# Effects of phytase supplementation to reduced-nutrient diets on performance, egg quality, and economic parameters in commercial layers

***Efectos de la suplementación con fitasa en dietas bajas en nutrientes sobre el rendimiento, la calidad del huevo y los parámetros económicos en ponedoras comerciales***

***Efeitos da suplementação de fitasse em dietas com nutrientes reduzidos sobre o desempenho, qualidade do ovo e parâmetros econômicos em poedeiras comerciais***

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**Abstract**

**Background:** Exogenous enzyme supplementation is an effective and cost-saving mechanism for increasing the availability of dietary nutrients by increasing digestion and reducing excretion. **Objective:** To evaluate the effects of phytase supplementation on performance, egg quality, and economic parameters in commercial laying hens fed reduced-nutrient diets from 70 weeks of age. **Methods:** Novogen White® commercial laying hens (n=256) were randomly allocated to four treatment groups, with eight replicates of eight hens in each group: PC (positive control): conventional diet not supplemented with phytase; diet with reduced levels (RN) of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg), and supplemented with 300 phytase units (FTU/kg; RN300FTU); diet with reduced levels of P (-0.16%), Ca (-0.13%), ME (-18 kcal/kg), CP (-8%), synthetic amino acids (-0.01%), and supplemented with 600 FTU phytase/kg (RN600FTU); and diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal/kg), CP (-20%), synthetic amino acids (-0.01%), and supplemented with 900 FTU phytase/kg (RN900FTU).

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**Results:** The layers fed the RN300FTU diet had 2.68% higher egg production than those fed the PC diet. Egg mass produced by PC- and RN300FTU-fed hens was statistically similar. Eggshell thickness was increased in PC-fed hens. Feed cost for the RN900FTU diet was approximately 9% lower compared with that of the PC diet. **Conclusion:** The best performance and economic results were achieved when layers were fed a reduced-nutrient diet supplemented with 300 FTU phytase.

**Keywords**: *available phosphorus; bone health; broiler; exogenous enzymes; feed conversion; growth performance; low phosphorus diet; mineral; nutrition; phytase; poultry; weight gain.*

**Resumen**

**Antecedentes:** La suplementación con enzimas exógenas es un mecanismo eficaz y económico para aumentar la disponibilidad de nutrientes dietéticos al aumentar la digestión y reducir la excreción. **Objetivo:** Evaluar los efectos de una dieta con reducción de nutrientes y suplementada con niveles incrementales de fitasa en ponedoras a partir de las 70 semanas de edad sobre su desempeño, calidad del huevo y parámetros económicos. **Métodos:** Un total de 256 ponedoras Novogen White® se asignaron al azar a cuatro grupos de tratamiento, con ocho réplicas de ocho gallinas en cada unidad experimental: Control positivo (PC) dieta convencional sin fitasa; dieta con niveles reducidos (RN) de P (-0,12%), Ca (-0,10%) y ME (-14 kcal/kg) y suplementada con fitasa (300 FTU/kg: NR300FTU); dieta con NR de P (-0,16%), Ca (-0,13%), ME (-18 kcal/kg), PB (-18%), aminoácidos sintéticos (-0,01%) y suplementada con fitasa (600 FTU/kg: NR600FTU); y dieta con RN de P (-0,18%), Ca (-0,15%), ME (-20 kcal/kg), PB (-20%), aminoácidos sintéticos (-0,01%) y suplementada con fitasa (900 FTU/kg: RN900FTU). **Resultados:** Las ponedoras alimentadas con RN300FTU presentaron una producción de huevos 2,68% mayor al PC. La masa de huevos producida por gallinas alimentadas con PC y RN300FTU fue estadísticamente semejante. El grosor de cáscara del huevo fue mayor para las gallinas alimentadas con dieta basal. El costo de alimentación de la dieta RN900FTU fue aproximadamente 9% menor que la dieta basal. **Conclusión:** Las ponedoras alimentadas con la dieta RN300FTU presentaron los mejores resultados económicos y zootécnicos.

**Palabras clave:** *ave de corral; conversión alimenticia; desempeño; dieta baja en fósforo; enzimas exógenas; fitasa; fósforo disponible; ganancia de peso; mineral; nutrición; pollo de engorde; salud ósea.*

**Resumo**

**Antecedentes:** A suplementação de enzimas exógenas é um mecanismo eficaz e econômico para aumentar a disponibilidade de nutrientes da dieta, aumentando a digestão e reduzindo a excreção. **Objetivo:** Avaliar os efeitos das dietas com redução de nutrientes suplementadas com fitasse a galinhas poedeiras comerciais a partir das 70 semanas de idade sobre desempenho, qualidade dos ovos e parâmetros econômicos. **Métodos:** Um total de256 poedeiras Novogen White® foram distribuídas aleatoriamente a quatro grupos de tratamento, com oito repetições de oito galinhas em cada unidade experimental: PC (controle positivo): dieta convencional, sem fitasse; dieta com níveis reduzidos (RN) de P (-0,12%), Ca (-0,10%) e ME (-14 kcal/kg) e suplementada com fitasse de 300 FTU/kg (RN300FTU); dieta com níveis reduzidos de P (-0,16%), Ca (-0,13%), ME (-18 kcal/kg), PB (-18%), aminoácidos sintéticos (-0,01%) e suplementados com 600 Fitasse de FTU/kg (RN600FTU); e dieta com níveis reduzidos de P (-0,18% P), Ca (-0,15%), ME (-20 kcal / kg), PB (-20%), aminoácidos sintéticos (-0,01%) e suplementados com fitasse de 900 FTU/kg (RN900FTU). **Resultados:** As poedeiras alimentadas com NR300FTU apresentaram produção de ovos 2,68% maior do que as com a dieta PC. A massa de ovos produzida por galinhas alimentadas com PC e RN300FTU foi estatisticamente semelhante. A espessura da casca dos ovos foi melhor para galinhas alimentadas com PC. O custo de alimentação da dieta RN900FTU foi aproximadamente 9% menor em comparação com o da dieta de PC. **Conclusão:** Este estudo mostrou que as poedeiras alimentadas com dieta RN300FTU apresentaram os melhores resultados econômicos e zootécnico.

**Palavras-chave:** *conversão alimentar; desempenho; dieta baixa em fósforo; enzimas exógenas; fitasa; fósforo disponível; galinha; ganancia de peso; mineral; nutrição; pollo de engorda; saúde óssea.*


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## Introduction

Exogenous enzyme supplementation in feed is used to counteract the effects of antinutritional factors present in raw materials, thus increasing nutrient availability. However, for appropriate enzyme supplementation, it is important to know the substrates on which enzymes act. The substrate of phytase is phytate, which contains 28.2% phosphorus (P), and it is the main form of P storage in plants. Phytate is poorly digested by monogastric animals, particularly poultry, which produce no, or very low levels, of endogenous phytase (Wu *et al.*, 2014). Phytase is a phosphatase that cleaves phosphate radicals from inositol, releasing P for absorption (Borrmann, 1999; Fukayama *et al.*, 2008). Layer diets are composed mainly of plant feedstuffs and phytase supplementation releases P from phytate, increasing its availability.

Phytate is a polyanionic molecule and, in plants, typically binds cationic minerals (calcium, copper, iron, magnesium, manganese, zinc), as well as protein and energy. Therefore, the inclusion of phytase in the diet of monogastric animals releases other nutrients in addition to P, potentially resulting in significant savings in feed costs (Santos-Viana *et al.*, 2009). A meta-analysis on the responses of layers to dietary phytase supplementation showed that diets based on corn and soybean meal containing 0.22% available P and not supplemented with phytase increased egg production, egg mass, and feed efficiency. Supplementation of diets with 150, 300, and 400 phytase units (FTU)/kg diet allowed available dietary P to be reduced to 0.18, 0.15, and 0.14%, respectively (Ahmadi *et al.*, 2012). The addition of 350 FTU to a layer diet with low available P (1.8 g/kg) promoted better eggshell quality than a diet with high available P (2.1 g/kg; Englmaierová *et al.*, 2012).

Several literature reports show that inclusion of phytase in the diet of both commercial broilers and layers promotes better performance. However, most of these studies focus only on Ca and P release from the raw materials, and there has been limited investigation of the possible release of energy, protein, and other nutrients. Consideration of the nutrient values released by phytase in the formulation of layer diets may permit changes to formulations which reduce feed costs, and therefore, production costs.

Therefore, the objective of this study was to evaluate the effects of reduced-nutrient diets supplemented with variable phytase levels on performance, egg quality, and economic parameters of layers.

## Materials and Methods

### *Ethical considerations*

All experimental procedures were reviewed and approved by the Committee of Ethics on Animal Use (CEUA) of the Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Universidade de São Paulo (USP), Pirassununga, SP, Brazil (Protocol n. 8091070617).

### *Animal care*

The birds were reared in brick sheds and housed in conventional battery cages (1.0×0.45×0.45 m) in two tiers (pyramid system). Each cage was equipped with a bowl drinker and external trough feeder placed in the front of the cage. Bird management, lighting program, environmental temperature and relative humidity followed recommendations of the genetic company manual (Novogen). Feed and water were supplied *ad libitum*.

### *Experimental diets*

A total of 256 Novogen White layers were evaluated between 70 to 86 weeks of age. Hens were randomly assigned to one of four dietary treatments with eight replicates of eight hens in each group.

The experimental diets were based on corn and soybean meal (Table 1). The positive control diet (PC) was formulated to supply the nutritional requirements of white layers during lay (Rostagno, 2011), and was not supplemented with phytase. The other three diets were formulated with reduced nutrient levels relative


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to the PC diet and supplemented with different phytase levels: RN300FTU —diet with reduced levels of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg) and supplemented with 300 FTU phytase/kg; RN600FTU —diet with reduced levels of P (-0.16% P), Ca (-0.13%), ME (-18 kcal ME/kg), crude protein (CP; -18%), synthetic amino acids (AA;-0.01%), and supplemented with 600 FTU phytase/kg; and RN900FTU —diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal ME/kg), crude protein (CP; -20%), and synthetic amino acids (-0.01%) and supplemented with 900 FTU phytase/kg.

The commercial phytase product contained phytase from *Escherichia coli* expressed in the yeast *Pichia pastoris*, with maximum activity at pH 4.5 and temperature up to 60ºC.

**Table 1.** Ingredients and calculated composition of the experimental diets.

| **Ingredients (g/kg)** | **PC** | **RN300FTU** | **RN600FTU** | **RN900FTU** |
|---|---|---|---|---|
| Corn (8.8% CP) | 68.332 | 68.396 | 68.422 | 68.442 |
| Soybean meal (45% CP) | 20.130 | 19.396 | 19.220 | 19.100 |
| Calcitic limestone[1] | 8.580 | 8.740 | 8.810 | 8.830 |
| Dicalcium phosphate | 1.360 | 0.720 | 0.500 | 0.390 |
| Wheat middlings | 0.850 | 2.020 | 2.340 | 2.530 |
| Salt | 0.440 | 0.420 | 0.420 | 0.420 |
| DL-Methionine (99%) | 0.160 | 0.160 | 0.150 | 0.150 |
| L-Lysine HCl (78.4%) | 0.030 | 0.030 | 0.020 | 0.020 |
| Vitamin and mineral premix[2] | 0.100 | 0.100 | 0.100 | 0.100 |
| Sand (inert material) | 0.018 | 0.012 | 0.006 | 0.000 |
| Commercial phytase[3] | 0.000 | 0.006 | 0.012 | 0.018 |
| Total | 100.000 | 100.000 | 100.000 | 100.000 |
| **Calculated composition, %** | | | | |
| Metabolizable energy (kcal/kg) | 2,800 | 2,786 | 2,782 | 2,780 |
| Crude protein | 15.280 | 15.140 | 15.100 | 15.08 |
| Calcium | 3.720 | 3.620 | 3.590 | 3.570 |
| Available phosphorus | 0.350 | 0.230 | 0.190 | 0.170 |
| Lysine | 0.670 | 0.660 | 0.650 | 0.650 |
| Sulfur amino acids | 0.610 | 0.600 | 0.590 | 0.590 |
| Sodium | 0.210 | 0.210 | 0.210 | 0.210 |
| Threonine | 0.440 | 0.430 | 0.430 | 0.420 |
| Valine | 0.680 | 0.670 | 0.670 | 0.660 |

PC (positive control): conventional diet, not supplemented with phytase; RN300FTU: diet with reduced levels of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg), and supplemented with 300 FTU phytase/kg; RN600FTU —diet with reduced levels of P (-0.16%), Ca (-0.13%), ME (-18 kcal/kg), CP (-18%), synthetic amino acids(-0.01%), and supplemented with 600 FTU phytase/kg; and RN900FTU —diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal/kg), CP (-20%), synthetic amino acids (-0.01%), and supplemented with 900 FTU phytase/kg.

[1]50% small particle size -50% large particle size.

[2]Vitamin and mineral supplement for layers. Guaranteed levels per kg product in 1 t of feed: vitamin A 7,500,000 IU; vitamin D3 2,000,000 IU; vitamin E 6,000 IU; vitamin K3 900 mg/kg; vitamin B1 350 mg; vitamin B2 4,000 mg; vitamin B6 2,500 mg; vitamin B12: 8,000 mg; niacin: 15 g; thiamine: 700 mg; pantothenic acid: 4, 000 mg, folic acid: 300 mg; biotin: 30 mg; copper: 6,000 mg; iron (min) 30g; manganese: 60g; zinc: 50 g; iodine: 800 mg; selenium: 200 mg.

[3]5,000 FTU/g product.

*Performance parameters*

Performance parameters were measured for the duration of the experimental period (16 weeks), and included feed intake (FI, kg/day), egg production (EP, %), egg weight (EW, g), egg mass (EM, g), and feed conversion ratio per egg mass (FCR/EM) and per dozen eggs (FCR/dz).

Daily egg production (%) was calculated as the total number of eggs and average daily egg production per hen, and then as a percentage relative to the total number of days. Daily average feed intake (g/d) was the difference between daily feed offered and feed residues. Egg mass (g) was calculated by multiplying egg production percentage by average egg weight (g) per replicate. Feed conversion ratio per dozen eggs was calculated by dividing feed intake (g) by egg mass (g) and multiplying the result by 12 (dozen eggs). Feed conversion ratio per egg mass was calculated by dividing feed intake (g) by egg mass (g).

*Egg quality traits*

The quality traits of three eggs collected during the last three days of the experimental period were measured. Egg weight (EW, g), albumen height (AH, mm), Haugh units (HU), and eggshell strength (ESS, kgf) and thickness (EST, mm) were determined using a digital egg tester (model DET6000, Nabel, Kyoto, Kansai, Japan). Eggs were then broken, and the eggshells were carefully washed to prevent eggshell membrane damage, and internal egg contents were removed. Eggshells were dried at room temperature for 7 days, then weighed on a digital scale (Shimadzu®, Kyoto, Kansai, Japan) at 0.01-g accuracy, and eggshell percentage was calculated by dividing eggshell weight by egg weight and multiplying by 100.

*Economic analysis*

The output considered for the economic analysis was egg production. Fixed costs were identical for all treatment groups. The cost of feed was the only variable cost considered. Feed cost per hen, gross margin of egg sales, and total revenue per treatment were analyzed (Gameiro, 2009).

Feed cost (FCi) per hen included feed and debeaking costs. The costs of diet per 30 dozen eggs were based on the historical average of monthly feedstuff prices during a 10-year period. Deflation of the monthly feedstuff prices between February 2007 and March 2017, according to the National Consumer Price Index (INPC, 2017), was applied. Debeaking costs included fees paid to the debeaking professional and the costs of two debeaking procedures. The price paid for each case of 30 dozen eggs (360 eggs) at the end of the study was US$ 22.65 dollars.

Total revenue per hen (TRi; equation 1) was calculated as the number of eggs produced per hen (NEi, egg production during the entire experimental period) multiplied by the price paid per case of eggs (PECi), and the result was divided by 360 to obtain the price per egg for each treatment. Gross margin per hen (GMi; equation 2) was calculated by dividing total revenue per hen (TRi) by feed cost per hen (FCi). Economic efficiency (EE; equation 3) was calculated by dividing TRi by FCi. The closer the TRi to FCi ratio to 1, the less efficient the treatment.

$$TRi=(NEi) \times PECi \div 360 \quad (1)$$

$$GMi=TRi-FCi \quad (2)$$

$$EEi=TRi/FCi \quad (3)$$

*Statistical analysis*

Performance, egg quality, and economic parameter results were analyzed using the SAS software package, version 9.4 (SAS Institute Inc., Cary, NC, USA; 2013). First, the normality of the residues and the homogeneity of the variances were tested by the Shapiro-Wilk and Bartlett's tests, respectively. When these assumptions were satisfied, data were submitted to analysis of variance (ANOVA) and means were compared by Tukey' test at 5% significance level.

**Results**

Feed intake and feed conversion ratio per dozen eggs and per egg mass (Table 2) were not influenced by treatment (p>0.05).

The highest egg production (p≤0.05) was observed in layers fed RN300FTU, which was 2.68% higher compared with those fed the PC diet. The hens fed RN600FTU had statistically similar egg production to those on the PC diet, and the lowest egg production was in hens fed RN900FTU.

The lowest egg production occurred in hens fed RN900FTU, whereas there was a statistically similar egg production in hens fed RN600FTU and those on the PC diet.

The heaviest eggs (p<0.05) were laid by hens fed the PC diet (66.80 g). RN300FTU supplementation enhanced (p<0.024) egg weight in comparison with RN900FTU and RN600FTU, respectively.

Egg mass was not different (p>0.05) between hens fed the PC and RN300FTU diets. This suggests that reduced-nutrient diets may have compromised performance of the layers. Except for eggshell thickness (p<0.05) the egg quality traits (Table 3) were not affected by diet (p>0.05). Thicker eggshells occurred in hens on the PC diet (p<0.05). RN300FTU supplementation significantly improved eggshell thickness in comparison with RN600FTU and RN900FTU, respectively.

The economic analysis was based on egg production and on the historical average monthly prices of feedstuffs during a 10-year period. The results are shown in Table 4 and presented in US dollars (USD). Feed cost per hen fed the PC diet was significantly higher (p>0.05) compared with those calculated for the reduced-nutrient diets supplemented with phytase. The feeding cost of the RN900FTU diet was approximately 9% lower compared with that of the PC diet due to reduced levels of expensive feedstuffs, such as dicalcium phosphate. Total revenue, gross margin, and total cost to gross revenue ratio (TC/TR) were not significantly different between treatments (p>0.05).

**Table 2.** Effects of phytase supplementation to reduced-nutrient diets on performance of laying hens from 70 to 86 weeks of age.

| Parameter | Dietary treatments | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | PC | RN300FTU | RN600FTU | RN900FTU | | |
| EP, % | 84.420[b] | 86.680[a] | 85.780[b] | 82.210[c] | 0.540 | 0.045 |
| FI, kg/day | 0.120 | 0.118 | 0.121 | 0.114 | 2.000 | 0.667 |
| EW, g | 66.800[a] | 65.750[b] | 64.880[d] | 65.490[c] | 0.190 | 0.024 |
| EM, g | 57.060[a] | 56.970[a] | 55.590[b] | 53.850[c] | 0.370 | 0.018 |
| FCR/dz | 1.693 | 1.644 | 1.701 | 1.670 | 0.024 | 0.951 |
| FCR/EM | 2.116 | 2.085 | 2.187 | 2.129 | 0.031 | 0.885 |

SEM: Standard error of the mean. Means within the same row followed by different superscript letters ([a, b, c, d]) are statistically different by the Tukey test (p<0.05). EP: egg production; FI; feed intake; EW: egg weight; EM: egg mass; FCR/dz: feed conversion ratio per dozen eggs and per egg mass (FCR/EM). PC (positive control): conventional diet, not supplemented with phytase; RN300FTU: diet with reduced levels of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg), and supplemented with 300 FTU phytase/kg; RN600FTU —diet with reduced levels of P (-0.16%), Ca (-0.13%), ME (-18 kcal/kg), CP (-18%), synthetic amino acids(-0.01%), and supplemented with 600 FTU phytase/kg; and RN900FTU —diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal/kg), CP (-20%), synthetic amino acids (-0.01%), and supplemented with 900 FTU phytase/kg.


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**Table 3.** Effects of phytase supplementation to reduced-nutrient diets on the quality egg of laying hens from 70 to 86 weeks of age.

| Parameter | Dietary treatments | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | PC | RN300FTU | RN600FTU | RN900FTU | | |
| AH, mm | 7.410 | 7.370 | 7.310 | 7.430 | 0.030 | 0.097 |
| HU | 83.860 | 83.750 | 83.540 | 83.990 | 0.220 | 0.265 |
| ESS, kgf | 3.560 | 3.630 | 3.650 | 3.540 | 0.030 | 0.546 |
| EST, mm | 0.394[a] | 0.392[b] | 0.386[c] | 0.383[d] | 0.001 | 0.020 |
| Eggshell % | 8.910 | 8.990 | 8.970 | 8.870 | 0.030 | 0.524 |
| FCR/EM | 2.116 | 2.085 | 2.187 | 2.129 | 0.031 | 0.885 |

SEM: Standard error of the mean. Means within the same row followed by different superscript letters ([a, b, c, d]) are statistically different by the Tukey test (p<0.05). AH: albumen height; HU: Haugh units; ESS: eggshell strength; EST: eggshell thickness. PC (positive control): conventional diet, not supplemented with phytase; RN300FTU: diet with reduced levels of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg), and supplemented with 300 FTU phytase/kg; RN600FTU —diet with reduced levels of P (-0.16%), Ca (-0.13%), ME (-18 kcal/kg), CP (-18%), synthetic amino acids(-0.01%), and supplemented with 600 FTU phytase/kg; and RN900FTU —diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal/kg), CP (-20%), synthetic amino acids (-0.01%), and supplemented with 900 FTU phytase/kg.

**Table 4.** Economic analysis of egg production of commercial layers fed diets supplemented with increasing phytase levels and formulated assuming nutrient release from feedstuffs by phytase.

| Parameter | Dietary treatments | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | PC | RN300FTU | RN600FTU | RN900FTU | | |
| Total cost (TC) | 2.336[a] | 2.252[b] | 2.234[b] | 2.190[b] | 0.045 | 0.005 |
| Total revenue (TR) | 6.029 | 6.117 | 6.052 | 5.802 | 0.187 | 0.337 |
| Gross margin (GM) | 3.659 | 3.834 | 3.784 | 3.638 | 0.191 | 0.765 |
| TC/TR | 0.388 | 0.368 | 0.369 | 0.377 | 0.012 | 0.648 |
| Eggshell % | 8.910 | 8.990 | 8.970 | 8.870 | 0.030 | 0.524 |
| FCR/EM | 2.116 | 2.085 | 2.187 | 2.129 | 0.031 | 0.885 |

SEM: Standard error of the mean. Means within the same row followed by different superscript letters ([a, b, c, d]) are statistically different by the Tukey test (p<0.05). Values are expressed in US dollars (USD). PC (positive control): conventional diet, not supplemented with phytase; RN300FTU: diet with reduced levels of P (-0.12%), Ca (-0.10%), and ME (-14 kcal/kg), and supplemented with 300 FTU phytase/kg; RN600FTU —diet with reduced levels of P (-0.16%), Ca (-0.13%), ME (-18 kcal/kg), CP (-18%), synthetic amino acids(-0.01%), and supplemented with 600 FTU phytase/kg; and RN900FTU —diet with reduced levels of P (-0.18% P), Ca (-0.15%), ME (-20 kcal/kg), CP (-20%), synthetic amino acids (-0.01%), and supplemented with 900 FTU phytase/kg.

## Discussion

These results indicate that layers fed diets with reduced nutrient levels and supplemented with phytase maintain the same feed intake and achieve the same FCR as those fed diets with standard nutrient levels. In a study evaluating diets with reduced available P (0.15%) supplemented with 200-600 FTU phytase, no differences in feed intake were observed (Viana *et al.*, 2009).

Phytase has shown to increase the availability of P and other nutrients, including phytate-bound cations (iron, magnesium, and zinc), energy, and amino acids (Fukayama *et al.*, 2008; Ferreira *et al.*, 2015). Phytase inclusion in our study may have contributed to the increase in egg production in layers fed the reduced-nutrient diet with 300 FTU supplemental phytase (RN300FTU) which produced 2.68% more eggs compared with those fed the PC diet. In the study of Borrmann (1999), commercial layers fed a diet with a commercial phytase (300 FTU/kg of feed) and reduced P levels (0.18% available P) had higher egg production compared with those fed 0.30 and 0.36% available P. Overestimation of nutrient release by dietary phytase supplementation can have deleterious effects on the performance of birds and, in our study, the layers fed RN900FTU had the lowest egg production. Further research into the effects of phytase on the availability of nutrients other than phosphorus is needed. Studies evaluating amino-acid digestibility in phytase-supplemented diets show variable results, and the underlying mechanisms of nutrient release by phytase are not well understood (Selle *et al.*, 2007). However, it is important to emphasize that optimal results with the supplementation of digestive enzymes can only be achieved with adequate provision of specific substrates, correct enzyme dosage, and specific pH and temperature conditions. These results suggest that the problem is complex in laying hens due to the high Ca requirement for eggshell development and the detrimental consequences of Ca:P metabolic interactions. The increase in feed conversion ratio and decrease in hen-day egg production and egg mass production in dietary treatments with 1,500 FTU/kg at a Ca concentration of 35 g/kg rather than 42 g/kg Ca revealed the dependence of phytase superdosing on the Ca content (Skřivan *et al.* 2018).

The egg quality results corroborate the findings of Costa *et al.* (2014), who reported no significant differences in egg quality traits when brown layers were fed diets with reduced available P and supplemented with several phytase levels. Eggshell thickness was, however, influenced ($p<0.05$) by the treatment. This result is consistent with the findings of Englmaierová *et al.* (2015), who fed layers with diets containing increasing levels of a phytase produced by *Aspergillus niger* (0, 150, 250, or 350 FTU/kg) and reduced non-phytate P (1.8 or 2.1 g/kg) and observed thinner eggshells in layers on the diet with the lowest non-phytate level and supplemented with phytase.

Based on the economic analysis results, reduced-protein diets supplemented with phytase decrease production costs. According to Plumstead (2008), the inclusion of 600 FTU phytase/kg and a 30% reduction of dicalcium phosphate in layer diets reduced feed costs by approximately US$ 0.89/t. In our study, the cost of the diet with 30% reduction of dicalcium phosphate and supplemented with 600 FTU phytase was US$ 0.103/100 g less than the PC diet.

Dietary inclusion of phytase is a common practice in the poultry industry, as it allows reduction of inorganic phosphorus sources, consequently reducing environmental contamination (Silva *et al.*, 2012). The analysis of the economic impacts (in terms of production) of different dietary formulations allows us to determine the optimum phytase supplementation to maximize economic benefits.

In conclusion, laying hens fed a reduced-nutrient diet supplemented with 300 FTU phytase improve egg production performance and reduce total cost production while egg quality traits are not affected by the diet. Nevertheless, the other diets formulated assuming higher nutrient release from the feedstuffs by phytase and supplemented with 600 and 900 FTU, do not improve layer performance compared with the conventional diet. However, neither of those diets have any negative effects on egg quality or economic results.

## Declarations

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*Conflicts of interest*

The authors declare they have no conflicts of interest regarding the work presented in this report.

*Author contributions*

FAR, conceptualization, methodology, investigation, and data curation. PSZ, BGSL, and CAG, methodology, research, and data curation. JCG, NACRG, NW, and URTM, methodology and research. CSSA, funding acquisition, writing, review, and editing. LFA, conceptualization, formal analysis, project administration, funding acquisition and writing of the original draft.

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