Nutritional cost reduction and increase profitability in commercial broiler production using phytase superdosing

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ABSTRACT - The objective of the present study was to evaluate the reduction of calcium (Ca), crude protein (CP), metabolizable energy (ME), and available phosphorus (aP) in the nutritional matrix of Brazilian commercial broiler diets supplemented with both phytase superdosing (1500 FTU/kg) or conventional doses (500 FTU/kg) on the feed cost and profitability, performance, bone mineralization, and carcass yield of broiler from 1 to 42 d. A total 1200 one-day-old chicks (Cobb 500) were randomly distributed in a commercial feeding program composed of a positive control diet and three diets with reduction of Ca, P, ME, and CP in the nutritional matrix supplemented with 500, 1000, and 1500 FTU/kg of phytase. The broilers subjected to diets with phytase superdosing presented similar performance, bone ash, and carcass yield, among the treatments. There was a linear effect in the total nutrition cost, gross margin, and estimated net margin per bird with the increase of dietary inclusion of phytase in all purposed scenarios. Thus, for each 500 FTU/kg of dietary phytase included in the diet with reduction of Ca, P, ME, and CP in the nutritional matrix, the total nutrition cost decreased R$ 0.072/bird, R$ 0.079/bird, and R$ 0.081/bird in scenarios of high, medium, and low prices of corn and soybean meal, respectively, that allowed an increase in the estimated net margin of R$ 0.20/bird, R$ 0.22/bird, and R$ 0.22/bird in the same scenarios.

Keywords: gross margin, net margin, nutritional value, production cost

1. Introduction

Among the factors that affect animal production costs, the amount invested in nutrition is the highest, accounting for approximately 74% of the total cost (Embrapa, 2017). In this context, energy, amino acid (Patience, 2013), and phosphorus sources (e.g., dicalcium phosphate; Arabi, 2013) are the ones with greatest impact in the dietary cost, and together, can account for more than 80% of diet costs. Thus, reducing the inclusion of these ingredients in the diet can contribute to the reduction of broiler chicken production costs. However, to maintain nutritional levels of diets, it is necessary to use exogenous enzymes, capable of releasing certain nutrients previously unavailable for animal absorption to maintain them nutritionally balanced, such as phytase. However, the inclusion of phytase in diet goes beyond economic improvement. Its use is correlated to animal efficiency, better use of nutrients, and...
greater growth performance (Cowieson et al., 2011), reducing nutrient excretion and, consequently, environmental pollution (Kumar et al., 2016).

In Brazil, its use is consensual in broiler nutrition and is employed at a dose of 200-500 FTU/kg. Phytase supplementation, associated to the reduction in the nutritional matrix of Ca and P, reduces diet costs without reducing animal performance (Kies et al., 2001; Adeola and Cowieson, 2011; Freitas et al., 2019). In recent years, the concept of superdosing has been shown to have beneficial effects on bird performance (Walk et al., 2014) and nutrient retention (Cowieson et al., 2017) in diets supplemented with doses of 1500 FTU/kg (Beeson et al., 2017) and greater levels (Manobhavan et al., 2016; Pieniazek et al., 2017). The expression “phytase superdosing” is designated to refer a high dose of dietary phytase inclusion that can be considered as ≥ 1500 FTU/kg (Walk et al., 2014) or ≥ 2500 FTU/kg (Adeola and Cowieson, 2011), depending on researchers. Studies evaluating how phytase impacts broiler production cost, to our knowledge, are scarce. The hypothesis presented is that dietary supplementation with phytase superdosing in diets presenting reduction of calcium (Ca), available phosphorus (aP), crude protein (CP), and metabolizable energy (ME) in the nutritional matrix would have lower feed costs and greater profitability, as compared with conventional broiler diets that present similar performance of broiler chickens.

The objective of this study was to evaluate the economic benefits of using phytase superdosing in commercial diets with reduction of Ca, P, ME, and CP in the nutritional matrix, as well as their effects on weight, weight gain, feed intake, gain-to-feed ratio, bone mineralization, carcass, and yield of noble cuts of broiler chickens from 1 to 42 d of age.

2. Material and Methods

All procedures and protocols were approved by the local Ethics Committee on Animal Use, according to the Arouca Law (No. 11.794 of October 8, 2008) under protocol number 7280161017. The microbial 6-phytase (Microtech 5000, Guangdong VTR Bio-Tech) used has enzyme activity of 5000 units/g of product, as reported by the manufacturer. The enzyme is derived from a modified E. coli strain in a culture of Pichia pastoris. For the present study, the enzymatic activity of the tested product was measured by estimating the number of micromoles of inorganic P released by sodium phytate in 1 min at a temperature of 37 °C and pH of 5.5 (1 FTU = 1 μmol inorganic P/min).

A total of 1200 one-day-old Cobb 500 chicks were obtained from a local commercial hatchery and randomly distributed into 100 floor pens. The environmental temperature was controlled by forced ventilation with axial fans, arranged in three lines with two fans each and 5-m spacing between lines, as well as heating provided by infrared lamps (one unit/pen). Each pen had an area of 1 m², respecting the animal density of 30 kg/m². Tubular feeder and nipple drinkers were used, providing feed and water ad libitum. All general practices were guided by manual recommendations of the genetic line.

Four dietary treatments were randomly assigned to 100 replicate pens of 12 birds each (n = 25 pens/treatment) in a completely randomized design. Diets were formulated in a three-phase feeding program: starter, 1 to 21 days; grower, 22 to 34 days; and finisher, 35 to 42 days.

Composition and nutritional levels of the experimental diets followed Rostagno et al. (2011) recommendations with adaptations to lower broiler performance: reducing the levels of Ca and aP, ME, and CP according to each treatment (Table 1). To attain the desired ME levels in each treatment, there was a reduction in soybean oil inclusion of 0.89, 1.13, and 1.32% in the starter phase; 0.92, 1.17, and 1.39% in the grower phase; and 0.92, 1.19, and 1.41% in the finisher phase, to treatments 500, 1000, and 1500 FTU/kg of phytase, respectively, corrected in corn inclusion. To attain the desired CP levels in each treatment, there was a reduction in soybean meal inclusion in 0.92, 1.16, and 1.36% in the starter phase; 0.97, 1.19, and 1.42% in the grower phase; and 0.98, 1.26, and 1.48% in the finisher phase, to treatments 500, 1000, and 1500 FTU/kg of phytase, respectively. The desired Ca and aP levels in each treatment were obtained...
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by increasing limestone dietary levels and decreasing dicalcium phosphate inclusion considering that 500, 1000, and 1500 FTU/kg of phytase supply 0.09, 0.12, and 0.14% aP, respectively, and 0.10, 0.13, and 0.15% total Ca, respectively.

Table 1 - Composition and nutritional levels of experimental diets

| Ingredient (%) | Control 100 | 500 | 1500 | Control 100 | 500 | 1500 | Control 100 | 500 | 1500 |
|----------------|------------|-----|------|------------|-----|------|------------|-----|------|
| Corn           | 59.59      | 62.43 | 62.9 | 64.00      | 66.35 | 68.98 | 67.55      | 68.75 | 71.79 | 72.34 |
| Soybean meal   | 34.73      | 33.57 | 33.37 | 30.25      | 29.28 | 29.06 | 28.83      | 25.89 | 24.91 | 24.41 |
| Soybean oil    | 1.55       | 0.42  | 0.23 | 2.41       | 1.49  | 1.24  | 1.02       | 2.22  | 1.30  | 1.03  |
| Dicalcium phosphate | 1.66 | 1.17  | 1.01  | 1.26       | 0.72  | 0.56  | 0.33       | 1.05  | 0.51  | 0.34  |
| Limestone      | 0.81       | 0.90  | 0.89  | 0.79       | 0.87  | 0.89  | 0.99       | 0.72  | 0.80  | 0.82  |
| Premix         | 0.80       | 0.80  | 0.80  | 0.60       | 0.60  | 0.60  | 0.60       | 0.60  | 0.60  | 0.60  |
| Salt           | 0.42       | 0.42  | 0.42  | 0.40       | 0.40  | 0.40  | 0.40       | 0.40  | 0.40  | 0.40  |
| L-Lysine       | 0.20       | 0.20  | 0.20  | 0.18       | 0.19  | 0.19  | 0.19       | 0.24  | 0.25  | 0.25  |
| L-Threonine    | 0.09       | 0.08  | 0.08  | 0.06       | 0.05  | 0.05  | 0.05       | 0.07  | 0.06  | 0.06  |
| DL-Methionine  | 0.08       | 0.08  | 0.08  | 0.00       | 0.00  | 0.00  | 0.00       | 0.00  | 0.00  | 0.00  |
| L-Valine       | 0.07       | 0.07  | 0.07  | 0.05       | 0.04  | 0.01  | 0.01       | 0.06  | 0.06  | 0.06  |
| Phytase        | 0.00       | 0.01  | 0.02  | 0.00       | 0.01  | 0.02  | 0.03       | 0.00  | 0.02  | 0.03  |
| Total          | 100.0      | 100.0 | 100.0 | 100.0      | 100.0 | 100.0 | 100.0      | 100.0 | 100.0 | 100.0 |

Calculated level (%)

| Ingredient (%) | Control 100 | 500 | 1500 | Control 100 | 500 | 1500 | Control 100 | 500 | 1500 |
|----------------|------------|-----|------|------------|-----|------|------------|-----|------|
| ME (kcal/kg)   | 2983       | 2960 | 2954 | 2954       | 2949 | 3100 | 3100       | 3100 | 3127 | 3120 | 3115 |
| Crude protein  | 21.27      | 21.04 | 20.93 | 21.04      | 20.93 | 20.93 | 20.93      | 20.93 | 20.93 | 20.93 | 20.93 |
| Ca             | 0.850      | 0.750 | 0.720 | 0.750      | 0.720 | 0.720 | 0.720      | 0.720 | 0.720 | 0.720 | 0.720 |
| Available P    | 0.340      | 0.240 | 0.210 | 0.300      | 0.240 | 0.210 | 0.300      | 0.240 | 0.210 | 0.300 | 0.240 |
| Na             | 0.20       | 0.20  | 0.20  | 0.20       | 0.20  | 0.20  | 0.20       | 0.20  | 0.20  | 0.20  | 0.20  |
| Lysine         | 1.219      | 1.207 | 1.203 | 1.207      | 1.203 | 1.203 | 1.207      | 1.207 | 1.207 | 1.207 | 1.207 |
| Methionine     | 0.476      | 0.475 | 0.474 | 0.476      | 0.475 | 0.474 | 0.476      | 0.475 | 0.474 | 0.476 | 0.475 |
| Met + Cys      | 0.879      | 0.875 | 0.873 | 0.872      | 0.754 | 0.749 | 0.747      | 0.746 | 0.670 | 0.665 | 0.663 |
| Threonine      | 0.793      | 0.779 | 0.775 | 0.773      | 0.701 | 0.687 | 0.683      | 0.681 | 0.656 | 0.654 | 0.652 |
| Valine         | 0.939      | 0.925 | 0.921 | 0.919      | 0.841 | 0.827 | 0.823      | 0.821 | 0.788 | 0.786 | 0.785 |

Control diet with adequate levels of Ca and P; 500 = control diet with reduced levels of Ca and P, supplemented with 500 FTU/kg of phytase; 1000 = control diet with reduced levels of Ca and P, supplemented with 1000 FTU/kg of phytase; 1500 = control diet with reduced levels of Ca and P, supplemented with 1500 FTU/kg of phytase.

Vitamin and mineral supplement plus anticoccidian agent, growth promoter and choline, supplied the following amounts of vitamin and minerals per kilogram of diet for the age of 1 to 21 d: choline, 0.261 g; vitamin A, 11,000 IU; vitamin D3, 3,600 IU; vitamin E, 16 IU; vitamin K3, 1.5 mg; thiamine, 1.2 mg; riboflavin, 4.5 mg; vitamin B6, 2 mg; vitamin B12, 16 mcg; pantothenic acid, 9.2 mg; niacin, 35 mg; biotin, 0.06 mg; folic acid, 0.4 mg; Mn, 60 mg; Fe, 30 mg; Zn, 60 mg; Cu, 9 mg; I, 1 mg; Se, 0.25 mg; avilamycin, 10 mg; nicarbazin, 40 mg; senduramicine, 15 mg; for the age of 22 to 34 d: choline, 0.22 g; vitamin A, 9,000 IU; vitamin D3, 3,600 IU; vitamin E, 14 IU; vitamin K3, 1.5 mg; thiamine, 1 mg; riboflavin, 4 mg; vitamin B6, 1.8 mg; vitamin B12, 12 mcg; pantothenic acid, 8.28 mg; niacin, 30 mg; biotin, 0.05 mg; folic acid, 0.3 mg; Mn, 0.06 g; Fe, 30 mg; Zn, 0.06 g; Cu, 9 mg; I, 1 mg; Se, 0.25 mg; avilamycin, 10 mg; salinomycin, 0.066 g; and for the age of 35 to 42 d: choline, 0.13 g; vitamin A, 2,700 IU; vitamin D3, 450 IU; vitamin E, 4.5 IU; vitamin K3, 0.45 mg; thiamine, 0.27 mg; riboflavin, 0.9 mg; vitamin B6, 0.36 mg; vitamin B12, 2.7 mcg; pantothenic acid, 3.312 mg; niacin, 4.5 mg; biotin, 0.014 mg; Mn, 0.06 g; Fe, 30 mg; Zn, 0.06 g; Cu, 9 mg; I, 1 mg; and Se, 0.18 mg.
The Ca:P ratio was determined according to the experience of the researchers and agroindustry experts.

Body weight and feed intake were determined on a per pen basis at the end of each growth phase (21, 34, and 42 d of age). Body weight gain and feed intake were corrected for mortality and calculated by phase consisting of days 1 to 21, days 22 to 33, and days 34 to 42, and by accumulated periods consisting of days 1 to 34 and day 1 to 42. The gain:feed ratio was calculated as feed intake (g)/body weight gain (g).

At the end of the trial (42 d), after a 12-h feed withdrawal period, three birds from each replicate were selected (n = 75 birds per treatment) using average body weight of the pen as criteria based on individual weighing (e = 0.002), banded, and then, transported to the slaughterhouse. All birds were slaughtered in the same day.

Broiler slaughter followed procedures described by Pillai et al. (2006). Birds were electrically stunned, manually bled by severing the left carotid artery and jugular vein, bled out, soft-scalded, and feathers were picked with the use of commercial defeathering machine. Broilers were then immediately eviscerated and moved to an immersion chiller for 45 min. Feet, neck, and head were removed, and carcasses taken to a cold chamber for 24 h at 4 °C.

Carcasses were individually weighed (e = 0.002) to obtain the carcass dressing data, separated into chest and legs (thighs and drumsticks), and weight again (e = 0.002). Then, the right tibia was removed from each animal at the time of the carcass evaluation, properly identified, and sent to the laboratory for tibia ash measurements (Silva and Queiroz, 2004).

To perform the economic calculations and establish corn and soybean meal price scenarios, the average prices of ingredients used in the formulation of diets were obtained from both a ten-year historical series of each ingredient in national databases (IEA-SP/ESALQ-USP/CONAB), which are companies specialized in agricultural consulting (Scot Consultoria), and from prices reported in the region of São Paulo State, according to Gameiro (2009).

All prices were deflated using the values of the National Consumer Price Index (INPC/IBGE) for the month of July 2016 (US$ 1 = R$ 3.28). Prices per kg (R$) of ingredients used were: soybean oil, R$ 3.28; dicalcium phosphate, R$ 1.86; calcitic limestone, R$ 0.12; vitamin-mineral supplement, R$ 12.73; common salt, R$ 0.73; L-lysine, R$ 6.60; L-threonine, R$ 10.46; DL-methionine, R$ 11.78; L-valine, R$ 21.77; and phytase, R$ 14.76.

Price paid per one-day-old chick was R$ 1.60. The average price practiced per kg of live bird was considered as R$ 2.78. To obtain the price of the diet, the inclusion value of each ingredient in a kg of diet/phase was considered. Diet costs per phase was obtained considering feed intake of bird per experimental unit, percental dietary inclusion of ingredient, and price of each ingredient in each phase; and the total nutrition cost (1) was determined from ingredient prices and average feed intake of each experimental unit.

\[
TNC_t = \sum_{p=1}^{3} CD_{pt} \forall t
\]

in which \(p\) is the phase (starter, grower, and finisher); \(CD\) is the cost of diet per bird for treatment \(t\) in phase \(p\); \(t\) is treatment (control, 500, 1000, 1500 FTU/kg); \(TNC\) is the total nutrition cost per bird for each treatment \(t\); and \(\forall\) is for each \(t\) treatment;

The total estimated broiler production cost (TEBPC; Equations 2 and 2.1) was obtained from information according to the Central de Inteligência de Aves e Suínos for June 2016 (Embrapa, 2017).

\[
TEBPC = \sum_{f=1}^{n} EBPC_f
\]

\[
EBPC_f = \frac{NC \times y}{N \times HCN}
\]

in which \(NC\) is the total nutrition cost per bird for each treatment \(t\); \(y\) is the yield of live bird per kilogram of feed intake; and \(N\) and \(HCN\) are the number of experimental units and number of carcasses, respectively.
in which $EBPC_f$ is the estimated broiler production cost (per bird) related to each production factor; $f$ is the production factor (e.g., depreciation, litter, labor, etc); $NC$ is the total nutrition cost in the whole experimental period; $y$ is the percentage participation of studied production factors $i$; $N$ is the percentage of nutrition in broiler production cost in June, 2016 (74.26%; Embrapa, 2017); and $HCN$ is the husbandry chicks number.

To estimate the participation of the other production factors in broiler production cost, nutrition cost was considered to be 74.26% of the total broiler production cost (ICPFRangos/Embrapa, June, 2016; Embrapa, 2017). The value of one-day-old chick was disregarded from the model because its real value was obtained at the time of purchase. The nutrition used in the study presented a total value of R$ 5,445.41.

The other production factors included in the total costs of broiler production and percentage of participation were: manpower (4.78%), transport (3.89%), depreciation (1.95%), electric energy/bedding/heating (1.75%), cost of capital (1.61%), maintenance/financial/Funrural (Brazilian social security tax) (0.65%), animal health (0.08%), and miscellaneous/others (0.20%). Production factors were included in the whole nutrition cost calculated to compose the total broiler cost production in this study. Therefore, the percentages of participation were applied to total cost in each treatment.

Thus, values were estimated from the total cost of diets used in the study, extrapolated to the other variables according to the percentage of participation suggested by the author, and divided by the number of birds used. The value obtained was R$ 0.91/bird for June 2016.

To determine the desired economic indicators (gross margin, net margin, and TNC), the following primary variables were considered: final body weight per bird (FBW), feed intake per bird per phase (FI), price paid per kg live bird (PP), price paid per chick (PPC), and total estimated broiler production cost per bird (TEBPC).

The following economic indicators were obtained: TNC (Equation 1), estimated net margin per bird (NM; Equation 3), and gross margin per bird (GM; Equation 4):

\[
NM_t = (FBW_t \times PP) - \left(PPC + \left[TNC_t + (TEBPC - NC_2)\right]\right) \quad (3)
\]

\[
GM_t = (FBW_t \times PP) - TNC_t \quad (4)
\]

in which $NC_2$ is the total nutrition cost in whole experimental period per bird.

To evaluate the impact of phytase dosage under the desired economic indicators, nine scenarios were proposed varying the soybean meal (SB) and corn (Co) prices. The proposed scenarios were: medium and medium price (MedCo + MedSB), minimum and medium price (MinCo + MedSB), high and medium price (MaxCo + MedSB), medium and low price (MedCo + MinSB), low and low price (MinCo + MinSB), medium and high price (MedCo + MaxSB), low and high price (MinCo + MaxSB), and high and high price (MaxCo + MaxSB) of corn and soybean meal, respectively. The medium price of corn and soybean meal was obtained from the average monthly price practiced in the historical series of ten years. For the low and high prices of corn and soybean meal, the highest and lowest real price practiced in the same historical series were considered. Also, evaluating scenarios of minimum and maximum prices is important since agricultural commodities in Brazil suffer a strong seasonal variation over time. Thus, considering only one average price level would provide a limited result among the other possibilities of price scenarios from market for these commodities. The prices were considered as: R$ 0.61 and R$ 1.33 for medium price; R$ 0.42 and R$ 0.87 for low price; and R$ 0.97 and R$ 1.99 for high price of corn and soybean meal, respectively.

For the impact of each ingredient measurement on the overall cost of the diets (Equation 5), the participation of corn, soybean meal, soybean oil, and dicalcium phosphate was considered separately within the average feed/bird intake per experimental unit, per phase in scenario 1, and total experiment in the proposed feeding program. The value of the ingredients was estimated as R$/bird.
in which $SA$ is the impact of ingredient on the overall cost in the dietary program in each treatment; $i$ is the ingredient in the feed program; $Pi$ is the price of the ingredient $i$; and $FI$ is the average feed intake of bird per experimental unit in the whole experimental period.

For statistical analysis of the performance index, each pen was considered as the experimental unit, using the average data of animals per pen. Performance parameters were pondered considering the dead bird weight, body weight of the remaining birds, and $FI$ for that pen on the death day.

For statistical analysis of carcass and yield of noble cuts and tibia ash analysis, each bird was considered as the experimental unit. In both performance parameters, carcass and yield of noble cuts and tibia ash statistical analysis were considered the following model described below:

$$Y_{ij} = \mu + A_i + e_{ij}$$

in which $Y$ is the observation to treatment $i$ (control, 500, 1000, and 1500 FTU/kg of the diet) in the experimental unit $j$; $\mu$ is the general data; $A$ is the fixed effect of phytase dosage; and $e$ is the residual error.

Data were subjected to ANOVA appropriate for a completely randomized design using the MIXED procedure of SAS (Statistical Analysis System, version 9.4). Pairwise differences between means were determined using PDIFF option of LSMEANS statement. Each pen was considered as random effects. The main effects of the four treatments with 25 replicates each on performance parameters (body weight gain, feed intake, and feed conversion), carcass and noble cuts, and tibia ashes were tested. The means of the treatments were compared using Tukey’s test, and differences were considered significant when $P<0.05$.

For statistical analysis of economic performance and impact of the ingredients on the TNC, mean data obtained from each experimental unit of each treatment were used. Data were subjected to analysis of variance by means of the MIXED procedure of SAS. Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response of the estimated NM, GM, and TNC (dependent variables) according to the increasing phytase level (independent variable). The differences were considered significant when $P<0.05$.

### 3. Results

There was no effect of treatment on performance variables (final body weight, body weight gain, feed intake, gain:feed ratio; Table 2, $P>0.05$). In addition, no difference was observed on carcass yield, leg yield, or bone ash of broilers among treatments (Table 2, $P>0.05$). However, there was a greater breast yield for broiler fed diets supplemented with 1500 FTU/kg of phytase ($P<0.05$).

In scenario of medium (MedCo + MedSB), low (MinCo + MinSB), and high prices (MaxCo + MaxSB) of corn and soybean meal, the inclusion of 1500 FTU/kg of phytase reduced the TNC in 5.6, 7.5, and 3.7%, respectively, compared with the control diet (Table 3, $P<0.05$). Each 500 FTU/kg of phytase inclusion in broiler diet reduced in R$ 0.079, 0.081, and 0.072 per bird the TNC in scenario of medium (MedCo + MedSB), low (MinCo + MinSB), and high prices (MaxCo + MaxSB) of corn and soybean meal, respectively (Figure 1).

In scenario of medium (MedCo + MedSB), low (MinCo + MinSB), and high prices (MaxCo + MaxSB) of corn and soybean meal, the inclusion of 1500 FTU/kg of phytase increased GM in 7.4, 5.1, and 22.7%, respectively, compared with the control diet (Table 4, $P<0.05$).

In scenario of medium (MedCo + MedSB), low (MinCo + MinSB), and high prices (MaxCo + MaxSB) of corn and soybean meal, the inclusion of 1500 FTU/kg of phytase increased the estimated NM in 48.1, 12.7, and 12.7%, respectively, compared with the control diet (Table 5, $P<0.05$).
There was linear effect in the inclusion cost of corn and soybean meal, while for soybean oil and dicalcium phosphate, there was cubic effect in the inclusion cost (Table 6, *P*<0.05). In each 500 FTU/kg of phytase inclusion, corn inclusion cost increased R$ 0.035/bird, while soybean meal, soybean oil, and dicalcium phosphate inclusion cost presented reduction of R$ 0.051/bird, R$ 0.145/bird, and R$ 0.047/bird, respectively. Limestone inclusion cost increased R$ 0.0004/bird, being disregarded in economic assessment.

### 4. Discussion

Studies demonstrating dietary inclusion of phytase superdosing in broiler diets with reduction in the nutritional matrix of aP (Pieniazek et al., 2017); aP and Ca (Poernama et al., 2020; Cowieson et al., 2015); aP, Ca, and AME (Ennis et al., 2020), and its combinations with other enzymes in diets with...
reduction in the nutritional matrix of aP, Ca, AME, and digestible aminoacids (Jlali et al., 2020) have been reported recently with benefits on growth performance and mineral deposition. In addition, results of studies using different sources of phytase (i.e., bacterial and fungal phytases) in non-ruminant diets observed in the last years were presented in reviews from Adeola and Cowieson (2011), Cowieson et al. (2011), and Walk et al. (2014). The results of this study showed that broilers fed diets with reduction in the nutritional matrix of Ca and P, supplemented with E. coli 6-phytase presented similar growth performance (Zhou et al., 2008; Poernama et al., 2020) and tibia ash (Zhou et al., 2008) when compared with broilers fed the positive control diet.

In this study, the evaluated diets presented reduction not only of Ca and P, but also of ME and CP. It is known that phytate dephosphorylation reduces chelating capacity and increases digestibility not only of Ca and P, but also of energy and amino acids (Cowieson et al., 2006), in addition to nutrients such as Ca, Fe, Zn (Carnovale et al., 1988), and proteins (Thompson, 1993; Slominski, 2011) in the digestive tract of monogastric animals (Slominski, 2011). Several studies have demonstrated the efficacy of phytase, alone or in combination with other enzymes, to provide bird performance and uniformity when subjected to nutrient-deficient diets (Ravindran et al., 2000; Cowieson and Adeola, 2005; Cowieson et al., 2006; Jlali et al., 2020). Thus, phytase, in addition to providing Ca and P, also increases the digestibility of energy and amino acids (Sandberg et al., 1993; Ravindran et al., 2001) that were previously encapsulated and, therefore, unavailable (Liebert et al., 1993). Thus, if on the one hand, the increase of amino acids and energy liberation improve broiler performance, on the other hand, the increase of mineral liberation improves bone mineralization. The results of this study agree with Freitas et al. (2019), who observed no differences in broiler growth performance nor in tibia ashes when compared with 500, 1000, and 1500 FTU/kg of phytase included in diets with reduction in the nutritional matrix of aP, Ca, and Na. Thus, similar results to bone mineralization could be assigned to

### Table 3 - Total nutrition cost (R$/bird) in scenarios of medium (Med), low (Min), and high (Max) price of corn (Co) and soybean meal (SB) in the sale of broilers subjected to diets supplemented with 500, 1000, and 1500 FTU/kg of phytase

|                   | Control | 500    | 1000   | 1500   | SEM | P-value | Linear  | Quadratic |
|-------------------|---------|--------|--------|--------|-----|---------|---------|-----------|
| MedCo + MedSB     | 4.80    | 4.61   | 4.62   | 4.53   | 0.0352 | <0.0001 | 0.170  |
| MinCo + MedSB     | 4.21    | 4.01   | 4.00   | 3.91   | 0.0304 | <0.0001 | 0.065  |
| MaxCo + MedSB     | 5.92    | 5.76   | 5.80   | 5.71   | 0.0443 | 0.004   | 0.499  |
| MedCo + MinSB     | 4.13    | 3.97   | 3.98   | 3.90   | 0.0303 | <0.0001 | 0.176  |
| MinCo + MinSB     | 3.55    | 3.36   | 3.36   | 3.28   | 0.0257 | <0.0001 | 0.052  |
| MaxCo + MinSB     | 5.25    | 5.12   | 5.16   | 5.08   | 0.0395 | 0.007   | 0.532  |
| MedCo + MaxSB     | 5.74    | 5.52   | 5.53   | 5.43   | 0.0419 | <0.0001 | 0.181  |
| MinCo + MaxSB     | 5.16    | 4.92   | 4.92   | 4.81   | 0.0371 | <0.0001 | 0.081  |
| MaxCo + MaxSB     | 6.86    | 6.67   | 6.72   | 6.61   | 0.0511 | <0.0001 | 0.437  |

SEM - standard error of the mean; aP - available phosphorus; ME - metabolizable energy.

Control diet with adequate levels of Ca and P; 500 = control diet with reduced levels of Ca and P, supplemented with 500 FTU/kg of phytase; 1000 = control diet with reduced levels of Ca and P, supplemented with 1000 FTU/kg of phytase; 1500 = control diet with reduced levels of Ca and P, supplemented with 1500 FTU/kg of phytase.

Corn and soybean meal price scenarios (respectively):

- Medium and medium (MedCo + MedSB): Y = 4.75773 - 0.00015888x, R² = 0.192; medium and low (MinCo + MedSB): Y = 4.17044 - 0.00008112x, R² = 0.292; high and medium (MaxCo + MedSB): Y = 5.88622 - 0.00011767x, R² = 0.072; medium and low (MedCo + MinSB): Y = 4.99876 - 0.00014120x, R² = 0.202; low and low (MinCo + MinSB): Y = 3.51079 - 0.00016201x, R² = 0.314; high and high (MaxCo + MaxSB): Y = 5.22630 - 0.00009888x, R² = 0.064; medium and high (MedCo + MaxSB): Y = 5.69547 - 0.00010675x, R² = 0.188; low and high (MinCo + MaxSB): Y = 5.10765 - 0.00020483x, R² = 0.268; high and high (MaxCo + MaxSB): Y = 6.82313 - 0.00014458x, R² = 0.082.

Commercial dollar = R$ 3.28.

Values based on total average intake/bird.

Reduction in the dietary treatments:

- 1 to 21 d: 500 = ME, 23 kcal; CP, 0.23%; Ca, 0.10%; aP, 0.09%; 1000 = ME, 29 kcal; CP, 0.29%; Ca, 0.13%; aP, 0.12%; 1500 = ME, 34 kcal; CP, 0.34%; Ca, 0.15%; aP, 0.14%.
- 22 to 42 d: 500 = ME, 23 kcal; CP, 0.24%; Ca, 0.11%; aP, 0.10%; 1000 = ME, 30 kcal; CP, 0.31%; Ca, 0.14%; aP, 0.13%; 1500 = ME, 35 kcal; CP, 0.36%; Ca, 0.16%; aP, 0.15%.
greater dephosphorylation of phytic acid and, consequently, to greater availability of P and Ca to the organism even in diets with large aP and Ca reductions.

The greater nutrient liberation can also be linked to similar carcass and legs yield among the dietary treatments. To our knowledge, there is little information about the combination among the use of phytase superdosing in broiler diets with reduction of aP, Ca, ME, and CP in the nutritional matrix and its effects on carcass characteristics of broilers described in the scientific literature. Recently, Freitas et al. (2019) observed no difference in broiler quantitative carcass characteristics when compared with phytase supplementation of 500, 1000, and 1500 FTU/kg of diet.
aP, Ca, and Na in the nutritional matrix. In this study, the average carcass yield was 71.5%. The same authors observed carcass yield of 75.4% in diets with reduction of 0.150 and 0.165% of Ca and aP, respectively, demonstrating a lower carcass yield of 3.9 percentual points among the results. The results of the present study are supported by Campasino et al. (2014), who observed carcass yield of 71.75% of broilers fed diets reduced in 0.14 and 0.13% of Ca and aP, respectively, and supplemented with 1600 FTU/kg of phytase.

Also, similar to this study, Campasino et al. (2014) observed higher breast yield in broilers subjected to diets with 1600 FTU/kg of phytase when compared with birds fed the control diet, reaching 22.19% of breast yield. In the present study, breast yield reached 33.68%, demonstrating 11.49 percentual points higher than the results proposed by these authors. According to Rosen (2002), the preponderant factor influencing the response of phytase supplementation is the dose used. Thus, with the increase in dietary phytase supplementation of 1500 FTU/kg, there may have been an increase in the release not only of Ca and P, but also of energy and protein, which are linked to phytic acid. The complete dephosphorylation of dietary phytic acid prevents it from aggregating to cationic components and forming insoluble complexes and myo-inositol liberation (Walk et al., 2014; Lee and Bedford, 2016). Knowing that current broiler lines have been developed for greater deposition of breast musculature, it is possible that the myo-inositol, resulting from the breakdown of phytate by the action performed by phytase superdosing, has been used for muscle deposition, mainly in the breast region.

Other benefit that should be correlated to the use of phytase superdosing as nutritional strategy is linked to the better economic performance in the broiler production system that uses it. To assess the economic performance in livestock systems, Gameiro (2009) suggested the use of nutrition cost, gross margin, and profitability measurement to compare different productive strategies. In addition,

### Table 4 - Gross margin (R$/bird) in scenarios of medium (Med), low (Min), and high (Max) price of corn (Co) and soybean meal (SB) in the sale of broilers subjected to diets supplemented with 500, 1000, and 1500 FTU/kg of phytase

| Control          | 500  | 1000 | 1500 | SEM  | P-value | Linear | Quadratic |
|------------------|------|------|------|------|---------|--------|-----------|
| MedCo + MedSB    | 2.96 | 3.13 | 3.12 | 3.18 | 0.038   | 0.000  | 0.185     |
| MinCo + MedSB    | 3.55 | 3.73 | 3.74 | 3.80 | 0.040   | <0.0001| 0.132     |
| MaxCo + MedSB    | 1.84 | 1.97 | 1.94 | 2.00 | 0.035   | 0.006  | 0.368     |
| MedCo + MinSB    | 3.63 | 3.77 | 3.76 | 3.81 | 0.039   | 0.002  | 0.276     |
| MinCo + MinSB    | 4.21 | 4.37 | 4.38 | 4.43 | 0.042   | 0.001  | 0.209     |
| MaxCo + MinSB    | 2.51 | 2.61 | 2.58 | 2.64 | 0.036   | 0.031  | 0.485     |
| MedCo + MaxSB    | 2.02 | 2.22 | 2.21 | 2.28 | 0.036   | <0.0001| 0.100     |
| MinCo + MaxSB    | 2.60 | 2.82 | 2.82 | 2.90 | 0.037   | <0.0001| 0.070     |
| MaxCo + MaxSB    | 0.90 | 1.06 | 1.03 | 1.10 | 0.035   | 0.001  | 0.247     |

SEM - standard error of the mean; aP - available phosphorus; ME - metabolizable energy. Control diet with adequate levels of Ca and P; 500 = control diet with reduced levels of Ca and P, supplemented with 500 FTU/kg of phytase; 1000 = control diet with reduced levels of Ca and P, supplemented with 1000 FTU/kg of phytase; 1500 = control diet with reduced levels of Ca and P, supplemented with 1500 FTU/kg of phytase.

Corn and soybean meal price scenarios, respectively:
- Medium and medium (MedCo + MedSB): Y = 2.99992 + 0.00013044x, R² = 0.124;
- Low and medium (MinCo + MedSB): Y = 3.58827 + 0.00015237x, R² = 0.151;
- High and medium (MaxCo + MedSB): Y = 1.87167 + 0.00008948x, R² = 0.066;
- Medium and low (MedCo + MinSB): Y = 3.64068 + 0.00011028x, R² = 0.083;
- Low and low (MinCo + MinSB): Y = 2.42407 + 0.00013727x, R² = 0.107;
- High and high (MaxCo + MaxSB): Y = 2.53140 + 0.00007092x, R² = 0.037;
- Medium and high (MedCo + MaxSB): Y = 2.65174 + 0.00017863x, R² = 0.216;
- High and high (MaxCo + MaxSB): Y = 0.93518 + 0.00016262x, R² = 0.110.

Commercial dollar = R$ 3.28.

Values based on total average intake/bird.

Price paid per kg/live bird = R$ 2.78 (Average weight × Price paid per kg/live bird).

Gross margin = price received/live bird – Cost of diet.

Reduction in dietary treatments:
- 1 to 21 d: 500 = ME, 23 kcal; CP, 0.23%; Ca, 0.10%; aP, 0.09%; 1000 = ME, 29 kcal; CP, 0.29%; Ca, 0.13%; aP, 0.12%; 1500 = ME, 34 kcal; CP, 0.34%; Ca, 0.15%; aP, 0.14%.
- 22 to 42 d: 500 = ME, 23 kcal; CP, 0.24%; Ca, 0.11%; aP, 0.10%; 1000 = ME, 30 kcal; CP, 0.31%; Ca, 0.14%; aP, 0.13%; 1500 = ME, 35 kcal; CP, 0.36%; Ca, 0.16%; aP, 0.15%.
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Table 5 - Estimated net margin (R$/bird) in scenarios of medium (Med), low (Min), and high (Max) price of corn (Co) and soybean meal (SB) in the sale of broilers subjected to diets supplemented with 500, 1000, and 1500 FTU/kg of phytase

|                  | Control | 500    | 1000   | 1500   | SEM   | Linear | Quadratic |
|------------------|---------|--------|--------|--------|-------|--------|-----------|
| MedCo + MedSB    | 0.45    | 0.62   | 0.61   | 0.67   | 0.038 | 0.000  | 0.185     |
| MinCo + MedSB    | 1.04    | 1.22   | 1.23   | 1.29   | 0.040 | <0.0001| 0.132     |
| MaxCo + MedSB    | -0.67   | -0.54  | -0.57  | -0.51  | 0.035 | 0.006  | 0.368     |
| MedCo + MinSB    | 1.12    | 1.26   | 1.25   | 1.30   | 0.040 | 0.002   | 0.276     |
| MinCo + MinSB    | 1.70    | 1.86   | 1.87   | 1.92   | 0.042 | 0.001   | 0.209     |
| MaxCo + MinSB    | 0.00    | 0.10   | 0.07   | 0.13   | 0.036 | 0.031   | 0.485     |
| MedCo + MaxSB    | -0.49   | -0.29  | -0.30  | -0.23  | 0.036 | <0.0001| 0.100     |
| MinCo + MaxSB    | 0.09    | 0.31   | 0.31   | 0.39   | 0.037 | <0.0001| 0.070     |
| MaxCo + MaxSB    | -1.61   | -1.45  | -1.48  | -1.41  | 0.035 | 0.001   | 0.247     |

SEM - standard error of the mean; aP - available phosphorus; ME - metabolizable energy. Control diet with adequate levels of Ca and P; 500 = control diet with reduced levels of Ca and P, supplemented with 500 FTU/kg of phytase; 1000 = control diet with reduced levels of Ca and P, supplemented with 1000 FTU/kg of phytase; 1500 = control diet with reduced levels of Ca and P, supplemented with 1500 FTU/kg of phytase. Corn and soybean meal price scenarios, respectively: Medium and medium (MedCo + MedSB): Y = 0.48992 + 0.00013044x, R² = 0.124; low and medium (MinCo + MedSB): Y = 1.07827 + 0.00015237x, R² = 0.151; high and medium (MaxCo + MedSB): Y = -0.63833 + 0.00008948x, R² = 0.066; medium and low (MedCo + MinSB): Y = 1.15086 + 0.00011028x, R² = 0.083; low and low (MinCo + MinSB): Y = 1.73807 + 0.00013277x, R² = 0.107; high and low (MaxCo + MinSB): Y = 0.02140 + 0.00007092x, R² = 0.037; medium and high (MedCo + MaxSB): Y = -0.44645 + 0.00015663x, R² = 0.184; low and high (MinCo + MaxSB): Y = 0.14174 + 0.00017863x, R² = 0.216; high and high (MaxCo + MaxSB): Y = -1.57482 + 0.00011622x, R² = 0.110. Commercial dollar = R$ 3.28. Values based on total average intake/bird. Price paid per kg/live bird = R$ 2.78 (Average weight × Price paid per kg/live bird). Estimated net margin = Price received/live bird − (Total cost of diet/bird + price paid/day old broiler chick + variable costs/bird); variable costs estimated from the percentage participation of the variables, provided by ICPFrangos for June 2016. Reduction in the dietary treatments: 1 to 21 d: 500 = ME, 23 kcal; CP, 0.23%; Ca, 0.10%; aP, 0.09%; 1000 = ME, 29 kcal; CP, 0.29%; Ca, 0.13%; aP, 0.12%; 1500 = ME, 34 kcal; CP, 0.34%; Ca, 0.15%; aP, 0.14%. 22 to 42 d: 500 = ME, 23 kcal; CP, 0.24%; Ca, 0.11%; aP, 0.10%; 1000 = ME, 30 kcal; CP, 0.31%; Ca, 0.14%; aP, 0.13%; 1500 = ME, 35 kcal; CP, 0.36%; Ca, 0.16%; aP, 0.15%.

Table 6 - Impact of the ingredients on the total nutritional cost of broiler diets supplemented with different phytase levels

|                  | Control | 500    | 1000   | 1500   | SEM   | Linear | Quadratic | Cubic |
|------------------|---------|--------|--------|--------|-------|--------|-----------|-------|
| Corn (R$/bird)   | 1.8684  | 1.9275 | 1.9702 | 1.9696 | 0.01528| <0.0001| 0.058     | 0.703 |
| Soybean meal (R$/bird) | 1.9108  | 1.8404 | 1.8493 | 1.8185 | 0.01375| 0.007  | 0.042     | 0.063 |
| Soybean oil (R$/bird) | 0.3366  | 0.1920 | 0.1538 | 0.1195 | 0.00162| <0.0001| <0.0001   | <0.0001|
| Dicalcium phosphate (R$/bird) | 0.1145  | 0.0673 | 0.0536 | 0.0352 | 0.00046| <0.0001| <0.0001   | <0.0001|
| Limestone (R$/bird)  | 0.0045  | 0.0049 | 0.0051 | 0.0056 | 0.00044| <0.0001| 0.018     | 0.016 |

SEM - standard error of the mean; aP - available phosphorus; ME - metabolizable energy. Control diet with adequate levels of Ca and P; 500 = control diet with reduced levels of Ca and P, supplemented with 500 FTU/kg of phytase; 1000 = control diet with reduced levels of Ca and P, supplemented with 1000 FTU/kg of phytase; 1500 = control diet with reduced levels of Ca and P, supplemented with 1500 FTU/kg of phytase. Equations to corn: Y = 1.88170 + 0.000069200x, R² = 0.195; soybean meal: Y = 1.91084 − 0.00029959x + 3.9665E−7x², R² = 0.180; soybean oil: Y = 0.33660 − 0.00046427x + 4.1843E−7x² − 1.3694E−10x³, R² = 0.989; dicalcium phosphate: Y = 0.11454 − 0.00015373x + 1.4404E−7x³ − 5.1212E−11x⁴, R² = 0.989; limestone: Y = 0.00451 + 0.00000139x − 1.388E−9x² + 6.1933E−13x³, R² = 0.772. Commercial dollar = R$ 3.28. Values based on total average intake/bird. Reduction in the dietary treatments: 1 to 21 d: 500 = ME, 23 kcal; CP, 0.23%; Ca, 0.10%; aP, 0.09%; 1000 = ME, 29 kcal; CP, 0.29%; Ca, 0.13%; aP, 0.12%; 1500 = ME, 34 kcal; CP, 0.34%; Ca, 0.15%; aP, 0.14%. 22 to 42 d: 500 = ME, 23 kcal; CP, 0.24%; Ca, 0.11%; aP, 0.10%; 1000 = ME, 30 kcal; CP, 0.31%; Ca, 0.14%; aP, 0.13%; 1500 = ME, 35 kcal; CP, 0.36%; Ca, 0.16%; aP, 0.15%.
it is important to assess the economic performance in different scenarios of ingredient prices. Ideally, scenarios of medium, high, and low price of the mainly energetic and proteic ingredients (i.e., corn and soybean meal) could be proposed to check the effectiveness of the given strategy in scenarios that hypothetically would not favor its use. In this study, nine scenarios were proposed considering medium, high, and low prices of corn and soybean meal. As expected, the increase of phytase inclusion allowed a reduction in TNC and an increase in GM and estimated NM in whole scenarios. The greater decrease in TNC was observed in scenario of low price of corn and high price of soybean meal (R$ 0.10/bird), while the lowest decrease in TNC was observed in scenarios of high price of corn and low price of soybean meal (R$ 0.049/bird), for each 500 FTU/kg of phytase inclusion. Moreover, as the growth performance of broilers was similar between treatments (mainly final body weight and feed intake), the highest (R$ 0.30/bird) and the lowest (R$ 0.13/bird) GM per bird were observed in the same scenarios of low price of corn and high price of soybean meal and high price of corn and low price of soybean meal, respectively. As expected, the same differences mentioned above were observed for the total estimated cost per bird, due to the inclusion of the estimated costs of other variables that affect production costs of broilers (e.g., electricity, heating, bedding, etc.) in the calculation. The value generated was added to the other costs and expressed per bird, becoming a constant. Thus, scenarios of high prices of soybean meal presented greater reduction in TNC and greater GM values and estimated NM. This was possible because the impact on the economic performance when decreasing soybean meal inclusion when high prices are practiced becomes more effective than when practiced in scenarios of low prices of soybean meal. However, there were no profits in the scenarios of high and medium prices of corn and soybean meal due to the very high TNC. In this scenario, the ingredient reductions plus phytase inclusion showed no beneficial effects on the economic indicator.

The results of the economic performance in the use of dietary phytase in broiler diets agree with those of Selle et al. (2003) and Santos et al. (2008). Both studies reported lower total cost when diet was supplemented with phytase and nutritionally reduced in Ca, P, ME, and CP. In addition, Santos et al. (2008) found a greater bioeconomic index for diets with phytase. Kies et al. (2001) proposed that, depending on some factors, the use of phytase can reduce feed cost by US$ 3.00 per ton. In this study, comparing diets with 1000 and 1500 FTU/kg to 500 FTU/kg of phytase supplementation, the nutrient reductions suggested in the nutritional matrix implied a decrease of US$ 3.58 and US$ 6.12 per ton, respectively (US$ 1.00 = R$ 3.28). For the present study, the ingredients that most affected the total cost of the diet were soybean meal and soybean oil. To achieve the better economic performance, all nutrition strategies were based on phytase use and reduction of aP, Ca, ME, and CP in the nutritional matrix by means of reduction of dicalcium phosphate, limestone, soybean oil, and soybean meal, respectively. For example, if one considers the scenario of medium prices of corn and soybean meal together, the reduction of ingredients promotes a decrease of R$ 0.079/bird in TNC for each 500 FTU/kg of dietary phytase inclusion. Moreover, the lower values for the inclusion of these were presented in the diets supplemented with 1500 FTU/kg. As suggested by Cowieson et al. (2017), supplementation with phytase superdosing is advantageous when the value of released nutrients is compared to the cost of phytase inclusion, which can be explained by changes in certain nutrients such as Ca, P, energy, and amino acids.

In summary, the search for strategies to increase the profitability in the production of broiler chickens is constant. The alternative proposed by the present study would be the reduction in the cost of feeding program adopted. However, drastic nutritional reductions can directly impact animal performance by reducing the availability of nutrients. Nevertheless, phytase supplementation is a good alternative to alleviate dietary deficiencies. In the present study, the efficacy of phytase superdosing can be observed (1500 FTU/kg) in a drastic nutrient reduction in the nutritional matrix. Possibly, this is because the total dephosphorylation of phytic acid is evidenced not only by similarity in the results of bone ash but also by performance and carcass yield. This may have been a result of a greater release and action of myo-inositol on muscle deposition. Another point to be addressed would correlate the ingredient and nutritional reductions proposed by the study and economic results. In the present scenario of modern poultry, reduction in costs or increase in revenue, even if small as...
demonstrated in the present study, are significant, because of the large volume, amount of capital generated, and number of broiler chickens slaughtered. Thus, it can be observed that, in addition to the reduction of the inorganic P source, the decrease of soybean meal and soybean oil—energy and protein sources used in the feeding program—, was a potential means for reducing diet costs. Also, the impact of ingredients in the nutrition cost demonstrated that dietary phytase inclusion allowed for reduction of the expensive ingredient even increasing the inclusion of some ingredients as corn and limestone. However, its cost is cheaper in comparison to reduced ingredients such as soybean meal, soybean oil, and phosphate dicalcium.

5. Conclusions

For each 500 FTU/kg of dietary phytase included in the diet with reduction of calcium, phosphorus, metabolizable energy, and crude protein in the nutritional matrix, the total nutrition cost decreased R$ 0.072/bird, R$ 0.079/bird, and R$ 0.081/bird in scenarios of high, medium, and low prices of corn and soybean meal, respectively, which allowed an increase in the estimated net margin of R$ 0.20/bird, R$ 0.22/bird, and R$ 0.22/bird in the same scenarios.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: R.A. Nacimento. Data curation: R.A. Nacimento and P.H. Pelissari. Formal analysis: R.A. Nacimento. Funding acquisition: U.R.T. Moraes, J.C. Gonçalves, N. Wen and L.F. Araújo. Investigation: R.A. Nacimento, P.H. Pelissari, U.R.T. Moraes, J.C. Gonçalves and N. Wen. Methodology: U.R.T. Moraes, J.C. Gonçalves, N. Wen, A.H. Gameiro and L.F. Araújo. Project administration: U.R.T. Moraes, J.C. Gonçalves, N. Wen. Supervision: C.S.S. Araújo and L.F. Araújo. Validation: L.F. Araújo. Writing-original draft: R.A. Nacimento, P.H. Pelissari, A.H. Gameiro and L.F. Araújo. Writing-review & editing: R.A. Nacimento, A.H. Gameiro and L.F. Araújo.

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