The Effect of Phytase and Organic Acid on Growth Performance, Carcass Yield and Tibia Ash in Quails Fed Diets with Low Levels of Non-phytate Phosphorus

P. Sacakli*, A. Sehu, A. Ergün, B. Genc and Z. Selcuk
Ankara University, Faculty of Veterinary Medicine, Department of Animal Nutrition, 06110 Ankara, Turkey

ABSTRACT : An experiment was conducted to investigate the effect of phytase, organic acids and their interaction on body weight gain, feed consumption, feed conversion ratio, carcass yield and tibia ash. A total of 680 three-day old Japanese quail chicks (*Coturnix coturnix japonica*) were assigned to 20 battery brooders, 34 chicks in each. The experimental period lasted 35 days. The treatment groups employed were: 1) a positive control which included 3.5 g available phosphorus (AP)/kg diet and 10 g Ca/kg diet; 2) a negative control which included 2 g AP/kg diet and 8 g Ca/kg diet, 3) negative control diet supplemented with either 300 FTU phytase/kg diet (phytase) or 4) 2.5 g organic acid (lactic acid+formic acid)/kg diet (organic acid); or 5) 300 FTU phytase/kg diet+2.5 g organic acid/kg diet (phytase+organic acid). All birds were fed with the positive control diet for a week and then transferred to the dietary treatments. At the end of the study, there were no differences ($p>0.005$) among the groups in body weight, weight gain, feed consumption, feed conversion ratio and carcass yield. Tibia ash, however, was reduced ($p<0.001$) for quails fed the negative control diet containing a low-level of AP compared to the positive control diet containing adequate AP. The addition of phytase, organic acid or phytase+organic acid to the diets containing the low-level of AP improved ($p<0.001$) tibia ash. On the other hand, an extra synergistic effect of phytase and organic acid on tibia ash was not determined. This study demonstrated that it may be possible to reduce supplemental level of inorganic P with phytase and/or organic acid supplementation for quail diets without adverse effect on performance and tibia ash. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 198-202)***

Key Words : Non-phytate Phosphorus, Organic Acid, Phytase, Quail, Tibia Ash

INTRODUCTION

About two-thirds of the total phosphorus (P) present in most feedstuffs used in poultry diets is in the form of phytate which is unavailable for monogastric animals due to insufficient quantities of endogenous phytate (Nelson, 1967). Thus, the bioavailability of P in feedstuffs of plant origin is generally very low. Bioavailability estimates of P in corn and soybean meal for pigs and poultry range from 10 to 30% (Nelson, 1967; Jongbloed and Kemme, 1990). In addition to low P availability, phytate limits availability of several other essential nutrients. Formation of insoluble phytate makes both Ca and P unavailable. Phytic acid has chelating potential forming a wide variety of insoluble salts with di- and trivalent cations at neutral pH (Vohra et al., 1965; Oberleas, 1973). Phytase, (myo-inositol hexakisphosphate phosphohydrolase), is the enzyme that releases P from phytate molecule (Gibson and Ullah, 1990). The efficacy of microbial phytase to improve dietary bioavailability has been reported by several researchers (Simons et al., 1990; Nair and Duvnjak, 1991; Denbow et al., 1995; Kornegay et al., 1996; Gordon and Ronald 1997; Sohail and Roland, 1999; Selle et al., 2003; Yan et al., 2003).

Organic acids are widespread in plant and animal tissues. It was reported that propionic, formic, citric, lactic and ascorbic acid increased nutrient digestibility without deleteriously affecting performance. It was reported that phosphorus utilization was improved by dietary citric acid in broilers (Boling et al., 2001) and dietary lactic acid or formic acid in pigs (Jangbloed et al., 2000). The main function of Ca and P is in the make up of the bones of the body. The young quail needs a minimum of 0.8% of the diet as Ca and 0.45% as available phosphorus. The use of phytase or organic acid alone or in combination could possibly reduce the need for inorganic P supplementation and increase nutrient bioavailability of corn-soybean meal quail diets. Thus, it may be possible to reduce environmental pollution resulted from phosphorus excreation.

The previous experiments clearly indicated that individually phytase and organic acids increase the availability of P in corn-soybean meal diets. There is no data on the use of phytase and organic acid combination in quail rations. The use of phytase in combination with organic acids may have synergetic effects on reducing inorganic P supplementation. The primary objective of the current study was to determine the effect of phytase, organic acid and their interaction on body weight gain, feed consumption, feed conversion ratio, carcass yield and tibia ash.

MATERIALS AND METHODS

A total of 680 three-day old Japanese quail chicks (*Coturnix coturnix japonica*) were weighed, and assigned to

* Corresponding Author: P. Sacakli. Tel: +90-312-517-25-65, Fax: +90-312-517-25-65, E-mail: psacakli@uark.edu
Received April 11, 2005; Accepted September 1, 2005

Table 1. Composition of the experimental diets fed to quails (g/kg)

Ingredient	Treatments				
	Positive control	Negative control	Phytase[1]	Organic acid[2]	Phytase+ organic acid[3]
Corn	533.7	538.0	538.0	538.0	538.0
Soybean meal	403.2	404.9	404.9	404.9	404.9
Vegetable oil	29.6	27.2	27.2	27.2	27.2
Limestone	12.9	18.2	18.2	18.2	18.2
Dicalcium phosphate	13.7	4.8	4.8	4.8	4.8
Salt	2.0	2.0	2.0	2.0	2.0
Vitamin premix*	2.5	2.5	2.5	2.5	2.5
Mineral premix**	1.0	1.0	1.0	1.0	1.0
Methionine	1.4	1.4	1.4	1.4	1.4
Analysed values					
Crude protein	216.5	213.5	213.5	213.5	213.5
Crude fat %	5.98	5.82	5.82	5.82	5.82
Metabolisable energy (MJ/kg)	13.17	13.07	13.07	13.07	13.07
Ca	10.5	8.0	8.0	8.0	8.0
Total P	6.0	4.3	4.3	4.3	4.3
Calculated values					
Available P	3.5	2.0	2.0	2.0	2.0
Lysine	13.5	13.5	13.5	13.5	13.5
Methionine+cystine	8.5	8.5	8.5	8.5	8.5

[1] diet was supplemented with 300 FTU phytase/kg diet.
[2] diet was supplemented with 2.5 g organic acid/kg diet.
[3] diet was supplemented with 300 FTU phytase/kg diet+2.5 g organic acid/kg diet.
* Provided per kg of diet: vitamin A: 15,000; vitamin D_3: 3,000 IU; vitamin E-15 IU; vitamin K_3-2.5 mg; vitamin B_1-1 mg. vitamin B_2- 10 mg; niacin-70 mg; calcium-D-pantothenat: 20 mg; vitamin B_{12}: 4 mg; folic acid: 2 mg; biotin: 0.1 mg; BHT: 125 mg.
** Provided per kg of diet: manganese: 80 mg; iron: 25 mg; zinc: 50 mg; copper: 7 mg; iodine: 0.3 mg; selenium: 0.15 mg; choline chlorite: 350 mg.

20 battery brooders, 34 chicks in each. The experimental period lasted 35 days. The experiment was conducted by using a corn-soybean meal based diet. Dietary treatments consisted of: 1) a positive control which included 3.5 g available phosphorus (AP)/kg diet and 10 g Ca/kg diet; 2) a negative control which included 2 g AP/kg diet and 8 g Ca/kg diet, 3) negative control diet supplemented with either 300 FTU phytase (Ronozyme™ P, ROCHE)/kg diet (Phytase); or 4) 2.5 g organic acid (Nutrilac™, NUTRI-AD INTERNATIONAL)/kg diet (mainly lactic acid+formic acid) (Organic Acid); or 5) 300 FTU phytase/kg diet+2.5 g organic acid/kg diet (Phytase+Organic Acid). All diets were isocaloric and isonitrogenous to supply the same amount of energy and protein to all the birds in those groups (Table 1).

All birds were fed with the positive control diet for a week and then transferred to the dietary treatments. Three-day old quail chicks (n = 680) were placed in electrically heated, wire-floored battery brooders (91 cm×45 cm×23 cm) which are 5 tiered and 2.35 cm of feeder space per bird. Continuous light was provided with day light and 20 watt light bulb. Heating was provided with rod radian (temperature was 35°C at first and reduced 3°C each week) Feed and water were offered *ad libitium*.

Chemical analyses of the diets used in the experiment were done using the methods of the AOAC (1984) and metabolisable energy of diets was determined according to the method suggested by Carpenter and Clegg (1956). Mean body weights of each brooder were determined at 3, 10, 17, 24, 31 and 38 d; cage feed consumption was measured for the same period. Mortality was recorded on a daily basis. At the end of the study, four male quails from each brooder were selected randomly and weighed and slaughtered. Tibias were obtained from two of the slaughtered quails and removed for bone ash determination as described by AOAC (1984).

Data on performance parameters and tibia ash were analysed by one-way ANOVA. The significance of differences between treatment means was tested according to Duncan, (1955). Differences in mortality rate were evaluated by Chi-Square test (Snedecor and Cochran, 1980). Statistical analyses were done using the SPSS program (version 10.0, USA). Statements of statistical significance are based on $p<0.05$.

RESULTS AND DISCUSSION

The supplementation of phytase or organic acid alone or in a combination with the low-level AP diets had no significant ($p>0.05$) effect on body weight, body weight gain, feed consumption, feed conversion ratio (Table 2) at any age during the study. Also, no interaction between phytase and organic acid were observed.

Reducing AP (to 2 g AP/kg diet) and Ca (to 8 g Ca/kg diet) without or with phytase or organic acid had no

Table 2. The effect of phytase and organic acid on body weight, feed consumption, body weight gain and feed conversion ratio of quails

Age (days)		Positive control	Negative control	Phytase	Organic acid	Phytase+ organic acid	P	LSD value
3	BW (g)	15.48±0.45	15.36±0.43	16.09±0.47	15.47±0.48	15.40±0.47	0.21	0.73
17		57.25±1.54	56.89±1.15	57.53±1.68	56.65±1.00	58.72±0.84	0.81	3.87
38		143.00±1.13	142.10±1.36	145.05±2.39	139.65±0.61	142.06±2.28	0.31	5.12
3-17	FC (g)	113.19±3.59	114.00±6.17	114.95±7.53	110.30±5.80	114.73±3.34	0.76	8.32
	BWG (g)	41.78±3.22	41.54±2.00	41.42±2.83	41.17±1.62	43.33±1.35	0.71	3.50
	FCR	2.72±0.15	2.74±0.04	2.78±0.09	2.68±0.08	2.65±0.08	0.38	0.14
17-38	FC (g)	330.00±19.81	343.96±13.93	350.23±21.52	335.80±8.69	339.28±10.7	0.46	23.74
	BWG	85.74±1.42	85.20±4.15	87.52±3.67	83.00±1.81	83.33±4.28	0.33	4.97
	FCR	3.85±0.27	4.04±0.31	4.00±0.15	4.05±0.05	4.08±0.34	0.71	0.38
3-38	FC (g)	443.19±11.13	457.96±9.95	465.18±13.91	446.10±2.62	454.01±5.72	0.50	28.79
	BWG (g)	127.52±1.14	126.74±1.50	128.94±2.23	124.17±0.34	126.66±2.18	0.38	4.93
	FCR	3.47±0.06	3.61±0.10	3.61±0.05	3.59±0.01	3.59±0.11	0.66	0.22

Values represent the mean±SE.

FC: Feed consumption; BWG: Body weight gain; FCR (FC/BWG): Feed conversion ratio; LSD: Least square deviation; p>0.05.

negative effects on growth parameters as compared to positive control diet containing 3.5 g AP/kg and 10 g Ca/kg. Similar to the present study, Waldroup et al. (1974) reported that in two of three trials, 1.2 g AP/kg diet (lowest level tested) was adequate for body weight gain and feed conversion ratio in broilers 4 to 8 weeks of age. In a third trial no more than 2.4 g AP/kg diet was required. More recently, it was reported that removal of supplemental phosphate sources from corn-soybean meal diets fed to broilers from 42 to 49 d or 42 to 56 d (Skinner et al., 1992a, 1992b) and 42 to 63 d (Yan et al., 2003) did not adversely affect body weight gain and feed conversion ratio. Singh et al. (2003) reported that phytase supplementation to low NPP (0.30%) diets improved the growth performance, relative retention of nutrients (N, Ca and P) and minerals (Ca, P) status of blood and bone in broiler chickens, with a better efficacy in maize based diets.

However, in numerous studies (Denbow et al., 1995; Qian et al., 1996; Cabahug et al., 1999; Sohail and Roland, 1999), while body weight gain, feed intake and feed conversion ratio were decreased by a low-level AP diet, supplementation of phytase to the diet improved performance of broilers. The results obtained from the present study were consistent with the previous reports that inclusion of 50 g lactic acid/kg diet did not affect live weight, weight gain (Yalçın et al., 1997). On the other hand, it was reported that the addition of 30 g (Cave, 1984; Pinchasov and Jensen 1989), 50 g (Lessard et al., 1993) and 75 g (Jacob et al., 1990) lactic acid/kg diet to the broiler rations numerically reduced body weight and weight gain. In contrast to results in our study, addition of 20, 40 and 60 g citric acid/kg diet resulted in increases in body weight gain and feed conversion ratio (Boling et al., 2000b, 2001).

In general, feed consumption and feed conversion ratio followed a similar pattern to that of body weight gains. In this study, feed consumption and feed conversion ratio were affected by neither low-level AP diet nor the addition of phytase or organic acid alone or in combination to the diet. It was reported that supplementation of 10, 30 g lactic acid/kg diet to the adequate level AP diets had no significant effect on feed consumption in broilers at 21 to 29 day (Cave, 1984) and 21 to 28 day (Pinchasov and Jensen, 1989) of age. In the study conducted by Jacob et al. (1990), while feed consumption was not affected by 25 g Ca-lactat/kg diet, it was decreased by 50 g and 75 g Ca-lactat/kg diet.

In this study, feed consumption for per kg weight gain was not affected by reducing AP with or without phytase or organic acid. The present results were in agreement with previous reports of studies that used lactic acid (Pinchasov and Jensen, 1989; Yalçın et al., 1997). In contrast to these results, Lessard et al. (1993) reported that feed conversion ratio was improved by 50 g lactic acid/kg diet supplementation at the rate of 7% compared to control group. All discrepancies reported in performance values can be attributed to changes in phosphorus requirement of chicks with the age, size and strain of birds in addition to dietary, management and environmental factors. At the end of the experiment, slaughtered quails had similar carcass weight and carcass yield.

The addition of phytase or organic acid alone or in combination to the low-level AP diet had no significant effect on carcass percentage of quails slaughtered at 38 days of age. Although live performance is an important measure of dietary changes, when available phosphorus and Ca levels or one of the two in the diet are changed, bone

Table 3. The effect of phytase and organic acid on carcass weights, carcass yield and tibia ash of quails

	Positive control	Negative control	Phytase	Organic Acid	Phytase+ Organic Acid	P	LSD value
Carcass weight (g)	100.06±1.57	99.13±1.13	100.19±1.54	100.75±2.09	99.87±1.61	0.97	4.55
Carcass yield (%)	71.35±0.51	70.76±0.59	71.27±0.55	71.89±0.53	71.60±0.44	0.64	1.48
Tibia ash (%)	41.35±0.63[b]	39.46±1.06 [a]	41.37±0.66 [b]	41.10±0.47 [b]	41.28±0.34 [b]	0.00***	0.69

Values represent the mean±SE.

[a, b] Row means with common superscript do not differ (p>0.05), *** p<0.001; LSD: least square deviation.

parameters are generally more sensitive than performance. A significant effect of phytase and organic acid supplementation was revealed on tibia ash. The inclusion of 300 FTU phytase/kg diet, 2.5 g organic acid/kg diet and 300 FTU phytase/kg diet+2.5 g organic acid/kg diet resulted in a significant ($p<0.001$) increase in tibia ash (Table 3). The present results are in agreement with numerous previous reports that using phytase (Denbow et al., 1995; Qian et al., 1996; Cabahug et al., 1999; Sohail and Roland, 1999; Yan, et al., 2003), and citric acid (Boling et al., 2000a, b; 2001). This study demonstrated that it may be possible to reduce supplemental level of inorganic P with phytase and/or organic acid supplementation for quail diets. However no synergetic effect was observed between phytase and organic acid on performance or tibia ash content.

Based on the results of this study, the following conclusions were drawn: 1) performance parameters were not adversely affected by a reduction of AP to 2 g/kg diet and Ca to 8 g/kg diet as compared to diet containing 3.5 g AP/kg diet and 10 g Ca/kg diet. 2) The negative effect on tibia ash associated with a dietary level of 2 g AP/kg diet and 8 g Ca/kg diet was completely reversed by the inclusion of 300 FTU phytase/kg diet or 2.5 g organic acid/kg diet either alone or in a combination. 3) Combination of phytase and organic acid in diet provided no additional benefit.

REFERENCES

AOAC. 1984. Official methods of analysis. 14th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Boling, S. D., M. W. Douglas, J. L. Snow, C. M. Parsons and D. H. Baker. 2000a. Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. Poult. Sci. 79:1335-1337.

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

Boling, 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.

Cabahug, S., V. Ravındran, P. H. Selle and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40 :660-666.

Carpenter, K. J. and K. M. Clegg. 1956. The metabolizable energy of poultry feedingstuffs in relation to their chemical composition. J. Sci. Food Agric. 7:45-51.

Cave, N. A. G. 1984. Effect of dietary propionic and lactic acids on feed intake by chicks. Poult. Sci. 63:131-134.

Denbow, D. M., V. Ravindran, E. T. Kornegay, Z. Yi and R. M. Hulet. 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci. 74:1831.

Duncan, D. B. 1955. Multiple Range and Multiple F tests. Biometrics, 11:1-42.

Gibson, D. M. and A. H. J. Ullah. 1990. Phytase and their actions on phytic acid. In: Inositol Metabolism in Plants. (Ed. D. J. Morre, W. F. Boss, F. A. Loewus) Wiley-Liss, Inc., New York. p. 77.

Gordon, R. W. and Sr. D. A. Roland. 1997. Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. Poult. Sci. 76:1172.

Jacob, J. P., R. Blair and E. E. Gardıner. 1990. Effect of dietary lactate and glucose on the incidence of sudden death syndrome in male broiler chickens. Poult. Sci. 69:1529-1532.

Jongbloed, A. W. and P. A. Kemme. 1990. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Netherlands J. Agric. Sci. 38:567.

Jongbloed, A. W., Z. Mroz, R. van der Weij-Jongbloed and P. A. Kemme. 2000. The effect of microbial phytase and organic acids and their interactions in diets for growing pigs. Livest. Prod. Sci. 1. 67(1-2):113-122.

Kornegay, E. T., D. M. Denbow, Z. Yi and V. Ravindran. 1996. Response of broilers to graded levels of microbial phytase added to maize-soyabean meal-based diets containing three levels of non-phytate phosphorus. Br. J. Nutr. 75:839.

Lessard, P., M. R. Lefrançois and J. F. Bernier. 1993. Dietary addition of cellular metabolic intermediates and carcass fat deposition in broilers. Poult. Sci. 72:535-545.

Nair, V. C. and Z. Duvnjak. 1991. Phytic acid content reduction in canola meal by various microorganisms in a solid state fermentation process. Acta Biotech. 11:211.

Nelson, T. S. 1967. The utilization of phytate P by poultry-A review. Poult. Sci. 46:862.

Oberleas, D. 1973. Phytats: Toxicants Occurring Naturally in Foods. National Academy Press. Washington, DC.

Pinchasov, Y. and L. S. Jensen. 1989. Effect of short chain fatty acids on voluntary feed of broiler chicks. Poult. Sci. 68:1612-1618.

Qian, H., H. P. Veit, E. T. Kornegay, V. Ravindran and D. M. Denbow. 1996. Effect of supplemental phytase and phosphorus on histological and other tibial bone characteristic and performances of broilers fed semi-purified diets. Poult. Sci. 75:618-626.

Selle, P. H., V. Ravindran, P. H. Pittolo and W. L. Bryden. 2003. Effects of Phytase Supplementation of Diets with Two Tiers of Nutrient Specifications on Growth Performance and Protein Efficiency Ratios of Broiler Chickens. Asian-Aust. J. Anim. Sci. 16(8):1158-1164.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and V. Verchoor. 1990. Improvement of phosphorus availability by microbial phytase in poultry and pigs. Br. J. Nutr. 64:525-540.

Singh, P. K., V. K. Khatta, R. S. Thakur, S. Dey and M. L. Sangwan. 2003. Effects of Phytase Supplementation on the Performance of Broiler Chickens Fed Maize and Wheat Based Diets with Different Levels of Non-Phytate Phosphorus. Asian-Aust. J. Anim. Sci. 16(11):1642-1649.

Skinner, J. T. A., A. L. Izat and P. W. Waldroup. 1992a. Effect of removal of supplemental calcium and phosphorus from broiler finisher diets. J. Appl. Poult. Res. 1:42-47.

Skinner, J. T. A., A. L. Waldroup and P. W. Waldroup. 1992b. Effect of removal of vitamin and trace mineral supplements from grower and finisher diets on live performance and carcass composition of broilers. J. Appl. Poult. Res. 1 :280-286.

Snedecor, G. W. and W. C. Cochran. 1980. Statistical Methods. 7th edn. Iowa State University Press, Ames, Iowa.

Sohail, S. S. and Sr. D. A. Roland. 1999. Influence of supplemental phytase on performance of broilers four to six weeks of age. Poult. Sci. 78:550-555.

Vohra, P., G. A. Gray and F. H. Kratzer. 1965. Phytic acid-metal complexes. Proceedings of the Society of Experimental Biology and Medicine. 120:447.

Waldroup, P. W., R. J. Mitchell and K. R. Hazen. 1974. The phosphorus needs of finishing broilers in relation to dietary nutrient density levels. Poult. Sci. 53:1655-1663.

Yalcin, S., I. Onbasilar and B. Kocaoglu. 1997. Bıldırcın besisinde laktik asit kullanımı. Ankara Üniv. Vet. Fak. Derg. 44:169-181.

Yan, F., J. H. Kersey, J. H. Fritts and P. W. Waldroup. 2003. Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. Poult. Sci. 82:294-300.