



■ Author(s)

Krabbe EL¹  <https://orcid.org/0000-0002-7520-058X>
Gopinger E²  <https://orcid.org/0000-0003-0833-2205>
Corassa R³  <https://orcid.org/0009-0004-6968-6079>
Budke RCK⁴  <https://orcid.org/0009-0008-6756-2645>
Naiorka A⁵  <https://orcid.org/0000-0001-5468-7731>

- ¹ Brazilian Agricultural Research Corporation-Embrapa Swine and Poultry, Concórdia, Santa Catarina, Brazil.
² Animal Science, Doctor in Animal Nutrition, Stimulus Fellow The Innovation of the Edmundo Gastal Agricultural Research and Development Support Foundation, Concórdia, Santa Catarina, Brazil.
³ Instituto Federal Catarinense, Curso de Agronomia, Concórdia, SC, Brasil.
⁴ Universidade Federal do Paraná, Curitiba, PR, Brasil.

■ Mail Address

Corresponding author e-mail address
Mohammad Sedghi
Department of Animal Sciences, College of
Agriculture, Isfahan University of Technology,
Isfahan, Iran.
Phone: +0098-3133913511
Email: mo.sedghi@iut.ac.ir

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Phytase as a Strategy to Reduce Broiler Feeding Costs During Scenario of High Ingredient Price

ABSTRACT

The use of enzymes is a concrete fact in broiler feeding. The global economic situation has been undergoing major changes, especially in the cost of agricultural commodities such as corn and soybean meal, the main ingredients in broiler diets in many parts of the world. This study aimed to assess the effect of increasing doses of phytase by assigning an optimized non-linear increasing nutritional matrix to the diet of broilers from 1 to 42 days of age, using economic production indicators as response variables. 900 one-day-old chicks were distributed in a randomized block design with six treatments and ten replications. Different nutritional matrices were studied: a positive control diet without phytase; diets with increasing levels of this enzyme (350, 500, 1,000, 1,500 FTU); and a negative control diet (-100 kcal/kg EMA) without phytase supplementation. Performance from 1 to 42 days of age and economic viability were assessed. It was observed that the negative control and the overdose (1,500 FTU) of phytase showed worse feed conversion than the positive control diet. In the economic analysis, there was a reduction in feed costs with increasing doses of the enzyme, as well as lower production costs per carcass kilo. The data found in this study shows that performance indicators do not always reflect economic efficiency responses. Specifically, the production cost was lower in broiler diets (1 to 42 d) formulated with increasing doses of phytase (up to 1,500 FTU kg⁻¹).

INTRODUCTION

The use of exogenous enzymes in poultry diets has been consolidated over the years and aims to increase the digestibility and absorption of nutrients by acting directly on indigestible compounds, many of which are anti-nutritional factors. Thereby, they optimize the use of nutrients, reducing production costs, increasing the variability of raw materials for formulations, and standardizing production (Lu *et al.*, 2017).

There are two ways to supplement these enzymes: (i) "on top", which means the nutritional contribution of the enzyme activity on the nutrients is not considered, or (ii) when this contribution is considered. In the latter case, a reformulation of the diet considering a nutritional matrix attributed to the enzyme is required (Pasquali *et al.*, 2017). Depending on the type of enzyme added to the feed, percentage reductions are recommended in the content of nutrients such as protein, amino acids, phosphorus, calcium, and metabolizable energy. This is due to the hydrolysis of glycosidic bonds, which breaks the cell membrane and starts the digestion of the cell's contents, which are then available for absorption.

Enzyme supplementation associated with the use of a nutritional matrix enables the formulation of diets with optimized reductions



in the use of nutrients. Advantages of enzyme supplementation include the possibility of reducing the input of high-cost ingredients in diet formulation, thus resulting in a cost reduction without losses in animal performance (Ravindran, 2014; Romero *et al.*, 2014).

Although the use of phytase is already established in the poultry sector, its use in diet supplementation is mainly limited to the values of Ca and P. The energy and protein fraction provided by it are not usually considered, as there are still doubts about the enzyme's potential in that regard. Phytase hydrolyzes phytic acid, making the nutrients complexed with this molecule available, such as cations, proteins, amino acids, starch and enzymes, thus being associated with increased nutrient digestibility (Bavaresco *et al.*, 2020). With all this in mind, the present study aimed to assess the effect of increasing doses of phytase by assigning an optimized non-linear increasing nutritional matrix to the diet of broilers from 1 to 42 days old, using economic production indicators as response variables.

MATERIALS AND METHODS

The experiment was conducted in the experimental poultry sector at Embrapa Suínos e Aves - CNPSA, in Concórdia, SC, Brazil. The project was approved by Embrapa's animal ethics committee.

We studied a total of 900 one-day-old male Cobb chicks from a commercial hatchery, with an initial average chick weight of 47.05g. The birds were housed in boxes with new pine wood shavings, at a stocking density of 12 birds per m² (15 birds/box). The boxes were equipped with tube feeders, nipple drinkers (three per box), and gas heating via hoods.

The birds were weighed and distributed in the experimental boxes in a randomized block design considering their initial weight; with six treatments and ten repetitions, totaling 60 experimental units.

The experimental diets followed a formulation with increasing doses of phytase of 0, 350, 500, 1,000, 1,500 FTU, assigning an optimized non-linear increasing nutritional matrix (as a function of the value of the enzyme, according to Table 1). A positive control diet (PC) was used as a reference, meeting all the nutritional requirements of the birds, according to the recommendations of Rostagno *et al.* (2017), without the use of phytase. The negative control diet (NC) had a reduction of apparent metabolizable energy (AME) of 100 kcal/kg, also without phytase

supplementation. Two diets were adopted: an initial diet (from 1 to 21 days old) (Table 2) and a final diet (from 22 to 42 days old) (Table 3), formulated from corn and soybean meal. Both pelleted and crushed until day 12, and subsequently, supplied in the form of whole pellets. The experimental period lasted 42 days.

Table 01 – Nutritional matrix adopted for increasing phytase levels.

Nutrient	Phytase Dosage (FTU/kg)			
	350	500	1000	1500
AME, Kcal/kg	1514286	1378000	901000	606673
Crude protein (%)	8866	7124	5092	4415
Calcium (%)	3614	3289	2151	1585
Available phosphorous (%)	3286	2990	1955	1441
Sodium (%)	44	40	26	20
Digestible Arginine (%)	386	380	338	291
Digestible Cysteine (%)	349	276	194	166
Dig. Phenylalanine + Tyrosine (%)	371	338	311	281
Digestible Phenylalanine (%)	371	338	311	281
Digestible Glycine +Serine (%)	732	656	578	504
Digestible Histidine (%)	177	156	133	125
Digestible Isoleucine (%)	354	370	351	306
Digestible Leucine (%)	757	692	540	442
Digestible lysine (%)	350	350	345	340
Digestible methionine (%)	85	82	81	80
Digestible methionine cystine (%)	429	358	275	247
Digestible threonine (%)	409	340	256	224
Digestible tryptophan (%)	86	78	59	49
Digestible valine (%)	429	390	378	340

Apparent metabolizable energy (AME).

The birds were weighed weekly, their feed intake was recorded and their zootechnical performance (average weight, average weight gain, feed consumption and feed conversion) was determined.

On the last day of the experimental period, 4 birds were selected from each experimental unit based on their average weight ($\pm 5\%$). These birds were slaughtered in a slaughterhouse inspected by the official veterinary service, following the industry's operational standards. Carcass and cut yields (breast, drumstick, thigh, back, and wing) were determined.

We calculated the production costs based on the cost per kg of live weight and per carcass. To do this, the diets' costs (R\$/kg) were determined for the two studied phases (1 to 21 d and 22 to 42 d). We used ingredient prices quoted in March 2022 in the southern region of Brazil converted to US dollars (USD) using the Ptax rate (USD 5.15) related to the average for the month, as shown in Table 4.



Table 02 – Composition of experimental diets from 1 to 21 days of age.

Ingredients	Phytase Dosage FTU/kg					
	PC, 0	350	500	1000	1500	NC, 0
Corn	60.18	63.37	64.32	66.09	66.89	66.89
Soybean meal	32.66	31.56	31.24	30.39	29.87	29.87
Soybean oil	3.28	1.78	1.33	0.66	0.49	0.49
Limestone	0.451	0.480	0.489	0.503	0.509	0.509
Dicalcium phosphate	1.721	1.107	0.923	0.680	0.572	0.572
Salt	0.447	0.443	0.442	0.440	0.439	0.439
DL- methionine (99%)	0.279	0.267	0.265	0.260	0.254	0.254
Mycotoxin Adsorbent	0.200	0.200	0.200	0.200	0.200	0.200
L- Lysine	0.254	0.266	0.264	0.262	0.262	0.262
L- Threonine	0.073	0.065	0.065	0.064	0.061	0.061
vit. and min. Premix *	0.300	0.300	0.300	0.300	0.300	0.300
L-Valina	0.027	0.023	0.022	0.013	0.009	0.009
Phytase 10000 FTU/g	0.000	0.004	0.005	0.010	0.015	0.000
Choline Chloride	0.100	0.100	0.100	0.100	0.100	0.100
Monensin 40%	0.046	0.046	0.046	0.046	0.046	0.046
Antioxidant (BHT 99%)	0.010	0.010	0.010	0.010	0.010	0.010
Total	100	100	100	100	100	100
Composition						
Phytase, FTU/kg	PC. 0	350	500	1000	1500	NC. 0
AME, Kcal/kg	3.000	3.000	3.000	3.000	3.000	2.909
Crude protein, %	19.90	19.95	19.93	19.82	19.80	19.13
Ca, %	0.67	0.67	0.67	0.67	0.67	0.67
tP, %	0.65	0.65	0.65	0.65	0.65	0.65

PC- Positive Control; NC - negative control. * levels per kg of the product (Min): Folic acid 300mg; Pantothenic acid 4317mg; B.H.T. 16700mg; Biotin 30mg; Niacin 13067mg; Vitamin A 3213.000 UI; Vitamin B1 864,40mg; Vitamin B12 5350 mg; Vitamin B2 2150,40mg; Vitamin B6 1204,50mg; Vitamin D3 803.500 UI; Vitamin E 12035 UI; Vitamin K3 643,75mg; Copper 2767mg; Iron 13900mg; Iodine 282mg; Manganese 19467mg; Selenium 83,30mg; Zinc 18070,15mg; choline 130667mg.

Table 03 – Composition of experimental diets from 22 to 42 days of age.

Ingredients	Phytase Dosage FTU/kg					
	PC, 0	350	500	1000	1500	NC, 0
Corn	62.94	66.09	67.19	68.81	69.48	69.49
Soybean meal	28.53	27.47	27.01	26.29	25.90	25.90
Soybean oil	5.08	3.59	3.12	2.47	2.32	2.32
Limestone	0.610	0.640	0.649	0.662	0.668	0.668
Dicalcium phosphate	1.509	0.894	0.711	0.467	0.358	0.358
Salt	0.423	0.419	0.418	0.416	0.415	0.415
DL- methionine (99%)	0.248	0.236	0.235	0.228	0.221	0.221
Mycotoxin Adsorbent	0.100	0.100	0.100	0.100	0.100	0.100
L- Lysine	0.220	0.231	0.236	0.234	0.224	0.224
L- Threonine	0.057	0.049	0.050	0.047	0.043	0.043
Premix vitam/min *	0.200	0.200	0.200	0.200	0.200	0.200
L-Valina	0.029	0.025	0.026	0.017	0.009	0.009
phytase 10000 FTU/g	0.000	0.004	0.005	0.010	0.015	0.000
Monensin 40%	0.046	0.046	0.046	0.046	0.046	0.046
Antioxidant (BHT 99%)	0.010	0.010	0.010	0.010	0.010	0.010
Total	100.00	100.00	100.00	100.00	100.00	100.00
Composition						
Phytase, FTU/kg	0	350	500	1000	1500	0
AME, Kcal/kg	3.150	3.150	3.150	3.150	3.150	3.059
Crude Protein, %	18.17	18.24	18.16	18.11	18.12	17.46
Ca, %	0.67	0.67	0.67	0.67	0.67	0.67
tP, %	0.59	0.59	0.59	0.59	0.59	0.59

PC- Positive Control; NC - negative control. * levels per kg of the product (Min): Folic acid 300mg; Pantothenic acid 4317mg; B.H.T. 16700mg; Biotin 30mg; Niacin 13067mg; Vitamin A 3213.000 UI; Vitamin B1 864,40mg; Vitamin B12 5350 mg; Vitamin B2 2150,40mg; Vitamin B6 1204,50mg; Vitamin D3 803.500 UI; Vitamin E 12035 UI; Vitamin K3 643,75mg; Copper 2767mg; Iron 13900mg; Iodine 282mg; Manganese 19467mg; Selenium 83,30mg; Zinc 18070,15mg; choline 130667mg.



Table 04 – Cost of ingredients – March/22 (U\$D/kg).

Ingredient	U\$D
Corn	0.334
Soybean meal	0.515
Soybean oil	1.340
Limestone	0.905
Dicalcium phosphate	0.058
Salt	0.078
Px Vit/Min	2.344
DL- methionine (99%)	4.031
L- Lysine	2.718
Mycotoxin Adsorbent	1.165
Choline Chloride	1.192
L- Threonine	3.062
L-Valina	5.825
Monensin 40%	7.767
Antioxidant (BHT 99%)	7.017
Phytase 10000 FTU/g	14.563

The feed cost per kg of the diets (Table 5) was obtained based on the number of ingredients in each diet. A reduction in the diets' costs was observed as the levels of phytase inclusion increased. This was due to the nutritional value attributed to the enzyme, which enabled a reduction in the input of high-cost ingredients into the matrix. Furthermore, in relation to the total cost of the diets, the inclusion of phytase represented 0.13%; 0.16%, 0.34; 0.51% of the cost for inclusions of 350, 500, 1000 and 1500 FTU/kg, respectively.

Table 05 – Cost of experimental diets (U\$D/kg), march/22.

Diet	U\$D/kg	
	1 - 21 d	22 - 42 d
Negative control (-100 kcal)	0.422	0.427
PC- Positive Control 0 FTU kg ⁻¹	0.464	0.468
Phytase 350 FTU kg ⁻¹	0.443	0.448
Phytase 500 FTU kg ⁻¹	0.437	0.441
Phytase 1000 FTU kg ⁻¹	0.428	0.432
Phytase 1500 FTU kg ⁻¹	0.425	0.429

Using performance and carcass yield results, we calculated the cost per kg of chilled carcasses, as

Table 06 – Body weight (BW), weight gain (WG), feed intake (FI) and feed conversion (FCR) of broiler chickens from 1 to 21 days age fed diets formulated with increasing doses of phytase, using a non-linear matrix.

Diet	BW (g)	WG (g)	FI (g)	FCR (g:g)
CN, 0 FTU kg ⁻¹	1047.3 B	1000.0 B	1365.1	1.3632 A
PC, 0 FTU kg ⁻¹	1111.6 A	1064.5 A	1415.8	1.3174 B
Phytase 350 FTU kg ⁻¹	1115.7 A	1069.9 A	1430.1	1.3328 AB
Phytase 500 FTU kg ⁻¹	1093.0 AB	1045.4 AB	1382.9	1.3233 B
Phytase 1000 FTU kg ⁻¹	1104.5 AB	1057.4 AB	1408.3	1.3325 AB
Phytase 1500 FTU kg ⁻¹	1100.0 AB	1052.6 AB	1427.8	1.3533 AB
Pr>f*	0.0148	0.0129	0.0612	0.0032
CV, %	3.82	3.99	3.68	1.93
SEM	6.42	6.43	7.21	0.0042

PC- Positive Control; NC- negative control. *Pr>f- Significance level by ANOVA at 5%. Different capital letters in the column differ from each other by the 5% Tukey test. CV- Coefficient of variation (%). EPM- Standard error of the mean.

well as the cost per kg of live weight, according to the methodology proposed by Miele *et al.* (2010). This methodology is based on defining the production system, surveying zootechnical production coefficients, and market prices. Costs were calculated per experimental unit. To determine costs, the following formulas were used: Cost Feed per bird = (cost of diet¹ x consumption in phase ¹/bird) + (cost of diet² x consumption in phase ²/bird), whereby: phase 1- 1 to 21 days, and phase 2- 22 to 42 days; cost feed /kg= cost feed/ live weight at 42 days; cost feed/kg carcass = cost feed/(carcass yield/100).

Total production cost equals the sum of all feed costs, other costs, labor costs, capital costs, and depreciation of the facilities. To determine the total cost of production, information from CIAS (EMBRAPA) was used for the month evaluated, which indicated that feed represented 73.37% of costs, and the remaining 26.63% represented other costs. It was determined using the following formulas: Total production cost = feed cost/(% feed cost of the month*/100); Production cost per bird = production cost/bird; Production cost /kg = production cost/ live weight at 42 days; Production cost /Kg carcass= production cost/(carcass yield/100).

The data was analyzed using SAS software (SAS, Inst. Inc., Cary, NC, 2002) and ANOVA at 5%. The means were compared using Tukey's test at 5% significance.

RESULTS AND DISCUSSION

The experimental model was validated through statistical difference among the evaluated parameters, as shown in the comparison between positive and negative controls. Broiler performance data up to 21 days is shown in Table 6. We observed that both the average weight and weight gain were lower in the



negative control diet, contrasting with the positive control and the diet with the inclusion of 350 FTU per kg⁻¹. Moreover, we noticed a worse feed conversion in the negative control diet, which differed from the positive control and the inclusion of 500 FTU of phytase.

In the performance evaluation of the total period, from 1 to 42 days of age (Table 7), no difference

could be found for the increasing doses of phytase on average weight, weight gain and feed consumption when compared with both the positive (PC) and negative controls (NC). However, a significant response was observed in the feed conversion variable, in which the negative control and the overdose (1500 FTU kg⁻¹) showed worse feed conversion compared to the positive control.

Table 07 – Body weight (BW), weight gain (WG), feed intake (FI) and feed conversion (FCR) of broiler chickens from 1 to 42 days age fed diets formulated with increasing doses of phytase, using a non-linear matrix.

Diet	BW (g)	WG (g)	FI (g)	FCR (g:g)
NC, 0 FTU kg ⁻¹	3046.90	2999.50	5172.40	1.731 A
PC, 0 FTU kg ⁻¹	3194.60	3147.50	5166.90	1.641 B
Phytase 350 FTU kg ⁻¹	3114.80	3069.40	5207.40	1.679 AB
Phytase 500 FTU kg ⁻¹	3157.60	3109.90	5193.70	1.680 AB
Phytase 1000 FTU kg ⁻¹	3136.90	3089.80	5085.70	1.691 AB
Phytase 1500 FTU kg ⁻¹	3116.70	3069.40	5193.70	1.693 A
Pr>f*	0.2144	0.2176	0.7628	0.0007
CV, %	3.67	3.73	3.34	2.00
SEM	121.9	122.1	167.8	0.050

PC- Positive Control; NC- negative control. *Pr>f- Significance level by ANOVA at 5%. Different capital letters in the column differ from each other by the 5% Tukey test. CV- Coefficient of variation (%). SEM- Standard error of the mean.

The main expected beneficial effect from adding phytase to the diets was better utilization of P from plant ingredients, as well as better digestion of other minerals such as Ca, which is credited to the enzyme through the nutritional matrix assigned to it. Various studies (Dersjant-Li *et al.*, 2015; Nissar *et al.*, 2017; Bavaresco *et al.*, 2021) have shown that, for each dose of phytase, different levels of minerals, amino acids, and energy can be applied to the phytase nutritional matrix, which is known as a non-linear matrix. Furthermore, many studies such as those by Boney & Moritz (2017), Oliveira *et al.* (2018), and Woyengo & Wilson (2019), recommend the overdose of phytase, since the anti-nutritional effects of phytate are thus

more efficiently reduced, while also improving the availability of nutrients complexed by phytate.

In our study, the doses up to 500 FTU/kg of phytase proved to be efficient in hydrolyzing the phytate molecule and maintained birds' performances, given the nutritional matrix applied. However, overdosing (more than 1,000 FTU kg⁻¹) negatively affected feed conversion, demonstrating that the contribution of the enzyme employed through the application of an increasing non-linear nutritional matrix has a performance limit.

Similarly, Bavaresco *et al.* (2021) observed that the use of phytase (500FTU kg⁻¹) is efficient at maintaining the performance of broilers up to 42 days of age that

Table 08 – Carcass and cut yields of broiler chickens at 42 days fed diets formulated with increasing doses of phytase, using a non-linear matrix.

Diet	Yields (%)					
	Carcass*	breast	thigh	drumstick	back	wing
CN, 0 FTU kg ⁻¹	85.44	36.73	12.63	14.10	14.20	9.08
CP, 0 FTU kg ⁻¹	85.19	37.07	12.50	13.85	14.14	9.44
Phytase 350 FTU kg ⁻¹	85.81	37.19	12.65	13.73	14.20	9.28
Phytase 500 FTU kg ⁻¹	85.68	37.77	12.43	13.58	14.38	9.14
Phytase 1000 FTU kg ⁻¹	85.57	37.73	12.22	13.75	14.15	9.40
Phytase 1500 FTU kg ⁻¹	85.91	37.30	12.65	13.84	14.11	9.29
Pr>f**	0.147	0.271	0.379	0.419	0.836	0.060
CV, %	1.30	5.33	7.52	7.03	5.85	5.79
SEM	0.08	0.14	0.07	0.07	0.06	0.04

**Carcass cooled with foot and head. PC- Positive control; NC- negative control. **Pr>f- Significance level by ANOVA at 5%. Different capital letters in the column differ from each other by the 5% Tukey test. CV- Coefficient of variation (%). SEM- Standard error of the mean.



are fed diets formulated with a reduction of up to 70 kcal kg⁻¹, 0.16% Ca, and 0.15% available P.

Our results show that the inclusion of phytase in diets is beneficial to birds, and that the enzyme can be effectively included in the formulation at up to 500 FTU kg⁻¹. Greater inclusion levels remain in a plateau, where larger quantities of the enzyme result in no meaningful contribution to performance.

Carcass and cut yield results are shown in Table 8. There was no significant effect of diets with increasing doses of phytase on the carcass, breast, drumstick, thigh, back, and wing yields.

When determining the cost of feed per bird and kilogram of live weight (Figure 1), the positive control diet without phytase showed the highest cost, differing from the diets with 500, 1,000 and 1,500 FTU and the negative control, without the enzyme. Considering the variable feed cost kg⁻¹ of live weight, the diets providing the lowest cost were those that included 1,000 and 1,500 FTU kg⁻¹ of phytase. The results show that formulating the nutritional matrix according to the value of the enzyme reduces the feed cost since an increase in the dose of enzyme reduced the inclusion of high-cost ingredients in the diet.

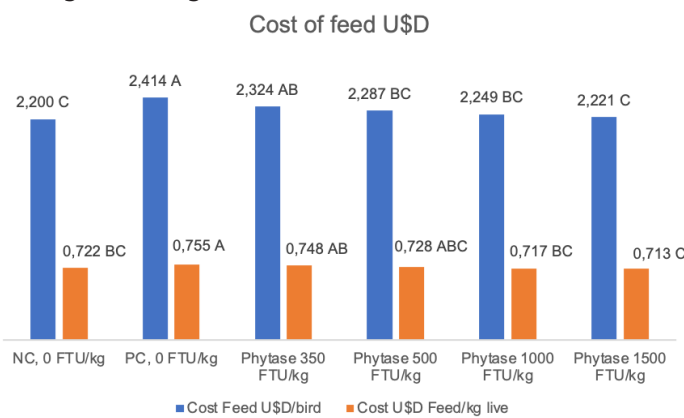


Figure 01 – Cost of feeding broilers at 42 days with diets formulated with increasing doses of phytase, using a non-linear matrix (\$ USD of feed/bird at 42 days - \$ USD of feed kg⁻¹ live at 42 days d). PC- Positive Control; NC- negative control. Capital letters differ from each other using the Tukey test at 5%.

When evaluating the total cost of production per bird (Figure 2), we observed that the negative control diet and the diet supplemented with 1,500 FTU kg⁻¹ had lower costs, contrasting with the positive control diet and the diet supplemented with 350 FTU kg⁻¹. Furthermore, considering the total cost kg⁻¹ of live weight, birds fed with diets supplemented with 1,000 and 1,500 FTU kg⁻¹ showed lower costs when compared to those consuming a positive control diet or the 350 FTU kg⁻¹ diet.

Regarding the feed cost and the total cost per kg of carcass (Figure 3), we noticed that the lowest-cost

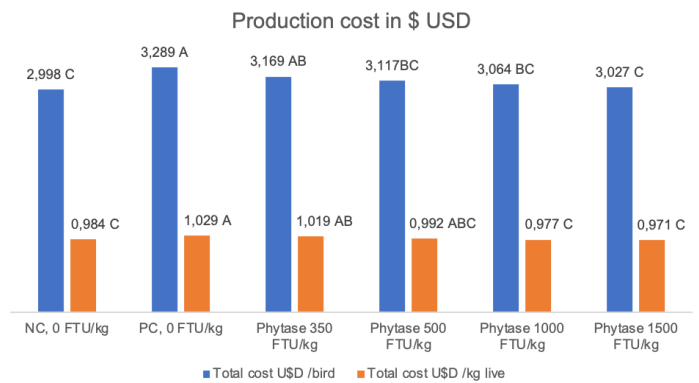


Figure 02 – Total production costs of broiler at 42 days fed with diets formulated with increasing doses of phytase, using a non-linear matrix (\$ USD/ bird - \$ USD/ kg live). PC- Positive Control; NC- negative control. Capital letters differ from each other using the Tukey test at 5%.

diet was the one with the inclusion of 1,500 FTU kg⁻¹, which differed from the positive control and 350 FTU kg⁻¹ diets. This represents, respectively, an expenditure of \$ 0.054 USD and \$ 0.040 less on feed per kg of carcass and \$ 0.073 USD and \$ 0.054 cents less on the total cost per kg of carcass, compared to the positive control and 350 FTU kg⁻¹ diets. Thus, an increase in the inclusion of phytase in diets formulated using a non-linear increasing nutritional matrix shows a lower cost of production per kilo of carcass. This demonstrates greater economic efficiency resulting from the use of the enzyme, which in turn implies less inclusion of high-cost ingredients in the diets.

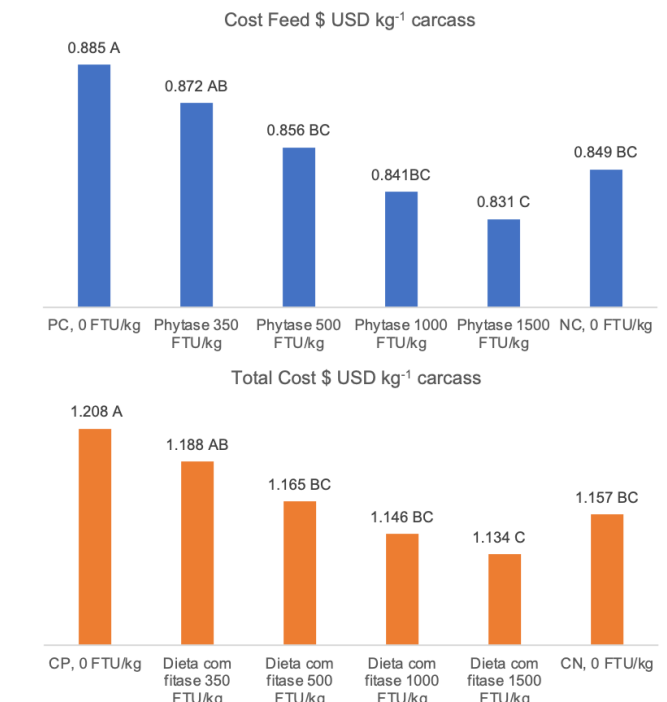


Figure 03 – (A) Feeding cost and (B) Production cost per kilogram of carcass of broiler chickens at 42 days fed with diets formulated with increasing doses of phytase, using a non-linear increasing matrix. PC- Positive Control; NC- negative control. Capital letters differ from each other using the Tukey test at 5%.



According to Shelton *et al.* (2004), supplementing diets with phytase while considering the enzyme's contribution to nutrients such as P, Ca, energy and amino acids, results in the reduction of some ingredients, such as limestone, phosphates, soybean meal, among others. Thus, it contributes to a reduction in the cost of the final feed. The present study showed that this cost reduction, both per kilo of live weight and per kilo of carcass, was indeed observed when the dietary inclusion of phytase was increased.

CONCLUSION

The supplementation of increasing doses of phytase in broiler diets applying a non-linear increasing nutritional matrix assigned to the enzyme at doses of 500, 1,000 and 1,500 FTU kg⁻¹ showed better economic viability and similar results to those observed in the NC, resulting in savings in both feed and production costs per kilogram of live weight and per kilogram of carcass. However, when evaluating the birds' performance in isolation, the inclusion of up to 500 FTU kg⁻¹ provided the best response. In times of high ingredient costs, we suggest that phytase users consider not only the performance of the birds, but especially the economic performance of their production system.

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