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Evaluation of liquid xylanase and phytase added after broiler feed pelletization

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ABSTRACT - The objective of this study was to evaluate whether the addition of the enzymes phytase and xylanase, isolated or associated, in the liquid form after feed pelletization could improve energy utilization and digestibility of calcium and phosphorus by broiler chickens. Three experiments were performed using 120 birds each, divided into five treatments with eight replicates per group (n = 3), identified as: experiment 1 (xylanase: control, 1000 IU, 1500 IU, 2000 IU, 2500 IU), experiment 2 (phytase: control, 500 FTU, 1000 FTU, 1500 FTU, 2000 FTU), experiment 3 (xylanase + phytase: control, 3000 IU + 500 FTU, 3000 IU + 1000 FTU, 3000 IU + 1500 FTU, 3000 IU + 2000 FTU). Samples for digestibility tests were collected at 14 to 21 days of age. Therefore, the inclusion of liquid phytase and liquid phytase + xylanase after pelletization in broiler diets has become a relevant way to reduce the inclusion of inorganic P, which can reduce the cost of feed and P excretion in the environment. Furthermore, it is an interesting strategy to avoid enzyme denaturation in the pelleting process.

Keywords: digestibility, exogenous enzymes, minerals, pelletizing

1. Introduction

Poultry diets are mainly based on corn, wheat, and soybean meal, which contain around 10 to 22.7% of non-starch polysaccharides (NSP) in their composition (Kim et al., 2021), classified as antinutritional factors that negatively impact the gastrointestinal tract of birds. Non-starch polysaccharides may increase the viscosity of the digesta, decrease the rate of intestinal passage of nutrients, interfere with the microbiota that colonizes the digestive tract (Cardoso et al., 2018), as well as increase the rate of mucosal cell turn-over, mucin secretion, and undigested content (Dong et al., 2018). Therefore, they negatively influence poultry performance and health.

Birds do not produce endogenous enzymes to break beta 1,4 and alpha 1,6 bonds of some components present in vegetables. In this way, several exogenous enzymes such as xylanases, alpha-amylases,

beta-glucanases, cellulases, and phytases are produced to aid in the digestion of nutrients, increase the proportion of beneficial bacteria in the intestinal tract, decrease intestinal viscosity, and save energy. These enzymes might be produced by some bacteria, yeasts, and fungi (Oliveira and Moraes, 2007). The addition of exogenous enzymes to feed can reduce the harmful effects caused by antinutritional factors such as NSP and phytic acid. It can also improve the digestive process and availability of energy in food, particularly in young birds (Olukosi et al., 2020).

In this context, phytase is the main exogenous enzyme used in the diet of birds, since they do not produce endogenous phytase (Dessimoni et al., 2019). Phytase breaks down phosphate from phytic acid in a sequential process, and the final product is myoinositol and phosphate, besides other nutrients that are trapped in phytic acid (Yu et al., 2012). Kim et al. (2021) reported that the addition of multiple enzymes with phytase improves the digestibility of dry matter (DM), crude energy, crude protein, calcium, and phosphorus. Another advantage of utilizing phytase is that it increases the availability of phosphorus, reducing the use of inorganic phosphate (Dessimoni et al., 2019).

In addition, one can mention xylanases, which are carbohydrases that act on the xylan skeleton, breaking the internal beta-(1,4) bonds, thus reaching the cell wall (Zhang et al., 2016). In this way, they release oligosaccharides, disaccharides, and monomeric pentose sugars such as xylose. Then, xylose can be absorbed in the small intestine and provide energy (Huntley and Patience, 2018). Therefore, the use of xylanase in poultry diets can reduce digesta viscosity and improve nutrient availability by NSP hydrolysis (Barasch and Grimes, 2021), in addition to aiding the phytase access to phytate stored in the cell wall membrane of vegetables, which also helps to improve the use of phosphorus and energy.

Broiler feed is usually pelletized, a process by which the ingredients are subjected to temperatures between 75 and 83 °C. Due to their thermolabile properties, these conditions may result in denaturation and inactivation of exogenous enzymes when subjected to pressure and heat (Pope et al., 2020). Given this fact, the application of enzymes after pelletization, in liquid form, might be a promising alternative to overcome this problem. Then, in a spraying process in the already pelleted feed, the enzymes can be added at room temperature without loss due to enzymatic denaturation that could occur at higher temperatures. Therefore, the objective of this study was to evaluate whether the addition of phytase and xylanase enzymes, isolated or associated, in liquid form after pelleting in broiler ration, could improve energy utilization and digestibility of calcium and phosphorus in the diets.

2. Material and Methods

2.1. Animals, experimental design, and diets

The experimental protocol was approved by the Institutional Ethics Committee on the Use of Animals (CEUA) under protocol number 27/2020.

Three experiments were performed with 360 Cobb 500 females and broiler chickens in Santa Catarina, Brazil (Latitude: −26.8364531149; Longitude: −52.4079407059). In the three trials, a completely randomized experimental design was adopted with five treatments (Table 1), eight replicates, and three animals per experimental unit. In the pre-experimental period (1 to 14 days), the birds were raised in shavings, according to the lineage manual.

The basal ration used in this study was based on crushed corn and soybean meal according to the nutritional requirements described in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017; Table 2), however, with a reduction in metabolizable energy levels (3150 to 3100 kcal/kg) and crude protein (19.71 to 19.00%). The rations were pelleted in a pelletizer model Koppers Junior C40 (Siemens), with a 50 horsepower (hp) engine and a ring with holes with a diameter of 3/16 inches, being processed at a temperature of 80 °C and device retention time of 30 s. The enzymes were added to the feed in liquid form, immediately after pelleting, with the aid of in-line sprinklers. The xylanase used had a concentration of 3,000 IU/g, and the phytase, a concentration of 10,000 FTU/g. Feed and water were provided *ad libitum* throughout the experimental period.

Table 1 - Treatments used – final levels in the feed

Treatment	Exp. 1 - Xylanase	Exp. 2 - Phytase	Exp. 3 - Xylanase + Phytase
T1	Control	Control	Control
T2	1000 IU	500 FTU	3000 IU + 500 FTU
T3	1500 IU	1000 FTU	3000 IU + 1000 FTU
T4	2000 IU	1500 FTU	3000 IU + 1500 FTU
T5	2500 IU	2000 FTU	3000 IU + 2000 FTU

FTU and/or IU per ton of feed.

Table 2 - Feed and nutritional composition of the basal experimental ration

Ingredient (g/kg)	Amount
Corn	655.52
Soybean meal	281.92
Soybean oil	21.34
Dicalcium phosphate	16.87
Limestone	8.05
Salt	4.25
Choline chloride	0.80
DL-Methionine	2.68
L-Lysine HCl	3.26
L-Threonine	1.01
Vitamin supplement[1]	2.00
Mineral supplement[2]	2.00
Antioxidant[3]	0.20
Calculated values (g/kg)	
Energy metabolizable (kcal/kg)	3100
Crude protein	190.00
Digestible lysine	11.00
Digestible met. + cys.	7.91
Digestible threonine	7.14
Digestible tryptophan	2.00
Calcium	8.37
Available phosphorus	4.18
Sodium	2.08

[1] Vitamin supplement containing per kg: vitamin A, 6,500,000 IU; vitamin D3, 2,500,000 IU; vitamin E, 30,000 IU; vitamin B1, 3.0 g; vitamin B2, 3.75 g; vitamin B6, 3.0 g; vitamin B12, 0.010 g; pantothenic acid, 12.0 g; biotin, 0.150 g; vitamin K3, 2.0 g; folic acid, 0.250 g; nicotinic acid, 35.0 g; excipient q.s.p, 1000 g.
[2] Mineral supplement containing per kg: iron, 50.0 g; cobalt, 2.0 g; copper, 10.0 g; manganese, 80.0 g; zinc, 66.60 g; iodine, 6.66 g; selenium, 233 mg; excipient q.s.p, 1000 g;
[3] Butylated hydroxytoluene 99% and ethoxyquin 66.6%.
[4] This mixture will contain the enzymes, and in treatments with smaller inclusions of enzymes, vehicle will be added without alteration of the nutritional composition of the feed.

2.2. Sampling and feed energy calculation

The total excreta collection method was used, with the birds housed in a metallic structure battery consisting of cages, distributed on two floors, equipped with a feeder and a nipple-type drinker. The experimental period was of ten days and consisted of five days for adapting the birds to cages, feed, and management, and five days for total excreta collection. Excreta from all experimental units were collected daily (08:00 and 17:00 h) in plastic-covered trays and conditioned in a freezer until the end of the experiment. At the end of the experimental period, the excreta were thawed, weighed, and homogenized, after which a 500 g sample was taken from each experimental unit and placed in a forced-circulation oven at 55 °C for pre-drying. They were analyzed according to the techniques described by Silva and Queiroz (2002) to determine the values of metabolizable energy (Matterson et al., 1965) and nitrogen (Kjieldahl's method), for subsequent use, obtaining the metabolizable energy values of the diets.

At the end of the experiment, the feed intake per experimental unit during the five days of the collection was determined. Once the results of the laboratory analyses of the rations and excreta were obtained, the apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen retention (AMENR) values were calculated using equations proposed by Matterson et al. (1965):

$$\text{Feed AME (kcal/kg)} = \frac{\text{GE ingested} - \text{GE excreted}}{\text{DM ingested}} \quad \text{(Eq. 1)}$$

$$\text{Feed AMENR (kcal/kg)} = \frac{\text{GE ingested} - (\text{GE excreted} - 8.22 \times \text{NB})}{\text{DM ingested}} \quad \text{(Eq. 2)}$$

in which GE = gross energy and NB = nitrogen balance (N ingested − N excreted).

2.3. Dietary calcium and phosphorus balance calculation

To determine the utilization of calcium and phosphorus in the diets, excreta and feed samples were subjected to absorption spectrophotometry analysis as described by Tedesco et al. (1995). From these data, the phosphorus digestibility coefficients were calculated according to the formula below:

$$\text{Digestibility coefficient (\%)} = \frac{\text{\% of nutrient in feces}}{\text{\% of nutrient in the diet}} \times 100 \quad \text{(Eq. 3)}$$

2.4. Statistical analysis

The results were subjected to the Shapiro-Wilk normality test. When normal, the analysis of variance used was parametric and if significant, subjected to linear and quadratic regression analysis and also to Dunnett's test. However, when there was no normality in the data, the variables were analyzed using the non-parametric Kruskal-Wails test and Dunnett's test to compare means. The different levels of the exogenous enzymes were subjected to regression analysis using linear and quadratic functions. All analyzes were performed at a 5% significance level, using the R statistical program (R Development Core Team, 2019). The statistical model was as follows:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}, \quad \text{(Eq. 4)}$$

in which Y_{ij} = dependent variable, μ = variable mean, β_i = fixed effect of broilers of the group, and ε_{ij} = experimental error associated with observation Y_{ij}.

3. Results

There was no statistical difference for the variables DM intake, DM excreta production, AME, and AMENR ($P>0.05$) for broilers supplemented with different levels of xylanase in liquid form after pelleting (Table 3).

There was no statistical difference for calcium balance variables for birds supplemented with different levels of phytase in liquid form ($P>0.05$; Table 4). However, for the phosphorus balance, a linear effect was observed for DM intake as the levels of phytase inclusion increased ($P = 0.01$). We also observed an increase in the total phosphorus ingested in the 1000 FTU group, followed by 2000 FTU group in relation to the 0 and 1500 FTU groups ($P<0.001$). In addition, there was this variable presented a linear ($Y = 0.3569x + 5.4174$) and also a quadratic effect ($Y = -0.3202x^2 + 2.2781x + 3.176$). For the apparent digestibility coefficient (ADCo) of the total phosphorus of the diet, an increase was observed in all groups that received liquid phytase in relation to the control ($P = 0.020$), and there was also it also presented a linear ($Y = 2.5486x + 60.347$) and quadratic effect ($Y = -1.822x^2 + 13.481x + 47.593$). All groups that received liquid phytase had lower phosphorus excretion compared with the control

(P = 0.020), besides the linear (Y = −2.5152x + 39.506) and quadratic effect (Y = 1.944x^2 − 13.282x + 52.067) (Table 5).

There was no statistical difference in the calcium balance of birds supplemented with different levels of liquid phytase + xylanase (P>0.05; Table 6). However, for the phosphorus balance, a decrease in DM intake was verified in birds that received 1000 FTU + 3000 IU in relation to the other groups (P<0.001). The largest amount of phosphorus ingested was observed in groups of 1500 and 2000 FTU, followed by groups 0 and 500 in relation to the group that received 1000 FTU of phytase + xylanase (P<0.001). The highest coefficient of total apparent digestibility of phosphorus was found in all groups that received liquid enzymes compared with the control (P = 0.046). Along with that, the lowest total phosphorus excretion was also observed in all groups that received the two enzymes associated with the control (P = 0.046; Table 7).

Table 3 - Metabolizable energy values of diets supplemented with different levels of liquid xylanase after pelleting

Variable	Xylanase (IU/kg)					CV (%)	Linear effect	Quadratic effect	P-value
	0	1000	1500	2000	2500				
DM intake (g/bird/d)	823.64	878.11	894.51	832.58	854.92	8.19	0.823	0.124	0.230
DM excreted (g/bird/d)	159.25	175.99	172.23	171.63	173.80	11.97	0.291	0.342	0.528
AME (kcal/kg)	3.259	3.285	3.273	3.271	3.258	1.52	0.233	0.981	0.523
AMENR (kcal/kg)	3.094	3.101	3.107	3.093	3.118	1.48	0.044	0.202	0.112

DM - dry matter; AME - apparent metabolizable energy; AMENR - apparent metabolizable energy corrected by nitrogen retention.

Table 4 - Mean values of calcium balance of broiler rations supplemented with liquid phytase at different levels after pelleting

Variable	Phytase (FTU)					CV (%)	Linear effect	Quadratic effect	P-value
	0	500	1000	1500	2000				
DM intake (g/bird/d)	113.24	119.58	118.08	118.34	119.95	4.00	0.102	0.180	0.130
DM excreted (g/bird/d)	23.74	23.88	27.46	23.09	25.23	22.39	0.730	0.590	0.520
Total Ca ingested (g/bird/d)	0.87	0.91	0.91	0.91	0.911	8.41	0.143	0.760	0.323
Ca excreted (g/bird/d)	0.34	0.34	0.40	0.33	0.38	25.86	0.503	0.730	0.809
Ca amount in excreta (%)	1.42	1.44	1.44	1.44	1.50	9.44	0.280	0.610	0.806
ADCo of total Ca in the diet (%)	61.28	62.95	56.45	63.36	59.05	16.63	0.710	0.800	0.380
Total Ca excreted (%)	38.77	37.01	43.94	36.60	40.95	25.38	0.710	0.800	0.380

DM - dry matter; ADCo - apparent digestibility coefficient.

Table 5 - Mean values of phosphorus balance in broiler rations supplemented with liquid phytase at different levels after pelleting

Variable	Phytase (FTU)					CV (%)	Linear effect	Quadratic effect	P-value
	0	500	1000	1500	2000				
DM intake (g/bird/d)	112.62	119.00	117.63	117.75	119.25	4.00	0.01	0.180	0.130
DM excreted (g/bird/d)	23.74	23.88	27.46	23.09	25.23	22.39	0.730	0.597	0.520
Total P ingested (g/bird/d)	0.532c	0.788ab	0.808a	0.704c	0.772b	14.43	<0.001[a]	<0.001[b]	<0.001
P excreted (g/bird/d)	0.227	0.218	0.253	0.202	0.228	11.83	0.836	0.823	0.650
Fecal amount of P (%)	0.954	0.912	0.912	0.872	0.918	11.83	0.368	0.362	0.698
ADCo of total P in the diet (%)	57.22b	72.32a	68.66a	71.28a	70.48a	9.21	0.009[c]	0.026[d]	0.020
Total P excreted (%)	42.59b	27.66a	31.38a	28.66a	29.51a	15.66	0.009[e]	0.026[f]	0.020

DM - dry matter; ADCo - apparent digestibility coefficient.
a,b,c - Means in the same row with different letters differ significantly (P<0.05) by Dunnett's test.
[a] Y = 0.3569x + 5.4174, R^2 = 0.311.
[b] Y = −0.3202x^2 + 2.2781x + 3.176, R^2 = 0.6615.
[c] Y = 2.5486x + 60.347, R^2 = 0.4265.
[d] Y = −1.822x^2 + 13.481x +47.593, R^2 = 0.7317.
[e] Y = −2.5152x + 39.506, R^2 = 0.4258.
[f] Y = 1.944x^2 − 13.282x + 52.067, R^2 = 0.7292.

Table 6 - Mean values of calcium balance of broiler rations supplemented with liquid xylanase + phytase at different levels after pelleting

Variable	Xylanase + phytase					CV (%)	Linear effect	Quadratic effect	P-value
	0	3000 IU + 500 FTU	3000 IU + 1000 FTU	3000 IU + 1500 FTU	3000 IU + 2000 FTU				
DM intake (g/bird/d)	132.22	132.27	131.44	132.85	132.87	0.97	0.187	0.194	0.183
DM excreted (g/bird/d)	25.16	23.28	24.47	24.00	23.36	15.35	0.503	0.874	0.840
Total Ca ingested (g/bird/d)	0.944	0.944	0.938	0.948	0.948	4.44	0.312	0.365	0.162
Ca excreted (g/bird/d)	0.296	0.243	0.293	0.298	0.270	16.55	0.95	0.94	0.080
Fecal amount of Ca (%)	1.193	1.048	1.203	1.270	1.159	15.97	0.45	0.93	0.150
ADCo of total Ca in the diet (%)	68.64	74.30	68.79	68.58	71.55	6.76	0.45	0.50	0.470
Ca excreted (%)	31.35	25.70	31.27	31.41	28.44	15.30	0.45	0.50	0.470

DM - dry matter; ADCo - apparent digestibility coefficient.

Table 7 - Phosphorus balance (mean values) in broiler rations supplemented with liquid xylanase + phytase at different levels after pelleting

Variable	Xylanase + phytase					CV (%)	Linear effect	Quadratic effect	P-value
	0	3000 IU + 500 FTU	3000 IU + 1000 FTU	3000 IU + 1500 FTU	3000 IU + 2000 FTU				
DM intake (g/bird/d)	132.22a	132.27a	131.44b	132.85a	132.87a	0.97	0.187	0.194	<0.001
DM excreted (g/bird/d)	25.16	23.28	24.47	24.00	23.36	15.35	0.50	0.87	0.840
Total P ingested (g/bird/d)	0.859b	0.860b	0.855c	0.864a	0.864a	0.97	0.187	0.194	<0.001
P excreted (g/bird/d)	0.271	0.199	0.234	0.220	0.213	34.04	0.286	0.448	0.440
Fecal amount of P (%)	1.088	0.854	0.966	0.928	0.919	29.09	0.418	0.438	0.770
ADCo of total P in the diet (%)	58.47a	76.83b	72.60b	74.45b	75.31b	12.25	0.270	0.344	0.046
Total P excreted (%)	41.52a	23.17b	27.49b	25.56b	24.69b	34.02	0.270	0.344	0.046

DM - dry matter; ADCo - apparent digestibility coefficient.
a,b,c - Means in the same row with different letters differ significantly (P<0.05) by Dunnett's test.

4. Discussion

The addition of liquid phytase (500 FTU) and phytase + xylanase (500 FTU + 3000 IU) after pelleting showed promising values in terms of improving the use of phosphorus in the feed and, with that, decreasing phosphorus excretion, which is desirable in production systems due to the environmental impact that this mineral may cause. Furthermore, there are not many studies in the literature on the use of liquid enzymes after pelleting and the impact of this process on the denaturation of exogenous enzymes used in animal production. However, it is known that the factor that most contributes to the denaturation of enzymes is the accumulation of forces necessary to bind the particles within the pelletizing matrix, such as moisture, heat, and pressure, in addition to exposure to steam inside the conditioning chamber (Pope and Fahrenholz, 2020).

In this context, when adding liquid xylanase after pelleting, no improvement in energy coefficients was observed. The results of the present study corroborate the results found by Denstadli et al. (2010), who pointed out that when supplementing broilers with 2500 IU/kg of xylanase in the liquid form after pelleting, there was no significant difference in AME. Thus, the inclusion levels used in this study were not efficient to cause the hydrolysis of the beta 1,4 and alpha 1,6 bonds of the NSP, so that there were significant changes in the metabolizable energy of the diet or there was not enough substrate for the enzyme to act efficiently. However, more studies should be carried out using different xylanases, as several authors demonstrate that the pelletization process decreases the activity of xylanases (Pope et al., 2020; Pope and Fahrenholz, 2020).

The use of phytase improved the apparent digestibility coefficient of P and decreased its excretion. Sens et al. (2021) found that the inclusion of 2500 FTU/kg phytase for broiler chickens increased phosphorus digestibility, plasma myoinositol levels, and tibia and toe ashes. Thus, by increasing the digestibility of this mineral, its excretion was reduced, and bone mineral composition was improved. Auereli et al. (2011) observed an increase in phosphorus digestibility at increasing levels of up to 2000 FTU/kg of liquid phytase. Ingelmann et al. (2019) found that the use of 500 FTU/kg of feed increased pre-caecal phosphorus digestibility by approximately 15% in broilers. Therefore, these data support the results found in the present study.

When supplying 750 FTU/kg of phytase to broiler chickens, Martins et al. (2013) observed a reduction of 12.7% in fecal phosphorus excretion without compromising animal performance. However, other studies reported a reduction of 33% (Applegate et al., 2003) and 55% (Laurentiz et al., 2009) in excreted phosphorus. However, in addition to the use of phytase, the authors also used the strategy of reducing dietary phosphorus. Thus, by combining the two strategies, the improvements in the reduction of excretion were significantly higher. However, in the present study, using only a phosphorus reduction strategy, we found a 35.05% reduction in phosphorus at the 500 FTU dose of phytase and a 44.19% at the 500 FTU + 3000 IU xylanase dose compared with the control. Therefore, xylanase also contributed to a decrease of phosphorus excretion in the environment.

Pontes et al. (2021) provided spray phytase in extruded diets for Nile tilapia, and confirmed that supplementation of this enzyme increases energy and nutrient digestibility, including aminoacids, phosphorus, and cationic minerals. Furthermore, the results were dose-dependent, which corroborates those found in our study. Also, the authors pointed out that phytase is a useful strategy to reduce the inclusion of inorganic phosphorus in diets and nitrogen. In this scenario, phytate increases the mucin section in the intestine and this increases the endogenous loss of aminoacids (Selle et al., 2012). In addition, phytate binds to cations such as Ca, Mg, Zn, Fe, and Cu, and the addition of phytase can break this complex and provide greater absorption of these minerals (Adeola and Cowieson, 2011). However, we found no difference in the digestibility of calcium as expected. This could be due to a lower concentration of minerals linked to phytic acid, such as calcium in this case, and also due to the retention time and pH of the gastrointestinal tract that may have influenced the action of the enzyme on the release of minerals. Furthermore, another possible explanation could be the high levels of calcium in the feed, which could have reduced its absorption in the intestine, even with the addition of phytase. Perhaps there was a need to reduce the inclusion of this mineral in the feed so that a difference in its digestibility could be observed.

In addition, most studies that used the two associated enzymes did not demonstrate an improvement in digestibility (Olukosi and Adeola, 2008; Olukosi et al., 2020), unlike what was seen in the present study, in which there was an improvement in the apparent digestibility coefficient of phosphorus and lower phosphorus excretion when using the association of liquid phytase + xylanase after feed pelletization. Therefore, both the use of isolated liquid phytase and that associated with liquid xylanase after pelletization were efficient in improving the use of phytic phosphorus present in the ingredients of plant origin. This fact may have happened because of the effect of temperature and humidity in the pelletization process, which causes expansion of starch granules (Cruz et al., 2020), and thus facilitates access of the enzymes to substrates, such as NSP and phytate.

5. Conclusions

The addition of phytase alone or in combination with xylanase is able to improve the apparent digestibility and reduce phosphorus excretion. Therefore, the use of liquid phytase and liquid phytase + xylanase after pelletization becomes interesting to reduce the inclusion of inorganic phosphorus, which can reduce the feed cost and phosphorus excretion in the environment. Furthermore, applying the enzymes after pelleting is a useful alternative since it prevents denaturation or inactivation of the enzymes.

Author Contributions

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