



## Available phosphorus levels in diets supplemented with phytase for male broilers aged 22 to 42 days kept in a high-temperature environment

Tarciso Tizziani<sup>1</sup>, Rita Flávia Miranda de Oliveira Donzele<sup>2</sup>, Juarez Lopes Donzele<sup>2</sup>, Eric Márcio Balbino<sup>3</sup>, Amanda Dione Silva<sup>1</sup>, Érika Martins de Figueiredo<sup>1</sup>, Jorge Cunha Lima Muniz<sup>1</sup>, Rodrigo de Freitas Jacob<sup>1</sup>

<sup>1</sup> Universidade Federal de Viçosa, Programa de Pós-graduação em Zootecnia, Viçosa, MG, Brasil.

<sup>2</sup> Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil.

<sup>3</sup> Universidade Federal do Recôncavo Baiano, Cruz das Almas, BA, Brasil.

**ABSTRACT** - This study was conducted to evaluate the effect of reduction of the available phosphorus (avP) in diets supplemented with 500 FTU/kg phytase on performance, carcass characteristics, and bone mineralization of broilers aged 22 to 42 days kept in a high-temperature environment. A total of 336 Cobb broilers with an average initial weight of 0.883±0.005 kg were distributed in a completely randomized design with six treatments — a positive control (0.354 and 0.309% avP without addition of bacterial phytase for the phases of 22 to 33 and 34 to 42 days, respectively), and another five diets with inclusion of phytase (500 FTU) and reduction of the level of avP (0.354, 0.294, 0.233, 0.173, and 0.112%; and 0.309, 0.258, 0.207, 0.156, and 0.106% for the phases of 22 to 33 and 34 to 42 days, respectively) — eight replicates, and seven birds per cage. The experimental diets were formulated to meet all nutritional requirements, except for avP and calcium. Birds were kept in climatic chambers at a temperature of 32.2±0.4 °C and air humidity of 65.3±5.9%. Phytase acted by making the phytate P available in diets with reduction in the levels of avP, keeping feed intake, weight gain, feed:gain, and carcass characteristics unchanged. Treatments affected ash and calcium deposition and the Ca:P ratio in the bone; the group fed the diets with 0.112 and 0.106%, from 22 to 33 and 34 to 42 days of age, respectively, obtained the lowest values, although the phosphorus deposition in the bone was not affected. Diets supplemented with 500 FTU of phytase, with available phosphorus reduced to 0.173 and 0.156%, and a fixed Ca:avP ratio of 2.1:1, meet the requirements of broilers aged 22 to 33 and 34 to 42 days, respectively, reared in a high-temperature environment.

Key Words: bone mineralization, carcass characteristics, heat stress, performance, thermal environment

### Introduction

Broiler diets consist basically of plant-derived ingredients. These feeds are characterized by having a large part of phosphorus in the form of phytate (Rostagno et al., 2011), which is poorly hydrolyzed by monogastric animals (Ravindran et al., 1995), which do not have a significant activity of enzymes such as phytase throughout the gastrointestinal tract.

However, in addition to complexing with phosphorus, the phytate molecule is also able to bind to other minerals and amino acids, forming insoluble complexes, which consequently decreases its availability and use and increases its excretion (Selle et al., 2009; Khalid et al., 2013). Because of these anti-nutritional effects, supplementation

with an inorganic source of phosphorus is necessary so that the requirements of animals for both growth and bone development are properly met. Based on that, the use of exogenous enzymes in diets has been proposed aiming to reduce the supplementation of inorganic sources and improve the use of the dietary nutrients.

Phytases are phosphatases that can hydrolyze one or more groups of phosphate from the phytate molecule (Tamim et al., 2004), and their activity varies according to the pH, moisture, temperature, presence of certain minerals such as calcium and other enzymes, besides the passage time of the digesta. Their activity is expressed as FTU or U/kg, which corresponds to the amount of enzymes necessary to release one micromole of inorganic phosphorus in one minute into a substrate of phytate sodium, at a temperature of 37 °C and pH of 5.5 (Lelis et al., 2010).

Another decisive factor in broiler farming concerns the environmental conditions in which these birds are reared, given that they are highly sensitive to heat; the climate in Brazil being predominantly hot characterizes a problem for poultry farming, especially as the birds age. Birds subjected to a heat stress environment reduced phosphorus absorption

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Corresponding author: tarciso@zootecnista.com.br

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(El-Husseiny and Creger, 1981) and retention (Belay et al., 1992). Moreover, under unfavorable rearing conditions at high temperatures, the phosphorus requirement may be altered (Persia et al., 2003). Additionally, decrease has been reported in feed digestibility after heat exposure (Bonnet et al., 1997), which might contribute to a decrease in the amounts of nutrients available for growth. Therefore, phytase can improve growth performance, bone quality, and P utilization in broiler chickens (Shang et al., 2015) under these conditions.

Considering that little research has focused on interactions between heat stress and low dietary phosphorus (Persia et al., 2003) and phytase in chicks, this subject needs to be further evaluated.

In view of the above considerations, the objective of this study was to evaluate the effect of diets with reduction in the levels of available phosphorus and calcium (keeping the Ca:avP fixed at 2:1) and inclusion of phytase on performance, yield of carcass and prime cuts, and concentrations of ash, calcium and phosphorus in the bones of broilers aged 22 to 42 days of age kept in a high-temperature environment.

## Material and Methods

The experiment was conducted in Viçosa/MG, Brazil.

The procedures adopted in this study were previously approved by the Animal Use Committee of Universidade Federal de Viçosa (27/2012), complying with the ethical principles of animal experimentation defined by Colégio Brasileiro de Experimentação Animal (COBEA, 1991).

A total of 336 male broilers of the Cobb strain with an initial weight of  $0.883 \pm 0.005$  kg, vaccinated against Marek's disease, aged 22 to 42 days of age, were housed in an environmentally regulated room for the experiment.

During the pre-starter period (1 to 7 days) and starter period (8 to 21 days of age), birds were reared in a conventional shed, and fed a diet formulated to meet or exceeded their respective nutritional requirements according to Rostagno et al. (2011).

Upon completing 22 days of age, broilers were weighed and transferred to the climatic chambers, where they were distributed and housed in  $0.72\text{-m}^2$  cages with screened floor, provided with gutter-type feeders and drinkers. The rooms were air-conditioned with air temperature and relative humidity that represented a high-temperature condition for the birds during the experimental period.

The temperature and relative air humidity inside the rooms were monitored twice daily (07.00 h and 18.00 h), using dry- and wet-bulb thermometers and a black-globe

thermometer installed in the center of the rooms. These data were later utilized to calculate the black globe-humidity index (BGHI), as proposed by Buffington et al. (1981). During the experimental period, the temperature and relative air humidity remained at  $32.2 \pm 0.42$  °C and  $65 \pm 5.9\%$ , respectively, and the black globe-humidity index (BGHI) calculated in the period was  $83 \pm 1.0$ , characterizing the heat stress environment in which the birds were maintained (Cobb-Vantress, 2008; Lator et al., 2008).

The adopted lighting program was continuous (24 h of artificial light), using three 45-W fluorescent bulbs per room. Birds were distributed in a completely randomized experimental design with six treatments — a positive control (0.354 and 0.309% of available phosphorus without addition of phytase for the phases of 22 to 33 and 34 to 42 days of age, respectively), and another five diets with inclusion of bacterial phytase Quantun Blue® (500 FTU/kg of diet) and reduction of the level of available phosphorus (0.354, 0.294, 0.233, 0.173, and 0.112%; and 0.309, 0.258, 0.207, 0.156, and 0.106 for the phases of 22 to 33 and 34 to 42 days of age, respectively) (Tables 1 and 2) — eight replicates, and seven birds per cage. The experimental unit was represented by the cage.

The basal diet, composed of corn and soybean meal, was formulated to meet the nutritional requirement of the birds, according to recommendations of Rostagno et al. (2011), except for available phosphorus and calcium. The treatments were obtained by removing the dicalcium phosphate and limestone from the diets in substitution of the inert, keeping the Ca:avP ratio fixed. Water and the diets were available *ad libitum* to the animals during the entire experimental period. The diet supplied, waste, and orts were weighed periodically throughout the experiment, and the values were utilized for the calculation of feed intake (FI). To determine weight gain (WG), the birds were weighed at the beginning (22 days) and end of the experiment (42 days of age). Feed:gain was calculated based on the data of feed intake and weight gain.

At the end of the experimental period (42nd day), the birds were weighed and four birds from each experimental period, with weight close to the average of the cage ( $\pm 10\%$ ), were subjected to a feed-deprivation period of 12 h. Subsequently, the birds were stunned by an electric shock (60 V) and slaughtered by bleeding by jugular venipuncture. After bleeding, these birds were plucked and eviscerated. The two birds with the weight closest to the average were weighed to determine the relative carcass weight. To calculate the relative weight of the prime cuts (breast, drumstick, and thigh), the weight of the whole carcass (including head and feet) was considered. The

other birds had their tibia removed, forming a pool of two samples per replicate, which were frozen for subsequent laboratory analyses.

Table 1 - Composition and nutritional values of experimental diets for 22 to 33 days and 34 to 42 days<sup>1</sup>

| Item (kg/100 kg)                     | 22-33 days | 34-42 days |
|--------------------------------------|------------|------------|
| Corn                                 | 56.35      | 60.08      |
| Soybean meal                         | 35.33      | 31.92      |
| Soybean oil                          | 4.87       | 4.88       |
| Dicalcium phosphate                  | 1.31       | 1.10       |
| Limestone                            | 0.89       | 0.80       |
| Inert <sup>2</sup>                   | 0.06       | 0.06       |
| Salt                                 | 0.46       | 0.45       |
| DL-methionine                        | 0.27       | 0.24       |
| L-lysine HCl                         | 0.15       | 0.16       |
| L-threonine                          | 0.03       | 0.03       |
| Mineral mix <sup>3</sup>             | 0.05       | 0.05       |
| Vitamin mix <sup>4</sup>             | 0.03       | 0.03       |
| Anticoccidial <sup>5</sup>           | 0.05       | 0.05       |
| Choline chloride                     | 0.13       | 0.13       |
| Antibiotic <sup>6</sup>              | 0.01       | 0.01       |
| Antioxidant <sup>7</sup>             | 0.01       | 0.01       |
| Total (kg)                           | 100.0      | 100.0      |
| Nutritional composition <sup>8</sup> |            |            |
| Metabolizable energy (kcal/kg)       | 3150       | 3200       |
| Crude protein (%)                    | 20.74      | 19.49      |
| Digestible methionine+cystine (%)    | 0.826      | 0.774      |
| Digestible lysine (%)                | 1.131      | 1.060      |
| Digestible threonine (%)             | 0.735      | 0.689      |
| Digestible valine (%)                | 0.882      | 0.827      |
| Digestible isoleucine (%)            | 0.813      | 0.757      |
| Sodium (%)                           | 0.200      | 0.195      |

<sup>1</sup> Treatments were achieved through the withdrawal of dicalcium phosphate and limestone in the diets replacing the inert, with a fixed Ca:avP ratio.

<sup>2</sup> Washed sand.

<sup>3</sup> Quantity per kg of product: vit. A - 5,600,000 IU; vit. D3 - 1,200,000 IU; vit. E - 10,000 IU; vit. B1 - 1,550 mg; vit. B2 - 4,000 mg; vit. B6 - 2,080 mg; pantothenic acid - 10,400 mg; vit. K3 - 1,200 mg; folic acid - 650 mg; niacin - 28,000 mg; vit. B12 - 8,000 µg; selenium - 300 mg; antioxidant - 0.50 g.

<sup>4</sup> Quantity per kg of feed: manganese - 150,000 mg; zinc - 140,000 mg; iron - 100,000 mg; copper - 16,000 mg; iodine - 1,500 mg.

<sup>5</sup> Salinomycin sodium - 60 ppm.

<sup>6</sup> Avilamicyn - 10 ppm.

<sup>7</sup> Butylated hydroxytoluene (BHT).

<sup>8</sup> According to Rostagno et al. (2011).

After thawing, the tibia were stripped out of the surrounding flesh and pre-dried in an oven at 65 °C for 72 h. Next, the samples were partially defatted in a Soxhlet extractor by the method described by Silva and Queiroz (2002) and dried in an oven at 65 °C. These were later ground in a ball mill. The mineral matter and P and Ca contents of the experimental diets and bones were analyzed in the laboratory.

The following variables were studied: feed intake, weight gain, feed:gain, absolute and relative weights of carcass and prime cuts (breast, drumstick, and thigh), and concentrations of ash, calcium, and phosphorus and the Ca:P ratio in the bones.

Statistical analyses of the studied variables were performed using SAEG (Sistema para Análises Estatísticas, version 8.0) software. The requirements of available phosphorus for male broilers aged 22 to 42 days were estimated by comparing the control treatment and the other treatments using Dunnett's test at 5% significance.

## Results and Discussion

As regards the performance results observed in the present experiment (Table 3), no difference ( $P>0.05$ ) was observed in the weight gain (WG) of birds fed the basal diet (0.354 and 0.309% avP from 22 to 33 and 34 to 42 days of age, respectively) as compared with the other groups fed diets with different levels of phosphorus supplemented with phytase (0.354 and 0.309, 0.294 and 0.258, 0.233 and 0.207, 0.173 and 0.156, and 0.112 and 0.106% in the periods from 22 to 33 and 34 to 42 days of age, respectively). In a study conducted during the grower and final stages, Santos et al. (2011) found that the use of phytase made it possible to reduce the levels of avP and Ca of the diet by 36.6 and 26.3%, respectively. The positive

Table 2 - Formulated (analyzed) nutrient content (%) of experimental diets

|                               | 22-33 days    |               |               |               |               |               |
|-------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                               |               |               |               |               |               |               |
| Available P (%)               | 0.354 (0.416) | 0.354 (0.420) | 0.294 (0.315) | 0.233 (0.220) | 0.173 (0.217) | 0.112 (0.150) |
| P (%)                         | 0.581 (0.621) | 0.581 (0.625) | 0.521 (0.520) | 0.460 (0.425) | 0.400 (0.422) | 0.338 (0.355) |
| Ca (%)                        | 0.758 (0.726) | 0.758 (0.776) | 0.629 (0.626) | 0.499 (0.500) | 0.370 (0.479) | 0.240 (0.298) |
| Phytic P (%)                  | 0.205         | 0.205         | 0.205         | 0.205         | 0.205         | 0.205         |
| Ca:avP ratio                  | 2.14          | 2.14          | 2.14          | 2.14          | 2.14          | 2.14          |
| Phytase (FTU/kg) <sup>1</sup> | -             | 500           | 500           | 500           | 500           | 500           |
|                               | 34-42 days    |               |               |               |               |               |
| Available P (%)               | 0.309 (0.329) | 0.309 (0.328) | 0.258 (0.267) | 0.207 (0.213) | 0.156 (0.173) | 0.106 (0.109) |
| P (%)                         | 0.532 (0.544) | 0.532 (0.543) | 0.481 (0.482) | 0.430 (0.428) | 0.379 (0.388) | 0.329 (0.343) |
| Ca (%)                        | 0.663 (0.714) | 0.663 (0.713) | 0.555 (0.584) | 0.445 (0.497) | 0.335 (0.437) | 0.228 (0.387) |
| Phytic P (%)                  | 0.215         | 0.215         | 0.215         | 0.215         | 0.215         | 0.215         |
| Ca:avP ratio                  | 2.15          | 2.15          | 2.15          | 2.15          | 2.15          | 2.15          |
| Phytase (FTU/kg) <sup>1</sup> | -             | 500           | 500           | 500           | 500           | 500           |

<sup>1</sup> Bacterial phytase (Quantum Blue - AB Vista Feed Ingredients, Marlborough, Wiltshire, UK).

effect of addition of phytase in hydrolyzing the phytate phosphorus in diets with reduced levels of avP for broilers was also demonstrated in the studies of Cardoso Júnior et al. (2010) and Donato et al. (2011), who detected that phytase supplementation in diets with low levels of avP and Ca provided similar results for WG as compared with the control treatment with consequent improvement in the retention and reduction of P excretion by the birds.

The consistent results found in different studies prove the action of phytase in increasing the availability of phytate P in a sufficient amount to ensure the growth rate of broilers irrespective of the rearing period, even when the levels of Ca are reduced concurrently.

In the present study, the use of phytase (500 FTU/kg) in the diets provided a reduction of 68.4% ( $0.354\% \times 0.112\%$ ) and 65.7% ( $0.309\% \times 0.106\%$ ) in the level of the dietary avP for the periods of 22 to 33 and 34 to 42 days of age, respectively, with no negative effects on the growth of the birds. In addition, Conte et al. (2003) found that the WG of broilers aged 1 to 42 days was not affected when they reduced the avP level in diets supplemented with phytase, confirming the suggestion of Tamim et al. (2004) that phytase supplementation makes 25 to 70% of the dietary phytate available.

Considering the likely beneficial effect of phytase on the availability of the dietary P (Yan et al., 2004), and given that the animals from the control group with phytase obtained a similar WG to the control without phytase, it can be inferred that the dietary avP level utilized was sufficient for proper growth of the birds.

These results probably reflect the greater release of phytate P from the diets due to the action of phytase. Therefore, the lower level of avP (0.112 and 0.106% from 22 to 33 and 34 to 42 days of age, respectively) in diets supplemented with 500 FTU of the enzyme was sufficient to maintain the WG of the broilers. However, it must be stressed that, among other factors, the alteration of the ideal temperature to raise the birds caused by the high temperature ( $32.2 \pm 0.42$  °C) at which they were kept may have been decisive for the results of requirement for animal

growth. Moreover, Persia et al. (2003) proposes that stress caused by chronic heat, as occurred in the present study, results in a lower requirement of phosphorus in growing broilers, probably because the heat stress limits their growth, decreasing the requirement of available phosphorus.

No effect ( $P > 0.05$ ) of treatments was detected on the FI of the groups of broilers that received the basal diet as compared with those fed the diet with reduction of the available phosphorus level and supplemented with phytase.

In a study with broilers aged 23 to 40 days, El-Sherbiny et al. (2010) did not find any significant differences in FI according to the P level in diets supplemented with phytase, demonstrating the positive effect of the enzyme on the availability of P. According to the reports of Persia et al. (2003), and Laurentiz et al. (2009), low levels of P in the diet caused a reduction in the FI of broilers, which corroborates Parmer et al. (1987), who suggested that broilers fed diets deficient in P reduce the serum levels of thyroid hormones (thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ )) and growth hormone due to the reduction of the synthesis of secretion of these hormones, consequently reducing FI. Thus, it can be stated that the results obtained in this study prove the action of phytase in hydrolyzing and releasing the dietary phytate, thereby making it available for absorption and use by broilers in an amount sufficient to maintain their FI and growth.

In this study, the maintenance of the Ca:avP ratio of the diets obtained by the reduction of the levels of avP and Ca did not compromise the efficiency of phytase in maintaining FI and consequently the growth rate of the broilers, thus confirming the proposal of Selle et al. (2009) and Delezie et al. (2012), that concurrent reduction of Ca and P in diets, maintaining a fixed Ca:P ratio, does not impair the FI of broilers. Similarly, Cardoso Júnior et al. (2010) observed that supplementing the diets with phytase, when the levels of Ca and avP were reduced together to maintain the Ca:avP close to 2:1, the FI of the diet was benefited as compared with the diets with a high Ca:avP ratio.

The effect of inclusion of phytase and the levels of avP on the FI observed in this study, coupled with the fact that

Table 3 - Performance of broiler chicks fed the dietary treatments from 22 to 42 days of age

| Parameter       | Treatment        |             |                              |             |             |             | P-value | CV (%) |
|-----------------|------------------|-------------|------------------------------|-------------|-------------|-------------|---------|--------|
|                 | Positive control |             | Available P (%) <sup>1</sup> |             |             |             |         |        |
|                 | 0.354/0.309      | 0.354/0.309 | 0.294/0.258                  | 0.233/0.207 | 0.173/0.156 | 0.112/0.106 |         |        |
| Weight gain (g) | 1132             | 1217        | 1249                         | 1227        | 1219        | 1203        | 0.9999  | 12.41  |
| Feed intake (g) | 2108             | 2122        | 2180                         | 2170        | 2221        | 2273        | 0.2790  | 6.98   |
| Feed:gain       | 1.88             | 1.75        | 1.76                         | 1.77        | 1.85        | 1.90        | 0.1899  | 8.33   |

Means of treatments with phytase followed by an asterisk in the same row differ from positive control by Dunnett's test ( $P < 0.05$ ).

CV - coefficient of variation.

<sup>1</sup> Available phosphorus: (22-33/34-42 days) with phytase (500 FTU/kg).

the experimental diets were isonutritive, help to explain the present results, since, according to Mendonça et al. (2008), the growth of broilers depends on their intake of nutrients and energy.

Feed:gain did not vary ( $P>0.05$ ) between the group of animals that received the basal diet and those fed the diet supplemented with the phytase enzyme. These results agree with those found by Powell et al. (2008), who detected a positive effect of inclusion of phytase in maintaining the FC of broilers aged 14 to 41 days fed diets containing reduced levels of Ca and avP (fixed Ca:avP ratio).

The results observed for FC may be related to the action of phytase in releasing the phytate P of diets with the lower avP levels in sufficient amounts to maintain the FI and growth rates of the birds.

Similarly to the present study, Santos et al. (2011) found an improvement in the digestibility of phosphorus with supplementation of 500 FTU/kg of diet, suggesting that in the experimental conditions of the present study, the level of phytase included in the diets was sufficient to improve the use of this nutrient.

Based on the results for FC, it can be stated that the inclusion of phytase (500 FTU/kg) enabled the release of sufficient amounts of avP to maintain the FI and consequently the appropriate intake of nutrients to sustain the growth of the birds, even for the birds fed diets with reduced avP levels.

The absolute and relative carcass weights did not vary ( $P>0.05$ ) among the broilers fed the basal diet as compared with the other groups of animals, which were fed diets supplemented with phytase and different levels of available phosphorus (Table 4). The results observed in the present experiment were similar to those obtained by Teixeira et al. (2013), who did not find significant variations in the relative

and absolute weights of the carcass of hens at the end of the phase from 22 to 42 days of age receiving diets with different levels of avP (0.3, 0.4, and 0.5%), supplemented with phytase.

In view of the positive effect of phytase in improving the utilization of the dietary nutrients (Fukayama et al., 2008; and Teixeira et al., 2013), predicting the negative effects of low levels of avP in the diet with the results found in the present study, it can be inferred that supplementation of 500 FTU/kg in diets with reduced levels of avP was efficient in making phytate P available to maintain the animal growth without affecting the deposition of meat and the carcass yield of the birds.

No variation ( $P>0.05$ ) was found in the absolute and relative weights of the prime cuts (breast, drumstick, and thigh) between the animals that received the basal diets and the birds that were fed diets supplemented with phytase with different levels of avP. Similarly, Teixeira et al. (2013) did not find differences in the absolute and relative weights of the prime cuts of broilers at 42 days of age fed diets with reduction of avP and supplemented with phytase, confirming the reports of Oliveira et al. (2009), in which the levels of avP (0.45, 0.38, and 0.31% from 1 to 21 days; and 0.41, 0.34 and 0.28% from 22 to 42 days of age) did not influence the yield of the prime cuts of 42-day-old birds.

Considering the existence of a positive correlation between weight gain and the weights of carcass and prime cuts of broilers during the growth phase, as was found by Silva et al. (2003), the data pertaining to carcass and prime cuts found in this study can be justified by the positive effect of phytase on the maintenance of the growth of the broilers fed diets with reduced levels of avP.

The results for absolute and relative weights of carcass and prime cuts are coherent with that of FC, which

Table 4 - Relative and absolute weights of carcass and prime cuts of broiler chicks fed the dietary treatments from 22 to 42 days of age

| Parameter | Treatment           |             |                              |             |             |             | P-value | CV (%) |
|-----------|---------------------|-------------|------------------------------|-------------|-------------|-------------|---------|--------|
|           | Positive control    |             | Available P (%) <sup>1</sup> |             |             |             |         |        |
|           | 0.354/0.309         | 0.354/0.309 | 0.294/0.258                  | 0.233/0.207 | 0.173/0.156 | 0.112/0.106 |         |        |
|           | Absolute weight (g) |             |                              |             |             |             |         |        |
| Carcass   | 1705                | 1774        | 1798                         | 1790        | 1793        | 1753        | 0.3110  | 7.3    |
| Breast    | 567                 | 574         | 581                          | 587         | 585         | 568         | 0.9999  | 10.2   |
| Thigh     | 216                 | 229         | 229                          | 230         | 221         | 234         | 0.4009  | 11.5   |
| Drumstick | 252                 | 270         | 271                          | 267         | 262         | 258         | 0.2056  | 9.3    |
|           | Relative weight (%) |             |                              |             |             |             |         |        |
| Carcass   | 87.4                | 88.0        | 86.8                         | 87.5        | 87.5        | 86.6        | 0.2376  | 2.0    |
| Breast    | 33.4                | 32.3        | 32.3                         | 32.8        | 32.6        | 32.4        | 0.9999  | 6.8    |
| Thigh     | 12.7                | 12.9        | 12.7                         | 12.8        | 12.3        | 13.4        | 0.1440  | 8.9    |
| Drumstick | 14.8                | 15.2        | 15.0                         | 15.0        | 14.6        | 14.7        | 0.3921  | 6.0    |

Means of treatments with phytase followed by an asterisk in the same row differ from positive control by Dunnett's test ( $P<0.05$ ).

CV - coefficient of variation.

<sup>1</sup> Available phosphorus: (22-33/34-42 days) with phytase (500 FTU/kg).

demonstrates that in addition to maintaining the growth rate, the use of phytase in diets with low levels of avP also maintained the composition of gain of the birds invariable.

Among the analyzed bone parameters (Table 5), the weight and concentration of P were not influenced ( $P>0.05$ ) by the reduction of the level of dietary avP; however, the weight and concentration of ash and Ca were lower ( $P<0.05$ ) in the bones of the broilers that received the diet with the lowest level of avP (0.112 and 0.106%), supplemented with phytase during the grower and final stages. In a study conducted by Santos et al. (2011), the reductions of 27.8% and 15.2% in the level of avP and 36.6% and 26.3% in the level of Ca in diets supplemented with 500 FTU phytase, in the periods from 22 to 35 and 36 to 42 days of age, respectively, provided an improvement in bone mineralization in the birds without affecting their performance. Nevertheless, Cardoso Júnior et al. (2010) observed that reducing Ca by 35.3% and avP by 26.7% (maintaining a Ca:avP ratio fixed at 2:1) in diets supplemented with phytase during the period from 8 to 35 days of age caused a decrease in the bone ash content, demonstrating the variability between different studies. In the present study, addition of 500 FTU of phytase/kg of diet provided a reduction of 0.354 and 0.309, and up to 0.173 and 0.156% avP, which corresponded to a reduction of 51.1 and 49.5% avP for the phases from 22 to 33 and 34 to 42 days of age, respectively, without affecting the ash content of the bones of the broilers.

The results found in the present study confirm those obtained by Yan et al. (2004), Bozkurt et al. (2006), and Cardoso Júnior et al. (2010), in which the deposition of bone ash was more sensitive to reduction in the levels of dietary minerals, and that modern broiler lines prioritize muscle growth over bone mineralization.

Determining the phosphorus content of the tibiae of broilers, Akyurek et al. (2005) and Shang et al. (2015)

found that the use of phytase in diets with low avP maintained the phosphorus deposition in the tibia of the broilers irrespective of the growth phase, which made it possible to infer that inclusion of phytase was efficient in making available sufficient amounts of phosphorus bound to phytate, maintaining its adequate absorption and deposition. Similar results were found by Catalá-Gregori et al. (2006), who reduced the avP level by 64.4% in a diet supplemented with phytase (600 FTU/kg) for broilers of 22 to 42 days of age, without affecting P deposition in the tibia.

Considering that the use of phytase increases P retention and reduces its excretion (Ahmad et al., 2000; Lelis et al., 2010), the positive results for the bone deposition of P suggest that the use of 500 FTU/kg of diet was sufficient to make phytate P available, thereby maintaining the performance and bone phosphorus deposition of the broilers.

However, the reduction of the deposition of bone ash observed with the lower levels of Ca and P may be indicative of a possible calcium deficiency in the diet, since the deposition of P in the tibiae was not influenced by the treatments. The exposure of animals to chronic heat stress may have triggered physiological effects such as respiratory alkalosis, reduction of the blood ionic calcium content, and reduction of the mobilization of Ca from the bone reserves, as described by Dagher (2008). These mechanisms associated with reduced intake of Ca might have led to a deficiency of this mineral in the broilers fed diets with lower levels of Ca and avP in diets with a fixed Ca:avP ratio (2.1:1), thus limiting bone deposition. Although there was a likely increase in availability of Ca due to the action of phytase, this release might have not been sufficient to keep bone deposition unchanged under the experimental conditions.

Because the animals were fed diets with a fixed Ca:avP ratio, the results for bone deposition probably reflect the

Table 5 - Bone parameters in the tibia of broiler chicks fed the dietary treatments from 22 to 42 days of age

| Parameter      | Treatment        |             |                              |             |             |             | P-value | CV (%) |
|----------------|------------------|-------------|------------------------------|-------------|-------------|-------------|---------|--------|
|                | Positive control |             | Available P (%) <sup>1</sup> |             |             |             |         |        |
|                | 0.354/0.309      | 0.354/0.309 | 0.294/0.258                  | 0.233/0.207 | 0.173/0.156 | 0.112/0.106 |         |        |
| Ash (g)        | 2.622            | 2.638       | 2.697                        | 2.695       | 2.574       | 2.318*      | 0.0314  | 13.6   |
| Ash (%)        | 49.1             | 49.0        | 48.8                         | 49.1        | 47.5        | 45.1*       | 0.0000  | 4.8    |
| Calcium (g)    | 0.925            | 0.931       | 0.987                        | 0.960       | 0.901       | 0.835*      | 0.0325  | 14.3   |
| Calcium (%)    | 17.4             | 17.3        | 17.8                         | 17.5        | 16.7        | 16.2*       | 0.0026  | 6.9    |
| Phosphorus (g) | 0.367            | 0.375       | 0.398                        | 0.396       | 0.406       | 0.373       | 0.9999  | 13.6   |
| Phosphorus (%) | 8.6              | 8.5         | 8.7                          | 8.6         | 8.6         | 8.4         | 0.9999  | 6.3    |
| Ca:P ratio     | 2.03             | 2.04        | 2.06                         | 2.04        | 1.94*       | 1.94*       | 0.0342  | 6.6    |

Means of treatments with phytase followed by an asterisk in the same row differ from positive control by Dunnett's test ( $P<0.05$ ).

CV - coefficient of variation.

<sup>1</sup>Available phosphorus: (22-33/34-42 days) with phytase (500 FTU/kg).

intake of these nutrients from the diet, demonstrating that the deposition of P in the bone occurs simultaneously with that of Ca, at the proportion of approximately 2:1, which characterizes a relationship of interdependence between these minerals. Thus, it can be inferred that maintaining the Ca:avP ratio of the diet according to the standards described in the literature seems to be crucial when animals are reared in a high-temperature environment and when the level of Ca does not meet the requirement of broilers.

## Conclusions

Diets supplemented with phytase, containing 0.173 and 0.156% available phosphorus, and with a ratio of calcium to available phosphorus fixed at 2.1:1, meet the requirements of broilers from 22 to 33 and 34 to 42 days of age reared under high temperatures, respectively.

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## References

- Ahmad, T.; Rasool, S.; Sarwar, M.; Haq, A. and Hasan, Z. 2000. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. *Animal Feed Science and Technology* 83:103-114.
- Akyurek, H.; Senkoylu, N. and Ozduven, M. L. 2005. Effect of microbial phytase on growth performance and nutrients digestibility in broilers. *Pakistan Journal of Nutrition* 4:22-26.
- Belay, T.; Wiernusz, C. J. and Teeter, R. G. 1992. Mineral balance and urinary and fecal mineral excretion profile of broilers housed in thermoneutral and heat-distressed environments. *Poultry Science* 71:1043-1047.
- Bonnet, S.; Geraert, P. A.; Lessire, M.; Carre, B. and Guillaumin, S. 1997. Effect of high ambient temperature on feed digestibility in broilers. *Poultry Science* 76:857-863.
- Bozkurt, M.; Çabuk, M. and Alçiçek, A. 2006. The effect of microbial phytase in broiler grower diets containing low phosphorus, energy and protein. *The Journal of Poultry Science* 43:29-34.
- Buffington, D. E.; Colazzo-Arocho, A.; Canton, G. H.; Pitt, D.; Thatcher, W. W. and Collier, R. J. 1981. Black globe-humidity index (BGHI) as comfort equation for dairy cows. *Transaction of the ASAE* 24:711-714.
- Cardoso Junior, A.; Rodrigues, P. B.; Bertechini, A. G.; Freitas, R. T. F.; Lima, R. R. and Lima, G. F. R. 2010. Levels of available phosphorus and calcium for broilers from 8 to 35 days of age fed rations containing phytase. *Revista Brasileira de Zootecnia* 39:1237-1245.
- Catalá-Gregori, P.; García, V.; Hernández, J. O.; Madrid, J. and Cerón, J. J. 2006. Response of broilers to feeding low-calcium and phosphorus diets plus phytase under different environmental conditions: body weight and tibiotarsus mineralization. *Poultry Science* 85:1923-1931.
- Cobb-Vantress. 2008. Guia de manejo para frangos de corte Cobb 500. Cobb Vantress, Guapiaçu.
- COBEA – Colégio Brasileiro de Experimentação Animal. 1991. Princípios éticos na experimentação animal. Available at: <[http://www.univap.br/ipd/docs/principios\\_eticos\\_na\\_experimentalcao\\_animal.pdf](http://www.univap.br/ipd/docs/principios_eticos_na_experimentalcao_animal.pdf)>. Accessed on: Nov. 14, 2013.
- Conte, A. J.; Teixeira, A. S.; Fialho, E. T.; Schoulten, N. A. and Bertechini, A. G. 2003. Efeito da fitase e xilanase sobre o desempenho e as características ósseas de frangos de corte alimentados com dietas contendo farelo de arroz. *Revista Brasileira de Zootecnia* 32:1147-1156.
- Daghir, N. J. 2008. Poultry production in hot climates. 2th ed. CAB International, London.
- Delezie, E.; Maertens, L. and Huyghebaert, G. 2012. Consequences of phosphorus interactions with calcium, phytase, and cholecalciferol on zootechnical performance and mineral retention in broiler chickens. *Poultry Science* 91:2523-2531.
- Donato, D. C. Z.; Albuquerque, R.; Garcia, P. D. S. R. and Balieiro, J. C. C. 2011. Desempenho de frangos de corte alimentados com rações contendo diferentes níveis de cálcio suplementadas com fitase. *Revista Brasileira de Zootecnia* 40:2161-2166.
- El-Husseiny, O. and Creger, C. R. 1981. Effect of ambient temperature on mineral retention and balance of the broiler chicks. *Poultry Science* 60(suppl. 1):1651.
- El-Sherbiny, A. E.; Hassan, H. M. A.; Abd-Elsamee, M. O.; Samy, A. and Mohamed, M. A. 2010. Performance, bone parameters and phosphorus excretion of broilers fed low phosphorus diets supplemented with phytase from 23 to 40 days of age. *International Journal of Poultry Science* 9:972-977.
- Fukayama, E. H.; Sakomura, N. K.; Dourado, L. R. B.; Neme, R.; Fernandes, J. B. K. and Morcato, S. M. 2008. Efeito da suplementação de fitase sobre o desempenho e a digestibilidade dos nutrientes em frangos de corte. *Revista Brasileira de Zootecnia* 37:629-635.
- Khalid, M. F.; Hussain, M.; Rehman, A. U.; Shazad, M. A.; Sharif, M. and Rahman, Z. U. 2013. Broiler performance in response to phytate and supplemented phytase. *Iranian Journal of Applied Science* 3:1-12.
- Laurentiz, A. C.; Junqueira, O. M.; Filardi, R. S.; Duarte, K. F.; Assuena, V. and Sgavioli, S. 2009. Desempenho, composição da cama, das tíbias, do fígado e das excretas de frangos de corte alimentados com rações contendo fitase e baixos níveis de fósforo. *Revista Brasileira de Zootecnia* 38:1938-1947.
- Lavor, C. T. B.; Fernandes, A. A. O. and Sousa, F. M. 2008. Efeito de materiais isolantes térmicos em aviários no desempenho de frangos de corte. *Revista de Ciência Agronômica* 39:308-316.
- Lelis, G. R.; Albino, L. F. T.; Silva, C. R.; Rostagno, H. S.; Gomes, P. C. and Borsatto, C. G. 2010. Suplementação dietética de fitase sobre o metabolismo de nutrientes de frangos de corte. *Revista Brasileira de Zootecnia* 39:1768-1773.
- Mendonça, M. O.; Sakomura, N. K.; Santos, F. R.; Freitas, E. R.; Fernandes, J. B. K. and Barbosa, N. A. A. 2008. Níveis de energia metabolizável para machos de corte de crescimento lento criados em semiconfinamento. *Revista Brasileira de Zootecnia* 37:1433-1440.
- Oliveira, M. C.; Marques, R. H.; Gravena, R. A.; Bruno, L. D. G.; Rodrigues, E. A. and Moraes, V. M. B. 2008. Qualidade óssea de frangos alimentados com dietas com fitase e níveis reduzidos de fósforo disponível. *Acta Scientiarum Animal Science* 30:263-268.
- Parmer, T. G.; Carew, L. B. and Alster, F. A. 1987. Thyroid function, growth hormone, and organ growth in broilers deficient in phosphorus. *Poultry Science* 66:1995-2004.
- Persia, M. E.; Parsons, C. M. and Koelkebeck, K. W. 2003. Interrelationship between environmental temperature and dietary nonphytate phosphorus in chicks. *Poultry Science* 82:1616-1623.

- Powell, S.; Johnston, S.; Gaston, L. and Southern, L. L. 2008. The effect of dietary phosphorus level and phytase supplementation on growth performance, bone-breaking strength, and litter phosphorus concentration in broilers. *Poultry Science* 87:949-957.
- Ravindran, V.; Bryden, W. L. and Kornegay, E. T. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poultry Avian Biology Reviews* 6:125-143.
- Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. L.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.
- Santos, L. M. S.; Rodrigues, P. B.; Alvarenga, R. R.; Naves, L. P.; Hespanhol, R.; Lima, G. F. R.; Lara, M. C. C. and Silva, L. R. 2011. Níveis de fósforo disponível e cálcio em rações suplementadas com fitase para frangos de corte nas fases de crescimento e final. *Revista Brasileira de Zootecnia* 40:2486-2495.
- Selle, P. H.; Cowieson, A. J. and Ravindran, V. 2009. Consequences of calcium interactions with phytase for poultry and pigs. *Livestock Science* 124:126-141.
- Silva, D. J. and Queiroz, A. C. 2002. Análise de alimentos: métodos químicos e biológicos. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.
- Silva, J. H. V.; Albino, L. F. T. and Nascimento, A. D. 2003. Estimativas da composição anatômica da carcaça de frangos de corte com base no nível de proteína da ração e peso da carcaça. *Revista Brasileira de Zootecnia* 32:344-352.
- Shang, Y.; Rogiewicz, A.; Patterson, R.; Slominski, B. A. and Kim, W. K. 2015. The effect of phytase and fructooligosaccharide supplementation on growth performance, bone quality, and phosphorus utilization in broiler chickens. *Poultry Science* 94:955-964.
- Tamim, N. M.; Angel, R. and Christman, M. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. *Poultry Science* 83:1358-1367.
- Teixeira, E. N. M.; Silva, J. H. V.; Goulart, C. C.; Jordão Filho, J. and Ribeiro, M. L. G. 2013. Suplementação da fitase em rações com diferentes níveis de fósforo disponível para frangos de corte. *Revista Ciência Agronômica* 44:390-397.
- Valerio, S. R.; Oliveira, R. F. M.; Donzele, J. L.; Gomes, P. C.; Apolônio, L. R. and Resende, W. O. 2003. Níveis de lisina digestível em rações, em que se manteve ou não a relação aminoacídica, para frangos de corte de 22 a 42 dias de idade, mantidos em estresse por calor. *Revista Brasileira de Zootecnia* 32:372-382.
- Yan, F.; Fritts, C. A. and Waldroup, P. W. 2004. Evaluation of modified dietary phosphorus levels with and without phytase supplementation on live performance and excreta phosphorus concentration in broiler diets. 2. Modified early phosphorus levels. *Journal Applied Poultry Research* 13:394-400.