



## Heat-Resistant Bacterial Phytase in Broiler Pelleted Diets

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### ■ Keywords

Performance, digestibility, enzyme, phytate, phosphorus, broiler nutrition.

### ABSTRACT

The objective of the study was to evaluate the effects of a heat-resistant bacterial phytase added to pelleted diets on mineral digestibility, live performance, carcass traits, and bone quality of broilers. Three treatments were evaluated: Positive control; negative control, with 0.10 points reduction in calcium level and 0.15 points reduction in available phosphorus level; and negative control + phytase at 500 FTU/kg. Mineral digestibility and bone quality results demonstrated that the evaluated phytase resisted pelleting as it increased the utilization of the minerals present in the diet.

### INTRODUCTION

Enzymes are protein that play specific roles in biochemical reaction, and are macromolecules known for their extraordinary specificity and catalytic power. Among exogenous enzymes studied and utilized in monogastric diets, phytase is applied to increase the availability of nutrient present in complexes with the phytate molecule of plant feedstuffs (Dari, 2004). Moreover, phytase reduces the environmental impact caused by excessive phosphorus excretion (Bess *et al.*, 2006), contributing for the sustainability of poultry production.

The objective of the study was to evaluate the effects of a heat-resistant bacterial phytase added to pelleted diets on mineral digestibility, live performance, carcass traits, and bone quality of broilers.

### MATERIAL AND METHODS

The experiment was carried out at the Center of Poultry Research of the School of Veterinary Medicine and Animal Science of Universidade de São Paulo, Pirassununga campus, Brazil. Two trials were performed: one to evaluate mineral digestibility and the other to evaluate broiler performance, carcass traits, and bone quality.

Mineral digestibility was evaluated using 240 one-d-old Cobb 500 broilers distributed according to a completely randomized experimental design consisting of three treatments (positive control; negative control, with 0.10 points reduction in calcium level and 0.15 points reduction in available phosphorus level; and negative control + phytase at 500 FTU/kg), with eight replicates of 10 birds each. Birds were housed in metabolic cages and reared from one to 21 days old.

The experimental diets were formulated to supply the nutritional requirements recommended by Rostagno *et al.* (2011) for the starter phase (1-21 days) and are presented in Table 1. The phytase product derived from the expression of the E. coli gene changed by mutagenesis to make the molecule resistant to pelleting temperature. The positive control feed was supplied to all birds until they were 13 days old. On



day 14, the experimental diets started to be fed until day 21, with five days of adaptation and three days of total excreta collection.

At the end of the trial, the feces collected per replicate were homogenized, and a sample was removed, weighed, and pre-dried in a forced-ventilation oven at 65°C for 72h. Samples were exposed to air until they reached environment temperature and humidity. Samples were then weighed, ground, properly packed, and submitted to the laboratory for calcium and phosphorus analyses, together with the experimental diets. Data were analyzed using the GLM procedure of SAS (2001) statistical package and means were compared by the test of Tukey at 5% significance level.

Live performance, carcass traits, and bone quality were evaluated using 350 one-d-old Cobb 500 broilers distributed according to a completely randomized experimental design consisting of three treatments (positive control; negative control, with 0.10 points reduction in calcium level and 0.15 points reduction in available phosphorus level; and negative control +

phytase at 500 FTU/kg), with eight replicates of 15 birds each. Birds were housed in 24 floor pens and reared from one to 42 days old.

The experimental diets were formulated to supply the nutritional requirements recommended by Rostagno *et al.* (2011). Three feeding phases were applied: starter (1-21 days), grower (21-33 days), and finisher (33-42 days), and are presented in Table 1. The phytase product derived from the expression of the E. coli gene changed by mutagenesis to make the molecule resistant to pelleting temperature.

Birds and feeds were weighed on days 1, 21, and 42 to determine feed intake, weight gain, and feed conversion ratio. At the end of the experiment, when broilers were 42 days old, two birds per replicate were sacrificed to determine carcass, breast, and leg yields. The right tibiae were collected for the analyses of calcium, phosphorus, and ash contents. Data were analyzed using the GLM procedure of SAS (2001) statistical package and means were compared by the test of Tukey at 5% significance level.

**Table 1** – Ingredients and nutritional composition of the experimental diets.

Ingredients, kg	Starter		Grower		Finisher	
	PC*	NC**	PC	NC	PC	NC
Corn	58.20	58.97	62.55	63.33	66.82	67.60
Soybean meal	35.58	35.35	30.55	30.32	26.60	26.43
Dicalcium phosphate	1.76	0.95	1.31	0.49	1.11	0.30
Soybean oil	2.00	2.00	3.26	3.26	3.19	3.19
Calcitic limestone	0.81	1.08	0.83	1.10	0.73	0.99
Salt	0.45	0.45	0.40	0.40	0.40	0.40
Mineral and vitamin supplement <sup>1</sup>	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.34	0.34	0.29	0.29	0.26	0.26
L-lysine	0.33	0.33	0.30	0.30	0.32	0.32
L-threonine	0.12	0.12	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional composition						
Metabolizable energy, kcal/kg	3,005	3,005	3,150	3,150	3,200	3,200
Crude protein, %	21.80	21.80	19.80	19.80	18.40	18.40
Calcium, %	0.88	0.78	0.76	0.66	0.66	0.56
Available phosphorus, %	0.44	0.29	0.35	0.20	0.31	0.16
Sodium, %	0.22	0.22	0.20	0.20	0.20	0.20
Sulfur amino acids, %	0.92	0.92	0.83	0.83	0.77	0.77
Digestible lysine, %	1.27	1.27	1.13	1.13	1.06	1.06
Digestible threonine, %	0.83	0.83	0.74	0.74	0.69	0.69
Digestible valine, %	0.88	0.88	0.80	0.80	0.74	0.74

\*PC, positive control; \*\*NC, negative control; <sup>1</sup>Guaranteed levels/ton of feed, vitamin-mineral supplement added at 4kg/ton: copper (min) 8000 mg; iron (min) 50 g; iodine (min) 1200 mg; manganese (min) 70 g; selenium (min) 200 mg; zinc (min) 50 g; folic acid (min) 1000 mg; pantothenic acid (min) 15 g; niacin (min) 40 g; Vitamin A (min) 8000000 IU; Vitamin B1 (min) 2400 mg; Vitamin B12 (min) 14000 mcg; Vitamin B2 (min) 6000 mg; Vitamin B6 (min) 4000 mg; Vitamin D3 (min) 2400000 IU; Vitamin E (min) 12000 IU; Vitamin K3 (min) 2000 mg; Coxistac 12%; zinc bacitracin 15%.



## RESULTS AND DISCUSSION

The results presented in Table 2 show that the applied treatments did not influence ash or calcium excretion ( $p>0.05$ ). However, when diets with phosphorus reduction were fed, excreta phosphorus content was also reduced when compared with the broilers fed the positive-control diet ( $p<0.05$ ).

**Table 2** – Excretion of nutrients in broilers fed the experimental diets.

	Treatments*			CV	SEM	P
	PC	NC	NC + phytase			
Ashes (g/bird/d)	4.0	3.6	3.3	13.51	0.7	0.094
Calcium (mg/bird/d)	428	428	405	18.04	73	0.202
Phosphorus (mg/bird/d)	321 <sup>b</sup>	250 <sup>a</sup>	232 <sup>a</sup>	11.15	34	0.012

\* PC – positive control; NC – negative control. ( $p<0.05$ )

The dietary addition of phytase promoted higher calcium and phosphorus retention relative to positive and negative control diet, as shown in Table 3. Ash retention was numerically different among treatments, with broilers fed the diet with phytase presenting higher retention, despite the lack of statistical difference ( $p>0.05$ ).

These results are consistent with those reported by Dourado *et al.* (2006), who evaluated the effect of phytase supplementation to broiler diets on nutrient digestibility and found higher phosphorus retention in birds fed diets with phytase supplementation.

**Table 3** – Retention of nutrients in broilers fed the experimental diets.

	Treatments*			CV	SEM	P
	PC	NC	NC + phytase			
Ashes, %	31.13	26.63	34.38	7.63	2.28	0.166
Calcium, %	50.00 <sup>b</sup>	45.63 <sup>c</sup>	55.14 <sup>a</sup>	4.99	4.02	0.037
Phosphorus, %	54.38 <sup>b</sup>	52.00 <sup>b</sup>	59.29 <sup>a</sup>	5.17	2.92	0.006

\* PC – positive control; NC – negative control. ( $p<0.05$ )

This is in agreement with the findings of Fukayama *et al.* (2008) from a study on the effect of phytase supplementation on nutrient digestibility in broilers that calcium digestibility was not different during the phase of one to 21 days between birds fed reduced nutritional levels and supplemented with phytase and those fed the positive control diet. Broilers fed the

diets with reduced nutritional levels presented lower phosphorus digestibility than those fed the positive control diet; however, the supplementation of phytase to the negative control diet improved phosphorus digestibility.

When evaluating the effect of the dietary supplementation of phytase on calcium and phosphorus digestibility in broilers, Lelis *et al.* (2010) obtained better phosphorus digestibility coefficient and higher phosphorus retention, as well as lower phosphorus excretion and higher tibial phosphorus content; however, calcium digestibility coefficient and tibial ash content was not statistically different.

Live performance results are presented in Table 4. Although phytase supplementation numerically improved broiler performance when compared to the negative control diet, results were not statistically different among treatments ( $p<0.05$ ). Carcass quality parameters were not influenced by the treatments (Table 5).

**Table 4** – Live performance of 1- to 42-d-old broilers fed the experimental diets.

	Treatments*			CV	SEM	P
	PC	NC	NC + phytase			
Average weight, g	3.224	3.145	3.176	2.58	165	0.632
Feed intake, g	4.858	5.052	4.846	2.21	518	0.675
Weight gain, g	3.176	3.096	3.126	2.65	165	0.631
Feed conversion ratio, g/g	1.53	1.63	1.55	1.88	0.12	0.388

\* PC – positive control; NC – negative control. ( $p<0.05$ )

**Table 5** – Carcass traits of broilers fed the experimental diets.

	Treatments*			CV	SEM	P
	PC	NC	NC + phytase			
Carcass, %	72.92	73.09	72.59	3.17	2.10	0.585
Breast, %	30.73	30.55	29.75	4.12	1.75	0.156
Leg, %	31.30	31.16	31.31	3.61	1.44	0.933

\* PC – positive control; NC – negative control. ( $p<0.05$ )

Broiler tibial calcium content was not influenced by the evaluated diets (Table 6). However, dietary phytase supplementation increased tibial ash content when compared with the negative control diet. Broilers fed the diet supplemented with phytase presented similar tibial phosphorus content compared with those fed the positive control diet and higher compared with those fed the negative control diet ( $p<0.05$ ).



**Table 6** – Ash, calcium, and phosphorus tibial contents of broilers fed the experimental diets.

	Treatments*			CV	SEM	p
	PC	NC	NC + phytase			
Ashes, %	53.59 <sup>a</sup>	50.41 <sup>c</sup>	52.39 <sup>b</sup>	3.94	1.00	<0.001
Calcium, %	20.72	19.90	20.02	5.18	1.17	0.333
Phosphorus, %	9.73 <sup>a</sup>	9.04 <sup>b</sup>	9.51 <sup>a</sup>	4.44	0.28	<0.001

\* PC – positive control; NC – negative control. (p<0.05)

The obtained results are different from those found by Laurentiz *et al.* (2005), who observed that broilers fed diets containing phytase and reduced phosphorus levels did not present higher mineral deposition in the tibia, but that tibial ash and phosphorus contents were reduced when a diet containing reduced phosphorus levels was fed.

## CONCLUSIONS

The supplementation of phytase to broiler diets increased dietary calcium and phosphorus digestibility, and consequently, improved bone quality.

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