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Exogenous enzyme strategies in broiler diets: impacts on performance, nutrient utilisation and metabolic efficiency

Rosa Aparecida Reis de Léo
Doctor Scientiae

VIÇOSA - MINAS GERAIS
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ROSA APARECIDA REIS DE LÉO

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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Assent:

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Dedication

To God, my family, and friends.

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Thank you very much!

Consagre ao Senhor tudo o que você faz, e os seus planos serão bem-sucedidos.
Provérbios 16:3

ABSTRACT

LÉO, Rosa Aparecida Reis de, D.Sc., Universidade Federal de Viçosa, March, 2026. **Exogenous enzyme strategies in broiler diets: impacts on performance, nutrient utilisation and metabolic efficiency**. Adviser: Melissa Izabel Hannas. Co-advisers: Arele Arlindo Calderano, Arele Arlindo Calderano and Jean Kaique Valentim.

This thesis investigated the effects of different exogenous enzyme supplementation strategies on the nutritional and metabolic efficiency of broiler chickens. Two complementary studies were conducted to evaluate phytase superdosing and multi-enzyme supplementation in nutritionally reduced diets containing plant- and animal-derived ingredients. In the first study, the effects of phytase superdosing were evaluated in diets formulated with corn, soybean meal, and meat and bone meal. Increasing phytase inclusion levels improved feed conversion ratio and enhanced bone mineralisation, with linear increases observed in metabolisable energy and nutrient metabolizability coefficients. Supplementation with 2000 and 2500 FTU/kg resulted in greater calcium and phosphorus deposition in bones, increased nutrient utilization by up to 29%, and reduced phosphorus excretion by more than 30%, demonstrating that phytase superdosing remains effective even in diets containing mixed phosphorus sources. The second study evaluated exogenous enzyme supplementation in nutritionally reduced diets, focusing on growth performance, metabolisable energy, and amino acid digestibility. Enzyme inclusion promoted nutritional recovery, increased metabolisable energy values, and improved amino acid digestibility, demonstrating that enzymatic strategies can mitigate the effects of dietary nutrient reduction without compromising bird performance. Collectively, the results demonstrate that exogenous enzyme supplementation, whether through phytase superdosing or multi-enzyme complexes, improves nutrient utilization, metabolic efficiency, and sustainability in broiler production systems. These findings reinforce the role of enzyme technology as a multifunctional nutritional tool capable of optimizing feed efficiency and reducing the environmental impact of modern poultry production.

Keywords: exogenous enzymes; phytase; broiler chickens; nutrient utilization; metabolisable energy; amino acid digestibility; bone mineralisation; sustainability

RESUMO

LÉO, Rosa Aparecida Reis de, D.Sc., Universidade Federal de Viçosa, março de 2026. **Estratégias com enzimas exógenas em dietas para frangos de corte: impactos no desempenho, utilização de nutrientes e eficiência metabólica**. Orientadora: Melissa Izabel Hannas. Coorientadores: Arele Arlindo Calderano, Arele Arlindo Calderano e Jean Kaique Valentim.

Esta tese investigou os efeitos de diferentes estratégias de suplementação com enzimas exógenas sobre a eficiência nutricional e metabólica de frangos de corte. Foram conduzidos dois estudos com o objetivo de avaliar a superdosagem de fitase e a suplementação de complexos enzimáticos em dietas nutricionalmente reduzidas, contendo ingredientes de origem vegetal e animal. No primeiro estudo, foram avaliados os efeitos da superdosagem de fitase em dietas formuladas à base de milho, farelo de soja e farinha de carne e ossos. O aumento dos níveis de inclusão de fitase promoveu melhora na conversão alimentar e aumento da mineralização óssea, com incrementos lineares na energia metabolizável e nos coeficientes de metabolizabilidade dos nutrientes. A suplementação com 2000 e 2500 FTU/kg resultou em maior deposição de cálcio e fósforo nos ossos, aumento da utilização de nutrientes em até 29% e redução da excreção de fósforo superior a 30%, demonstrando que a superdosagem de fitase permanece eficaz mesmo em dietas contendo fontes mistas de fósforo. O segundo estudo avaliou a suplementação de enzimas exógenas em dietas nutricionalmente reduzidas, com foco no desempenho produtivo, energia metabolizável e digestibilidade de aminoácidos. A inclusão enzimática promoveu recuperação nutricional, aumento dos valores de energia metabolizável e melhora na digestibilidade de aminoácidos, evidenciando que estratégias enzimáticas podem mitigar os efeitos da redução nutricional das dietas sem comprometer o desempenho das aves. De forma integrada, os resultados demonstram que a suplementação com enzimas exógenas, seja por meio da superdosagem de fitase ou da utilização de complexos enzimáticos, melhora a utilização de nutrientes, a eficiência metabólica e a sustentabilidade dos sistemas de produção de frangos de corte. Esses achados reforçam o papel da tecnologia enzimática como ferramenta nutricional multifuncional, capaz de otimizar a eficiência alimentar e reduzir o impacto ambiental da produção avícola moderna.

Palavras-chave: enzimas exógenas; fitase; frangos de corte; utilização de nutrientes; energia metabolizável; digestibilidade de aminoácidos; mineralização óssea; sustentabilidade

SUMMARY

INITIAL CONSIDERATIONS

Modern poultry production has become one of the most efficient animal production systems worldwide, supplying a substantial proportion of the global demand for high-quality animal protein. Continuous genetic progress, improvements in management practices, health programs, housing systems, and nutritional strategies have contributed to remarkable advances in growth performance, feed conversion efficiency, and carcass yield. However, the rapid expansion of poultry production has also intensified the need for sustainable production practices capable of meeting increasing consumer demand while minimizing environmental impacts and maintaining economic viability.

Among the various factors influencing poultry productivity, nutrition plays a central role due to its direct effects on bird performance and its substantial contribution to production costs. Feed expenses represent the largest component of broiler production costs, making the efficient utilization of dietary nutrients a major objective for nutritionists and producers. Consequently, nutritional strategies that improve nutrient digestibility and maximize feed efficiency have become increasingly important for maintaining the competitiveness and sustainability of poultry production systems.

Commercial broiler diets are predominantly formulated using plant-derived ingredients, particularly corn and soybean meal, which provide most of the dietary energy and protein required for growth. Despite their nutritional value, these feed ingredients contain several antinutritional factors that can impair nutrient digestion and absorption. Such compounds reduce nutrient availability, increase endogenous nutrient losses, and may negatively affect growth performance and feed efficiency. As a result, overcoming the limitations imposed by antinutritional factors remains a significant challenge in modern poultry nutrition.

Among these compounds, phytate is recognized as one of the most important antinutritional factors present in poultry diets. Phytate serves as the principal storage form of phosphorus in plant tissues, but a large proportion of this phosphorus is unavailable to poultry due to the limited endogenous phytase activity in the gastrointestinal tract. In addition to reducing phosphorus availability, phytate can interact with proteins, amino acids, minerals, and digestive enzymes, forming complexes that further impair nutrient utilization. These interactions may compromise growth performance, reduce feed efficiency, and increase nutrient excretion into the environment.

The introduction of exogenous phytase has transformed poultry nutrition by improving the utilization of phytate-bound phosphorus and reducing the need for inorganic phosphorus supplementation. Beyond increasing phosphorus availability, phytase supplementation has been associated with several additional benefits related to nutrient digestion and metabolism. The

degradation of phytate reduces its antinutritional effects, thereby improving the digestibility of amino acids, increasing mineral utilization, enhancing energy availability, and promoting more efficient nutrient absorption. These responses, often described as extra-phosphoric effects, have expanded the role of phytase from a phosphorus-releasing enzyme to a multifunctional nutritional tool capable of improving overall feed efficiency.

As research in enzyme technology has progressed, the concept of phytase superdosing has gained considerable attention. Superdosing involves the inclusion of phytase at levels substantially higher than those required solely to satisfy phosphorus requirements, aiming to achieve more complete phytate degradation throughout the gastrointestinal tract. This strategy may further reduce the antinutritional effects of phytate and enhance nutrient utilization beyond traditional matrix values. Consequently, phytase superdosing has emerged as a promising nutritional approach for maximizing feed efficiency and supporting sustainable poultry production.

In parallel with the development of phytase technology, other classes of exogenous enzymes have become increasingly important in broiler nutrition. Carbohydrases, proteases, and multi-enzyme complexes are widely used to improve the digestion of feed components that are not fully utilized by poultry. Carbohydrases facilitate the degradation of non-starch polysaccharides and plant cell wall structures, increasing nutrient accessibility and reducing digesta viscosity. Proteases enhance protein hydrolysis, improve amino acid availability, and may reduce the negative effects of protein-associated antinutritional factors. The combined use of different enzyme classes has created opportunities for more efficient nutrient recovery and improved digestive function.

The growing adoption of exogenous enzymes is closely associated with advances in precision nutrition and the search for more sustainable feeding programs. By increasing nutrient availability and improving digestive efficiency, enzyme supplementation allows nutritionists to formulate diets with reduced nutrient specifications while maintaining productive performance. Such approaches can decrease feed costs, improve resource utilization, reduce dependence on non-renewable feed ingredients, and minimize nutrient excretion into the environment. Therefore, enzyme technology represents an important strategy for balancing economic performance with environmental responsibility in modern poultry production.

Despite substantial advances in the understanding of enzyme supplementation, several aspects related to nutrient recovery, digestive responses, and productive performance under different dietary conditions remain incompletely understood. Variations in ingredient composition, nutrient density, enzyme inclusion levels, and dietary formulation strategies may influence the magnitude of enzymatic

responses. Consequently, further investigation is required to better characterize the mechanisms through which exogenous enzymes affect nutrient utilization and to optimize their application under commercial production conditions.

Within this context, the present thesis was developed to investigate different enzymatic strategies aimed at improving nutrient utilization and productive efficiency in broiler chickens. The first chapter evaluates the effects of phytase superdosing in diets formulated with corn, soybean meal, and meat and bone meal, focusing on growth performance, feed efficiency, carcass characteristics, bone mineralization, and nutrient metabolizability. The second chapter examines the supplementation of exogenous enzyme complexes in nutritionally reduced diets, assessing growth performance, metabolizable energy, and amino acid digestibility as indicators of nutrient recovery and productive efficiency.

Collectively, the studies presented in this thesis contribute to the understanding of the physiological, nutritional, and productive responses associated with enzyme supplementation in broiler diets. By integrating information related to nutrient utilization, digestive efficiency, productive performance, and sustainability, this research provides scientific evidence supporting the use of enzyme technology as an effective nutritional strategy for optimizing poultry production and promoting more sustainable and efficient feeding systems.

CHAPTER 1

Phytase superdosing in broiler diets containing animal-derived protein: effects on performance, bone mineralisation and nutrient metabolisability

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Chapter 1: Phytase superdosing in broiler diets containing animal-derived protein: effects on performance, bone mineralisation and nutrient metabolisability

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Abstract:

This study evaluated the effects of phytase superdosing in broiler chicken diets based on corn, soybean meal and meat and bone meal on performance, carcass yield, pectoral myopathies, bone mineral composition and the metabolisability of energy and nutrients. Two experiments were conducted: one focused on performance and the other on nutrient metabolism. In both experiments, birds were randomly assigned to a completely randomised experimental design. The performance trial included 1600 male Cobb 500 broilers, whereas the metabolism trial included 480 birds of the same strain. In the performance trial, birds were allocated to one of eight treatments with 10 replicates of 20 birds per experimental unit from 1 to 42 d of age. In the metabolism trial, each experimental unit consisted of six birds. Linear and quadratic regression analyses were performed to estimate the optimal phytase supplementation level. Higher phytase inclusion levels, particularly 2000 phytase units/kg feed, resulted in greater weight gain and better feed conversion ratio. Diets supplemented with 2000 and 2500 phytase units/kg caused the highest calcium and phosphorus concentrations in tibia bones. In the nutrient metabolisability trial, phytase supplementation promoted linear increases in metabolisability coefficients for gross energy, organic matter and zinc, along with linear reductions in excreted mineral matter and zinc. Overall, phytase superdosing, especially at 2000 and 2500 phytase units/kg, enhanced broiler performance, increased bone mineral deposition, improved nutrient metabolisability by 7 to 29% and reduced phosphorus excretion by up to 31.6%. These findings demonstrated that high-dose phytase can be an effective strategy to improve feed efficiency and nutrient utilisation in broiler diets containing mixed phosphorus sources, including animal-derived ingredients.

Keywords: Available phosphorus; bone quality; enzyme supplementation; nutrient metabolizability

1. Introduction

Poultry nutrition has significantly evolved over the past few decades, driven by the need to optimize production efficiency while mitigating environmental impact. The inclusion of exogenous enzymes, particularly phytase, has proven to be an effective practice for enhancing nutrient digestibility, improving animal performance, and reducing phosphorus excretion (Soares et al., 2024; Gomes et al., 2025). These benefits are especially important in diets based on corn and soybean meal, the main components of broiler chicken feeding, which contain antinutritional compounds, such as phytate, responsible for limiting the absorption of essential minerals like phosphorus (Valente Junior et al., 2024).

Phosphorus is one of the most costly and environmentally sensitive minerals in poultry nutrition, being essential for bone formation, energy metabolism, and various enzymatic reactions (Selle et al., 2023). However, much of the phosphorus in plant ingredients is in the form of phytate, and its availability to monogastric animals is limited, as chickens lack endogenous phytase capable of efficiently hydrolyzing this molecule (Liu et al., 2022). In addition to reducing phosphorus availability, phytate acts as a potent antinutritional factor by complexing minerals such as calcium, zinc, and iron, as well as interacting negatively with proteins and starches, decreasing nutrient digestibility and feed efficiency (Abd El-Hack et al., 2018).

Exogenous phytase degrades phytate, releasing inorganic phosphorus and other nutrients that would otherwise not be available for absorption by the birds (Costa et al., 2024). This results in increase nutrient utilization and greater feed efficiency, reducing the need for inorganic mineral sources in the diet and, consequently, production costs (Souza Sanches, et al., 2025). Furthermore, the use of phytase reduces phosphorus excretion into the environment, contributing to the environmental sustainability of poultry production (Nuamah et al., 2024).

In recent years, the concept of phytase superdosing has emerged as a strategy to maximize these benefits by promoting more complete phytate degradation and exploring its extra-phosphoric effects, which include enhanced digestibility of amino acids, metabolisable energy, and mineral absorption (Kryukov et al., 2021; Li et al., 2024; Paul et al., 2025). Although the effectiveness of phytase in corn and soybean meal-based diets is widely recognized, there is increasing interest in investigating the impact of phytase superdosing (Cowieson et al., 2011), particularly in diets that also include animal-derived ingredients such as meat and bone meal (Zanu et al., 2021).

These ingredients provide high-quality nutrients, but their combination with high levels of phytase has been underexplored (De Léo et al., 2025). According to Sung et al. (2024), phytase supplementation at levels above those traditionally used can offer additional benefits regarding productive performance, feed efficiency, and mineral deposition in broiler chickens. Furthermore, the magnitude of phytase effects may vary depending on the supplementation level, ingredient type, and diet composition, with diets formulated with enhanced nutritional value and mixed phosphorus sources (vegetal and animal) potentially responding differently to phytase superdosing (Sampath et al., 2023; Nuamah et al., 2024).

Although animal-derived ingredients, such as meat and bone meal, provide high-quality nutrients, their interaction with phytase has not yet been widely studied. Most research focuses on plant-based diets, and the variability in animal-derived ingredients (Zanu et al., 2020) makes it difficult to generally predict the effects of phytase superdosing (Nuamah et al., 2024). Further studies are needed to understand how this combination can be optimized and to maximize its benefits for nutrient digestibility and absorption.

Thus, the hypothesis of this study is that the use of high phytase levels in diets formulated with reduced nutritional matrix, combined with meat and bone meal, improves or maintains feed efficiency, productive performance, bone development, energy availability, and mineral digestibility in poultry. Therefore, the objective of this study was to evaluate the effects of phytase superdosing with enhanced nutritional value in diets based on corn-Soybean meal, and meat and bone meal on productive performance, carcass yield, pectoral myopathy, calcium and phosphorus deposition in bones, and nutrient metabolizability.

2. Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (CEUAP-08/2021), and followed the guidelines set by the National Council for the Control of Animal Experimentation (CONCEA).

2.1. *Experiment I* – Performance, Carcass Yield, Cuts, Pectoral Myopathies, and Bone Mineral Composition

2.1.1. Animals and Housing

For this experiment, 1600 male Cobb 500® broiler chicks, 1 day old (44 ± 1 g), were used. The chicks were housed in a conventional shed with lateral curtains, concrete pens (1.0 x 2.0 m), and bedding made of wood shavings. The pens were equipped with trough feeders and nipple drinkers, and feed and water were provided ad libitum throughout the experimental period. The temperature in the experimental shed was controlled, and the lighting program followed the recommendations provided in the Cobb 500 manual (Cobb 500 Manual, 2008).

The chicks were randomly assigned to 8 treatments with 10 replicates of 20 birds, for a 42-day experiment, divided into two phases: the starter phase (1 to 21 days) and the grower phase (22 to 42 days). The phytase used was a commercial 6-phytase product (Natuphos® E 10000 G, BASF, Ludwigshafen, Germany), supplied in granular form, with a guaranteed activity of 10,000 FTU/g. The activity of phytases is commonly expressed in FTU or FYT (Phytase Unit), indicating the amount of phytase capable of releasing 1 μmol of inorganic phosphorus per minute from 0.0051 mol/L of sodium phytate, at pH 5.5 and a temperature of 37°C (ISO 30024).

The experimental treatments (Table 1) included phytase enzyme at different inclusion levels, as follows: Positive Control (PC) with a corn, soybean meal, and meat and bone meal-based diet; Negative Control 1 (NC1) with a reduction of 0.164% calcium (Ca) and 0.163% available phosphorus (P); Negative Control 2 (NC2) with a complete matrix reduction (NC1 + reduction of 0.42% crude protein and 40 Kcal/kg of metabolisable energy); NC2 + Phytase 500 FTU/kg; NC2 + Phytase 1000 FTU/kg; NC2 + Phytase 1500 FTU/kg; NC2 + Phytase 2000 FTU/kg; and NC2 + Phytase 2500 FTU/kg.

All experimental diets were formulated based on corn, soybean meal, and meat and bone meal, following the nutritional recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017), considering the nutritional levels for male broilers with superior average performance (Tables 2 and 3).

The enzymatic recovery of phytase in broiler chicken diets was evaluated using five phytase inclusion levels (500, 1,000, 1,500, 2,000, and 2,500 FTU/kg). Using the colorimetric method MA-110 Rev.0 ISO 30024, conducted at CBO, Valinhos–SP, the following enzymatic activity values were obtained: 480, 1,300, 2,170, 2,900, and 3,040 FTU/kg, respectively.

2.1.2. Productive Performance

At 21 and 42 days of age, all broilers and feed leftovers were weighed to obtain body weight (BW, kg) and feed intake (FI, kg/bird). The average daily weight gain (WG, kg/bird) and feed conversion ratio (FCR, g/g) were calculated based on these data. FCR was corrected for mortality during the experimental period.

2.1.3. Carcass Yield, Cuts, and Bone Composition

At 42 days of age, two birds closest to the average body weight of each experimental unit (EU) were selected and fasted for 12 hours (water provided ad libitum). After fasting, the birds were weighed (pre-slaughter weight – PSW) and euthanized following approved procedures. For carcass yield (CY), the weight of the clean, eviscerated carcass, excluding feet, head, and neck, was considered. The cuts were weighed (breast – B, thigh – T, and drumstick – D), and the yield of cuts (breast yield – BY, thigh yield – TY, and drumstick yield – DY) was calculated as a percentage of the eviscerated carcass. The tibia and the middle toe (phalange) were collected, weighed, and frozen for later drying in an oven at 55°C and subsequent analysis of ash, calcium, and phosphorus (Detmann et al., 2021).

2.1.4. Pectoral Myopathies

After the cuts were obtained, the breast muscles were used for the analysis of pectoral myopathies. To assess the occurrence of white striping (WS), the fillets were classified as normal (score 0), mild (score 1), moderate (score 2), or severe (score 3), based on the presence and size of white striations, according to Kuttapan et al. (2012).

2.2. ***Experiment II*** – Nutrient Metabolizability and Determination of Metabolisable Energy Values of Experimental Diets

2.2.1. Animals and Housing

One-day-old chicks were housed in a conventional masonry shed and raised according to the lineage guidelines until they reached the experimental age of 14 days. The lighting program was 24 hours of light, and the ambient temperature was set at 32ºC during the first week of life and gradually reduced according to the recommendations of the Cobb® lineage manual. A pre-starter diet (1 to 14 days) based on corn and soybean meal was formulated according to the recommendations of Rostagno et al. (2017) and provided ad libitum to the animals. At 14 days of age, 480 male broilers weighing 501g ± 24g were transferred to wire-floored metal battery cages (600 cm² per bird) in a room with controlled environmental conditions and continuous light supply.

The cages were equipped with trough feeders and nipple drinkers, and feed and water were provided ad libitum throughout the experimental period. The birds were randomly assigned to 8 treatments, with 10 replicates of 6 birds per experimental unit (EU), for a 10-day experiment, with five days of adaptation to the diet and five days for fecal collection.

2.2.2. Excreta Collection

The excreta collection method used was total excreta collection. Excreta were collected twice daily (early morning and late afternoon) using aluminum trays lined with plastic placed under the cages. After collection, the excreta were stored in plastic bags, identified by experimental unit, and kept in a freezer until the end of the experimental period. At the end of the experimental period, the excreta were thawed at room temperature, weighed, and homogenized. A 200g sample was taken from each experimental unit for pre-drying at 55ºC for 72 hours in a forced-air oven.

After drying, the feed, ingredient, and excreta samples were sent to the laboratory for evaluation of gross energy, dry matter, crude protein, mineral matter, Ca, P, and Zn

(Sakomura and Rostagno, 2016). Gross energy was determined using an adiabatic bomb calorimeter. Dry matter, crude protein, and mineral matter were analyzed according to Detmann et al., (2021). Calcium, phosphorus, and zinc were determined by spectrophotometry (Detmann et al., 2021).

The following equations were used to calculate metabolisable energy:

$$\text{AME} = \frac{\text{GEingested} - \text{GEexcreted}}{\text{DM ingested}}$$

$$\text{AMEn} = \frac{(\text{GEingested} - \text{GEexcreted})}{\text{DM ingested}} - 8.22 \times \text{NB}$$

Where:

AME = Apparent metabolisable energy, Kcal/g

AMEn = Apparent metabolisable energy corrected for nitrogen balance, Kcal/g

GE ingested = Gross energy ingested, Kcal; GE ingested = GE of the feed (Kcal/kg) x Feed consumption in kg

GE excreted = Gross energy excreted, Kcal; GE excreted = GE of the excrete (Kcal/kg) x Excrete produced in kg

DM ingested = Dry matter ingested, g; DM ingested = DM of the feed x Feed consumption in kg

NB = Nitrogen balance, g; NB= N ingested – N excreted

The calculation of the nutrient metabolizability coefficient (NMC) from total excreta collection was performed using the following equation:

NMC (%) = (Nutrient in feed - Nutrient in excreta) / (Nutrient in feed) × 100

3. Statistical Analyses

Model assumptions were verified, including normality of residuals by the Shapiro-Wilk test, homogeneity of variances by the Bartlett test, and error independence by the Durbin-Watson test. After confirming assumptions, means were subjected to analysis of variance (ANOVA) with a significance level of 5%. When significant effects were observed, means were compared to the positive control using Dunnett's test at the same significance level. Linear and quadratic regressions were performed to estimate the optimal phytase dose.

Data from the evaluation of myopathy incidence were grouped and the percentage of occurrence of each score within each experimental unit was calculated. Subsequently, treatment effects were verified through non-parametric analysis of variance using the Kruskal-Wallis test with Dunn-Bonferroni post hoc correction at a 5% significance level.

4. Results

4.1. Performance

There was a decreasing linear effect (P=0.002) of phytase levels on the feed conversion ratio (FCR) in the 1 to 21-day phase and the total period, with the 2500 FTU phytase level showing the lowest FCR observed, 1.252 g/g and 1.518 g/g, respectively. Treatments with 2000 FTU and 2500 FTU had lower FCR compared to the positive control (PC) by Dunnett's test (P=0.001). A quadratic effect (P=0.002, for both respectively) was observed for weight gain (WG) and body weight (BW) in the 1 to 21-day phase, with a level of 1618.671 FTU of phytase resulting in 1.062 kg of WG and 1.106 kg of BW (Table 4; Figure 1).

4.2. Carcass Yield and Pectoral Myopathies

No effect of treatments was observed for the variables of carcass yield and cuts (Table 5). The analysis of white striping in the breast muscle did not show differences between treatments (P=0.430), suggesting that phytase supplementation, even at high doses, does not influence the occurrence of this specific myopathy (Table 6).

4.3. Bone Mineral Composition

A quadratic effect (P<0.001) of phytase levels was observed on calcium (Ca) concentration in the tibias and a linear increasing effect (P<0.001) on phosphorus (P) concentration in the tibias and Ca (P<0.001) and P (P<0.001) in the phalanges. A quadratic effect for Ca in the tibias was observed, with a level of 1930.03 FTU of phytase resulting in 32.29% Ca in the tibia. The 1500, 2000 and 2500 FTU levels promoted higher concentrations of Ca and P in the tibia compared to the PC. The 2500 FTU level showed higher phosphorus levels in the phalanges compared to the PC (P=0.013) (Table 7; Figure 2).

4.4. Metabolisable Energy of Diets

A linear increasing effect (P=0.014 and P=0.009, respectively) of phytase levels was observed on apparent metabolisable energy (AME) and nitrogen-corrected apparent metabolisable energy (AMEn). The NC2 treatment had the lowest AMEn compared to the PC (P=0.012). All treatments, except NC1, differed from the PC, showing lower values of excreted nitrogen (Table 8; Figure 3).

4.5. Nutrient Metabolizability Coefficients

A linear increasing effect (P=0.014, P<0.001, P=0.010, P<0.001 and P<0.001, respectively) was observed for the coefficients of metabolizability of gross energy (CMGE), organic matter (CMOM), retained mineral matter (MM retained), zinc (Zn retained), and the coefficient of zinc metabolizability (CMZn). A linear decreasing effect (P=0.010 and P<0.001, respectively) was observed on the excretion of mineral matter (MM) and zinc (Zn). The use of phytase at all levels resulted in (P<0.001, P<0.001, P<0.001 and P=0.005, respectively) lower excretion of MM, phosphorus (P), calcium (Ca), and crude protein (CP), as well as higher (P<0.001, P<0.001 and P=0.002, respectively) crude protein metabolizability (CMCP), retention of CP, and zinc retention compared to the PC treatment. However, lower intake (P<0.001 and P<0.001, respectively) of calcium (Ca) and phosphorus (P) was observed compared to the PC (Table 9; Figure 3).

5. Discussion

The use of phytase in broiler diets is a well-established practice in modern poultry production due to the enzyme's beneficial effects on nutrient digestibility, particularly phosphorus (P), which leads to increase bird performance (Selle et al., 2023). However, determining the ideal supplementation level remains a challenge for nutritionists, as there are discrepancies in the literature regarding the dosage and application method of the enzyme. In this study, phytase supplementation above 2000 FTU resulted in the best performance and bone mineral composition outcomes, as well as the highest nutrient metabolizability coefficients, indicating that phytase enhances nutrient utilization, especially in diets with nutritional restrictions, thereby reinforcing its efficacy in optimizing nutrient metabolizability and subsequent animal performance.

The decreasing linear effect on the feed conversion ratio (FCR), along with increasing linear concentrations of calcium (Ca) and phosphorus (P) in the tibia and phalanges, apparent metabolisable energy (AME), nitrogen-corrected apparent metabolisable energy (AMEn), coefficients of metabolizability for gross energy (CMGE), organic matter (CMOM), and zinc (CMZn), and retention of mineral matter (MM) and zinc (Zn), suggests that phytase superdosing improves dietary utilization efficiency. The performance improvement observed at higher supplementation levels (2000 and 2500 FTU/kg) may be attributed to the enhanced hydrolysis of phytic acid, releasing nutrients complexed to the molecule, such as cations, proteins, amino acids, and starches, which favor digestibility in the gastrointestinal tract (Krabbe et al., 2024).

These results highlight the positive impact of phytase superdosing, particularly above 2000 FTU/kg. Similarly, Silva et al. (2015) reported that phytase supplementation in phosphorus-reduced diets maintained productive efficiency, while Mulvenna et al. (2022) observed increased weight gain and feed efficiency in broilers fed diets containing between 1500 and 4000 FTU/kg of phytase.

Regarding bone mineral composition, it was observed that increasing levels of phytase, especially 2000 FTU/kg and 2500 FTU/kg, resulted in higher concentrations of calcium and phosphorus in the tibias and medium phalanges, indicating that enzyme supplementation favors mineral retention and bone mineralization. Similar findings were reported by Vaz et al. (2013), who observed increased bone mineral concentrations of Ca and P in broilers fed diets containing phytase (1200 FTU) and reduced phosphorus.

The findings of this study demonstrate that nutrient metabolizability is strongly influenced by the phytase supplementation level in diets with nutritional reductions for broilers. The increased CMGE, CMOM, and CMZn, as well as greater retention of mineral matter and crude protein, coupled with lower excretion of mineral matter,

phosphorus, calcium, and zinc, especially at 2000 and 2500 FTU levels, underscore the positive impact of phytase superdosing on nutritional efficiency. The increased metabolizability and reduced excretion of nutrients not only improve productive efficiency but also reduce the environmental impacts associated with poultry production (Selle et al., 2023).

The practical application of these findings can contribute to formulating more efficient and sustainable diets in poultry production. For AME and AMEn values, a similar behavior was observed, with higher values found at higher phytase supplementation levels, corroborating previous studies linking phytase supplementation to increased metabolisable energy in diets (Selle et al., 2009; Bernardes et al., 2022).

In addition to the benefits on performance, nutrient digestibility, and energy, the use of phytase in diets can reduce costs related to mineral supplementation and improve the efficiency of phosphorus utilization in feed ingredients (El-Wahab et al., 2024). Similarly to the findings of Borges et al. (2025), the present results reinforce the multifunctional role of phytase as a nutritional tool capable of sustaining broiler performance under nutrient-reduced conditions, as the authors reported that phytase supplementation restored growth performance, bone strength, and mineral balance to levels comparable to a nutritionally adequate diet, even when phosphorus, calcium, metabolisable energy, crude protein, and digestible amino acids were simultaneously reduced.

This aspect is particularly relevant in a global context that demands greater sustainability and reduced environmental impacts from animal production (Iqbal et al., 2023). Phytase, by releasing phytic phosphorus and reducing its excretion, decreases the dependence on inorganic mineral sources, making feed formulation more economical and environmentally responsible (Borges et al., 2025).

In diets containing meat and bone meal, the response to phytase superdosing may differ from that of conventional corn–soybean meal diets due to the contribution of non-phytate phosphorus and calcium, which alters phytate availability and mineral interactions (Nuamah et al., 2024). Under these conditions, extra-phosphoric effects of phytase become more relevant than its classical phosphorus-releasing role.

In this study, phytase superdosing increased metabolisable energy, nutrient metabolizability, mineral retention, feed efficiency, and bone mineralization, even in the presence of animal-derived ingredients, particularly at 2000 and 2500 FTU/kg. These results indicate that phytase acts as a multifunctional enzyme, enhancing nutrient utilization beyond phytate hydrolysis in diets with mixed phosphorus sources.

Taken together, these findings demonstrate that phytase acts as a multifunctional nutritional tool, capable of improving not only phosphorus efficiency but also energy utilization, nutritional balance, and sustainability in modern poultry production systems. Therefore, phytase superdosing appears as an efficient and sustainable nutritional strategy for modern poultry production, offering productive, economic, and ecological benefits.

6. Conclusion

Phytase superdosing improved broiler performance, nutrient utilization, and bone mineralization in diets containing corn, soybean meal, and meat and bone meal. Increasing phytase levels resulted in linear improvements in feed conversion, metabolisable energy, nutrient metabolizability, and mineral retention, while quadratic responses were observed for early growth performance and tibial calcium deposition. Supplementation at 2000 and 2500 FTU/kg enhanced calcium and phosphorus deposition in bones, increased nutrient metabolizability by up to 29.02%, and reduced phosphorus excretion by up to 31.63%. These results demonstrate that phytase superdosing remains

effective in diets containing animal-derived protein sources and represents a viable strategy to improve feed efficiency and sustainability in broiler production.

Author statement

All authors have reviewed and approved the final version of the manuscript submitted. This article represents the original work of the authors, has not been previously published, and is not currently being considered for publication in any other venue.

CRediT authorship contribution statement

Rosa Aparecida Reis de Léo: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. Jean Kaique Valentim: Writing – review & editing, Methodology. Rafael de Sousa Ferreira: Investigation, Formal analysis. Alexander Alexandre de Almeida: Writing – review & editing, Investigation. Maria Rogervânia Silva de Farias: Investigation, Conceptualization and Data curation. Melissa Izabel Hannas: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary materials associated with this article can be made available upon request to the corresponding author.

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Figures

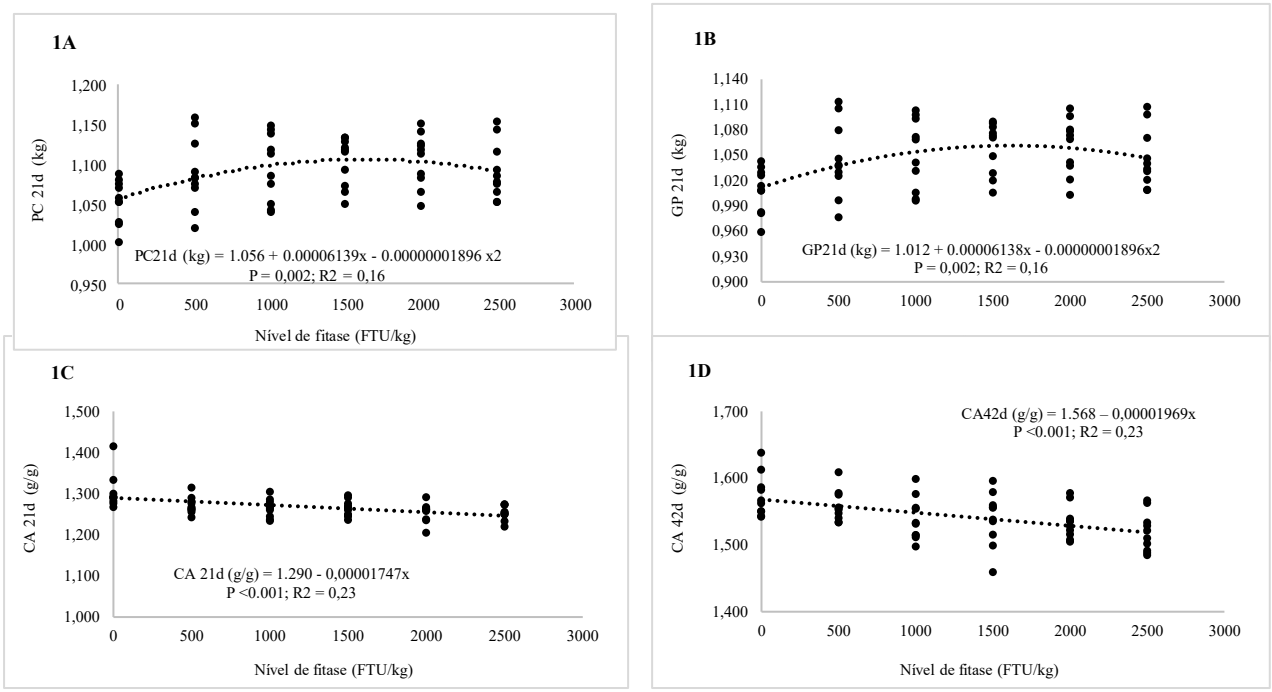


Figure 1. Effect of the level of phytase supplementation on the performance of broilers. Body weight – BW, weight gain – GP, feed conversion – WC.

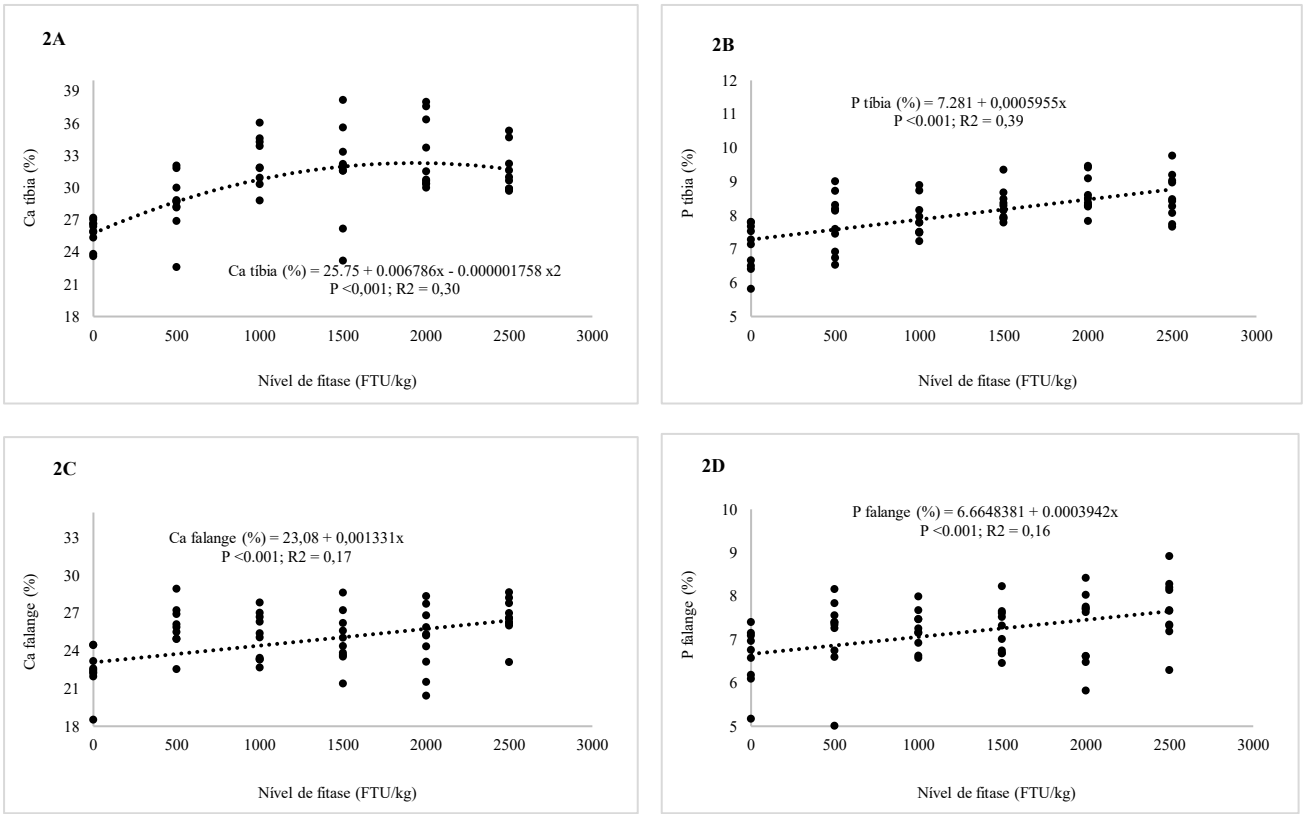
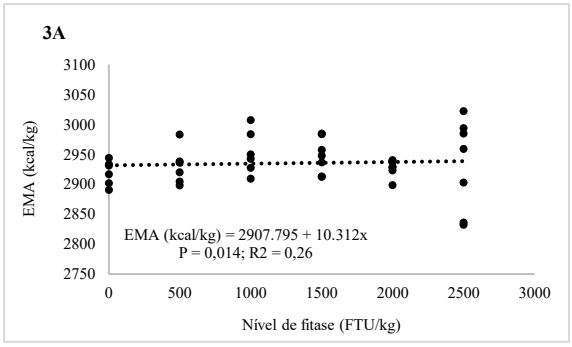
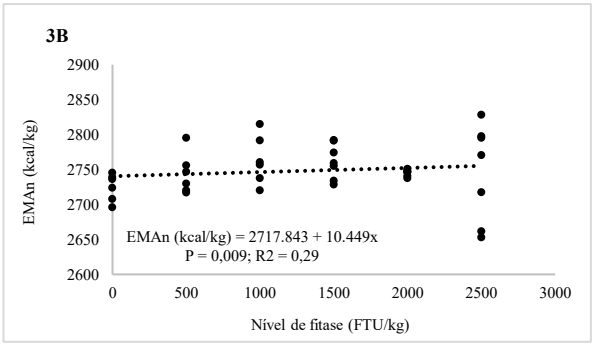
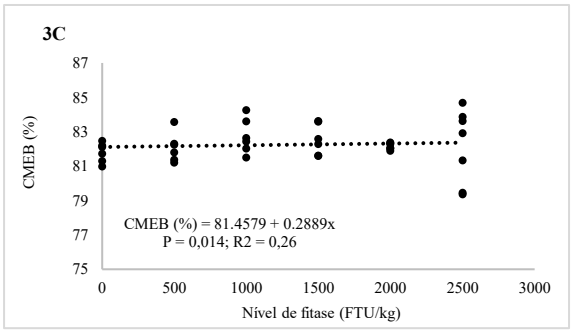
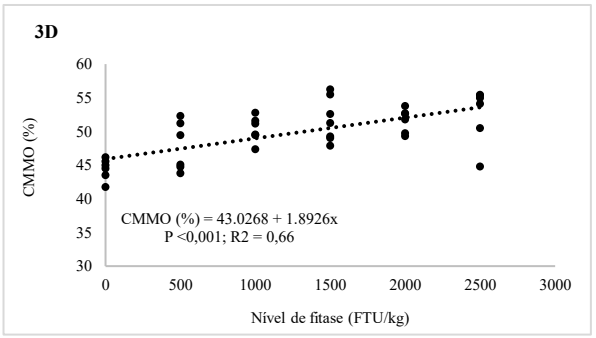


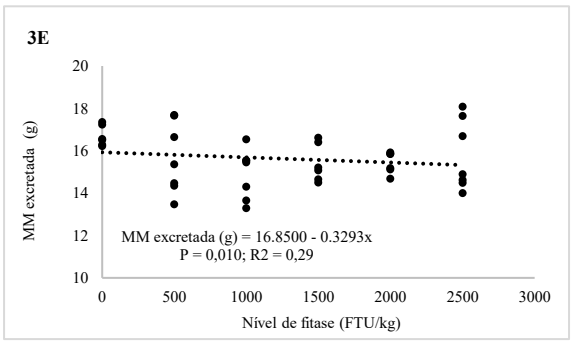
Figure 2. Effect of the level of phytase supplementation on the bone composition of broilers.

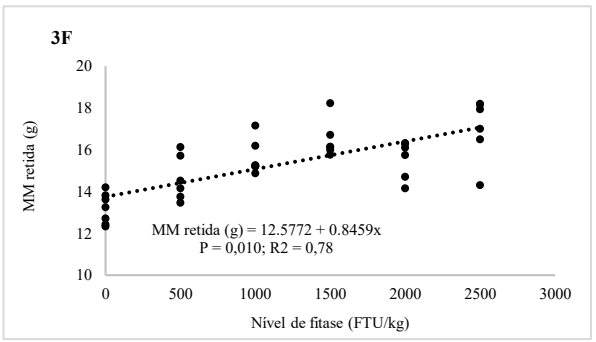

3A
EMA (kcal/kg)
EMA (kcal/kg) = 2907.795 + 10.312x
P = 0,014; R2 = 0,26
Nível de fitase (FTU/kg)

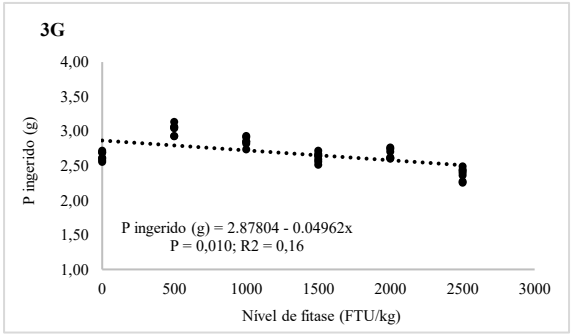

3B
EMAn (kcal/kg)
EMAn (kcal/kg) = 2717.843 + 10.449x
P = 0,009; R2 = 0,29
Nível de fitase (FTU/kg)

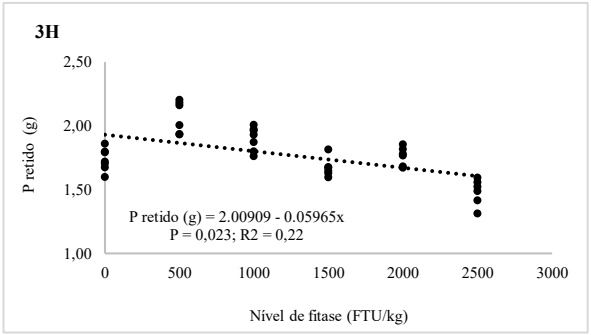

3C
CMEB (%)
CMEB (%) = 81.4579 + 0.2889x
P = 0,014; R2 = 0,26
Nível de fitase (FTU/kg)

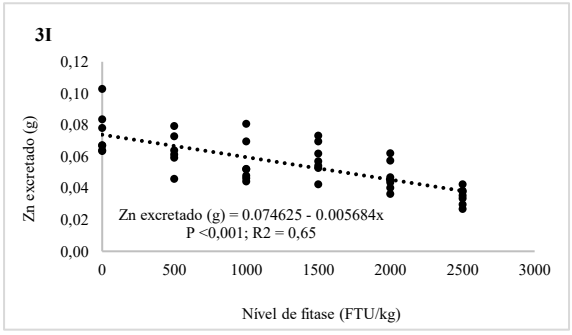

3D
CMMO (%)
CMMO (%) = 43.0268 + 1.8926x
P <0,001; R2 = 0,66
Nível de fitase (FTU/kg)

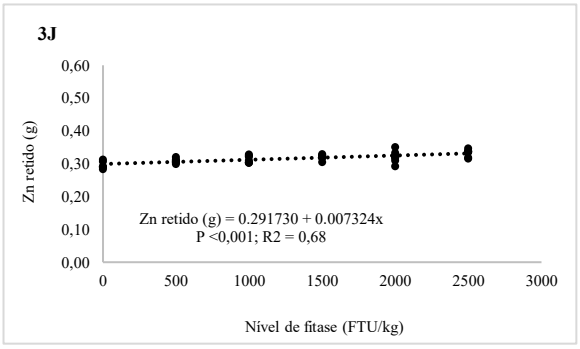

3E
MM excretada (g)
MM excretada (g) = 16.8500 - 0.3293x
P = 0,010; R2 = 0,29
Nível de fitase (FTU/kg)


3F
MM retida (g)
MM retida (g) = 12.5772 + 0.8459x
P = 0,010; R2 = 0,78
Nível de fitase (FTU/kg)


3G
P ingerido (g)
P ingerido (g) = 2.87804 - 0.04962x
P = 0,010; R2 = 0,16
Nível de fitase (FTU/kg)


3H
P retido (g)
P retido (g) = 2.00909 - 0.05965x
P = 0,023; R2 = 0,22
Nível de fitase (FTU/kg)


3I
Zn excretado (g)
Zn excretado (g) = 0.074625 - 0.005684x
P <0,001; R2 = 0,65
Nível de fitase (FTU/kg)


3J
Zn retido (g)
Zn retido (g) = 0.291730 + 0.007324x
P <0,001; R2 = 0,68
Nível de fitase (FTU/kg)

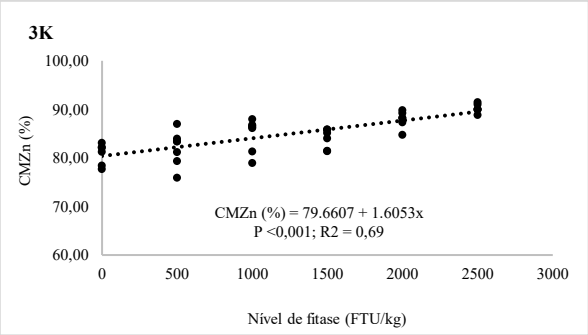


Figure 3. Effect of the level of phytase supplementation on AME and AMEn values, metabolizability coefficients, intake, excretion and nutrient retention of broilers.

Tables

Table 1. Experimental Treatments

Treatment 1	Positive control (PC) with Corn, Soybean Meal and Meat and Bone Meal.
Treatment 2	Negative control 1 (NC1) - reduction of 0.164% of Ca and 0.163% of available P.
Treatment 3	Negative control 2 (NC2) - reduction of the complete matrix (NC1 + reduction of 0.42% of CP, 40 Kcal/kg of ME).
Treatment 4	NC2 + phytase (500 FTU/kg).
Treatment 5	NC2 + phytase (1000 FTU/kg).
Treatment 6	NC2 + phytase (1500 FTU/kg).
Treatment 7	NC2 + phytase (2000 FTU/kg).
Treatment 8	NC2 + phytase (2500 FTU/kg).

FTU – Phytase Unit

Table 2. Composition Calculated and nutritional analysis of the experimental diets – Period from 1 to 21 days

Ingredients	Treatments[1]							
	PC	NC1	NC2	NC2 + 500	NC2 + 1000	NC2 + 1500	NC2 + 2000	NC2 + 2500
Corn	56.71	55.47	57.67	57.67	57.66	57.66	57.65	57.65
Soybean meal, 46%	32.97	36.21	34.95	34.95	34.95	34.95	34.95	34.95
Meat and bone meal, 45%	6.541	3.718	3.734	3.734	3.734	3.734	3.734	3.734
Soybean oil	2.102	2.469	1.517	1.517	1.517	1.517	1.517	1.517
Salt	0.408	0.458	0.458	0.458	0.458	0.458	0.458	0.458
DL-Methionine, 98%	0.315	0.305	0.289	0.289	0.289	0.289	0.289	0.289
Limestone, 38%	0.253	0.721	0.724	0.724	0.724	0.724	0.724	0.724
L-Lysine HCl, 78%	0.168	0.133	0.147	0.147	0.147	0.147	0.147	0.147
Choline Chloride, 60%	0.079	0.074	0.078	0.078	0.078	0.078	0.078	0.078
L-Threonine, 98.5%	0.045	0.030	0.023	0.023	0.023	0.023	0.023	0.023
Mineral Supplement[2]	0.130	0.130	0.130	0.130	0.130	0.130	0.130	0.130
Vitamin Supplement[3]	0.130	0.130	0.130	0.130	0.130	0.130	0.130	0.130
Commercial Phytase[4]	-	-	-	0.005	0.010	0.015	0.020	0.025
Antioxidant (BHT)	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Anticoccidial[6]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Inert	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional Composition[5]								
Crude protein, %	23.00	23.00	22.58	22.58	22.58	22.58	22.58	22.58
Calcium, %	0.990	0.826	0.826	0.826	0.826	0.826	0.826	0.826
Available Phosphorus, %	0.495	0.332	0.332	0.332	0.332	0.332	0.332	0.332
Ca/PAv	2.000	2.489	2.489	2.489	2.489	2.489	2.489	2.489
Sodium, %	0.223	0.223	0.223	0.223	0.223	0.223	0.223	0.223
Choline Chloride, ppm	1800	1800	1800	1800	1800	1800	1800	1800
Metabolisable energy, kcal/kg	3000	3000	2960	2960	2960	2960	2960	2960
SID Lysine, %	1.197	1.197	1.180	1.180	1.180	1.180	1.180	1.180
SID Methionine + Cystine, %	0.746	0.746	0.737	0.737	0.737	0.737	0.737	0.737
SID Threonine, %	0.645	0.645	0.636	0.636	0.636	0.636	0.636	0.636

SID Tryptophan, %	0.188	0.197	0.195	0.195	0.195	0.195	0.195	0.195
SID Isoleucine, %	0.680	0.702	0.697	0.697	0.697	0.697	0.697	0.697
SID Valine, %	0.767	0.779	0.775	0.775	0.775	0.775	0.775	0.775
SID Arginine, %	1.164	1.180	1.171	1.171	1.171	1.171	1.171	1.171

[1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg).

[2] Mineral supplement (Amount per kg of feed): Manganese - 82 mg; Iron – 58.4 mg; Selenium - 0.35 mg; Copper – 11.6 mg; Iodine – 1.18 mg.

[3] Vitamin supplement (Amount per kg of feed): vitamin A – 13,493 IU; vitamin D3 – 3,374 IU; Vitamin E – 50.5 IU; Vitamin B1 - 3.64 IU; vitamin B2 – 9.0 IU; vitamin B6 – 5.05 IU; Pantothenic Acid - 18.1 mg; Biotin - 0.126 mg; Vitamin K3 – 2.71 mg; Folic acid – 1.26 mg; Nicotinic acid - 54.9 mg; Vitamin B12 – 0.022 mg.

[4] Commercial phytase: Natuphos® E (BASF, Ludwigshafen, Germany), guaranteed activity of 10,000 FTU/g.

[5]Nutritional composition was calculated according to Rostagno et al. (2017).

[6]: Salinomycin, 12 %.

BHT: Hydroxy butyl toluene.

SID: Standardized ileal digestibility.

Table 3. Calculated and nutritional composition of experimental diets – Period from 22 to 42 days

Ingredients	Treatments[1]							
	PC	NC1	NC2	NC2 + 500	NC2 + 1000	NC2 + 1500	NC2 + 2000	NC2 + 2500
Corn	62.29	61.05	63.25	63.25	63.24	63.24	63.23	63.23
Soybean meal, 46%	27.25	30.48	29.22	29.22	29.22	29.22	29.22	29.22
Meat and bone meal, 45%	4.905	2.083	2.098	2.098	2.098	2.098	2.098	2.098
Soybean oil	4.009	4.376	3.424	3.424	3.424	3.424	3.424	3.424
Salt	0.354	0.404	0.403	0.403	0.403	0.403	0.403	0.403
DL-Methionine, 99%	0.260	0.250	0.235	0.235	0.235	0.235	0.235	0.235
Limestone, 38%	0.258	0.726	0.729	0.729	0.729	0.729	0.729	0.729
L-Lysine HCl, 78%	0.148	0.113	0.127	0.127	0.127	0.127	0.127	0.127
Choline Chloride, 60%	0.087	0.082	0.086	0.086	0.086	0.086	0.086	0.086
L-Threonine, 98.5%	0.039	0.024	0.017	0.017	0.017	0.017	0.017	0.017
Mineral Supplement[2]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin Supplement[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Commercial phytase[4]	-	-	-	0.005	0.010	0.015	0.020	0.025
Antioxidant (BHT)	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Anticoccidial[6]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Inert	0.260	0.260	0.260	0.260	0.260	0.260	0.260	0.260
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional Composition[5]								
Crude protein, %	19.90	19.90	19.48	19.48	19.48	19.48	19.48	19.48
Calcium, %	0.780	0.616	0.616	0.616	0.616	0.616	0.616	0.616
Available Phosphorus, %	0.390	0.227	0.227	0.227	0.227	0.227	0.227	0.227
Ca/PAv	2.000	2.715	2.715	2.715	2.715	2.716	2.716	2.716
Sodium, %	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
Choline Chloride, ppm	1685	1685	1685	1685	1685	1685	1685	1685
Metabolisable energy, kcal/kg	3180	3180	3140	3140	3140	3140	3140	3140
SID Lysine, %	1.015	1.015	0.998	0.998	0.998	0.998	0.998	0.998
SID Methionine + Cystine, %	0.765	0.765	0.755	0.755	0.755	0.755	0.755	0.755
SID Threonine, %	0.658	0.658	0.647	0.647	0.647	0.647	0.647	0.647

SID Tryptophan, %	0.189	0.200	0.198	0.198	0.198	0.198	0.198	0.198
SID Isoleucine, %	0.690	0.717	0.711	0.711	0.711	0.711	0.711	0.711
SID Valine, %	0.786	0.799	0.795	0.795	0.795	0.795	0.795	0.795
SID Arginine, %	1.171	1.190	1.179	1.179	1.179	1.179	1.179	1.179

[1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg).

[2] Mineral supplement (Amount per kg of feed): Manganese - 82 mg; Iron – 58.4 mg; Selenium - 0.35 mg; Copper – 11.6 mg; Iodine – 1.18 mg.

[3] Vitamin supplement (Amount per kg of feed): vitamin A – 13,493 IU; vitamin D3 – 3,374 IU; Vitamin E – 50.5 IU; Vitamin B1 - 3.64 IU; vitamin B2 – 9.0 IU; vitamin B6 – 5.05 IU; Pantothenic Acid - 18.1 mg; Biotin - 0.126 mg; Vitamin K3 – 2.71 mg; Folic acid – 1.26 mg; Nicotinic acid - 54.9 mg; Vitamin B12 – 0.022 mg.

[4] Commercial phytase: Natuphos® E (BASF, Ludwigshafen, Germany), guaranteed activity of 10,000 FTU/g.

[5]Nutritional composition was calculated according to Rostagno et al. (2017).

[6]: Salinomycin, 12 %.

BHT: Hydroxy butyl toluene.

SID: Standardized ileal digestibility.

Table 4. Performance of broilers from 1 to 21 days, 22 to 42 days and 1 to 42 days receiving diets with overdose of corn-based phytase, soybean meal and meat and bone meal

Variables	Treatments[1]								SEM	p-value	Regression
	PC	NC1	NC2	NC2 + 500	NC2 + 1000	NC2 + 1500	NC2 + 2000	NC2 + 2500			
	1 to 21 days										
FI, kg	1.33	1.30	1.31	1.32	1.32	1.33	1.32	1.31	0.013	0.709	-
WG, Kg	1.04	1.01	1.00	1.04	1.05	1.05	1.06	1.04	0.010	0.026	Q
FCR	1.28	1.28	1.30	1.27	1.26	1.26	1.25*	1.25*	0.009	0.001	L
BW, kg	1.08	1.05	1.05	1.08	1.09	1.10	1.10	1.09	0.010	0.027	Q
	22 to 42 days										
FI, kg	3.97	3.78	3.87	3.88	3.82	3.77	3.82	3.79	0.043	0.330	-
WG, kg	2.39	2.25	2.29	2.30	2.30	2.26	2.29	2.31	0.025	0.735	-
FCR	1.66	1.67	1.69	1.68	1.66	1.66	1.66	1.63	0.014	0.117	-
BW, kg	3.47	3.31	3.34	3.39	3.39	3.36	3.40	3.40	0.030	0.632	-
	1 to 42 days										
FI, kg	5.31	5.08	5.19	5.21	5.15	5.10	5.15	5.10	0.050	0.576	-
WG, kg	3.43	3.27	3.29	3.34	3.35	3.32	3.35	3.36	0.030	0.631	-
FCR	1.54	1.55	1.57	1.55	1.53	1.53	1.53	1.51	0.010	0.004	L
BW, kg	3.47	3.31	3.34	3.39	3.39	3.36	3.40	3.40	0.030	0.632	-

Means in the same row followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$). Linear Effect (L); Quadratic effect (Q). SEM = standard error of the mean. [1] PC- Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg). FI - Feed Intake; WG - Weight gain; FCR - Feed conversion. BW – Body weight. WG 1 to 21d (kg) = $1.012 + 0.00006138x - 0.00000001896x^2$ ($P = 0.002$; $R^2 = 0.16$; Maximum point: 1618.67); BW 1 to 21d (kg) = $1.056 + 0.00006139x - 0.00000001896\ x^2$ ($P = 0.002$; $R^2 = 0.16$; Maximum point: 1618.93); FCR 1 to 21d = $1.290 - 0.00001747x$ ($P < 0.001$; $R^2 = 0.23$); FCR 1 to 42d = $1.568 – 0.00001969x$ ($P < 0.001$; $R^2 = 0.23$).

Table 5. Carcass and meat cuts (%) yields of broilers fed diets with overdose of phytase based on corn, soybean meal and meat and bone meal

Variables (%)	Treatments[1]								SEM	p-value	Regression
	PC	NC1	NC2	NC2 + 500	NC2 + 1000	NC2 + 1500	NC2 + 2000	NC2 + 2500			
Carcass	86.77	86.92	87.23	86.33	86.54	87.26	86.25	87.00	0.619	0.907	-
Breast	36.29	37.34	37.07	37.36	37.12	36.17	37.48	36.16	0.434	0.109	-
Thigh	11.33	11.50	11.58	11.47	11.49	11.58	11.46	11.64	0.136	0.843	-
Drumstick	14.06	13.66	13.65	13.89	13.81	13.97	13.93	13.91	0.170	0.667	-

Means in the same row followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$). Not significant (ns); SEM = standard error of the mean. [1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg).

Table 6. Analysis of *White Striping* of broiler breasts receiving diets with overdose of corn-based phytase, soybean meal and meat and bone meal

Treatments[1]	White striping [ns]
PC	1.00
NC1	0.80
NC2	0.80
NC2+500	0.90
NC2+1000	0.95
NC2+1500	0.75
NC2+2000	1.00
NC2+2500	0.95
SEM	0.396
p-value	0.430

SEM = standard error of the mean; ns: not significant by the Kruskal-Wallis test (P>0.05) (N = 10 per treatment). [1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg).

Table 7. Mineral composition, in percentage, of calcium and phosphorus of the tibiae of broilers at 42 days receiving diets with overdose of phytase based on corn, soybean meal and meat and bone meal

Treatment[1]	Bone mineral composition					
	Tibia			Middle phalanx		
	% DM[2]	%Ca[2]	%P[2]	% DM[2]	%Ca[2]	%P[2]
PC	92.82	27.74	7.81	93.97	24.28	6.56
NC1	92.90	25.84	7.30	93.54	23.11	6.54
NC2	92.65	25.83	7.05*	93.35	21.89	6.40
NC2+500	92.26	28.58	7.75	93.45	24.98	7.13
NC2+1000	92.30	30.83	7.89	93.11	25.11	7.23
NC2+1500	92.13	31.57*	8.28	92.72	24.95	7.19
NC2+2000	91.04	32.90*	8.55*	92.67	24.87	7.28
NC2+2500	91.43	31.46*	8.60*	91.87	26.65	7.70*
SEM	-	1.037	0.209	-	0.784	0.258
p-value	-	<0.001	<0.001	-	0.002	0.013
Regression	-	Q	L	-	L	L

Means in the same column followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$). Linear Effect (L); Quadratic effect (Q). SEM = standard error of the mean. [1]Treatments: PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg). [2]DM: Dry Matter [2] Ca: Calcium, [2]P: Phosphorus. Tibia Ca (%) = 25.75 + 0.006786x - 0.000001758 x^2 ($P < 0.001$; $R^2 = 0.30$; Maximum point: 1930.03); Tibia P (%) = 7.281 + 0.0005955x ($P < 0.001$; $R^2 = 0.39$); Ca phalanx (%) = 23.08 + 0.001331x ($P < 0.001$; $R^2 = 0.17$); P phalanx (%) = 6.6648381 + 0.0003942x ($P < 0.001$; $R^2 = 0.16$).

Table 8. Dry matter intake, nitrogen balance, and metabolisable energy in broilers fed diets with overdose of phytase based on corn, soybean meal and meat and bone meal

Treatments[1]	Variables					
	DMI (g)	N cons. (g)	N excr. (g)	NB	AME MN[2] (kcal/kg)	AMEn MN[2] (kcal/kg)
PC	468.66	17.20	4.88	12.32	2976	2795
NC1	465.11	17.59	5.00	12.70	2997	2808
NC2	468.14	16.84	3.57*	13.12*	2919*	2724*
NC2+500	487.24	17.47	4.17*	13.09*	2929	2744
NC2+1000	476.78	17.11	3.95*	13.16*	2953	2763
NC2+1500	488.31	17.41	4.07*	13.10*	2948	2762
NC2+2000	465.23	16.80	4.18*	12.68	2927	2745
NC2+2500	470.15	17.00	4.23*	12.99	2933	2746
SEM	1.992	0.068	0.074	0.071	5.939	5.780
p-value	0.441	0.129	0.005	0.078	0.011	0.012
Regression	-	-	ns	-	L	L

Means in the same column followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$). Linear Effect (L); Quadratic effect (Q). Not significant(s). SEM = standard error of the mean. [1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg). DMI: Dry matter intake (g); AME in MN[2]: Apparent metabolisable energy (kcal/kg) in Natural Matter; AMEn in MN[2]: Metabolisable energy corrected by nitrogen balance (kcal/kg) in Natural Matter; N cons.: Nitrogen consumed (g); N excr.: Excreted nitrogen (g); NB: Nitrogen Balance (g); N: Nitrogen. AME (kcal/kg) = 2907.795 + 10.312x ($P = 0.014$; $R^2 = 0.26$); AMEn (kcal/kg) = 2717.843 + 10.449x ($P = 0.009$; $R^2 = 0.29$).

1

Table 9. Nutrient metabolizability coefficients of broilers fed diets with overdose of phytase based on corn, soybean meal and meat and bone meal

Variables	Treatments[1]								SEM	p-value	Regression
	PC	NC1	NC2	NC2 + 500	NC2 + 1000	NC2 + 1500	NC2 + 2000	NC2 + 2500			
					GE (kcal/kg)						
Ingested	2051.00	2058.00	1996.00	2078.00	2016.00	2082.00	1984.00	2005.00	8.533	0.121	-
Excreted	379.40	395.60	362.10	362.10	341.20*	354.60	357.50	357.30	3.937	0.007	ns
Retained	1681.00	1658.00	1615.00	1712.00	1674.00	1708.00	1626.00	1668.00	7.309	0.993	-
CMGE (%)	81.24	80.93	81.78	82.07	82.73*	82.54	82.15	82.16	0.161	0.008	L
					DM (g)						
Ingested	468.60	465.10	468.10	487.20	472.60	488.30	465.20	470.10	2.015	0.481	-
Excreted	95.26	101.25	90.68	89.13	84.65*	91.05	88.40	93.50	1.057	0.039	ns
Retained	375.70	367.60	373.70	397.10	388.00	394.00	376.80	376.60	2.065	0.144	-
CMDM (%)	79.48	78.63	80.38	81.50*	81.69*	81.22*	81.14*	80.73	0.191	<0.001	ns
					OM (g)						
Ingested	435.00	433.00	438.00	456.10	442.10	450.70	434.50	437.90	1.849	0.485	-
Excreted	76.12	82.26	74.00	71.92	69.75	75.34	73.31	77.73	0.854	0.303	-
Retained	361.20	351.20	360.60	381.80	372.40	372.30	361.20	365.30	1.784	0.099	-
CMOM (%)	42.83	40.70	44.39	47.75*	50.29*	51.66*	51.64*	52.51*	0.700	<0.001	L
					MM (g)						
Ingested	33.62	32.07	30.13	31.08	30.74	32.72	30.64	32.15	0.190	0.241	-
Excreted	19.62	19.19	16.68*	15.65*	14.89*	15.40*	15.42*	15.76*	0.276	<0.001	L
Retained	14.70	13.34	13.18*	14.61	15.58	16.48*	15.54	17.00*	0.218	<0.001	L
CMMM (%)	82.33	81.38	82.75	83.71*	83.86*	83.16	83.22	82.80	0.160	0.019	ns
					CP (g)						
Ingested	102.10	109.90	105.20	101.30	106.90	108.10	105.00	106.30	0.515	0.301	-
Excreted	30.52	31.29	22.70*	26.09*	24.34*	25.58*	26.13*	26.47*	0.454	0.005	ns
Retained	71.58	79.43*	81.86*	74.04	81.70*	81.81*	80.25*	81.24*	0.637	<0.001	ns
CMCP (%)	70.61	71.81	78.13*	73.95*	77.39*	75.69*	75.43*	75.87*	0.405	<0.001	ns
					P (g)						
Ingested	4.32	2.89*	2.64*	3.02*	2.85*	2.62*	2.67*	2.38*	0.078	<0.001	L
Excreted	1.38	1.14*	0.87*	0.88*	0.92*	0.89*	0.92*	0.97*	0.025	<0.001	ns
Retained	3.03	1.77*	1.73*	2.07*	1.90*	1.67*	1.76*	1.48*	0.063	<0.001	L
CMP (%)	69.19	60.90	66.93	69.43	67.38	65.83	66.51	59.60	0.627	0.066	-
					Ca (g)						
Ingested	7.75	4.84*	4.81*	5.01*	4.86*	5.02*	4.78*	4.83*	0.132	<0.001	ns
Excreted	2.33	2.12*	1.83*	1.73*	1.72*	1.79*	1.84*	1.72*	0.034	<0.001	ns

Retained	5.55	2.77*	2.96*	3.26*	3.07*	3.08*	2.97*	3.05*	0.119	<0.001	ns
CMCa (%)	70.24	56.73	61.86	65.02	64.30	61.97	62.57	64.15	0.582	0.509	-
					Zn (g)						
Ingested	0.44	0.49*	0.37*	0.38*	0.37*	0.38*	0.36*	0.37*	0.005	<0.001	ns
Excreted	0.09	0.09	0.07	0.06*	0.06*	0.06*	0.05*	0.03*	0.002	<0.001	L
Retained	0.35	0.40*	0.29*	0.30*	0.31*	0.32*	0.32*	0.33*	0.004	0.002	L
CMZn (%)	79.95	81.01*	80.76*	82.00*	84.86*	83.91*	87.90*	90.19*	0.573	<0.001	L

Means in the same row followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$). Linear Effect (L); Quadratic effect (Q). Not significant(s). SEM = standard error of the mean. [1] PC - Positive control; NC1- Negative control 1 with a reduction of 0.164% of Ca and 0.163% of available P; NC2 - NC1+ reduction of 0.42% of crude protein, 40 Kcal/kg of metabolisable energy; NC2 + 500 - Negative control 2 + Phytase (500 FTU/kg); NC2 + 1000 - Negative control 2 + Phytase (1000 FTU/kg); NC2 + 1500 - Negative control 2 + Phytase (1500 FTU/kg); NC2 + 2000 - Negative control 2 + Phytase (2000 FTU/kg); NC2 + 2500 - Negative control 2 + Phytase (2500 FTU/kg). CMDM: Dry Matter Metabolizability Coefficient, CMOM: Organic Matter Metabolizability Coefficient, CMMM: Mineral Matter Metabolizability Coefficient, CMCP: Crude Protein Metabolizability Coefficient, CMP: Phosphorus Metabolizability Coefficient, CMCa: Calcium Metabolizability Coefficient, CMZn: Zinc Metabolizability Coefficient. CMGE (%) = 81.4579 + 0.2889x ($P = 0.014$; $R^2 = 0.26$); CMOM (%) = 43.0268 + 1.8926x ($P <0.001$; $R^2 = 0.66$); MM excreted (g) = 16.8500 - 0.3293x ($P = 0.010$; $R^2 = 0.29$); MM retained (g) = 12.5772 + 0.8459x ($P = 0.010$; $R^2 = 0.78$); P ingested (g) = 2.87804 - 0.04962x ($P = 0.010$; $R^2 = 0.16$); P retained (g) = 2.00909 - 0.05965x ($P = 0.023$; $R^2 = 0.22$); Zn Excreted (g) = 0.074625 - 0.005684x ($P <0.001$; $R^2 = 0.65$); Zn Retained (g) = 0.291730 + 0.007324x ($P <0.001$; $R^2 = 0.68$); CMZn (%) = 79.6607 + 1.6053x ($P <0.001$; $R^2 = 0.69$).

CHAPTER 2

EXOGENOUS ENZYME SUPPLEMENTATION IN NUTRITIONALLY REDUCED DIETS: EFFECTS ON PERFORMANCE, METABOLISABLE ENERGY, AND AMINO ACID DIGESTIBILITY IN BROILER CHICKENS

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Chapter 2: Exogenous enzyme supplementation in nutritionally reduced diets: effects on performance, metabolisable energy, and amino acid digestibility in broiler chickens

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Abstract

An experiment was conducted to evaluate the effects of phytase (PHY), protease (PRO), xylanase (XYL), and an enzyme blend (BLE) on growth performance, metabolisable energy, amino acid digestibility, carcass traits, and bone mineralization of broiler chickens fed nutritionally reduced diets formulated according to enzyme matrix values. A total of 1,440 male Cobb 500 chicks were randomly assigned to nine treatments, with eight replicates of 20 birds each. Treatments included a positive control (PC), based on corn and soybean meal and formulated to fully meet nutritional requirements, and four negative controls with specific nutrient reductions: NC-PHY (0.48% reduction in crude protein, 35 kcal/kg reduction in metabolisable energy, 0.120% reduction in calcium, and 0.120% reduction in available phosphorus); NC-PRO (0.75% reduction in crude protein and 40 kcal/kg reduction in metabolisable energy); NC-XYL (0.30% reduction in crude protein and 65 kcal/kg reduction in metabolisable energy); and NC-BLE (full matrix reduction with 0.80% reduction in crude protein, 85 kcal/kg reduction in metabolisable energy, 0.120% reduction in calcium, and 0.120% reduction in available phosphorus). From these reduced diets, the respective enzyme-supplemented treatments were evaluated: NC+PHY, NC+PRO, NC+XYL, and NC+BLE. Additional metabolism (n = 432) and digestibility (n = 480) trials were conducted to determine apparent metabolisable energy (AME), nitrogen-corrected apparent metabolisable energy (AMEn), and standardized ileal amino acid digestibility (SIAAD). Body weight gain, feed conversion ratio, AME, AMEn, and SIAAD of birds fed the PC were higher than those fed the average of the negative controls without enzymes ($P \leq 0.05$). Enzyme supplementation improved these parameters compared with their respective negative controls ($P \leq 0.05$), although performance recovery was partial relative to the PC. Protease supplementation resulted in the highest AME and AMEn values and the most consistent increases in SIAAD ($P \leq 0.05$). Carcass yield and body composition were largely unaffected, except for bone ash content. In conclusion, supplementation with exogenous enzymes in

nutritionally reduced diets improves digestive efficiency and partially mitigates performance losses, with responses dependent on enzyme type and the magnitude of nutrient restriction applied.

Keywords: Broiler chickens, Exogenous enzymes, Ileal amino acid digestibility, Metabolisable energy, Nutritionally reduced diets.

1. Introduction

The modern nutrition of broiler chickens is driven by the need to maximize productive performance and optimize feed efficiency, while also aiming to reduce costs and increase the sustainability of production systems (Zampiga et al., 2021). In this context, the formulation of diets with reduced nutritional density is a promising strategy to lower costs and minimize environmental impacts associated with nutrient excretion (Gomes et al., 2026). However, the adoption of this technique in cereal-based diets presents additional challenges, primarily due to the high presence of antinutritional factors in these ingredients, such as phytic acid and non-starch polysaccharides (NSP), which reduce nutrient digestibility and energy utilization by the birds (Bavaresco et al., 2021). As a consequence, there may be a compromise in zootechnical performance, highlighting the need for the development of nutritional strategies that improve the utilization of the nutrients present in the diet.

In this context, supplementation with exogenous enzymes, such as xylanases, proteases, and phytases, has been used to increase nutrient and energy utilization efficiency in diets. Xylanases act on the degradation of non-starch polysaccharides, while proteases promote the hydrolysis of resistant protein fractions and the inactivation of protease inhibitors, and phytases act on the degradation of phytic acid, favoring the release of phosphorus and other complexed nutrients. As a result, these enzymes can enhance nutrient digestibility and improve the utilization of metabolisable energy in diets (Sureshkumar et al., 2023). Moreover, the use of these enzymes may promote a balanced intestinal microbiota, improve gastrointestinal tract integrity, and contribute to the productive performance of the birds (Moita and Kim, 2022). Although the individual effects of these enzymes are well established, there are still gaps related to the effects of combined enzyme supplementation, especially when associated with diets with reduced nutritional density.

Studies show that the inclusion of exogenous enzymes in diets with reduced nutrition can partially compensate for the decrease in energy, protein, and mineral levels, maintaining or even

improving zootechnical performance and feed efficiency. Borges et al. (2025), when evaluating increasing levels of phytase supplementation in diets with reduced metabolisable energy, crude protein, digestible amino acids, sodium, calcium, and available phosphorus for broilers, observed that the enzyme was able to restore productive performance, as well as improve bone characteristics and body composition of the birds. Similarly, Gomes et al. (2026), working with diets reduced in metabolisable energy, crude protein, calcium, and available phosphorus supplemented with carbohydrases, proteases, and phytase, found that the combined supplementation of carbohydrases, proteases, and phytase mitigated the negative effects of reduced nutrition on broiler performance.

Additionally, the effects of exogenous enzymes may vary depending on the type of enzyme used, diet composition, bird age, and rearing conditions, highlighting the need for further investigations to better understand these interactions. Therefore, the hypothesis of this study is that the inclusion of exogenous enzymes in broiler diets improves nutrient digestibility, energy efficiency, and productive performance. Thus, the aim was to evaluate the effect of different exogenous enzymes on zootechnical performance, metabolisable energy values, amino acid digestibility, as well as their influence on carcass yield and bone mineralization.

2. Material and methods

2.1. Ethics committee

All procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (CEUAP-91/2022) and followed the guidelines of the National Council for Animal Experimentation Control (CONCEA).

Experiment I

2.2. Common procedures, experimental design, housing and experimental diets

In this experiment, 1,440 male Cobb 500® broiler chicks, 1 day old (45 ± 1 g), were used. The chicks were housed in a conventional barn with side curtains and concrete pens (1.0 x 2.0 m) covered with wood shavings. The pens were equipped with trough feeders and nipple drinkers, and feed and water were provided ad libitum throughout the experimental period. The temperature of the experimental barn was controlled, and the lighting program was provided as recommended by the lineage manual. Upon hatching, the chicks were randomly allocated into 9 treatments, with 8 repetitions of 20 birds, for a 35-day experiment, divided into two rearing phases: starter (1 to 21 days) and grower (22 to 35 days).

The experimental treatments (Table 1) consisted of a basal diet as the positive control, to meet the nutritional requirements of the birds according to the recommendations of Rostagno et al. (2017). Four other diets were formulated by reducing the nutritional values recommended in the enzyme matrices (Table 2) as negative controls. From the nutrient-reduced diets, different enzymes were added, resulting in a total of 9 treatments. All experimental diets (Tables 3 and 4) were formulated based on corn and soybean meal, following the nutritional recommendations of Rostagno et al. (2017).

2.3. Enzyme traits

The commercial enzymes used in this study were provided by Vaccinar - Animal Nutrition and included Biozyme Carbo (xylanase), Biozyme PRO (protease), Biozyme Fit (phytase), and Biozyme Max (enzyme blend). The enzymes were incorporated into the diets according to the manufacturer's recommendations, with inclusions of 1 kg/t for each enzyme in their respective treatments. The aim of supplementation was to optimize the digestion of carbohydrates, proteins, and phytate, improving nutrient utilization in the diets. The technical specifications of the enzymes are presented in Table 2.

2.4. Growth performance

The offered diets and leftovers were weighed on days 1, 21, and 35 to calculate the cumulative feed intake (FI) for the periods of 1 to 21 days and 1 to 35 days. The body weight of the birds was recorded on the same dates to determine body weight gain (BWG), and the feed conversion ratio (FCR) was calculated as the ratio between FI and BWG. In cases of mortality, the feeders were weighed on the day of death to estimate the average intake up to that point, based on the number of birds present, according to Sakomura and Rostagno (2016). After the removal of deceased birds, intake was recalculated for the remaining phase, excluding the deceased birds. Total feed intake was determined by summing both intake intervals.

2.5. Slaughter procedures

In the 35^{th} d of age, two male broiler chickens per experimental unit (total number = 144 male broiler chickens) were selected on the basis of the average live-body weight (LBW) of the experimental unit, and the animals were fasted for 12 h and slaughtered by cervical dislocation for further analysis of carcass traits and body composition. The left and right tibias of these birds were collected, weighed, and frozen for later freeze-drying, followed by analysis of ash, calcium, and phosphorus (Detman et al., 2021).

2.5.1. Carcass characteristics and body composition

After slaughter, two male broiler chickens per experimental unit were manually defeathered, eviscerated, and had their heads, necks, and feet removed according to standard carcass processing procedures. The eviscerated carcasses were weighed using a digital scale with an accuracy of ± 1.0 g, and the carcass yield was calculated as a percentage of the pre-slaughter live body weight (LBW).

The breasts (pectoralis major and minor), thigh and drumstick were then separated and weighed individually, and their yields were calculated as percentages of the eviscerated carcass weight.

As described by Aguiar et al. (2024), total body composition was assessed using dual-energy X-ray absorptiometry (DEXA) with a Lunar Prodigy Advance scanner (GE Healthcare, Madison, WI), equipped with enCORE software version v18SP2. All scans were performed at the body composition and densitometry laboratory of the Federal University of Viçosa. Equipment calibration was conducted prior to scanning using a manufacturer-provided anthropomorphic phantom to ensure measurement accuracy. The broiler chickens were positioned dorsally on the scanning platform with wings extended laterally and legs fully aligned to minimize anatomical superimposition. A rectangular region of interest (ROI), encompassing the entire broiler chicken, was manually defined for each scan. The DEXA system provided raw data for soft-lean-tissue mass (SLTDEXA), fat-tissue mass (FATDEXA), and bone mineral content (BMCDEXA), which were expressed in grams per broiler chicken. The body composition results were subsequently converted to chemically equivalent estimates (in g/broiler chicken) of body fat weight (BFW), soft-lean tissue (SLT), body protein (BP), body water (BW), and body ash (BA) content using prediction equations (Eqs. 1 to 5) previously validated for broiler chickens (Aguiar et al., 2024).

$$BFW = 11.817 + (0.756 \times FATDEXA);\ R^2 = 0.98 \quad (1)$$

$$SLT = -31.807 + (1.157 \times SLTDEXA);\ R^2 = 0.99 \quad (2)$$

$$BP = -8.8705 + (0.2167 \times SLTDEXA);\ R^2 = 0.98 \quad (3)$$

$$BW = -22.94 + (0.940 \times SLTDEXA);\ R^2 = 0.99 \quad (4)$$

$$BA = 7.139 + (1.578 \times BMCDEXA);\ R^2 = 0.96 \quad (5)$$

Experiment II

2.6. Nutrient Metabolizability and Determination of Metabolisable Energy Values of Experimental Diets

2.6.1. Animals and Housing

One-day-old chicks were housed in a conventional masonry shed and raised according to the lineage guidelines until they reached the experimental age of 14 days. The lighting program was 24 hours of light, and the ambient temperature was set at 32ºC during the first week of life and gradually reduced according to the recommendations of the Cobb® lineage manual. A pre-starter diet (1 to 14 days) based on corn and soybean meal was formulated according to the recommendations of Rostagno et al. (2017) and provided ad libitum to the animals. At 14 days of age, 432 broilers weighing 501g ± 24g were transferred to wire-floored metal battery cages (800 cm² per broiler) in a room with controlled environmental conditions and continuous light supply.

The cages were equipped with trough feeders and nipple drinkers, and feed and water were provided ad libitum throughout the experimental period. The birds were randomly assigned to 9 treatments, with 8 repetitions of 6 birds per experimental unit (EU), for a 10-day experiment, with five days of adaptation to the diet and five days for fecal collection.

2.6.2. Excreta Collection

Excreta were collected twice daily (early morning and late afternoon) using aluminum trays lined with plastic placed under the cages. After collection, the excreta were stored in plastic bags, identified by experimental unit, and kept in a freezer until the end of the experimental period. At the end of the experimental period, the excreta were thawed at room temperature, weighed, and homogenized. A 200g sample was taken from each experimental unit for pre-drying at 55ºC for 72 hours in a forced-air oven. After drying, the samples were sent to the laboratory for evaluation of gross energy, dry matter, crude protein, mineral matter, Ca, P, and Zn (Sakomura and Rostagno,

2016).

The following equations were used to calculate metabolisable energy:

$$\text{AME} = \frac{\text{GEingested} - \text{GEexcreted}}{\text{DM ingested}}$$

$$\text{AMEn} = \frac{(\text{GEingested} - \text{GEexcreted})}{\text{DM ingested}} - 8.22 \times \text{NB}$$

Where:

AME = Apparent metabolisable energy.

AMEn = Apparent metabolisable energy corrected for nitrogen balance.

GE ingested = Gross energy ingested.

GE excreted = Gross energy excreted.

DM ingested = Dry matter ingested.

NB = Nitrogen balance.

The calculation of the nutrient metabolizability coefficient (NMC) from total excreta collection was performed using the following equation:

NMC (%) = (Nutrient in feed - Nutrient in excret) / (Nutrient in feed) × 100

Experiment III

2.7. Determination of Amino Acid Digestibility Coefficients

The 1-day-old chicks were housed in a conventional masonry barn and raised according to lineage guidelines until they reached the experimental age of 24 days. At 24 days of age, 480 male broiler chickens were weighed and distributed in a completely randomized design with 10 treatments, with 8 replications of 6 birds per experimental unit, for a 5-day experiment. The treatments consisted of the 9 experimental diets used in Experiment I, and a protein-free diet (DIP) (Table 5). All diets included 1% Celite (insoluble acid ash) as an indigestible marker.

At the end of the experimental period, all birds in the experimental unit were euthanized by cervical dislocation. The ileum was exposed through abdominal incision, and the ileal content was collected by pressure, with the composite ileal digesta from the 6 birds being considered the sample for this analysis. The samples were immediately collected, stored in plastic containers, frozen, and later freeze-dried for further analysis of dry matter, N, and total amino acids (Sakomura and Rostagno, 2016).

The amino acid digestibility was calculated using the indigestible marker (CIA). The method for determining apparent ileal amino acid digestibility was carried out according to the methodology proposed by Sakomura and Rostagno (2007) and is presented below.

$$FI = (\text{Marker in the diet}) / (\text{Marker in the digesta})$$

$$CDIap = ((\%AA\ diet - (\%AA\ digesta \times FI))) / (\%AA\ diet) \times 100$$

Where:

FI: Indigestibility factor.

CDIap: Apparent ileal digestibility coefficient.

% AA diet = Percentage of amino acids in the diet.

% AA dig = Percentage of amino acids in the ileal digesta.

Amino acid digestibility calculations were made using the insoluble acid ash (IAA) indigestible marker to estimate the acid indigestibility factor with the equations proposed by Sakomura and Rostagno (2016):

Ileal indigestibility factor:

$$FI\ 1 = ([IAA] \text{ in the diet}) / ([IAA] \text{ in the ileal digesta})$$

$$FI\ 2 = ([IAA] \text{ in DIP}) / ([IAA] \text{ in the ileal digesta})$$

Ileal digestibility coefficient (CDI):

$$CDAIAA\ (\%) = ((\%AA \text{ diet} - (\%AA \text{ digesta} \times FI\ 1))) / (\%AA \text{ diet}) \times 100$$

$$CDITAA\ (\%) = ((\%AA \text{ diet} - (\%AA \text{ digesta} \times FI\ 1) - (\text{endogenous AA} \times FI\ 2))) / (\%AA \text{ diet}) \times 100$$

Where:

FI = Indigestibility fator.

[IAA] = Insoluble acid ash concentration.

DIP = Protein-free diet.

CDAIAA = Apparent ileal amino acid digestibility coeficiente.

CDITAA = True ileal amino acid digestibility coeficiente.

% AA diet = Percentage of amino acids in the diet.

% AA dig = Percentage of amino acids in the ileal digesta.

Endogenous AA = Endogenous amino acid.

2.8. Statistical Analysis

The assumptions of the model were checked, with the normality of residuals tested by the Shapiro-Wilk test, the homogeneity of variances tested by the Bartlett test, and the independence of

errors tested by the Durbin-Watson test. After confirming the assumptions, the means were subjected to analysis of variance (ANOVA) with a significance level of 5%, using R software (R Studio, 2023). When significant effects were observed, means were compared using the Tukey test, adopting the same significance level.

3. Results

Experimento I

3.1. Performance

From 1 to 21 days of age, significant differences were observed between treatments for body weight gain (BWG), feed conversion ratio (FCR), and final average weight (FAW) ($p \leq 0.05$; Table 6), while feed intake (FI) was not influenced ($p > 0.05$). The positive control (PC) treatment showed higher BWG and BW, as well as better FCR, compared to the negative controls.

In the 22 to 35-day phase, all variables were influenced by the treatments ($p \leq 0.05$; Table 6). Birds in the PC treatment showed higher BWG compared to NC-PRO, while the other treatments showed intermediate performance. For FCR, the PC and NC+XYL treatments showed the best results, with no differences between them. And the highest FI was observed in the CN-BLE.

For the total period from 1 to 35 days, FI remained similar across treatments ($p > 0.05$), but significant differences were observed for BWG, FCR, and FAW ($p \leq 0.05$; Table 6). The PC treatment showed higher BWG and FAW and better FCR. The negative control treatments showed the worst zootechnical indices, while the enzyme-supplemented treatments promoted partial recovery of productive performance.

3.2. Carcass Characteristics and body composition

Weight slaughter and carcass weight were influenced by the treatments ($p \leq 0.05$; Table 7), with higher slaughter weight values observed in the PC treatment and higher carcass weight in the

PC and PN + PHY treatments compared to NC-PRO. No effect of the treatments was observed on the weights and yields of breast, thigh, and drumstick, as well as on the percentage yields of carcass and cuts ($p > 0.05$).

In the body composition evaluation by DXA, a significant effect was observed only for the body ash content ($p = 0.0123$; Table 8), which was lower in the NC-PRO treatment compared to the PC and NC-XYL treatments. The other variables were not influenced by the treatments ($p > 0.05$).

Regarding the mineral composition of the tibias at 35 days, a significant effect was observed only for the bone ash percentage ($p \leq 0.05$; Table 9), with the highest value in the NC+PRO treatment and the lowest in the NC-BLE treatment. The calcium and phosphorus levels did not differ between treatments ($p > 0.05$).

Experiment II

3.3. Metabolism Trial, Determination of the Metabolisable Energy Values of the Experimental Diets

Significant differences were observed between treatments for the apparent metabolisable energy (AME) values ($p = 0.001$) and the nitrogen-corrected apparent metabolisable energy (AMEn) values ($p = 0.001$) (Table 10). For AME, the NC+PRO treatment showed the highest value, statistically differing from the NC-PHY and NC-XYL treatments ($p = 0.001$). Similarly, for AMEn, the NC+PRO treatment showed the highest value, differing from the NC-PHY and NC-PRO treatments ($p = 0.001$). The other treatments showed intermediate values, with no significant differences among them ($p > 0.05$) (Table 10).

Experiment III

3.4. Determination of the Amino Acid Digestibility Coefficients of the Experimental Diets

Significant differences were observed between treatments for the standardized ileal digestibility coefficients (SIAAD) of all the amino acids evaluated ($p < 0.001$), indicating the effect of dietary treatments on the ileal digestibility of amino acids (Table 11).

In general, the negative controls (NC-PHY, NC-PRO, NC-XYL, and NC-BLE) showed lower digestibility coefficients for most amino acids when compared to the positive control (PC) ($p < 0.05$), although this response was not uniformly observed for all amino acids (Table 11).

Supplementation with protease (NC+PRO) resulted in the highest standardized ileal digestibility coefficients for most of the amino acids evaluated, including aspartic acid, glutamic acid, alanine, glycine, proline, serine, tyrosine, arginine, phenylalanine, histidine, isoleucine, leucine, methionine, threonine, and valine, statistically differing from the respective negative controls ($p < 0.05$) (Table 11).

The treatments NC+PHY and NC+XYL showed intermediate digestibility values, with statistical differences in relation to the negative controls for some amino acids, but without a consistent effect for all the amino acids evaluated ($p < 0.05$) (Table 11). Supplementation with the enzyme blend (NC+BLE) resulted in significant increases in the digestibility coefficients of some of the amino acids evaluated, but did not consistently show the highest values among the treatments when compared to other supplemented treatments ($p < 0.05$) (Table 11).

4. Discussion

The combined analysis of the three experiments shows that supplementation with exogenous enzymes in nutritionally reduced diets consistently improved ileal amino acid digestibility and metabolisable energy values. However, these digestive improvements were not reflected, to the same extent, in improvements in the zootechnical performance of the birds. This difference between greater

nutrient availability and productive response suggests that simple digestive release does not, by itself, guarantee greater body deposition. Probably, there are physiological and metabolic limits that restrict the utilization of nutrients released by enzymes, especially when diets have more severe nutritional restrictions. Furthermore, the magnitude of the response varied according to the type of enzyme used, the composition of the nutritional matrix, and the rearing phase of the birds. This indicates that the effects of enzyme supplementation are not universal, but result from the interaction between the substrate available in the diet, the intestinal environment, and the metabolic capacity of the animal in each productive phase.

Experiment I

4.1. Performance

In the initial phase (1–21 days), enzyme supplementation was not able to restore the performance of birds subjected to reduced diets. Although greater sensitivity of young chicks to enzyme supplementation is frequently reported due to digestive immaturity (Cao et al., 2023), our results indicate that, under the experimental conditions adopted, the magnitude of nutritional restriction may have exceeded the compensatory capacity promoted by the enzymes. Furthermore, the absence of a difference in FI throughout the experimental period may suggest that the partial recovery of BWG and FCR is mostly associated with improved nutrient utilization efficiency, and not with behavioral adjustments in intake. In the growth phase (22–35 days), the response to enzymes became more evident, with partial recovery of the performance of supplemented birds, indicating that the development of the gastrointestinal tract may potentiate the effects of exogenous enzymatic hydrolysis (Cowieson et al., 2019). Similar results were reported by Gomes et al. (2026) working with diets with reduced metabolisable energy, crude protein, Ca and P, supplemented with carbohydrases, proteases and phytase, demonstrated that supplementation with multiple enzymes was able to mitigate the negative effects of energy and nutritional restriction on broiler performance.

4.2. Carcass Characteristics and body composition

Although differences were observed in SW and CW, cut yields were not altered by enzyme supplementation. This indicates that improvements in performance were accompanied by proportional tissue growth, without alteration in body distribution (Selle & Ravindran, 2021; Zanu et al., 2022; Lee et al., 2022). When analyzing the body composition and bone mineralization of the tibias, we observed an effect of the treatments only on ash content, with no effect on Ca and P. Although studies indicate that enzymes, especially phytase, can favor bone mineralization (Selle & Cowieson, 2020; Liu et al., 2020; Ravindran et al., 2022), our results suggest that these effects were limited, not demonstrating a consistent improvement in bone mineral deposition under the evaluated conditions. Different results were described by Borges et al. (2025), who demonstrated that phytase supplementation in nutritionally reduced diets was able to restore bone quality in broiler chickens to levels comparable to the positive control, without a linear response to increasing dose. It is possible that the level of mineral reduction adopted in our research was not severe enough to limit bone deposition in a detectable way, or that homeostatic mechanisms partially compensated for the variation in mineral availability.

Experiment II

4.3. Metabolism Trial, Determination of Metabolisable Energy Values of the Experimental Diets

Supplementation with protease resulted in the highest AME and AMEn values, indicating that protein hydrolysis contributed significantly to the energy utilization of the diet. The improvement in energy values may be associated with greater protein solubilization and release of amino acids for absorption (Angel et al., 2011; Cowieson & Roos, 2016). However, the increase in metabolisable energy values was not proportionally reflected in superior performance. These results reinforce that

metabolisable energy does not necessarily represent net energy available for growth (Musigwa et al., 2021). Thus, the results suggest that the increase in AME and AMEn represents an improvement in digestive utilization, but does not, in isolation, guarantee greater productive efficiency. In general, treatments without enzyme supplementation showed lower AME and AMEn values, reinforcing the role of enzymes in the partial recovery of dietary energy in diets with nutritional restrictions (Selle & Ravindran, 2007). These results confirm the potential of enzyme supplementation, although they highlight that its effects are not uniform, requiring individualized assessment for each nutritional strategy.

Experiment III

4.4. Determination of the Amino Acid Digestibility Coefficients of the Experimental Diets

Our results demonstrated that standardized ileal amino acid digestibility responses were dependent on both the amino acid evaluated and the type of enzyme supplemented. Protease supplementation resulted in the most consistent improvements across a broader spectrum of amino acids, including Met, Asp, Leu, Ser, and several other essential and non-essential amino acids, confirming its direct role in protein hydrolysis and potential reduction of endogenous nitrogen losses (Angel et al., 2011; Cowieson & Roos, 2016).

Phytase supplementation also enhanced the digestibility of selected amino acids, such as Glu, Ala, Gly, Ser, Tyr, Phe, Ile, Leu, Lys, Thr, and Val, which may be associated with the degradation of phytate-protein complexes and the release of nutrients previously bound to phytic acid (Lima et al., 2021). However, its effects were less consistent compared to protease under the present experimental conditions. Xylanase supplementation showed amino acid–specific responses, suggesting that disruption of the plant cell wall matrix may improve enzymatic accessibility in a heterogeneous manner (Cowieson, 2005).

Despite significant improvements in the digestibility of several essential and non-essential amino acids, performance gains were only partially restored. This indicates that, under nutritionally restrictive conditions, improvements in digestive efficiency do not necessarily translate proportionally into growth responses. Such dissociation may be associated with metabolic utilization limits or with the magnitude of nutrient restriction applied. Therefore, reassessment of enzyme matrix values should consider not only improvements in nutrient digestibility but also the physiological capacity of birds to utilize the additional nutrients released, particularly in highly restrictive formulations (Choi et al., 2024).

5. Conclusions

Supplementation with exogenous enzymes in nutritionally reduced diets significantly improved ileal amino acid digestibility and metabolisable energy values, reflecting greater digestive efficiency. However, the recovery of zootechnical performance was only partial, indicating that the increase in digestive nutrient availability does not automatically translate into greater body composition. The enzymatic response proved to be dependent on the type of enzyme and the nutritional matrix adopted, reinforcing the need for strategic adjustments in matrix allocation and the intensity of nutritional restriction.

Declaration of Competing Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Authors' contributions

All authors contributed to the conception and design of the study. Conceptualization: J.K. Valentim, R.A.R. de Léo, M.I. Hannas. Methodology: R.A.R. de Léo, J.K. Valentim, A.M. Ribeiro, A.A. de Almeida, M.I. Hannas. Investigation: R.A.R. de Léo, J.K. Valentim, A.M. Ribeiro, M.R. Farias. Data curation: R.A.R. de Léo, J.K. Valentim. Formal analysis: J.K. Valentim, R.A.R. de Léo. Resources: R.F. Jacob, M.I. Hannas. Supervision: J.K. Valentim, M.I. Hannas. Project administration: R.A.R. de Léo, M.I. Hannas. Funding acquisition: M.I. Hannas. Writing – original draft: R.A.R. de Léo. Writing – review & editing: J.K. Valentim, R.A.R. de Léo, A.A. de Almeida, A.M. Ribeiro, M.I. Hannas. All authors read and approved the final manuscript.

Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1

Description of the experimental dietary treatments, including positive controls, negative controls, and supplementation with exogenous enzymes.

	Treatments
Treatment 1	Positive control (PC) with Corn and Soybean Meal.
Treatment 2	Negative control for Phytase (NC Phyt) - reduction of the full matrix (0.48% reduction in crude protein, 35 Kcal/kg of metabolisable energy, 0.120% Ca, and 0.120% available P).
Treatment 3	Negative control for Protease (NC Pro) - 0.75% reduction in crude protein, 40 Kcal/kg of metabolisable energy.
Treatment 4	Negative control for Xylanase (NC Xyl) - 0.30% reduction in crude protein, 65 Kcal/kg of metabolisable energy.
Treatment 5	Negative control for Enzyme Blend (NC Blend) - reduction of the full matrix (0.80% reduction in crude protein, 85 Kcal/kg of metabolisable energy, 0.120% Ca, and 0.120% available P).
Treatment 6	NC Phyt + Phytase Enzyme.
Treatment 7	NC Prot + Protease Enzyme.
Treatment 8	NC Xyl + Xylanase Enzyme.
Treatment 9	NC Blend + Enzyme Blend.

Ca – Calcium; P – Phosphorus; Kcal/kg – Kilocalories per kilogram.

Table 2

Nutritional Matrix Considered in the Formulation of Diets for Phytase, Protease, Xylanase, and Enzyme Blend

Enzyme	Phytase (500 FTU) [1]	Protease[2]	Xylanase[3]	Enzyme Blend
ME (kcal/kg)	35	40	65	85
CP	0.48	0.75	0.30	0.80
Ca	0.12	-	-	0.12
PAv	0.12	-	-	0.12
Lysine	0.0225	0.0326	-	0.0753
Methionine	0.0065	0.0090	-	0.0200
Methionine +Cysteine	0.0100	0.0150	-	0.0380
Threonine	0.0135	0.0310	-	0.0575
Tryptophan	0.0050	0.0050	-	0.0050
Valine	0.0230	0.0960	-	0.1150
Isoleucine	0.0190	0.1020	-	0.1000
Arginine	0.0242	0.0420	-	0.0880

The matrix considers the digestible amino acids; Enzyme inclusion: 0.1% or 1.0 kg/ton. [1]Phytase: derived from *Aspergillus niger*. [2]Protease: Blend of acidic and neutral proteases derived from *Aspergillus niger* and *Bacillus subtilis*, respectively. [3]Xylanase: derived from *Aspergillus niger*. CP: Crude Protein; Ca – Calcium; PAv – Available Phosphorus.

Table 3

Percentage and nutritional composition of the experimental diets during the initial phase (1 to 21 days)

Ingredients / Treatments[1]	**PC**	**NC-PHY**	**NC-PRO**	**NC-XYL**	**NC-BLE**
Corn	56.85	60.51	60.22	59.51	62.52
Soybean meal, 46% CP	37.80	36.10	35.60	36.60	35.20
Soybean oil	3.595	2.285	2.425	2.140	1.180
Limestone, 38% Ca	0.750	0.900	0.800	0.750	0.900
Dicalcium phosphate	1.750	1.100	1.750	1.750	1.100
Salt	0.440	0.440	0.440	0.440	0.440
Choline chloride, 60%	0.100	0.100	0.100	0.100	0.100
DL-Methionine, 98%	0.360	0.360	0.360	0.365	0.335
L-Lysine HCl, 78%	0.230	0.240	0.250	0.255	0.200
L-Threonine, 98.5%	0.095	0.100	0.090	0.110	0.065
L-Valine, 99%	0.040	0.035	-	0.550	-
Coccidiostat 12[2]	0.055	0.055	0.055	0.055	0.055
Antioxidant (BHT)[3]	0.010	0.010	0.010	0.010	0.010
Mineral/Vitamin Supplement[4]	0.300	0.300	0.300	0.300	0.300
Total, %	100	100	100	100	100
Calculated composition of nutrients (%) and metabolisable energy (kcal/kg)					
Metabolisable energy	3050	3016	3009	2986	2966
Crude protein	22.01	21.50	21.26	21.68	21.19
Total Methionine	0.671	0.665	0.662	0.672	0.638
Total Methionine + Cysteine	1.021	1.010	1.003	1.019	0.980
Total Lysine	1.417	1.383	1.376	1.408	1.331
Total Tryptophan	0.271	0.262	0.259	0.265	0.258
Total Threonine	0.924	0.907	0.888	0.924	0.863
Total Valine	1.097	1.066	1.020	1.094	1.019
Total Arginine	1.435	1.392	1.375	1.405	1.370
SID[5] lysine	1.252	1.224	1.219	1.247	1.175
SID Methionine	0.647	0.642	0.639	0.649	0.6159
SID Methionine + Cysteine	0.940	0.930	0.924	0.938	0.901
SID Tryptophan	0.257	0.249	0.246	0.251	0.245
SID Threonine	0.827	0.813	0.795	0.829	0.770
SID Valine	0.975	0.949	0.904	0.975	0.905
Sodium, mg/Kg	1908	1912	1910	1910	1915
Calcium	0.889	0.773	0.902	0.886	0.770
Total phosphorus	0.679	0.561	0.675	0.679	0.562
Available phosphorus	0.417	0.299	0.415	0.416	0.299

[1]Experimental Treatments PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend.

[2]Coxistac 12%.

[3]Butylated hydroxytoluene.

[4]Composition per kg of product: Ether extract – 1.5934%; Mineral matter – 47.9328%; Sodium – 248.0401 mg; Chlorine – 47.5692 mg; Calcium – 8.3802%; Total phosphorus – 0.0048%; Total selenium – 100.0000 mg; Potassium – 190.2767 mg; Sulfur – 11,344.8200 mg; Magnesium – 4,092.7290 mg; Fluorine – 249.7381 mg; Iodine – 333.3651 mg; Iron – 17,498.6600 mg; Copper – 3,333.3340 mg; Manganese – 21,666.7100 mg; Zinc – 20,000.00 mg; Vitamin A – 2,100 KUI, Vitamin D3 – 750.0001 KUI; Vitamin E – 4,000.0010 mg; Vitamin K3 Menadion – 666.6948 mg; Thiamine (B1) – 500.0001 mg; Riboflavin (B2) – 1,666.6670 mg; Pyridoxine (B6) – 666.6668 mg; Vitamin B12 – 4,000.0010 mg; Niacin – 11,666.7500 mg; Pantothenic Acid – 4,000.0010 mg; Biotin – 8.3333 mg; Folic acid – 250.0000 mg. [5]Standardized ileal digestibility.

Table 4

Percentage and nutritional composition of the experimental diets during the growth phase (21 to 35 days)

Ingredients / Treatments[1]	**PC**	**NC-PHY**	**NC-PRO**	**NC-XYL**	**NC-BLE**
Corn	59.30	62.70	62.72	61.80	64.96
Soybean meal, 46% CP	31.50	29.90	29.30	30.40	28.90
Soybean oil	5.075	3.765	3.910	3.620	2.670
Limestone, 38% Ca	0.750	0.900	0.750	0.750	0.900
Dicalcium phosphate	1.700	1.050	1.700	1.700	1.050
Salt	0.390	0.390	0.390	0.390	0.390
Choline chloride, 60%	0.100	0.100	0.100	0.100	0.100
DL-Methionine, 98%	0.340	0.340	0.340	0.345	0.315
L-Lysine HCl, 78%	0.290	0.305	0.310	0.320	0.260
L-Threonine, 98.5%	0.115	0.120	0.110	0.125	0.085
L-Valine, 99%	0.070	0.065	0.005	0.085	-
Coccidiostat 12[2]	0.055	0.055	0.055	0.055	0.055
Antioxidant (BHT)[3]	0.010	0.010	0.010	0.010	0.010
Mineral/Vitamin Supplement[4]	0.300	0.300	0.300	0.300	0.300
Total, %	100	100	100	100	100
Calculated composition of nutrients (%) and metabolisable energy (kcal/kg)					
Metabolisable energy	3198	3163	3159	3134	3114
Crude protein	19.50	19.03	18.76	19.21	18.68
Total Methionine	0.619	0.615	0.611	0.621	0.587
Total Methionine + Cysteine	0.934	0.924	0.916	0.933	0.893
Total Lysine	1.292	1.265	1.252	1.290	1.206
Total Tryptophan	0.234	0.226	0.223	0.229	0.222
Total Threonine	0.843	0.828	0.807	0.839	0.782
Total Valine	1.001	0.973	0.900	1.001	0.894
Total Arginine	1.245	1.205	1.185	1.218	1.180
Total Glycine	0.714	0.678	0.664	0.689	0.655
SID[5] lysine	1.151	1.129	1.118	1.152	1.073
SID Methionine	0.598	0.594	0.591	0.601	0.567
SID Methionine + Cysteine	0.861	0.852	0.845	0.860	0.822
SID Tryptophan	0.222	0.215	0.211	0.217	0.210
SID Threonine	0.758	0.746	0.727	0.757	0.701
SID Valine	0.899	0.875	0.804	0.901	0.799
Sodium, mg/Kg	1710	1714	1712	1712	1717
Calcium	0.858	0.742	0.852	0.855	0.739
Total phosphorus	0.646	0.528	0.642	0.646	0.528
Available phosphorus	0.400	0.282	0.398	0.400	0.282

[1]Experimental Treatments PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend. [2]Coxistac 12%. [3]Butylated hydroxytoluene.

[4]Composition per kg of product: Ether extract – 1.5934%; Mineral matter – 47.9328%; Sodium – 248.0401 mg; Chlorine – 47.5692 mg; Calcium – 8.3802%; Total phosphorus – 0.0048%; Total selenium – 100.0000 mg; Potassium – 190.2767 mg; Sulfur – 11,344.8200 mg; Magnesium – 4,092.7290 mg; Fluorine – 249.7381 mg; Iodine – 333.3651 mg; Iron – 17,498.6600 mg; Copper – 3,333.3340 mg; Manganese – 21,666.7100 mg; Zinc – 20,000.00 mg; Vitamin A – 2,100 KUI, Vitamin D3 – 750.0001 KUI; Vitamin E – 4,000.0010 mg; Vitamin K3 Menadion – 666.6948 mg; Thiamine (B1) – 500.0001 mg; Riboflavin (B2) – 1,666.6670 mg; Pyridoxine (B6) – 666.6668 mg; Vitamin B12 – 4,000.0010 mg; Niacin – 11,666.7500 mg; Pantothenic Acid – 4,000.0010 mg; Biotin – 8.3333 mg; Folic acid – 250.0000 mg.

[5]Standardized ileal digestibility.

Table 5

Composition of the protein-free diet used for the determination of ileal amino acid digestibility

Ingredients / Diets	DIP[1]
Starch	80.31
Sugar	5.00
Soybean oil	5.00
Dicalcium phosphate	2.10
Limestone	0.70
Salt	0.45
Potassium carbonate (K_2CO_3)	1.00
Corn cob	4.00
Mineral supplement[2]	0.08
Vitamin supplement[3]	0.15
Choline chloride	0.20
Antioxidant (BHT)	0.01
Insoluble acid ash (Celite™)	1.00
Total	**100.00**

[1]DIP: protein-free diet.

[2]Mineral supplement – Guaranteed levels per kg of product: Selenium – 250.0 mg; Manganese – 58.36 g; Iron – 41.68 g; Zinc – 54.21 g; Copper – 8.31 g; Iodine – 843.0 mg.

[3]Vitamin supplement – Guaranteed levels per kg of product: Vitamin A – 9,638,000 IU; Vitamin D_3 – 2,410,000 IU; Vitamin E – 36,100 IU; Vitamin B_1 – 2,600 mg; Vitamin B_2 – 6,450 mg; Vitamin B_6 – 3,610 mg; Vitamin B_{12} – 15.9 mg; Nicotinic acid – 39.2 g; Pantothenic acid – 12.95 g; Vitamin K_3 – 1,936 mg; Folic acid – 903.9 mg; Biotin – 89.8 mg. [3]Butylated hydroxytoluene.

Table 6

Productive performance of broiler chickens during the initial, growth, and total periods, fed with nutritionally reduced diets supplemented with exogenous enzymes

Treatments[1]	Variables										
	1 - 21 days				22 - 35 days			1 - 35 days			
	FI	BWG	FCR	BW	FI	BWG	FCR	FI	BWG	FCR	BW
PC	1.38	1.08a	1.27b	1.13a	2.41ab	1.53a	1.57d	3.78	2.61a	1.45c	2.66a
NC-PHY	1.40	1.02b	1.37a	1.06b	2.40ab	1.44bc	1.67abc	3.80	2.46bc	1.54a	2.50bc
NC-PRO	1.37	1.01b	1.36a	1.05b	2.37ab	1.39c	1.70a	3.74	2.40c	1.55a	2.45c
NC-XYL	1.38	1.02b	1.35a	1.07b	2.40ab	1.47abc	1.62abcd	3.78	2.50b	1.51ab	2.54b
NC-BLE	1.39	1.01b	1.37a	1.06b	2.46a	1.46abc	1.69ab	3.86	2.47bc	1.56a	2.52bc
NC+PHY	1.37	1.03b	1.34ab	1.07b	2.40ab	1.51ab	1.59cd	3.77	2.54ab	1.49bc	2.58ab
NC+PRO	1.38	1.03b	1.34ab	1.08b	2.37ab	1.49ab	1.59cd	3.75	2.53b	1.48bc	2.57b
NC+XYL	1.41	1.04b	1.35a	1.09b	2.34b	1.49ab	1.57d	3.75	2.53ab	1.48bc	2.57ab
NC+BLE	1.39	1.04b	1.34ab	1.08b	2.42ab	1.47abc	1.65abcd	3.81	2.51b	1.52ab	2.55b
SEM	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.02	0.01	0.02
P-value	0.855	<0.001	0.002	<0.001	0.078	<0.001	<0.001	0.331	<0.001	<0.001	<0.001

Different letters in the same column indicate a statistical difference by Tukey's test ($p \leq 0.05$).

FI, feed intake (kg/broiler); BWG, body weight gain (kg/broiler); FCR, feed conversion ratio (kg/kg); BW, body weight (kg).

SEM: Standard Error of the Mean.

[1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend.

Table 7

Body weight, carcass weight, and yield of meat cuts from broilers at 35 days of age

Treatments[1]	Variables								
	SW	Carcass		Breast		Thigh		Drumstick	
	Kg	Kg	%	Kg	%	Kg	%	Kg	%
PC	2.49a	1.94a	78.04	0.73	37.70	0.22	11.34	0.28	14.60
NC-PHY	2.42ab	1.89ab	78.08	0.71	37.70	0.22	11.61	0.28	15.05
NC-PRO	2.37b	1.83b	77.28	0.70	38.40	0.22	11.98	0.28	15.10
NC-XYL	2.45ab	1.90ab	77.44	0.72	37.69	0.22	11.78	0.28	14.94
NC-BLE	2.41ab	1.86ab	76.93	0.72	38.11	0.22	11.68	0.28	15.05
NC+PHY	2.48ab	1.94a	78.34	0.74	38.16	0.23	12.14	0.29	15.21
NC+PRO	2.43ab	1.89ab	77.91	0.74	39.33	0.22	11.78	0.27	14.19
NC+XYL	2.40ab	1.87ab	77.84	0.70	37.66	0.22	11.81	0.28	15.21
NC+BLE	2.42ab	1.90ab	78.48	0.73	38.71	0.22	11.85	0.27	14.50
SEM	0.02	0.02	0.55	0.01	0.61	0.01	0.24	0.01	0.28
P-value	0.036	0.008	0.633	0.227	0.495	0.062	0.232	0.235	0.152

Different letters in the same column indicate a statistical difference by Tukey's test ($p \leq 0.05$). SEM: Standard Error of the Mean.

[1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend.

Table 8

Estimated body composition (in g) by DXA and corrected by equations in broilers at 35 days of age

Treatments[1]	Corrected Values by Equation – DXA					
	Weight	Body Fat	Body Ash	Soft lean Mass	Body Water	Body Protein
PC	2548	279	58	2237	1820	416
NC-PHY	2439	246	53	2173	1768	404
NC-PRO	2449	240	52	2194	1785	408
NC-XYL	2542	256	58	2265	1843	422
NC-BLE	2528	276	56	2219	1806	413
NC+PHY	2524	252	54	2255	1835	420
NC+PRO	2534	266	56	2243	1825	417
NC+XYL	2449	240	56	2189	1781	407
NC+BLE	2461	249	53	2192	1784	408
SEM	31.62	10.97	1.38	34.35	27.90	6.44
P-value	0.122	0.086	0.012	0.586	0.586	0.586

Different letters in the same column indicate a statistical difference by Tukey's test ($p \leq 0.05$). CV: Coefficient of Variation, SEM: Standard Error of the Mean. [1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend.

Table 9

Mineral composition (%) of the tibias of broilers at 35 days of age

Treatments[1]	Bone Mineral Composition		
	Ash %	Phosphorus %	Calcium %
PC	40.20abc	10.43	23.66
NC-PHY	39.73bc	10.26	23.43
NC-PRO	40.13abc	10.20	23.47
NC-XYL	40.08abc	10.35	23.29
NC-BLE	39.59c	10.27	23.45
NC+PHY	40.32abc	10.54	23.62
NC+PRO	40.88a	10.32	23.72
NC+XYL	40.63ab	10.48	23.69
NC+BLE	40.61ab	10.35	23.84
SEM	0.20	0.13	0.36
P-value	0.001	0.327	0.988

Different letters in the same column indicate a statistical difference by Tukey's test ($p \leq 0.05$). CV: Coefficient of Variation, SEM: Standard Error of the Mean. [1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend.

Table 10

Apparent Metabolisable Energy (AME) and Metabolisable Energy corrected by nitrogen balance (AMEn) of experimental diets supplemented with exogenous enzymes

Treatments[1]	AME[2]	AMEn[3]
PC	3330abc	3135abc
NC-PHY	3114c	2984c
NC-PRO	3199abc	2981bc
NC-XYL	3170bc	2991bc
NC-BLE	3222abc	3040abc
NC+PHY	3274abc	3107abc
NC+PRO	3417a	3227a
NC+XYL	3310abc	3111abc
NC+BLE	3403ab	3186ab
SEM	52.60	48.85
P-value	0.001	0.001

Different letters in the same column indicate a statistical difference by Tukey's test ($p \leq 0.05$). [2]AME: Apparent Metabolisable Energy (kcal/kg Dry matter); [3]AMEn: Metabolisable Energy corrected by nitrogen balance (kcal/kg Dry matter). CV: Coefficient of Variation, SEM: Standard Error of the Mean. [1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend.

Table 11

Standardized ileal digestibility coefficients of amino acids in experimental diets for broilers

AA[2]	Treatments [1]										
	PC	NC-PHY	NC-PRO	NC-XYL	NC-BLE	NC+PHY	NC+PRO	NC+XYL	NC+BLE	SEM	*P*-value
Asp	96.25ab	94.50cde	94.50cde	94.75bcde	93.25e	95.87abc	96.37a	95.75abcd	94.25de	0.32	<0.001
Glu	97.00abcd	96.25cde	96.00de	96.25cde	95.50e	97.50a	97.37ab	97.12abc	96.37bcde	0.24	<0.001
Ala	95.12abc	93.37cd	92.62d	94.50abc	92.25d	95.62a	95.37ab	95.37ab	93.75bcd	0.38	<0.001
Cys	98.37ab	96.25cd	95.87d	97.00abcd	96.50bcd	98.00abc	98.37ab	98.37ab	96.37cd	0.40	<0.001
Gly	93.12ab	90.75cd	89.87d	91.37bcd	90.25d	93.87a	93.12ab	93.37ab	90.12d	0.50	<0.001
Pro	94.12bc	93.25cd	92.25d	93.12cd	92.50d	95.37ab	95.25ab	95.00ab	93.12cd	0.31	<0.001
Ser	98.25a	95.62bcd	95.00cd	96.62abc	94.25d	98.12a	97.50a	97.25ab	96.62abc	0.35	<0.001
Tyr	97.50abcd	96.87cde	96.00e	97.12abcde	96.37de	98.37a	98.25ab	98.00abc	97.00bcde	0.27	<0.001
Arg	93.87abcd	93.00cde	91.62e	93.75bcd	91.87e	95.37ab	94.50abc	95.62a	92.37de	0.39	<0.001
Phe	94.75abc	94.00bcde	93.00e	94.62abcd	93.37de	95.75a	95.12ab	94.87ab	93.50cde	0.29	<0.001
His	96.62abc	95.25cd	93.75e	96.12abcd	93.50e	96.75ab	96.00bcd	97.12ab	94.87de	0.31	<0.001
Ile	95.62abc	94.62cde	93.62e	95.25bcd	93.87de	96.87a	96.37ab	96.37ab	93.62e	0.31	<0.001
Leu	95.00abc	94.25bcde	93.00e	94.75abcd	93.50de	95.70a	95.62a	95.55ab	94.00cde	0.29	<0.001
Lys	96.50a	95.50abc	94.62cd	96.00ab	93.87d	96.62a	96.12ab	96.37a	95.00bcd	0.25	<0.001
Met	99.62ab	98.75bcd	98.37cd	99.12abc	97.87d	99.62ab	99.75a	99.50ab	99.25abc	0.20	<0.001
Thr	97.62abc	96.00cd	95.00d	97.37abc	95.00d	98.75a	97.87ab	98.25a	96.25bcd	0.39	<0.001
Val	95.37bc	94.37cd	93.62d	95.12bcd	93.75d	97.00a	96.37ab	96.50ab	94.37cd	0.33	<0.001

Different letters in the same row indicate a statistical difference by Tukey's test ($p \leq 0.05$). SEM: standard error of the mean. CV (%): Coefficient of Variation in percentage. [1]Experimental Treatments - PC: Positive Control; NC-Phy: Negative Control for Phytase; NC-Pro: Negative Control for Protease; NC-Xyl: Negative Control for Xylanase; NC-Ble: Negative Control for Enzyme Blend; NC+Phy: Negative Control for Phytase + Phytase; NC+Pro: Negative Control for Protease + Protease; NC+Xyl: Negative Control for Xylanase + Xylanase; NC+Ble: Negative Control for Enzyme Blend + Blend. [2]AA: Amino acids; Asp = Aspartic Acid; Glu = Glutamic Acid; Ala = Alanine; Cys = Cystine; Gly = Glycine; Pro = Proline; Ser = Serine; Tyr = Tyrosine; Arg = Arginine; Phe = Phenylalanine; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Thr = Threonine; Val = Valine.

FINAL CONSIDERATIONS

The present thesis contributes to the advancement of knowledge regarding the use of exogenous enzymes as nutritional tools for improving nutrient utilization and production efficiency in broiler chickens. Considering the increasing demand for sustainable poultry production systems and the need to maximize the efficiency of feed resource utilization, the results obtained reinforce the strategic importance of enzyme technology within modern precision nutrition programs.

The findings demonstrate that exogenous enzymes can positively influence digestive processes and nutrient utilization through distinct but complementary mechanisms. The evaluation of phytase superdosing and multi-enzyme supplementation revealed that enzymatic strategies are capable of enhancing nutrient recovery, improving metabolic efficiency, and maintaining productive performance even under nutritional challenges associated with reduced dietary nutrient levels. These responses highlight the capacity of enzymes to optimize the use of feed ingredients and increase the biological value of poultry diets.

The results obtained with phytase superdosing confirm that the benefits of phytase extend beyond phosphorus release. Improvements in feed efficiency, nutrient metabolizability, metabolisable energy utilization, and bone mineralization demonstrate the broad physiological impact of phytate degradation and reinforce the relevance of maximizing phytate hydrolysis in practical feeding programs. Likewise, the positive responses observed with multi-enzyme supplementation indicate that the strategic combination of enzymatic activities can improve nutrient accessibility and digestive efficiency, allowing the successful implementation of nutritionally reduced diets without impairing productive performance.

Importantly, the outcomes of both studies converge toward a common concept: maximizing nutrient utilization through enzymatic supplementation represents an effective approach to improve production efficiency while simultaneously reducing nutrient losses. Such improvements have implications that extend beyond animal performance, contributing to more sustainable production systems through the reduction of nutrient excretion and the more efficient use of feed resources. Therefore, enzyme technology should be regarded not merely as a feed additive, but as an integral component of nutritional strategies designed to enhance productivity, profitability, and environmental sustainability.

From a practical perspective, the results support the application of phytase superdosing and multi-enzyme supplementation as viable nutritional approaches for commercial broiler production. The ability of these enzymes to improve nutrient recovery and sustain performance under reduced

nutritional specifications may provide opportunities for greater flexibility in feed formulation, potentially reducing feeding costs while maintaining productive efficiency.

Although the present thesis provides important insights into the nutritional and productive effects of enzyme supplementation, further investigations are warranted to deepen the understanding of the biological mechanisms underlying these responses. Future studies exploring the interactions between enzymatic supplementation, intestinal physiology, nutrient transport systems, gut microbiota, inositol metabolism, and long-term economic outcomes may contribute to the refinement of enzyme application strategies and the continued evolution of precision poultry nutrition.

In summary, the evidence generated in this thesis demonstrates that exogenous enzyme supplementation constitutes a robust and sustainable nutritional strategy capable of improving nutrient utilization, supporting productive performance, and enhancing the efficiency of broiler production systems. By integrating productive, metabolic, and sustainability-related responses, this research reinforces the central role of enzyme technology in the development of more efficient and environmentally responsible poultry production systems.