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Effects of Microbial Phytase on Growth Performance, Carcass Yield, Biochemical Parameters, Oxidative Stress and Faecal Phosphorus Content of Japanese Quails

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Abstract: This study was carried out to investigate the use of different levels of phytase enzyme supplementation on the growth performance, carcass yield, oxidative stress, faecal phosphorus and biochemical parameters of Japanese quails. Total of 220 Japanese quails *(Coturnix coturnix japonica)* at three-day old age were used. The birds were randomly assigned to one control and three experimental groups based on their initial body weight, comprising five replicates with 11 birds each. They were fed a basal diet (Control) or the basal diet supplemented with either 500 FTU kg^{-1} (Group I), 750 FTU kg^{-1} (Group II) or 1000 FTU kg^{-1} (Group III) of microbial phytase (Peniophora lycii, containing 500 FTU kg^{-1} phytase activities). As a result, phytase supplementation to diets of quails didn't affect growth performance and oxidative stress parameters ($p>0.05$); however, it decreased faecal phosphorus content ($p<0.05$) and serum potassium level ($p<0.01$) and increased triglycerides and serum VLDL levels ($p<0.05$).

Key words: Phytase, quail, faecal phosphorus, biochemical parameters, microbial phytase

INTRODUCTION

Phosphorus (P) is an essential mineral for growing broilers. Because of the demands for adequate skeletal development of the rapidly growing birds and the deleterious consequences of P deficiencies on productivity, it is necessary to provide an adequate margin of safety for this mineral in broiler diets (Yan and Waldroup, 2006).

About two-thirds of the total 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 phytase (Nelson, 1967). Thus, the bioavailability of P from feedstuffs of plant origin is generally very low. In standard corn-soybean diets, available P from corn and soybean ingredients is limited to one third of the total P to be utilized by the chickens. The rest of the total P (two thirds) is tied in the form of an organic compound called phytate (Ravindran *et al*., 1995). Phytase enzyme, lacking in the chicken GI tract, could hydrolyze the phytate compound and release P and other minerals for use by birds (Gibson and Ullah, 1990). Earlier literature showed that phytase enzyme supplementation to low-phosphorus diets significantly improved body weight gain and feed intake of broiler chicks and improved bone mineralization. However, feed conversion ratio was not affected by phytase supplementation (Ahmed *et al*., 2000).

Adding phytase enzyme to broiler diets to improve utilization of P and other minerals could help lower the need to add expensive commercial inorganic phosphate (dicalcium phosphate) to poultry rations, thus reducing the cost of poultry diets. Using P from the organic phytate compound could help reduce P contamination to ground water and provides a benefit for improving the environment (Kornegay *et al*., 1996; Aksakal and Bilal, 2002).

The objective of this study, was to investigate the use of different levels of phytase enzyme supplementation on the growth performance, carcass, oxidative stress, faecal phosphorus and biochemical parameters of Japanese quails.

MATERIALS AND METHODS

In this research, a total of 220 Japanese quails (*Coturnix coturnix japonica*) at three-day old age were used. The birds were randomly assigned to one control and three experimental groups based on their initial body weight, comprising five replicates of 11 birds each. They were fed a basal diet (Control) or the basal diet supplemented with either 500 FTU kg^{-1} (Group I), 750 FTU kg^{-1} (Group II) or 1000 FTU kg^{-1} (Group III) of microbial phytase (Peniophora lycii, containing 500 FTU kg^{-1} phytase activities). Ingredients and chemical compositions of the basal diets are shown in

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Table 1: Composition of basal diet fed to quails (g kg^{-1})

Ingredients (g kg^{-1})	g kg^{-1}
Ground corn	437
Wheat	133.5
Soybean meal	340
Fish meal	50
Vegetable oil	20
Dicalcium phosphate	3.5
Limestone	11
Salt	2.50
Vitamin-mineral premix*	2.50
Total	1000
Chemical analysis, (g kg^{-1} dry matter)	
Crude protein	238
Calcium	8.0
Available phosphorus	3.0
Calculated nutrient composition	
ME (Kcal kg^{-1})	2923
Lysine(g kg^{-1})	13.3
Methionine+cystine(g kg^{-1})	7.9

|Vitamin premix provided the following per kg diet: Vitamin A, 12500IU; Vitamin D3, 1500 IU; Vitamin E, 31.25 mg; Vitamin K3, 3.75 mg; Vitamin Bl, 2.5 mg; Vitamin B2, 7.5 mg; Niacin 25 mg; Cal. D-pantothenate 10 mg; Vitamin B6, 5mg; Vitamin B12, 0.019 mg; Folic acid 1 mg; Choline chloride 250 mg; Mn 100 mg; Fe 75 mg; Zn 75 mg; Cu 6.25 mg; Co 0.25 mg; I, 1.25 mg;Se 0.19 mg

Table 1. Chemical compositions of the diets were analyzed using the international procedures of AOAC (1994). Small amounts of the basal diet were first mixed with the respective amount of phytase as a small batch and then with a larger amount of the basal diet until the total amounts of the respective diets were homogeneously mixed.

The diets and water were given for ad libitum basis consumption throughout the experiment lasting five weeks. Continuous light were provided with day light and 40 Watt fluorescent lamp in the birdhouse. Body weights and feed consumption data were recorded at weekly intervals. Body weight gain and feed conversion ratio were also calculated.

At the end of the trial, the birds were held for 4-6 h without food and water prior to the determining of final body weights. Each bird was weighed live and slaughtered. The carcass was immersed in water 4°C and washed. Upon removal from water, the carcass was drained for 10 min, weighed for hot carcass yield, bagged and stored at 3±0.5°C for 24 h (Yalcin *et al*., 1999). Upon removal from the bag, carcass was weighed to determine a cold carcass yield. Carcass procedures mentioned above was performed by two experienced people according to Brake *et al*. (1993).

At the end of five weeks, ten animals of each group were decapitated and blood samples were collected into heparinized tubes. Fasting plasma blood calcium (Ca), P, potassium (K), iron (Fe), Total Protein (TP), Glucose (Gl), Cholesterol (Ch), Triglyceride (T), VLDL concentrations were measured by automated chemistry analyzer (Aeroset, Abbott, USA) using commercial kits (Abbott). Copper (Cu) and Zinc (Zn) concentrations were determined by a Spectra A 250 plus Zeeman Atomic Absorption Spectrometer (Varian, Australia) with a deuterium background correction. Excreta was dried and ground to pass through a 1 mm sieve and feed samples were also ground to pass through a 1 mm sieve. Phosphorus concentrations in faeces were determined calorimetrically by the molybdo vanadate method (AOAC, 1995).

Total antioxidant capacity, seruloplazmin:feroxidase, sülphidril, arilesterase, total oxidation status and paraoxanase were determined according to the methods described by Erel (2004).

Experimental data were analyzed in a completely randomized design using GLM procedure of SAS (1989). The means were compared using Duncan mean comparison test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of phytase enzyme supplementation on the performance of control and phytase treatment groups of Japanese quails are shown in Table 2. Body weights, feed consumption and feed conversion of control and phytase treatment groups were not significantly different ($p>0.05$) which is in a line with previous reports (Pizzolante *et al*., 2002; Saricicek *et al*., 2005). In contrast, some researchers indicated some improvements with supplementation on same parameters (Midilli *et al*., 2003; Karimi, 2006). Body weight gain, feed intake and feed conversion ratio were decreased by a low-level available P diet, supplementation of phytase to the diet improved performance of broilers (Cabahug *et al*., 1999; Sohail and Roland, 1999). In current study, it can be concluded that control diet of quails is not a P deficient thus increment P availability had no effect on performance. The carcass characteristics values of current study are shown in Table 3. As shown in the Table 3, the differences among groups in terms of all carcass characteristics were not found significant ($p>0.05$). The results of this study were in agreement with results reported by Sacakli *et al*. (2006).

Effect of phytase enzyme supplementation on the biochemical parameters, feacal P content and oxidative stress parameters of Japanese quails was given in Table 4. Biochemical parameters such as Ca, P, Cu, Zn, Fe, Gl, TP and cholesterol of Japanese quails were not affected by the dietary phytase supplementation. But, serum K, triglyceride and VLDL concentrations in the experimental groups were significantly affected. When compared with the control group, phytase is decreased serum potassium concentrations ($p<0.01$), increased triglyceride and VLDL concentrations ($p<0.05$).

Table 2: Effect of phytase enzyme supplementation on the performance parameters of Japanese quails

	Groups	1st wk Mean±SEM	2nd wk Mean±SEM	3rd wk Mean±SEM	4th wk Mean±SEM	5th wk Mean±SEM	6th wk Mean±SEM
BW(g)	Control	12.76±0.21	36.54±0.43	73.65±1.37	110.39±2.13	141.05±1.94	167.39±4.43
	Group 1	12.66±0.14	37.82±0.52	76.13±0.76	113.76±2.32	145.58±1.99	173.32±1.69
	Group 2	12.62±0.14	36.51±0.43	73.42±1.13	109.47±1.55	141.90±1.14	168.66±1.04
	Group 3	12.51±0.18	36.67±0.70	74.36±1.52	112.36±2.00	139.69±1.72	164.29±1.47
	P	ns	ns	ns	ns	ns	ns
		2^{nd} wk	3^{rd} wk	4^{th} wk	5^{th} wk	6^{th} wk	2-6^{th} wk
BWG	Control	23.78±0.28	27.18±1.46	36.74±1.21	30.66±1.16	26.34±2.92	154.63±4.28
(g week^{-1})	Group 1	25.16±0.44	27.80±0.76	37.63±1.60	31.82±1.07	27.74±1.60	160.66±1.56
	Group 2	23.89±0.46	26.49±1.55	36.05±0.83	32.43±0.98	26.76±1.43	156.04±1.02
	Group 3	24.16±0.73	27.96±1.16	38.00±0.66	27.34±0.81	24.60±1.58	151.78±1.40
	P	ns	ns	ns	ns	ns	ns
FC	Control	46.47±1.13	80.62±1.82	110.04±3.88	141.40±3.30	164.08±7.84	542.60±9.60
(g week^{-1})	Group 1	48.33±0.70	78.29±3.67	109.51±4.15	146.60±1.61	171.67±5.63	554.39±8.01
	Group 2	46.93±1.31	72.18±3.19	110.00±5.41	147.42±4.66	168.42±4.39	544.96±12.97
	Group 3	46.40±1.09	75.53±2.43	109.27±4.99	143.67±2.52	162.61±3.68	537.49±9.14
	P	ns	ns	ns	ns	ns	ns
FCR	Control	1.95±0.04	2.99±0.15	3.01±0.16	4.63±0.13	6.44±0.50	3.52±0.06
	Group 1	1.92±0.02	2.82±0.11	2.92±0.12	4.63±0.14	6.27±0.40	3.45±0.03
	Group 2	1.96±0.03	2.78±0.25	3.05±0.12	4.57±0.24	6.39±0.46	3.49±0.09
	Group 3	1.92±0.04	2.71±0.09	2.88±0.14	5.28±0.24	6.71±0.42	3.54±0.09
	P	ns	ns	ns	ns	ns	ns

BWG: Body Weight Gain; FC: Feed Consumption; FCR: Feed Conversion Ratio, a-b: means in the same parameter with different letters are significantly different ($p<0.05$), ns: $p>0.05$

Table 3: Effect of phytase enzyme supplementation on the carcass characteristics of Japanese quails (n = 10)

Treatments	Live weight at slaughter (g) Mean±SEM	Hot carcass yield (%) Mean±SEM	Chilled carcass yield (%) Mean±SEM
Control	156.40±1.83	76.98±0.86	74.29±0.71
Group 1	155.20±3.50	76.82±0.49	74.22±0.27
Group 2	152.80±3.14	77.73±0.92	74.84±0.85
Group 3	155.20±1.62	77.58±0.82	74.75±0.57
P	ns	ns	ns

ns: $p>0.05$

Table 4: Effect of phytase enzyme supplementation on the biochemical parameters, feacal phosphorus content and oxidative stress parameters of Japanese quails

	Control	Group 1	Group 2	Group 3	P
Ca (mg dL^{-1})	6.66±0.62	6.25±0.35	6.64±0.07	6.00±0.26	ns
P (mg dL^{-1})	6.70±0.41	6.18±0.46	6.79±0.36	6.45±0.34	ns
K (mEq L^{-1})	5.94±0.29^{a}	4.34±0.21^{b}	5.23±0.40^{ab}	4.18±0.33^{b}	**
Cu (µg dL^{-1})	23.68±3.38	20.00±1.35	16.84±2.05	20.32±2.45	ns
Zn (µg dL^{-1})	316.48±26.75	345.43±36.27	257.36±11.25	267.18±22.34	ns
Fe (µg dL^{-1})	118.00±5.59	122.75±5.76	121.80±4.95	123.60±6.89	ns
Glucose (mg dL^{-1})	292.20±11.20	274.25±10.18	309.00±5.84	302.00±6.79	ns
Total protein (g dL^{-1})	2.30±0.22	2.18±0.14	2.22±0.11	2.16±0.05	ns
Triglyceride (mg dL^{-1})	56.00±4.12^{b}	59.20±4.93^{ab}	71.80±3.72^{a}	70.25±1.11^{a}	*
Cholesterol (mg dL^{-1})	122.60±3.36	145.00±18.85	164.80±10.16	156.80±13.63	ns
VLDL (mg dL^{-1})	11.25±0.75^{b}	11.60±1.03^{b}	14.40±0.75^{a}	13.75±0.25^{ab}	*
Faecal phosphorus, %	1.21±0.10^{a}	1.14±0.10^{ab}	0.93±0.11^{ab}	0.84±0.04^{b}	*
Total antioxidant capacity	0.70±0.03	0.62±0.05	0.77±0.03	0.72±0.04	ns
Seruloplazmin:Feroxidase	0.69±0.01	0.67±0.01	0.69±0.00	0.71±0.01	ns
Sülphidril	0.09±0.00	0.09±0.00	0.09±0.00	0.08±0.00	ns
Arilesterase	68.60±1.75	65.00±8.72	84.00±13.93	68.00±7.73	ns
Total oxidation status	8.08±0.48	7.33±0.45	8.01±0.30	7.99±0.30	ns
Paraoxanase U L^{-1}	1.72±0.14	1.85±0.05	1.72±0.38	1.48±0.14	ns

a-b: means in the same parameter with different letters are significantly different ($p<0.05$), ns: $p>0.05$, *: $p<0.05$, **: $p<0.01$

Midilli *et al.* (2002) reported that addition of dietary phytase increased Ca, P and Mg content of tibia, but not serum contents of same minerals. Perney *et al.* (1993) indicated that increment of available P in diet elevated inorganic P content of serum, but phytase supplementation had no similar effect. Increasing phytase levels (500, 750 ve 1000 FTU kg^{-1}) decreased significantly faecal P content ($p<0.05$) and lowest value was observed with the highest phytase supplementation. Since adding phytase broken down the pyhtate-bound P and made it available for use of broilers, feacal P contents were diminished. Therefore, it is clear that exogenous phytase addition to quail diets will help to reduce P pollution to environment (Yan *et al.*, 2003; Afsharmanesh and Pourreza, 2005). Total antioxidant capacity, seruloplazmin, sülphidril, sülphidril, total oxidative status and paraoxanase parameters were not significant different ($p>0.05$) between control and phytase treatment groups.

CONCLUSION

As a result, phytase supplementation to diets of quails didn't affect growth performance and oxidative stress parameters; however, it decreased faecal P content and serum K level and additionally increased triglycerides and VLDL levels of serum. Low-level available P containing diets should be chosen for future phytase supplementation studies with quails to decreasing P supplementation and protecting environment from P pollution.

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