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Original Article

Superdosing Phytases in the Diets of Light Laying Hens: Productive Performance and Bone Quality

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ABSTRACT

Two experiments were carried out with the objective of evaluating the effect of superdosing of two phytases on performance, egg quality, digestive organ biometry and bone quality of light hens in the first (58 weeks) and second (87 weeks) productive cycle. In the first cycle, 270 light hens were used, in which a completely randomized design was adopted in a 2 x 2 + 1 factorial scheme (bacterial phytase or fungal phytase x 450 FTUs or 900 FTUs + control diet). In the second cycle, 270 hens were used, following the same design as the previous experiment. The performance and quality of the eggs were evaluated in both cycles, and the biometry of the digestive organs and bone characteristics were also evaluated in the first experiment. There was no effect in the comparison between the means for the performance and egg quality (in both experiments), or for the biometric variables and bone characteristics (first experiment). There was no significant interaction between the factors for any of the variables in the two experiments. In the first experiment, egg production was higher with bacterial phytase and egg weight with fungal phytase. The mineral matter showed greater value with 450 FTUs. It is recommended to use bacterial phytase produced from Escherichia coli as it improves the performance of light laying hens. The dosage of 450 FTUs improves the mineral content of light laying hens and the use of phytase in the laying hen diet implies a lower feed cost.

INTRODUCTION

The use of exogenous enzymes in poultry diets is widely used today, with phytase standing out among these for having the main benefit of an ability to promote the release of phytic phosphorus present in foods of plant origin, improving digestibility and consequently reducing the inorganic phosphorus supplementation in the feed and minimizing the environmental impact caused by its excess in bird excreta (Żyła *et al.*, 2013). Its use also provides some extra phosphoric effects such as the release of amino acids, proteins, carbohydrates and other minerals which are complexed to phytate (Dersjant-Li *et al.*, 2014), in addition to providing an improvement in the use of food energy by the animal organism (Leyva-Jimenez *et al.*, 2019).

Fungi are the most used in industrial phytase production, however the new generation of bacterial phytase has better thermostability, pH specificity (Kathirvelan *et al.*, 2015) and high resistance to proteolytic degradation when compared to fungal phytases (Adeola & Cowieson, 2011), thus promoting greater release of phytic phosphorus present in food, mainly due to the fact that the birds' gastrointestinal tract provides adequate conditions for the optimal activity of the bacterial enzyme (Jain *et al.*, 2016).

The term phytase superdosing refers to using the enzyme in concentrations two to three times above those normally recommended by suppliers (Dersjant-Li *et al.*, 2014). According to Cowieson *et al.* (2011), the use of phytase doses above conventional has aroused the interest of researchers, since superdosing enables more complete and rapid degradation of the phytate molecule in the dietary ingredients, along with a consequent release of nutrients.

Phytase superdosing in the poultry diet has shown consistent beneficial effects, mainly in relation to animal performance (Pirgozliev *et al.*, 2011; Pieniazek *et al.*, 2017) and improvement in bone characteristics (Manobhavan *et al.*, 2016; Sharma *et al.*, 2016), in addition to the quality of eggs, especially the shells (Rojas *et al.*, 2017).

Given the commercial interest of phytases and the different types on the market, there is a need for research which provides information on such enzymes; furthermore, there is also a need to obtain information on its use in laying hens due to the beneficial results obtained by using high phytase doses in broilers. Thus, the objective was to evaluate the effect of superdosing of two different phytases on performance, egg quality, biometry of digestive organs and bone quality of light laying hens of the Hy-Line White lineage in the first and second productive cycles.

MATERIALS AND METHODS

All procedures performed in this study were approved by the Ethics Committee on the Use of Animals (CEUA) Protocol No. 002.02.018.UVA.504.03.

Two experiments were carried out in Sobral, CE, Brazil (3º 36 ʼʼ S, 40º 18 ʼʼ W and 56 m asl). In the first experiment, 270 laying hens of the Hy Line White lineage were used at 58 weeks of age, weighing 1.645 ± 0.06kg and with an average egg production of 77.29 ± 3.62%, observed during 112 days divided into four periods of 28 days. In the second experiment, 270 laying hens of the Hy-Line White lineage were used at 87 weeks of age, weighing 1.665 ± 0.05 kg and with average egg production of 71.43 ± 13.16%, observed for 140 days divided into five periods of 28 days.

The birds were housed in a closed shed with flat galvanized wire screens in galvanized wire cages with dimensions of 90x45x45 cm, with three 30 cm subdivisions and a density of 450 cm²/bird-1 in both experiments. The birds were weighed and selected before the beginning of the experiments to obtain experimental plots with body weight and uniform egg production, according to the recommendations proposed by Sakomura & Rostagno (2007).

A completely randomized design was adopted in a 2 X 2 + 1 factorial scheme with two types of phytases (bacterial produced from Escherichia coli and fungal produced from Aspergillus oryzae (the physical form of both phytases was grainy), two dosages (450 Active Phytase Unit-FTUs and 900 FTUs) and a control diet (without phytases), thus totaling 5 treatments with 6 repetitions of 9 birds each in the two experiments. The diets in the treatments with phytase addition were reformulated according to the nutritional matrix of the phytases, with a nutritional reduction in relation to the control diet. The nutritional and energetic contributions of the enzymes used to formulate the experimental diets were recommended by the manufacturers and are shown in Table 1.

The experimental diets (Table 2) were formulated according to nutritional requirements suggested by the lineage manual (Hy-line, 2016) and the composition of the ingredients used followed the recommendations of Rostagno *et al.* (2017).

The performance and egg quality parameters were evaluated in both experiments. Thus, egg production together with feed consumption was recorded daily until the end of each 28-day period and were then used to calculate the following performance variables: feed consumption (g/bird/day), egg production (%), egg weight (g), egg mass (g/bird/day), conversion by egg mass (kg/kg), and conversion by dozen eggs (kg/dz).

The following egg quality parameters were also evaluated at the end of each 28-day period: percentage of albumen, yolk, and shell, thickness of shell (mm) and specific gravity of the eggs (g/cm3). Thus, four eggs were selected per repetition for these measures, and the selection of the eggs occurred by weighing all the eggs produced on the day of analysis by selecting the eggs closest to the average weight, and eliminating those which were furthest from the average, in addition to broken, cracked or dirty eggs. Two of these eggs were destined to perform specific gravity using the salt fluctuation method (Bezerra *et al.*, 2015), in which the eggs were immersed in different saline solutions with the necessary adjustments for a volume of 25 liters of water with densities ranging from 1.060 to 1.100 with a 0.0025 interval, while the other two eggs were used in the other quality analyzes.

The eggs were manually broken and their components weighed separately. The percentage of each egg component was obtained by dividing the component weight by the egg weight, then multiplying

Table 1 – Nutritional and energetic contribution of phytase enzymes.

Nutritional matrix	Phytases			
	Bacterial[1] 450 FTUs	Bacterial[1] 900 FTUs	Fungal[2] 450 FTUs	Fungal [2] 900 FTUs
Inclusion (g/ton)	45	90	60	120
Metabolizable energy (Kcal/kg)	61	77	75	95
Crude protein (g/kg)	4.91	6.23	2.50	3.10
Calcium (g/kg)	1.92	2.44	1.80	2.30
Available phosphorus (g/kg)	1.75	2.22	1.50	1.80
Sodium (g/kg)	0.40	0.51	0.00	0.00
Digestible methionine (g/kg)	0.04	0.06	0.04	0.05
Digestable Met + cystine (g/kg)	0.44	0.58	0.06	0.08
Digestible lysine (g/kg)	0.20	0.25	0.11	0.14
Digestible threonine (g/kg)	0.39	0.49	0.06	0.08
Digestible tryptophan (g/kg)	0.22	0.28	0.02	0.02
Digestible valine (g/kg)	0.26	0.34	0.11	0.14
Digestible arginine (g/kg)	0.16	0.19	1.00	0.13
Digestible isoleucine (g/kg)	0.30	0.38	0.10	0.13

[1]Quantun Blue 10000 (thermostability up to 90 °C; ideal pH 5.5); [2]Hiphos GT Posture (thermostability up to 90°C; ideal pH 3.5).

the result by 100. The egg shells were put to dry for 24 hours, weighed and then three measurements were taken with a digital caliper in the equatorial region of the egg and extremities to calculate the average value of the shell thickness (mm).

Finally, 20 birds were euthanized at the end of the first experiment consisting of 4 birds from each treatment using the universal method of cervical dislocation (according to Normative Resolution No. 37/2018 - CONCEA) to perform biometry of the digestive organs (proventricle, gizzard, pancreas, liver, intestines and bowel length) and bone analysis. In the biometric analysis, the intestines and gizzards were properly emptied to determine the weight of the empty organs. All organs were weighed on a 0.01 g precision scale and the weight data expressed as a percentage

Table 2 – Percentage and nutritional composition calculated from the experimental diet.

Ingredients (g/kg)	Control	Bacterial		Fungal	
		450 FTUs	900 FTUs	450 FTUs	900 FTUs
Grain corn	568.2470	590.1270	594.3360	588.3130	592.7820
Soybean meal 45%	238.0190	253.5360	259.2870	253.9570	259.2320
Limestone	87.2200	92.3820	94.0550	90.8750	91.9580
Meat and bone meal	60.0680	28.3930	24.0370	33.8270	26.3190
Soy oil	38.0060	26.5420	19.0620	24.0880	20.5820
Vitamin-mineral supplement*	4.0000	4.0000	4.0000	4.0000	4.0000
Common salt	2.9400	3.4750	3.6330	3.3800	3.5070
DL methionine	1.5000	1.5000	1.5000	1.5000	1.5000
Phytase	0.0000	0.0450	0.0900	0.0600	0.1200
Total	1000	1000	1000	1000	1000
Cost per kg of feed (R$/kg)	1.35	1.31	1.30	1.31	1.31
Metab Energy (Kcal/ kg)	2950	2890	2875	2875	2855
Crude protein (g/kg)	183.00	178.10	177.00	180.50	180.00
Calcium (g/kg)	41.00	39.10	38.60	39.20	38.70
Available phosphorus (g/kg)	4.30	2.60	2.10	2.90	2.50
Sodium (g/kg)	1.80	1.80	1.80	1.80	1.80
Digestable Met + Cis (g/kg)	6.93	6.95	6.96	6.99	7.01
Digestible methionine (g/kg)	4.42	4.40	4.39	4.42	4.42
Digestible lysine (g/kg)	8.34	8.21	8.20	8.32	8.33
Digestible threonine (g/kg)	5.92	5.89	5.89	5.95	5.97
Digestible tryptophan (g/kg)	1.77	1.82	1.84	1.83	1.85

*PX POSTURA 0.4% 500 TEC - Product warranty levels (composition per kg of product): Iron:10.00 g, Copper: 2,500.00 mg, Zinc: 20.00 g, Manganese: 20.00 g, Iodine: 208.00 mg, Selenium: 75.15 mg, Retinol (Vit A): 600.00 mg, Cholecalciferol (Vit. D):15.63 mg, Tocopherol (Vit. E): 3370.79 mg, Menadione sodium: 395.92 mg, Folic acid: 74.25 mg, Choline: 100.00 g, Niacin: 5.025,74 mg, Pantothenic acid: 1,805.16 mg, Thiamine (Vit. B1): 250.09 mg, Riboflavin (Vit. B2): 1,000.00 mg, Pyridoxine (Vit. B6): 250.1 mcg, Cyanocobalamin (Vit. B12): 2,400.00 mcg, Methionine: 125.00 g, Colistin: 1,750.00 mg of body weight. The length of the birds' intestines was also measured.

The tibiae of these birds were removed and used to analyze the following bone characteristics: weight (g), length (mm), resistance (kgf/cm2), bone deformity (mm), Seedor Index (mg/mm) and mineral matter (g/kg). The bone length was measured with a digital caliper and the weight was obtained on an electronic scale with an accuracy of 0.01g. Bone density was assessed using the Seedor index obtained by dividing the weight (mg) by the length (mm) of the evaluated bone (Seedor *et al.*, 1991).

Bone strength and deformity analyzes were performed with one mechanical press, in which the left tibiae were placed in a horizontal position, and a compression force was applied in the center of each of them. The maximum amount of force applied to the bone until its rupture was considered its resistance to breaking (kgf/cm²), as measured through a digital extensometer. The deformity (mm) was observed through an analog extensometer until the moment of bone rupture.

After boning, the right tibiae were weighed and placed in a forced ventilation oven at 105ºC for 72 hours. Then they were weighed and crushed with a mortar and pistil. The ground samples were then identified for determining mineral matter (MM) according to the methodology described by Silva & Queiroz (2002).

The feed cost was calculated by multiplying the quantity of each ingredient used according to the diet composition by the following prices, and not including the feed cost: corn (R$ 1.04); soybean meal (R$ 1.60); limestone (R$ 0.625); meat and bone meal (R$ 1.5); soybean oil (R$ 3.7); common salt (R$ 1.00); DL-Methionine (R$ 14.44); mineral and vitamin supplement (R$ 16.25); Quantun Blue phytase (R$ 89.32) and HiPhos phytase (R$ 120.00), obtaining the final cost per kilogram of feed.

Except for the cost of the feed, in both experiments, the data were first analyzed according to a completely randomized model and the Dunnett test was applied with a 5% probability to compare the means of the control and the other treatments. Then an analysis was carried out considering the factorial model in which the effects of the phytase types and dosages were included, as well as the interaction between these factors.

RESULTS

Diets with the addition of phytase, regardless of type or dosage, resulted in productive performance, egg quality, bone quality and biometrics of digestive organs in both experiments, being similar (P>0.05) to those observed with the control diet without phytase addition (Tables 3, 4, 5, 6 and 7). Likewise, there was no interaction between the type of phytase and the dosages used for any of the variables studied in either experiment. However, egg production was higher for treatments which contained bacterial phytase and egg weight was higher for treatments with fungal phytase in the first experiment; in both cases there was no influence of the phytase dosage used (Table 3). Furthermore, the variables were not influenced by the phytase type or by the dosages used in the second experiment (Table 4).

Although mineral matter was not influenced by the type of phytase used, it was influenced by the dosage, presenting the highest value in treatments with the 450 FTUs dosage of phytase (Table 8). There was no effect of the type or dosage of phytase used for all other variables.

Regarding the cost of the feed (Table 2), It can be seen that the use of phytase provided a reduction in the feed cost, and all treatments using phytase showed lower values compared to the control treatment as the control treatment showed higher feed cost, while the lowest value was obtained by treatment with bacterial phytase superdosing.

DISCUSSION

The use of both fungal and bacterial phytase in the different dosages studied in both experiments provided productive performance and egg quality results similar to those obtained with the control diet, even though these diets were reformulated with nutritional reduction, indicating that the phytases were efficient in not only providing phosphorus, but also other nutrients such as calcium and amino acids, as well as energy.

It is possible to mainly relate the reduced feed cost with the use of phytases to the reduction in the meat and bone meal and soy oil ingredients. This fact demonstrates that reformulating diets using a nutritional matrix of enzymes enables a decrease in the feed cost, as it enables a reduction in the amount of certain ingredients used in the feed formulation, consequently reducing the production cost and improving the profitability of poultry activity. (Junqueira *et al.*, 2010).

In the first experiment, bacterial phytase proved to be effective in promoting improvement in egg

Table 3 – Feed Intake (g/bird/day), production (%), weight (g) and egg mass (g/bird/day), conversion by egg mass (kg/kg) and conversion by a dozen (kg/dz) light laying eggs fed with diets containing two types of phytase with different dosages from 58 to 74 weeks of age.

Diet	Intake	Production	Egg weight	Egg mass	CM[1]	CDZ[2]
Control	95.10	75.25	63.02	47.42	2.00	1.51
Bacterial 450	96.98	79.17	61.92	49.02	1.98	1.47
Bacterial 900	97.47	77.84	62.24	48.42	2.01	1.50
Fungal 450	96.24	75.81	63.24	47.93	2.00	1.52
Fungal 900	96.63	76.07	63.79	48.51	1.99	1.52
Mean	96.49	76.83	62.84	48.26	2.00	1.50
CV[3] (%)	3.40	3.52	2.10	3.36	4.32	4.96
Phytases						
Bacterial	97.23	78.50A	62.08B	48.72	1.99	1.48
Fungal	96.44	75.94B	63.51A	48.22	2.00	1.52
Dosages						
450 FTUs[4]	96.61	77.49	62.58	48.47	1.99	1.49
900 FTUs	97.05	76.95	63.01	48.46	2.00	1.51
ANOVA[5]			*p-value*			
*Diet	0.77	0.10	0.12	0.50	0.95	0.70
Phytase (F)	0.56	0.025	0.020	0.43	0.92	0.26
Dosage (D)	0.74	0.61	0.45	0.99	0.81	0.59
F x D	0.96	0.45	0.83	0.36	0.47	0.60

[1]Conversion by egg mass; [2]Conversion by dozen eggs; [3]Coefficient of Variation; [4]Active Phytase Unit; [5] Analysis of Variance * Dunnett's test. Means followed by different capital letters in the same column differ by the Tukey's test at 5% probability.

Table 4 – Feed Intake (g/bird/day), production (%), weight (g) and egg mass (g/bird/day), conversion by egg mass (kg/kg) and conversion by a dozen (kg/dz) light laying eggs fed with diets containing two types of phytase with different dosages from 87 to 107 weeks of age.

Diet	Intake	Production	Egg weight	Egg mass	CM[1]	CDZ[2]
Control	96.08	69.67	67.32	46.40	2.07	1.67
Bacterial 450	97.99	71.63	66.01	47.22	2.07	1.64
Bacterial 900	97.39	71.48	66.21	47.34	2.05	1.63
Fungal 450	99.59	70.94	66.86	47.44	2.10	1.68
Fungal 900	98.83	73.21	66.12	48.35	2.04	1.62
Mean	97.98	71.44	66.50	47.35	2.07	1.65
CV[3] (%)	4.13	3.66	2.32	5.01	4.79	4.92
Phytases						
Bacterial	97.69	71.55	66.11	47.28	2.06	1.63
Fungal	99.21	72.08	66.50	47.90	2.07	1.65
Dosages						
450 FTUs[4]	98.79	71.28	66.43	47.33	2.09	1.66
900 FTUs	98.11	72.35	66.16	47.85	2.05	1.62
ANOVA[5]			*p-value*			
*Diet	0.62	0.28	0.53	0.72	0.86	0.54
Phytase (F)	0.40	0.64	0.56	0.55	0.84	0.66
Dosage (D)	0.70	0.34	0.68	0.61	0.38	0.29
F x D	0.96	0.28	0.48	0.69	0.64	0.41

[1]Conversion by egg mass; [2]Conversion by dozen eggs; [3]Coefficient of Variation; [4]Active Phytase Unit; [5] Analysis of Variance * Dunnett's test.

production compared to fungal phytase. This fact possibly occurred due to the enzyme action in making the phosphorus and calcium available which were complexed to the phytate molecule, in addition to the release of other nutrients which were assimilated by the layers and supported a good egg production rate (Abreu *et al.*, 2018). This is because the amount of calcium and phosphorus are not the only factors which guarantee good quality of the eggs, but also an adequate balance of minerals (Albino, 2014).

Fungal phytases, mainly of the Aspergillus genus, are widely used in the industrial production of phytase (Alves-Campos *et al.*, 2017), however the use of bacterial phytases (Escherichia coli) in animal feed is increasing (Mapa, 2018). According to Adeola & Cowieson (2011), the new generation of bacterial

Table 5 – Percentage (%) of albumen, yolk and shell, shell thickness (ST; mm) and specific gravity (SG; g/cm³) of light-weight hens fed diets containing two types of phytases with different dosages from 58 to 74 weeks of age.

Diet	Albumen	Yolk	Shell	ST[1]	SG[2]
Control	60.54	28.14	9.08	0.35	1.091
Bacterial 450	60.21	28.87	8.86	0.35	1.089
Bacterial 900	60.76	28.23	8.71	0.35	1.088
Fungal 450	60.94	28.10	8.74	0.34	1.087
Fungal 900	60.34	28.77	8.76	0.35	1.088
Mean	60.56	28.42	8.83	0.35	1.089
CV[3] (%)	1.03	2.35	3.00	3.73	0.21
Phytases					
Bacterial	60.57	28.55	8.79	0.350	1.088
Fungal	60.74	28.58	8.75	0.348	1.087
Dosages					
450 FTUs[4]	60.67	28.64	8.80	0.34	1.088
900 FTUs	60.64	28.50	8.74	0.35	1.087
ANOVA[5]			*p-value*		
*Diet	0.27	0.15	0.13	0.93	0.07
Phytase (F)	0.51	0.90	0.73	0.66	0.61
Dosage (D)	0.92	0.61	0.56	0.73	0.78
F x D	0.10	0.05	0.47	0.61	0.25

[1] Shell thickness; [2]Specific gravity; [3]Coefficient of Variation; [4]Active Phytase Unit; [5] Analysis of Variance * Dunnett's test.

phytase has high resistance to proteolytic degradation when compared to fungal phytases, which partially explains the high efficiency of its use reported in several studies. Jain *et al.* (2016) state that phytases of bacterial origin are more efficient in promoting the release of phytic phosphorus present in food in relation to fungal phytases, mainly due to the fact that the gastrointestinal tract of birds provides adequate conditions for the optimal activity of the bacterial enzyme.

Treatments with fungal phytase provided heavier eggs, however there was a reduction in egg production which possibly occurred due to the relationship between egg production and size, so that a reduction in the laying rate increases egg weight (Bain *et al.*, 2016).

Although the eggs from bacterial phytase treatments had a significantly lower weight than with fungal phytase, they also remained within the commercial standards, being classified in the category

Table 6 – Percentage (%) of albumen, yolk and shell, shell thickness (ST; mm) and specific gravity (SG; g/cm³) of light-weight hens fed diets containing two types of phytases with different dosages from 87 to 107 weeks of age.

Diet	Albumen	Yolk	Shell	ST[1]	SG[2]
Control	61.22	27.96	8.66	0.31	1.083
Bacterial 450	61.40	28.13	8.66	0.31	1.085
Bacterial 900	61.93	27.61	8.61	0.32	1.082
Fungal 450	61.20	27.77	8.70	0.32	1.084
Fungal 900	61.21	27.78	8.46	0.31	1.083
Mean	61.39	27.85	8.62	0.31	1.083
CV[3] (%)	1.23	2.62	2.54	3.10	0.21
Phytases					
Bacterial	61.66	27.87	8.64	0.31	1.083
Fungal	61.20	27.77	8.58	0.32	1.084
Dosages					
450 FTUs[4]	61.30	27.95	8.68	0.32	1.084
900 FTUs	61.57	27.69	8.53	0.31	1.082
ANOVA[5]			*p-value*		
*Diet	0.41	0.76	0.35	0.62	0.30
Phytase (F)	0.16	0.74	0.51	0.54	0.65
Dosage (D)	0.41	0.40	0.08	0.88	0.07
F x D	0.43	0.39	0.26	0.40	0.42

[1] Shell thickness; [2]Specific gravity; [3]Coefficient of Variation; [4]Active Phytase Unit; [5] Analysis of Variance * Dunnett's test.

Table 7 - Relative weight of the digestive organs of light-weight hens fed diets containing two types of phytases with different dosages from 58 to 74 weeks of age.

Diet	Proventricle (%)	Gizzard (%)	Liver (%)	Pancreas (%)	Intestines (%)	Intestines lenght (cm)
Control	0.32	0.96	2.09	0.15	1.89	142.50
Bacterial 450	0.38	1.09	2.36	0.18	1.82	151.75
Bacterial 900	0.38	1.05	2.62	0.17	1.67	153.25
Fungal 450	0.37	1.28	3.01	0.18	2.12	141.25
Fungal 900	0.29	0.96	2.08	0.16	1.71	144.75
Mean	0.35	1.07	2.43	0.17	1.84	146.70
CV[1] (%)	22.51	20.04	23.71	23.41	18.27	8.89
Phytases						
Bacterial	0.39	1.07	2.49	0.17	1.75	114.81
Fungal	0.34	1.12	2.54	0.16	1.91	116.17
Dosages						
450 FTUs[2]	0.38	1.17	2.68	0.18	1.97	115.73
900 FTUs	0.34	1.00	2.35	0.16	1.69	115.25
ANOVA[3]			*p-value*			
*Diet	0.39	0.25	0.17	0.70	0.39	0.60
Phytase (F)	0.28	0.69	0.86	0.76	0.36	0.54
Dosage (D)	0.34	0.15	0.32	0.53	0.13	0.82
F x D	0.38	0.26	0.08	0.64	0.48	0.72

[1]Coefficient of Variation; [2]Active Phytase Unit; [3] Analysis of Variance * Dunnett's test.

of extra type eggs (60 to 65g), according to MAPA/DIPOA Resolution No. 01 of 01/09/2003 (Brasil, 2003).

The performance variables in the second experiment were similar with the supplementation of both types of phytases and dosages, thus showing that they are adequate to maximize the production parameters, and therefore provide additional effects with the use of bacterial phytase in this productive phase of the birds, as observed in the first experiment. The results found occurred due to the laying hens used in the experiment being in the post-peak production phase, where the nutritional requirements are lower.

Different results were reported by Skřivan *et al.* (2018) when working with the superdosing (1,500 FTUs) of a bacterial phytase (Escherichia coli) in young laying hens (24 weeks of age), in which the authors found that egg mass and feed conversion were negatively influenced by the superdosing used. The

Table 8 – Weight (g), length (mm), Seedor index (mg/mm), resistance (kgf/cm^2), deformity (mm) and mineral matter (g/kg) of the tibiae of light laying hens fed diets containing two types of phytases with different dosages from 58 to 74 weeks of age.

Diet	Weight	Length	SI[1]	BR[2]	BD[3]	M.M[4]
Control	6.75	112.60	59.84	8.76	3.08	52.09
Bacterial 450	7.00	115.45	60.55	8.16	3.01	51.90
Bacterial 900	6.65	114.17	58.24	8.50	3.02	47.31
Fungal 450	7.30	116.01	62.81	9.02	3.00	53.12
Fungal 900	6.85	116.33	58.74	9.87	3.45	51.36
Mean	6.91	114.92	60.04	8.86	3.11	51.16
CV[5] (%)	11.38	3.67	8.67	16.82	13.84	5.57
Phytases						
Bacterial	6.82	114.81	59.39	8.33	3.02	49.61
Fungal	7.07	116.17	60.78	9.45	3.22	52.24
Dosages						
450 FTUs[6]	7.15	115.73	61.68	8.59	3.00	52.51A
900 FTUs	6.75	115.25	58.49	9.19	3.24	49.35B
ANOVA[7]				*p-value*		
*Diet	0.79	0.72	0.75	0.57	0.54	0.08
Phytase (F)	0.54	0.54	0.61	0.17	0.37	0.06
Dosage (D)	0.34	0.82	0.25	0.45	0.32	0.031
F x D	0.90	0.72	0.74	0.74	0.35	0.30

[1] Seedor index; [2]Bone resistance; [3]bone conformity; [4]Mineral matter; [5]Coefficient of Variation; [6]Active Phytase Unit; [7] Analysis of Variance * Dunnett's test. Means followed by different capital letters in the same column differ by the Tukey's test at 5% probability.

aforementioned results differ from those found in this research, mainly due to the superdosing used and the age of the birds.

Cowieson *et al.* (2011) reported a great release of phosphorus and calcium from food among the effects of phytase superdosing, and consequently greater availability of these nutrients for absorption. It is a fact that these nutrients are important for forming eggs and for the mineralization of bones in birds (Gongruttananun, 2011). Thus, it can be inferred that the addition of phytases, despite the nutritional reductions carried out in these studies, was able to meet the demands of the birds and promote good egg quality along with an emphasis on the quality of the shells, especially for the age at which the experimental birds were found (74 and 107 weeks after the first and second experiment, respectively). This is because as a bird's age increases, there is a reduction in the capacity for dietary absorption and calcium bone mobilization, in addition to an increase in egg size without proportional increase in the amount of shell (Jiang *et al.*, 2015).

Taking into account that the specific gravity of eggs is one of the parameters used to assess the shell quality (Duman *et al.*, 2016) and that values equal to or greater than 1080 g/cm3 show good quality eggs (Roque & Soares, 1994), the values of this variable found in the present study indicate that these eggs showed good shell quality, and the results for the two phytase types and the two dosages were similar to those obtained in the control group, where nutritional levels were adequate. Thus, such phytases present themselves as viable enzymes on the market for feeding commercial hens.

Similar results have been reported by Brunelli *et al.* (2012), who also found no effects of phytases (500 FTUs and 1000 FTUs) on the shell thickness and specific gravity of light laying hens' eggs (28 to 44 weeks of age). On the other hand, the results were different from those reported by Viana *et al.* (2009) in working with laying hens (24 to 36 weeks of age) and finding an increase in the weight of eggshells with the supplementation of bacterial phytase (Escherichia coli) with the dosages of 200 FTUs, 400 FTUs and 600 FTUs.

The biometric analysis of the digestive organs makes it possible to identify probable changes arising from the nutritional management to which the birds were subjected, as the development of these organs is directly related to the animal's food (Farias *et al.*, 2019). Considering the use of superdosing (900 FTUs) of enzymes in the diets, changes in the digestive organs, could evidence adverse effects of such ingredients, therefore making their use unfeasible. However, no changes in the digestive organs were observed with the use of superdosing, so it can be inferred that both phytases and dosages did not cause adverse effects.

Phosphorus is an essential component in the metabolic and structural processes of the animal organism, thus making it indispensable for the bird to reach its maximum performance potential (Gautier *et al.*, 2018). It is known that Ca and P are directly related, since the storage of Ca in the body is almost entirely like hydroxyapatite crystals of Ca phosphate in bone (Bougouin *et al.*, 2014). The increase in the bone ash percentage found with the use of phytase at the dosage of 450 FTUs indicates that there was an improvement in bone mineralization, possibly caused by the greater availability of P and Ca.

Both dosages (450 FTUs and 900 FTUs), especially that of 900 FTUs, were expected to promote improvement in bone characteristics, since superdosing enables greater degradation of the phytate molecule (Cowieson *et al.*, 2011), resulting in greater availability of essential nutrients for bone matrix mineralization, when compared to conventional doses. However, this event was not observed in this study, since the other bone variables (notwithstanding the positive results obtained with the dosage of 450 FTUs for MM) were not influenced by either dosage, and it is not possible to associate the improvement in bone quality based on just one indicator, although MM is a good parameter for assessing bone mineralization. (Gomide *et al.*, 2011).

According to Sakomura *et al.* (2014) divergent effects regarding the use of enzymes may be related to the physiological interactions that occur in response to environmental changes, animal characteristics, nutritional requirements and nutrient levels in the diet. Thus, the lack of consistent effect of the dosages, especially the dosage of 900 FTUs, on bone quality can be attributed to the fact that the bird has met its Ca and P requirements, and therefore there is no response to the minerals released by the action of phytase, since these would no longer be necessary because the Ca and P levels would already be adequate (Pongmanee *et al.*, 2020).

In working with supplementation of 300 FTUs of bacterial phytase (Escherichia coli) in laying hens at 56 weeks of age with reduced levels of protein and energy, Lei *et al.* (2011) observed that there was an improvement in the tibial ash percentage, indicating greater bone integrity.

It is recommended to use bacterial phytase produced from Escherichia coli as it improves the performance of light laying hens. The dosage of 450 FTUs improves the mineral content of light laying hens and the use of phytase in the laying hen diet implies a lower feed cost.

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