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Phytase in diets with crude protein levels for commercial layers

Adriana Aparecida Pereira[1*], Otto Mack Junqueira[2], Dirceu Neutzling Griep Júnior[3], Dorgival Morais de Lima Júnior[1], Sarah Sgavioli[2] and Socorro da Silva Moraes[1]

*[1]Universidade Federal de Alagoas, Arapiraca, Avenida Manoel Severino Barbosa, s/n, 57309-005, Alagoas, Brazil. [2]Faculdade de Ciências Agrícolas e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [3]Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil. *Author for correspondence: E-mail: adri_zoo@hotmail.com*

ABSTRACT. The aim of this study was to evaluate the action of the phytase enzyme in diets formulated with variations in the crude protein content on performance and egg quality of commercial layers. A total of 336 commercial layers were housed at eight birds per cage in a 3 × 2 factorial arrangement with seven replicates. Treatments consisted of four crude-protein levels (17, 15, and 13%) and two enzyme levels (0 and 500 FTU kg^{-1} diet), distributed in a completely randomized design. Feed intake, egg production, feed conversion per dozen eggs and per kilogram, egg weight and mass, specific gravity, eggshell thickness, and Haugh unit were measured over five 28-day periods. The performance of commercial layers fed the diet containing 13% crude protein worsened, irrespective of phytase addition. However, at 15% crude protein, phytase inclusion did not affect feed conversion or egg weight. Inclusion of phytase leads to equal egg-quality and performance results at the dietary crude protein levels of 17 and 15%. The crude protein content of 13%, with or without phytase, is not recommended for the diet of brown-egg layers.

Keywords: phosphorus, chicken, nitrogen, eggs.

Fitase em rações com níveis de proteína bruta para poedeiras comerciais

RESUMO. Objetivou-se avaliar a ação da enzima fitase em rações formuladas com variação na proteína bruta sobre o desempenho e a qualidade de ovos de poedeiras comerciais. Foram alojadas 336 poedeiras comerciais, em esquema fatorial 3 x 2, com sete repetições e oito aves em cada gaiola. Os tratamentos constituíram de três níveis de proteína bruta (17, 15 e 13%) e dois níveis da enzima fitase (0 e 500 FTU kg^{-1} ração), distribuídos em delineamento inteiramente casualizado. Durante cinco períodos, de 28 dias cada, foram avaliados o consumo de ração, a produção de ovos, a conversão alimentar por dúzia e por quilograma, o peso e a massa de ovos, a gravidade específica, a espessura de casca e a unidade Haugh. Constatou-se piora no desempenho das poedeiras comerciais alimentadas com 13% de proteína bruta na ração, independente da adição da fitase. Porém, em 15% de proteína bruta, a inclusão da fitase não afetou a conversão alimentar e o peso dos ovos das aves. A inclusão da fitase proporciona resultados de qualidade de ovos e desempenho iguais para os níveis de 17 e 15% de proteína bruta na ração. Não se recomenda o nível de 13% de proteína bruta, com ou sem fitase, na ração de poedeiras semipesadas.

Palavras-chave: fósforo, aves, nitrogênio, ovos.

Introduction

Poultry diets are based mainly on plant ingredients, especially corn and soybean meal. Approximately two-thirds of the phosphorus in these ingredients form complexes with the phytic acid (or phytate) molecule, which prevents their use by non-ruminants (Diarra, Usman, Igwebuike, & Yisa, 2010). This fact implies addition of inorganic phosphorus to the diets, originating from non-renewable sources in the nature, to meet the bird requirements of this mineral. As a result, the feed becomes costlier and birds eliminate larger amounts of phosphorus in their excreta.

The phytase enzyme produced by *Aspergillus niger* has been successfully used in poultry and swine diets to release part of the complexed phosphorus in the form of phytate; improve the nutrient digestibility; and consequently excrete less phosphorus into the environment (Selle, Cowieson, & Ravindran, 2009, Gomide et al., 2011a, Selle, Cowieson, Cowieson, & Ravindran, 2012).

Crude protein must also be investigated by nutritionists so that the least possible amount of nitrogen is excreted into the environment. A greater use efficiency of dietary protein and amino acids by birds may reduce the required amount of dietary protein to meet the same production level, in addition to lessening nitrogen excretion and production costs (Cowieson, Bedford, Selle, & Ravindran, 2009, Nagata et al., 2009).

Silva et al. (2006) and Gomide et al. (2011b) observed that, through phytase addition, it is possible to reduce the protein content of hen diets without affecting their performance and also reduce the excretion of minerals in the birds' excreta. However, there is little research with phytase for layers fed high-crude protein diets.

Therefore, this study was conducted to evaluate phytase supplementation in diets formulated with different levels of crude protein on the performance and egg quality of commercial layers.

Material and methods

The birds were housed in a conventional shed (3 m width × 2 m ceiling height) covered with French tiles, containing galvanized-wire cages with four compartments measuring 25 × 40 × 40 cm each. Cages were distributed side-by-side, on two floors, at 0.80 m above the floor, and were equipped with plastic-cup drinkers and metal trough feeders along their entire front length.

Hens were selected according to their body weight and egg production for two weeks, for a subsequent standardization of the lot and equalization of the plots. A single diet was provided during this period.

A total of 336 brown-egg Isa Brown layer hens with an average initial weight of 1,750 kg and a laying rate of 92.0 ± 4.76% were used from 24 to 44 weeks of age during five 28-day production periods. Birds were distributed into six treatments and seven replicates with eight hens per plot. A 3 × 2 factorial arrangement with crude protein and phytase as the factors was adopted, in a completely randomized design.

Treatments consisted of three levels of crude protein (17, 15, and 13%), with or without phytase supplementation (500 FTU kg^{-1}). The diets with phytase had their dicalcium phosphate reduced by around 72%.

During the five egg-laying periods, the hens received water and feed *ad libitum*, under a lighting program of 17 hours of natural + artificial light per day.

Diets were corn- and soybean meal-based, and formulated so as to meet the nutritional requirements as recommended by Rostagno et al. (2011); they were iso-calcium (4.200% Ca), iso-phosphorus (0.375% Pd, considering the effect of phytate on the dietary phosphorus availability) and iso-energetic (2,900 kcal ME kg^{-1}). The diets with reduced protein levels were supplemented with industrial sources of lysine (L-lysine HCl = 78.4%), methionine (DL-methionine = 98%), threonine (L-threonine), and tryptophan (L- tryptophan). The phytase enzyme was obtained from fermentation with *Aspergillus niger* fungi, containing an activity of 10,000 FTU g^{-1} of the enzyme as declared by the manufacturer (Natuphos® from BASF company) (Table 1).

Table 1. Centesimal composition and calculated levels of nutrients in the experimental diets.

Ingredient	Experimental diet					
	Without phytase			With phytase		
	17%	13%	15%	15%	17%	13%
Ground corn	56.767	63.053	69.132	59.303	65.561	71.634
Soybean meal	26.831	21.264	15.735	25.861	20.299	14.772
Soy oil	4.248	3.285	2.390	3.528	2.574	1.682
Limestone	9.806	9.829	9.852	9.976	9.999	10.022
Dicalcium phosphate	1.432	1.474	1.516	0.409	0.450	0.492
Salt	0.498	0.506	0.515	0.498	0.507	0.515
Vitamin-mineral supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200
DL -methionine (98%)	0.191	0.241	0.292	0.190	0.241	0.292
L-lysine HCl (78.4%)	0.000	0.090	0.275	0.000	0.103	0.288
L-tryptophan	0.027	0.058	0.089	0.029	0.060	0.092
L-threonine	0.000	0.000	0.004	0.000	0.000	0.007
Phytase	0.000	0.000	0.000	0.005	0.005	0.005
Total	100.000	100.000	100.000	100.000	100.000	100.000
	Calculated level					
Met. energy (kcal kg^{-1})	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein (%)	17.000	15.000	13.000	17.000	15.000	13.000
Nitrogen (%)	2.753	2.453	2.167	2.716	2.419	2.133
Calcium (%)	4.200	4.200	4.200	4.200	4.200	4.200
Total phosphorus (%)	0.584	0.572	0.559	0.393	0.380	0.377
Available phosphorus (%)	0.375	0.375	0.375	0.375	0.375	0.375
Sodium (%)	0.230	0.230	0.230	0.230	0.230	0.230
Lysine (%)	0.907	0.834	0.836	0.885	0.823	0.825
Dig. lysine (%)	0.875	0.800	0.800	0.864	0.800	0.800
Methionine (%)	0.460	0.484	0.509	0.457	0.482	0.507
Dig. methionine (%)	0.446	0.471	0.496	0.445	0.467	0.495
Methionine + cystine (%)	0.758	0.757	0.756	0.752	0.753	0.752
Dig. methionine +cystine (%)	0.740	0.740	0.740	0.740	0.740	0.740
Threonine (%)	0.662	0.584	0.510	0.653	0.576	0.505
Dig. threonine	0.577	0.507	0.440	0.582	0.511	0.448
Tryptophan (%)	0.197	0.194	0.190	0.193	0.190	0.187
Dig. tryptophan (%)	0.180	0.180	0.180	0.180	0.180	0.180
Valine (%)	0.807	0.712	0.616	0.796	0.619	0.606
Dig. valine (%)	0.715	0.629	0.544	0.705	0.700	0.534
Isoleucine (%)	0.728	0.629	0.531	0.715	0.616	0.518
Dig. isoleucine (%)	0.663	0.572	0.482	0.663	0.572	0.482
Phenylalanine (%)	0.227	0.252	0.277	0.237	0.262	0.287
Dig. phenylalanine (%)	0.209	0.232	0.254	0.218	0.241	0.264
Arginine (%)	1.079	0.922	0.766	1.057	0.899	0.744
Dig. arginine (%)	1.035	0.885	0.735	1.014	0.863	0.714

[1]Enriched with the following elements per kilogram of feed: vitamin A - 6,250 IU; vitamin D_3 - 2,500 IU; vitamin E - 13 mg; vitamin K_3 – 1 mg; vitamin B_1 - 1.5 mg; vitamin B_2 - 3.4 mg; vitamin B_6 - 1 mg; vitamin B_{12} - 20 mg; folic acid - 0.25 mg; pantothenic acid 2.85 mg; niacin - 10 mg; biotin - 0.1 mg; choline - 0.24 mg; copper - 7.5 mg; zinc - 60 mg; manganese - 46 mg; iodine - 1 mg; selenium - 0.2 mg; antioxidant - 0.4 mg.

The number of eggs produced was recorded daily, including cracked, broken, and abnormal eggs, relative to the number of layers in each plot. At the beginning and end of each period, the diets supplied and leftovers from feeders and buckets were weighed per plot to quantify the feed consumed by the bird per day.

Feed conversion per kilogram of eggs was calculated by dividing the average feed intake by the average weight of the eggs produced in the last two days of each period. Egg mass was determined by multiplying the percentage of eggs produced by the weight of these eggs.

Egg quality-related traits were assessed during the last two days of each period; specific gravity was measured immediately after the eggs were laid, and cracked eggs were discarded, as suggested by Voisey and Hunt (1974). The eggs were then immersed in containers with NaCl solutions in ascending order of density (1.065 to 1.100 g cm^{-3}), with 0.005 g cm^{-3} increases. Egg density was considered the solution of lowest density in which the egg floated (Hamilton, 1982).

Immediately afterwards, the three eggs with weight closest to the average weight of each plot were used to determine the internal egg quality. For this step, they were cracked and their content (egg white + yolk) was placed on a flat and leveled surface. Albumen height was then measured by reading the value indicated by the AMES S-6428 tripod micrometer.

With the egg weight and thick egg white height values, the formula below was applied to calculate the Haugh Unit (Eisen, Bohren, & McKean, 1962), according Equation 1.

$$HU = 100 \log (hour + 7.57 – 1.7 W^{0.37}) \quad (1)$$

where:
hour = height of the thick white (mm);
W = egg weight.

After being dried, shells were measured with a precision micrometer (0.01-mm divisions) in three different points in the center-transverse area to determine their thickness.

Results were subjected to analysis of variance, using the General Linear Model (GLM) procedure of SAS (version 2002 - Statistical Analysis System [SAS], 2002), at the 5% probability level. The crude protein levels were compared by polynomial regression models.

Results and discussion

Mean values obtained during the entire experimental period (24 to 44 weeks) for performance traits are described in Table 2.

There was no influence ($p > 0.05$) of CP levels or the interaction between CP levels and phytase on feed intake during the entire experimental period. However, a lower feed intake was observed ($p < 0.05$) when phytase was added to the diet.

It is likely that phytase improved the availability of dietary energy (Assuena et al., 2009) and protein to the birds.

These results contrast with the findings reported by Assuena et al. (2009), who observed higher feed intake in hens receiving 600 FTU phytase kg^{-1} diet, when compared with 0 and 300 FTU kg^{-1} diet. The author attributed this result to the lower availability of energy than what is considered during diet formulation when the phytase matrix is valued, and birds consumed more feed to meet their phosphorus and energy requirements.

As regards the dietary CP level, the present findings disagree with the reports of Silva et al. (2010a, b), who stated that diets with a low CP level may result in a lower feed intake due to the occurrence of an effective protein deficiency. Thus, it is suggested that the crude protein level of 13%, supplemented with the main essential amino acids (methionine, lysine, threonine, and tryptophan), does not compromise the feed intake of commercial brown-egg layers (Jardim Filho et al., 2011).

Egg production decreased linearly ($p < 0.05$) as the protein level in the diet was reduced, according to the following Equation 2:

$$y = 26.701 + 3.569x \ (R^2 = 0.97) \quad (2)$$

Moreover, the diets with phytase addition showed a significant difference ($p < 0.05$) between the treatments with and without the enzyme. However, there was no interaction between the two sources of variation.

Table 2. Mean values for performance traits of layer hens from 24 to 44 weeks of age.

Treatment	FI (g bird day^{-1})	EP (% egg bird day^{-1})	FC kg^{-1} (kg kg^{-1})	EM (g egg day^{-1})	EW (g)
17% CP	94.53	88.10	2.00	52.71	59.04
15% CP	93.26	84.75	2.05	50.30	58.80
13% CP	92.08	78.16	2.18	46.63	59.00
0 FTU	95.33	85.71	2.06	52.03	58.65
500 FTU	91.29	81.69	2.10	47.72	59.24
		Probability (%)			
CP	0.556	0.000*	0.009*	0.011*	0.971
P	0.032*	0.004*	0.303	0.006*	0.516
CP × P interaction	0.651	0.072	0.653	0.092	0.172
SEM	0.000	18.574	0.007	7.689	3.465

FI = feed intake; EP = egg production; FC kg^{-1} = feed conversion per kilogram of eggs; EM = egg mass; EW = egg weight. * = significant ($p < 0.05$); SEM = standard error of the mean.

Analyzing the average egg production obtained with 13, 15, and 17% CP with phytase addition, we observed lowest egg production in the treatment containing 13% CP, which might have induced the average of the other treatments (17 and 15%) to be lower than those of treatments without phytase addition. In this case, the low dietary CP level (13%) could not be offset by phytase addition (Onyango, Asem, & Adeola, 2009).

Compared with the birds from the treatment with 17% CP, those fed the diets with 15 and 13% CP showed, respectively, losses of 3.8 and 11.3% in egg production. This might have been caused by the amino acid imbalance and the antagonism between lysine and arginine, since the amount of arginine is compromised when a larger amount of lysine is provided for absorption. To prevent this effect, the diet should also be supplemented with arginine, which did not occur in the present study (Jardim Filho et al., 2011).

During the entire experimental period, there was no significant difference ($p > 0.05$) between the treatments with or without phytase inclusion, and there was no interaction effect between CP levels and presence or absence of phytase in the diet on FC per kilogram of eggs. However, FC (kg kg^{-1}) responded linearly ($p < 0.05$) to a decrease in the dietary CP levels, as estimated by the following Equation 3:

$$y = 1.820 - 0.023x \ (R^2 = 0.97) \qquad (3)$$

These data are in line with the results for FI and EP, which were respectively similar and lower as the dietary CP was reduced, implying worse FC at the CP level of 13%. Additionally, the fact that FC was not influenced by phytase contributed to the lower FI and EP values with the inclusion of the enzyme, given that FC is the best parameter to check the viability of production.

These results differed from the findings of Pavan, Móri, and Garcia (2005), who did not observe differences in the FC (kg dz^{-1}) of layers fed diets containing 14 to 17% crude protein. The similarity between the treatments with absence and presence of phytase on FC (kg kg^{-1}) also suggests that the phytase level recommended by the manufacturer (0.005%) was not sufficient to optimize the feed conversion per kilogram of eggs of the birds, which should require, as described by Costa et al. (2004), approximately 0.02% phytase in the diet.

There was no significant interaction effect ($p > 0.05$) between CP levels and phytase in the diet on egg mass, but these sources of variation revealed an impact when analyzed separately. The decrease in dietary CP levels (17 to 13%) linearly reduced egg mass, according to the following Equation 4:

$$y = 28.590 - 1.419x \ (R^2 = 0.99) \qquad (4)$$

The presence of phytase provided a reduction in egg mass.

Given that egg mass is the ratio between egg weight and egg production, these findings are in line with the previously described data, which showed lower egg production, and consequently a lower egg mass, when phytase and 13% CP were included.

Similarly, Leeson and Summers (2001) reported that layer hens fed diets with 10.0% crude protein and supplemented with synthetic amino acids produced 11.0% less egg mass when compared with hens consuming diets with 17.0% protein.

These results suggest that the lower egg mass of the birds fed diets with a low crude protein content (13%) may be due to an inadequate intake of some essential amino acids; e.g., valine, isoleucine, and arginine, which were not supplemented in the experimental diets (Kamisoyama, Honda, Kubo, & Hasegawa, 2010).

The influence of phytase on egg mass was similar to the effect on egg weight, decreasing with the presence of the enzyme. This is also due to the lower average egg mass in birds fed diets with a reduced level of CP (13%) and inorganic phosphorus.

There was no significant effect ($p > 0.05$) of the treatments or a significant interaction effect ($p > 0.05$) between the studied factors for egg weight. Similar results were found by Punna and Roland (1999), who fed diets with 0.1 to 0.4% available phosphorus, with and without phytase inclusion (0 and 300 FTU), to layers between the 37^{th} and 48^{th} weeks of age and also did not find differences in egg weight with inclusion of phytase.

With respect to CP, the results of this experiment agree with those found by Keshavarz and Austic (2004), with similar egg weights to those obtained with a conventional diet with 16% crude protein.

The similar egg weights can be explained by the existence of a positive correlation between the sulfur amino acids of the diet and egg weight, and the fact that the digestible methionine + cystine levels of all treatments in the present study were within the birds' requirements (Kamisoyama et al., 2010, Silva et al., 2010b, Domingues et al., 2012).

Mean values for the quality traits of eggs from brown-egg layers from 24 to 44 weeks of age are shown in Table 3.

Table 3. Mean values for quality traits of eggs from brown-egg layers from 24 to 44 weeks of age.

Treatment	Eggshell thickness (mm)	Specific gravity (g cm^{-3})	Haugh unit
17% CP	0.400	1.095	90.86
15% CP	0.399	1.095	90.04
13% CP	0.401	1.093	91.52
0 FTU	0.401	1.094	89.73
500 FTU	0.401	1.094	91.67
	Probability (%)		
CP	0.261	0.018*	0.500
P	0.902	0.942	0.073
CP × P interaction	0.929	0.656	0.040*
SEM	0.000	0.000	4.675

* = significant effect (p < 0.05); SEM = standard error of the mean.

No significant effects (p > 0.05) of the crude protein levels, phytase, or protein × phytase interaction were found on shell thickness throughout the entire experimental period. This finding is similar to the reports of Vieira, Bertechini, Fialho, Santos, and Teixeira (2001), who did not detect differences in shell thickness of eggs from white-egg (Hy-Line W36) layers when the birds were fed diets with different phytase levels (100, 200, 300, and 400 FTU).

No significant difference was detected (p > 0.05) between the phytase levels or interaction between phytase levels and CP on the specific gravity (SG) of the eggs. However, specific gravity decreased linearly (p < 0.05) with the reduction of the dietary CP levels.

There was no significant difference (p > 0.05) between the CP levels and addition of phytase to the diet or lack thereof on the Haugh unit (HU) of eggs from commercial layers from 24 to 44 weeks of age. However, there was an interaction effect between these sources of variation on this variable.

Decomposing the interaction between CP levels and presence or absence of phytase on the average HU revealed that phytase improved (p < 0.05) HU for the birds receiving diets with 15 and 13% when compared with those that did not consume phytase in their diet (Figure 1).

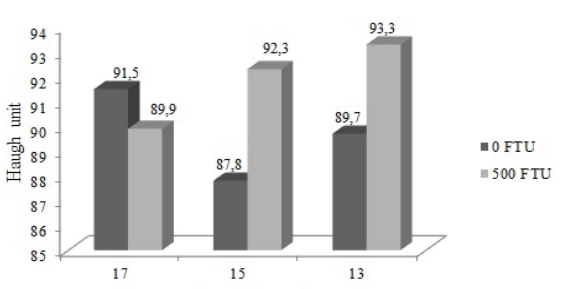

Figure 1. Interaction between crude protein levels and phytase enzyme on average Haugh unit.

These results demonstrate that phytase addition favored the use of the lowest CP level (13%) tested in this study and might have made available nutrients present in corn and/or soybean meal that were previously unavailable to the birds (Singh, 2008, Cowieson et al., 2009, Selle et al., 2012).

Conclusion

Inclusion of the phytase enzyme (500 FTU kg^{-1}) provided equal egg-quality results for the dietary crude protein levels of 17 and 15%. Diets with 13% crude protein, with or without phytase inclusion, are not recommended for commercial layers.

References

Assuena, V., Junqueira, O. M., Duarte, K. F., Laurentiz, A. C., Filardi, R. S., & Sgavioli, S. (2009). Effect of dietary phytase suplementation on the performance, bone densitometry, and phosphorus and nitrogen excretion of broilers. *Brazilian Journal of Poultry Science, 11*(1), 25-30.

Costa, F. J. P., Souza, H. C., Gomes, C. A. V., Barros, L. R., Brandão, P. A., Nascimento, G. A. J., ... Amarante Junior, V. S. (2004). Níveis de proteína bruta e energia metabolizável na produção e qualidade dos ovos de poedeiras da linhagem Lohmann Brown. *Ciência Agrotécnica, 28*(6), 1421-1427.

Cowieson, A. J., Bedford, M. R., Selle, P. H., & Ravindran, V. (2009). Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. *World's Poultry Science Journal, 65*(3), 401-418.

Diarra, S. S., Usman, B. A., Igwebuike, J. U., & Yisa, A. G. (2010). Breeding for eficiente phytate-phosphorus utilization by poultry. *International Journal of Poultry Science, 9*(10), 923-930.

Domingues, C. H. F., Sgavioli, S., Praes, M. F. F. M., Duarte, K. F., Castiblanco, D. M. C., Santos, E. T., ... Junqueira, O. M. (2012). Lysine and methionine + cystine for laying hens during the post-molting phase. *Brazilian Journal of Poultry Science, 14*(3), 159-232.

Eisen, E. J., Bohren, B. B., & McKean, H. E. (1962). The haugh unit as a measure of egg albumen quality. *Poultry Science, 41*(5), 1461-1468.

Gomide, E. M., Rodrigues, P. B., Bertechini, A. G., Freitas, R. T. F., Fassani, E. J., Reis, M. P., ... Almeida, E. C. (2011a). Rações com níveis reduzidos de proteína bruta, cálcio e fósforo com fitase e aminoácidos para frangos de corte. *Revista Brasileira de Zootecnia, 40*(11), 2405-2414.

Gomide, E. M., Rodrigues, P. B., Zangeronimo, M. G., Bertechini, A. G., Santos, L. M., & Alvarenga, R. R. (2011b). Nitrogen, calcium and phosphorus balance of broilers fed diets with phytase and crystalline amino acids. *Ciência e Agrotecnologia, 35*(3), 591-597.

Hamilton, R. M. G. (1982). Methods and factors that affect the measurement of egg shell quality. *Poultry Science, 61*(10), 2022-2039.

Jardim Filho, R. M., Stringhini, J. H., Café, M. B., Leandro, N. S. M., Andrade, M. A., & Carvalho, F. B. (2011). Níveis de lisina digestível para poedeiras Lohmann LSL na fase de 16 a 25 semanas de idade. *Revista Brasileira de Zootecnia, 40*(9), 1947-1954.

Kamisoyama, H., Honda, K., Kubo, S., & Hasegawa, S. (2010). Effects of dietary proteins levels on amino acid digestibility at different site of male adult chicken intestines. *Japan Poultry Science, 47*(3), 220-226.

Keshavarz, K., & Austic, R. E. (2004). The use of low-protein, low-phosphorus, amino acid and phytase supplemented diets on laying hen performance and nitrogen and phosphorus excretion. *Poultry Science, 83*(1), 83-75.

Leeson, S., & Summers, J. D. (2001). *Scott's nutrition of the chicken* (4a ed.). Ghelph, CAN: University Books.

Nagata, A. K., Rodrigues, P. B., Rodrigues, K. F., Frietas, R. T. F., Albino, L. F. T., & Fialho, E. T. (2009). Uso do conceito de proteína ideal em rações com diferentes níveis energéticos, suplementadas com fitase para frangos de corte de 1 a 21 dias de idade. *Ciência e Agrotecnologia, 33*(2), 599-605.

Onyango, E. M., Asem, E. K., & Adeola, O. (2009). Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. *British Journal of Nutrition, 101*(6), 836-842.

Pavan, A. C., Móri, C., & Garcia, E. A. (2005). Níveis de proteína bruta e de aminoácidos sulfurosos totais sobre o desempenho, a qualidade dos ovos e a excreção de nitrogênio de poedeiras de ovos marrons. *Revista Brasileira de Zootecnia, 34*(2), 568-574.

Punna, S., & Roland, D. A. (1999). Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus-deficient diets from day one of age. *Poultry Science, 78*(10), 1407-1411.

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., & Gomes, P. C. (2011). *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais* (3a. ed.). Viçosa, MG: Universidade Federal de Viçosa.

Selle, P. H., Cowieson, A. J., & Ravindran, V. (2009). Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livestock Science, 124*(1), 126-141.

Selle, P. H., Cowieson, A. J., Cowieson, N. P., & Ravindran, V. (2012). Protein-phytate interactions in pig and poultry nutrition: a reappraisal. *Nutrition Research Reviews, 25*(1), 1-17.

Silva, M. F. R., Faria, D. E., Rizzoli, P. W., Santos, A. L., Sakamoto, M. I., & Souza, H. R. B. (2010a). Desempenho, qualidade dos ovos e balanço de nitrogênio de poedeiras comerciais alimentadas com rações contendo diferentes níveis de proteína bruta e lisina. *Revista Brasileira de Zootecnia, 39*(6), 1280-1285.

Silva, M. F. R., Faria, D. E., Rizzoli, P. W., Santos, A. L., Sakamoto, M. I., & Souza, H. R. B. (2010b). Desempenho e qualidade dos ovos de poedeiras comerciais alimentadas com rações contendo diferentes níveis de metionina e lisina. *Revista Brasileira de Zootecnia, 39*(10), 2246-2252.

Silva, Y. L., Rodrigues, P. B., Freitas, R. T. F., Bertechini, A. G., Fialho, E. T., Fassani, E. J., & Pereira, C. R. (2006). Redução de proteína e fósforo em rações com fitase para frangos de corte no período de 1 a 21 dias de idade. Desempenho e teores de minerais na cama. *Revista Brasileira de Zootecnia, 35*(3), 840-848.

Singh, P. K. (2008). Significance of phytic acid and supplemental phytase in chicken nutrition: a review. *World's Poultry Science Journal, 64*(4), 553-580.

Statistical Analysis System. (2002). *User' guide: statistics*. Cary, NC: SAS.

Vieira, R. S. A., Bertechini, A. G., Fialho, E. T., Santos, C. D., & Teixeira, A. S. (2001). Desempenho e qualidade de ovos de poedeiras comerciais de segundo ciclo alimentadas com rações contendo fitase. *Ciência e Agrotecnologia, 25*(6), 1413-1422.

Voisey, P. W., & Hunt, J. R. (1974). Messurements of eggshell strength. *Textile Studies, 5*(1), 135-182.

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