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The effect of dietary phytase and various available phosphorus levels on performance and egg quality in Japanese quail

Fitase e diferentes níveis de fósforo disponível em dietas de codornas japonesas sobre a produção e qualidade de ovos

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SUMMARY

Birds show poor utilization of phytic phosphorus in their diets because of the lack of endogenous enzymes to digest phosphorus and make it bioavailable. Therefore, the objective of this study was to evaluate phytase supplementation in the diets of laying quails. Eighty quails were used in a completely randomized experimental design with five treatments and four replicates of four birds each. The treatments were as follows: 0.35% Pd; 0.20% Pd; 0.20% Pd + 500FTUs of phytase; 0.09% Pd; 0.09% Pd + 500 FTUs of phytase. The birds were housed in metallic batteries during a trial period of 21 days, for evaluation of egg production, egg weight, feed intake and feed conversion. After being collected, the eggs were sent to the nutrition laboratory of the department of zootechnics of the CEO/Udesc, where analyses of albumin, yolk and shell quality were performed. The results were subjected to analysis of variance, and the differences between the averages were compared using the Tukey test at 5% of significance. No differences (P> 0.05) were observed in terms of performance and egg quality parameters. We conclude that the inclusion of phytase in the diet of laying quails can be accomplished without compromising the performance and quality of the eggs. Additionally, 0.09% of 22 disponible phosphorus can be used to grant adequate performance for 21 days.

Keywords: Enzymes, minerals, nutrition, phosphorus, quail production

RESUMO

As aves possuem baixo aproveitamento do fósforo fítico das dietas, devido à falta de enzimas endógenas que consigam realizar a quebra e aproveitamento do mesmo. Objetivou-se no presente estudo avaliar a adição de fitase em dietas de codornas poedeiras. Foram utilizadas 80codornas, em delineamento experimental inteiramente casualizado composto por cinco tratamentos e quatro repetições, de quatro aves cada. Os tratamentos foram: 0,35% Pd; 0,20% Pd; 0,20% Pd + 500FTUs de fitase; 0,09% Pd; 0,09% Pd + 500 FTUs de fitase. As aves foram alojadas em baterias metálicas, em período experimental de 21 dias, para avaliação da produção de ovos, peso dos ovos, consumo de ração e conversão alimentar. Após coletados, os ovos foram encaminhados ao laboratório de nutrição do departamento de zootecnia do CEO/Udesc, onde foram realizadas as análises de qualidade de albúmen, de clara e casca. Os resultados obtidos foramsubmetidos à análise de variância e as diferenças entre as médias foram comparadas pelo teste de Tukey a 5% de significância. Não foram observadas diferenças (P>0,05) nos parâmetros de desempenho e qualidade de ovos avaliados. Conclui-se que a inclusão da fitase na dieta de codornas poedeiras pode ser empregada sem causar comprometimento do desempenho e qualidade dos ovos das aves.Adicionalmente, 0,09% de fósforo disponível pode ser utilizado, garantindo o desempenho em período de 21 dias de produção.

Palavras-chave: Coturnicultura, enzimas, minerais, nutrição

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INTRODUCTION

Phosphorus is an expensive element in quail diets, as it a mineral that profoundly impacts the final cost of bird rations. The primary sources currently available for quail feeding include phosphates in animal meal, with higher bioavailability for birds, and plant phosphorus, with lower bioavailability. Lower use of plant phosphorus occurs because of complexation in the phytic form, rendering it unavailable for birds (Franceschina et al., 2016), with consequent elimination of the mineral in feces, further increasing the negative impact this residue on the environment.

Intake of phytic acid increases the excretion of amino acids, energy and minerals(Ribeiro et al., 2015), forming a wide variety of insoluble salts with cations such as calcium, zinc, copper, cobalt, manganese, iron and magnesium, negatively influencing digestion and increasing the excretion of these nutrients(Lima et al., 2010).However, the greatest environmental impact occurs on the environmental excretion of phosphorus, not only because it is the mineral present in highest quantities in phytic acid, but also because it leaches to surface water and to groundwater, exacerbating the serious problem of environmental pollution(Lima et al., 2010).

There is now strong pressure to reduce phosphorus levels in feed because lower levels allow lower costs and minimize excretion. The use of phytase is an alternative that allows plant phosphates to be used, hydrolyzing the molecule in the intestinal lumen and making the phosphorus bioavailable, decreasing its excretion in the environment (Teixeira et al., 2013; Shewita & Ahmed, 2015).Since quails do not express this enzyme endogenously, supplementation constitutes an efficient alternative for improving phosphorus utilization from vegetable grains (Lima et al., 2011). Several studies on the use of phytase have been developed with broilers and laying hens; however, there are few reports on the use of phytase in quail diets, and many professionals in the area extrapolate results obtained with other types of birds, giving rise to unsatisfactory zootechnical indexes in quails (Ribeiro et al., 2015).

Therefore, the objective of this study was to evaluate the effect of phytase supplementation in Japanese quail diets on the performance and quality parameters of eggs destined for commercial sale.

MATERIALSAND METHODS

This study was conducted in the poultry sector of the Department of Animal Science of the State University of Santa Catarina, Chapecó, SC, Brazil, using a completely randomized design, consisting of five treatments and four replications, including four birds in each unit experimental, totaling 80 quails. Os tratamentos foram: 0,35% Pd; 0,20% Pd; 0,20% Pd + 500FTUs de fitase; 0,09% Pd; 0,09% 89 Pd + 500 FTUs de fitase. The birds were housed in galvanized wire batteries with feed and water provided at will throughout the experimental period, with rations (Table 1) formulated according to the recommendations of Rostagno et al. (2011).

Feed and weight were weighed throughout the experimental period for the determination of feed intake and feed conversion (kg/dz). The experimental period was 21 days, including the phase from 60 to 81 days of age, when eggs were collected daily, twice a day, and placed in plastic trays for the quantification of percentage egg production per day. During the last four days of the experiment, all eggs were identified and stored for later analysis of the qualitative parameters. For this evaluation, all eggs were weighed on a precision scale, then were broken on a special glass table and immediately subjected to albumin, yolk and shell quality measurements.

For albumen quality, we evaluated Haugh units, following the methodology proposed by Card and Nesheim (1978), calculated according to the following equation: UH= 100 log (H + 7.57 – 1.7W $^{0.37}$), where UH = Haughunits; H = albumen height (in millimeters); W = egg weight (in grams). The height measurement of the albumen was obtained through the use of a special egg altimeter (egg quality micrometer), and weight was measured using a precision scale. The pH of the albumen was evaluated with a digital probe, by inserting the electrode into the respective beakers containing the previously separated albumen samples.

Table1. Food and nutritional composition of the rations

Ingredient (g/kg)	**0.35%Pd**	**0.20%Pd**	**0.20%Pd + 500FTU**	**0.09%Pd**	**0.09%Pd + 500FTU**
Corn	510.35	526.80	52..80	537.09	537.09
Soybean meal (46%)	353.34	350.05	350.05	348.69	348.69
Soy oil	31.59	26.00	26.00	22.51	22.51
Dicalcium phosphate	13.06	4.93	4.93	-	-
Limestone	55.70	55.70	55.70	55.70	55.70
Salt (NaCl)	4.85	4.85	4.85	4.84	4.84
DL-Methionine (99%)	2.13	2.12	2.12	2.12	2.12
L-Lysine HCl	0.95	1.00	1.00	1.02	1.02
L-Threonine	1.26	1.25	1.25	1.25	1.25
Premix	2.00	2.00	2.00	2.00	2.00
Phytase[1]	-	-	0.05	-	0.005
Vehicle	0.87	1.00	0.95	0.88	0.083
Calculated values					
Metabolic Energy kcal/kg	2950	2950	2950	2950	2950
Crude protein g/kg	200	200	200	200	200
Lysine dig. g/kg	10.83	10.83	10.83	10.83	10.83
Methionine dig. g/kg	4.87	4.87	4.87	4.87	4.87
Met. + Cis. dig. g/kg	8.88	8.88	8.88	8.88	8.88
Threonine dig. g/kg	6.50	6.50	6.50	6.50	6.50
Calcium g/kg	30.00	30.00	30.00	30.00	30.00
Available phos. g/kg	3.50	2.00	2.00	0.90	0.90
Sodium g/kg	1.80	1.80	1.80	1.80	1.80

[1] Phytase was added in place of the vehicle: Phytase Genophos®.

Yolk quality was evaluated by calculating the yolk index, which consists of the relationship between the height and the diameter of the yolk, through the formula: YI = YH/YD, where YI = yolk index; YH = yolk height (in mm); YD = yolk diameter (in mm). The height of the yolk was measured with an altimeter, with value obtained in millimeters, and the diameter of the yolk was measured with a digital caliper, also in millimeters. Then, the pH of the yolk was measured with a digital probe, by inserting the electrode into the respective beakers containing the The specific gravity, weight, thickness and strength were evaluated for the shell quality evaluation. For the specific gravity, the method was based on the Archimedean principle, in which we weighed the eggs, intact, in and out of the water, and the specific gravity being obtained according to formula: SG = WA/WW x Cf where: SG = specific gravity, PA = egg weight in air (g), PAG = egg weight in water (g)and Cf = correction factor of gravity as a function of water temperature. After break the eggs, the shells were weighed on a precision scale, and their thickness was obtained with a digital caliper. Subsequently, the peels were analyzed in a texturometer, to evaluate the resistance of the shell, with the results expressed in kgf.

The data of egg production, feed intake, feed conversion and egg quality were subjected to analysis of variance, and in the case of significant effects, the means were compared by the Tukey test at 0.05 of previously separated yolks.
significance, using the statistical software R.

RESULTS AND DISCUSSION

The performance results are presented in Table 2, demonstrating no differences in production(P=0.920) or egg weight (P=0.166).The behavior observed in the present study corroborates the efficacy of phytase effects in making phytic phosphorus available, and it is possible to reduce the levels of total phosphorus of the rations without altering egg production. A similar result was reported by Lima et al. (2011), who tested different levels of phytase in basal diets containing 0.13% of available phosphorus, and found that inclusion above 463 FTU allowed for adequate use of phytic phosphorus in amounts compatible with daily nutritional requirements.

Table2. Performance of laying quails fed various levels of phosphorus, whether or not supplemented with phytase

Treatment	Egg production (%)	Egg weight (g)
0.35% Pd	78.27	10.84
0.20% Pd	82.73	11.15
0.20% Pd + 500FTU	77.97	9.99
0.09% Pd	78.87	11.05
0.09% Pd+ 500FTU	75.59	10.86
Valor P	0.920	0.166
CV (%)	13.86	6.19

* Means followed by different letters on the same line differ, at 0.05 significance, by the Tukey test.

The results agree with those of Zahran et al. (2012) and Ribeiro et al. (2015) who worked with laying quails, with those of Resende (2013) and Ferreira et al. (2015), who worked with light laying hens, and with those of Sacakli et al. (2005), Blake & Hess (2013) and Pirzado et al. (2016) who worked with broiler quails; none found differences in terms of egg production or egg weight, supplemented or not with phytase. The absence of significant differences in performance data can be explained in part by the 21-day experimental period used, because birds have phosphorus reserves in the bones, and the period may not have been long enough to promote reduction or phosphorus deficiency in quails, with a consequent fall in yield.

There is strong pressure to reduce phosphorus levels in feed because of the possibility of environmental excretion; however, as it is a concern because of the large number of biochemical reactions in the organism (Xue et al., 2016). Nutritionists resist reducing available phosphorus levels because of the impact on the performance of the animals.

There were no significant changes (P > 0.05) in albumen height, Haugh units or albumen pH (Table 3). The albumen consists of the protein fraction of the egg, with almost no mineral content. Therefore, it is understandable that there were no differences in the evaluations of this component, which is more sensitive to changes in protein and amino acid levels in rations, especially the amino acid methionine, than to changes in phosphorus levels. Furthermore, because the evaluations were done with fresh eggs, the effect of the storage time on these eggs could not be evaluated, because in general, albumen quality is more influenced by time and storage resistance. The data agree with those reported by Ribeiro et al. (2015), who found no changes in the percentage parameters of clear areas when adding phytase in the diet of laying quails. Similarly, Rezende et al. (2013), when evaluating the inclusion of phytase in the diet of light laying hens, found no differences in the evaluation of the Haugh units of poultry eggs.

Table3. Quality index of laying quail eggs fed with various levels of phosphorus, whether or not supplemented with phytase

Treatment	Albumen height	Haugh units	Albumen pH	Yolk height	Yolk width	Yolk index	Yolk pH
0.35% Pd	3.92	86.83	8.54	11.59	22.54	0.51	6.23
0.20% Pd	4.19	88.10	8.68	11.79	22.12	0.53	6.08
0.20% Pd + 500 FTU	3.72	86.37	8.77	11.32	21.55	0.52	6.09
0.09% Pd	4.03	87.31	8.68	11.54	21.59	0.53	6.18
0.09% Pd+ 500FT	3.88	86.60	8.69	11.54	21.99	0.52	6.07
P-value	0.417	0.757	0.233	0.551	0.337	0.711	0.688
CV (%)	8.73	2.29	1.52	3.29	3.35	4.18	3.15

* Means followed by different letters on the same line differ, at 0.05 significance, by the Tukey test.

Similar values (P > 0.05) were found in the assessments of yolk height, yolk width, yolk index and yolk pH (Table 3). The yolk corresponds to the lipid fraction of the egg, that is altered more substantially by levels of fatty acids in the diet, especially linoleic acid. Therefore, the various levels of available phosphorus were not able to generate an influence on yolk quality parameters.

The yolk contains large amounts of phospholipids and phosphoproteins, important components for embryonic development. In general, phosphorus deficiency leads to a reduction in the qualitative parameters of the yolk (Costa et al., 2011), a fact not observed in the present study. This evidence demonstrates the efficacy of phytase in making phosphorus available while maintaining adequate poultry

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performance. Nevertheless, because birds fed a diet containing 0.09% of available phosphorus without addition of phytase produced eggs of the same quality compared to other evaluated treatments, we believe that, for the 21-day period evaluated, quails had bodily reserves of phosphorus, and at the moment when the reserves diminished there would have been depletion in the qualitative values of the yolk.

There were no differences (P > 0.05) in weight, thickness and resistance of eggshells (Table 4). Furthermore, no changes were observed (P > 0.05) in terms of the specific gravity of eggshells subjected to various treatments. These data agree with those of Ribeiro et al. (2015), who found no differences in the specific weight of eggshells, an element corresponding to the specific gravity evaluated in the present study. Lima et al. (2011), who worked with Japanese quail, and Silva et al. (2012), who worked with semi-heavy laying hens, also found no differences in the evaluation of shell weight, shell thickness or egg specific gravity when hens were supplemented with various levels of phytase.

Table4. Egg quality parameters of laying quails fed with various levels of phosphorus, whether or not supplemented with phytase

Treatment	Shell weight	Shell thickness	Shell resistance	Specific gravity
0.35% Pd	0.90	0.16	1.30	1.072
0.20% Pd	0.94	0.18	1.35	1.072
0.20% Pd + 500FTU	0.84	0.17	1.22	1.081
0.09% Pd	0.90	0.16	1.21	1.075
0.09%Pd + 500FTU	0.92	0.18	1.30	1.073
P-value	0.360	0.138	0.869	0.345
CV (%)	7.73	6.04	16.98	0.65

The composition of the eggshell is mostly calcium carbonate; therefore, the mineral that exerts the greatest influence on its composition and quality is calcium. However, levels of dietary phosphorus may interfere with intestinal calcium absorption, leading to imbalances that could impair the metabolism of calcium in the body, with negative consequences on the deposition of this mineral in eggshell.

The influence of phosphorus on egg shell formation and composition is related to the reduction of metabolic acidosis, because when serum levels are higher, renal excretion of phosphates occurs (Carvalho & Fernandes, 2013), carrying protons, reducing acidosis and helping to maintain levels of circulating bicarbonates essential for egg shell composition (Bertechini, 2012). Rezende et al. (2013), who worked with Bovans White strainlaying hens, stated that shell weight, shell thickness and specific gravity reduced in linear fashion according to the increase in the available phosphorus level of the rations or even with the increases in inclusion of phytase in diets, also causing levels of available phosphorus in the diets to increase. Nevertheless, as in the present study, phosphorus levels reduced according to phytase inclusion, and shell quality parameters remained unchanged.

According to the results obtained, levels of 0.09% of available phosphorus, with or without phytase addition in the diets, on 60-81 day-old phase, are sufficient to guarantee the performance and egg quality, including adequate formation of quail egg shells.

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