



Dietary phytase levels on performance and egg quality of Japanese quails

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ABSTRACT - The experiment was conducted to evaluate the addition of phytase on productive performance and egg quality of Japanese quails fed diets containing different levels of phytase. It was used 320 Japanese quails at 167 days of age, weighing 182.3 ± 3.8 g distributed in a completely randomized design with four experimental diets and eight repetitions of ten birds per experimental unit. Diets were formulated from a basal diet (control) and they were corn and soybean meal based, without supplementation, added with 200, 400 or 600 phytase activity unit (ftu). Supplementation of phytase in the diets improved productive performance and quality of eggs. Efficiency of phosphorus use for egg mass, from 0.13% of availability of this mineral in the ration, improved with supplementation of 463 ftu/kg. Optimum levels of phytase for the other performance traits and egg quality were also reached with 463 ftu/kg.

Key Words: *Coturnix coturnix japonica*, enzyme, nutritional profile, phosphorus, phytate

Níveis de fitase na dieta sobre o desempenho e a qualidade dos ovos de codornas japonesas

RESUMO - O experimento foi realizado para avaliar a adição de fitase sobre o desempenho produtivo e a qualidade dos ovos de codornas japonesas alimentadas com rações contendo diferentes níveis de fitase. Foram utilizadas 320 codornas japonesas, com 167 dias de idade, peso de $182,3 \pm 3,8$ g, distribuídas em delineamento inteiramente casualizado com quatro dietas experimentais e oito repetições de dez aves por unidade experimental. As dietas foram formuladas a partir de uma dieta basal (controle), à base de milho e farelo de soja, sem suplementação, e foram acrescidas de 200, 400 ou 600 unidades de atividade de fitase (uft). A suplementação de fitase na ração melhorou o desempenho produtivo e a qualidade dos ovos. A eficiência do uso de fósforo para massa de ovos, a partir de 0,13% de disponibilidade deste mineral na ração, melhorou com a suplementação de 463 uft/kg. Os níveis ideais de fitase para as demais características de desempenho produtivo e qualidade dos ovos também foram alcançados com 463 uft/kg.

Palavras-chave: *Coturnix coturnix japonica*, enzima, fitato, fósforo, perfil nutricional

Introduction

Phytase is commonly used for production of broilers and laying hens to increase bioavailability of phytic acid in the diet, to save phosphorus sources and to reduce environmental pollution (Bertechini, 2006; Broz & Ward, 2007).

Although phytate is a reactive anion that can form a wide variety of insoluble salts, many studies have shown the efficacy of phytase in improving the availability of minerals, protein and energy. The performance of animals depends largely on the availability of nutrients in the diets inasmuch as these nutrients can be absorbed and metabolically used (Nagashiro, 2007).

Enzyme supplementation in diet can improve the activity of endogenous enzymes, improving nutrient utilization and bird performance. Diets with reduced levels of minerals, protein or amino acids and energy and supplemented with phytase can provide the same performance for broilers fed diet with adequate nutritional levels (Zanella et al., 1999).

Nutritionists have been engaged in researching diets that meet the nutritional requirements of quails because their fast growth and high productivity have demanded better food and additives so these birds can make better use of the nutrients. Thus, the objective of this research is to evaluate the performance and egg quality of Japanese quails fed diets of poor nutritional value containing different phytase levels.

Material and Methods

The experiment was conducted at the Setor de Avicultura of the Departamento de Zootecnia of the Universidade Federal de Viçosa (UFV) from September 2 to November 25, 2007, totaling 84 days. It was used 320 Japanese quails (*Coturnix coturnix japonica*) at 167 days of age, weighing 182.3 ± 3.8 g and with an egg production rate of 89.0%. The birds were distributed in a completely randomized design with four treatments and eight replicates of ten birds each. The experimental diets were formulated from a corn-soybean meal based diet to meet the nutritional requirements of the quail and nutritional matrix values for phytase. In the basal diet 0, 200, 400 or 600 units of phytase activity (ftu/kg) were added.

Birds were housed in galvanized wire cages arranged in batteries, so each battery was composed of five cages measuring $96 \times 37 \times 16$ cm (width \times height \times depth) with a cage for walkings and three divided cages, thus the experimental unit measured $1,184$ cm². Ten birds were housed per division, providing 118.4 cm² per bird. Under the floor of the cages, trays for excreta collection were placed. The cages were equipped with drinkers and feeding troughs, both covering the entire length of the cage, the feeder positioned in the front and the trough at the rear of the cage. Each feeder was equipped with two wooden partitions, coinciding with the width of each experimental unit.

The batteries were installed in a room, with maximum ($27.5^\circ\text{C} \pm 1.9$) and minimum ($19.3^\circ\text{C} \pm 2.1$) temperatures recorded once a day at 4 p.m.; average temperature ($23.8^\circ\text{C} \pm 2.5$) and relative humidity (81.2 ± 4.6) were recorded twice (8 a.m. and 4 p.m) daily.

For the lighting program, it was adopted a natural photoperiod combined with artificial lighting, totaling 17 hours of light.

The experimental diets were formulated based on corn and soybean meal, according to the nutritional requirements recommended by NRC (1994), except for lysine, methionine + cystine, threonine and digestible tryptophan, which were used by Pinto et al (2003), Umigi et al. (2008) and Pinheiro et al. (2008), respectively. Calcium was determined by Barreto et al. (2007), available phosphorus was determined by Costa et al. (2007) and metabolizable energy was determined by Moura et al. (2008), maintaining the same ratio between metabolizable energy and nutrients. From the nutritional requirements, it was formulated a basal diet (Table 1) with reductions of 0.36% crude protein, 0.115% calcium, 45 kcal of metabolizable energy, 0.01% lysine, 0.015% sulfur amino acids 0,03% of threonine and adopting a level of 0.13% available P of 43% of the requirement of this

mineral. The reduction of nutrients and energy in the diet was based on recommendations of the phytase matrix for laying hens. Thus, it was adopted 0, 0.008, 0.016 and 0.024% phytase in diets for composing the levels of 0, 200, 400 and 600 (ftu/kg), respectively. Phytase was used to replace starch. The chemical composition and nutritional value of ingredients used to formulate diets were those recommended by Rostagno et al. (2005). Diets and water were supplied ad libitum and food was offered twice daily at 8 a.m. and 4 p.m.

During the experiment, the following parameters were observed and evaluated: feed intake (g/bird/day) and phosphorus (mg/bird/day), phosphorus use efficiency for egg mass (mg/bird/day), daily egg production per bird (%), egg production per housed bird (%), commercial egg production (%), daily production of viable eggs per bird (%), egg weight (g), egg mass (g/bird/day), feed conversion by egg mass (kg feed/kg eggs), feed conversion per egg dozen (kg feed/dozen eggs), egg components, yolk (g%), albumen (g%) and shell (g%), body weight variation (g) birds viability (%) and specific gravity (g/cm³).

Every 21 days, it was evaluated the amount of food consumed in relation to the number of birds in each diet in

Table 1 - Composition in the ingredients and nutrients of the basal diet

Ingredient	Percentage
Corn	61.270
Soybean meal (45.0%)	30.000
Limestone	7.410
Dicalcium phosphate	0.150
Salt	0.320
Mineral mixture ¹	0.050
Vitamin mixture ²	0.100
DL-methionine (98.2%)	0.270
L-lysine (78.8%)	0.220
Antioxidant ³	0.010
Choline chloride (60.0%)	0.100
Starch	0.100
Phytase	0.000
Nutritional composition calculated	
Metabolizable energy (kcal/kg)	2755
Crude protein (%)	18.950
Digestible lysine (%)	1.070
Digestible methionine + cystine (%)	0.854
Digestible tryptophan (%)	0.226
Digestible threonine (%)	0.563
Calcium (%)	2.980
Available phosphorus (%)	0.130
Total phosphorus (%)	0.330
Sodium (%)	0.145
Crude fiber (%)	2.690

¹ Composition/kg product: Mn - 160 g; Fe - 100 g; Zn - 100 g; Cu - 20 g; Co - 2 g; I - 2 g; excipients q.s.p.: 1.000 g.

² Composition/kg of product: vit. A - 12,000,000 UI; vit. D₃ - 3,600,000 UI; vit. E - 3,500 UI; vit. B₁ - 2,500 mg; vit. B₂ - 8,000 mg; vit. B₆ - 5,000 mg; pantothenic acid - 12,000 mg; Biotin - 200 mg; vit. K - 3,000 mg; folic acid - 1,500 mg; nicotinic acid - 40,000 mg; vit. B₁₂ - 20,000 mg; Se - 150 mg; vehicle q.s.p. - 1,000 g.

³Butyl-hydroxy-toluene.

the 21-day period and the value expressed in grams of feed consumed per bird per day. For death of birds during the experimental period, it was proceeded the correction of feed intake to obtain the true average intake in the experimental unit. Leftovers were weighed and subtracted from the amount of diet weighted in the experimental period for evaluation of feed intake.

The efficiency of phosphorus utilization on egg mass (EUPM) was obtained by measuring phosphorus consumption (mg/bird/day). Accordingly, the values were expressed in relation to egg mass (g/bird/day) for each repetition.

Eggs were collected daily at 8 a.m. to determine average production for the period, which was obtained by registering the number of eggs produced daily, including broken, chipped and abnormal eggs, expressed as a percentage of the number of birds of the period (egg/bird/day) and on the number of birds housed at the start of the experiment (egg/housed bird). The average number of commercial eggs (expressed in percentage) during the experimental period was also calculated, discounting broken, cracked and abnormal eggs. Considering the percentage of commercially viable eggs), the production of viable eggs per bird per day was calculated.

All intact eggs produced in each repetition on the experimental days 19, 20, 21, 40, 41, 42, 61, 62, 63, 82, 83 and 84 were weighed on a 0.001-g readability balance scale and the total weight obtained was divided by the number of eggs used for weighing, resulting in the average weight of eggs.

The average weight of eggs was multiplied by the total number of eggs produced during the experimental period, obtaining the total eggs mass, which was divided by the total number of birds per day of the period and expressed as grams of eggs per bird per day (egg g/bird/day).

To evaluate the components of the eggs, weights of yolk, albumen and shell were compared to egg weight on the experimental days 19, 20, 21, 40, 41, 42, 61, 62, 63, 82, 83 and 84. For that, it was used four eggs randomly chosen from each experimental unit and individually weighed on a 0.001-g readability balance scale. The yolk of each egg was weighed and recorded and the shell was washed and air dried to obtain the weight. The weight of albumen was obtained by subtracting the weight of the egg yolk from the weight of the shell.

It was evaluated feed conversion per egg dozen, expressed as total feed intake in kilograms divided by the dozen eggs (kg/dozen) and egg mass, which was obtained by the feed intake in kilograms divided by egg mass kilogram (kg/kg).

All birds were weighed at the beginning and in the end of the experiment for determining body weight variation. The total number of dead birds was recorded daily and the cumulative number was subtracted from the total number of live birds, and these values were converted into percentage at the end of the experiment to determine the viability of the birds.

On days 16, 17, 18, 37, 38, 39, 58, 59, 60, 79, 80 and 81 of the experimental period, specific gravity of all intact eggs collected was evaluated. The eggs were immersed in salt solutions (water + NaCl) with densities varying from 1.055 to 1.100 g/cm³, with variations of 0.005 g/cm³ among them. The solution density was measured using a hydrometer model INCOTERM - OM - 5565.

The parameters were subjected to analysis of variance at 5% probability by SAEG Program - System for Genetic Analysis and Statistics (UFV, 2007). Afterwards, the effects of phytase levels were estimated by analysis of variables in linear regression models, quadratic and linear response plateau, with the best fit obtained for each variable and considering the biological behavior of the birds.

Results and Discussion

For laying quails, the range of thermal comfort or thermoneutral zone is from 18 to 22°C and relative humidity is from between 65 and 70% (Oliveira, 2004). Thus, according to the values recorded for mean temperature and relative humidity during the experiment, the quails were submitted to periods of heat stress.

There was no effect ($P>0.05$) of phytase supplementation on feed intake by birds (Table 2). Results in this work are similar to those reported by Boling et al. (2000), Vieira et al. (2001), Liebert et al. (2005) and Ferreira et al. (2008), who did not observe any significant variations in feed intake in laying hens supplemented with phytase. On the other hand, the results from this work differed from those of Savietto et al. (2007), who observed effect of phytase supplementation on feed intake in laying hens. In addition, there was not significant effect ($P>0.05$) of phytase levels on phosphorus intake because rations were isophosphoric and feed intake did not vary among enzyme levels.

For egg production, the level at which a plateau occurred was estimated ($P<0.01$) at 335 ftu/kg according to the equation $\hat{Y} = 85.1520 + 0.016X$, $R^2 = 0.99$, $y = 90.5205$. The linear response plateau (LRP), was the best fit to the data, although the egg production per bird per day varied linearly.

Table 2 - Japanese quails performance submitted to diet supplemented with phytase

Parameter	Phytase level (ftu/kg)				
	0	200	400	600	CV (%)
Feed intake (g/bird/day) ^{ns}	26.62	26.44	26.49	26.95	2.23
Phosphorus intake (mg/bird/day) ^{ns}	34.61	34.37	34.44	35.03	2.32
Egg production per hen day (%) ¹	85.15	88.35	90.57	90.47	3.92
Egg mass (g/bird/day) ²	10.24	10.86	10.97	11.22	4.40
Phosphorus efficiency use on egg mass (mg/g) ³	3.38	3.17	3.14	3.12	3.89
Feed conversion by egg mass (kg/kg) ¹	2.591	2.437	2.408	2.416	3.80
Feed conversion per dozen eggs (kg/dozen) ⁴	0.372	0.360	0.349	0.358	4.19
Egg production per hen housed (%) ^{ns}	82.96	88.35	88.07	87.38	6.66
Viable eggs production per bird day (%) ⁴	82.84	85.29	89.28	88.60	4.80
Birds viability (%) ^{ns}	92.50	100.00	96.25	95.00	8.21
Body weight variation (g) ⁵	4.47	4.92	5.51	2.99	-

¹Linear response plateau (P<0.01); ²Linear effect (P<0.01); ³Quadratic effect (P<0.05); ⁴Linear response plateau (P<0.05); ^{ns}No significant effect (P>0.05); ⁵Descriptive analysis because the data did not follow normal distribution.

There was a linear increase (P<0.01) in the egg mass when concentration of phytase in the diets increased according to the equation $\hat{Y} = 10.3626 + 0.00153460 X$, $R^2 = 0.89$. A similar effect of phytase supplementation on egg mass was reported by Soto-Salanova & Wyatt (1997) when working with laying hens. According to these authors, the improvement in egg mass is related to an increasing amount of albumen and yolk in eggs. Possibly, there is greater availability of metabolic phosphorus for physiological and productive use by birds. From the results, it can be inferred that the egg mass was the variable most sensitive to the level of phosphorus.

Difference of dietary phytase levels (P<0.05) on phosphorus efficiency use on egg mass, which quadratically increased up to the level 463 ftu/kg, according to the equation $\hat{Y} = 3.37943 - 1.16787 \times 10^{-3} X + 1.26127 \times 10^{-6} X^2$; $R^2 = 0.97$, was observed. Because there was no effect of phytase (P>0.05) on phosphorus intake, phytase may have promoted the availability of phosphorus from plant ingredients of the diets, possibly by catalyzing metabolic reactions that improved the use efficiency of this mineral in the egg mass.

Feed conversion per egg mass was influenced (P<0.05) by the use of phytase in the diet and improved quadratically up to the level 437 ftu/kg, according to the equation $\hat{Y} = 2.58669 - 8.80722 \times 10^{-4} X + 1.00664 \times 10^{-6} X^2$, $R^2 = 0.98$. Positive effect of phytase on feed conversion by egg mass was also observed by Costa et al. (2004), Savietto et al. (2007) and Silva et al. (2008) in laying hens. Conversely, Noebauer (2006) found no effect of phytase supplementation on this variable in laying hens.

There was a significant quadratic effect (P<0.05) of phytase levels on feed conversion per egg dozen, which increased up to the level 400 ftu/kg, according to the equation $\hat{Y} = 0.373508 - 1.05748 \times 10^{-3} X + 1.32335 \times 10^{-7} X^2$,

$R^2 = 0.95$. Because isophosphoric rations and feed intake did not vary among the levels of phytase, this result is possibly an indication that efficiency of utilization of phosphorus in the diet improved, with higher metabolic availability of this mineral for egg production.

In the provided diets, when the level of phytase increased (P<0.05), production of viable eggs per bird per day increased up to the level 368 ftu/kg, estimated by linear response plateau, according to the equation $\hat{Y} = 82.3452 + 0.015 X$, $R^2 = 0.99$, $y = 87.7138$. There was no significant differences (P>0.05) among percentages of eggs per housed bird, bird viability and body weight variation related to levels of phytase in the feed.

Phytase levels in diets influenced (P<0.05) egg yolk weight. Although a linear variation occurred, the linear response plateau was that best fit for the data, and when enzyme levels increased, that variable also increased up to the level 180 ftu/kg, according to the equation $\hat{Y} = 3.7530 + 0.001 X$, $R^2 = 0.99$, $y = 3.8855$ (Table 3). This increase in yolk weight can be attributed to a greater metabolizable energy availability of feed ingredients by the phytase action. Selle & Ravindran (2007) and Remus (2007) commented that phytase was effective in increasing the metabolizable energy food. According to Cavalheiro et al. (1983), the smallest amount of phosphorus used by hens during egg formation is used in the albumen formation and the largest portion is directed to the yolk in the form of phospholipids and phosphoproteins, which may also explain the results found for yolk weight, due to the increased availability of metabolic phosphorus provided by the enzyme in the rations.

The characteristics of eggs quality (egg weight, albumen weight, shell weight, specific gravity, yolk percentage, albumen and shell and commercial eggs) were not influenced (P>0.05) by phytase in the ration. Ligeiro et al. (2007) observed that the use of phytase in diets for laying hens,

Table 3 - Japanese quail egg quality submitted to diet supplemented with phytase

Parameter	Phytase level (ftu/kg)				
	0	200	400	600	CV (%)
Egg weight (g) ^{ns}	12.02	12.29	12.12	12.41	2.32
Yolk weight ¹ (g)	3.75	3.90	3.84	3.93	3.49
Albumen weight (g) ^{ns}	7.57	7.88	7.71	7.84	2.85
Shell weight (g) ^{ns}	0.98	1.03	1.01	1.03	3.54
Yolk percentage ^{ns}	30.50	30.47	30.58	30.70	2.11
Albumen percentage ^{ns}	61.50	61.48	61.38	61.27	1.10
Shell percentage ^{ns}	8.00	8.05	8.04	8.03	2.33
Specific gravity (g/cm ³) ^{ns}	1.071	1.071	1.072	1.072	0.12
Commercial eggs (%) ^{ns}	97.22	97.10	98.59	97.92	1.49

¹ Linear response plateau (P<0.05).

^{ns} No significant effect (P>0.05).

except for egg weight, did not significantly affect other parameters of egg quality. The result obtained for egg weight agrees with that of Jalal & Scheideler (2001) and Punna & Roland (2001), who did not observe any differences in this parameter when using phytase in diets for laying hens.

Unlike what was obtained in this study, Noebauer (2006) found that the specific gravity of eggs increased significantly with the inclusion of phytase in the diet of laying hens. Among the quality parameters of eggs that were not influenced by phytase levels in the diet, it can be observed improvement in absolute values of the diets containing phytase, compared to non-use of the enzyme. Accordingly, lower values for egg weight, shell and albumen were observed when nutritional level of the diets was reduced.

Data indicate that it was possible to reduce the nutritional value of feed for Japanese quail concomitantly with the addition of phytase. The use of this enzyme in the diet showed an improvement in production rates, which indicates the effect of phytase on nutrient release associated with the phosphate ions present in the ingredients of the diet. The analysis showed better feed conversion per egg mass and dozen eggs at levels 437 and 400 ftu/kg, respectively. However, considering the daily egg production per bird and production of viable eggs per bird per day, the better levels were 335 and 368 ftu/kg, respectively. For egg mass, the highest level of phytase provided better results. However, for efficient use of phosphorus, the level 463 ftu/kg was the best for the composition of the egg mass with 0.13% of available phosphorus in the diet. For the other parameters, performance and egg quality, optimal levels of phytase were also achieved with 463 ftu/kg.

Conclusions

Phytase supplementation in the diet improves performance and egg quality of Japanese quails. The best level can be set at 463 ftu/kg for phosphorus use efficiency by the birds for egg mass with 0.13% available phosphorus in the diet.

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