aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 6 (7): 678-687, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.678.687

The Effect of Organic Acid and Zeolite Addition Alone and in Combination on the Bone Mineral Value in Broiler Fed Different Dietary Phosphorus Levels

[1]Ismail Abas, [1]Tanay Bilal, [2]Erol Ercag and [1]Onur Keser

[1]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University, Avcilar, Istanbul, Turkey

[2]Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, Istanbul, Turkey

Corresponding Author: Ismail Abas,Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University, Avcilar, Istanbul, Turkey

ABSTRACT

The aim of this study was to investigate the effects of the addition of Organic Acid (OA) and Zeolite (ZE) alone or combined to the diet with microbial phytase contained low (3.9 g kg^{-1}) and adequate (7.0 g kg^{-1}) levels of Phosphorus (P) on bone mineral concentration. In this study, 480 one-day old broiler chicks were used. Chicks were assigned equally to 8 groups for the study carried out 6 replicates during 42 days. Basal ration contained 600 phytase enzyme unit (FTU kg^{-1}) was formulated separately for starter and grower period. On day 21, tibia ash rate ($p<0.001$) and ash P levels ($p<0.03$) decreased in chicks fed low P ration. Similarly, the addition of ZE also decreased tibia ash rate on day 21 ($p<0.01$). The use of OA+ZE increased the ash P level on day 42 ($p<0.05$). Depending on P level of ration, the level of P in tibia ash samples decreased only on day 21 ($p<0.03$). On day 42, tibia P level was decreased and increased by the addition of ZE and OA+ZE, respectively ($p<0.05$). The addition of OA+ZE increased the bone Zn level on day 21. Cu level of bone decreased ($p<0.05$) depending on the low P content of the diet. Also, tibia Mn level increased ($p<0.05$) depending on the increase in the P level of diet at the end of the experiment. P level of litter material increased depending on the additives ($p<0.02$) and the P level of the diet ($p<0.01$).

Key words: Poultry, propionic acid, clinoptilolite, phosphorus, broiler, organic acid, zeolite tibia mineral level

INTRODUCTION

The supplementation of phytase enzyme to diets of monogastric animals for phytate-phosphorus utilization is an effective and practical method (Waldroup, 1999). Phytase makes phytate Phosphorus (P) and other cations usable for birds (Payne *et al.*, 2005). It was reported that dietary phytase supplementation increased the amount of tibia ash in broilers and there was a positive interaction between phytase supplementation and non-phytate P levels (Waldroup *et al.*, 2000; Onyango *et al.*, 2005). However, the positive effects of microbial phytase on mineral metabolism were determined in diets with low P content (Viveros *et al.*, 2002).

There are many alternative methods to increase the P utilization. One of these is methods the use of Organic Acid (OA) supplementation in the diets. It was indicated that organic acid and phytase enzyme decreased the amount of inorganic calcium, phosphorus, magnesium and zinc supplementation (Brenes *et al.*, 2003). The use of citric acid (4-6%) in broiler diets improved the utilization of phytate-phosphorus (Boling *et al.*, 2000), decreased the P requirement and increased the P utilization without any effects on Ca utilization or Ca requirement (Boling-Frankenbach *et al.*, 2001). Similarly, Capdevielle *et al.* (1998) reported that acidified diet increased the rate of tibia ash and this was the result of the increase in P utilization. Also, Boling *et al.* (2000) indicated that the addition of citric acid and phytase had a positive effect on tibia ash.

The other feed additive, which has effects on Ca and P utilization, is Zeolite (ZE). Zeolites are commonly used as commercial adsorbents because of their adsorptive ability on toxic agents, especially aflatoxins, in feeds (Oguz and Kurtoglu, 2000). However, it was determined that zeolites repressed P utilization by making indigestible compound with P through its aluminosilicate component, increased Ca utilization (Elliot and Edwards, 1991) and had an indirect effect on P absorption and metabolism (Leach *et al.*, 1990). In another study it is claimed that the addition of zeolite had increased Ca utilization and increased the rate of bone ash during growth (Watkins and Southern, 1992). Furthermore, while Edwards *et al.* (1992) reported that the addition of zeolite to broiler diets significantly decreased the retention of phosphorus, this effect of zeolite was attributed to insufficient use of phytate and non-phytate phosphorus (Moshtaghian *et al.*, 1991).

The previous experiments clearly indicated beneficial effects of phytase on P utilization in poultry. Therefore, the objectives of this study were (1) to evaluate effects of OA, ZE and OA+ZE on the beneficial effects of microbial phytase in broiler chickens fed different P levels and (2) to evaluate the effects of OA, ZE and OA+ZE on tibia mineral composition and the interaction of these additives in broiler chickens was also examined.

MATERIALS AND METHODS

Experimental procedure was conducted under the guidelines of University Istanbul of Ethics Committee.

A total of 480 one-day old broiler chicks (Ross 308 strain) were obtained from a commercial hatchery. The chicks were placed in trial pens of the Vocational High Schools, Balikesir University, Bandirma, Turkey. The birds were housed in starter pen in an environmentally controlled room. The fluorescent lights were on 24 h each day. Diets in mash form and water were provided for *ad libitum* consumption. Chicks were assigned equally to 48 pens, each pen containing 10 chicks, to receive eight dietary treatments of wheat-soybean based diets for six replicates of each treatment. Diets were formulated by considering nutrient requirements of broilers for starter and grower periods (NRC, 1994) (Table 1). Treatment structure consisted of two levels of phosphorus (3.9 g kg^{-1} P or 7.0 g kg^{-1} P) (Table 2). As feed additives, 600 FTU kg^{-1} phytase enzyme [fermented substance from *Aspergillus oryzae* (Natuphos 500, 500.000 FTU kg^{-1} phytase, BASF Corp.)], organic acid, 20 g kg^{-1}, (Salb Curb, Kemin Industries, Inc., a mold inhibitor for processed feed ingredients and animal feeds contains primarily propionic acid) and natural zeolite, 20 g kg^{-1}, (containing 98% clinoptilolite) were used in experimental diets. At the end of each experimental period (21 or 42 d) two chicks from each subgroup were randomly selected. After the birds were killed by cervical dislocation, the right tibias were removed. Also, litter samples from each group were collected on day 21 and 42.

Tibia samples were prepared according to Wisser *et al.* (1990) for the mineral and ash analysis. Diets were analyzed for Crude Protein (CP), dry matter (by drying in an oven at 105°C for 10 h). Tibia and litter samples were ashed at 550°C degrees for 12 h to calculate the rate of tibia and litter

Table 1: Ingredient and nutrient composition (g kg^{-1}) of broiler starter and grower diet

	Starter (0-21day)		Grower (22-42 day)	
Items	Low P	Adequate P	Low P	Adequate P
Feed stuffs				
Wheat	565.5	540.5	595.5	595.5
Soybean meal	341.5	356.5	301.5	301.5
Wheat bran	10	-	20	-
Sunflower oil	60	60	60	60
Salt	4	4	4	4
Dicalcium phosphate	-	20	-	20
Limestone	11	11	11	11
DL-Methionine	2	2	2	2
Lysine	1	1	1	1
Premix[1]	5	5	5	5
Nntritional content (data on dry matter)				
ME (kcal kg^{-1})	3183	3120	3201	3157
CP (g kg^{-1})	230	231	214	211
Lysine (g kg^{-1})	12	12	11	11
Met+Cys. (g kg^{-1})	9	9	8	8
Ca (g kg^{-1})	5.5	11	5.3	11
P (g kg^{-1})	3.9	7	3.9	7

[1]Vitamin and mineral mix supplied/kg diet: Vitamin A; 10.000.000 IU, Vitamin D_3; 1.500.000 IU, Vitamin E;40.000 mg, Vitamin K_3; 3000 mg, Vitamin B_1; 2200 mg, Vitamin B_2; 4500 mg, Niasine; 30.000 mg, Cal.D-Pant.;13.000 mg, Vitamin B_6; 3000 mg, Vitamin B_{12}; 15 mg, folic asit; 1500 mg, Biotin;100 mg, choline chloride; 250.000 mg, , Vitamin C; 12.000 mg, Mn; 80.000 mg, Zn; 60.000 mg, Fe; 30.000 mg, Cu; 5000 mg, I; 1000 mg, Co; 200 mg, Se; 150 mg

Table 2: Experimental design

Group	OA	ZE	OA+ZE
Low P (3.9 g kg^{-1})			
Control	-	-	-
OA	20 g kg^{-1}	-	-
ZE	-	20 g kg^{-1}	-
OA+ZE	-	-	20 g kg^{-1}+20 g kg^{-1}
Adeqnate P (7.0 g kg^{-1})			
Control	-	-	-
OA	20 g kg^{-1}	-	-
ZE	-	20 g kg^{-1}	-
OA+ZE	-	-	20 g kg^{-1}+20 g kg^{-1}

OA: Organic acid, ZE: Zeolite, P: Phosphorus

ash. Ca and P contents of feed, tibia and litter were determined as colorimetric and spectrophotometric, respectively (AOAC, 1984). Bone samples were wet acid digested with the nitric and perchloric acid mixture (AOAC 1984). Cu, Zn and Mn concentrations in bone and litter were analyzed with an atomic absorption spectrophotometer (Varianspectra AA, 220 fast seqvential), the laboratory of Chemistry Department of the Faculty of Engineering, University of Istanbul.

A factorial arrangement of treatment was used to investigate the effect of two levels of phosphorus and four diets. The statistical model included phosphorus levels and diets as main effects. Data were evaluated to analyses variance using the General Linear Models in statistical package software (SPSS, 1999). Mean differences were determined by Duncan's multiple range test (Snedecor and Cochran, 1980).

RESULTS

Tibia ash values increased ($p<0.001$) depending on the level of P in diet whereas the addition of zeolite resulted in a decrease ($p<0.01$) in ash rates (39.80% vs. 46.37%) within the first 21 days (Table 3). Also, it was determined that there were positive effects of the addition of organic acid and OA+ZE on tibia ash rate on day 21 ($p<0.01$). At the end of the experiment, there were no significant differences between groups for tibia ash rates (Table 4).

Tibia Ca concentrations were not different in both periods. On the other hand tibia P levels increased ($p<0.001$) depending on diet P levels on day 21 (Table 3). On day 42, tibia P level was significantly higher in-group supplemented with OA+ZE compared to control and group supplemented with ZE ($p<0.05$) (Table 4).

Tibia Cu concentration was higher in-group fed adequate P diet than those of other groups on day 21 ($p<0.05$). On the other hand, the addition of OA, ZE and OA+ZE decreased the level of Cu compared to control ($p>0.05$) whereas Mn and Zn concentrations were not affected (Table 3). At the end of the experiment, while Mn concentration increased depending on P level ($p<0.001$) and the addition of ZE ($p<0.05$), Zn concentration increased due to the addition of ZE and OA+ZE to diet ($p<0.05$) (Table 4).

Table 3: The effects of the addition of organic acid and/or zeolite to low and adequate phosphorus diets on tibia ash rate and mineral levels on day 21

		Parameters						
Analysis	Level of P (g kg^{-1})	Tibia (g)	Ash (%)	Ca (%)	P (%)	Mn (%)	Cu (%)	Zn (μg g^{-1})
Group								
Control	3.9	1.25^{bc}	44.57^{bcd}	31.43	15.26	3.33	1.91^{ab}	271.72
OA	3.9	1.17^{c}	44.05^{cd}	30.21	15.74	3.03	1.39^{ab}	260.93
ZE	3.9	1.31^{abc}	39.80^{e}	30.89	15.46	2.84	1.45^{ab}	283.94
OA+ZE	3.9	1.41^{ab}	42.34^{c}	30.21	15.85	2.75	1.32^{b}	277.29
Control	7.0	1.49^{a}	47.04^{ab}	30.51	17.03	3.06	1.96^{a}	292.81
OA	7.0	1.41^{ab}	46.87^{ab}	32.13	16.64	2.62	1.70^{ab}	283.64
ZE	7.0	1.47^{ab}	46.37^{abc}	32.24	16.47	3.20	1.57^{ab}	254.26
OA+ZE	7.0	1.40^{abc}	47.85^{a}	32.96	16.61	3.35	1.84^{ab}	287.60
SEM		0.036	0.39	0.404	0.147	0.24	0.135	13.66
P levels								
3.9		1.28^{b}	42.64^{b}	30.68	15.58^{b}	2.97	1.51^{b}	273.50
7.0		1.44^{a}	46.97^{a}	31.97	16.69^{a}	3.06	1.77^{a}	279.58
Diet								
Control		1.37	45.80^{a}	30.97	16.15	3.20	1.94^{a}	282.30
OA		1.28	45.37^{a}	31.17	16.19	2.82	1.55^{ab}	272.28
ZE		1.39	43.08^{b}	31.57	15.96	3.01	1.49^{b}	269.13
OA+ZE		1.40	44.97^{a}	31.59	16.24	3.05	1.58^{ab}	282.44
Probabilities								
Level of P		0.01	0.001	NS	0.001	NS	0.05	NS
Diet		NS	0.05	NS	NS	NS	0.05	NS
P X Diet		NS	NS	NS	NS	NS	0.05	NS

OA: Organic acid, ZE: Zeolite, P: Phosphorus. Within columns, means with no common letter differ significantly ($p<0.05$). NS: Non significant ($p>0.05$)

Table 4: The effects of the addition of organic acid and/or zeolite to low and adequate phosphorus diets on tibia ash rate and mineral levels on day 42

		Parameters						
Analysis	Level of P (g kg^{-1})	Tibia (g)	Ash (%)	Ca (%)	P (%)	Mn (%)	Cu (%)	Zn (μg g^{-1})
Group								
Control	3.9	5.28	55.71	34.93	16.56	2.55^{c}	1.40	270.71^{ab}
OA	3.9	5.48	54.50	35.70	17.12	2.77^{bc}	1.39	258.98^{ab}
ZE	3.9	5.47	55.48	35.40	15.84	3.04^{abc}	1.23	226.68^{b}
OA+ZE	3.9	5.73	55.87	36.33	17.30	2.84^{abc}	1.26	243.14^{ab}
Control	7.0	5.11	55.00	33.79	15.73	3.44^{ab}	1.37	254.32^{ab}
OA	7.0	5.72	54.31	37.58	16.27	3.34^{abc}	1.27	259.07^{ab}
ZE	7.0	6.04	54.97	36.21	15.90	3.63^{a}	1.13	242.44^{ab}
OA+ZE	7.0	5.48	55.70	37.97	17.45	3.40^{ab}	1.19	319.81^{a}
SEM		0.09	0.23	0.18	0.20	0.183	0.11	12.09
P levels								
Low P	3.9	5.48	55.24	35.59	16.70	2.80^{b}	1.32	249.88
Adequate P	7.0	5.58	55.00	36.39	16.34	3.46^{a}	1.24	268.91
Diet								
Control		5.19	55.35	34.56	16.15b	2.91^{a}	1.39	262.52^{ab}
OA		5.60	54.40	36.64	16.70^{ab}	3.06^{ab}	1.33	259.03^{ab}
ZE		5.75	54.92	35.81	15.87^{b}	3.34^{b}	1.18	234.56^{b}
OA+ZE		5.59	55.79	35.06	17.37^{a}	3.12^{ab}	1.23	281.47^{a}
Probabilities								
Level of P		NS	NS	NS	NS	0.001	NS	NS
Diet		NS	NS	NS	0.01	0.05	NS	0.05
P X Diet		NS	NS	NS	NS	0.05	NS	NS

OA: Organic acid, ZE: Zeolite, P: Phosphorus. Within columns, means with no common letter differ significantly ($p<0.05$). NS = Non significant ($p>0.05$)

Table 5: The effects of the addition of organic acid and/or zeolite to low and adequate phosphorus diets on litter Ca and P levels

		Parameters			
		Ca (%)		P (%)	
Analysis	Level of P (g kg^{-1})	Day 21	Day 42	Day 21	Day 42
Group					
Control	3.9	3.37	8.96	2.01	2.44^{c}
OA	3.9	4.35	8.42	2.59	3.02^{bc}
ZE	3.9	4.70	9.22	2.23	3.11^{b}
OA+ZE	3.9	3.79	8.68	2.63	3.01^{bc}
Control	7.0	3.48	8.40	2.43	3.16^{b}
OA	7.0	3.59	10.53	2.42	3.56^{ab}
ZE	7.0	5.69	14.35	2.48	3.30^{ab}
OA+ZE	7.0	4.30	10.23	2.73	3.80^{a}
SEM		0.32	0.48	0.096	0.066
P levels					
Low P		4.06	8.82	2.36	2.89^{b}
Adequate P		4.27	10.12	2.51	3.46^{a}

Table 5: Continued

		Parameters			
		Ca (%)		P (%)	
Analysis	Level of P (g kg^{-1})	Day 21	Day 42	Day 21	Day 42
Diet					
Control		3.42	8.69	2.22	2.80[b]
OA		3.97	9.47	2.51	3.29[a]
ZE		5.19	10.28	2.35	3.20[a]
OA+ZE		4.05	9.45	2.68	3.41[a]
Probabilities					
Level of P		NS	NS	NS	0.01
Diet		NS	NS	NS	0.05
P X Diet		NS	NS	NS	NS

OA: Organic acid, ZE: Zeolite, P: Phosphorus. Within columns, means with no common letter differ significantly (p<0.05). NS: Non significant (p>0.05)

The results of the analysis of litter samples collected on day 21 and 42 were presented in Table 5. The levels of Ca in litter samples were not different during the experiment. However, it was determined that P contents in litter samples increased depending on the diet P level (p<0.01) and the addition of OA and/or ZE to diet (p<0.02).

DISCUSSION

Tibia ash rates were increased at 10% by rising of P level from 3.9 g kg^{-1} to 7.0 g kg^{-1} in the group supplemented with phytase alone (p<0.001). The addition of zeolite decreased ash rate at low P level (p<0.01), but this negative effect decreased depending on the increase in P level. At adequate P level, it was determined that the use of OA+ZE combination had more positive effects on tibia ash rather than the other groups (p<0.01) (Table 3).

It was reported by many researchers that the increase in the tibia ash rate was a useful indicator in the evaluation of bone mineralization in poultry (Qian *et al*., 1996; Ahmad *et al.*, 2000). In a study conducted by Brenes *et al.* (2003) to investigate the effects of citric acid and microbial phytase on mineral utilization in broilers, it was determined that the decrease in available P level of diet decreased the tibia ash rate significantly. Similarly, in our study, reducing of P level from 7.0 to 3.9 g kg^{-1} decreased the ash rate from 46.97 to 42.64%. Waldroup *et al.* (2000) reported that there was no effect of phytase if the level of P was high and this result was agree with our findings.

Bone Ca concentration was not affected in both periods of experiment. On day 21, tibia P level increased depending on diet P level (p<0.001) whereas it increased by the addition of OA+ZE on day 42 (p<0.05). Mitchell and Edwards (1996) stated that the use of phytase enzyme in low P diet increased the tibia ash rate at any level of dietary calcium. On the contrary, tibia ash rate was higher in group fed 7.0 g kg^{-1} dietary P than group fed 3.9 g kg^{-1} P in our study, but these changes observed on 21 day period and they were not observed on 42 day period. These differences may be due to the age of broilers. Because, the necessity of phytase supplementation decreases due to the level of phytase enzyme in poultries increased by the age (Maenz and Classen, 1998).

In this study, it was determined that tibia Ca contents were constant, but tibia P contents changed depending on the dietary P level. It was reported that the increase in the percentage of tibia ash was relevant to the increase in the retention of minerals such as Ca, P and Zn by releasing these minerals from phytate-mineral complex through phytase (Viveros *et al.*, 2002; Brenes *et al.*, 2003).

In current study, the percentages of tibia ash of broilers fed low P diet supplemented with phytase and zeolite were lower than those of other groups. Similarly, Chung and Baker (1990) reported that supplementation of diet with zeolite (sodium-calcium-aluminium silicate) decreased tibia ash percentage and weight. As a reason for this, it was indicated that aluminium silicate type zeolites reduced the use of phytate and inorganic P whereas increased the utilization of Ca and played role as a P antagonist. In a study on turkeys, Qian *et al.* (1996) reported that phytin, a cation chelator, decreased Ca availability, but the supplementation of diet with phytase increased Ca availability by increasing Ca retention. Therefore, in this study, as a reason for the decrease of tibia ash rate and P content in the group supplemented with ZE, it was considered that Ca retention was increased by phytase enzyme and Ca/P balance could be impaired due to the decrease of Ca utilization by supplementation of ZE and the negative effect of ZE on P, consequently tibia mineral concentration could be affected negatively from this situation.

While the negative effect of zeolite supplementation alone on tibia ash rate and tibia P level on day 21 and 42 were determined, the supplementation of OA+ZE to both dietary P levels improved tibia ash percentage and tibia P level. Boling-Frankenbach *et al.* (2001) reported that complex formation between citric acid and Ca decreased binding capacity of Ca to phytate and increased the utilization of phytate-phosphorus. Similar mechanism of action could be valid for the current study. In addition, it has been thought that OA+ZE supplementation affected P utilization positively by increasing the P utilization and decreasing Ca/P rate (Qian *et al.*, 1997). Also, it is possible to say that modifier effect of zeolite on the digestibility of minerals and several nutrients in digestive tract due to its adsorbent and ion-exchange capacity (Roland *et al.*, 1993) could be affected positively in the presence of organic acid.

On day 21, OA+ZE and ZE supplementation increased and decreased tibia Zn concentration, respectively ($p<0.05$). It is known that, phytin binds Zn and prevents its utilization. It was suggested that the increase of bone Zn concentration was related to the supplementation of phytase enzyme (Sebastian *et al.*, 1996) or insufficient Zn in diet (Qian *et al.*, 1997; Mohanna and Nys, 1999).

Tibia Cu concentration decreased on day 21 depending on the decrease of dietary P level and ZE supplementation ($p<0.05$). Phytase supplementation increased Cu retention in this study and this result was also agreed with the findings of Sebastian *et al.* (1996).

Manganese availability decreases with the increasing levels of Ca and P (Baker and Oduho, 1994). In this study, tibia Mn concentration increased depending on the increase in the level of P at the end of the study. The indifference between groups and the increase in Mn concentration depending on the increase in the level of P on day 21 showed that phytase enzyme was not effective enough. The main reason of this may be the sensibility of Mn to phytic acid and the formation of insoluble complex between them, as reported by Mohanna and Nys (1999).

The effects on litter Ca and P levels are presented in Table 5. There were no differences between groups for litter Ca levels during the study. Litter P level in group fed low dietary P was lower ($p<0.01$) than the group fed adequate dietary P (2.80% vs. 3.46%). As reported by Li *et al.* (2000), restriction of dietary P intake induced more retention of P in body for the continuation of physiological functions. It was determined that the supplements used in this study increased litter P level compared to control group ($p<0.02$). Selle and Ravindran (2007) reported that P excretion was related to the increase in dietary P level, indigestible inorganic P level and the loss of endogenous P activity of phytase in digestive system.

CONCLUSIONS

In this study, the effects of the addition of organic acid and/or zeolite in the presence of phytase enzyme were investigated. Tibia ash rate decreased on day 21 depending on ZE supplementation and the decrease in the dietary P level. As a result of this study, it was determined that OA+ZE supplementation had an effect on the increase of tibia ash P level percentage. Thus, phytase activity was increased by OA+ZE supplementation to diet and an alternative method, which would be useful for the solution of bone problems originated from mineral imbalance, was introduced.

ACKNOWLEDGMENTS

This research was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No: 105 O 195).

REFERENCES

AOAC, 1984. Official Methods of Analysis. 14th Edn., Association of Official Analytical Chemists, Washington, DC.

Ahmad, T., S. Rasool, M. Sarwar, A.U. Haq and Z.U. Hasan, 2000. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. Anim. Feed Sci. Technol., 83: 103-114.

Baker, D.H. and G.W. Oduho, 1994. Manganese utilization in the chick: Effects of excess phosphorus on chicks fed manganese deficient diets. Poult. Sci., 73: 1162-1165.

Boling, S.D., D.M. Webel, I. Marromichalis, C.M. Parsons and D.H. Baker, 2000. The effects of citric acid on phytate phosphorus utilization in young chicks and pigs. J. Anim. Sci., 78: 682-689.

Boling-Frankenbach, S.D., J.L. Snow, C.M. Parsons and D.H. Baker, 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci., 80: 783-788.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro and C. Braro, 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol., 110: 201-219.

Capdevielle, M.C., L.E. Hart, J. Goff and C.G. Scanes, 1998. Aluminum and acid effects on calcium and phosphorus metabolism in young growing chickens (*Gallus gallus domesticus*) and mallard ducks (Anas platyrhynchos). Arch. Environ. Contam. Toxicol., 35: 82-88.

Chung, T.K. and D.H. Baker, 1990. Phosphorus utilization in chicks fed hydrated sodium calcium aluminosilicate. J. Anim. Sci., 68: 1992-1998.

Edwards, H.D., M.A. Elliot and S. Sooncharernying, 1992. Effect of dietary calcium on tibial dyschondroplasia. Interaction with light, cholecalciferol, 1,25 dihydroxycholecalciferol, protein and synthetic zeolite. Poult. Sci., 71: 2041-2055.

Elhot, M.A. and H.M. Edwards, 1991. Comparison of the effects of synthetic and natural zeolite on laying hen and broiler chicken performance. Poult. Sci., 70: 2115-2130.

Leach, R.M., S. Brenda, S. Heinrichs and J. Burdetti, 1990. Broiler chicks fed low calcium diets. 1. Influence of zeolite on growth rate and parameters of bone metabolism. Poult. Sci., 69: 1539-1543.

Li, Y.C., D.R. Ledoux, T.L. Veum, V. Raboy and D.S. Ertl, 2000. Effects of low phytic acid corn on phosphorus utilization, performance and bone mineralization in broiler chicks. Poult. Sci., 79: 1444-1450.

Maenz, D.D. and H.L. Classen, 1998. Phytase activity in the small intestinal brash border membrane of the chicken. Poult. Sci., 77: 557-563.

Mitchell, R.D. and Jr. H.M. Edwards, 1996. Effects of phytase and 1,25 dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci., 75: 95-110.

Mohanna, C. and Y. Nys, 1999. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. Anim. Feed Sci. Technol., 77: 241-253.

Moshtaghian, J., C.M. Parsons, R.W. Leeper, P.C. Harrison and K.W. Koelkebeck, 1991. Effect of sodium aluminosilicate on phosphorus utilization by chicks and laying hens. Poult. Sci., 70: 955-962.

NRC, 1994. Nutrient Requirement of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC.

Oguz, H. and V. Kurtoglu, 2000. Effect of clinoptilolite on performance of broiler chickens during experimental aflatoxicosis. Br. Poult. Sci., 41: 512-517.

Onyango, E.M., M.R. Bedford and O. Adeola, 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. Poult. Sci., 84: 248-255.

Payne, R.L., T.K. Lavergne and L.L. Southern, 2005. A comparison of two sources of phytase in liquid and dry forms in broilers. Poult. Sci., 84: 265-272.

Qian, H., E.T. Kornegay and D.M. Denbow, 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. Poult. Sci., 75: 69-81.

Qian, H., E.T. Kornegay and D.M. Denbow, 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium: Total phosphorus ratio in broiler diets. Poult. Sci., 76: 37-46.

Roland, D.A. Sr., H.W. Jr. Rabon, K.S. Rao, R.C. Smith, J.W. Miller, D.G. Barnes and S.M. Laurent, 1993. Evidence for absorption of silicon and aluminium by hens fed sodium zeolite A. Poult. Sci., 72: 447-455.

SPSS, 1999. SPSS for Windows. Standard Version 10.0.1, SPSS Inc., Headquarters, Chicago, Illinois.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean diets. Poult. Sci., 75: 729-736.

Selle, P.H. and V. Ravindran, 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol., 135: 1-41.

Snedecor, G.W. and W.G. Cochran, 1980. Statical Methods. 7th Edn., The Iowa State University Press, Ames, Iowa.

Viveros, A., A. Brenes, I. Arija and C. Centeno, 2002. Effects of phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci., 81: 1172-1183.

Waldroup, P.W., 1999. Nutritional approaches to reducing phosphorus excretion in poultry. Poult. Sci., 78: 683-691.

Waldroup, P.W., J.H. Kersey, E.A. Saleh, C.A. Fritts and F. Yan *et al*., 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci., 79: 1451-1459.

Watkins, K.L. and L.L. Southern, 1992. Effect of dietary sodium zeolite A and graded levels of calcium and phosphorus on growth, plasma and tibia characteristics of chicks. Poult. Sci., 71: 1048-1058.

Wisser, L.A., B.S. Heinrichs and R.M. Leach, 1990. Effect aluminum on performance and mineral metabolism in young chicks and laying hens. J. Nutr., 120: 493-498.