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Research Article

Efficacy of Two Bacterial Phytases in the Release of Phosphorus in Diets for Growing Broilers

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Abstract

The efficacy of two phytases (*Citrobacter braakii* and *Escherichia coli*) used in different doses (500 and 1,000 FTU) were evaluated in a diet deficient in phosphorus (0.15% available P) formulated with sorghum-soybean meal. To estimate the efficacy of the release of P evaluated phytases, inorganic phosphorus supplemented by mono-phosphate (MDP) to reach a final concentration of available P of 0.23, 0.31 and 0.39% was used as reference. The basal diet deficient in P was supplemented as follows: 1) Without phytases, without MDP; 2) 0.08% MDP; 3) 0.16% MDP; 4) 0.24% MDP; 5) *Citrobacter*-500 FTU; 6) coli FTU-500; 7) *Citrobacter*- 1,000 FTU and 8) FTU coli-1,000. The treatments were applied to Ross 216 chickens (27 birds/treatment) 7 to 21 d old. Weight gain, feed efficiency and concentration of tibia ash and P was better in chickens receiving 0.39% PMD and 1,000 FTU of both phytase, with no differences in these levels between MDP and phytase treatments. The amount of phosphorus released was not affected (P > 0.05) by type of phytase (average 0.142%), with effect to level of phytase used (0.102 vs 0.182% for 500 and 1,000 FTU respectively). The results indicate that supplementation 1,000 FTU/kg in both phytase in diets deficient in P results in weight gain and feed efficiency similar when the diets are supplemented with 0.24% MDP. Both phytases improved to 78.4% utilization of phytate phosphorus in sorghum-soybean meal diets on growing chicks.

***Keywords**: Phytases; Citrobacter braakii; Escherichia coli; Phosphorus tibia; Tibia ashes; Broilers*

Introduction

Phosphorus is an important element to sustain the production of food of animal origin, the world reserves of phosphates are limited and in the future it will be a problem to obtain sufficient quantities to provide this compu this to the animal feed industry, resulting in a challenge to sustain this demand [1,2].

For the poultry sector this implies that the use of phosphorus by animals must be explored for a maximum use of this mineral in diets. This includes further studies on the use of additives, especially phytases as a tool in efficiency to extract more phosphorus in plant-based diets, as 50 to 85% of the phosphorus stored in grains is bound to phytic acid and its salts [3,4].

The use of phytases in the feed industry has been favored by the attractiveness of their prices, the improvement in the use of phytic phosphorus, in the increase in animal yield (meat and bone) and the decrease in the degree of environmental damage (less elimination of phosphorus to the environment through excreta) [5].

Phytases release phosphorus from phytic phosphorus, and as a result of the destruction of this anti-nutritional factor, they allow to reduce the use of inorganic phosphorus sources in the diet [6]. As a result, the use of phytases improves the digestibility not only of phosphorus and divalent cations such as Ca, Mg and Zn, but also of energy and protein [7].

At present, recent phytases of bacterial origin provide greater stability at low pHs and withstand high temperatures to which poultry feed is subjected without losing its properties. Among the bacteria used to obtain phytases, those from *Escherichia coli* and *Citrobacter braakii* [8,9] standout. Each phytase offers benefits in its use and there are even different presentations of the commercial product.

A dose equivalent to 500 FTU/kg of *E. coli* releases 20 to 30% more nutrients than the same dose of an *Aspergillus* spp phytase, and its effect is more consistent due to its pH and greater stability during pepsin digestion in the intestinal tract [10].

Using a high concentration of phytase (megadoses) in diets, in search of a rapid reduction of the concentration of phytate in the stomach or proventricle, thus reduces the anti-nutritional effect of phytate, and increases even plus the release of phosphorus [11]. In this way the maximum destruction of the phytate is achieved, in such away that the type of phytases, dosage levels and the phytate content of the diet will have an important role in the productive response [12]. Recent studies indicate that elevated levels of phytase (megadoses) in the diet of chickens (1,000 and 2,000 FTU/kg) improve the release of phosphorus (0.15%) and calcium (0.18%) and increase productive parameters [9].

With this background, the present study was proposed to know the release of phosphorus in relation to monodicalcium phosphate in sorghum + soy diets for growing chickens deficient in phosphorus available, through the supply of two sources of phytases (*Citrobacter braakii* and *Escherichia coli*) at different doses (500 and megadoses of 1,000 FTU/kg).

Materials and Methods

The experiment was carried out in a booth of natural environment, located in the Center for Teaching, Research and Extension in Poultry Production of the National Autonomous University of Mexico. We used 216 male chickens of the seven-day-old Ross 308 lineage, distributed in eight treatments with three repetitions of nine birds cada one (27 animals per treatment). The chickens were housed in cage floors in Petersime-type batteries, with electric heating and temperature regulated with thermostat. Food and drinking water were offered free access. Chickens from 1 to 6 days of age, were fed sorghum-soy diets, formulated to cover all the nutritional recommendations of the lineage. At seven days of age they were distributed completely to the tsar in eight treatments:

1) Basal diet sorghum-soy, deficient in phosphorus available (0.15%).
2) As 1 + 0.08% inorganic phosphorus from monodygalic phosphorus (FMD).
3) Like 1 + 0.16% inorganic phosphorus from FMD.
4) Like 1 + 0.24% inorganic phosphorus from FMD.
5) As 1 + 500 FTU* of microbial phytase of *Citrobacter braakii*.
6) As 1 + 500 FTU of microbial phytase of *Escherichia coli*.

7) As 1 + 1,000 FTU of microbial phytase of *Citrobacter braakii*.

8) As 1 + 1,000 FTU of microbial phytase of *Escherichia coli.*

*FTU= amount of enzyme needed to release 1 μmol of inorganic phosphorus per minute at a pH of 5.5 to 37°C.

For the estimation of the efficacy in the release of P, inorganic phosphorus (Pi) supplemented with monodycalcium phosphate (FMD) was used as a reference to achieve a final concentration of available Pi of 0.23, 0.31 and 0.39% (treatments 2, 3 and 4). The basal diet covers all requirements with the exception of Pi (Table 1); the additions of FMD and phytases were replacing cellulose in the rodete as an experimental unit. The phosphorus content and ash percentage in tibias were analyzed as a completely random design (SPSS for Windows version 17.0). The bioavailability of phosphorus was estimated with standard curves through simple first-degree linear regressions (Y=βo+β1x), using an SSPS computer package version 17.0(14), from the percentage content of phosphorus and ash in tibias. For the standard curve, treatments 1, 2, 3 and 4 were used. For the *Citrobacter braakii* curve the treatments 1, 5 and 7. Finally for the *Escherichia coli* were treatments 1, 6 and 8. Diet basal for No alter the balance nutritional of the different experimental diets.

Ingredient	Medical history
Sorghum	627.06
Soy paste	327.13
Calcium carbonate	22.91
Soybean oil	6.65
L-Lysine HCl	4.80
Will	3.80
DL-Methionine	3.30
Cellulose Choline Chloride 60% Vitamin Premix* Mineral Premix** Zinc Bacitracin 10% Antioxidant***	1.40 1.00 1.00 0.50 0.30 0.15
Total	1000.00
Calculated analysis	
Metabolizable energy, Kcal/kg	2950
Crude protein, %	22.00
Determined non-phytic available phosphorus, %	0.15
Total calcium, %	1.02
Methionine + cystine digestible, %	0.92
Lysine digestible, %	1.39
Threonine digestible, %	0.82
Sodium, %	0.18

Table 1: *Composition of the experimental basal diet for chickens from 7 to 21 days of age.*

**Premezcla of vitamins per kg: Vitamin A 12'000,000 IU; vitamin D32'500,000 IU; vitamin E 15,000 IU; vitamin K3 2,000 mg/kg; vitamin B1 2,250 mg/kg; vitamin B2 7,500 mg/kg; vitamin B3 45,000 mg/kg; vitamin B5 12,500 mg/kg; vitamin B6 3,500 mg/kg; vitamin B12 20 mg/kg; folic acid 1,500 mg/kg; biotin 125 mg/kg.*

***Mineral premix per 0.5 kg: iodine 300 mg/kg; selenium 200 mg/kg; cobalt 200 mg/kg; iron 50,000 mg/kg; copper 12,000 mg/kg; zinc 50,000 mg/kg; manganese 110,000 mg/kg.*

****BHT (1.2%), BHA (9.0%), Etoxiquin (4.8%).*

Feed consumption was recorded per replica (for every nine chickens) and daily weight gain was recorded individually on a Torrey Mod Brand electronic scale. L-EQ 5/10. At 21 days of age, 60% of the chickens were slaughtered with the method indicated in the NOM-033-ZOO-1995 standard, by means of the safe fastening of the animal and immediately the cervical dislocation in a single movement to avoid unnecessary pain; the left tibia was drawn to determine the percentage content of ash and phosphorus (according to the AOAC, 2006) [13] respectively.

Growth, consumption and feed efficiency data were analyzed.

To know the percentage of inorganic phosphorus released by the enzymes used, the standard curves of percentage of ash and phosphorus in tibias were used and the values obtained with the phytase of the next model (X=(βo–Y)/β1). Where: X= percentage of Pi released by the enzyme, βo=ordered to origin, Y=weight gain, feed efficiency, percentage of ash in tibias and percentage of phosphorus in tibias and β1 = regression coefficient. The comparison of means with the Tukey test was also carried out, considering a significant difference when P < 0.05.

Results

The average results of weight gain, feed efficiency and percentages of ash and P in tibias in 14 days of experimentation, are shown in table 2. It can be seen that phosphorus supplementation from FMD or phytase supplementation improved (P < 0.05) the productive variables by increasing the phosphorus content in the diets and by including the phytases. It is also seen that the percentages of ash and phosphorus in tibias with the two bacterial phytases, released similar amounts (P > 0.05) of Pi in both the 500 FTU and the 1,000 FTU. The percentage content of ash and phosphorus in tibias, was used to calculate therelease of phosphorus obtained with phytases in relation to monodycalcium phosphate.

Treatments	Weight gain (g)	Feed efficiency (kg:kg)	Ash in tibias (%)	Phosphorus in tibias (%)
1	586 ± 11.0^{a}	0.69 ± 0.001^{a}	42.78 ± 0.52^{a}	7.07 ± 0.10^{a}
2	649 ± 10.1^{from}	0.71 ± 0.001^{d}	46.45 ± 0.46^{b}	7.29 ± 0.11^{b}
3	713 ± 9.9^{b}	0.73 ± 0.001^{b}	48.62 ± 0.49^{c}	7.95 ± 0.09^{c}
4	737 ± 10.7^{c}	0.76 ± 0.001^{c}	48.87 ± 0.51^{d}	8.00 ± 0.09^{d}
5	673 ± 10.8^{from}	0.72 ± 0.001^{d}	46.60 ± 0.48^{b}	7.51 ± 0.07^{b}
6	668 ± 10.9^{from}	0.72 ± 0.001^{a}	46.50 ± 0.49^{b}	7.43 ± 0.08^{b}
7	705 ± 10.6^{b}	0.75 ± 0.001^{c}	48.71 ± 0.47^{c}	7.82 ± 0.09^{c}
8	684 ± 11.5^{b}	0.74 ± 0.001^{bc}	48.15 ± 0.45^{c}	7.91 ± 0.09^{c}
EEM	± 10.68	± 0.001	± 0.48	± 0.09

Table 2: *Average results of productive yield and percentages of ash and inorganic phosphorus in warmths of chickens 7-21 days of age.*

The results of the percentage of ash in tibias showed a linear response to the different levels of phosphorus from FMD added in the diet (P < 0.05). The percentage of ash increased linearly (ash,% = 43,993 + 24,429 ×%Pi added, R2 = 0.34) as the percentage of phosphorus in the diet increased (Figure 1). By transposing into the equation the percentage of ash in tibias, the dose of 500 FTU of the phytases of *Citrobacter braakii* and *Escherichia coli* was obtained on average a release of 0.105%. However, for the inclusion of 1,000 RETUs from both phytase sources, phosphorus release was found to be 0.180%.

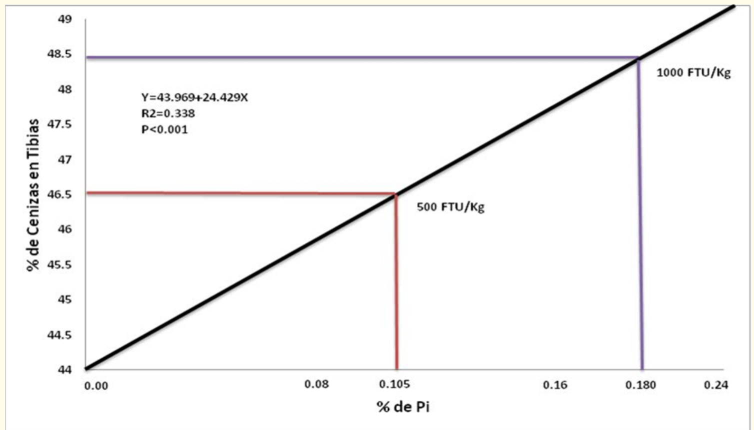

Figure 1: *Percentages of phosphorus released based on ash content in tibias with 500 and 1,000 FTU/kg of Citrobacter braakii or Escherichia coli phytases.*

When analyzing the amount of phosphorus deposited in the tibias, it was determined that the basal diet (without added Pi), obtained the lowest percentage of phosphorus (7.07%), which graduallycreed with increasing the amount of Pi in the diet (P in tibia, % = 7.062 + 4.28 ×% Pi added), and the treatment 4 obtained 8.0% phosphorus in the tibia (Table 2). Similarly, the addition of phytase to the basal diet increased the percentage of P in the tibias.

When transposing into the equation the percentage of Pi in tibias, it was observed that when adding 500 FTU/kg of the two phytases (*Escherichia coli* and *Citrobacter braakii*) there was an average phosphorus release of 0.097%. On the other hand, increasing the dose to 1,000 FTU/kg of both phytase sources increased the amount of phosphorus released by an average of 0.185% (Figure 2).

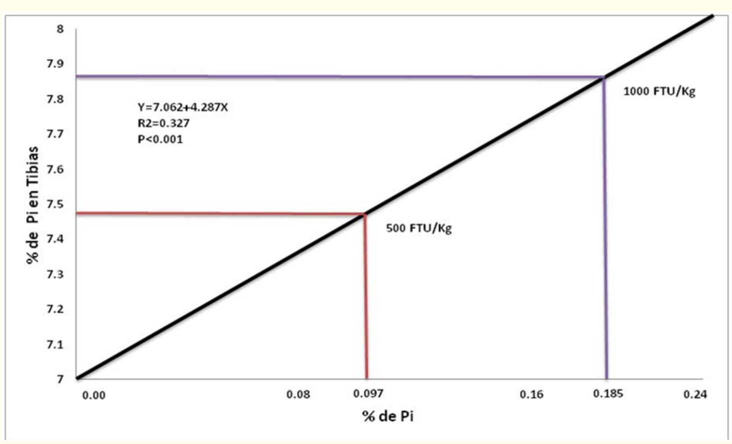

Figure 2: *Percentages of phosphorus released based on phosphorus content in tibias with 500 and 1,000 FTU/kg of Citrobacter braakii or Escherichia coli phytases.*

When averaging the release values of P in the percentage ash content and P in the tibias, with the two phytases, the amount released was 0.102% with 500 FTU/kg and 0.182% for 1,000 FTU/kg of feed in sorghum-soy diets.

Discussion

The main purpose of supplementing phytases in diets for birds is to improve the use of phytic phosphorus and reduce the amount of phosphate in the diet, since phytase hydrolyzes phytate and releases inorganic phosphorus, which is used by the bird to cover the requirement of this mineral.

In thisstudy, weight gain was improved as the amount of Pi in the diet deficient in this mineral increased; this effect was similar to that found by other authors [15], who evaluated this parameter with different levels of Phosphorus in the diet (less than 0.45% Pi), the birds had a higher consumption of feed and therefore phosphorus, which resulted in greater weight gain when approaching the requirement of this mineral. Driver., *et al.* [16] conducted a study, using phosphorus-deficient diets (less than 0.42%) in chickens aged 14 and 28 days, and production parameters improved when birds had higher dietary phosphorus intake.

The biological optt level of phosphorus available in the diet for maximum weight gain in the period 1 to 21 days of age suggested by Julian and Summers [17] and Perney., *et al.* [18], is0.46%. This result is similar to the 0.45% suggested by the NRC [19], but13.3% higher than the P used as the maximum level in this experiment. Other authors [20] recommend 0.5% as the optimal, however they used phosphate rock that perhaps had a lower phosphorus availability. The gain and efficiency results for the maximum levels of P supplemented in our experiment are in line with those expected for the type of chicken used when fed without restriction. in P in the diet.

Olukosi and Fru-Nji [8] evaluated three phytase niveles of *Citrobacter braakii* (0, 1,000 and 2,000 FTU) in chickens for 14 days in corn-wheat-soy diets with the phytase matrix in the diet and another diet without the phytase matrix, with two ratios of Ca:P (2:1 and 2.5:1). The results indicated that there was only a significant effect on the use of phosphorus, calcium and energy, by adding both doses of phytases in diets without the phytase matrix, and also with beneficial effect on diets containing a ratio of calcio:phosphorus of 2:1. In a similar study [9] they report better results for weight gain, feed efficiency; as well as ash, phosphorus, calcium, magnesium, potassium and zinc in tibias, when supplementing 1,000 and 2,000 FTU/kg of phytase in the diet, without any difference between the doses used.

In a meta-analysis work with articles published up to 2013, where information was collected from 286 articles on the retention of phosphorus in broilers, they summarized that phytase supplementation (1,039 FTU/kg diet) for broilers increased phosphorus retention by 0.086% [21].

Reducing inorganic phosphorus levels in the diet decreases the percentage of ash in tibias, this is because 80% of the body's total phosphorus is deposited in bone tissue [22].

The results showed that the addition of microbial phytases from *Citrobacter braakii* or *Escherichia coli* similarly improved the bioavailability of phosphorus from phytic phosphorus from soy paste and sorghum; Similar results report that the use of 500 FTU/kg was effective in improving growth, ash in tibias and phosphorus retention in broilers, with the release of 0.08% of phosphorus available [23]. Augspurger., *et al.* [10] when evaluating 500 FTU/kg of E phytase *E. coli* in diets for chickens mention releases of 0.125% phosphorus.

In the present study, both phytases were found to release similar amounts of phosphorus, and phytase when used in high doses has been shown to improve productive performance [24]. However, Cortes., *et al.* [4] found greater weight gain, feed conversion and apparent metabolizable energy in chickens from 1 to 19 days of age with the use of phytase from *Peniophora lycii*, in addition to allowing to reduce diet (sorghum-soy) 0.1% phosphorus without affecting productive performance.

It is also mentioned [11] that when evaluating a dose of 500 FTU of a microbial phytase in diet as with 0.22% of Pi, they obtained an increase in weight gain, and mineralization in tibias; by increasing the dose to 1,000 FTU the amount of Phosphorus that released the enzyme increased by 0.07%, obtaining results very similar to those found in the present study. The same authors used a third dose of microbial phytase of 2,000 FTU with the same amount of phosphorus in the diet (0.22%) without obtaining significant differences between this dose and the utilization of 1,000 FTU.

In this study, phosphorus release from phytate was increased by 78.4% by increasing the dose from 500 FTU to 1,000 FTU for both phytases from *Citrobacter braakii* and *Escherichia coli*. To achieve maximum hydrolysis of phytic phosphorus, dosage levels and dietary phytate content will play an important role in the productive response [25].

Conclusion and Implications

With the results obtained in the present study, it can be concluded that the use of both *Citrobacter braakii* or *Escherichia coli* phytases at the dose of 500 FTU/kg released 0.102% phosphorus and with the inclusion of 1,000 FTU/kg (megadoses) released 0.182%, equivalent to 78.4% more approving of phytic phosphorus in sorghum-soy diets for growing chickens.

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Volume 7 Issue 3 March 2022