

# Standardized ileal phosphorus digestibility of meat and bone meal and poultry byproduct meal for broilers

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**ABSTRACT** - Two experiments were conducted using direct method to determine the standardized ileal phosphorus (P) digestibility coefficient (SIPDC) of meat and bone meal (MBM) and poultry byproduct meal (PBM) for broilers. In the first experiment, three MBM from different batches were evaluated, with four treatments (MBMI, MBMII, MBMIII, and a P-free diet) and nine replicates of seven birds each, totaling 252 birds distributed across a completely randomized design. The P-free diet was used to determine endogenous P losses. Titanium dioxide ( $5 \text{ g kg}^{-1}$ ) was used as an indigestible indicator. In the second experiment, three PBM from different batches were evaluated, with 189 birds distributed over three treatments (PBMI, PBMII, and PBMIII) and nine replicates of seven birds each. These two experiments were conducted when birds were 19-22 days old. The birds were euthanized to collect the ileal content. Using the P-free diet, endogenous P losses were determined at  $94.9 \text{ mg kg}^{-1}$  dry matter intake. Standardized ileal P digestibility of MBMI (0.65) and MBMIII (0.69) was higher compared with MBMII (0.49). The SIPDC determined for three PBM sources were similar and 0.96, 0.96, and 0.93 for PBMI, PBMII, and PBMIII, respectively. The present study demonstrates that P in MBM is not as available as it is believed and that the variability between batches should be considered by nutritionists, while PBM can be considered an excellent P source to broiler diets.

**Keywords:** direct method, meat and bone meal, poultry byproduct meal

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## 1. Introduction

Phosphorus (P) is an essential macro-mineral for birds that plays an important role in the development and maintenance of the animal's skeletal system. It is also an important component of phosphate in DNA and RNA molecules (Adedokun and Adeola, 2013; Nie et al., 2013).

Inorganic P sources are finite, costly, and potential environmental pollutants. For that reason, there is an increasing interest in alternative P sources to be used in animal feed (Neset and Cordell, 2012). For each tonne of meat prepared for human consumption, approximately 300 kg is discarded as inedible, and 200 kg is converted into animal meal (Lesson and Summers, 1997). Meat and bone meal (MBM) and poultry byproduct meal (PBM) are largely produced and available to be used in animal feed industries (ABRA, 2018). A high variability exists in the nutritional composition of these ingredients, owing to the characteristics of the material used, the way it is obtained, and the different sources of meat used (Dozier et al., 2003).

Poultry diets have traditionally been formulated in non-phytic P (nPP) values (NRC, 1994; Rostagno et al., 2017), which is calculated based on total P and phytic P chemical analyses and do not consider the absorptive capacity of a given P source by the bird. Nutritionists have been seeking solutions to more accurately meet the P requirements of animals. Knowledge of P availability for birds is fundamental for efficiently using this mineral. The WPSA (2013) indicated that ileal P digestibility is the preferable method for determining the P availability of ingredients for poultry.

Several studies have been conducted to determine the true digestibility of P from ingredients for birds (Witzig et al., 2018; Verardi et al., 2019), which includes animal byproduct meals (Zarei et al., 2014; Mutucumarana et al., 2015; Mutucumarana and Ravindran, 2016). Among the methodologies applied to determine the digestibility of P, the direct method proved to be viable for evaluating ingredients of animal origin (Mutucumarana and Ravindran, 2016). In the direct method, the test ingredient must be the only source of P in the diet. The digestibility measured is apparent, so it is necessary to make corrections for endogenous losses. Determination of P digestibility values allows the migration of the formulation of nPP content to digestible P contents in broiler diets.

The objective of this research was to determine the coefficients of standardized ileal P digestibility of three meat and bone meal (MBM) sources and three poultry byproduct meal (PBM) sources, with a hypothesis that broilers may have different P absorption response when ingredients of varying nutritional composition are offered.

## 2. Material and Methods

Two experiments were conducted: the first to determine the coefficient of standardized P ileal digestibility (SIPDC) of three MBM and the second to determine the SIPDC of three PBM for broilers.

Both experiments were approved by the Ethics Committee for the Use of Animals (CEUA), approved under case numbers 23083.008228/2018-16 and 23083.021049/2018-66. The experiments were conducted in Seropédica (22°44'29" S, 43°52'19" W, and 36 m altitude), in the state of Rio de Janeiro, Brazil.

### 2.1. Pre-experimental period

For each experiment, 400 male day-old broilers (Cobb 500) were purchased. The birds were raised in floor pens and fed a ration formulated to meet the nutritional requirements, according to the Brazilian tables for poultry and swine (Table 1) (Rostagno et al., 2017).

### 2.2. Meat and bone meal experiment

Meat and bone meals were purchased from a local supplier (Grande Rio Reciclagem Ambiental, Nova Iguaçu, RJ, Brazil). Three meals from different manufacturing batches (called MBMI, MBMII, and MBMIII) were purchased to obtain variability in the composition of ingredients. The MBM were analyzed to determine their chemical composition.

Four diets were prepared for this experiment (Table 2). A purified diet free of P and calcium (Ca), was formulated to determine endogenous P loss. Except in the P-free diet, MBM served as sole dietary P source. The diets were formulated to contain the nPP content close to requirements of the birds (4 g kg<sup>-1</sup>) and contained 58.1, 56.1, and 53.2 g kg<sup>-1</sup> of MBMI, MBMII, and MBMIII, respectively. Titanium dioxide (TiO<sub>2</sub>) was added to all diets at a level of 5 g kg<sup>-1</sup> as an indigestible indicator.

The design used was completely randomized, with the four treatments (experimental diets) consisting of nine replicates. Each replicate had seven birds; in total, there were 252 birds (606±15.3 g) and 36 experimental units used in the experiment. These birds were selected at 14 days of age based on their average weight. They were distributed randomly in metabolic cages where a cage served as the experimental unit. Each experimental cage was equipped with a 90-cm trough-type feeder and an automatic cup drinker. The dimensions of each cage were 90 cm long, 45 cm wide, and 40 cm high.

**Table 1** - Pre-experimental diet provided to birds up to 18 days of age

| Item   | g kg <sup>-1</sup> |
|--|--------------------|
| Ingredient                                   |                    |
| Corn   | 506.6              |
| Soybean meal                                 | 422.0              |
| Soybean oil                                  | 33.6               |
| Dicalcium phosphate                          | 16.7               |
| Limestone                                    | 8.1                |
| Salt   | 5.1                |
| DL-methionine                                | 3.2                |
| L-lysine HCl                                 | 1.1                |
| Mineral premix <sup>1</sup>                  | 1.0                |
| Vitamin premix <sup>2</sup>                  | 1.0                |
| Choline chloride                             | 0.83               |
| L-threonine                                  | 0.47               |
| Butylated hydroxytoluene                     | 0.1                |
| Calculated nutrients                         |                    |
| Metabolizable energy (cal g <sup>-1</sup> )  | 2,975              |
| Crude protein (g kg <sup>-1</sup> )          | 233.1              |
| Calcium (g kg <sup>-1</sup> )                | 8.78               |
| Non-phytate phosphorus (g kg <sup>-1</sup> ) | 4.19               |
| Sodium (g kg <sup>-1</sup> )                 | 2.18               |
| Digestible lysine (g kg <sup>-1</sup> )      | 12.52              |
| Digestible methionine (g kg <sup>-1</sup> )  | 6.27               |
| Digestible Met+Cys (g kg <sup>-1</sup> )     | 9.37               |
| Digestible threonine (g kg <sup>-1</sup> )   | 8.35               |
| Digestible tryptophan (g kg <sup>-1</sup> )  | 3.00               |
| Digestible valine (g kg <sup>-1</sup> )      | 11.94              |

<sup>1</sup> Composition per kg of product: iron (min), 60 g kg<sup>-1</sup>; copper (min), 13 g kg<sup>-1</sup>; manganese (min), 120 g kg<sup>-1</sup>; zinc (min), 100 g kg<sup>-1</sup>; iodine (min), 2,500 mg kg<sup>-1</sup>; selenium (min), 500 mg kg<sup>-1</sup>.

<sup>2</sup> Composition per kg of product: vitamin A (min), 7,500,000 IU kg<sup>-1</sup>; vitamin D3 (min), 2,500,000 IU kg<sup>-1</sup>; vitamin E (min), 1,200 mg kg<sup>-1</sup>; vitamin K3 (min), 1,200 mg kg<sup>-1</sup>; thiamine (min), 1,500 mg kg<sup>-1</sup>; riboflavin (min), 5,500 mg kg<sup>-1</sup>; pyridoxine (min), 2,000 mg kg<sup>-1</sup>; vitamin B12 (min), 12,000 mcg kg<sup>-1</sup>; niacin, 35 g kg<sup>-1</sup>; calcium pantothenate (min), 10 g kg<sup>-1</sup>; biotin (min), 67 mg kg<sup>-1</sup>.

Birds received the pre-experimental ration until they were 19 days old while they were adapted to the experimental cages.

At 19 days of age, the birds were weighed to determine their initial weight, and the provision of experimental diets began. The diets, in mash form, were given *ad libitum*. Water was freely available throughout the three-day experimental period. When they were 22 days old, the birds were weighed again, and feed intake was computed to calculate the variables weight gain and feed intake. After weighing, birds were euthanized by asphyxiation in CO<sub>2</sub>. Two hours before being euthanized, the birds were encouraged to consume feed through mechanical and visual stimuli.

After asphyxiation, the celomic cavity of the birds was exposed for intestinal collection. Meckel's diverticulum and the ileocecolic junction were identified, these being the structures that define the beginning and end of the ileum of the birds, respectively. The ileum was exposed, and contents of the distal half (the half close to the ileocecolic junction) were collected by applying light pressure and deposited in labeled plastic containers for each experimental unit. Therefore, each sample was obtained from the ileal content of seven birds.

Samples were placed in a ventilated oven (Tecnal, model TE-394, Piracicaba, SP, Brazil) at 55 °C for pre-drying, and were then mechanically crushed using a pistil and ceramic grail until the contents could pass through a sieve with a mesh size of 1 mm. The ground material was kept for later analysis of dry matter (DM), P, and titanium (Ti).

### 2.3. Poultry byproduct meal experiment

Poultry byproduct meal was also obtained from a local supplier (Creaves, São José do Vale do Rio Preto, RJ, Brazil), and consisted of the ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, feet, undeveloped eggs, and intestines, exclusive of feathers. Three PBM were purchased from different manufacturing batches (called PBMI, PBMII, and PBMIII) to obtain variability in the composition of ingredients. The PBM were analyzed to determine their chemical composition.

In this study, three semipurified diets were formulated (Table 2). For the correction of endogenous losses, the data generated using the free diet obtained from the MBM experiment was used. The PBM served as sole dietary P source. The diets were formulated to contain nPP value close to requirement of the birds ( $4 \text{ g kg}^{-1}$ ) and contained 295, 313, and  $307 \text{ g kg}^{-1}$  of PBMI, PBMII, and PBMIII, respectively. The indigestible indicator added to all diets was  $\text{TiO}_2$  at a level of  $5 \text{ g kg}^{-1}$ .

The design used was completely randomized, with three treatments of nine replicates each. Each replicate contained seven birds, such that 189 birds and 27 experimental units were used in total.

When the birds were 14 days old, 189 were selected based on their average weight ( $597 \pm 9.7 \text{ g}$ ) and distributed randomly in metabolic cages to compose the experimental units. The provision of experimental diets started at 19 days of age. At 22 days of age, the birds were euthanized, and ileal contents collected, as previously described. Samples obtained were processed and sent for P, DM, and Ti analyses.

**Table 2** - Experimental diets used to determine the ileal phosphorus digestibility of meat and bone meal (MBM) and poultry byproduct meal (PBM)

| Item                              | Experimental diet |       |       |        |       |       |        |
|-----------------------------------|-------------------|-------|-------|--------|-------|-------|--------|
|                                   | P-free            | MBMI  | MBMII | MBMIII | PBMI  | PBMII | PBMIII |
| Ingredient ( $\text{g kg}^{-1}$ ) |                   |       |       |        |       |       |        |
| Cornstarch                        | 608.7             | 549.0 | 551.0 | 553.9  | 315.8 | 298.6 | 304.6  |
| Sugar                             | 300               | 300   | 300   | 300    | 300   | 300   | 300    |
| MBMI                              | -                 | 58.1  | -     | -      |       |       |        |
| MBMII                             | -                 | -     | 56.1  | -      |       |       |        |
| MBMIII                            | -                 | -     | -     | 53.2   |       |       |        |
| PBMI                              |                   |       |       |        | 295.5 | -     | -      |
| PBMII                             |                   |       |       |        | -     | 313.0 | -      |
| PBMIII                            |                   |       |       |        | -     | -     | 307.0  |
| Dietetic fiber <sup>1</sup>       | 50.0              | 50.0  | 50.0  | 50.0   | 50.0  | 50.0  | 50.0   |
| Soybean oil                       | 20.0              | 20.0  | 20.0  | 20.0   | 20.0  | 20.0  | 20.0   |
| Potassium carbonate               | 9.1               | 10.0  | 10.0  | 10.0   | 7.9   | 7.7   | 7.8    |
| Sodium bicarbonate                | 3.0               | 2.9   | 2.9   | 2.9    | 2.7   | 2.7   | 2.7    |
| Salt                              | 0.17              | 0.25  | 0.25  | 0.25   | 0.05  | 0.04  | 0.04   |
| $\text{TiO}_2$                    | 5.0               | 5.0   | 5.0   | 5.0    | 5.0   | 5.0   | 5.0    |
| Vitamin premix <sup>2</sup>       | 1.0               | 1.0   | 1.0   | 1.0    | 1.0   | 1.0   | 1.0    |
| Mineral premix <sup>3</sup>       | 1.0               | 1.0   | 1.0   | 1.0    | 1.0   | 1.0   | 1.0    |
| Choline chloride                  | 0.54              | 0.54  | 0.54  | 0.54   | 0.54  | 0.54  | 0.54   |
| Calculated values                 |                   |       |       |        |       |       |        |
| Crude protein                     | 3.50              | 24.1  | 23.7  | 21.5   | 187.9 | 201.8 | 197.0  |
| Calcium                           | -                 | 6.4   | 7.3   | 6.7    | 10.4  | 8.2   | 10.9   |
| Non-phytate P                     | -                 | 4.0   | 4.0   | 4.0    | 4.0   | 4.0   | 4.0    |
| Analyzed values                   |                   |       |       |        |       |       |        |
| Phosphorus                        | 0.13              | 4.39  | 4.18  | 4.06   | 3.54  | 3.80  | 3.88   |

<sup>1</sup> Opticell® C5 eubiotic fibre (BIOSEN, Salto, SP, Brazil). Composition: 65% crude fiber.

<sup>2</sup> Composition per kg of product: iron (min),  $60 \text{ g kg}^{-1}$ ; copper (min),  $13 \text{ g kg}^{-1}$ ; manganese (min),  $120 \text{ g kg}^{-1}$ ; zinc (min),  $100 \text{ g kg}^{-1}$ ; iodine (min),  $2,500 \text{ mg kg}^{-1}$ ; selenium (min),  $500 \text{ mg kg}^{-1}$ .

<sup>3</sup> Composition per kg of product: vitamin A (min),  $7,500,000 \text{ IU kg}^{-1}$ ; vitamin D3 (min),  $2,500,000 \text{ IU kg}^{-1}$ ; vitamin E (min),  $1200 \text{ mg kg}^{-1}$ ; vitamin K3 (min),  $1200 \text{ mg kg}^{-1}$ ; thiamine (min),  $1500 \text{ mg kg}^{-1}$ ; riboflavin (min),  $5500 \text{ mg kg}^{-1}$ ; pyridoxine (min),  $2000 \text{ mg kg}^{-1}$ ; vitamin B12 (min),  $12,000 \text{ mcg kg}^{-1}$ ; niacin,  $35 \text{ g kg}^{-1}$ ; calcium pantothenate (min),  $10 \text{ g kg}^{-1}$ ; biotin (min),  $67 \text{ mg kg}^{-1}$ .

## 2.4. Chemical analysis

The DM contents of MBM and PBM sources, experimental diets, and ileal digesta were determined by drying the ileal contents in a sterilization oven for 24 h at 105 °C (method 930.15; AOAC, 2005) and weighing these after allowing them to stand for 1 h in a desiccator. The crude protein (CP) was determined using the Kjeldahl method (method 954.01; AOAC, 2005), using a correction factor of 6.25. The fat contents of test ingredients were determined by the Soxhlet method (method 991.36; AOAC, 2005) and extracting it with petroleum ether. Ash was determined by burning porcelain crucibles in a muffle furnace for 4 h after reaching a temperature of 580 °C (method 942.05; AOAC, 2005).

To determine the levels of calcium (Ca) in MBM and PBM sources and P in the test ingredients, ashes of diets and digesta were subjected to acid digestion to prepare the mineral solution (method 965.17; AOAC, 2005). Phosphorus was determined using the colorimetric method (Fiske and Subbarow, 1925), using ammonium molybdate and vitamin C. The standard curve was determined using the  $K_2PO_4$  solution, corresponding to 0.1 mg mL<sup>-1</sup> of P. Five milliliters of acid ammonium molybdate, 1 mL of mineral solution, and 2 mL of 4 % vitamin C were added to a 50-mL flask. The volume was then volumetrically completed, and 6 min were allowed for color development and reading in a UV colorimeter (visible at 725 nm).

The Ti contents in the diet and digesta was determined by adapting the colorimetric methodology described by Myers et al. (2004), in which 250 mg of sample was digested with sulfuric acid and a catalytic mixture in a micro Kjeldahl tube, at 370 °C for 1 h. After digestion, 10 mL of hydrogen peroxide 30% and 20 mL of distilled water were added into the tube and the contents were filtered into a 100-mL flask using quantitative rapid filter paper. Once filtered, three drops of hydrogen peroxide were added to obtain an orange color. Reading was performed on a visible UV spectrophotometer at 410 nm. The standard curve was prepared with the same  $TiO_2$  used in the experiment, using 11 tubes, each with an amount of  $TiO_2$  ranging from 0 to 10 mg.

## 2.5. Granulometry

To determine the granulometry of ingredients, 200 g of duplicate samples were inserted in a set of six sieves of different diameters in a sieve shaker (Bertel, Caieras, SP, Brazil) and stirred for 10 min (Zanotto et al., 2016). The weight of the empty sieves and sieves with the retained sample were determined on a precision scale, and the mean geometric diameter (DGM) was calculated for each sample using Granucalc® software (EMBRAPA, 2013).

## 2.6. Calculations

In the direct method, the digestibility of the diet is considered as the digestibility of ingredient, once the ingredient is the only P source in the diet. As only the apparent digestibility is measured, it is necessary to carry out corrections and calculations of endogenous losses (Mutucumarana and Ravindran, 2016) to obtain the standardized value.

The coefficient of apparent ileal P digestibility (AIPDC) of the diet was calculated according to the following equation (Anwar et al., 2018):

$$AIPDC = 1 - [(Ti/Tf) \times (Pf/Pi)],$$

in which  $Ti$  is the indicator concentration in the diet,  $Tf$  is the indicator concentration in the ileal digesta,  $Pf$  is the P concentration in the ileal digesta, and  $Pi$  is the P concentration in the diet.

The calculations for the determination of endogenous losses (EPL), in grams lost per kilogram of dry matter intake (g kg<sup>-1</sup> DMI), were carried out according to data obtained from the P-free diet by applying the following equation (Anwar et al., 2018):

$$EPL = P_{digesta} \times (Ti/Tf),$$

in which  $P_{digesta}$  is the P content of digesta in g kg<sup>-1</sup> DM in birds that received the free diet.

The coefficient of standardized ileal P digestibility of P (SIPDC) of the test ingredient was calculated according to the following formula, proposed by Fan et al. (2008):

$$SIPDC = AIPDC + (EPL/Pi)$$

## 2.7. Statistical analysis

The analysis of variance was performed according to the statistical model to a completely randomized design:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}$$

in which  $Y_{ij}$  = observed value for treatment  $i$ , in repetition  $j$ ,  $\mu$  = average of the experiment,  $\beta_i$  = effect of different diets, and  $\varepsilon_{ij}$  = random error associated to each observation.

The performance data and the apparent and standardized digestibility of the experimental diets were subjected to ANOVA at  $\alpha = 0.05$ , using SISVAR software. When significant, the data were analyzed with the Student-Newman-Keuls test.

## 3. Results

### 3.1. Chemical composition of ingredients and experimental diets

The CP of MBM was 404.5 to 422.0 g kg<sup>-1</sup>, the P content varied from 69.8 to 76.2 g kg<sup>-1</sup>, the calcium contents from 111.5 to 131.9 g kg<sup>-1</sup>, and the Ca:P ratio from 1.60 to 1.83 (Table 3). For PBM, CP was 629.2 to 638.3 g kg<sup>-1</sup>, P from 12.8 to 13.5 g kg<sup>-1</sup>, Ca from 26.2 to 35.6 g kg<sup>-1</sup>, and Ca:P ratio from 2.04 to 2.73. Geometric mean diameter of test MBM were close, from 561 to 603  $\mu$ m, while PBM showed higher GMD differences, ranging from 654 to 871  $\mu$ m.

The P-free diet showed a low analyzed P value, only 0.13 g kg<sup>-1</sup> (Table 2). The P levels in the experimental diets ranged from 3.54 to 4.39 g kg<sup>-1</sup>, differing from the values calculated (nPP, 4 g kg<sup>-1</sup>) at -0.46 to 0.39 g kg<sup>-1</sup>.

### 3.2. Broiler performance

Birds that received the experimental diets with MBM lost weight (Table 4); however, there were no significant differences ( $P > 0.05$ ) among treatments. Feed intake was also not affected by the supply of different MBM ( $P > 0.05$ ). Birds that received the experimental diets with viscera meal gained weight, although there were no significant differences among PBM diets ( $P > 0.05$ ).

### 3.3. Phosphorus digestibility

Endogenous P losses were determined at 94.9 mg kg<sup>-1</sup> DMI using the P-free diet.

Diets MBMI and MBMIII showed similar AIPDC and SIPDC, which were higher than those of MBMII ( $P < 0.05$ ). The AIPDC values were 0.6410, 0.4869, and 0.6779, and the SIPDC values were 0.6495, 0.4928, and 0.6858 for MBMI, MBMII, and MBMIII, respectively (Table 5). There were no significant differences in AIPDC and SIPDC of PBM ( $P > 0.05$ ). The AIPDC values were 0.9105, 0.9094, and 0.8919, and the SIPDC were 0.9599, 0.9606, and 0.9339, for PBMI, PBMII and PBMIII, respectively.

**Table 3** - Chemical composition of the meat and bone meal (MBM) and poultry byproduct meal (PBM) used in the study<sup>1</sup>

| Nutrient                             | MBMI  | MBMII | MBMIII | PBMI  | PBMII | PBMIII |
|--------------------------------------|-------|-------|--------|-------|-------|--------|
| Dry matter (g kg <sup>-1</sup> )     | 921.6 | 953.7 | 954.9  | 926.9 | 947.9 | 951.9  |
| Crude protein (g kg <sup>-1</sup> )  | 415.0 | 422.0 | 404.5  | 629.2 | 638.3 | 635.7  |
| Mineral matter (g kg <sup>-1</sup> ) | 399.1 | 425.1 | 443.3  | 87.6  | 80.9  | 83.0   |
| Ether extract (g kg <sup>-1</sup> )  | 95.6  | 95.0  | 94.2   | 83.9  | 80.6  | 58.5   |
| Phosphorus (g kg <sup>-1</sup> )     | 69.8  | 72.2  | 76.2   | 13.5  | 12.8  | 13.0   |
| Calcium (g kg <sup>-1</sup> )        | 111.5 | 131.9 | 128.1  | 35.1  | 26.2  | 35.6   |
| Ca:P ratio                           | 1.60  | 1.83  | 1.68   | 2.60  | 2.04  | 2.73   |
| GMD ( $\mu$ m)                       | 561   | 603   | 579    | 654   | 794   | 871    |

GMD - geometric mean diameter.

<sup>1</sup> Data expressed as fed basis.

**Table 4** - Performance of broilers fed the experimental diets from 19 to 22 days of age

| Diet                              | Weight variation<br>(g bird <sup>-1</sup> day <sup>-1</sup> ) | Intake<br>(g bird <sup>-1</sup> day <sup>-1</sup> ) | P diet<br>(g kg <sup>-1</sup> DM) | P output<br>(g kg <sup>-1</sup> DMI) |
|-----------------------------------|---|---|-----------------------------------|--------------------------------------|
| Meat and bone meal experiment     |   |   |                                   |                                      |
| MBMI                              | -12.77  | 53.34   | 4.39                              | 1.576                                |
| MBMII                             | -13.67  | 54.28   | 4.18                              | 2.145                                |
| MBMIII                            | -13.73  | 54.97   | 4.06                              | 1.308                                |
| SEM                               | 0.589   | 1.67  | -                                 | 0.23                                 |
| P-value                           | 0.449   | 0.789   | -                                 | 0.071                                |
| P-free diet                       | -19.09  | 53.27   | 0.13                              | 0.0949                               |
| Poultry byproduct meal experiment |   |   |                                   |                                      |
| PBMI                              | 38.97   | 78.95   | 3.82                              | 0.342                                |
| PBMII                             | 39.96   | 77.28   | 4.11                              | 0.372                                |
| PBMIII                            | 39.47   | 78.51   | 4.19                              | 0.453                                |
| SEM                               | 1.69  | 1.37  | -                                 | 0.04                                 |
| P-value                           | 0.917   | 0.679   | -                                 | 0.155                                |

DM - dry matter; DMI - DM intake; SEM - standard error of the mean.

**Table 5** - Apparent (AIPDC) and standardized (SIPDC) ileal phosphorus digestibility coefficients from meat and bone meal (MBM) and poultry byproduct meal (PBM)

| Ingredient                        | AIPDC   | SIPDC   |
|-----------------------------------|---------|---------|
| Meat and bone meal experiment     |         |         |
| MBMI                              | 0.6410a | 0.6495a |
| MBMII                             | 0.4869b | 0.4928b |
| MBMIII                            | 0.6779a | 0.6858a |
| SEM                               | 0.049   | 0.049   |
| P-value                           | 0.025   | 0.025   |
| Poultry byproduct meal experiment |         |         |
| PBMI                              | 0.9105  | 0.9599  |
| PBMII                             | 0.9094  | 0.9606  |
| PBMIII                            | 0.8919  | 0.9339  |
| SEM                               | 0.010   | 0.013   |
| P-value                           | 0.358   | 0.291   |

SEM - standard error of the mean.

a,b,c - Means within a column with different letters are significantly different (P<0.05).

## 4. Discussion

The reduction in protein levels coincided with the increase in the ash content of MBM, indicating the variability in the manufacture of this ingredient and indicated how different proportions of soft tissues and bones were used when processing the byproduct into animal meal. There are MBM with CP content from 440 to 620 g kg<sup>-1</sup> and ash from 200 to 400 g kg<sup>-1</sup> reported in literature (Garcia et al., 2006). According to the Compêndio Brasileiro de Alimentação Animal (2017), MBM must have a minimum of 40 g kg<sup>-1</sup> P, and Ca must not exceed 2.2 times the level of P. Therefore, the MBM of the current study fit the desired characteristics.

The CP of the PBM used in this assay ranged from 629.2 to 638.3 g kg<sup>-1</sup>, varying by only 1.5%. According to Silva et al. (2011), the PBM can present values between 544 and 646 g kg<sup>-1</sup> with meals that have a higher protein content considered to be of higher quality. The P levels were close to the variability of 15 to 32 g kg<sup>-1</sup> observed by Dozier et al. (2003).

Due to the high P content of MBM, the inclusion was near 55 g kg<sup>-1</sup> in the test diets. This was reflected in the low protein content of the MBM diet that affected the performance of poultry, since MBM was the only protein source in the experimental diet. Other authors have reported weight loss in birds when

working with semipurified diets to determine digestible P (Mutucumarana et al., 2015). One of the proposed solutions to prevent bird weight loss is to include a protein source that is rich in essential amino acids and poor in P, such as dried egg albumen or casein (WPSA, 2013).

In the PBM evaluation, broilers gained weight during the three-day experimental period. The inclusion of the test ingredient was close to 300 g kg<sup>-1</sup>, and in addition, the PBM diets had high protein content. The values of average weight gain obtained under the experimental diets (39 g bird<sup>-1</sup> day<sup>-1</sup>) were below those described in the Brazilian tables for poultry and swine (78 g bird<sup>-1</sup> day<sup>-1</sup>) (Rostagno et al., 2017), since the provided diet was not balanced to meet the ideal protein profile, with PBM as the only protein source. However, even in studies with an additional supply of 10% dried egg albumen, birds showed weight gain below their genetic potential (Mutucumarana and Ravindran, 2016).

Endogenous P losses were determined at 94.9 mg kg<sup>-1</sup> DMI. Endogenous losses using purified diets have been reported at 272 mg kg<sup>-1</sup> DMI by Rutherford et al. (2002), and 354 mg kg<sup>-1</sup> DMI by Mutucumarana and Ravindran (2016). The factors that can cause these variations in determining endogenous losses are unknown. Using the isotope dilution technique, Dias et al. (2019) determined the endogenous losses in excreta to be between 240 and 420 mg kg<sup>-1</sup> DMI.

The differences found in the MBM P digestibility in this study are difficult to justify. The P content of the MBM was close, with MBMII with intermediate P content (72.2 g kg<sup>-1</sup>) being the one with the lowest digestibility. According to Sulabo and Stein (2013), the higher the P level in MBM, the lower its digestibility for pigs. Another characteristic that might possibly influence the use of P in meal is particle size. Sell and Jeffrey (1996) reported that there was no difference in the bioavailability of P from MBM of different particle sizes for poultry. However, the difference in the granulometry of MBMI, II and III was small, so this does not explain the difference in digestibility of the MBMII.

It should be noted that protein deficiency in diets may have influenced the determination of P digestibility. The results of the present study were close to those described by Mutucumarana and Ravindran (2016), who also used the direct method to determine the SIPDC of four meat and bone flours, but also used dried egg albumen as a protein source in experimental diets. They found SIPDC values of 0.623, 0.617, 0.615, and 0.444. Similarly, the digestibility of P from soybean meal was not influenced by the addition of casein in semipurified diets for broilers (Liu et al., 2014) and pigs (Liu and Adeola, 2016). In determining the ileal P digestibility of monocalcium phosphate in pigs, diets with low protein content impaired P digestibility, while P digestibility was higher in diets with higher protein levels (Xue et al., 2017). Studies comparing the influence of protein in determining the P digestibility for broilers are necessary to elucidate this issue.

The P digestibility of PBM was particularly high, even higher than that described by Zarei et al. (2014). These authors evaluated two PBM for broilers and reported AIPDC from 0.38 to 0.47 and SIPDC from 0.66 to 0.68. The Brazilian tables for poultry and swine (Rostagno et al., 2017) and NRC (2012) suggested P digestibility of 0.53 for poultry and pigs, respectively.

The variability of digestibility found between PBM and MBM may be related to the way in which P is present in these ingredients. In vertebrates, most P (85%-88%) exists as bone P, while 10 to 15% of P is in the organic form, and only a small amount is present as free ions or soluble inorganic phosphates (Hua et al., 2005). According to the same authors, there is a negative correlation between bone P content and CP and lipid values. The PBM used in our experiment had a high protein content and a low ash content when compared with MBM, indicating that the proportion of bone P is lower in PBM, which could partly explain the high levels of digestibility observed. In a study with broilers, van Harn et al. (2017) found greater P digestibility in bone meal that underwent a chemical process to dissolve bone matrix when compared with bone meal *in natura*, which allows us to assume that the P present in the bone matrix is less digestible for poultry.

## 5. Conclusions

The P availability from meat and bone meal is lower than that described in the nutritional composition tables, and poultry byproduct meal can be considered an excellent P source in feed for broilers.



## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: F. Dilelis, L.W. Freitas and C.A.R. Lima. Data curation: F. Dilelis, L.W. Freitas, D.V. Quaresma and T.L. Reis. Formal analysis: F. Dilelis, L.W. Freitas, D.V. Quaresma, N.J.B. Machado, T.L. Reis and C.S. Souza. Funding acquisition: C.A.R. Lima. Investigation: F. Dilelis, D.V. Quaresma, T.L. Reis and C.S. Souza. Methodology: L.W. Freitas, D.V. Quaresma, N.J.B. Machado, C.S. Souza and C.A.R. Lima. Project administration: C.A.R. Lima. Supervision: C.A.R. Lima. Writing-original draft: F. Dilelis, T.L. Reis and C.A.R. Lima. Writing-review & editing: L.W. Freitas, N.J.B. Machado, T.L. Reis and C.S. Souza.

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