



Phytase Superdosing in the Diet of Lightweight Replacement Pullets: Performance, Organ Biometry and Bone Characteristics

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ABSTRACT

The aim of this study was to evaluate the effect of two types of phytases (the 1st produced from *Escherichia coli* and the 2nd from *Aspergillus oryzae*) with different dosages (300 and 900 Unit Phytase or FTU) on performance, organ biometry and bone quality of replacement pullets in the period of 8 to 17 weeks of age. A total of 288 *Hy-Line White* laying hens were used at 8 weeks of age. A completely randomized design was adopted in a 2 x 2 factorial scheme, totaling 4 treatments with 8 replicates of 9 birds each. Performance, biometric variables and bone characteristics were evaluated. There was no significant interaction between the factors for any of the evaluated variables. The conversion and the accumulated consumption were influenced ($p < 0.05$) by the phytase dosages, with the highest results being observed for the consumption obtained with 300 FTU, and the best results for the feed conversion obtained with the dosage of 900 FTU. The relative weight of the liver was influenced by the treatments ($p < 0.05$), presenting higher values with bacterial phytase and a dosage of 900 FTU. The sternum length and tibial deformity were influenced by fungal phytase ($p < 0.05$). It is concluded that the use of 900 FTU superdosing and fungal phytase improves the performance and bone characteristics of light replacement pullets.

INTRODUCTION

Due to the great productivity of today's poultry on the market, there is a need for research which provides relevant information on the nutrition of replacement pullet laying hens. Nutrition is one of the main factors influencing productivity within the poultry segment (Rezende *et al.*, 2013), being of fundamental importance to the growth of the laying pullets and their productive longevity during the adult phase.

Success in the production phase of laying hens is associated with adequate performance and sexual maturity obtained during the growth phase. Thus, it is necessary to provide adequate nutrients which meet the requirements of the laying pullets, so that they can develop adequate body structure and reproductive tract formation, allowing greater productivity in this phase (Rezende *et al.*, 2013; Bastos-Leite *et al.*, 2016; D'Agostini *et al.*, 2017).

The use of additives in monogastric diets is a common practice and often necessary in animal nutrition, with the use of exogenous enzymes among them. These enzymes are part of the group of zootechnical additives and their use in diets has grown in recent years. Such use is justified by the beneficial effects they present such as in the removal of antinutritional factors, improved digestibility and availability of phosphorus and other minerals, reducing their excretion and their deposition in the environment (Viana *et al.*, 2009; Leite *et al.*, 2011).



Phytases are enzymes which have the ability to provide phosphorus as well as other nutrients which are complexed to phytic acid molecules (Lima *et al.*, 2010), with the latter being an antinutritional factor present in foods of plant origin. Thus, the effects of phytases on making phosphorus available and reducing the antinutritional activity of phytate is undeniable. In addition, phytases also provide the release of other molecules chelated to phytate such as aminoacids, other minerals and energy (Dersjant-Li *et al.*, 2015; Ribeiro Junior *et al.*, 2015).

Phytases produced from microorganisms such as fungi (*Aspergillus sp.*) and bacteria (*Escherichia coli*) are more commonly used commercially in the diets of poultry and pigs. Phytase produced from *Aspergillus sp.* presents the best characteristics for commercial scale production (Dersjant-Li *et al.*, 2015), where bacterial phytases have shown better results in many studies because they are thermally more stable and resistant to proteolytic action, presenting a similar pH to that of the proventriculus and gizzard of birds, where enzymes have higher action (Igbasan *et al.*, 2000; Pereira *et al.*, 2012; Wu *et al.*, 2015).

The recommended phytase dosage for broiler diets is 500 FTU/kg (Phytase Unit), while the *Superdosing* concept of phytase is the addition of higher levels of this enzyme in the diets, being double or triple the usual value (Meneghetti *et al.*, 2011; Dersjant-Li *et al.*, 2015;). Research has reported that phytase *superdosing* has beneficial effects on animal performance (Cowieson *et al.*, 2011; Pirgozliev *et al.*, 2011).

Broilers are normally used in researches carried out with phytase *superdosing*, with limited information on the application of this concept in laying hens. Due to the beneficial results obtained by the use of high doses of phytase in chickens, there is also a need to obtain information about its use in replacement pullets (Cowieson *et al.*, 2011; Pirgozliev *et al.*, 2011; Kim *et al.*, 2017). In this context, the objective of this study was to evaluate the effect of two different phytases at two dosages on performance, biometry of digestive and reproductive organs, and bone quality of light replacement pullets.

MATERIAL AND METHODS

All procedures performed in this study were approved by the Ethics Commission on Animal Use (CEUA) of the State University of Acaraú Valley – UVA, Ceará, Brazil (Protocol No. 004.05.017.UVA.504.03).

The study was carried out during the rainy period lasting 56 days in the Poultry Sector of the Experimental

Farm of Acaraú Valley, Sobral, Ceará, Brazil. A total of 288 *Hy-Line White* strain pullets with an average weight of 639.60 ± 6.05 g, clinically healthy at eight weeks of age, were used. All birds were housed in a closed shed with smooth galvanized wire, kept in galvanized wire cages with dimensions of 90x45x45 cm, with three subdivisions of 30 cm and density of 675 cm²/bird⁻¹.

The experiment lasted 56 consecutive days and the birds were weighed and selected to obtain experimental plots with uniform mean weight before starting, according to the recommendations proposed by Sakomura & Rostagno (2016).

A completely randomized design was used in a 2 x 2 factorial scheme, with two types of phytases (1st produced from *Escherichia coli* and 2nd produced from *Aspergillus oryzae*) x two dosages (300 FTU and 900 FTU), totaling four treatments with eight replicates of nine birds each. The phytase types evaluated were bacterial phytase produced from *Escherichia coli* and fungal phytase produced from *Aspergillus oryzae* at the 300 and 900 FTU dosages, supplemented in the diet without altering the standard formulation, i.e. *on top*.

The rations for growth stage I and II (Table 1) were isonutrient and formulated according to the suggestions of the lineage manual (Hy-Line, 2016), while the composition of the ingredients used in the formulation followed the recommendation of Rostagno *et al.* (2017).

Both the birds and the rations were weighed at 8, 12 and 17 weeks of age for calculating the performance variables. The evaluated performance variables were the mean weight (g), weight gain (g), feed consumption (g/bird) and feed conversion (g/g).

Next, 20 pullets were drawn, identified and euthanized by the cervical dislocation method at the end of the experiment (according to Normative Resolution No. 37/2018 - CONCEA). The animals were then weighed individually and necropsied. The organs were removed and emptied for biometric analysis of the gizzard, liver, pancreas, intestines, ovary and oviduct using a precision balance of 0.01 g. All weight data were expressed as a percentage of body weight.

Crest height and sternum bone length were measured with a digital caliper. A tape measure was used to measure the length of the intestines.

The tibia and femur of the pullets were used to evaluate bone quality. Their lengths were measured with a digital caliper and the weights were obtained on an electronic scale with an accuracy of 0.01 g.



Bone density assessment was performed using the Seedor index, obtained by dividing the weight (mg)

by the length (mm) of the evaluated bone (Seedor *et al.*, 1991).

Table 1 – Percentage and nutritional composition of experimental diet in growth phase I and II.

Ingredients	Growth phase I				Growth phase II			
	Bacterial		Fungal		Bacterial		Fungal	
	300FTU	900FTU	300FTU	900FTU	300FTU	900FTU	300FTU	900FTU
Grain corn (kg)	56.921	56.9096	56.9191	56.9039	59.8075	59.7961	59.8056	59.7904
Soybean meal (45%) (kg)	21.5827	21.5847	21.583	21.5857	15.8857	15.8877	15.8861	15.8887
Wheat bran (kg)	10.0000	10.0000	10.0000	10.0000	12.0000	12.0000	12.0000	12.0000
Limestone (kg)	0.4239	0.4239	0.4239	0.4239	1.0909	1.0908	1.0909	1.0908
Meat flour (kg)	5.7766	5.7767	5.7766	5.7767	5.8471	5.8472	5.8472	5.8472
PX Posture* (kg)	0.4000	0.4000	0.4000	0.4000	0.4000	0.4000	0.4000	0.4000
Common salt (kg)	0.2739	0.2739	0.2739	0.2739	0.2978	0.2978	0.2978	0.2978
Soybean oil (kg)	4.4108	4.4142	4.4114	4.4159	4.4609	4.4642	4.4614	4.4659
L-Lysine (kg)	0.0954	0.0953	0.0954	0.0953	0.1091	0.1090	0.1091	0.1090
DL-Methionine (kg)	0.1127	0.1127	0.1127	0.1127	0.0980	0.0980	0.0980	0.0980
Phytase (g/t)	0.0030	0.0090	0.0040	0.0120	0.0030	0.0090	0.0040	0.0120
Calculated nutritional composition								
ME** (Mcal/kg)	2.8999	2.8999	2.8999	2.8999	2.8999	2.8999	2.8999	2.9000
Crude Protein (%)	18.5000	18.5000	18.5000	18.5000	16.5000	16.5000	16.5000	16.5000
Calcium (%)	0.9500	0.9500	0.9500	0.9500	1.2000	1.2000	1.2000	1.2000
Available phosphorus (%)	0.4400	0.4400	0.4400	0.4400	0.4400	0.4400	0.4400	0.4400
Sodium (%)	0.1700	0.1700	0.1700	0.1700	0.1800	0.1800	0.1800	0.1800
Digestible methionine + cystine (%)	0.6514	0.6514	0.6514	0.6514	0.5900	0.5900	0.5900	0.5900
Digestible methionine (%)	0.4000	0.4000	0.4000	0.4000	0.3621	0.3621	0.3621	0.3621
Digestible lysine (%)	0.8800	0.8800	0.8800	0.8800	0.7600	0.7600	0.7600	0.7600
Digestible threonine (%)	0.5738	0.5738	0.5738	0.5738	0.4994	0.4994	0.4994	0.4994
Digestible tryptophan (%)	0.1795	0.1795	0.1795	0.1795	0.1517	0.1517	0.1517	0.1517

* PX POSTURE 0.4% 500 TEC - Provides per kg: iron (min) - 10.00 g/kg; copper (min) - 2,500.00 mg/kg; zinc (min) - 20.00 g/kg; manganese (min) - 20.00 g/kg; iodine (min) - 208.00 mg/kg; selenium (min) - 75.15 mg/kg; vit. A (min) - 2,000,000.00 IU/kg; vit. D3 (min) - 625,000.00 IU/kg; vit. E (min) - 3,000.00; vit. K3 (min) - 395.92 mg/kg; folic acid (min) - 74.25 mg/kg; choline (min) - 100.00 g/kg; niacin (min) - 5,025.74 mg/kg; pantothenic acid (min) - 1,805.16 mg/kg; vit. B1 (min) - 250.09 mg/kg; vit. B2 (min) - 1,000.00 mg/kg; vit. B6 (min) - 250.10 mg/kg; vit. B12 (min) - 2,400.00 mcg/kg; methionine (min) - 125.00 g/kg; colistin (min) - 1,750.00 mg/kg.** ME: Metabolizable energy.

Bone strength and deformity analyzes were performed by mechanical press, where the left tibia and femur were placed horizontally, and a compression force was applied to the center of each bone. The maximum amount of force applied to the bone until its rupture was considered the resistance to breaking (kgf/cm²), which was measured through a digital extensometer. The deformity (mm) was measured through an analog replacement gauge until the rupture moment of each bone. These analyzes were carried out at the *Laboratory of Soil Mechanics at the Department of Transportation Engineering of the Federal University of Ceará*.

Next, mineral matter was determined at the *Animal Nutrition Laboratory (LANUT)* of the State University of Acaraú Valley. After deboning, the right tibias and femurs were weighed and placed in a forced ventilation oven at 105 °C for 72 hours. They were then weighed again and crushed with a mortar and pestle. Afterwards, these ground samples were identified for

determining mineral matter (MM) according to the methodology described by Silva & Queiroz (2002).

The data were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability using the Statistical Analysis System (SAS, 2000) program, considering the factorial model that included the effects of treatments, phytases and dosages, and the interaction between factors.

RESULTS AND DISCUSSION

No significant interaction between the studied factors was observed for any of the evaluated performance variables. The initial, final weight and weight gain were not influenced by the evaluated treatments. However, feed conversion and cumulative consumption were influenced (P <0.05) by phytase dosages, with the highest accumulated consumption being obtained with 300 FTU, and the best feed conversion with the dosage of 900 FTU (Table 2).



Table 2 – Performance of replacement pullets fed diets containing two types of phytases with different dosages in the period from 8 to 17 weeks of age.

Factors	Variables				
	Starting weight (g/bird)	Final weight (g/bird)	Wheight gain (g/bird)	Accrued consumption (g/bird)	Conversion (g/g)
Phytases					
Bacterial	639.29	1248.68	609.39	3040.6	4.995
Fungal	639.92	1251.15	611.22	2960.9	4.855
Dosage					
300 FTU	639.50	1247.67	608.17	3056.8 ^A	5.038 ^A
900 FTU	639.72	1252.15	612.43	2944.7 ^B	4.813 ^B
CV ¹ (%)	0.99	2.04	4.14	4.20	5.63
Mean	639.61	1249.91	610.30	3000.7	4.925
ANOVA²					
	<i>p</i> -value				
Phytase (P)	0.7812	0.7865	0.8387	0.0844	0.1644
Dosage (D)	0.9243	0.6232	0.6367	0.0178	0.0302
F x D	0.9291	0.2751	0.2802	0.1286	0.0502

^{A, B} Means followed by different capital letters in the same column differ from each other by the Tukey test (5%); ¹CV (%) – Coefficient of Variation; ²ANOVA – Analysis of Variance.

Considering that feed conversion depends on food intake, it can be inferred that the dosage of 900 FTU, regardless of the phytase origin, was able to release more nutrients adhered to the phytate molecule, increasing its use by pullets and optimizing performance, leading to improvement in feed conversion as observed with this dosage, according to studies (Karadas *et al.*, 2010; Cowieson *et al.*, 2011; Beeson *et al.*, 2017) where *superdosing* of phytase in the diets of birds can provide even better performance responses.

Different results were reported by Viana *et al.* (2009), in which they did not observe an effect on the diet consumption of commercial laying hens when

they evaluated the nutritional matrix of a bacterial phytase (*Escherichia coli*) with doses of 200, 400 and 600 FTU.

No significant interaction was observed between the factors studied for the relative weight of the digestive organs, or for the length of the intestines of the replacement pullets. The relative weight of the proventriculus, gizzard, pancreas, intestines and intestinal length were not influenced by the phytase type or the dosages. However, the relative weight of the liver was influenced by the treatments, presenting higher mean values with bacterial phytase and the dosage of 900 FTU (Table 3).

Table 3 – Relative weight of digestive organs and length of intestines of replacement pullets fed diets containing two types of phytases with different dosages in the period from 8 to 17 weeks of age.

Factors	Variables					
	Proventriculus (%)	Gizzard (%)	Liver (%)	Pancreas (%)	Intestines(%)	Intestinal lengths (%)
Phytases						
Bacterial	0.31	1.80	1.98 ^a	0.20	2.53	1.37
Fungal	0.28	1.72	1.78 ^b	0.19	2.37	1.39
Dosage						
300 FTU	0.30	1.81	1.72 ^B	0.19	2.57	1.38
900 FTU	0.29	1.71	2.04 ^A	0.18	2.33	1.38
CV ¹ (%)	12.04	11.72	9.90	12.16	10.20	5.39
Mean	0.30	1.76	1.88	0.19	2.45	1.38
ANOVA²						
	<i>p</i> -value					
Phytase (P)	0.1515	0.4406	0.0270	0.2955	0.1585	0.5572
Dosage (D)	0.3776	0.3089	0.0012	0.4438	0.0515	1.0000
P x D	0.9074	0.8229	0.1984	0.0817	0.1683	0.5969

^{a, b} Means followed by different lowercase letters in the same column differ from each other by the Tukey test (5%). ^{A, B} Means followed by different capital letters in the same column differ from each other by the Tukey test (5%); ¹CV (%) – Coefficient of Variation; ²ANOVA – Analysis of Variance.

According to Hazelwood (1986), laying hens' livers increase in size before the laying period, due to greater activity by this organ to synthesize the yolk material which will be deposited in the ovary for the production period. Therefore, the increase in liver weight in this

study may be associated with better activity of bacterial phytase and *superdosing* of 900 FTU, providing an increase in the nutritional supply of the pullets, thereby favoring the best liver development and potentiating its activity in the laying period.



There was no significant interaction between the studied factors for the relative weight of the reproductive organs, height and length of ridges, and length of sternum of the replacement pullets. The relative weight of ovary, oviduct, height and

crest length were not influenced by any of the evaluated treatments. However, the length of the sternum presented a higher value with fungal phytase, not differing between the evaluated dosages (Table 4).

Table 4 – Relative weight of reproductive organs, height and length of ridges and sternum length of replacement pullets fed diets containing two types of phytases with different dosages in the period from 8 to 17 weeks of age.

Factors	Variables				
	Ovary (%)	Oviduct (%)	Crest height (mm)	Crest length (%)	Sternum length (mm)
Phytases					
Bacterial	0.08	0.20	24.41	41.85	116.18 ^b
Fungal	0.08	0.24	22.30	41.35	120.14 ^a
Dosage					
300 FTU	0.09	0.22	22.74	39.87	118.75
900 FTU	0.07	0.23	23.96	43.33	117.57
CV ¹ (%)	33.53	66.56	19.22	15.17	2.17
Mean	0.08	1.76	23.35	41.60	118.16
ANOVA ²					
	<i>p</i> -value				
Phytase (P)	0.7323	0.9514	0.3073	0.8605	0.0032
Dosage (D)	0.2038	0.8634	0.5503	0.2375	0.3185
P x D	0.7969	0.8201	0.5857	0.9400	0.3939

^{a, b} Means followed by different lowercase letters in the same column differ from each other by the Tukey test (5%). ¹CV (%) – Coefficient of Variation; ²ANOVA – Analysis of Variance.

Phytic acid can also influence the use of proteins and the absorption of amino acids in the diet which can affect the metabolism and biometry of the organs by forming insoluble complexes, reducing the availability and digestibility of these nutrients (Sousa *et al.*, 2015). In contrast, the phytases and dosages used in this study did not influence changes related to the biometrics of the reproductive organs, nor at the crest height of the pullets.

The distance between the pelvic bones and the development of the sternum bone (preferably more elongated) must be observed in selecting quality laying hens, being considered of great importance for the production period of the birds (Ani & Nnamani, 2011;

Hy Line, 2016), among other characteristics. In this study, it was observed that fungal phytase was more efficient for the sternum bone growth (120.14 mm). Thus, this phytase probably provided greater availability of nutrients such as calcium and phosphorus for developing this bone.

No significant interaction between the studied factors was observed for any of the evaluated variables in the bone quality of the tibias. The bone quality variables of the tibia were not influenced by the treatments, except for bone deformity, which showed higher averages in treatments which contained fungal phytase (Table 5).

Table 5 – Mean values of weight, length, Seedor index (SI), resistance, deformity and mineral matter of tibia for replacement pullets fed diets containing two types of phytases with different dosages in the period from 8 to 17 weeks of age.

Factors	Variables					
	Weight (g)	Length (mm)	SI ¹ (mg/mm)	Resistance (kgf/cm ²)	Deformity (mm)	Mineral matter (g/kg)
Phytases						
Bacterial	6.36	115.58	54.99	10.31	3.03 ^b	43.02
Fungal	6.42	116.65	54.99	11.40	3.66 ^a	43.70
Dosage						
300 FTU	6.26	115.87	54.00	11.10	3.40	43.40
900 FTU	6.52	116.36	55.98	10.62	3.29	43.31
CV (%) ²	11.04	1.73	9.96	19.86	19.24	5.11
Mean	6.39	116.11	54.99	10.86	3.35	43.36
ANOVA ³						
	<i>p</i> -value					
Phytase (P)	0.8515	0.2537	1.0000	0.2749	0.0437	0.5073
Dosage (D)	0.4219	0.5968	0.4307	0.6245	0.7103	0.9287
P x D	0.4955	0.9948	0.4372	0.5367	0.9428	0.9054

^{a, b} Means followed by different lowercase letters in the same column differ from each other by the Tukey test (5%). ¹SI – Seedor index; ²CV (%) – Coefficient of Variation; ³ANOVA – Analysis of Variance.



Deformity measures the flexibility of the bone as a function of a force applied to it until the moment of its rupture (Oliveira *et al.*, 2013), and is directly related to the breaking resistance of the bone. As noted, fungal phytase provided an improvement in tibial bone deformity, regardless of the dosage used. Thus, due to the relationship between deformity and bone resistance, it was expected that the latter would also be influenced by fungal phytase, but this was unobserved. Thus, both phytases were efficient in releasing the nutrients necessary for proper bone development, resulting in the bone resistance values found.

Such results corroborate those of Powell *et al.* (2011), who reported that the addition of phytase

in the diet provides more calcium and phosphorus (essential elements for bone growth and formation), thereby increasing weight and concentration of mineral matter.

Moreover, Lima *et al.* (2010) performed experiments with a reduction in nutritional levels of laying hens diet with the addition of phytase at the level of 600 FTU, and did not observe effects of the nutritional reductions on the evaluated bone parameters; however, bone resistance improved when they added phytase at the dosage of 600 FTU.

There was no significant interaction between the factors studied for the bone quality variables of the femurs. The bone quality variables of the femurs were also not influenced by the treatments (Table 6).

Table 6 – Mean values of weight, length, Seedor index (SI), resistance, deformity and mineral matter of the replacement femora femurs fed diets containing two types of phytases with different dosages in the period from 8 to 17 weeks of age.

Factors	Variables					
	Weight (g)	Length (mm)	SI ¹ (mg/mm)	Resistance (kgf/cm ²)	Deformity (mm)	Mineral matter (g/kg)
Phytases						
Bacterial	5.31	78.30	67.79	14.84	3.04	38.96
Fungal	5.31	79.61	66.73	15.60	2.93	40.04
Dosage						
300 FTU	5.20	79.16	65.75	15.78	2.98	39.63
900 FTU	5.42	78.76	68.76	14.65	2.99	39.37
CV ² (%)	9.97	3.34	9.26	13.81	23.17	4.38
Mean	5.31	78.96	67.26	15.22	2.98	39.50
ANOVA ³						
	p-value					
Phytase (P)	1.0000	0.2841	0.7079	0.4341	0.7382	0.1800
Dosage (D)	0.3668	0.7374	0.2967	0.2463	0.9857	0.7372
P x D	0.8680	0.2020	0.7853	0.5945	0.4666	0.3619

¹SI – Seedor index; ²CV (%) – Coefficient of Variation; ³ANOVA – Analysis of Variance.

Our results corroborate the study by Rezende *et al.* (2013) who used three levels of phytase (0, 500 and 1000 FTU/kg of feed) of fungal origin (*Aspergillus niger*) in an experiment with commercial laying hens of the *Hy-line White* line with reduced phosphorus (0.10; 0, 20; 0.30 and 0.40%) and also did not find significant results for bone characteristics of the femur.

Some studies report that bacterial phytases are more efficient than phytases of fungal origin in hydrolyzing phytate, as they have greater resistance to protease (Igbasan *et al.*, 2000; Pereira *et al.*, 2012; Dersjant-Li *et al.*, 2015). Although phytase supplementation in the diet is considered the most effective way of releasing and using the minerals linked to phytate, there are divergent results regarding the exact dosage, duration, species of birds, the best microbial form of phytase, among other issues which need to be better studied (El-Hack *et al.*, 2018).

In conclusion, 900 FTU of phytase of fungal origin is recommended in the diet due to the better performance and bone characteristics of light replacement pullets.

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