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The Active Role of Spray-Dried Phytase Produced by *Rhizopus microsporus* var. *microsporus* Biofilm in Feeding Broiler Chickens

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Abstract

Objective: Phytases are enzymes that can catalyze the hydrolysis of phytic acid, and have potential for use in the formulation of broiler chickens' diets. As such, we evaluated the effects of supplementation of broiler chicken diets with a spray-dried phytase derived from the fungal *Rhizopus microsporus* var. *microsporus* biofilm on the animal performance.

Methods: Cobb 500 male chicks were housed for 42 days under controlled environment conditions and provided with three different dietary regimes. These included a control diet meeting nutritional requirements, a diet with phosphorus reduction of 0.175% over days 1-21 and 0.225% over days 22-42, and diets with spray-dried phytase added at 250 FTU/kg, 500 FTU/kg and 750 FTU/kg. Animal performance was assessed based on their body weight, feed intake, weight gain, feed conversion rate, and viability.

Results: At the first phase of development (1-21 days of age), performance was not improved by phytase addition, but at the latter phase (22-42 days of age); performance improved significantly, especially in birds fed phytase at 750 FTU/kg. In addition, phytase promoted an increase in villous height for animals of both 21- and 42 day-old animals, improving nutrient absorption and performance at the end phase of development.

Conclusion: Phytase supplementation positively affected animal development and jejunum morphology, highlighting its potential to be used as an additive for broiler chicken diet formulation.

Keywords: Broiler chickens; Diet formulation; Jejunum morphology; Phosphorus; Phytase; *Rhizopus microsporus* var. *microsporus* biofilm

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Introduction

Phytic acid, or inositol hexaphosphate, is a large phosphate storage molecule in most plants, grains, and oilseed [1], and can be used for feed formulation. Due to its lack of molecular specificity, it has high potential to complex with positively charged molecules such as proteins [2,3], carbohydrates, and amino acids [2,4], as well as with cations (Zn^{+2}, Fe^{+2}, Fe^{+3}, Mg^{+2}, and Ca^{+2}) in the intestine, promoting negative effects on animal development and performance. Physical and chemical methods can be employed to remove the phytic acid from food, but the use of these methods can reduce its nutritional value [5]. Alternatively, the anti-nutritional effects of phytic acid can be minimized by the use of enzymes such as phytases, which can catalyze the hydrolysis of phytate to myo-inositol and inorganic phosphate, especially

when added in the diets of non-ruminants such as pigs, fishes, poultries and humans [5]. Phytases can be applied in numerous fields such as the animal feed industry, the food industry, bread making, production of plant protein isolates, corn wet milling, and fractionation of cereal bran [6].

During the last two decades, phytases have attracted the attention of scientists and entrepreneurs of different areas such as biotechnology, environmental protection and nutrition. Despite the potential application of phytase in different fields, it's most important commercial value is associated with the food and feed industries [5]. Different works have examined the usefulness of fungal phytases for swine and poultry nutrition. Several fungal strains have been used for the commercial production of phytases, such as *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, and *Penicillium funiculosum*. For this purpose, submerged fermentation is the main process for enzyme production, using both wild-type and recombinant strains [5]. The effects of fungal phytase supplementation to feeds on performance and nutrient utilization by poultry, especially chickens, have been reported in different studies [7-9]. Improvement of growth and performance, as well as improvement in the absorption capacity of the intestine and retention of phosphorus, were observed for broiler chickens. Fukuyama et al. found positive effects on different parameters during animal development when a commercial phytase (Phytase 5000) from Ouro Fino (Brazil) was added to diets with reduced amounts of phosphate [10]. This strategy may reduce the cost of animal production and the environmental impact caused by phosphate pollution [5,7,8].

Animal diets have been experimentally supplemented with different phytase levels, in order to achieve the same or better performance of a standard nutritional diet with normal levels of nutrients and to promote the disruption of the maximum P-phytic acid complex and allow better mineral absorption [10-12]. Brazil has an important stake in this effort because of its strong production in the agricultural sector. The poultry industry has experienced intense development in recent years, which makes the search for new sources of phytases with biotechnological properties promising for generating new products for improving animal nutrition. Economic and environmental contexts make the use of phytase increasingly relevant in modern poultry production [13]. However, the use of spray-dried phytase in feed formulation for broiler chickens has not been previously investigated. Sato et al. [14] reported that spray-drying of phytase produced by *Rhizopus microsporus* var. *microsporus* biofilm yielded high enzyme activity. Accordingly, we aimed to investigate the active role of spray-dried phytase (using cornmeal as adjuvant) from the filamentous fungus *R. microsporus* var. *microsporus*, added to a corn and soybean meal diet, on the performance and jejunum morphology of broiler chickens.

Materials and Methods

Microorganism and culture conditions

A record of the filamentous fungus *R. microsporus* var. *microsporus* is deposited in the fungal culture collection of the Laboratory of Microbiology from the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo - USP, Ribeirão Preto, São Paulo, Brazil. Fungal colonies were maintained on potato-dextrose-agar slants held at 4°C. After cultivation, slants were scraped, and the scrapings were added to distilled water to obtain a (10^6 spores/mL) spore suspension for use in producing a fungal biofilm as described by Sato et al. [14]. The *Rhizopus* biofilm was obtained by immersing polyethylene sheets in the spore suspension for 2 h at 30°C under 100 rpm agitation. After spore adhesion, sheets were washed twice with distilled water to remove unadhered spores, then incubated for 96 h at 30°C under agitation 50 rpm in NBRIP modified medium [15], using sugarcane bagasse as a carbon source.

Obtaining the enzymatic fraction

After cultivation, the fungal biofilm was removed from the culture medium and the free cell solution containing the enzyme was used to quantify phytase activity and for spray drying, as described by Sato et al. [14], using a bench-top spray dryer (model SD-05; Lab-Plant, Huddersfield, UK), with a concurrent flow regime. Air was heated to 100°C and injected into the spray dryer, whereas crude extract, in presence of cornmeal as adjuvant, was fed past at a flow rate of 4 g/min. The atomizing air was adjusted at a pressure of 141 kPa and flow rate of 17.0 L/min.

Animals and housing

A total of 1,080 Cobb 500 male chicks, one day old at the beginning of the experiment, were housed for 42 days in an experimental facility with a tunnel-type system to control environmental conditions, in the Poultry sector of the Department of Animal Science of the Faculty of Agriculture and Veterinary Sciences of Jaboticabal, São Paulo, Brazil. Temperature, humidity and air exchange were automatically regulated by exhaust fans and climate control system, according to the age of the animals (Broiler Management Guide COBB 500, 2008). The air temperature values remained between 32.33 ± 0.57°C and 26.66 ± 1.15°C and relative humidity between 84.33 ± 13.21% and 24.66 ± 4.93%. For the period from 1 to 21 days of age, the ideal temperature recommended for breeding of broilers is 32-34°C. In the second week of life, the recommended temperature varies from 28°C to 32°C, and in the third week, from 26°C to 28°C [16]. For older animals, the indicated temperature is between 20°C and 27°C [17].

Chicks were provided ocular vaccination against coccidiosis on the 1st day of life. Water-administered vaccines were given against low strain Gumboro disease at 6 days of age, New Castle disease at 11 days of age, and strong strain Gumboro at 16 days of age. All experimental procedures were approved by the Ethics Committee on Animal Use of the Faculty of Agriculture and Veterinary Sciences of Jaboticabal - UNESP (authorization nº 0184337/13).

Diets and experimental design

All experimental diets were based on corn and soybean meal, and were formulated according to the Brazilian Tables for Poultry and Swine [18]. Diet composition and nutritional levels fed during the starter (1 to 21 days of age) and grower (22 to 42 days of age) phases are presented in **Table 1**.

Table 1 Composition of experimental diets for the phases from 1 to 21 and 22 to 42 days of age of the birds[1].

Item	1-21 days of age			22-42 days of age		
	NC	PC1	PC2	NC	PC1	PC2
Ingredients, %						
Corn	63.050	62.802	62.553	65.095	64.846	64.597
Soybean meal (45%)	32.888	32.939	32.990	29.367	29.418	29.469
Soy oil	0.610	0.694	0.778	2.768	2.851	2.935
Dicalcium phosphate	0.606	0.931	1.256	0.363	0.688	1.013
Limestone	1.543	1.333	1.123	1.337	1.127	0.917
NaCl	0.433	0.433	0.433	0.399	0.400	0.400
DL-Methionine	0.321	0.321	0.322	0.255	0.255	0.255
L-Lysine	0.317	0.316	0.315	0.234	0.233	0.232
L-Threonine	0.122	0.122	0.122	0.072	0.072	0.072
Premix[2,3]	0.100	0.100	0.100	0.100	0.100	0.100
Phytase	-	-	-	-	-	-
Inert	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
Nutritional levels, %						
ME, kcal/kg	3,000	3,000	3,000	3,170	3,170	3,170
Crude protein	21.6	21.6	21.6	20.0	20.0	20.0
Calcium	0.867	0.867	0.867	0.717	0.717	0.717
Phosphorus	0.225	0.285	0.345	0.175	0.235	0.295
Sodium	0.213	0.213	0.213	0.198	0.198	0.198
Potassium	0.794	0.794	0.794	0.735	0.735	0.735
Methionine	0.606	0.606	0.606	0.524	0.524	0.524
Methionine+Cystine	0.902	0.902	0.902	0.804	0.804	0.804
Lysine	1.253	1.253	1.253	1.101	1.101	1.101
Threonine	0.814	0.814	0.814	0.715	0.715	0.715
Tryptophan	0.218	0.218	0.218	0.199	0.199	0.199

[1]PC – positive control (diet meeting the nutritional requirements of the birds); NC – negative control (phosphorus reduction of 0.175% for 1-21 days and phosphorus reduction of 0.225% for 22-42 days). [2]Inicial: folic acid 1,000 mg; pantotenico acid 15,000 mg; antioxidant 0.5 g; niacin 40,000 mg; selenium 300 mg; biotin 60 mg; VB_1 1,800 mg; VB_{12} 12,000 mg; VB_2 6,000 mg; VB_6 2,800 mg; VD_3 2,000,000 IU; VE 15,000 mg; VK_3 1,800 mg; Mn 150,000 mg; Zn 100,000 mg; Fe 100,000 mg; Cu 16,000 mg; I 1,500 mg. [3]Final: folic acid 700 mg; pantotenico acid 13,000 mg; antioxidant 0.5 g; niacin 3,5000 mg; selenium 300 mg; VB_1 1,600 mg; VB_{12} 10,000 mg; VB_2 5,000 mg; VB_6 2,600 mg; VD_3 1,500,000 IU; VE 12,000 mg; VK_3 1,500 mg; Mn 150,000 mg; Zn 100,000 mg; Fe 100,000 mg; Cu 16,000 mg; I 1,500 mg.

All broiler chicks were weighed (± 45 g each) and randomly distributed into each treatment group according to the similarities of their body weight means, described as follows. Six treatments were analyzed, with 6 replicates for each, totaling 36 treatments housed in boxes of 3.0 m^2 with 30 animals per box.

Treatments were applied as follows. **PC** diets, or positive control diet, met the nutritional requirements of the animals. Two positive controls were used: **PC1**, or the basal diet+inorganic phosphate at 0.06%, and **PC2**, or the basal diet+inorganic phosphate 0.12%. **NC** diets, or negative control diets, had phosphorus content reduced by 0.175% for the initial phase (1 to 21 days) and by 0.225% for the final phase (22 to 42 days). **T** diets, or treatment diets, consisted of **T1**, with NC+phytase at 250 FTU/kg; **T2**, with NC+phytase at 500 FTU/kg; and **T3**, with NC+phytase at 750 FTU/kg.

Performance

Performance was assessed at 21 and 42 days of age, taking into account animal weight and leftover feed mass, as well as body weight (**BW**), feed intake (**FI**), body weight gain (**BWG**), feed conversion rate (**FCR**), and viability (**VIA**). Mortality was recorded daily and used to adjust the feed intake calculation and feed conversion ratio as described by Sakomura and Rostagno [19].

Analysis of the intestinal morphology

Jejunum samples from 21- and 42-day-old broilers were collected and stored in formal saline until required for analysis, as follows. After collecting, samples were fixed in Bouin's solution for 4 h, and then added to 10% formalin for 24 h. Then, samples were dehydrated in different alcohol solutions (70%, 80%, 90%, 95%, and 100%) for 1 h each. They were then cleared with xylene and impregnated with paraffin wax. Tissue sections of 4-5 µm depth were cut on a microtome (LUPETEC Modelo MRP2015), fixed on plain glass slides (26 mm × 76 mm), and stained using hematoxylin and eosin for 5 min. Slides were then washed in running water for 5 min and stained with eosin acid dye for 30 s.

Histological preparations were observed using a light microscope (Leica DM5000 B) and imaged using a digital camera (Leica 5000 B). Morphometric analysis was performed using Leica QWin software (Leica Image Processing and Analysis Software, Leica Microsystems Ltd, Heerbrugg, Switzerland). Villus height

measurements, defined as the villus tip to the villus-crypt junction, were obtained from six consecutive intact villi in each sample. Crypt depth was estimated by measuring six crypts per section.

Statistical Analysis

Statistical analysis were performed using SAS version 9.1 [20], using three replicates. Significant effects were determined based on analysis of variance with a $P<0.05$ threshold. Linear and quadratic polynomial regression analyses were carried out, and Tukey's test at 5% probability was performed when significant linear effects were detected.

Results and Discussion

Influence of the spray-dried phytase on broilers' performance

Initial phase of development (1-21 days): For broiler chickens in the initial phase of development (1-21 days), maximum BWG was achieved in animals receiving a diet with an appropriate nutritional level of phosphorus (PC2: inorganic phosphate 0.12%) and consequently high media for body weight (BWG) **(Table 2)**. Addition of different proportions of spray-dried phytase did not yield improvement in the BWG and BW, with animals receiving a phosphorus-deficient diet showing similar values (NC: phosphorus reduction of 0.175%) and differing from the positive controls ($P<0.05$). In addition, no significant differences were observed among the treatments containing phytase at different concentrations (250, 500 and 750 FTU/kg) ($P>0.05$). These results indicate that spray-dried phytase, independent of the proportion used, had no positive effect on animal development during the initial period (1-21 days). Previous researchers have similarly found no significant effect for the addition of phytase in diets on the performance of animals prior to 21 days of age [4,21,22]. Similarly, Murata et al. [23] found that adding 750 and 1,000 FTU of phytase/kg of feed did not yield significant effects on broiler performance during the initial phase. In the same study, no difference in weight gain was observed when 1,500-10,000 FTU of phytase/kg of feed was used in comparison to controls.

In terms of feed intake (FI), animals that received appropriate phosphorus level (PC2: inorganic phosphate 0.12%) showed optimum values for this parameter **(Table 2)**. However, there was no significant difference ($P>0.05$) among the animals fed phytase treatments and those fed positive control diets, or among animals fed phytase treatments and those fed negative control diets. The same was also observed for feed conversion ratio (FCR) parameter, indicating that the spray-dried phytase added to the diet did not affect these parameters. Previous studies [2,4,24-31] have similarly found no significant improvement in feed conversion parameters for broilers fed with phytase dietary supplements. According to Sebastian et al. [4] and Viveros et al. [32], diets with lower nutritional level, especially those deficient in phosphorus content, cause a reduction in the feed intake, which could explain the lower feed intake observed in animals receiving phytase-supplemented diets. In this case, addition of phytase was not able to recover the FI values such that they would be similar to the FI values obtained for animals receiving the appropriate levels of phosphorus (PC2: inorganic phosphate 0.12%) in spite of the absence of statistical significant difference. Reduced feed intake caused reduction in the BWG as also observed for the animals 1-21 days old. Viability was not affected by the addition of inorganic phosphate (controls) or phytase at different concentrations.

Latter phase of development (21-42 days): BWG for animals receiving PC diets was significantly higher than in those receiving NC and NC+250 FTU/kg diets ($P<0.05$). Diet supplementation with 500 FTU/kg and 750 FTU/kg of spray-dried phytase significantly affected BWG and BW in comparison to diets with a low nutritional level of phosphorus (NC) ($P<0.05$). The diet containing 750 FTU of phytase/kg of feed also induced an increase in BWG, similar to that observed in positive controls. In terms of FI, similar effects were observed for the treatments using different phytase concentrations and both positive controls. According to Santos et al. [12], when feed diets were supplemented with phytase, animals showed feed intake and weight gain similar to those of the positive control. In our study, addition of spray-dried phytase promoted a positive effect ($P<0.05$) on FI when compared to NC (with reduced inorganic phosphate amount), indicating its potential to eliminate the negative FI effect of the lower nutritional level of the diet. Addition of 750 FTU of phytase/kg of feed promoted the highest FI. We suggest that addition

Table 2 Effect of spray-dried phytase from *R. microsporus* var. *microsporus* on the performance of broilers at 1-21 days.

Treatments[1]	Body weight (kg)	Body weight gain, kg	Feed conversion	Feed intake, kg	Viability, %
PC2	0.76[a]	0.72[a]	1.54	1.11[a]	98.3
PC1	0.71[a]	0.67[a]	1.58	1.06[ab]	99.4
NC	0.60[b]	0.56[b]	1.63	0.91[b]	100.0
T1	0.61[b]	0.57[b]	1.72	0.98[ab]	97.2
T2	0.61[b]	0.56[b]	1.71	0.97[ab]	99.4
T3	0.60[b]	0.56[b]	1.64	0.92[b]	98.9
MSD	0.04	0.04	0.20	0.14	3.13
F value[2]	55.38**	56.19**	2.15[ns]	5.21**	1.90[ns]
CV, %	3.5	3.7	7.0	8.2	1.8

MSD=Minimal significant difference; CV=Coefficient of variation. a, b, c Values with the same letter for each column do not differ by Tukey test at 5% probability ($P \leq 0.05$). 1PC – Positive control (diet meeting the nutritional requirements of the birds); NC – Negative control (phosphorus reduction of 0.175%); T1-NC+250 FTU/kg; T2-NC+500 FTU/kg; T3-NC+750 FTU/kg. 2 ns=not significant, **=Significant at 1% probability.

of appropriate levels of phytase can overcome the suppressive effect of the poorer nutritional diet by disrupting the P-phytic acid complex, releasing phosphorus for absorption and reducing the negative effect of its deficiency on feed intake [4,10]. The FI values observed for treatments using 250 and 500 FTU of phytase/kg of feed were lower than that observed for positive controls, though not significantly so. This fact could be due to inadequate levels of phytase for achieving maximal hydrolysis of the phytate complex.

The highest FCR value was observed in animals fed a diet supplemented with 750 FTU/kg of spray-dried phytase **(Table 3)**. This result reinforces that an appropriate level of phytase should be used to obtain maximal hydrolysis of phytate. Statistically, no significant difference was observed among the NC, PC and spray-dried phytase treatments. Similar to the initial phase of development, viability of the animals was not affected in the final phase by the addition of phytase, or inorganic phosphate in the positive controls. However, viability (± 84.3%) was reduced in the final phase (21-42 days) in comparison with the initial phase (1-21 days) (± 98.6%).

Overall, the results obtained for the final phase of development differed from those obtained for the initial phase of development. Similarly, Lelis et al. [28] observed that supplementation with 250 and 500 FTU of phytase/kg of feed in diets with reduced nutritional levels improved broiler performance in the final phase of development. Other studies found positive effects of increasing levels of phytase in the diet on growth in broiler performance [21,29]. For example, Santos et al. [30] observed an improvement in the broiler performance using 500, 1000, 1500 FTU/kg in diets with reduced levels of phosphorus and calcium, similar to the results obtained using diets containing normal levels of nutrients in feed. According to Tejedor et al. [21] the optimum amount of phytase inclusion is 500-700 FTU of phytase/kg of feed. Strada et al. [31] added an enzymatic complex in corn-based feed and soybean meal, and found no significant improvements in performance indices for broilers up to 42 days old.

In general, when an improvement of animal performance is observed for broilers receiving diets supplemented with phytase, a multifactorial explanation can be presented, such as the utilization of discharged minerals from the mineral-phytate complex, usage of inositol obtained from phytate hydrolysis, improvement of starch digestibility and protein availability, and impediment of the saponification reaction among lipids and mineral-phytate complex [10]. Additionally, for phytase to be effective, the presence of the specific substrate in the diet formulation as well as its addition at ideal level must be determined.

Influence of phytase supplementation on the morphology of jejunum cells

At 21 days of age **(Figure 1A and 1C)**, villus length increased significantly with the addition of phytase ($P<0.05$), especially in animals receiving 500 FTU of phytase/kg of feed, similar to the values observed under PC treatment **(Table 4)**. Shorter villus length was observed under NC treatment, while crypt length was not affected by the various diets. At 42 days of age **(Figure 1B and 1D)**, there was significant difference in villus length between treatments with reduction of phosphorus content (NC) and PC

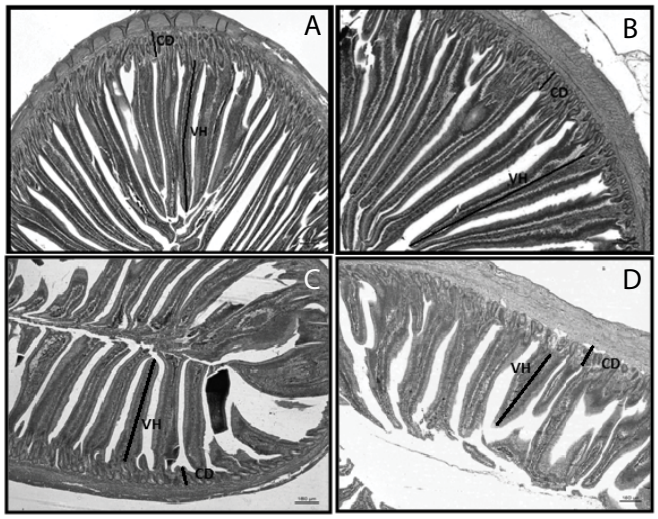

Figure 1 Photomicrograph of the jejunum intestinal cross section from a broiler receiving phytase supplementation (A and B) and negative control (NC) (C and D) at 21 (A and C) and 42 (B and D) days-old broilers. VH=Villous height; CD=Crypt depth. Size measurement 160 µm.

Table 3 Effect of phytase produced by biofilm *R. microsporus* var. *microsporus* on the performance of broilers at 22-42 days.

Treatments[1]	Body weight, kg	Body weight gain, kg	Feed conversion	Feed intake, kg	Viability, %
PC2	2.80^{a}	2.77^{a}	1.25	3.44^{ab}	87.8^{a}
PC1	2.55^{abc}	2.52^{abc}	1.29	3.23^{bc}	89.5^{a}
NC	2.17^{d}	2.12^{d}	1.25	2.64^{d}	87.8^{a}
T1	2.41^{cd}	2.37^{cd}	1.35	3.20^{bc}	82.8^{a}
T2	2.53^{bc}	2.49^{bc}	1.24	3.07^{c}	81.7^{a}
T3	2.69^{ab}	2.65^{ab}	1.39	3.63^{a}	79.5^{a}
MSD	0.26	0.26	2.07	0.34	10.65
F value[2]	14.03**	14.01**	0.18^{ns}	18.22**	2.74*
CV, %	5.77	5.87	8.02	6.07	7.06

MSD=Minimal significant difference; CV=Coefficient of variation. a, b, c, d Values with the same letter for each column do not differ by Tukey test at 5% probability ($P \leq 0.05$). 1PC- positive control (diet meeting the nutritional requirements of the birds); NC- negative control (phosphorus reduction of 0.225%); T1-NC+250 FTU/kg; T2-NC+500 FTU/kg; T3-NC+750 FTU/kg. 2ns=not significant, *=Significant at 5% probability, **=significant at 1% probability.

Table 4 Measurements of villous height (µm) and crypts (µm) height obtained from broilers slaughtered at 21 and 42 days of age receiving different dietary phytase treatments.

Treatments[1]	Villous		Crypts	
	21 days	42 days	21 days	42 days
PC2	512.27[a]	750.75[a]	87.61	82.82[ab]
PC1	491.02[a]	670.85[ab]	102.56	78.55[ab]
NC	397.82[b]	607.39[b]	92.63	77.73[b]
T1	467.95[ab]	680.35[ab]	103.14	95.50[a]
T2	524.93[a]	700.82[ab]	85.47	84.23[ab]
T3	495.99[a]	596.32[b]	97.60	86.48[ab]
MSD	90.19	131.65	25.27	17.22
F value[2]	4.81**	3.70*	1.67[ns]	2.67*
CV (%)	10.53	11.09	14.98	11.50

MSD=Minimal significant difference; CV=Coefficient of variation. a, b Values with the same letter for each column do not differ by Tukey test at 5% probability (P ≤ 0.05). 1PC - positive control (diet meeting the nutritional requirements of the birds); NC - negative control (phosphorus reduction of 0.175% for 1-21 days and phosphorus reduction of 0.225% for 22-42 days); T1-NC+250 FTU/kg; T2-NC+500 FTU/kg; T3-NC+750 FTU/kg. 2 ns=not significant, *=significant at 5% probability, **=significant at 1% probability.

treatment (P<0.05). Addition of 250 and 500 FTU of phytase/kg of feed also promoted an increase in villus height compared with the NC treatment, although not significantly so. Depth of the crypts under supplementation with 250 FTU of phytase/kg of feed was greater than that obtained under the NC treatment, but this was also not statistically significant.

It seems likely that improved performance resulted from the increased digestibility of nutrients based on a positive effect of phytase on the morphology of the intestine, with increased the villi height and crypt depth. According to Smulikowska et al. [33], morphological analysis of the mucosa of the small intestines of chicks after phytase supplementation indicates increases of villus height and crypt depth. The surface of the small intestine contains finger-like projections known as villi, which increase the surface area and therefore increases its absorptive capacity (Kadhim et al.) [34]. Phytase supplementation in poultry diets breaks the phytate-mineral complex to release minerals for absorption, which reduces mineral excretion and improves digestibility, as well as reduced intestinal stress (Sebastian et al.) [4].

There are few studies reporting the effect of phytase on intestinal morphology of animals. According to Pirgozliev et al. [35], the supplementation of feed with *Escherichia coli*-derived phytase did not affect morphometry of ileal villi of broiler chickens. This was further demonstrated by Langhout et al. [36] and Pirgozliev et al. [37]. However, Emami et al. [38] reported an increase in villus height and villus height/crypt depth ratio in the duodenum and jejunum, with decreased jejunum crypt depth, under a maize/soybean-based diet poor in available phosphorus but supplemented with phytase. The addition of 1,000 units of dietary phytase and citric acid stimulated villus and crypt modifications, suggesting an increased rate of nutrient absorption and a reduced rate of enterocyte cell migration from crypts to villi [39]. Smulikowska et al. [33] reported that jejunum and ileum villi were shorter, and crypt depth was greater, in chickens fed with diets with lower level of dietary phosphorus compared to diets with normal phosphorus levels. Organic acids further suppressed jejunum villus height, but phytase supplementation was able to increase both villus height and crypt depth. Similar to our study, Wu et al. [40] also observed an increase in villus height in the duodenum of broilers given phytase supplementation. They suggested that the effects of exogenous phytase on the morphology of the gastrointestinal tract may have contributed to improved animal performance. This suggests that the phytase from *Rhizopus microsporus* var. *microsporus* contributed to a good performance of broilers.

Conclusion

Supplementation with spray-dried phytase from *R. microsporus* var. *microsporus* promoted an improvement in the performance of animals at the final phase of development, especially when 750 FTU of phytase/kg of feed was used, but did not have effect on chickens in the initial phase of development. Phytase supplementation also positively affected jejunum morphology by increasing villus height, allowing better nutrient absorption and consequently improving performance. The spray-dried phytase from *R. microsporus* var. *microsporus* presented good biotechnological potential for application in broilers' diets, and future studies can potentially develop its use for industrial employment.

Conflict of Interest

All authors declare that they have no conflict of interest.

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