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Adding Phytase Enzyme to Low Phosphorus Broiler Diets and its Effect upon Performance, Bone Parameters and Phosphorus Excretion

[1]M.O. Abd-Elsamee, [1]A.E. El-Sherbiny, [2]H.M.A. Hassan, [2]A. Samy and [2]M.A. Mohamed
[1]Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt
[2]Department of Animal Production, National Research Centre, 12622, Dokki, Egypt

Corresponding Author: M.O. Abd-Elsamee, Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt

ABSTRACT

A broiler growth experiment was conducted using 360 one-day old Ross 308 chicks to study the effect of reducing dietary phosphorus and adding phytase enzyme. Two diets were formulated in starting period (1-20 days): a control diet contained 0.50% Available Phosphorus (AP) and a low P diet contained 0.40% AP. Such low P diet was fed without or with phytase supplementation (500 IU kg^{-1}). At the growing period (21-35 days) every group of birds of the first period was divided into two sub-groups. Two grower diets were formulated, a control diet contained 0.40% AP and a low P diet contained 0.30% AP. The low AP diet was offered with phytase supplementation (500 IU kg^{-1}). Growth performance, bone parameters and P excretion were measured at 20 and 35 day of age. No significant differences on chick performance among dietary treatments during the starting and growing periods were observed. The best FCR value was recorded for birds fed 0.50% AP diet in the first period then grown on 0.30% AP diet+phytase. Reducing dietary AP content did significantly ($p<0.05$) affect bone parameters at 20 and 35 day of age. Addition of phytase did alleviate such effect. Tibia Ca and P content significantly ($p<0.001$) increased by phytase supplementation. Phosphorus excretion decreased more than 20% at staring period and more than 30% at growing period when broilers were fed low P diets supplemented with phytase. The results showed pronounced beneficial effect regarding the excreted P. Supplementing phytase enzyme to broiler diets renders the dietary phosphorus contents more available to the birds. Therefore, the amount of supplemental phosphorus could be remarkably reduced. It could be concluded that reducing dietary P level and using phytase enzyme could limit quantity of excreted P from broilers without adverse effect on performance. This reduces such impact in environmental pollution.

Key words: Broilers, low available phosphorus, phytase, bone, phosphorus excretion

INTRODUCTION

Broiler diets contain large amounts of P in form of phytates that accounts for 60-80% of the total phosphorus in plant feeds. The phytate-bound phosphorus is largely unavailable to monogastric animals as they do not naturally have the enzyme needed to break it down-phytates (Bozkurt *et al.*, 2006). Thus, inorganic phosphorus is added to their feed to meet phosphorus requirements for optimal growth and production. Therefore, a large portion of dietary phosphorus is excreted. Such excess P in the manure has posed an environmental concern (Ravindran *et al.*, 2000). The high levels of phosphorus in surface waters increase the growth of algae and bacteria that in turn consume a greater proportion of the oxygen in the water, resulting in the death of many aquatic species through hypoxia or anoxia (Correll, 1999).

Several ways can be adopted on diet formulation to reduce the concentration of P in excreta. In conjunction with feeding closer to requirements, the addition of exogenous enzymes enhances P availability and thus P use by the broiler from diets fed can substantially decrease P in excreta (Angel *et al.*, 2005, 2006).

Such manipulation of P levels in diets supplemented with phytase can reduce P excretion and minimizing environmental pollution (Angel *et al.*, 2006; Selle and Ravindran, 2007). Meanwhile, phytase addition to broiler diets containing lower Ca and P increased mineral absorption and increased their utilization (Santos *et al.*, 2008; Zhou *et al.*, 2008; Bingol *et al.*, 2009). Also, adding phytase to broiler diet increased tibia ash, Ca and P percentages, while decreased Ca and P% in the manure (El-Deek *et al.*, 2009). So, dietary supplementation of phytase improved minerals utilization, thus playing an important role in reducing P environmental pollution. Woyengo *et al.* (2010) studied the effect of supplemented a corn-soybean meal-based diet with phytase (600 IU kg^{-1}) on nutrient utilization. Phytase improved ($p<0.05$) ileal digestibility of P and improved P utilization in broiler.

The objective of this study was to further investigate the influence of reducing dietary available phosphorus and phytase supplementation on performance, bone measurements and percentage of Ca and P excretions of broiler chicks.

MATERIALS AND METHODS

A broiler growth experiment was conducted using total 360 (one-day old Ross 308) chicks to study the effect of reducing dietary phosphorus and phytase supplementation on performance, bone parameters and calcium and phosphorus excretion. Phyzyme™ XP contained 500 IU activity/g, produced by Danisco Animal Nutrition, United Kingdom, was used.

The experiment lasted two periods. At the starting period (from 1-20 days), two diets were formulated: a control diet contained 0.50% AP and a low P diet contained 0.40% AP. Such low P diet was fed without or with phytase (500 IU kg^{-1}). Though, 3 dietary treatments were performed. Table 1 shows the formulation and nutrient composition of the different starter diets. These diets were fed to three groups of 120 (one-day old Ross 308) broiler chicks (6 replicates of 20 chicks each).

At the growing period (21-35 days) every group of birds of the starting period was divided into two sub-groups. Two grower diets were formulated, a control diet containing 0.40% AP and a low P diet containing 0.30% AP. The low AP diet was offered with phytase (500 IU kg^{-1}). Though, six dietary treatments were performed. Every dietary treatment was fed to six groups of 50 chicks each (5 replicates of 10 chicks each). Formulation and nutrient composition of the growing diets are shown in Table 2.

Replicates were randomly allocated in batteries and gas heaters were used to keep the required temperature for the brooding period. Light was provided 23 h daily throughout the experimental period. Feed and water were allowed for *ad libitum* consumption.

Throughout the experiment, birds were vaccinated against avian influenza, New Castle, IB and IBD. After such medical treatments a dose of vitamins (A, D_3 and E) was offered in the drinking water for the successive 3 days.

Table 1: Formulation and nutrient composition of the starter (1-20 days) diets

Items	Control diet (0.5% AP)	Low-P diet (0.4% AP)	Low-P diet +phytase (0.4% AP +phytase)
Yellow corn	56.30	56.64	56.54
Soybean meal (48%)	31.50	31.50	31.50
Corn gluten meal (60%)	6.00	6.00	6.00
Soybean oil	1.80	1.70	1.70
Dicalcium phosphate	2.00	1.44	1.44
Limestone	1.20	1.52	1.52
Vitamin and Mineral mix[1]	0.30	0.30	0.30
NaCl	0.30	0.30	0.30
L-lysine HCl	0.24	0.24	0.24
DL-methionine	0.26	0.26	0.26
Threonine	0.10	0.10	0.10
Phytase	-	-	0.10
Total	100.00	100.00	100.00
Calculated composition[2] (%)			
Crude protein (%)	23.90	23.98	23.98
ME (kcal kg^{-1})	3058	3060	3060
Lysine (%)	1.40	1.40	1.40
Methionine (%)	0.66	0.66	0.66
Methionine+Cystine (%)	1.07	1.07	1.07
Threonine (%)	0.92	0.92	0.92
Calcium (%)	1.00	1.00	1.00
Nonphytate P (%)	0.50	0.40	0.40

[1]Vitamin-mineral mixture supplied per kg of diet: Vit. A: 12000 IU, Vit. D_3: 2200 IU, Vit. E: 10 mg, Vit. K_3: 2 mg, Vit. B_1: 1 mg, Vit. B_2: 4 mg, Vit. B_6: 1.5 mg, Vit. B_{12}: 10 µg, Niacin: 20 mg, Pantothenic acid: 10 mg, Folic acid: 1 mg, Biotin: 50 µg, Choline chloride: 500 mg, Copper: 10 mg, Iodine: 1 mg, Iron: 30 mg, Manganese: 55 mg, Zinc: 50 mg, Selenium: 0.1 mg, [2]According to NRC (1994)

At 20 and 35 days of age, after fasting overnight, birds were individually weighed and feed consumption was recorded per replicate. Body weight gain and feed conversion ratio were calculated for the starting and growing period.

Determination of calcium and phosphorus excretion: At 20 and 35 days of age, samples of excreta from all treatments were collected to determine calcium and phosphorus excretion. The dried excreta from each replicate for the successive 3 days collection period were pooled, finely ground, well mixed and placed in a screw-top glass jar for Ca and P determination. Calcium and phosphorus were determined in excreta and dried fat-free bones ash based on the Official Methods of Analysis (AOAC, 1995).

Determination of bone parameters: At 20 and 35 days of age five birds per treatment were slaughtered and processed for bone measurements. The right tibia were removed and prepared for the different measurements as described by Potter *et al.* (1995) and Ravindran *et al.* (1995). Tibia was cleaned of all adhering flesh, extracted with ethanol and then with diethyl ether. After recording the overall weight, length and width of tibia, bones were oven dried at 105°C for constant weight. The dried fat-free bones were ashed in a muffle furnace at 600°C for 6 h. Samples of dried fat-free tibia were kept for wet ashing to determine P content. Tibia ash was expressed as a percentage of the fat-free dry weight. Tibia breaking strength was measured on apparatus Digital Force Gauge and expressed in kilograms force necessary for bone to be broken (Masic *et al.*, 1985).

Table 2: Formulation and nutrient composition of the grower (21-35 days) diets

	0.5% AP in the first period		0.4% AP in the first period		0.4% AP+phytase in the first period	
Ingredients (%)	Control diet (0.4% AP)	Low P diet (0.30% AP+phytase)	Control diet (0.4% AP)	Low P diet (0.30% AP+phytase)	Control diet (0.4% AP)	Low P diet (0.30% AP+phytase)
Yellow corn	62.47	62.72	62.47	62.72	62.47	62.72
Soybean meal (48%)	26.00	26.00	26.00	26.00	26.00	26.00
Corn gluten meal (60%)	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.70	2.60	2.70	2.60	2.70	2.60
Dicalcium phosphate	1.52	0.97	1.52	0.97	1.52	0.97
Limestone	1.30	1.60	1.30	1.60	1.30	1.60
Vitamin and Mineral mix[1]	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.16	0.16	0.16	0.16	0.16	0.16
DL-methionine	0.19	0.19	0.19	0.19	0.19	0.19
Threonine	0.06	0.06	0.06	0.06	0.06	0.06
Phytase	-	0.10	-	0.10	-	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition[2] (%)						
Crude protein (%)	21.08	21.10	21.08	21.10	21.08	21.10
ME (kcal kg^{-1})	3165	3165	3165	3165	3165	3165
Lysine (%)	1.17	1.17	1.17	1.17	1.17	1.17
Methionine (%)	0.55	0.55	0.55	0.55	0.55	0.55
Methionine+Cystine (%)	0.90	0.90	0.90	0.90	0.90	0.90
Threonine (%)	0.78	0.78	0.78	0.78	0.78	0.78
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate P (%)	0.40	0.30	0.40	0.30	0.40	0.30

[1]Vitamin-mineral mixture supplied per kg of diet: Vit. A: 12000 IU, Vit. D_3: 2200 IU, Vit. E: 10 mg, Vit. K_3: 2 mg, Vit. B_1: 1 mg, Vit. B_2: 4 mg, Vit. B_6: 1.5 mg, Vit. B_{12}: 10 µg, Niacin: 20 mg, Pantothenic acid: 10 mg, Folic acid: 1 mg, Biotin: 50 µg, Choline chloride: 500 mg, Copper: 10 mg, Iodine: 1 mg, Iron: 30 mg, Manganese: 55 mg, Zinc: 50 mg and Selenium: 0.1 mg, [2]According to NRC (1994)

Data were statistically analyzed for analysis of variance using the General Liner Model of SAS (2000). Significant differences among treatment means were separated by Duncan's new multiple rang test (Duncan, 1955) with a 5% level of probability.

RESULTS AND DISCUSSION

Table 3 shows the effect of reducing dietary Available Phosphorus (AP) and phytase supplementation on performance of broiler chicks. At the end of the starting period (20 days of age), no significant differences were detected on performance among dietary treatments. Birds fed 0.40% AP diet with phytase supplementation gave slight ($p>0.05$) increase in Body Weight Gain (BWG) compared to the other treatments.

At the end of the experiment (35 days of age), also no significant differences were observed on performance among dietary treatments. The best value of Feed Conversion Ratio (FCR) was recorded for birds fed with 0.50% AP diets in the first period then grown on diet contained 0.30% AP supplemented with phytase.

These results revealed that dietary AP could be reduced to 0.40% in the starting period and to 0.30% in the growing period without adverse effect on broiler performance. During the growing period, addition of phytase to 0.30% AP diet did enhance FCR (Table 3).

Table 3: Performance of broiler at starting (1-20 days) and growing (21-35 days) period as affected by phosphorus level and phytase supplementation

Item		Body weight gain (g)	Feed intake (g)	Feed conversion ratio (feed g/ gain g)
Starting period				
AP (%)				
0.50		456	620	1.36
0.40		451	620	1.37
0.40+		466	642	1.38
SE of means		±1.96	±14.25	±0.01
Significance		ns	ns	ns
Growing period				
AP% starting	AP% growing	Body weight gain (g)	Feed intake (g)	Feed conversion ratio (feed g/ gain g)
0.50	0.40	1035	1739	1.68
	0.30+	1005	1651	1.64
0.40	0.40	1006	1673	1.66
	0.30+	1036	1730	1.67
0.40+	0.40	1012	1679	1.66
	0.30+	1013	1721	1.70
SE of means	±5.30	±14.06	±0.01	
Significance	ns	ns	ns	

Means within each column for each effect with no common superscript are significantly different at p<0.05, ns: Not significant, +Plus 500 IU phytase

Different results have been reported on reducing dietary AP and/or using phytase. El-Sherbiny *et al.* (2005) and Mohamed *et al.* (2005) found that increasing dietary AP level by 0.1% or adding phytase at a level of 500 U kg^{-1} feed significantly ($p<0.05$) improved growth performance of broiler chicks. Also, Mondal *et al.* (2007) reported that FCR of broilers fed low phosphorus diet plus phytase was significantly better ($p<0.05$) than that of broilers fed low phosphorus without phytase. Bingol *et al.* (2009) concluded that dietary AP can be reduced down to 30% in broiler diets with phytase supplementation without affecting broiler performance.

Results of bone parameters (tibia length, weights, width, breaking strength, tibia ash percentages, Ca and P content) measured at 20 and 35 days of age are presented in Table 4. At 20 days of age, reducing dietary AP level from 0.50 to 0.40% significantly decreased length ($p<0.01$), weight ($p<0.01$), width ($p<0.001$) and breaking strength ($p<0.05$) of tibia. Addition of phytase did alleviate such effect. No significant differences were detected on such measurements between birds fed 0.50% AP diet and those fed 0.40% AP plus phytase.

Reducing dietary AP levels decreased tibia ash content. This effect disappeared when phytase was added to the diet. The results of Ca and P content of tibia followed the same trends as ash %. Reducing AP levels significantly ($p<0.001$) decreased Ca of tibia and this effect disappeared when phytase was added to the diet.

Birds fed 0.40% AP diet with phytase showed significant ($p<0.001$) higher tibia Ca compared to birds fed the control diet (0.50% AP) without phytase. Reducing dietary AP without or with phytase did not significantly affect tibia P content.

At 35 days of age, feeding low AP diets without or with phytase did not significantly affect weight, length, width and breaking strength of tibia compared to the control. Tibia ash percentage showed significant ($p<0.001$) differences among dietary treatments. Birds fed 0.40% AP with phytase in the starting period and 0.30% AP with phytase in the growing period recorded the highest ash percentage of tibia while, those fed the control diet (0.50% AP in the starting period

Table 4: Bone measurements of broiler at 20 and 35 days old as affected by phosphorus level and phytase supplementation

Item		Tibia length (cm)	Tibia weigh (g)	Tibia width (cm)	Tibia breaking strength (kgf)	Tibia ash (%)	Tibia Ca (%)	Tibia P (%)
At 20 days old								
AP (%)								
0.50		5.41^{a}	1.27^{a}	0.45^{a}	8.93^{a}	44.67^{a}	34.68^{b}	15.45
0.40		4.69 ^{b}	0.72 ^{b}	0.36 ^{b}	5.30^{b}	43.54^{b}	30.92^{c}	15.33
0.40^{+}		5.19^{a}	1.07^{a}	0.41^{a}	8.29^{a}	44.29^{a}	41.54^{a}	15.53
SE of means		±0.10	±0.09	±0.01	±0.69	±0.18	±1.57	±0.09
Significance		**	**	***	*	*	***	ns
AP% (1-20 days)	AP% (21-35 days)	Tibia length (cm)	Tibia weigh (g)	Tibia width (cm)	Tibia breaking strength (kgf)	Tibia ash (%)	Tibia Ca (%)	Tibia P (%)
At 35 days old								
0.50	0.40	8.23	4.76	0.78	23.96	41.91^{e}	31.67^{b}	16.75^{b}
	0.30+	8.19	4.45	0.78	24.66	46.96^{b}	38.07^{a}	16.88^{ab}
0.40	0.40	8.10	4.83	0.82	24.54	43.55^{d}	31.58^{b}	16.46^{c}
	0.30+	8.29	4.68	0.78	24.24	43.89^{d}	37.36^{a}	16.94^{ab}
0.40^{+}	0.40	8.19	4.83	0.77	23.63	45.41^{c}	37.88^{a}	16.79^{b}
	0.30+	8.26	4.32	0.80	24.55	48.94^{a}	36.95^{a}	17.12^{a}
SE of means	±0.09	±0.05	±0.01	±0.78	±0.58	±0.71	±0.06	
Significance	ns	ns	ns	ns	***	***	**	

Means within each column for each effect with no common superscript are significantly different at p<0.05, ns: Not significant, *p<0.05, **p<0.01, ***p<0.001, $^{+}$Plus 500 IU phytase

and 0.40% AP in the growing period without phytase) recorded the lowest tibia ash percentage. Tibia Ca content significantly (p<0.001) increased by phytase supplementation. The highest value of tibia Ca and P were recorded for birds fed 0.30% AP diet with phytase and the lowest value was recorded for birds fed 0.40% AP diet without phytase (Table 4).

The results of bone parameters are in agreement with some previous studies. Onyango *et al.* (2004), El-Sherbiny *et al.* (2005) and Mohamed *et al.* (2005) found that added phytase to low AP diets improved bone parameters measured as length of both femur and tibia or ash percentages in the femur, tibia and toe and bone mineral content. Mondal *et al.* (2007) and Rezaei *et al.* (2007) reported that percentage of tibia ash, Ca and P and retention of Ca and P significantly (p<0.05) increased by the addition of phytase to low phosphorus diet. Santoyo *et al.* (2009) proved that phosphorus and phytase levels influenced (p<0.05) percent of tibia bone ash. Also, Zaghari (2009) found that toe ash and toe ash Ca and P percentage of broiler chickens increased with the addition of phytase.

Results of Ca and P excretion at 20 and 35 days of age as affected by dietary AP and phytase supplementation are presented in Table 5. At 20 days of age, no significant differences were detected on Ca excretion due to reducing dietary AP levels and phytase supplementation. Meanwhile, P excretion significantly (p<0.001) decreased with reducing dietary AP level. More pronounced effect on reducing P excretion was obtained when phytase was added to the low AP diet. Phosphorus excretion decreased by 14.81 and 22.22% for birds fed on diet containing 0.40% AP without and 0.40% AP with phytase supplementation, respectively, compared to birds fed the control diet.

The results at 35 days of age showed that reducing AP levels and phytase supplementation significantly decreased Ca (p<0.01) and P (p<0.001) excretion. The excreted phosphorus decreased by 14.19, 21.94 and 31.61% for those fed 0.50, 0.40 or 0.40% AP with phytase in the starting period and 0.30% AP with phytase in the growing period, respectively, compared to birds fed the control

Table 5: Calcium and phosphorus excretion of broiler at 20 and 35 days old as affected by phosphorus level and phytase supplementation

Item		Ca excretion (%)	P excretion (%)	P reduction (%)
At 20 days old				
AP %				
0.50		2.03	1.62^{a}	-
0.40		2.12	1.38^{b}	14.81
0.40^{+}		2.05	1.26^{c}	22.22
SE of means		±0.03	±0.05	-
Significance		ns	***	-
At 35 days old				
AP% 1-20 days	AP% 21-35 days	Ca excretion (%)	P excretion (%)	P reduction (%)
0.50	0.40	2.79^{a}	1.55^{a}	
	0.30^{+}	2.38^{c}	1.33^{b}	14.19
0.40	0.40	2.72^{ab}	1.54^{a}	0.65
	0.30^{+}	2.47^{bc}	1.21^{c}	21.94
0.40^{+}	0.40	2.85^{a}	1.52^{a}	1.94
	0.30^{+}	2.46^{bc}	1.06^{d}	31.61
SE of means	±0.05	±0.05		
Significance	**	***		

Means within each column for each effect with no common superscript are significantly different at $p<0.05$, **$p<0.01$, ***$p<0.001$, ns: Not significant, $^{+}$Plus 500 IU phytase

diet. These results proved that Ca and P excretion significantly ($p<0.05$) decreased by reducing AP and/or reducing AP with phytase supplementation.

Decreasing calcium and phosphorus excretion as a results of phytase supplementation may be attributed to increase in the availability of P and Ca. The obtained results are in agreement with those of Ahmad *et al*. (2000), Kornegay (2001), Viveros *et al*. (2002), Bingol *et al*. (2009) and El-Deek *et al*. (2009) who concluded that adding phytase to broiler diets increased tibia ash, Ca and P% and decreased Ca and P% in the excreta. So, dietary supplementation of phytase improved minerals utilization, thus playing an important role in reducing P environmental pollution.

CONCLUSION

It could be concluded that using low P diets supplemented with phytase has pronounced beneficial effect regarding P excreted and its impact on environmental pollution that limits soil and water contamination. Supplementing phytase enzyme to corn-soybean meal diet renders the dietary phosphorus contents more available to the birds. Therefore, the amount of supplemental phosphorus could be remarkably reduced.

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