# Influence of Different Levels of Phytase Enzyme on Japanese Quail (*Coturnix japonica*) Performance

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#### Summary

An experiment was conducted to study the effect of phytase enzyme (Natuphos 500) supplementation in Japanese quail (*Coturnix japonica*) (3 to 45 days of age) on body weight, carcass performance, percentage of tibia bone ash and tibia phosphorus. Data were analyzed as a completely randomized design arranged with five levels (150, 300, 600, 1200 and 2400 FTU/Kg) of phytase enzyme as treatments 1, 2, 3, 4 and 5, respectively, and two control groups (positive and negative controls). None of the phytase levels had effect on carcass yield at 45 days of age (p>0.05), except first treatment with 70.06% of carcass yield that was significantly different than treatment 5 (p<0.01). The treatment 5 for percentage of tibia ash was 59.83% that was significantly different compared to the other treatments (p<0.01). Treatment 3 increased (p<0.01) phosphorus of tibia ash at 45 days of age (240.22 mg). Experimental replicates were not significantly different in any stage (p>0.05). Female quails at body weight, slaughter weight, carcass weight without viscera and legs weight were better than male (p<0.01), but male quails at carcass yield (69.06%) were significantly different (p<0.01) in contrast to the females (66.30%).

Key words

Japanese quail, phosphorus, phytase enzyme

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#### Introduction

Approximately two-thirds of the total phosphorus (P) in plants, which are the major constituents of poultry diets, is in the form of phytate (Punna and Roland, 1999: Viveros et al., 2000) and it is unavailable or poorly available when utilized by humans and other monogastric animals. This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994). Therefore, diets of monogastric animals are often supplemented with sources of inorganic P to meet the P requirements of the animal, which increases the cost of the diets and contributes to environmental pollution. Dietary supplementation with sources of microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals. Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. Phytases are naturally found in a number of seeds including cereals, legume, by-products, and other feedstuffs (Eeckhout and De Paepe, 1994; Viveros et al., 2000) and in microbial sources (Wyss et al., 1999).

Nelson et al. (1968) demonstrated that the addition of the enzyme phytase to grains and feeds was an effective way to increase phosphorus availability to poultry. Numerous reports have shown that phytate phosphorus present in cereal grains could be made available to both poultry and swine by treating the grains or supplementing the diets with the enzyme phytase, reducing the need to supplement these diets with inorganic phosphorus and decreasing phosphorus excretion (Nelson et al., 1971; Jongbloed and Kemme, 1990; Simons et al., 1990; Jongbloed et al., 1992; Ketaren et al., 1993; Mitchell and Edwards, 1996a, b). The commercial development of phytase has provided the animal industry with a tool to decrease the need to supplement animal feeds

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with inorganic phosphorus, which will decrease phosphorus in animal manures, and consequently decrease phosphorus loading of agricultural lands.

Phytase research that has focused on the effect of varying levels of supplemental phytase has shown increases in broiler performance, bone ash, and phytate P utilization with each additional unit or level of phytase (Shirley and Edwards, 2003).

Most of the investigations on phytase are with chickens while quails were less considered. Therefore, the target of this study is the investigation of different levels of phytase enzyme (Natuphos) on Japanese quail (*Coturnix japonica*) performance.

#### Materials and methods

A total of 168, 3-d-old Japanese quail chicks, (Coturnix japonica) were housed in pen with the newspaper litter until 12 days of age. Then birds were moved into one-tier cage (60×50×40 cm) in an environmentally controlled room, with 23-h constant overhead fluorescent lighting. The temperature was decreased gradually from 35°C to 20°C on the weekly bases. The chicks were allocated to 21 cages, each cage containing 8 chicks that were receiving seven diets with three replication of each diet. The experimental diets are presented in Table 1 and were formulated to meet the requirements of quail as established by the NRC (1994). Experimental diets were arranged with five levels 150, 300, 600, 1200 and 2400 phytase enzyme unit (FTU/Kg) with commercial name (Natuphos 500) as treatments 1, 2, 3, 4 and 5, respectively, and two control groups (positive and negative controls). One FTU is defined as the amount of enzyme that liberates micromole inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at  $37^{\circ}$ C and at pH = 5.5 (Hall et al.,

Diets Ingredients (%)	0 (positive control)	0 (negative control)	Phytase Levels (FTU/Kg) <sup>1</sup>					
			150	300	600	1200	2400	
Corn (8.5% CP)	49	49	49	49	49	49	49	
Soybean meal(44% CP)	44.91	44.91	44.91	44.91	44.91	44.91	44.91	
Vegetable oil (8.8 kcal/g)	2.865	2.865	2.865	2.865	2.865	2.865	2.865	
Limestone	1.304	1.304	1.304	1.304	1.304	1.304	1.304	
Salt (NaCl)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	
Dicalcium phosphate	0.747	0.212	0.212	0.212	0.212	0.212	0.212	
DL-Methionine	0.136	0.136	0.136	0.136	0.136	0.136	0.136	
Mix vitamins <sup>2</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
Trace mineral <sup>3</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
Sand	0.337	0.873	0.87	0.867	0.861	0.849	0.825	
Phytase (5000 FTU/ g)	0	0	0.003	0.006	0.012	0.024	0.048	
Total	100	100	100	100	100	100	100	
Nutrient (Calculated)								
Metabolizable energy (Kcal/Kg)	2900	2900	2900	2900	2900	2900	2900	
Crude protein (%)	24	24	24	24	24	24	24	
Methionine + cystine (%)	0.884	0.884	0.884	0.884	0.884	0.884	0.884	
Lysine (%)	1.33	1.33	1.33	1.33	1.33	1.33	1.33	
Calcium (%)	0.800	0.682	0.682	0.682	0.682	0.682	0.682	
Total phosphorus (%)	0.569	0.469	0.469	0.469	0.469	0.469	0.469	
Available phosphorus (%)	0.300	0.200	0.200	0.200	0.200	0.200	0.200	

<sup>1</sup> Danisco Animal Nutrition, Carol Stream, IL, FTU/kg feed = phytase units/kg feed. One phytase unit (FTU) is defined as the amount of enzyme that liberates micromole inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at  $37^{\circ}$ C and at pH = 5.5; <sup>2</sup> Provided per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (as DL-a-tocopheryl acetate), 8 IU; vitamin B12, 2800.02 mg; riboflavin, 5.5 mg; D-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B1 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; d-biotin, 0.05 mg; vitamin K (menadione sodium bisulfate complex), 2 mg; <sup>3</sup> Trace mineral mix provided the following (per kg of diet): manganese (MnSO<sub>4</sub>\_H<sub>2</sub>O), 60 mg; iron (FeSO<sub>4</sub>\_7H<sub>2</sub>O), 30 mg; zinc (ZnO), 50 mg; copper (CuSO<sub>4</sub>\_5H<sub>2</sub>O), 5 mg; iodine (ethylene diamine dihydroiodide), 1.5 mg.

2003). Small amounts of the basal diet were first mixed with the respective amounts of phytase as a small batch and then with a larger amount of the basal diets until the total amounts of the respective diets were homogeneously mixed. Diets in mash form and water were provided ad libitum from 3 to 45 days of age.

At the age of 45 days, four chicks were randomly selected from each treatment and weighed, and were killed for carcass performance in agreement with the method of Yalcin et al. (1999). Three quails were randomly selected from each treatment and a total of 21 left legs were banded for identification percentage tibia ash (TA) and phosphorus samples of bones tibia in agreement with the method of AOAC (1990). Data were subjected to analysis of variance using the general linear models procedures of SAS software (SAS Institute, 1990). Statements of statistical significance are based on a probability of  $P \le 0.05$ . The statistical model used was:

## $Y_{ijk} = \mu + T_i + S_j + TS_{ij} + R_k + e_{ijk}$

where  $Y_{ijk}$  is the individual observation,  $\mu$  is the overall mean,  $T_i$  is the treatment effects (i=1, 2..., 7),  $S_j$  is the sex effects (j=1,2),  $TS_{ij}$  Interaction effect between  $i^{th}$  dietary treatment and  $j^{th}$  sex,  $R_k$  is the *replication* effects, and  $e_{ijk}$  is the error term.

### Result and discussion

The effects of different levels of dietary phytase on the body weight (BW), slaughter weight (SW), carcass weight without viscera (CWV), carcass yield (CY), percentage of tibia bone ash (Tash) and tibia phosphorus (TP) of quails at 45 days of age are summarized in Table 2. The data indicated that body weights at 45 days of age were affected by phytase enzyme (p<0.01). So that quails of treatment 5 significantly increased body weight (195.16 g) compared to those on the negative control group that had weight 173.90 g. These results were nearly to those obtained by Huff et al. (1998), Viveros et al. (2002) and Hall et al. (2003) in broiler. Likewise sex was a significant source of variation for body weights at 45 days of age (p<0.01). Females showed 196.12 and male quails showed 169.74 g. The superiority of female versus male agreed with previous studies of Vali et al. (2005) and Vali

et al. (2006). It is necessary to notify that the hydrolysis and absorption of phytate phosphorus (P) by monogasteric animals are complex processes that are influenced by many factors. Dietary ingredients and feed processing seem to be the most important factors related to the diet, while age and type of birds could also affect phytate (P) utilization (Reddy et al., 1982; Sebastian et al., 1998; Attia et al., 2003). This possesses a problem to nonruminant animals because they do not produce sufficient amounts of intrinsic phytase which is necessary to hydrolyze the phytic acid complex EI-Badry et al. (2008).

The effects of experimental replications 1, 2 and 3 on body weight at 45 days of age 180.04, 184.90 and 181.73 g, respectively were not significantly different (p>0.05).

Carcass yield of quails treatment 1 was 70.06% and treatment 5 was 66.73% that was significantly different (p<0.01), but treatment 1 had not significant difference (p>0.05) with the rest of treatments. Also carcass yield of male quails with 69.06% had better performance than females (66.30%) that was significantly different (p<0.01).

These results demonstrated that treatment 3 had significantly different percentage of tibia ash (59.83%) compared with the rest of treatments (p<0.01), but in comparison with positive control (57.72%) there was no significant difference (p>0.05). This increment has been reported by several authors working with chickens and it is considered a good indication of bone mineralization (Broz et al., 1994; Qian et al., 1996; Sebastian et al., 1996a, b). The improvement in ash percentage in tibia probability can be related to the increase in Ca, P, Mg, and Zn retentions from the phytate-mineral complex by the action of phytase. In contrast, Keshavarz (2000) observed in pullets and Rama-Rao et al. (1999) in chickens that tibia ash was not influenced by phytase in the diets.

These results demonstrated that quails treatments 2 and 3 improved performance of P in tibia 217.38 and 240.22 mg respectively, and they were significantly different compared to other treatments (p<0.01).

Table 2. Influence of dietary phytase levels (FTU) on the body weight at 45 days of ages (BW), slaughter weight (SW), carcass weight without viscera (CWV), carcass yield (CY), percentage of tibia bone ash (Tash) and tibia phosphorus (TP) of Japanese quails (*Coturnix japonica*)

Main effects		BW (g)	SW (g)	CWV (g)	Cy (%)	Lw (g)	Tash (%)	TP(mg)
Overall mean		182.18	173.31	123.13	67.75	32.23	55.75	171.34
Treatment	1	175.25 <sup>abc</sup> 177.71 <sup>abc</sup>	166.39 <sup>bc</sup> 169.38 <sup>abc</sup>	$122.71^{ab}$ $122.12^{ab}$	70.06 <sup>a</sup> 68.83 <sup>ab</sup>	30.58 <sup>bc</sup> 32.65 <sup>abc</sup>	53.61 <sup>c</sup> 55.54 <sup>bc</sup>	132.46 <sup>d</sup>
	2 3	177.71 <sup>abc</sup> 190.00 <sup>abc</sup>	169.38 <sup>abc</sup> 178.48 <sup>abc</sup>	122.12 <sup>ab</sup> 127.28 <sup>ab</sup>	67.27 <sup>ab</sup>	32.65 <sup>ab</sup>	59.83 <sup>a</sup>	217.38 <sup>ab</sup> 240.22 <sup>a</sup>
	4 5	170.62 <sup>c</sup> 195.16 <sup>a</sup>	161.62 <sup>c</sup> 186.91 <sup>a</sup>	115.45 <sup>b</sup> 130.00 <sup>a</sup>	67.72 <sup>ab</sup> 66.73 <sup>b</sup>	29.13 <sup>c</sup> 34.74 <sup>a</sup>	55.60 <sup>bc</sup> 55.62 <sup>bc</sup>	164.94 <sup>cd</sup> 172.07 <sup>c</sup>
Control <sup>1</sup>	+	191.12 <sup>ab</sup> 173.90 <sup>bc</sup>	182.62 <sup>ab</sup> 165.79 <sup>bc</sup>	126.14 <sup>ab</sup> 116.53 <sup>ab</sup>	$66.14^{ab}$ $67.12^{ab}$	33.09 <sup>abc</sup> 31.80 <sup>abc</sup>	57.72 <sup>ab</sup> 52.36 <sup>c</sup>	190.45 <sup>bc</sup> 81.93 <sup>e</sup>
Replication	1 2 3	180.04 <sup>a</sup> 184.9 <sup>a</sup> 181.73 <sup>a</sup>	171.35 <sup>a</sup> 175.81 <sup>a</sup> 172.88 <sup>a</sup>	121.76 <sup>a</sup> 124.71 <sup>a</sup> 123.04 <sup>a</sup>	67.68 <sup>a</sup> 67.71 <sup>a</sup> 67.87 <sup>a</sup>	31.57 <sup>a</sup> 32.63 <sup>a</sup> 32.6 <sup>a</sup>	55.10 <sup>a</sup> 55.61 <sup>a</sup> 56.54 <sup>a</sup>	185.24 <sup>a</sup> 163.63 <sup>a</sup> 165.17 <sup>a</sup>
Sex	f m	196.12 <sup>a</sup> 169.74 <sup>b</sup>	186.10 <sup>a</sup> 161.64 <sup>b</sup>	129.65 <sub>a</sub> 117.19 <sub>b</sub>	66.30 <sup>b</sup> 69.06 <sup>a</sup>	34.33 <sup>a</sup> 30.32 <sup>b</sup>	55.88 <sup>a</sup> 55.58 <sup>a</sup>	175.55 <sup>a</sup> 165.74 <sup>a</sup>

 $^{1}$  Positive and negative control groups;  $^{abcde}$  Values with in a column with no common superscript differ significantly (p $\leq$ 0.05).

Data also showed that carcass yield, tibia ash, and tibia phosphorus were not affected at any stage by experimental replications (p>0.05; Table 2).

#### References

- AOAC (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Association of Official Analytical Chemists Washington, DC.
- Attia Y.A., Qota E.M.A., Aggor F.A.M., Kies A.K. (2003). Values of rice bran, its maximal utilization and upgrading by phytase and other enzymes and diet formulation based on available amino acid for broiler chicks. Archiv fur Gelfugelkunde 67: 157.
- Broz J., Oldale P., Perrin-voltz A.H., Tychen G., Schulze J., Simoes Nunes C. (1994). Effects of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. British poultry Science 35: 273–280.
- Eeckhout W., De Paepe M. (1994). Total phosphorus, phytate- phosphorus and phytase activity in plant feedstuffs. Animal Feed Science Technology 47: 19–29.
- EI-Badry A.S.O., Mahrousa M.H., Fatouh M.H., EI-Hakim Abd A.S. (2008). Role of Phytase Supplementation Into Muscovy Duckg Diet In Thermo – And Osmoregulation During Summer Season Egypt. Poultry Science Vol 28, IV: 1059-1081.
- Hall L.E., Shirley R.B., Bakalli R.I., Aggrey S.E., Pesti G.M., Edwards Jr H.M. (2003). Power of Two Methods for the Estimation of Bone Ash of Broilers. Poultry Science 82: 414–418.
- Huff W.E., Moore P.A., Waldroup Jr P.W., Waldroup A.L., Balog J.M., Huff G. R., Rath N.C., Daniel T.C., Raboy V. (1998). Effect of Dietary Phytase and High Available Phosphorus Corn on Broiler Chicken Performance. Poultry Science 77: 1899–1904.
- Jongbloed A.W., Kemme P.A. (1990). Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Animal Feed Science Technology 28: 233–242.
- Jongbloed A.W., Mroz Z., Kemme P.A. (1992). The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. Journal Animal Science 70: 1159–1168.
- Keshavarz K. (2000). Reevaluation of nonphytate phosphorus equirement of growing pullets with and without phytase. Poultry Science 70: 1143–1153.
- Ketaren P.P., Batterham E.S., Dettmann E.B., Farrell D.J. (1993).
  Phosphorus studies in pigs. 3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs. British Journal Nutrition 70: 289–311.
- Mitchell R.D., Edwards Jr H.M. (1996a). Effects of phytase and 1, 25-dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poultry Science 75: 95–110.
- Mitchell R.D., Edwards Jr H.M. (1996b). Additive effects of 1, 25-dihydroxycholecalciferol and phytase on phytatephosphorus utilization and related parameters in broiler chickens. Poultry Science 75: 111–119.
- Nelson T.S., Shieh T.R., Wodzinski R.J., Ware J.H. (1968). The availability of phytate phosphorus in soybean meal befor and after treatment with a mold phytase. Poultry Science 47: 1842- 1848.
- Nelson T.S., Shieh T.R., Wodzinski R.J., Ware J.H. (1971). Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. Journal Nutrition 101: 1289–1294.
- NRC (1994). Nutrient Requirements of poultry 9<sup>th</sup> rev. ed. National Academy Press, Nation Research Council Washington D.C.

- Pallauf J., Rimbach G., Pippig S., Schindler B., Most E. (1994).
  Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the bioavailability of dietary phosphorus, calcium,magnesium, zinc and protein in piglets. Agrobiologic Research 47: 39–48. Punna S, Roland Sr DA. (1999). Variation in phytate phosphorus utilization within the same broiler strain. Journal Applied Poultry Research 8: 10–15.
- Qian H., Veit H.P., Kornegay E.T., Ravindran V., Denbow D.M. (1996). Effects of supplemental phytase and phosphorus on histological and other tibial bone characteristics and performances of broilers fed semi-purified diets. Poultry Science 75: 618–626.
- Rama-Rao S. V., Ravindra Reddy V., Ramasubba Reddy V. (1999). Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. Animal Feed Science Technology 79: 211–222.
- Reddy N.R., Sathe S.K., Salunkhe D.K. (1982). Phytates in legumes and cereals. pp. 1-92 In: "Advances in Food Research." C. O. Chichester, E. M. Mark and G. F. Steward (eds), Academic Press, N.Y.
- SAS Institute. (1990). SAS User's Guide: Statistics. Version 8 edition SAS Institute Inc., Cary, NC.
- Sebastian S., Touchburn S.P., Chavez E.R. (1998). Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. World's Poultry Science Journal 54: 27-47.
- Sebastian S., Touchburn S. P., Chavez E.R., Lague P. C. (1996a). 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. Poultry Science 75: 729–736.
- Sebastian S., Touchburn S. P., Chavez E.R., Lague P. C. (1996b). Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poultry Science 75: 1516–1523.
- Shirley R.B., Edwards, Jr H.M. (2003). Graded levels of phytase past industry standards improves broiler performance. Poultry Science 82: 671–680.
- Simons P.C.M., Versteegh H.A.J., Jongbloed A.W., Kemme P.A., Slump P., Bos K.D., Wolters M.G.E., Beudeker R.F., Verschoor G.J. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. Journal Nutrition 64: 525–540.
- Vali N., Edriss M.A., Rahmani H.R. (2005). Genetic Parameters of Body and Some Carcass Traits in Two Quail Strains International Journal of Poultry Science 4 (5): 296-300.
- Vali N., Edriss M.A. and Moshtaghi H. (2006). Comparison of Egg Weight Between Two Quail Strains. International Journal of Poultry Science 5 (4), 398-400.
- Viveros A., Centeno C., Brenes A., Canales R., Lozano A. (2000). Phytase and acid phosphatase activities in plant feedstuffs. Journal of Agriculture Food Chemistry 48: 4009–4013.
- Viveros A., Brenes A., Arija I., Centeno C. (2002). Effects of Microbial Phytase Supplementation on Mineral Utilization and Serum Enzyme Activities in Broiler Chicks Fed Different Levels of Phosphorus Poultry Science 81: 1172–1183.
- Wyss M., Brugger R., Kronenberger A., Remy R., Fimbeld R., Oesterhelt G., Lehmann M., Van Loon A. P. (1999). Biochemical characterization of fungal phytases (*myo*-inositol hexakisphosphate phosphohydrolases): Catalytic properties. Apply Environ Microbiology 65: 367–373.
- Yalcin S., Ozkan S., Acikoz Z., Ozkan K. (1999). Effect of dietary methionine on performance, carcass characteristics and breast meat composition of heterozygous naked neck (Na/na+) birds under spring and summer conditions. British Poultry Science 40: 688-694.

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