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Effect of Phytase Superdoses and Citric Acid on Growth Performance, Plasma Phosphorus and Tibia Ash in Broilers Fed Canola Meal-Based Diets Severely Limited in Available Phosphorus

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Abstract

This experiment was conducted to investigate the effect of phytase superdoses alone or in combination with citric acid (CA) in canola meal-based diets severely limited in available phosphorus (P_a) on growth performance, plasma phosphorus (P), and tibia ash (TA) in broilers from 22 to 42 d of age. Two hundreds and eighty 21-d-old male broilers were used in 28 pens of 10 birds per each. The experimental diets consisted of a positive control (PC) diet and six negative control (NC) diets which consisted of two levels of CA (0 and 20 g/Kg) and three levels of phytase (0, 1000 and 4000 U/Kg) in a 2 × 3 factorial arrangement. The PC diet contained 4.3 g/Kg P_a, but all NC diets contained 1.5 g/Kg P_a. Results indicated that the birds fed the PC diet had a significantly higher average daily gain (ADG), plasma P and TA, but a lower feed conversion ratio (FCR) than those fed the NC diet. The ADG, FCR and plasma P values in birds fed NC diets supplemented with 4000 U/Kg phytase enzyme (with or without CA) significantly reached those of birds fed the PC diet. But, addition of phytase enzyme at 1000 U/Kg only plus CA to the NC diet could significantly improve FCR and plasma P. A significant interaction was observed between phytase and CA for FCR and plasma P. Although TA values in NC + 1000 U/Kg phytase treatments (with or without CA) were similar to the PC treatment, TA values of NC + 4000 U/Kg phytase treatments (with or without CA) was greater than that of the PC treatment. Results of this study showed that, in severely limited P_a corn-canola meal-based diets, supplementing 4000 U/Kg phytase or also 1000 U/Kg phytase plus CA will be sufficient to obtain the comparable feed efficiency in broilers to those fed the adequate P_a diet.

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Introduction

Approximately two-third of P in feedstuffs of plant origin presents in the form of phytate which is poorly digested by endogenous enzymes of monogastric animals. Consequently, inorganic sources of P, which are expensive, are supplemented to the diets to meet P needs for poultry. Additionally, phytate decreases nutrient digestibility and is excreted to environment, leading to environmental pollution. The negative effects of phytate on nutrients utilization can be partly ameliorated by exogenous phytase, which degrades phytate (Selle and Ravindran, 2007). Phytase increases P digestibility and utilization and hence reduces P excretion into the environment (Shirley and Edwards, 2003). The standard use of exogenous phytase as a source of dietary P often limits its inclusion in broiler diets to approximately 500 U/Kg. Obviously, P is not completely removed from phytate by phytase supplementation to poultry diets (Olukosi *et al.,* 2007; Woyengo *et al.,* 2008).

Several methods including dietary supplementation with phytase superdoses and citric acid (CA), which has a potential to increase the release of P from phytate, have been investigated. It has been shown that CA improves the efficiency of microbial phytase, because it can chelate cations like Ca and Mg that form insoluble complexes with phytate, thereby increasing phytate solubility (Maenz *et al.,* 1999). CA reduces the pH of the digesta (Radcliffe *et al.,* 1998), which then increases the dissociation between phytate and minerals (Maenz *et al.,* 1999) and enhances the activity of phytase, which expresses its optimal activity at a low pH (Simon and Igbasan, 2002). The improvement of P utilization in broilers due to addition of CA to a phytase-supplemented corn-soybean meal-based diet has been reported in the literature (Snow *et al.,* 2004; Woyengo *et al.,* 2010).

For increasing the release of P from phytate, many studies have focused on the use of phytase superdoses in soybean meal-based diets limited in available P up to 1000 (Dilger *et al.,* 2004; Jendza *et al.,* 2006; Liu *et al.,* 2008, 2010), 1500 (Walk *et al.,* 2013, 2014), 2000 (Shaw *et al.,* 2011; Rutherfurd *et al.,* 2012; Gehring *et al.,* 2013); 2500 (Zhang *et al.,* 2000; Pirgozliev *et al.,* 2007, 2008, 2009; Walk *et al.,* 2012; Pirgozliev and Bedford, 2013;), 4000 (Han *et al.,* 2009) or 12000 (Shirley and Edwards, 2003) U/Kg. It seems that addition of phytase at 6000 (Shirley and Edwards, 2003) or 4000 (Taheri *et al.,* 2015) U/Kg of the diet severely limited in available phosphorus improves growth performance of the birds to the similar extent to those fed the control. However, high levels of phytase may not be always cost effective. On the other hand, based on our information, there is a lack of information of phytase superdoses in canola-meal-based diets limited in available P.

Canola meal is compromised of 8.7 (NRC, 1994) or 8.22 (Leske and Coon, 1999) g/Kg phytate whereas soybean meal contains only 3.8 (NRC, 1994) or 3.96 (Leske and Coon, 1999) g/Kg. With regard to the high content of phytate in canola meal, it was hypothesized that, canola meal-based diets may increase the chance of

detecting more improvement in performance of broilers at phytase doses lower than 4000 U/Kg of the diet. There is a lack of information regarding phytase superdoses in canola-meal-based diets limited in available P. In addition, there is a lack of information on the effect of supplementing a combination of phytase superdoses and CA to a diet limited in available P on the performance of poultry.

Therefore, in the present study, the effect of phytase supplementation alone or in combination with CA in corn-canola meal-based diets contained insufficient P_a was evaluated compared with a control diet containing sufficient P_a in broilers from 22 to 42 d post hatch.

Materials and Methods

Birds, husbandry and experimental diets

Two hundred eighty 21-d-old Ross 308 male broilers were distributed into 28 pens and reared from 22 to 42 d of age. All birds received a regular diet containing 5.0 and 4.5 g/Kg P_a, respectively, from 1-10 and 11-21 d of age. Mean body weight of the birds in all pens was similar (750 ± 15 g) at the beginning of the experiment (22 d of age). Each treatment had 4 replicates of 10 broilers each. Birds were reared in floor pens (1.5 × 1.5 m) and in an environmentally controlled house and they were exposed to a 23L:1D lighting cycle. The experimental birds were given *ad libitum* access to water and mash diets.

The National Research Council's nutrient values for ingredients (NRC, 1994) were used to formulate the diets with the exception of CP. Since the CP values of the ingredients can be variables, therefore, it was analyzed for corn, soybean meal and canola meal, and the amino acids compositions of these ingredients were calculated based on equations (NRC, 1994). Two corn-canola meal-based diets were formulated to meet Ross 308 broiler nutrient requirements with an adequate (positive control, PC) or inadequate (negative control, NC) quantity of P_a from 22 to 42 d of age (Aviagen, 2009; Table 1). The NC diet was supplemented with two levels of CA (0 and 20 g/Kg) and three levels of phytase (0, 1000 and 4000 U/Kg) in a 2 × 3 factorial arrangement making six diets. All six diets were kept at a P_a level of 1.5 g/Kg. In addition, the PC diet was composed of 4.3 g/Kg P_a. The supplemental phytase was a commercially available Natuphos phytase (BASF, Mt. Olive, NJ) that was derived from *Aspergillus niger* and had an enzyme activity of 1,000 U/g phytase.

Sample collection

Birds were weighed before placement (d 21) and on d 42 to calculate ADG. Feed intake was also measured during d 22 to 42 and used to calculate average daily feed intake (ADFI) and FCR. On d 32, two birds/pen (eight birds/treatment) were randomly selected to obtain the blood samples via wing vein. The plasma was prepared and stored at -20°C for the further analysis. The concentration of inorganic P in the plasma samples were analyzed in duplicate using a

spectrophotometer following the instructions of corresponding reagent kits (Pars Azmoon, Iran).

On d 42, one bird/pen (four birds/treatment) was randomly slaughtered to obtain the tibia samples. Soft tissue was stripped off the bone, and the tibias were dried overnight at 100°C, extracted in ether for 6 h, and ashed in a muffle furnace for 15 h at 540°C. Tibia ash (TA) percentage was calculated as: [tibia ash weight/dry defatted tibia weight] × 100.

Table 1. Ingredients and chemical composition of control diets (as fed-basis)

Ingredients (g/Kg)	Positive control	Negative control
Corn (8.5 g/Kg CP)	457.4	457.4
Soybean meal (41.5 g/Kg CP)	105.0	105.0
Canola meal (31.5 g/Kg CP)	300.0	300.0
Corn oil	85.0	85.0
Dicalcium phosphate	15.5	-
Calcium carbonate	7.5	16.4
Sand	15.0	21.6
Common Salt	2.0	2.0
Sodium bicarbonate	4.0	4.0
L-Threonine	0.2	0.2
DL-Methionine	1.2	1.2
L-Lysine HCl	2.2	2.2
Vitamin premix[1]	2.5	2.5
Mineral premix[2]	2.5	2.5
Calculated composition		
ME (Kcal/Kg)	3150	3150
Crude protein (g/Kg)	180	180
Lysine (%)	10.6	10.6
Methionine + Cystine (g/Kg)	8.3	8.3
Threonine (g/Kg)	7.3	7.3
Calcium (g/Kg)	9.0	9.0
Available phosphorus (g/Kg)	4.3	1.5
Total phosphorus (g/Kg)	8.3	5.5
Sodium (g/Kg)	2.1	2.1

[1]The vitamin premix supplied the following per Kg of complete feed: Vitamin A, 9,000 IU (retinyl acetate); Cholecalciferol, 2,000 IU; Vitamin E, 18 IU (dl-α-tocopheryl acetate); Vitamin B_{12}, 0.015 mg; Menadione, 2 mg; Riboflavin, 6.6 mg; Thiamine, 1.8 mg; Pantothenic acid, 30 mg; Niacin, 10 mg; Choline, 500 mg; Folic acid, 1 mg; Biotin, 0.1 mg; Pyridoxine, 3 mg.

[2]The mineral premix supplied the following per Kg of complete feed: Manganese ($MnSO_4 \cdot H_2O$), 80 mg; Zinc (ZnO), 80 mg; Iron ($FeSO_4 \cdot 7H_2O$), 80 mg; Copper ($CuSO_4 \cdot 5H_2O$), 10 mg; Selenium (Na_2SeO_3), 0.3 mg; Iodine (Iodized NaCl), 0.8 mg; Cobalt ($CoCl_2$), 0.25 mg.

Statistical analysis

All data were analyzed in a completely randomized design using GLM procedure of SAS (SAS, 2003). Pen was the experimental unit, except for TA in

which the individual bird was the experimental unit. Ash data presented as percentages were transformed to their arcsine square root before statistical analysis, and the non-transformed data are presented in the table. The data of six NC treatments were additionally analyzed as a 2 × 3 factorial arrangement to determine the main effects of CA, phytase and their interactions. The treatment means were compared using LSD at $P<0.05$.

Results and discussion

The effects of phytase and CA on growth performance are presented in Table 2. Overall (d 22 to 42) mortality was approximately less than 4%, and not related to dietary treatments ($P>0.05$; data not shown). Birds fed the PC diet had a higher ADG, and also lower FCR than those of the birds fed the NC diet, confirming that the NC diet was indeed P_a-deficient. Phytase supplementation significantly affected ADG and FCR, while CA addition did not influence these parameters. The beneficial effect of phytase superdoses on feed efficiency or weight gain have been observed when supplemented to soybean meal-based diets limited in P_a (Shirley and Edwards, 2003; Han *et al.,* 2009; Liu *et al.,* 2010; Shaw *et al.,* 2011; Walk *et al.,* 2012, 2013, 2014; Pirgozliev and Bedford, 2013). However, there are a few research that show no significant effect on feed efficiency when phytase was supplemented to the NC diet (Pirgozliev *et al.,* 2007; Rutherfurd *et al.,* 2012; Gehring *et al.,* 2013).

There was no significant difference of ADFI among treatments. A deficiency of P_a in chickens is characterized by a reduction in feed intake and reduced circulating levels of growth hormone (Parmer *et al.,* 1987). The lack of significant difference in ADFI between treatments may indicate that young birds may be more susceptible to an P_a deficiency than older birds which were used in our trials. Walk *et al.* (2013) also found P_a deficiency and phytase addition did not affect feed intake even in chicks from 1 to 21 d of age. There are some studies that showed no significant difference of feed intake when phytase was supplemented to P_a deficient diets (Pirgozliev *et al.,* 2010; Shaw *et al.,* 2011; Chung *et al.,* 2013; Walk *et al.,* 2013), however, other researchers found that feed intake was influenced when phytase was supplemented to NC (Shirley and Edwards, 2003; Han *et al.,* 2009; Liu *et al.,* 2010; Rutherfurd *et al.,* 2012; Walk *et al.,* 2012, 2014; Pirgozliev and Bedford, 2013).

The ADG and FCR values of the NC diets supplemented with 4000 U/Kg (with or without CA) significantly reached those of the PC diet. However, addition of phytase at 1000 U/Kg plus CA to the NC diet improved FCR significantly compared to the PC diet. An interaction effect was observed between phytase and CA for FCR. In the present study, it had been assumed that high phytate content of canola meal may increase diets potential responding more to lower doses of phytase supplementation, but the results of ADG and FCR were similar to those obtained from soybean meal-based diets (Taheri *et al.,* 2015) which contained lower level of phytate. This observation may be similar to the findings of Leske and Coon

(1999) who showed that phytase more readily hydrolyses phytate in soybean meal (72.4%) than in canola meal (55.8%), although the latter contained higher levels of phytate. On the other hand, the results of FCR show that, if CA is supplemented (at 20 g/Kg) to severely limited P_a diets based on corn-canola meal, a lower level of phytase (1000 U/Kg) can be sufficient to obtain the comparable FCR to those fed the NC + 4000 U/Kg phytase or the PC diet. In agreement with our results, Snow *et al.* (2004) reported the improved growth performance of broilers due to the addition of CA to a phytase (300 U/Kg) supplemented low P_a diet.

Table 2. Effect of phytase superdoses and citric acid on growth performance of male broilers from 22 to 42 d post hatch

	ADG[1] (g)	ADFI[2] (g)	FCR[3]
Treatments:			
Negative control (NC)	52.7^{c}	98.4	1.86^{b}
NC + 20 g/Kg citric acid	52.0^{c}	108.3	2.08^{c}
NC + 1000 U/Kg phytase	57.5^{bc}	107.7	1.86^{b}
NC + 1000 U/Kg phytase + 20 g/Kg citric acid	57.2^{bc}	96.7	1.70^{ab}
NC + 4000 U/Kg phytase	62.7^{ab}	105.1	1.68^{ab}
NC + 4000 U/Kg phytase + 20 g/Kg citric acid	61.5^{ab}	104.4	1.69^{ab}
Positive control (PC)	65.9^{a}	102.8	1.55^{a}
SEM	2.55	6.18	0.072
Phytase (U/Kg):			
0	52.3^{b}	103.4	1.97^{b}
1000	57.4^{ab}	102.2	1.78^{a}
4000	62.1^{a}	104.7	1.69^{a}
SEM	1.74	4.51	0.054
Citric acid (g/Kg):			
0	57.6	104.2	1.80
20	56.9	103.2	1.82
SEM	1.43	3.69	0.044
P-values			
Treatments	0.005	0.74	0.001
Phytase effect	0.005	0.93	0.007
Citric acid effect	0.73	0.91	0.750
Phytase × Citric acid	0.98	0.24	0.040

[1]Average daily gain; [2]Average daily feed intake; [3]Feed conversion ratio.

[a-c] Means, within a column, with different superscripts differ significantly ($P<0.05$).

Table 3. Effect of phytase superdoses and citric acid on plasma P (on d 32) and tibia ash (on d 42) of male broilers

	Plasma P (mg/dL)	Tibia ash (%)
Treatments:		
Negative control (NC)	3.7[c]	32.7[c]
NC + 20 g/Kg citric acid	3.6[c]	35.9[c]
NC + 1000 U/Kg phytase	3.9[bc]	41.5[b]
NC + 1000 U/Kg phytase + 20 g/Kg citric acid	4.7[a]	41.0[b]
NC + 4000 U/Kg phytase	4.4[ab]	46.8[a]
NC + 4000 U/Kg phytase + 20 g/Kg citric acid	4.8[a]	47.1[a]
Positive control (PC)	4.8[a]	42.3[b]
SEM	0.18	1.43
Phytase (U/Kg):		
0	3.7[b]	34.3[c]
1000	4.3[a]	41.2[b]
4000	4.6[a]	46.9[a]
SEM	0.12	1.09
Citric acid (g/Kg):		
0	4.0[b]	40.2
20	4.4[a]	41.4
SEM	0.10	0.90
P-values		
Treatments	0.0002	<0.001
Phytase effect	0.0001	<0.001
Citric acid effect	0.02	0.490
Phytase × Citric acid	0.02	0.370

[a-c] Means, within a column, with different superscripts differ significantly (*P<0.05*).

Table 3 presents the plasma P (on d 32) and TA content (on d 42) of broilers. Birds fed the PC diet had significantly higher plasma P and TA than those of the birds fed the NC diet. Phytase supplementation significantly affected plasma P and TA, while CA addition increased only the plasma P. The plasma P value of the NC diets supplemented with 4000 U/Kg (with or without CA) significantly reached that of the PC diet. However, addition of phytase at 1000 U/Kg plus CA to the NC increased plasma P significantly comparable to that of the PC deit. A significant interaction effect between phytase and CA for plasma P was observed. The effect of phytase supplementation on plasma P has been reported in the previous research (Shirley and Edwards, 2003; Han *et al.,* 2009; Liu *et al.,* 2010). Contrary to our results, phytase supplementation even at 4000 U/Kg did not increased plasma P in soybean meal-based diets containing 1.4 g/Kg P_a to the similar extent of those fed the PC (Taheri *et al.,* 2015). Shirley and Edwards (2003) also showed that phytase supplementation up to 6000 U/Kg in P_a deficient diets did not increase

plasma P compared with the PC. They found phytase addition only at 12000 U/Kg to the NC enhanced the plasma P to the similar extent of that obtained by the PC. With regard to the high content of phytate in canola meal (NRC, 1994; Leske and Coon, 1999), it seems that 4000 U/Kg phytase or 1000 U/Kg phytase plus CA are sufficient to increase plasma P in broilers fed severely P_a limited diets. In the present study, the improvement in feed efficiency seems to be due to the phytase that increases the plasma P. Nevertheless, in soybean meal based diets, reduction of anti-nutritional effects of phytate may be involved in improvement of broilers performance as well (Taheri *et al.,* 2015).

Although TA values of the NC + 1000 U/kg phytase treatments (with or without CA) were significantly similar to the values of the PC treatment, TA of the NC + 4000 U/Kg phytase treatments (with or without CA) were significantly more than that of the PC treatment. The results of TA were in agreement with those obtained by soybean meal-based diets (Taheri *et al.,* 2015). Other studies also have shown that bone ash content was increased by graded levels of phytase supplementation (Zhang *et al.,* 2000; Dilger *et al.,* 2004; Han *et al.,* 2009; Shaw *et al.,* 2011; Rutherfurd *et al.,* 2012; Walk *et al.,* 2012, 2013, 2014). Although the overall response of phytase addition to low P_a diets between the previous research (Shirley and Edwards, 2003; Han *et al.,* 2009) and this study are similar, the degree of response differs. These differences are most likely due to the differences in processing of the tibia for ash analysis, Ca, total P and P_a contents of the diets used, variable levels of supplemental vitamin D, the environment in which the experiment was conducted, type of strains used, or a combination of them. Greater response of TA in NC + 4000 U/Kg phytase treatment may be related to the non-phosphoric and inositol effects of phytase superdoses (Cowieson *et al.,* 2011).

In conclusion, the results of this study showed that, in severely limited P_a corn-canola meal-based diets, supplementing 4000 U/Kg phytase or also 1000 U/Kg phytase plus CA will be sufficient to obtain comparable feed efficiency in broilers to those fed the adequate available P diet.

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