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Phytate Phosphorus Hydrolysis As Influenced by Dietary Calcium and Micro-Mineral Source in Broiler Diets

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Phytate phosphorus (PP) hydrolysis by a 3-phytase was studied in vitro at pH 2.5 and 6.5 with either 0, 1.0, 4.0, or 9.0 g of Ca/kg diet, or 0, 1.0, 5.0, 7.5, or 10.0 g/kg diet of micro-mineral premix added as inorganic (IMM) or an equivalent level as micro-mineral−amino acid complexes (MAAC). Adding Ca or micro-minerals reduced ($P < 0.05$) PP hydrolysis at both pHs; however, the effect was greater at pH 6.5. An in vivo experiment was conducted in which broilers were fed one of six diets for 30 h. The experimental design was a factorial of three micro-mineral forms (0 added, IMM, and MAAC) and two Ca levels (0 or 5 g/kg). Adding Ca reduced ($P < 0.05$) PP disappearance and increased Ca apparent absorption. No micro-minerals effect ($P > 0.05$) was seen. Therefore, in poultry diets, it is Ca that inhibits PP hydrolysis and decreases P availability.

KEYWORDS: Phytate phosphorus; calcium; absorption; micro-minerals; broilers

INTRODUCTION

Phytic acid (PA) (myo inositol hexaphosphoric acid) occurs naturally in plants, primarily in seeds, and serves as a storage form of phosphorus (P) (*1*). Poultry diets are composed mainly of plant based ingredients that have 60−90% of their P as phytate P (PP) (*1*, *2*), which is poorly available (*3*, *4*). This poor availability of the P present naturally in plant-based diets means that inorganic sources of P have to be added to the diet to meet the animal's P requirement. The addition of inorganic P to diets that already may contain enough P, albeit in an unavailable form, results in diets that contain total P levels well in excess of requirement. This results in excess P, regardless of dietary source, being excreted. The presence of high levels of P in poultry excreta is currently a concern, especially when excreta is applied to soil as a fertilizer, because high excreta P can lead to soil P accumulation and increased P leaching or runoff into waterways. Poultry excreta management, primarily due to its proportionately high P content, has become a cost for poultry production and one of the factors for regulation of animal feeding operations.

In its acidic form, PA has the capacity to bind or chelate multivalent cations including calcium (Ca), zinc (Zn), iron (Fe), magnesium (Mg), manganese (Mn), cobalt (Co), and copper (Cu) (*1*, *5*, *6*). Several researchers have observed that solubility of PA metal complexes is pH dependent (*7*, *8*). Most phytate−mineral complexes are soluble at low pHs (less than 3.5) with maximum insolubility occurring between pH 4 and 7 (*7*). Calcium and Mg phytate complexes precipitate at pHs between 4 and 6, and 5 and 7, respectively (*8*). The approximate pH of the intestine, where absorption of metal ions takes place, coincides with the pHs at which these complexes precipitate (*8*). Precipitated phytate−mineral complexes are not accessible for hydrolysis or absorption in the intestine. The order of stability of metal−phytate complexes was found to be Cu > Zn > Co > Mn > Fe > Ca (*2*). Other researchers (*6*) reported the order of mineral potency as inhibitors of PP hydrolysis at neutral pH to be $Zn^{2+} \gg Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$. Even though Ca has one of the lowest affinities for phytate, it has the greatest impact, because it is the mineral present at the highest concentration in the diet. Taylor (*9*) suggested that the primary factor determining PP utilization is the Ca ion concentration in the small intestine, where insoluble Ca-phytate complexes form. Increasing Ca level in the broiler diet from 1.2 to 5.2 g/kg decreased ($P < 0.05$) PP hydrolysis (*10*). Furthermore, high levels of Ca or Mg were found to lower the activities of intestinal alkaline phosphatase and phytase in broiler chicks, but Ca had a much more pronounced effect (*11*).

Phytate P availability has been found to improve by the addition of some amino acids (*1*). The addition of certain amino acids, including cysteine and histidine, was found to alleviate some of the Zn deficiency syndrome signs (*12*). In general, amino acids, which possess one amino and one carboxyl group and can form neutral chelates, have the potential to be and fulfill the requirements of a chelating agent (*13*). Specific amino acid metal chelates have the metal embedded in a cyclic configuration bearing no electrochemical charges to bind any other compound, thus eliminating metal to phytate complexing potential (*13*). The improvement in mineral absorption seen by Nielsen (*12*) may be due to the amino acids having higher affinity for the minerals, therefore minimizing phytate mineral interactions. Fouad (*13*) reported that mineral amino acid chelates, if properly prepared, would be absorbed intact through the intestinal mucosa. This absorption of intact mineral amino acid complexes would be important to minimize any potential interaction between mineral ions and phytate if minerals are released while still in the digesta.

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The present study was designed to test the hypothesis that decreasing Ca in the diet as well as the use of micro-mineral−amino acid complexes will increase PP hydrolysis both in vitro and in vivo. Experiments were conducted to determine the effect of Ca level and micro-mineral source (inorganic or amino acid-mineral complexes) on PP hydrolysis in vitro at 2 different pHs and in vivo.

MATERIALS AND METHODS

Materials. Sodium acetate, glycine, calcium carbonate, sodium phytate (dodecasodium salt from rice), phytase enzyme (3-phytase from *Aspergillus ficuum*), and inorganic P were purchased from Sigma, St. Louis, MO. Inorganic micro-mineral premix (IMM) was purchased from Southern States Cooperative Inc., Richmond, VA, and was labeled to contain, per kg, 98 g of Ca from $CaCO_3$, 210 g of Zn from ZnO, 120 g of Mn, of which 50% came from MnO and 50% from $MnSO_4$, 40 g of Fe from $FeSO_4$, 20 g of Cu from CuO, 3 g of I from $Ca(IO_3)_2$, and 0.05 g of Co from $CoCO_3$. The IMM was analyzed by inductive coupled plasma (*14*) and was found to contain on an as-is basis: 116.4 g of Ca, 192.0 g of Zn, 107.5 g of Mn, 34.9 g of Fe and 17.3 g of Cu per kg of premix. Dry matter content was also determined to be 995 g/kg.

Each of the following micromineral products was obtained as individual mineral products: Availa-Zn 100, zinc−amino acid complex; Availa-Mn 80, manganese−amino acid complex; Availa-Fe 60, iron−amino acid complex; and Availa-Cu 100, copper−amino acid complex; from Zinpro Corporation, Eden Prairie, MN; CaI_2O_6 and $CoCl_2$ from Sigma, St. Louis, MO. To make the micro-mineral−amino acid complex premix (MAAC), the amino acid complexes as well as CaI_2O_6 and $CoCl_2$ were mixed such that the proportions of Zn, Mn, Fe, Cu, I, and Co were the same as those in the IMM. The MAAC was analyzed by inductive coupled plasma and found to contain, per kg, 60.6 g of Ca, 47.2 g of Zn, 24.9 g of Mn, 10.7 g of Fe and 5.3 g of Cu per kg of premix. Dry matter content was also determined to be 981 g/kg.

By calculation, 4.5 g of MAAC would provide the same amount of micro-minerals (Fe, Mg, Cu, Zn, I, and Co) as 1 g of IMM.

In Vitro Phytate Phosphorus Hydrolysis. For the in vitro work, the general procedures of Chen (*15*) were followed with some modifications, including incubation times (15, 30, 60, and 120 min, instead of 60 min only), sodium phytate concentration (4.62 g/L instead of 8.4 g/L), as well as the use of inorganic P as a standard, instead of P liberated by a phytase enzyme with known activity. All incubations were done in quadruplicate, and each one of these quadruplicate incubations served as the experimental unit.

Phytate P hydrolysis by a 3-phytase enzyme (from *Aspergillus ficuum*) with a pH optimum of 5 (*16*) was determined over a 120 min period at pH 2.5, simulating gastric pH, and at pH 6.5, simulating small intestinal pH. Assuming typical PP content in a corn−soybean meal broiler starter diet of 2.7 g PP/kg and an expected feed to water consumption ratio of 2:1, a 4.62 g/L sodium phytate solution (929 mg PP/L) was prepared in a 200 mM glycine buffer (pH 2.5) or 200 mM sodium acetate buffer (pH 6.5), and this solution was used as the substrate. Buffers were chosen because of their pH specificities, with the glycine buffer being effective between pH 2.0 and 3.0 and the sodium acetate buffer between pH 3.5 and 6.5 (*16*). Phytase enzyme was suspended in a buffer and then diluted such that a 100-μL volume would contain the equivalent of 500 units (U) phytase/kg diet when added to the substrate solutions. The chosen level of phytase, 500 U phytase/kg diet, is within the range (300−800 U phytase/kg diet) recommended to be added to poultry diets (*17, 18*). A U of phytase activity, as defined by the manufacturer, is the amount of phytase needed to liberate 1.0 μmole of inorganic P from 4.2×10^{-2} M Mg · K phytate per min at 37 °C and pH 2.5.

Effect of Calcium. Four Ca levels were selected (0, 0.86, 3.44, and 7.74 g of $CaCO_3$/L) to be equivalent to 0, 1.0, 4.0, or 9.0 g/kg diet. The highest level chosen, 9.0 g/kg, is the level most commonly used in broiler starter diets. The two other levels (4.0 and 1.0 g/kg) were chosen as intermediate and low levels. The four Ca levels were added to the Na-phytate substrate, and the pH of the solutions was checked and readjusted to the two test pHs of 2.5 or 6.5, prior to the start of the incubations. To a test tube with 3.0 mL of the substrate solution and added Ca, a 100-μL volume of phytase enzyme was added. The resulting mixtures were incubated at 37 °C for 0, 15, 30, 60, or 120 min. A 2-mL volume of ammonium molybdate-metavanadate reagent prepared according to Chen (*15*) was added to stop the reaction, and liberated P was measured spectrophotometrically at 410 nm (*19*), using inorganic P as a standard.

Table 1. Ingredient Composition and Nutrient Levels in the Basal Diet

ingredient	basal g/kg
corn	541.8
soybean meal, 48%	398.7
crude soy oil	51.1
vitamin mix[a]	0.8
choline chloride, 60%	0.8
salt	4.6
DL methionine	2.2
formulated (analyzed) nutrient level	
crude protein (g/kg)	23.6
crude fat (g/kg)	7.02
energy (kcal ME/kg)	3230
calcium (g/kg)	1.8 (1.8)
total phosphorus (g/kg)	4.1 (4.0)
non pp[b] (g/kg)	1.3 (1.1)

[a] Supplied the following per kilogram of feed: vitamin A, 14991 IU as retinyl acetate; vitamin D, 5291 ICU as cholecalciferol; vitamin E, 52.9 IU as DL-α-tocopheryl acetate; vitamin B_{12}, 0.026 mg as cyanocobalamin; riboflavin, 17.64 mg as riboflavin; niacin, 70.55 mg as nicotinic acid; D-pantothenic acid, 24.6 mg as D-pantothenic acid; vitamin K, 3.2 mg as menadion sodium bisulfite complex; folic acid, 2.12 mg as folic acid; vitamin B6, 6.17 mg as pyridoxine hydrochloride; thiamine, 4.4 mg as thiamine mononitrate; and vitamin H, 0.149 mg as D-biotin. [b] Non phytate phosphorus (PP), determined by subtracting analyzed phytate phosphorus from analyzed total phosphorus.

Effect of Inorganic Micro-Mineral Premix. Five levels of IMM were used (0, 0.34, 1.7, 2.5, and 3.4 g/L). The levels used were chosen to reflect dietary additions of IMM at 0, 1.0, 5.0, 7.5, or 10.0 g/kg diet. These levels were selected based on the recommended inclusion level of this IMM in poultry diets of 1.0 g/kg and three levels higher (5.0, 7.5, and 10.0 g/kg), to exacerbate the potentially negative effect of micro-minerals. The solutions were incubated with the phytase enzyme and PP hydrolysis determined as described previously.

Effect of Micro-Mineral−Amino Acid Complexes. The MAAC was added to the substrate at levels that supplied the same concentrations of Zn, Mn, Fe, Cu, I, and Co as those supplied by 0, 1.0, 5.0, 7.5, or 10.0 g/kg IMM in the diet (0, 4.5, 22.5, 33.75, and 45 g/kg MAAC). Phytate P hydrolysis in the presence of MAAC was determined as described previously for IMM. For the micro-mineral studies (IMM and MAAC), micro-mineral levels studied are identified as 0, 1, 5, 7.5, and 10X; where 1X is equal to 1 g/kg in the case of IMM and 4.5 g/kg in the case of MAAC. The 1X level of either IMM or MAAC supplied the same amount of micro-minerals (Fe, Mn, Zn, Cu, I, and Co).

In Vivo Phytate Phosphorus Hydrolysis. *Animals and Diets.* A mash starter diet was mixed such that it met or exceeded National Research Council (*20*) broiler recommendations for all nutrients. For the experimental diets, a corn-soybean meal grower basal with no added micro-minerals, inorganic Ca, or P (**Table 1**) was mixed and analyzed for Ca, P, and PP, as described in the sample analysis section, before the experimental diets were formulated.

The experimental design was a 2 × 3 factorial with two added Ca levels (0, 5.0 g/kg) and three micro-mineral sources (none, 1X IMM, and 1X MAAC; 1X of each as defined previously). The basal diet was used at 970 g/kg of the diet, and $CaCO_3$, IMM, MAAC, and Celite were added to achieve levels desired in the experimental diets. Celite was added to all diets as an indigestible marker (*21*) and as a filler, to achieve 100% without affecting diet nutrient density, with a minimum inclusion rate of 10 g/kg. This level is the minimum needed for effective use of Celite as a marker. The dietary treatments were (1) basal, (2)

basal plus 5 g/kg Ca (from $CaCO_3$), (3) basal plus 1X IMM, (4) basal plus 1X IMM, plus 5.0 g/kg Ca (from $CaCO_3$), (5) basal plus 1X MAAC, and (6) basal plus 1X MAAC plus 5.0 g/kg Ca (from $CaCO_3$).

Day-old male (Ross 308) broiler chicks were raised in floor pens from hatch to 20 days of age and fed the starter diet, ad libitum. On day 20, birds were randomly assigned to eight replicate battery pens per dietary treatment, four birds per pen. The six dietary treatments were assigned to battery pens, using a completely randomized design. Birds were fasted for 16 h and then fed the experimental diet ad libitum for 30 h, sacrificed by cervical dislocation, ileum separated, and ileal contents collected by expressing gently. The ileum was defined as the segment from the Meckel's diverticulum to 3 cm before the ileocecal junction. Ileal contents were pooled by pen (the experimental unit), dried at 80 °C for 24 h, and stored at 10 °C for later analysis. The experimental period (30 h) was chosen to minimize functional adaptations of the gastrointestinal tract to the diets (*22*, *23*). All guidelines of the Animal Care and Use Committee of the University of Maryland were followed.

*Sample Analyses***.** Diets and ileal samples were ground to pass through a 0.5 and 0.25 mm screen, respectively. On all samples, moisture (*24*), acid insoluble ash (*25*), Ca (*26*), P (*19*), and PP (according to the method of Rounds and Nielsen (*27*) as modified by Newkirk and Classen (*28*)) were determined. All analyses were done in duplicate.

*Statistical Analyses***.** For the in vitro experiment, the Ca effect was studied using a 4 × 2 factorial (four Ca levels, two pHs) design. The data were analyzed using mixed procedures of SAS (*29*). Pairwise comparisons were done to compare means within each time period/pH combination, using Tukey's HSD test (*30*) to control experiment-wise error rate. For the micro-mineral study, the design was a 2 × 5 × 2 factorial (two pHs, five micro-mineral levels, two sources), and the data were analyzed separately for each time period, using mixed procedures of SAS (*29*). To test the effect of micro-mineral level, pairwise comparisons were done to compare means within each combination of pH, time, and source. The effect of micro-mineral source was also tested, by doing pairwise comparisons across sources within each time, pH, and level combination. Significance was accepted at $P < 0.05$.

For the in vivo experiment, the design was a completely randomized 3 × 2 factorial (three micro-mineral sources and two Ca levels), resulting in six dietary treatments. Each pen served as an experimental unit (eight pens/treatment), and data were analyzed using the General Linear Models procedures of SAS (*29*). When the model was significant, treatment means were separated using Tukey's HSD test (*30*). Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

In Vitro Phytate Phosphorus Hydrolysis. The results of the in vitro studies are summarized in **Table 2** and **Figures 1**, **2,** and **3**. In the Ca study, significant Ca, pH, and Ca × pH interaction was present at all time periods. Pairwise comparisons were done to test simple effect means, and results are summarized in **Figure 1**. At pH 2.5 (**Figure 1A**), PP hydrolysis was reduced ($P < 0.05$) by the addition of 4.0 g/kg or 9.0 g/kg Ca at all incubation times. A 1.0-g/kg Ca level resulted in significant reduction in PP hydrolysis only after 120 min of incubation. At pH 6.5, however, addition of Ca at levels as low as 1.0 g/kg resulted in a decrease ($P < 0.05$) in PP hydrolysis at 60 and 120 min, versus the no-Ca-added treatment (**Figure 1B**). Addition of Ca decreased PP hydrolysis to a greater extent at pH 6.5 than at 2.5, which explains the significant Ca × pH interaction. The addition of Ca at 1.0, 4.0, or 9.0 g/kg reduced PP hydrolysis during a 60 min incubation period by 2, 25, and 56%, respectively, at pH 2.5. At pH 6.5, the reductions were 23, 48, and 46%, respectively. These findings agree with published work, which showed that mineral cations, including Ca, negatively influenced PP hydrolysis and that this effect was more pronounced at higher pHs (*6*, *31*, *32*).

Table 2. *P* Values from the ANOVA Analysis for the Effect of Micro-Mineral Level and Source on in Vitro Phytate Phosphorus Hydrolysis at pH 2.5 and 6.5

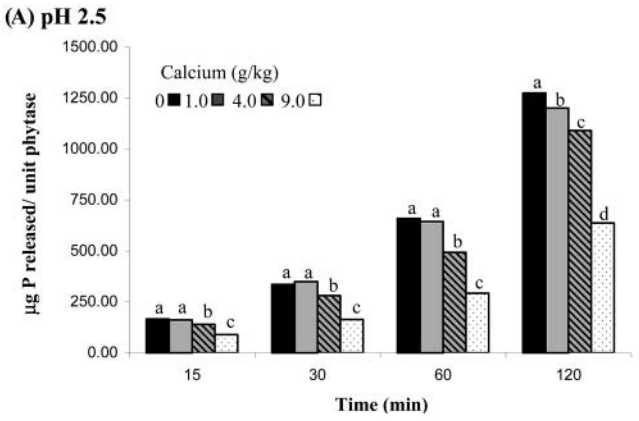
	time (min)			
sources of variation	15	30	60	120
level[a]	<0.0001	<0.0001	<0.0001	<0.0001
source[b]	0.3805	0.0103	<0.0001	0.0008
pH[c]	<0.0001	<0.0001	<0.0001	<0.0001
level × source	0.0071	0.0088	<0.0001	<0.0001
level × pH	<0.0001	<0.0001	<0.0001	<0.0001
source × pH	0.1157	0.0243	0.4982	<0.0001
level × source × pH	0.2763	0.5150	0.0389	0.0115

[a] Five micro-mineral levels were used; 0, 1, 5, 7.5, and 10X; where 1X was equivalent to 1 g/kg diet for the IMM and 4.5 g/kg diet for the MAAC. [b] Two micro-mineral sources were used. 1. Inorganic micro-mineral premix (IMM), which supplied per 1X: 98 mg Ca from $CaCO_3$, 210 mg Zn from ZnO; 120 mg Mn from MnO and $MnSO_4$, 40 mg Fe from $FeSO_4$, 20 mg Cu from CuO, 3.0 mg I from $Ca(IO_3)_2$, and 0.05 mg Co from $CoCO_3$. 2. Micro-mineral amino acid complex (MAAC), which supplied per 1X: 210 mg Zn from Availa-Zn 100, Zn amino acid complex; 120 mg Mn from Availa-Mn 80, Mn amino acid complex; 40 mg Fe from Availa-Fe 60, Fe amino acid complex; 20 mg Cu from Availa-Cu 100, Cu amino acid complex; 3.0 mg I from $Ca(IO_3)_2$; and 0.05 mg Co from $CoCl_2$. (X is equivalent to 0.45% in the diet). [c] Two pHs tested, 2.5 and 6.5.

One important factor that primarily influences PP hydrolysis is the Ca/phytate molar ratio (*33*). At high molar Ca concentrations and at near neutral pH, Ca binds to phytate, forming a low solubility complex. Under in vitro conditions at pH 7.5, when Ca was added to sodium phytate at equimolar ratios, very little Ca and phytate precipitated. However, when the Ca/phytate molar ratio was increased to 2:1, 40.8 and 47.6% of Ca and phytate, respectively, precipitated (*34*). Grynspan and Cheryan (*32*) reported maximum precipitation of PP at a pH higher than 6 and Ca/PA molar ratio of 6.5 or 12.67. A precipitated phytate is not available to the phytase enzyme. This can partially explain the inhibition in PP hydrolysis seen in this study.

In the present study, the molar ratios of Ca/PA studied were 1.7:1, 6.9:1, and 15.5:1. At pH 6.5, Ca/PA molar ratios of 6.9:1 and 15.5:1 resulted in 45 and 57% inhibition in PP hydrolysis, respectively, during a 30 min incubation period. At pH 2.5 however, 50% inhibition in PP hydrolysis in 30 min incubation was observed only at a Ca/PA molar ratio of 15.5:1. In a similar study, 50% inhibition in PP hydrolysis occurred at Ca/PA molar ratio of 35.9:1 at a 6.5 pH, while at pH 4.0, even a 7042:1 molar ratio did not result in 50% PP hydrolysis inhibition (*6*). The differences in the Ca/PA molar ratio required for a 50% inhibition between the Maenz et al. (*6*) work and the present study can be due to several key components that were different between the two studies, including concentration of the substrate (sodium phytate), the enzyme used and its pH optimum, incubation temperature, and incubation times. Finally, it is important to note that the addition of Ca to the phytate solution, as well as the duration of mixing and stirring, has an impact on its final pH, which in turn may affect PP hydrolysis.

In the micro-mineral study, the *P* values, from the ANOVA analyses, for the effect of micro-mineral level and source on PP hydrolysis at pH 2.5 and 6.5, are given in **Table 2**. Significant three way interactions (level × source × pH) were noted for incubation times of 60 and 120 min, while for the 15 and 30 min incubations only two-way interactions were significant. Pairwise comparisons were done to study simple effects and results are shown in **Figures 1** and **2**. Irrespective of pH or micro-mineral source, addition of micro-minerals at levels as low as 1X reduced ($P < 0.05$) PP hydrolysis at all time periods versus the no micro-minerals added treatments.



	P value			
Ca level	<0.0001	<0.0001	<0.0001	<0.0001
PH	<0.0001	<0.0001	<0.0001	<0.0001
Ca level x pH	0.0002	<0.0001	<0.0001	<0.0001

Figure 1. Effect of calcium addition from $CaCO_3$ on in vitro phytate phosphorus hydrolysis (μg P released/unit phytase): (A) pH 2.5; (B) pH 6.5; (a–d) means within the same time period within each pH with no common superscript letter differ ($P < 0.05$), $n = 4$.

However, when higher levels of micro-minerals were added, the response of PP hydrolysis differed between micro-mineral sources and pHs.

At pH 2.5 (**Figure 2**), addition of IMM or MAAC at levels higher than 1X resulted in an additional decrease ($P < 0.05$) in PP hydrolysis. At this pH (2.5), addition of micro-minerals at 1, 5, 7.5, and 10X and incubating for 60 min, resulted in 20, 29, 41, and 54% reduction in PP hydrolysis, respectively when IMM was used, and 11, 47, 61 and 59%, respectively when MAAC was used. Differences ($P < 0.05$) in micro-mineral source were noted only for 5 and 7.5X micro-mineral additions at the 60 min incubation period, in which PP hydrolysis was lower when MAAC was added than when IMM was added.

At pH 6.5 (**Figure 3**), addition of IMM at levels in excess of 1X did not result in any additional reduction in PP hydrolysis. While addition of MAAC at levels in excess of 1X resulted in further reduction ($P < 0.05$) in PP hydrolysis, such that when 7.5 and 10 X MAAC were added no PP hydrolysis was detected. The differential effects between micro-mineral sources at different levels explain the source by level interaction that was noted. At this pH (6.5), addition of micro-minerals at levels of 1, 5, 7.5, and 10X and incubation for 60 min resulted in 60, 50, 70, and 68% reduction in PP hydrolysis, respectively when IMM was used and 66, 91, 100, and 100%, respectively when MAAC was used. A significant difference between the two micro-mineral sources was noted mainly for the 5, 7.5, and 10X at 120 min incubation.

In contrast to what was hypothesized, addition of micro-minerals, as amino acid complexes, did not improve PP hydrolysis as compared to addition of micro-minerals in an inorganic form. These findings were unexpected and can be partially explained by a weakness in the hypothesis. When the hypothesis was made, the ionization potential of the micro-mineral−amino acid complexes was not considered. It could be that, under the conditions studied, the MAAC minerals were ionized resulting in free mineral cations that were available to bind phytate, thus reducing PP hydrolysis. Several factors, including the micro-mineral and its concentration, the concentration of the chelating agent, and the stability of the complex at different pHs, can affect mineral availability (*35*). Also, it could be that the micro-mineral cations have higher affinity for the phytate molecule than for the chelating amino acid and can therefore dissociate from the amino acid and bind to phytate.

In Vivo Phytate Phosphorus Hydrolysis. Results of the in vivo experiment are summarized in **Table 3**. No Ca by micro-mineral interactions were observed for any of the parameters studied, and only Ca main effects were found. The addition of Ca to the diets resulted in less PP disappearing from the intestine, irrespective of micro-mineral addition (68.6% when Ca was not added vs 21.3% when Ca was added). Other researchers have reported similar results, in which Ca has reduced PP hydrolysis. An increase in dietary Ca in chick diets, from 1.2 to 5.2 g/kg, reduced PP hydrolysis from 55.0% to 5.6% (*10*). Furthermore, a reduction of dietary Ca level from 10.0 to 5.0 g/kg diet increased PP hydrolysis in chicks by 15% (*34*). Similar results were also reported by Sebastian et al. (*4*), who found that broiler diets with 12.5 g/kg Ca resulted in lower P retention compared to that of birds fed diets containing 10.0 g/kg Ca. These authors (*4*) attributed the effect of reduced P utilization at higher Ca concentration to one of three factors: (1) precipitation of phytate by Ca through Ca-phytate complex formation (*33*); (2) increased intestinal pH caused by Ca, which reduces mineral solubility, and therefore availability (*36*); or (3) the direct effect of Ca on phytase by competing for the active sites of phytase (*11*).

Apparent absorption of P followed the same trend as PP hydrolysis, where the addition of 5.0 g/kg Ca resulted in a 24.9% apparent P absorption, compared to 65.2% when Ca was not added. Because P comprises both PP and nPP, and because there was no addition of any other source of P to these diets, it was expected that apparent P absorption would increase as PP hydrolysis increased. Others have reported similar findings, in which an increase in apparent absorption of P was found when the Ca level in the diet of rats and hamsters was reduced from 10.8 g/kg to 5.1 g/kg (*37*).

Apparent absorption of Ca was higher ($P < 0.05$) when Ca was added to the diet than when Ca was not added (51.0 versus 45.1%, respectively). Similar findings have been reported, in which increasing dietary Ca from 5.1 g/kg to 10.8 g/kg increased apparent absorption of Ca (*37*). However, contrasting findings have also been reported, in which increased levels of dietary Ca reduced Ca retention, with maximum retention being observed at 6.0 g/kg Ca, compared to 10.0 g/kg (*38*). In the present study, the only source of Ca in the diets with no added Ca was that from feed ingredients. This Ca can be bound to phytates and oxalates, and this may reduce bioavailability as compared to that of supplemental inorganic sources such as $CaCO_3$ (*39*). The short experimental period used in the present work, in which intestinal Ca absorption and transport mecha-

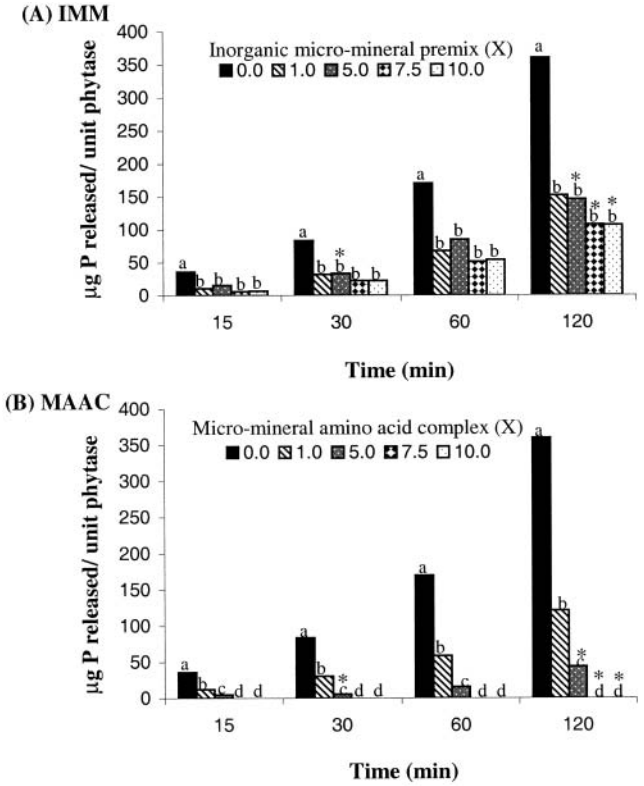


Figure 2. Effect of micro-mineral source and level on phytate phosphorus hydrolysis (μg P released/unit phytase) at pH 2.5: (A) inorganic micro-mineral premix (IMM); (B) micro-mineral–amino acid complex (MAAC). IMM supplied per 1X: 98 mg Ca from $CaCO_3$, 210 mg Zn from ZnO, 120 mg Mn from MnO and $MnSO_4$, 40 mg Fe from $FeSO_4$, 20 mg Cu from CuO, 3.0 mg I from $Ca(IO_3)_2$, and 0.05 mg Co from $CoCO_3$. (X is equivalent to 1 g/kg diet). MAAC supplied per 1X: 210 mg Zn from Availa-Zn 100, Zn–amino acid complex; 120 mg Mn from Availa-Mn 80, Mn–amino acid complex; 40 mg Fe from Availa-Fe 60, Fe–amino acid complex; 20 mg Cu from Availa-Cu 100, Cu–amino acid complex; 3.0 mg I from $Ca(IO_3)_2$; and 0.05 mg Co from $CoCl_2$.(X is equivalent to 4.5 g/kg diet). (a–d) Means within the same micro-mineral source and time period with no common superscript letter differ ($P < 0.05$), $n = 4$. * Designates significant difference between micro-mineral source within the same level and time period.

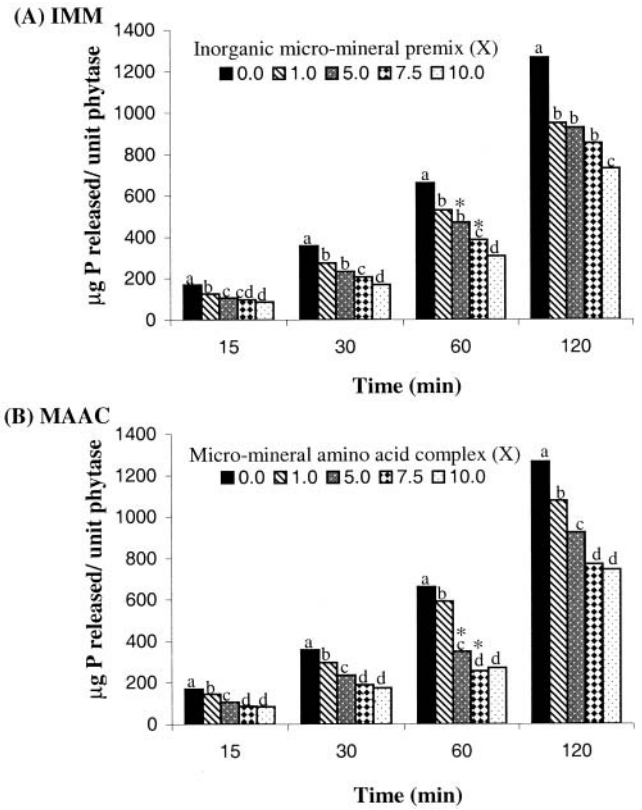


Figure 3. Effect of micro-mineral source and level on phytate phosphorus hydrolysis (μg P released/unit phytase) at pH 6.5: (A) inorganic micro-mineral premix (IMM); (B) micro-mineral–amino acid complex (MAAC). IMM supplied per 1X: 98 mg Ca from $CaCO_3$, 210 mg Zn from ZnO, 120 mg Mn from MnO and $MnSO_4$, 40 mg Fe from $FeSO_4$, 20 mg Cu from CuO, 3.0 mg I from $Ca(IO_3)_2$, and 0.05 mg Co from $CoCO_3$. (X is equivalent to 1 g/kg diet). MAAC supplied per 1X: 210 mg Zn from Availa-Zn 100, Zn–amino acid complex; 120 mg Mn from Availa-Mn 80, Mn–amino acid complex; 40 mg Fe from Availa-Fe 60, Fe–amino acid complex; 20 mg Cu from Availa-Cu 100, Cu–amino acid complex; 3.0 mg I from $Ca(IO_3)_2$; and 0.05 mg Co from $CoCl_2$. (X is equivalent to 4.5 g/kg diet). (a–d) Means within the same micro-mineral source and time period with no common superscript letter differ ($P < 0.05$), $n = 4$. * Designates significant difference between micro-mineral source within the same level and time period.

nisms did not have enough time to adapt to the lower Ca level in the diet, may also explain the contrasting findings of this study and those of Sebastian et al. (*38*). This time period was chosen based on reports that it takes more than 48 h for the chicken intestine to exhibit any functional changes in response to treatments (*22*, *23*). The main aim of this study was to examine the effect of Ca and micro-mineral source on PP hydrolysis in the intestinal tract, and thus it was essential that functional changes in the intestinal tract and whole body metabolic changes be minimized during the measured response period.

The fact that micro-minerals had a significant effect on PP hydrolysis in vitro but not in vivo may be attributed primarily to the use of sodium phytate as a substrate in the in vitro study compared to the phytate source used in the in vivo experiment. In the in vivo experiment, phytate was from corn and soybean meal, which would exist as mixed salts of K, Mg, and Ca phytate, where the PA molecule is already bound to the minerals and may be less available to bind additional diet minerals. Furthermore, sodium phytate exists only as IP6 (inositol ring with six phosphate groups), while in corn−soybean diets there is a small proportion of total PP present as IP5, IP4, IP3, or IP2 (inositol ring with five, four, three, and two phosphate groups, respectively). Such forms are more available to monogastric animals due to a higher solubility of the complexes formed between phytates and cations as the number of phosphate groups on the phytate molecule decreases (*40*) and are more easily hydrolyzed by non specific acid phosphatases. One more factor is that phytase was added in the in vitro study, while in the in vivo study, any phytase present would have come from ingredients, microbial, or intestinal origin. Finally, it is important to note that the in vivo system is more complex than the in vitro system, and the concentrations of micro-minerals added (1X) may not be high enough to give a measurable effect on PP disappearance.

From the results of the in vitro work, Ca and micro-minerals affected PP hydrolysis in a pH-dependent manner. While addition of Ca resulted in a large decrease in PP hydrolysis in vivo, the micro-minerals tested did not affect PP hydrolysis. Therefore, it would appear that birds have the potential to utilize PP; however, this ability is greatly influenced by the level of Ca in the diet. Because Ca is an important mineral needed by all animals, it would be important to find a delivery system or form of Ca to the animal that would not be reactive with the

Table 3. Effect of Added Calcium and Micro-Minerals on Apparent Calcium and Phosphorus Absorption and Phytate Phosphorus (PP) Disappearance in the Gastrointestinal Tract (up to the Ileum) of 22-Day-Old Broilers[a]

calcium added[b] g/kg	micro-mineral premix	PP disappearance[c] (%)	apparent phosphorus absorption (%)	apparent calcium absorption (%)
0	none	67.1	65.1	44.7
5.0	none	18.9	22.4	52.3
0	IMM[d]	70.3	66.5	47.4
5.0	IMM	21.3	25.1	50.8
0	MAAC[e]	68.5	64.0	43.2
5.0	MAAC	23.7	27.3	50.0
SEM		2.3	1.9	2.3
statistical analysis			probability > *F*	
calcium added		<0.0001	< 0.0001	0.0017
micro-minerals		0.39	0.50	0.48
calcium × micro-minerals		0.63	0.27	0.58
main effect means added calcium				
0		68.6[f]	65.2[f]	45.1[g]
0.5		21.3[g]	24.9[g]	51.0[f]
micro-mineral form				
none		43.0	43.8	43.8
IMM		45.8	45.8	45.8
MAAC		46.1	45.6	45.6

[a] All birds were fed the same starter diet from hatch to 20 days of age. Each mean represents eight replicate pens with 4 birds each. [b] Added from calcium carbonate. [c] Determined as PP in feed minus PP in ileal content, using Celite as a marker. [d] Supplied per kilogram of diet: 98 mg Ca from $CaCO_3$, 210 mg Zn from ZnO, 120 mg Mn from MnO and $MnSO_4$, 40 mg Fe from $FeSO_4$, 20 mg Cu from CuO, 3.0 mg Ca from $Ca(IO_3)_2$, and 0.05 mg Co from $CoCO_3$. Southern States Cooperative Inc., Richmond, VA. [e] Supplied per kilogram of diet: 210 mg Zn from Availa-Zn 100, Zn amino acid complex; 120 mg Mn from Availa-Mn 80, Mn amino acid complex, 40 mg Fe from Availa-60 Fe, Fe amino acid complex; 20 mg Cu from Availa-Cu 100 Cu, Cu amino acid complex; 3.0 mg I from $Ca(IO_3)_2$; and 0.05 mg Co from $CoCl_2$. Zinpro Availa series Zinpro Corporation, Eden Prairie, MN. [f−g] Means within a column with no common superscript letter differ ($P < 0.05$).

PA molecule. A Ca chelate, in which Ca has stronger affinity to the chelating molecule than to PA, would be a potential delivery system in which Ca remains complexed to the chelate during passage through the gastrointestinal tract. It is also very important that the Ca chelate either releases Ca close to the absorptive sites minimizing potential for complexing with phytate, or be absorbed as a complex, in which case, Ca must be released after absorption, so that it can be used by the animal. Increasing PP utilization by broilers would result in lower levels of inorganic P needed to be added to broiler diets and thus in lower total dietary P levels being used. The decreased need for inorganic P sources would result in less P being imported into regions where broiler production is high, lower dietary costs, and lower levels of excreta P; thus decreasing the environmental impact of broiler production as well as production costs.

ABBREVIATIONS USED

PA, phytic acid; PP, phytate phosphorus; IMM, inorganic micro-mineral premix; MAAC, micro-mineral−amino acid complexes; U, unit.

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