1	1.9
Fe µg/g	172.5	188.0	171.5	171.9	164.0	180.0
Cu µg/g	21.0	19.5	20.5	19.0	18.5	18.0

[1]Feed supplement provides per kg diet: vitamin A 10 IU, vitamin D_3 3 I U, vitamin E 0.008 IU, Copper (Cupric sulphate) 0.005mg, vitamin B_1 0.01g, vitamin B_2 0.005g, vitamin B_6 0.001g, vitamin B_{12} 0.008 mg, vitamin K 0.002g , nicotinic acid 0.02g, pantothenic acid 0.o1g, folic acid 0.001g, biotin 0.05mg, choline 0.075, iron 0.01g, cobalt 0.025g, manganese 0.08g, zinc 0.06g, iodine 0.001g, selenium 0.00015g.
[2]Celite was used as inert filler

Sample Collection and Measurement

At 28th day of the trial, 5birds per each treatment were randomly selected, weighed and sacrificed by cervical dislocation. Just prior to the sacrifice, blood samples were taken by cardiac puncture using sterile 22-gauge disposable syringes and individually collected into tagged heparinized (50 units/ml) tubes, then centrifuged at 3000 rpm for 10 minutes and kept frozen at –25 ºC pending alkaline phosphatase, inorganic phosphorus, copper, and zinc determinations.

The birds were dissected and weighed. Liver, spleen, pancreas and bursa of Fabricius were then expressed as relative to body weight. The left tibias were defleshed and observed for any abnormality, whilst, toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end; both the right and the left middle-toes were pooled for each bird, all the samples were kept frozen.

Chemical and Statistical Analyses

Both frozen (-25 ºC) tibias and toes samples were oven-dried at 105 ºC for approximately 24h then weighed and ashed in a muffle furnace at 600 ºC for 24h, their ashes weights were expressed relative to their dried bone weights. Ash samples were wet-digested using 16 M HNO_3 and 12 M $HClO_3$ (5:3 v/v), Ca, Mg and Zn were determined using atomic absorption spectrophotometer, while, P content was determined using an auto-analyser. Liver samples were approximately halved, dried in an oven for 48 h at 100 ºC. Then 30ml of HNO_3 (70%) was added to the cooled samples and allowed to stand for 24h (to prevents spurting and frothing) prior heating, permitting the liver sample to dissolve in the acid, leaving a dark brown solution. The samples covered with watch glasses were heated gently at 60 ºC, not allowing the mixture to boil (for 18 or 21 h) until the solutions became a clear yellow in colour and were evaporated down to dryness. 30 ml of nitric acid (70%) and 4 ml of perchloric acid (60%) were added to the partially digested samples allowed to simmer slowly down to dryness over two day period. Then, the temperature of the hot plate was raised from 259 to 300 ºC, until the crystalline samples were completely dried out. Liver mineral contents were determined using atomic absorption spectrophotometer, but their phosphorus content was determined in auto-analyser as in [10], [11] and [12].

Duplicate samples of the diets were ground through a Wiley Mill, using a 40 mesh screen, and then the diets' crude protein was determined according to a macro-kjeldahl procedure. P was determined using an auto-analyser, while, Ca, Zn, Cu, Mg, Mn and Fe was determined using an atomic

Table 2 .Effects of dietary supplements on weekly and overall chicks' performance (g/bird/day).

Parameter	Dietary supplements						
	Control	Phytase	EDTA	Citrate + EDTA	Phytase	Phytase + Citrate	±SEM
Week I							
Feed intake	23.2	21.8	21.1	19.4	19.5	21.4	1.50^{ns}
Body weight gain	8.3	8.8	8.7	8.7	8.5	9.3	0.66^{ns}
F C R	2.9	2.5	2.5	2.3	2.3	2.3	0.21^{ns}
Week II							
Feed intake	31.4	27.1	30.5	26.4	28.3	33.6	2.49^{ns}
Body weight gain	10.8	11.0	10.6	10.1	11.0	13.3	1.12^{ns}
F C R	2.9	2.5	2.9	2.6	2.6	2.5	0.18^{ns}
Week III							
Feed intake	37.2	34.8	32.2	30.8	33.0	38.8	3.66^{ns}
Body weight gain	11.7^{cd}	14.3^{ab}	13.1^{bc}	10.7^{d}	12.2^{bcd}	15.6^{a}	1.67
F C R	3.2^{a}	2.5^{c}	2.5^{c}	2.9^{ab}	2.7^{bc}	2.5^{c}	0.19
Week IV							
Feed intake	37.4^{a}	25.1^{b}	36.1^{a}	30.2^{b}	39.9^{a}	39.1^{a}	2.70
Body weight gain	12.6^{c}	12.9^{bc}	13.8^{abc}	11.5^{c}	15.0^{ab}	15.8^{a}	1.23
F C R	3.0^{a}	2.0^{c}	2.7^{ab}	2.6^{ab}	2.7^{ab}	2.5^{b}	0.19
Overall performance							
Final live wt, g/bird	406.1^{b}	411.8^{b}	410.2^{b}	368.9^{b}	408.4^{b}	467.6^{a}	27.1
Feed intake	32.3^{a}	27.2^{b}	30.0^{ab}	26.7^{b}	30.2^{ab}	33.2^{a}	2.1
Body gain	10.9^{a}	1.7^{a}	11.6^{a}	10.3^{a}	11.6^{a}	13.5^{b}	0.8
FCR, g/g	3.0^{a}	2.3^{c}	2.6^{b}	2.6^{b}	2.6^{b}	2.4^{bc}	0.1

Values are means of 6 replicates per treatment ±SEM. ^{ns}, insignificant P>0.05.
$^{a-d}$Means within a row lacking a common superscript letter differ significantly (P<0.05)

absorption spectrophotometer. Duplicated samples of diets, tibia and toes mineral contents were analysed according to AOAC (1990). Serum copper and zinc contents were determined by atomic absorption, while, serum alkaline phosphatase and inorganic phosphorus were analysed following Sigma Diagnostics ALP (ALP optimised, EC 3.1.3.1 colorimetric test) and method of Fiske and SubbaRow (1925), respectively.

The generated data were subjected to analysis of variance for complete randomised design using Minintab (1993) statistical software, and the significant effects of treatment for means were compared using *lsd* at 5% as in [15].

Results

Weekly and Overall Growth Performance

Effect of plant-based diets supplemented with phytase, EDTA and or citrate on chick's growth performance showed no effect (P>0.05) on the amount of food consumed throughout the first, second and third week of the experiment, as well as, weight gain and food conversion ratio throughout the first and second week (Table 2). However, in third and fourth weeks dietary treatments had a significant effect on weight gain and food conversion ratio (P<0.05) and in fourth week food intake was also significantly affected. In the third week, phytase with or without citrate showed increase in weight gain compared to control diet with relatively improved food conversion depicted by citrate diet. In the fourth week, phytase supplemented with EDTA and citrate showed improvement in weight gain.

The effect of supplementary phytase and mineral chelators on the overall chick growth performance (Table 2) showed a significant effect on total amount of food consumed, final live weight, final body gain and the food conversion ratio (P<0.05). Despite the insignificant difference between diet supplemented with 0.03% phytase + 0.5% EDTA and that containing 0.03% phytase + 1.5% citrate (P>0.05) the associative effect of phytase plus citrate yielded higher final live weight, food consumed and weight gain (P<0.05), compared to the rest of the treatments.

Effects of Dietary Treatments on Organ Relative Weights, Serum IP, Cu, Zn and APL

Effects of dietary treatments on organ relative weights, serum IP, Cu, Zn and APL presented in Table 3 showed that chicks' liver, spleen and pancreas relative weights were not affected by the dietary treatments (P>0.05). However, feeding supplementary phytase with or without mineral chelators had a significant effect on bursa of Fabricius relative weight, citrate treated diet had a vast increase in bursa of Fabricius relative weight, followed by phytase and phytase combined with citrate diets. Supplementary phytase with or without EDTA and citrate showed a significant difference in serum (IP) inorganic phosphorus (P<0.05) amongst the dietary treatments, with a vast increase when phytase combined either with EDTA or citrate (Table 3), however, the latter showed the highest level of serum IP. Despite the apparent differences in serum alkaline phosphatase they were not significantly affected by the dietary treatments.

The effect of feeding phytase with or without EDTA or citrate on serum copper and zinc levels (Table 3), have resulted in insignificant differences among the measured serum copper levels (P>0.05) but showed significant differences among serum zinc concentrations. Phytase combined with citrate showed significantly higher zinc level compared to the rest of the treatments (P<0.05) than either EDTA or citrate diets.

Effect of the Dietary Treatments on Liver Ca, Mg, Cu, Mn and Zn Concentrations

Effect of supplementary phytase with or without EDTA or citrate in plant-based diet on liver Ca, Mg, Cu, Mn and Zn concentrations presented in Table 3 showed significant differences (P<0.05) among dried liver weights, with the highest value for phytase combined with citrate and the lowest value for EDTA diet. Liver magnesium, manganese and copper concentrations were significantly different

Table 3. Effects of Dietary Supplements on Organ Relative Weight, Serum's IP, Cu, Zn, *ALP* and Liver's Ca,Mg, Mn, Cu & Zn content.

Parameter	Dietary variables						
	Control	Phytase	EDTA	Citrate	Phytase + EDTA	Phytase + Citrate	±SEM
Liver g/kg body wt	27.7	26.6	29.1	26.6	26.1	27.9	0.4ns
Spleen g/kg body wt	1.5	1.6	1.7	1.7	1.5	1.6	0.3ns
Bursa, g/kg body wt	3.1b	4.5a	3.8b	4.7a	3.8b	4.1a	0.5
Serum IP, µg/ml	21.8cd	19.6d	32.6ab	31.5bc	37.4ab	41.7a	4.8
Serum Cu, mg/l	0.23	0.27	0.20	0.18	0.20	0.31	0.07ns
Serum Zn, mg/l	1.60c	1.74cb	1.85b	1.89b	1.71cb	2.32a	0.218
Serum ALP, µg/l	3789	1877	3088	4053	3317	2766	1457ns
Liver mineral conc.							
Liver, DM%	28.3bc	28.5b	27.0e	28.1d	28.3c	29.1a	0.60
Ca, DM%	0.01	0.01	0.01	0.01	0.01	0.01	0.001ns
Mg, DM%	0.07c	0.08b	0.09a	0.09a	0.08b	0.07c	0.004
Mn, µg/g	7.0c	7.4b	6.8d	8.1a	7.1c	5.8e	0.60
Cu, µg/g	15.4a	14.4b	14.8b	4.4c	4.4c	9.6d	1.61
Zn, µg/g	87.6	90.1	87.6	91.7	91.6	89.7	2.73 ns

Values are means of 6 replicates per treatment ±SEM.
a-d Means within a row lacking a common superscript letter differ significantly (P<0.05)
ns insignifiant (P>0.05)

Table 4. Effects Dietary Supplements on Ash and Minerals Contents in Tibia and Toes.

Parameter	Dietary supplements						
	Control	Phytase	EDTA	Citrate	Phytase + EDTA	Phytase +Citrate	±SEM
Tibia							
Ash, g/kg body wt	1.3^{c}	1.5^{b}	1.1^{c}	1.2^{c}	1.7^{a}	1.5^{b}	0.06
Ca, ash%	31.3^{c}	34.1^{b}	40.0^{a}	37.7^{a}	33.1^{bc}	31.7^{c}	1.10
P, ash%	11.1^{b}	12.9^{a}	11.2^{b}	11.2^{b}	4.9^{c}	3.4^{c}	0.80
Mg, ash%	0.5^{d}	0.6^{bc}	0.6^{bc}	0.6^{bc}	0.8^{a}	0.7^{b}	0.04
Zn, µg/g ash	389.6^{b}	377.9^{b}	574.6^{a}	517.1^{a}	539.2^{a}	458.6^{ab}	61.80
Fe, µg/g ash	435.8^{b}	402.8^{b}	582.2^{a}	561.5^{a}	286.1^{d}	324.1^{bd}	40.90
Cu, µg/g ash	10.5^{a}	5.6^{b}	12.4^{a}	11.7^{a}	10.1^{a}	7.3^{b}	1.79
Mn, µg/g ash	11.6^{b}	16.6^{a}	19.3^{a}	7.7^{c}	18.5^{a}	12.5^{b}	1.39
Toe							
Ash, mg/kg body wt	54.1^{b}	73.7^{a}	46.9^{b}	49.4^{b}	71.9^{a}	72.6^{a}	8.2
Ca, ash%	32.4	30.5	32.1	33.3	35.3	33.5	2.8^{ns}
P, ash%	13.7^{bd}	15.6^{b}	13.1^{d}	14.2^{bd}	19.8^{a}	19.3^{a}	1.1
Mg, ash%	0.5^{dc}	0.6^{c}	0.5^{de}	0.5^{e}	0.9^{a}	0.9^{b}	0.4
Zn, µg/g ash	790.2	705.2	1117.5	916.3	1451.5	999.2	271.2^{ns}
Fe, µg/g ash	491.8	427.5	620.9	545.7	532.8	493.7	146.4^{ns}
Cu, µg/g ash	188.5	132.7	142.3	164.8	163.2	149.7	31.8^{ns}

Values are means of 6 replicates per treatment ±SEM.
$^{a-d}$ Means within row lacking common superscript letter differ significantly (P<0.05)

among the dietary treatments (P<0.05).Citrate dietary treatment showed the highest liver magnesium and manganese levels compared to the rest of the treatments. Liver copper level was significantly reduced compared to the control diet when diets were supplemented with phytase in combination with both EDTA and citrate or citrate alone, however, the experimental diets had no effect (P>0.05) on concentrations of calcium and zinc in liver.

Effect of the dietary treatments on ash and mineral contents of tibia and toes

The effect of supplementary phytase with or without EDTA and citrate in plant-based diets on ash and mineral content of both tibia and toes of growing chicks are presented in Table 4, it shows significant differences among the tibia ash (P<0.05). Diets supplemented with phytase, phytase combined with EDTA or citrate acid had a significant increase in tibia ash compared to the control diet. Tibia contents of calcium, phosphorus, magnesium, zinc, iron, copper and manganese relative to its ash weight were significantly different (P<0.05) among the dietary treatments.

Data presented in Table 4 showed that feeding supplementary phytase with or without EDTA and citrate in plant based diet caused a significant increase in toes ash, as well as, phosphorus and magnesium relative to ash weight compared to the control diet, the three parameters depicted the same pattern (P<0.05) in response to phytase or phytase in combination with EDTA or citrate compared to the rest of the treatments. However, plant-based diets supplemented with phytase and or without EDTA and citrate did not differ significantly (P>0.05) with respect to their effects on toes calcium, zinc, iron and copper.

Discussion

Supplementary phytase in association with citrate diet resulted in significant improvement in chick growth performance; it improve growers live weight, weight gain, feed intake and the feed efficiency, this combination seems to enhance chicks feed intake a main factor in mediating or weight gain enhancement. Over the four weeks experimental

period, inclusion of citrate (1.5%) reduced feed intake compared to the rest of the dietary treatments and consistent with the observation that adding citric acid to diet causes feed intake to decrease [16] and that weight gain and tibia ash responded more dramatically to citrate than did gain: feed ratio [17]. Citrate at levels of 4 to 6% of the diet was found to be markedly efficacious in improving utilization of phytate phosphorus in broiler chicks [17] which seem to contradict with the current findings, since the realised improvement in growth performance in this study could be ascribed to efficiency in nutrient utilisation rather than the increased feed intake.

Though phytase diet seemed to enhance feed conversion efficiency, supplementing phytase with citrate showed significant increase in final body weight, feed intake and body weight gain. Citrate supplemented diet had the highest bursa of Fabricius relative weight, but phytase combined with either citrate or EDTA had the highest serum concentration of inorganic phosphorus. It was also observed that diet containing combination phytase and citrate had the highest serum zinc concentration. The enhancement in growth associated with dietary fortifications, as well as serum concentrations of inorganic phosphorus and zinc are supportive to the proposal that addition of a chelator may serve to remove cations from binding to phytic acid and as such may increase the relative level of phytic acid susceptibility to enzyme in the meal.

Both EDTA and citrate were shown earlier to improve the efficacy of microbial phytase in forming inorganic phosphorus [18], similar improvement were reported but with pigs in availability of phosphorus as well as other minerals [19]. In the latter studies it was concluded that addition of 1.5% citric acid in a corn-soy diet for young pigs provides a better environment for phytase function, but in determining the synergistic effect between microbial phytase and or citrate on the total phosphorus utilisation, they did not observe any interactive effect of citric acid and microbial phytase on total dietary phosphorus utilisation and growth performance, they have concluded that factors such as differences in the ingredient's composition, variations in the pH of gastrointestinal tract contents, as well as, response of gastrointestinal tract pH to diet composition might account for some of the inconsistencies in their study. However, dietary inclusion of either citric acid or EDTA might have created a digesta pH conducive for phytate hydrolysis or alternatively EDTA might bind with calcium and so lessen its inhibitory effect. However, EDTA being a strong metal chelator poses a concern over its effect on metabolism of other nutritionally important trace elements and minerals, but separate results shows that supplementation of a diet containing soy protein isolate with EDTA decreases dietary mineral requirements as in [20] and [21]. Also it was concluded that using $NaFe^{3+}$EDTA as a food fortificant would have no detrimental effect on metabolism of zinc, copper and calcium and in some situations could improve zinc absorption and retention from low-bioavailability diets [22]. Earlier studies [23] have shown that copper at a concentration of 1mM forms soluble chelates with 10mM phytate while 1mM zinc forms phytate chelates that slowly precipitate out of solution over a 48 h period. But addition of 5mM EDTA completely overcame the inhibitory effect of Ca^{2+} on phytic acid hydrolysis by microbial phytase. Presumably, EDTA functioned as a competitive chelator removing calcium from the medium and shifting equilibrium to minimize formation of enzyme-resistant mineral-phytate complexes. However, in infants high gastric pH was reported to facilitate the formation of phytate-calcium-zinc chelates [24].

Phytase, at levels supplemented here seems to prevent a fall in serum or plasma phosphorus and rise in serum alkaline phosphatase and severe loss of appetite, which are the first clinical signs of phosphorus deficiency. The observed improvement in tibia and toes bone mineralisation is probably due to enhancement in calcium absorption which lessens the opportunity for calcium to form un-absorbable phytate in the gut. Subsequently this improvement in retention of calcium in the skeleton is accompanied by retention of phosphorus, therefore, the reported hypophosphataemic rickets in broilers was associated with low bone ash [25] were not seen in this study. Earlier studies as in [26] and [27] but in pigs have indicated that plasma inorganic phosphorus concentration is highly correlated with bone strength and growth in young pigs, which is consistent with the current findings. It was suggested [28] that plasma inorganic phosphorus concentration could be a good indicator of body phosphorus status in non-terminal pig studies.

There are two possible mechanisms that have been reported for citrate to promote dietary phosphorus utilisation and growth performance. First, supplemental citric acid may enhance the solubility of digesta phosphorus and the transit time of digesta in the small intestine by lowering digesta pH thereby improving total phosphorus absorption [27]. Secondly, both microbial phytase and wheat phytases used in the previous study have pH optimum around 2.5. Also, their experimental diet contained calcium phosphate or wheat middlings which had a pH of 6.0 and the gastric secretion of HCl in young pigs may be relatively insufficient. Thus, acidifying the diets by adding 1.5% citric acid, reducing the pH of the stomach digesta may enhance hydrolysis by phytases [27]. The same explanations may stand for this study, as alternatively the observed improvement in grower performance as a result of phytase augmented with mineral chelators might be explained in terms of competitive chelation, which might account for the effects of chelators in improving mineral bioavailability. Presumably, mineral-chelator complexes exist in a soluble form that can be absorbed intact or can release minerals to binding sites on the brush border membrane of the intestinal epithelium [18]. Furthermore, *in vitro* studies [29] have shown that trace metals are able to precipitate phytate at intestinal pH only when they are in unphysiologically high concentration relative to phytate, a situation that hardly exists in practical diets. But in the acidic condition of the stomach, phytate can react with positively charged amino groups on protein as in [1] which seems of less significance since below pH 4 calcium phytate itself is soluble and the absorption of some trace elements is most likely to occur. However, at neutral intestinal pH, phytate could complex with metal ions and negatively charged carboxyl groups on protein but these are disestablished by increasing calcium [1].

In the present studies it seems that dietary inclusion of either citric acid or EDTA might have created a digesta pH conducive for phytate hydrolysis and that addition of exogenous phytase fortified with mineral chelators generates adequate phosphorus and enhances nutrient utilisation. Also it seems that competitive chelating by either citric acid or EDTA has the potential to decrease the enzyme resistant forms of phytate and subsequently improves the efficiency of microbial phytase. It could also be noted that phytase-citrate combination was superior to that of phytase-EDTA and that

cirate seems to increase susceptibility of phytate to phytase hydrolysis. In general, the obtained results depicted a positive influence of acidifying-plant diet (with citric acid) on phytate hydrolysis or chick growth performance that rated next to supplementary phytase. The appropriate supplementary levels of citric acid with or without supplementary phytase need a further study in dose response feeding trials.

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