UNIVERSIDADE FEDERAL DE VIÇOSA

Evaluation of alternative feed ingredients and phytase supplementation for broiler chickens

Samuel Oliveira Borges
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2026

SAMUEL OLIVEIRA BORGES

Evaluation of alternative feed ingredients and phytase supplementation for broiler chickens

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*.

Adviser: Arele Arlindo Calderano

VIÇOSA - MINAS GERAIS
2026

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

B732e
2026

Borges, Samuel Oliveira, 1997-
Evaluation of alternative feed ingredients and phytase supplementation for broiler chickens / Samuel Oliveira Borges. – Viçosa, MG, 2026.
1 tese eletrônica (68 f.): il.

Texto em inglês.
Orientador: Arele Arlindo Calderano.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2026.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2026.251
Modo de acesso: World Wide Web.

1. Frango de corte - Nutrição. 2. Suplementos nutricionais. 3. Frango de corte - Alimentação e rações. I. Calderano, Arele Arlindo, 1981-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.50855

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

SAMUEL OLIVEIRA BORGES

Evaluation of alternative feed ingredients and phytase supplementation for broiler chickens

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*.

APPROVED: June 26, 2026.

Assent:

____
Samuel Oliveira Borges
Author

____
Arele Arlindo Calderano
Adviser

ACKNOWLEDGMENTS

To God, for the constant presence, guidance, protection, and for granting me health and wisdom throughout every stage of my life.

To my wife, Carolina Crissafe dos Santos Lemos, for her love, support, inspiration, companionship, and for transforming my life completely. You are my pride, and I love you.

To my parents, Silas Ferreira Borges and Lusimar do Nascimento Oliveira Borges, for their unconditional support, care, prayers, and encouragement throughout my journey. To my brother, Thiago Oliveira Borges, for always believing in me and supporting my academic path.

To the Federal University of Viçosa, for the opportunity to complete my graduate studies, and to the Instituto Nacional de Ciência e Tecnologia em Ciência Animal (INCT–CA), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant No. 425168/2025-5), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Grant No. APQ-08688-25), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES; Finance Code 001), for the financial support of this study.

To my advisors, Prof. Dr. Arele Arlindo Calderano and Prof. Dr. Luiz Fernando Teixeira Albino (in memoriam), for their guidance, trust, and for being the greatest professional examples in my career.

To Dr. Ideraldo Luiz Lima, for his support and valuable assistance.

To the members of my examining committee for their availability, attention, and valuable contributions during the evaluation of this thesis. It is an honor to have the participation of such highly qualified professionals at such an important moment in my academic journey.

To the First Baptist Church of Viçosa and all the friends who have been part of this journey, especially Alex, Mauro, Ítalo, Antônio, Sávio, Bruno, Elias, Romário, Carlos, Kelly, the interns, undergraduate students, and all colleagues from the Poultry Sector, thank you for the friendship, shared knowledge, support, and companionship throughout these years.

To the employees and technicians of the Poultry Sector, especially Carlos, André, Wilson, Daniel, João, and Gilberto, for the excellent work environment, trust, and support.

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research

Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).

ABSTRACT

BORGES, Samuel Oliveira, D.Sc., Universidade Federal de Viçosa, June, 2026. **Evaluation of alternative feed ingredients and phytase supplementation for broiler chickens**. Adviser: Arele Arlindo Calderano.

Modern poultry production requires nutritional strategies capable of improving productive efficiency, reducing feed costs, and promoting the sustainable use of available resources. In this context, the evaluation of alternative feed ingredients and the use of exogenous enzymes have emerged as relevant tools for optimizing broiler nutrition. Accordingly, this thesis was developed to contribute to the nutritional management of broiler chickens through two complementary approaches: the determination of the nutritional value and metabolizable energy values of alternative feed ingredients and the evaluation of phytase supplementation in diets with planned nutritional reductions. The first study aimed to determine the apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) values of alternative feed ingredients used in broiler diets. Eight ingredients were evaluated: whole annatto seed meal, spray-dried porcine plasma, poultry viscera meal, three sources of soybean acid oil, distillers corn oil, and degummed rice bran oil. The determined AME and AMEn values were, respectively, 7,023 and 6,841 kcal/kg, 7,743 and 7,466 kcal/kg, and 8,318 and 7,913 kcal/kg for the three soybean acid oil sources; 8,554 and 8,457 kcal/kg for distillers corn oil; 8,273 and 7,910 kcal/kg for degummed rice bran oil; 999 and 975 kcal/kg for whole annatto seed meal; 3,388 and 2,753 kcal/kg for spray-dried porcine plasma; and 3,691 and 3,216 kcal/kg for poultry viscera meal. These findings demonstrated substantial variation among the evaluated ingredients, reinforcing the importance of the specific nutritional characterization of each feedstuff to improve the precision of diet formulation. The second study evaluated the effects of phytase supplementation in nutritionally deficient diets on growth performance, blood biochemical parameters, bone characteristics, and body composition of broiler chickens. Phytase supplementation restored body weight gain, feed conversion ratio, bone mineralization, serum calcium and phosphorus concentrations, as well as lean tissue and bone deposition, achieving results comparable to those obtained with nutritionally adequate diets, even under reductions in available phosphorus, calcium, sodium, metabolizable energy, crude protein, and digestible amino acids. Overall, the results demonstrate that nutritional efficiency in broiler production can be enhanced through the combination of accurate nutritional evaluation of alternative feed ingredients and the use of technologies that improve nutrient utilization. The use of alternative feed ingredients increases formulation flexibility and may reduce dependence on conventional feedstuffs, whereas phytase supplementation enhances nutrient availability and sustains productive performance in more cost-effective feeding programs.

Keywords: agro-industrial coproducts; exogenous enzymes; metabolizable energy

RESUMO

BORGES, Samuel Oliveira, D.Sc., Universidade Federal de Viçosa, junho de 2026. **Avaliação de ingredientes alternativos e suplementação de fitase para frangos de corte**. Orientador: Arele Arlindo Calderano.

A avicultura moderna demanda estratégias nutricionais capazes de elevar a eficiência produtiva, reduzir custos alimentares e promover o uso sustentável dos recursos disponíveis. Nesse contexto, a avaliação de ingredientes alternativos e a utilização de enzimas exógenas têm se destacado como ferramentas relevantes para a otimização da nutrição de frangos de corte. Assim, esta tese foi desenvolvida com o objetivo de contribuir para o manejo nutricional de frangos de corte por meio de duas abordagens complementares: a determinação do valor nutricional e dos valores de energia metabolizável de ingredientes alternativos e a avaliação da suplementação de fitase em dietas com redução nutricional planejada. No primeiro estudo, objetivou-se determinar os valores de energia metabolizável aparente (EMA) e energia metabolizável aparente corrigida pelo balanço de nitrogênio (EMAn) de ingredientes alternativos utilizados na alimentação de frangos de corte. Foram avaliados oito ingredientes: farelo integral de urucum, plasma suíno desidratado, farinha de vísceras de aves, três fontes de óleo ácido de soja, óleo de milho de destilaria e óleo degomado de farelo de arroz. Os valores de EMA e EMAn determinados foram, respectivamente, de 7.023 e 6.841 kcal/kg, 7.743 e 7.466 kcal/kg e 8.318 e 7.913 kcal/kg para as três fontes de óleo ácido de soja; 8.554 e 8.457 kcal/kg para o óleo de milho de destilaria; 8.273 e 7.910 kcal/kg para o óleo degomado de farelo de arroz; 999 e 975 kcal/kg para o farelo integral de urucum; 3.388 e 2.753 kcal/kg para o plasma suíno desidratado; e 3.691 e 3.216 kcal/kg para a farinha de vísceras de aves. Os resultados evidenciaram ampla variação entre os ingredientes, reforçando a importância da caracterização nutricional específica de cada matéria-prima para formulações mais precisas. No segundo estudo, avaliaram-se os efeitos da suplementação de fitase em dietas nutricionalmente deficientes sobre o desempenho, parâmetros bioquímicos sanguíneos, características ósseas e composição corporal de frangos de corte. A inclusão de fitase restabeleceu o ganho de peso, a conversão alimentar, a mineralização óssea, os níveis séricos de cálcio e fósforo, bem como a deposição de tecido magro e ósseo, alcançando resultados comparáveis aos observados em dietas nutricionalmente adequadas, mesmo sob reduções de fósforo disponível, cálcio, sódio, energia metabolizável, proteína bruta e aminoácidos digestíveis. Em conjunto, os resultados demonstram que a eficiência nutricional na produção de frangos de corte pode ser ampliada pela associação entre a avaliação precisa de ingredientes alternativos e o uso de tecnologias capazes de melhorar o aproveitamento dos nutrientes dietéticos. A utilização de ingredientes alternativos aumenta a flexibilidade das formulações e pode reduzir a dependência de insumos convencionais, enquanto a suplementação com fitase eleva a disponibilidade nutricional e sustenta o desempenho em programas alimentares mais econômicos.

Palavras-chave: coprodutos agroindustriais; energia metabolizável; enzimas exógenas

Summary

GENERAL INTRODUCTION

Broiler production plays an essential role in the global supply of animal protein, standing out for its high biological efficiency (OKE *et al*., 2024) and ability to meet both highly demanding and less demanding markets (NEETESON *et al*., 2023). Maharjan *et al*. (2021) reported that advances in genetics, housing conditions, health management, and husbandry practices over recent decades have resulted in substantial productive gains, leading to broilers with faster growth rates and improved feed efficiency. However, these productive advances have also increased the demand for more precise nutritional programs capable of supporting the genetic potential of modern broilers (TAVÁREZ; SOLIS DE LOS SANTOS, 2016), under scenarios characterized by the need to align economic sustainability with environmental responsibility (BEAL *et al*., 2023), as well as increasing competition for conventional feed ingredients (BABATUNDE *et al*., 2021).

Among the factors that determine the competitiveness of the poultry industry, nutrition represents the most significant proportion of production costs (GUNASEKAR, 2007; MALLICK *et al*., 2020). In this context, even small improvements in diet formulation may generate substantial economic benefits on a large scale. Traditionally, corn and soybean meal constitute the main energy and protein sources in practical broiler diets (HANNAS *et al*., 2024), supplemented with lipid sources, minerals, industrial amino acids, and feed additives. Despite the widespread use of these conventional feed ingredients, seasonal supply fluctuations, climatic events, industrial demand, and logistical instability frequently affect their price and availability (PENG *et al*., 2025). As a result, increasing attention has been directed toward alternative feed ingredients and agro-industrial coproducts that may be incorporated into economically diets (DIAS *et al*., 2023) without compromising final product quality.

The rational inclusion of alternative ingredients depends on careful nutritional characterization; however, this becomes challenging when the nutrient composition of feed ingredients is highly variable (MOSS *et al*., 2020). Unlike highly standardized raw materials, many coproducts exhibit considerable variability in nutrient composition and in the metabolizability of nutrients and energy (RECH *et al*., 2024). This heterogeneity may result from factors such as ingredient origin, industrial processing, and storage conditions (PAHM *et al*., 2008; BELYEA *et al*., 2010; SHARMA *et al*., 2025). Consequently, tabulated values may lead to overestimation or underestimation of their nutritional and energetic value. In intensive production systems, errors of this nature may compromise the economic sustainability of the entire production chain (MOSS *et al*., 2021).

In broiler diet formulation, energy assumes a central and critical role. Voluntary feed intake is strongly influenced by dietary energy density (LEESON; CASTON; SUMMERS, 1996), and the balance between energy and other nutrients directly affects productive and reproductive performance (WANG *et al*., 2024). From a practical perspective, the metabolizable energy system remains widely adopted in poultry nutrition, as it estimates the fraction of energy effectively utilized by birds and can be determined through feasible experimental procedures (DURAIN *et al*., 2025).

The determination of apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) enables the estimation of the energy fraction available after fecal and urinary losses, constituting an essential tool for more precise diet formulation (FAO, 2003), and reflecting the digestive and absorptive capacity of the animals.

Despite its widespread use, the energetic value of a feed ingredient is not a fixed constant. In poultry, energy utilization is influenced by multiple factors, including lipid profile (MURUGESAN; KERR; PERSIA, 2017), thermal processing (OLIVEIRA *et al*., 2022), the presence of antinutritional compounds (BERNARDES *et al*., 2022), and interactions with other dietary components (CERRATE *et al*., 2019).

Lipid sources, for instance, may differ markedly in energy utilization depending on the degree of fatty acid saturation, the proportion of free fatty acids (GENG *et al*., 2022), and product quality or purity (SILVA *et al*., 2024). Similarly, animal by-product meals and plant coproducts exhibit considerable variability as a function of raw material origin and processing intensity (VARGAS *et al*., 2023; DE OLIVEIRA *et al*., 2025). Therefore, the continuous updating of energetic and compositional values is essential to support accurate and efficient feed formulation in modern nutritional programs.

Within this context, alternative feed ingredients have gained increasing attention due to their economic potential and their capacity to add value to industrial residues (BHOSALE *et al*., 2025). Oils and lipid by-products from vegetable oil refining and biofuel production can serve as concentrated energy sources (MURUGESAN; KERR; PERSIA, 2017), while animal by-product meals and functional ingredients may simultaneously supply protein and essential minerals (KERR *et al*., 2019). In parallel, plant coproducts traditionally used for pigmentation or other specific purposes may also offer additional nutritional benefits that remain insufficiently explored (AL-MASOUDI; AL-KAFAJI, 2023). The efficient incorporation of these resources into poultry diets contributes to waste reduction and enhances the overall competitiveness of the sector. Based on these characteristics, the ingredients evaluated in the present study were selected because they represent alternative feedstuffs with practical relevance to the poultry industry, yet still exhibit limited or highly variable information regarding their metabolizable energy values, highlighting the need for updated nutritional characterization.

Nevertheless, the inclusion of alternative ingredients alone does not ensure improved productive performance. Nutrient utilization efficiency is influenced by the bird's digestive capacity (RAVINDRAN; ABDOLLAHI, 2021) as well as by the chemical form in which nutrients are present in the diet (RAM *et al*., 2020). Plant-derived ingredients, particularly cereals, contain considerable amounts of phosphorus in the form of phytate, which serves as the primary storage form of this mineral in plants (BORGES *et al*., 2025). In non-ruminants, the endogenous capacity to hydrolyze phytate is minimal (SOMMERFELD; SANTOS, 2021), limiting phosphorus availability and promoting antagonistic interactions with calcium, proteins, amino acids, and trace minerals (MADSEN; BRINCH-PEDERSEN, 2019). Additionally, phytate can interfere with digestive enzyme activity and increase endogenous nutrient losses (SELLE *et al*., 2000), ultimately reducing overall nutritional efficiency.

The inclusion of exogenous enzymes, particularly phytase, has become a key nutritional strategy to overcome these constraints (JACOB *et al*., 2021). Phytase promotes the stepwise hydrolysis of phytate, thereby releasing bound phosphorus and attenuating its antinutritional properties (MARTÍNEZ-VALLESPÍN *et al*., 2025). Consequently, improvements are consistently observed in phosphorus utilization, along with enhanced availability of calcium, amino acids, and energy, as well as potential benefits to intestinal integrity (LU *et al*., 2020). From an environmental standpoint, phytase supplementation also contributes to reduced nutrient excretion (ABBASI *et al*., 2019), particularly in regions with intensive production systems.

Recent advances have demonstrated that the benefits of phytase extend well beyond the replacement of inorganic phosphorus sources. In contemporary nutritional programs, the enzyme is routinely incorporated into matrix-based formulation strategies, whereby nutritional credits are assigned for the release of phosphorus, calcium, sodium, amino acids, and metabolizable energy (BEDFORD; COWIESON, 2020). This approach enables the formulation of more cost-efficient diets (SELLE *et al*., 2023), as nutrient specifications can be reduced without compromising performance when enzymatic responses are accurately accounted for. Nonetheless, the magnitude of these responses is largely influenced by factors such as enzyme inclusion level and substrate availability (VAN KRIMPEN *et al*., 2016).

In modern broiler chickens, adequate bone mineralization is intrinsically linked to productive performance (NAEEM, 2025). Intensive genetic selection for rapid growth has substantially increased the requirement for skeletal support capable of sustaining accelerated tissue deposition (DIBNER *et al*., 2007). Consequently, mineral deficiencies or nutritional imbalances during early life can compromise bone integrity and negatively affect overall performance (WOOD *et al*., 2013). Within this context, strategies that enhance the availability of phosphorus and calcium—such as phytase supplementation, gain particular relevance, as they support proper skeletal development and promote optimal growth from the earliest stages of production.

Likewise, body composition is a key indicator of nutritional efficiency, as it reflects how dietary energy and amino acids are partitioned between lean tissue accretion and fat deposition (CALDAS *et al*., 2019). Balanced diets should promote lean tissue accretion and carcass yield while limiting excessive lipogenesis arising from imbalances between dietary energy and amino acid supply, which can impair the deposition of high-value cuts (WANG *et al*., 2025). In this context, the strategic selection of energy-yielding ingredients, combined with enhanced nutrient utilization through enzymatic supplementation, plays a central role in determining the metabolic fate of absorbed nutrients.

From a scientific standpoint, the nutritional evaluation of alternative ingredients and the use of phytase should be viewed not as isolated topics, but as complementary elements within a unified strategy for nutritional optimization.

Accurate determination of the energetic and compositional value of emerging feed ingredients expands formulation flexibility and supports the refinement of feed composition databases, such as the Brazilian Tables for Poultry and Swine. Concurrently, enhancing nutrient and energy digestibility through enzymatic supplementation enables more efficient utilization of these ingredients, particularly in diets formulated with tighter nutritional margins—an approach aligned with the current demands of intensive poultry production. Collectively, these efforts contribute to the generation of applied knowledge that underpins the development of diets that are more efficient, economically competitive, and technically sustainable for modern broiler systems.

REFERENCES

ABBASI, F.; FAKHUR-UN-NISA, T.; LIU, J.; LIU, X.; ABBASI, I. H. R. Low digestibility of phytate phosphorus, its environmental impacts, and phytase application in poultry nutrition. **Environmental Science and Pollution Research**, v. 26, p. 9469–9479, 2019. DOI: https://doi.org/10.1007/s11356-018-4000-0.

AL-MASOUDI, Z. A. A. A.; AL-KAFAJI, F. R. Effect of adding annatto seed powder (Bixa orellana) and astaxanthin on broiler performance. **IOP Conference Series: Earth and Environmental Science**, v. 1158, p. 052021, 2023. DOI: https://doi.org/10.1088/1755-1315/1158/5/052021.

BABATUNDE, O. O.; PARK, C. S.; ADEOLA, O. Nutritional potentials of atypical feed ingredients for broiler chickens and pigs. **Animals**, v. 11, p. 1196, 2021. DOI: https://doi.org/10.3390/ani11051196.

BEAL, C. M.; ROBINSON, D. M.; SMITH, J.; VAN DOREN, L. G.; TABLER, G. T.; ROCHELL, S. J.; LEI, X. Economic and environmental assessment of US broiler production: Opportunities to improve sustainability. **Poultry Science**, v. 102, p. 102887, 2023. DOI: https://doi.org/10.1016/j.psj.2023.102887.

BEDFORD, M. R.; COWIESON, A. J. Matrix values for exogenous enzymes and their application in the real world. **Journal of Applied Poultry Research**, v. 29, p. 15–22, 2020. DOI: https://doi.org/10.1016/j.japr.2019.10.011.

BELYEA, R. L.; RAUSCH, K. D.; CLEVENGER, T. E.; SINGH, V.; JOHNSTON, D. B.; TUMBLESON, M. E. Sources of variation in composition of DDGS. **Animal Feed Science and Technology**, v. 159, p. 122–130, 2010. DOI: https://doi.org/10.1016/j.anifeedsci.2010.05.005.

BERNARDES, R. D.; OLIVEIRA, C. H.; CALDERANO, A. A.; FERREIRA, R. S.; DIAS, K. M. M.; ALMEIDA, B. F.; ALEIXO, P. E.; ALBINO, L. F. T. Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets. **Revista Brasileira de Zootecnia**, v. 51, p. e20210211, 2022. DOI: https://doi.org/10.37496/rbz5120210211.

BHOSALE, T. R.; DHAGE, S. A.; GAIKWAD, U. S.; ADANGALE, S. B.; BIRARI, D. R. Nutritional perspectives and innovations in alternative feed resources for sustainable poultry production: a review. **International Journal of Veterinary Science and Animal Husbandry**, v. 10, n. 9, p. 168–173, 2025. DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i9c.2549.

BORGES, S. O.; PETROLLI, T. G.; HANNAS, M. I.; BERNARDES, R. D.; OLIVEIRA, C. H.; DIAS, K. M. M.; ALMEIDA, B. F.; FONSECA, L.; SOUSA, A. F. R.; CALDERANO, A. A. Effects of phytase supplementation on broiler chickens fed nutrient-deficient diets. **Livestock Science**, v. 301, p. 105823, 2025. DOI: https://doi.org/10.1016/j.livsci.2025.105823.

CALDAS, J. V.; BOONSINCHAI, N.; WANG, J.; ENGLAND, J. A.; COON, C. N. The dynamics of body composition and body energy content in broilers. **Poultry Science**, v. 98, n. 2, p. 866–877, 2019. DOI: https://doi.org/10.3382/ps/pey422.

CERRATE, S.; EKMAY, R.; ENGLAND, J. A.; COON, C. Predicting nutrient digestibility and energy value for broilers. **Poultry Science**, v. 98, n. 9, p. 3994–4007, 2019. DOI: https://doi.org/10.3382/ps/pez142.

DE OLIVEIRA, C. R. C.; RABELLO, C. B. V.; RODRIGUES, P. B.; COSTA LOPES, C.; SILVA, B. A.; LOPES, E. C.; LUDKE, M. C. M. M.; SOUZA, L. F. A.; NASCIMENTO, J. C. S.; SILVA, W. A.; RIBEIRO, A. G.; CARVALHO, L. R. A.; BATISTA DOS SANTOS, M. J. Nutritional composition and prediction of energy values of poultry offal meal and meat and bone meal for broilers. **Tropical Animal Health and Production**, v. 57, n. 7, p. 335, 2025. DOI: https://doi.org/10.1007/s11250-025-04580-8.

DIAS, K. M.; OLIVEIRA, C. H.; CALDERANO, A. A.; BERNARDES, R. D.; RIBEIRO, A. M.; LIMA, I. L.; ALBINO, L. F. Research note: Nitrogen-corrected apparent metabolizable energy and standardized ileal amino acid digestibility determination of high-protein dried distiller's grains and corn bran with solubles for broilers. **Poultry Science**, v. 102, p. 102757, 2023. DOI: https://doi.org/10.1016/j.psj.2023.102757.

DIBNER, J. J.; RICHARDS, J. D.; KITCHELL, M. L.; QUIROZ, M. A. Metabolic challenges and early bone development. **Journal of Applied Poultry Research**, v. 16, n. 1, p. 126–137, 2007. DOI: https://doi.org/10.1093/japr/16.1.126.

DURAIN, A. M.; SULABO, R. C.; ADIOVA, C. B.; MERCA, F. E. Nitrogen-corrected apparent metabolizable energy, nutrient and dietary fiber digestibilities of banana meal in broilers. **International Journal of Agricultural Technology**, v. 21, n. 3, p. 855–864, 2025. DOI: https://doi.org/10.63369/ijat.2025.21.3.855-864.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). Food energy - methods of analysis and conversion factors. **FAO Food and Nutrition Paper**, n. 77, p. 3–6, 2003.

GENG, S.; ZHANG, Y.; CAO, A.; LIU, Y.; DI, Y.; LI, J.; LOU, Q.; ZHANG, L. Effects of fat type and exogenous bile acids on growth performance, nutrient digestibility, lipid metabolism and breast muscle fatty acid composition in broiler chickens. **Animals**, v. 12, n. 10, p. 1258, 2022. DOI: https://doi.org/10.3390/ani12101258.

GUNASEKAR, K. R. Formulating feed for broiler performance. Gurgaon, India: Avitech Animal Health Pvt. Ltd., 2007.

HANNAS, M. I.; ROSTAGNO, H. S.; SCHULTZ, E. B.; VIANA, G. S. Nutritional requirements of broiler chickens. In: ROSTAGNO, H. S.; ALBINO, L. F. T. (eds.). **Brazilian Tables for Poultry and Swine: Nutritional Requirements of Broiler Chickens**. 5. ed. Visconde do Rio Branco, MG: Suprema, 2024. p. 231–272.

JACOB, R. D. F.; ALBINO, L. F. T.; CALDERANO, A. A.; BERNARDES, R. D.; ROSTAGNO, H. S.; XAVIER JÚNIOR, M. D. L.; LINO, R. H.; ALVES, W. J.; BORGES, S. O.; DIANA, T. F. Broiler feed formulated with phosphorus, calcium, and energy deficiencies supplemented with exogenous enzymes. **Semina: Ciências Agrárias**, v. 42, p. 3029–3046, 2021. DOI: https://doi.org/10.5433/1679-0359.2021v42n5p3029.

KERR, B. J.; URRIOLA, P. E.; JHA, R.; THOMSON, J. E.; CURRY, S. M.; SHURSON, G. C. Amino acid composition and digestible amino acid content in animal protein by-product meals fed to growing pigs. **Journal of Animal Science**, v. 97, n. 11, p. 4540–4547, 2019. DOI: https://doi.org/10.1093/jas/skz294.

LU, H.; SHIN, S.; KUEHN, I.; BEDFORD, M.; RODEHUTSCORD, M.; ADEOLA, O.; AJUWON, K. M. Effect of phytase on nutrient digestibility and expression of intestinal tight junction and nutrient transporter genes in pigs. **Journal of Animal Science**, v. 98, n. 7, p. skaa206, 2020. DOI: https://doi.org/10.1093/jas/skaa206.

MADSEN, C. K.; BRINCH-PEDERSEN, H. Molecular advances on phytases in barley and wheat. **International Journal of Molecular Sciences**, v. 20, p. 2459, 2019. DOI: https://doi.org/10.3390/ijms20102459.

MAHARJAN, P.; MARTINEZ, D. A.; WEIL, J.; SUESUTTAJIT, N.; UMBERSON, C.; MULLENIX, G.; HILTON, K. M.; BEITIA, A.; COON, C. N. Review: physiological growth trend of current meat broilers and dietary protein and energy management approaches for sustainable broiler production. **Animal**, v. 15, p. 100284, 2021. DOI: https://doi.org/10.1016/j.animal.2021.100284.

MALLICK, P.; MUDULI, K.; BISWAL, J. N.; PUMWA, J. Broiler poultry feed cost optimization using linear programming technique. **Journal of Operations and Strategic Planning**, v. 3, p. 31–57, 2020. DOI: https://doi.org/10.1177/2516600X19896910.

MARTÍNEZ-VALLESPÍN, B.; ADER, P.; ZENTEK, J. Effect of increasing levels of phytase on performance, prececal nutrient digestibility, intestinal mucosa physiology and immune response in broiler chickens from 1 to 21 days of age. **Poultry Science**, p. 105473, 2025. DOI: https://doi.org/10.1016/j.psj.2025.105473.

MOSS, A. F.; CHRYSTAL, P. V.; CROWLEY, T. M.; PESTI, G. M. Raw material nutrient variability has substantial impact on the potential profitability of chicken meat production. **Journal of Applied Poultry Research**, v. 30, p. 100129, 2021. DOI: https://doi.org/10.1016/j.japr.2020.100129.

MOSS, A. F.; CROWLEY, T. M.; CHOCT, M. Compilation and assessment of the variability of nutrient specifications for commonly used Australian feed ingredients. **Proceedings of the Australian Poultry Science Symposium**, v. 31, p. 52, 2020.

MURUGESAN, G. R.; KERR, B. J.; PERSIA, M. E. Energy content of select dietary supplemental lipids for broilers, turkeys, and laying hens. **Journal of Applied Poultry Research**, v. 26, n. 4, p. 536–547, 2017. DOI: https://doi.org/10.3382/japr/pfx027.

NAEEM, M. Improving bone health in broiler chickens: integrating population density, housing, and nutritional strategies for enhanced welfare and productivity. **Poultry Science Management**, v. 2, n. 1, p. 14, 2025. DOI: https://doi.org/10.1186/s44364-025-00017-y.

NEETESON, A.-M.; AVENDAÑO, S.; KOERHUIS, A.; DUGGAN, B.; SOUZA, E.; MASON, J.; RALPH, J.; ROHLF, P.; BURNSIDE, T.; KRANIS, A.; BAILEY, R. Evolutions in commercial meat poultry breeding. **Animals**, v. 13, p. 3150, 2023. DOI: https://doi.org/10.3390/ani13193150.

OKE, O. E.; AKOSILE, O. A.; UYANGA, V. A.; OKE, F. O.; ONI, A. I.; TONA, K.; ONAGBESAN, O. M. Climate change and broiler production. **Veterinary Medicine and Science**, v. 10, p. e1416, 2024. DOI: https://doi.org/10.1002/vms3.1416.

OLIVEIRA, L. M. S.; SILVA, P. G.; SILVA, M. R. S.; CORDEIRO, D. A.; SOUZA, L. P.; MINAFRA, C. S.; SANTOS, F. R. Effect of moisture, particle size and thermal processing of feeds on broiler production. **Brazilian Journal of Poultry Science**, v. 24, n. 4, p. eRBCA-2020, 2022. DOI: https://doi.org/10.1590/1806-9061-2020-1391.

PAHM, A. A.; PEDERSEN, C.; HOEHLER, D.; STEIN, H. H. Factors affecting the variability in ileal amino acid digestibility in corn distillers dried grains with solubles fed to growing pigs. **Journal of Animal Science**, v. 86, p. 2180–2189, 2008. DOI: https://doi.org/10.2527/jas.2007-0731.

PENG, D.; ZHANG, H.; ZHANG, Y.; YU, L.; CHEN, M.; CHEN, J. M.; YOU, L.; LI, P.; LIU, J.; ZHANG, X.; ARVOR, D.; KUCHLER, P.; HUANG, J.; ZHANG, H.; HAO, P.; HUANG, J.; SHU, Z.; WANG, F.; SONG, K.; PEI, Z.; LI, C.; XIE, Y.; ZHANG, Q.; LIANG, M.; LI, H.; HU, J.; LOU, Z.; ZHENG, S.; FENG, X.; PENG, H.; LI, X.; HUETE, A.; ZHANG, B. Global soybean trade dynamics: Drivers, impacts, and sustainability. **The Innovation**, 2025. DOI: https://doi.org/10.1016/j.xinn.2025.101124.

RAM, S.; NARWAL, S.; GUPTA, O. P.; PANDEY, V.; SINGH, G. P. Anti-nutritional factors and bioavailability: approaches, challenges, and opportunities. In: **Wheat and Barley Grain Biofortification**. p. 101–128, 2020. DOI: https://doi.org/10.1016/B978-0-12-818444-8.00004-3.

RAVINDRAN, V.; ABDOLLAHI, M. R. Nutrition and digestive physiology of the broiler chick: state of the art and outlook. **Animals**, v. 11, n. 10, p. 2795, 2021. DOI: https://doi.org/10.3390/ani11102795.

RECH, H.; ZEM FRAGA, A.; HAUBERT FRANCESCHI, C.; BONADIMAN MARIANI, A.; ROMEIRO DE OLIVEIRA, C.; MIOTTO GALLI, G.; LÉTOURNEAU-MONTMINY, M.-P.; HAUSCHILD, L.; BERTOL, T. M.; ANDRETTA, I. Variability in distillers' co-product compositions and their nutritional availability for pigs: Insights from a systematic literature review. **Animals**, v. 14, p. 3455, 2024. DOI: https://doi.org/10.3390/ani14233455.

SELLE, P. H.; MACELLINE, S. P.; CHRYSTAL, P. V.; LIU, S. Y. The contribution of phytate-degrading enzymes to chicken-meat production. **Animals**, v. 13, n. 4, p. 603, 2023. DOI: https://doi.org/10.3390/ani13040603.

SELLE, P. H.; RAVINDRAN, V.; CALDWELL, A.; BRYDEN, W. L. Phytate and phytase: consequences for protein utilisation. **Nutrition Research Reviews**, v. 13, n. 2, p. 255–278, 2000. DOI: https://doi.org/10.1079/095442200108729098.

SHARMA, S.; GAUTAM, P.; KUMAR, A.; JOSHI, H. C.; DOBHAL, A.; LOHANI, U. C.; KUMAR, S. Effect of storage conditions on the proximate composition, mineral retention, and phenolic contents of millet and chicken-based nutri-cereal mix. **Food and Humanity**, p. 100718, 2025. DOI: https://doi.org/10.1016/j.foohum.2025.100718.

SILVA, E. D. D.; VALENTIM, J. K.; OLIVEIRA, C. H.; DIAS, K. M. M.; ALMEIDA, B. F.; VIEIRA, M. J.; CALDERANO, A. A.; ALBINO, L. F. T. Metabolizable energy values and metabolizability coefficients of ether extracts of different types of corn oil for broilers. **Ciência Animal Brasileira**, v. 25, p. e77849, 2024. DOI: https://doi.org/10.1590/1809-6891v25e-77849E.

SOMMERFELD, V.; SANTOS, R. R. In vitro assays for evaluating phytate degradation in non-ruminants: chances and limitations. **Journal of the Science of Food and Agriculture**, v. 101, n. 8, p. 3117–3122, 2021. DOI: https://doi.org/10.1002/jsfa.11020.

TAVÁREZ, M. A.; SOLIS DE LOS SANTOS, F. Impact of genetics and breeding on broiler production performance: A look into the past, present, and future of the industry. **Animal Frontiers**, v. 6, p. 37–41, 2016. DOI: https://doi.org/10.2527/af.2016-0042.

VARGAS, J. I.; GULIZIA, J. P.; BONILLA, S. M.; SASIA, S.; PACHECO, W. J. Apparent metabolizable energy and amino acid digestibility of corn of different origin fed to male broilers. **Animals**, v. 13, n. 19, p. 3111, 2023. DOI: https://doi.org/10.3390/ani13193111.

VAN KRIMPEN, M. M.; VAN EMOUS, R. A.; SPEK, J. W.; KWAKERNAAK, C. Phytate degradation in broilers. Wageningen: **Wageningen Livestock Research**, 2016. DOI: http://dx.doi.org/10.18174/391336.

WANG, M.; MACELLINE, S. P.; LIU, S. Y.; SELLE, P. H. Fat deposition and growth performance in broiler chickens are diversely influenced by maize or wheat following dietary crude protein reductions plausibly involving insulin. **Journal of Animal Science and Biotechnology**, v. 16, p. 179, 2025. DOI: https://doi.org/10.1186/s40104-025-01314-6.

WANG, Y.; WANG, Q.; YAO, X.; GOU, Z.; LIN, X.; LUO, Q.; JIANG, S. Effects and interactions of dietary lysine and apparent nitrogen-corrected metabolizable energy on yellow-feathered broiler breeder hens. **Journal of Animal Science and Biotechnology**, v. 15, p. 143, 2024. DOI: https://doi.org/10.1186/s40104-024-01095-4.

WOOD, C. L.; WOOD, A. M.; HARKER, C.; EMBLETON, N. D. Bone mineral density and osteoporosis after preterm birth: the role of early life factors and nutrition.

International Journal of Endocrinology, v. 2013, p. 902513, 2013. DOI: https://doi.org/10.1155/2013/902513.

Chapter 1: Metabolizable energy values of alternative feed ingredients for broilers

Samuel Oliveira Borges*, Fernando de Castro Tavernari†, Herbert Rech‡, Beatriz Alvarez de Honório*, Giovanna Lima Vieira*, Romário Duarte Bernardes*, Arele Arlindo Calderano*

*Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Brazil

†Embrapa Suínos e Aves, Concórdia, Brazil

‡Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Paper history:

Submitted to the journal Animal Feed Science and Technology on April 20, 2026. Status date (on July 04, 2026): *Under Review.*

ABSTRACT

Accurate estimates of apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) are essential for the efficient use of alternative feed ingredients in broiler diet formulation. This study aimed to determine the AME and AMEn values of eight alternative feed ingredients used in broiler nutrition. A total of 400 male broilers (Cobb 500), 16 days of age, were allocated to a completely randomized design with ten treatments, eight replicates per treatment, and five birds per replicate. Two reference diets were formulated, and the test ingredients were evaluated using the substitution method. The evaluated ingredients included three meal-type feedstuffs (whole annatto seed meal, spray-dried porcine plasma, and poultry viscera meal) and five lipid sources (three sources of soybean acid oil, distillers corn oil, and degummed rice bran oil). The AME and AMEn values determined in the present study, respectively, were 7,023 and 6,841 kcal/kg for soybean acid oil source one, 7,743 and 7,466 kcal/kg for soybean acid oil source two, 8,318 and 7,913 kcal/kg for soybean acid oil source three, 8,554 and 8,457 kcal/kg for distillers corn oil, 8,273 and 7,910 kcal/kg for degummed rice bran oil, 999 and 975 kcal/kg for whole annatto seed meal, 3,388 and 2,753 kcal/kg for spray-dried porcine plasma, and 3,691 and 3,216 kcal/kg for poultry viscera meal.

Keywords: annatto; corn and rice oil; plasma; soybean acid oil; viscera meal

1. INTRODUCTION

Energy is not considered a nutrient per se but rather the result of the digestion and metabolism of macronutrients (NRC, 1994). In poultry nutrition, metabolizable energy of ingredients is widely used for diet formulation because it represents the energy available after excreta losses. The nitrogen-corrected apparent metabolizable energy (AMEn) system is one of the most widely adopted because it accounts for nitrogen retention (ABDOLLAHI *et al*., 2021). This correction assumes that if retained body protein were oxidized, its nitrogen would be excreted as uric acid, which has a known gross energy value of 8.22 kcal per gram of nitrogen (HILL; ANDERSON, 1958). Thus, AMEn values adjust the metabolizable energy of feed ingredients by removing the influence of protein retention, allowing meaningful comparisons across different physiological stages without assuming identical energy utilization throughout the animal's life (LOPEZ; LEESON, 2007).

Although net energy is considered the most accurate estimate of the usable energy of feed ingredients, its determination requires respirometry chambers and strict experimental control (BARZEGAR *et al*., 2020). Thus, AMEn remains the most practical and broadly applied approach in poultry research (DURAIN *et al*., 2025), as it can be obtained, for example, using total excreta collection, a simpler and more cost-effective technique.

The recent increase in prices of conventional ingredients such as corn, soybean meal, and soybean oil has encouraged the adoption of more economical alternatives capable of maintaining adequate dietary AMEn levels (KAKHKI *et al*., 2024). In this context, lipid by-products including soybean acid oil, distillers corn oil, and degummed rice bran oil have gained attention because to their energy value and market availability.

Alternative meal sourcesmay also contribute to cost reduction and dietary energy supply. Whole annatto seed meal is used as a natural pigment in Brazilian alternative poultry production systems (ALBINO *et al*., 2016), although its energy value is often disregarded in diet formulation. Poultry viscera meal is a cost-effective ingredient with a favorable amino acid profile (SILVA *et al*., 2014). Spray-dried porcine plasma, despite its elevated cost, presents high nutritional quality (KIM *et al*., 2022) and therefore can be used in a restricted manner during early phases or in elite genetic lines.

Given the potential of these alternative ingredients, accurate estimates of their apparent metabolizable energy (AME) and AMEn values are essential to support their efficient incorporation into broiler diets. Updated information also enhances the applicability of nutritional databases used by the poultry industry, such as the Brazilian Tables for Poultry and Swine. Accordingly, this study aimed to determine the AME and AMEn values of eight alternative feed ingredients used in broiler nutrition, comprising three meals (whole annatto seed meal, spray-dried porcine plasma, and poultry viscera meal) and five lipid sources (three sources of soybean acid oil, distillers corn oil, and degummed rice bran oil).

2. MATERIAL AND METHODS

2.1 Ethical matters

The Animal Care and Use Committee of the Federal University of Viçosa, Brazil, approved all animal procedures (protocol no. 39/2024). The experiment was conducted according to the experimental protocols for using live broilers from the Brazilian College of Animal Experimentation.

2.2 Birds and experimental design

One-day-old male broiler chickens (Cobb 500) were obtained from a commercial hatchery and reared on the floor pens in accordance with the strain's management guidelines until the beginning of the experiment. The birds had free access to water and were fed a standard corn-soybean meal-based diet in mash form. At 16 days of age, a total of 400 broilers (679.6 ± 14.0 g) were distributed in a completely randomized design consisting of ten treatments, eight replicates per treatment, and five birds per experimental unit. The birds were housed in metabolic cages (600 cm²/bird). Each cage was equipped with a nipple drinker and a trough feeder to ensure free access to water and feed throughoutt the experimental period. Temperature and light programs were set according to the strain’s management guidelines.

Two reference diets based on corn and soybean meal were formulated (Table 1). Reference diet 1 (REF1) was used to evaluate three alternative meal-type ingredients: whole annatto seed meal, spray-dried porcine plasma, and poultry viscera meal. Reference diet 2 (REF2) was used to evaluate five lipid sources: three sources of soybean acid oil, degummed rice bran oil, and distillers corn oil.

Treatments T1 and T2 corresponded to REF1 and REF2, respectively, without the inclusion of test ingredients. Treatments T3, T4, and T5 were formulated by partially replacing REF1 with the test ingredients, resulting in diets composed of 70% REF1 + 30% whole annatto seed meal, 80% REF1 + 20% spray-dried porcine plasma, and 80% REF1 + 20% poultry viscera meal, respectively. Treatments T6, T7, T8, T9, and T10 were formulated by partially replacing REF2, resulting in diets composed of 91.5% REF2 + 8.5% of the following ingredients: three soybean acid oil, degummed rice bran oil, and distillers corn oil, respectively.

Table 1. Ingredients and nutritional composition of the reference diet 1 and 2.

Ingredients (g/kg)	REF1	REF2
Corn	703.32	558.65
Soybean meal	249.34	401.34
Soy oil	9.44	2.37
Dicalcium phosphate	14.93	16.74
Limestone	6.40	7.30
Salt	4.80	5.30
DL-Methionine – 99%	3.33	3.56
Mineral premix	1.20	1.20
Vitamin premix	1.30	1.30
Choline chloride – 60%	0.80	0.80
L-Lysine HCl – 78.8%	3.26	1.14
L-Threonine – 98.5%	1.01	0.30
L-Valine – 98.5%	0.88	-
Calculated nutritional values		
Crude protein (g/kg)	170.5	230.0
AMEn Kcal/kg	3050	2830
Calcium (g/kg)	7.70	9.00
Available Phosphorus (g/kg)	3.70	4.30
Sodium (g/kg)	2.00	2.20
Digestible Lysine (g/kg)	10.50	12.50
Digestible Met + Cys (g/kg)	8.10	9.60
Digestible Threonine (g/kg)	6.90	8.20
Digestible Valine (g/kg)	8.10	9.68

[1]Vitamin premix provided per kg of diet: vitamin A. 9638000 UI; vitamin D3. 2410000 UI; vitamin E. 36100 UI; vitamin K3. 1936 mg; vitamin B1. 2600 mg; vitamin B2. 6450 mg. vitamin B12. 159 mg; vitamin B6. 3610 mg; vitamin B5. 12.95 g; vitamin B3. 39.2 g; vitamin B9. 903.0 mg; biotin. 89.8 mg.

[2]Trace mineral premix provided per kg diet: Mn. 58.36 g; Zn. 54.21 g; Fe. 41.68 g; Cu. 8.31 g; I. 843.00 mg; Se. 250.00 mg.

2.3 Metabolism trial

The trial was conducted from 16 to 25 days of age to determine the AME and AMEn. The experimental period lasted 10 days; the first 5 days were used to adapt the birds to the experimental diets, and the final 5 days were dedicated to total excreta collection and measurement of feed intake. A metal tray lined with plastic mesh, which allowed individual excreta collection for each experimental unit, was placed beneath the metabolic cages. Excreta were collected twice daily, at 08:00 and 16:00 h.

After each collection, excreta were placed in labeled plastic bags according to their respective experimental unit and stored at -20 °C. At the end of the final collection day, excreta were thawed and individually weighed to determine the total amount produced. Homogenous and representative samples of excreta, experimental diets, and alternative feed ingredients were analyzed according to the Association of Official Analytical Chemists (AOAC) procedures for dry matter (DM; AOAC, 934.01, 2006) and nitrogen (N; AOAC, 990.03, 2006). Gross energy (GE) was determined using a C500 adiabatic bomb calorimeter (IKA-Werke GmbH & Co, KG, Staufen, Germany). Nitrogen balance (NB) was calculated as the difference between N intake and N excreted.

The AME and AMEn were calculated as follows, respectively:

$$AME_{RD} = (GE_{ing} - GE_{exc}) / DMI$$

$$AME_{TD} = (GE_{ing} - GE_{exc}) / DMI$$

$$AME_{ing} = AME_{RD} + ((AME_{TD} - AME_{RD}) / \text{Ingredient inclusion level})$$

$$AMEn_{RD} = ((GE_{ing} - GE_{exc}) - (8.22 \times N_{ret})) / DMI$$

$$AMEn_{TD} = ((GE_{ing} - GE_{exc}) - (8.22 \times N_{ret})) / DMI$$

$$AMEn_{ing} = AMEn_{RD} + ((AMEn_{TD} - AMEn_{RD}) / \text{Ingredient inclusion level})$$

Where:

- AME_{RD} = apparent metabolizable energy of the reference diet

- AME_{TD} = apparent metabolizable energy of the test diet containing the evaluated ingredient at the specified inclusion level

- AME_{ing} = apparent metabolizable energy of the ingredient

- $AMEn_{RD}$ = nitrogen-corrected apparent metabolizable energy of the reference diet

- AMEn_TD = nitrogen-corrected apparent metabolizable energy of the test diet containing the evaluated ingredient at the specified inclusion level

- AMEn_ing = nitrogen-corrected apparent metabolizable energy of the ingredient

- GE_ing = gross energy intake

- GE_exc = gross energy excreted

- N_ret = nitrogen retention (N_ret = N_ing − N_exc)

- DMI = dry matter intake

3. RESULTS AND DISCUSSION

The AME and AMEn values of the evaluated ingredients are presented in Table 2.

Table 2. Chemical composition and metabolizable energy values of the evaluated ingredients.

	Lipid Sources					Meals Feedstuffs		
	SAO1	SAO2	SAO3	DRO	DCO	WASM	SDPP	PVM
Dry matter (g/kg)	960.2	975.5	973.0	992.7	994.6	890.8	933.0	958.8
Crude protein (g/kg)	-	-	-	-	-	132.0	803.0	635.0
Crude fiber (g/kg)	-	-	-	-	-	136.7	-	-
Ash (g/kg)	-	-	-	-	-	69.3	70.4	184.0
Ether extract (g/kg)	938.2	956.9	957.5	969.0	997.8	66.0	0.00	113.4
Gross energy (kcal/kg)	8590	8765	8717	9046	9004	4107	4829	4612
Acid index (as oleic acid, %)	83,17	65,99	50,21	15,11	13,97	-	-	-
AME (kcal/kg)	**7023**	**7743**	**8318**	**8273**	**8554**	**999**	**3388**	**3691**
AMEn (kcal/kg)	**6841**	**7466**	**7913**	**7910**	**8457**	**975**	**2753**	**3216**

SAO: soybean acid oil; DRO: degummed rice bran oil; DCO: distillers corn oil; WASM: whole annatto seed mea; SDPP: spray-dried porcine plasma; PVM: poultry viscera meal; AME: apparent metabolizable energy; AMEn: nitrogen-corrected apparent metabolizable energy.

The metabolizable energy values obtained in the present study demonstrated substantial variability among both lipid sources and alternative meal feedstuffs, reinforcing the importance of determining ingredient-specific AME and AMEn values for accurate broiler diet formulation.

Among the lipid sources evaluated in the present study, distillers corn oil exhibited AME and AMEn values that are consistent with those reported in the literature for corn-derived oils used in broiler nutrition, indicating its adequacy as an energy source for broiler chickens. Previous reports have shown that oils obtained from the ethanol industry are characterized by a high proportion of unsaturated fatty acids, particularly oleic and linoleic acids (DI LENA *et al*., 2020), which are more efficiently digested and absorbed by poultry (OKETCH *et al*., 2023). In addition, the elevated ether extract content and the favorable unsaturated-to-saturated fatty acid ratio are factors known to contribute to the metabolizable energy utilization of this type of lipid source (SERPA *et al*., 2023).

The soybean acid oil sources exhibited wide variability in energetic values, with AMEn ranging from 6,841 to 7,913 kcal/kg. These values partially overlap with those reported by Vieira *et al*. (2002), who observed AMEn values between 7,821 and 8,407 kcal/kg, highlighting the well-documented variability associated with the chemical composition and quality of soybean acid oil. According to Freitas *et al*. (2005), this variability is associated with the non-standardized composition of the product and the predominance of free fatty acids.

This variation highlights the strong influence of the chemical quality of ingredients, including fatty acid composition, oxidation level, acidity, and processing characteristics, on metabolizable energy (SONG *et al*., 2025). Furthemore, the acid index (expressed as oleic acid, %) indicates the amount of free fatty acids resulting from degradation present in an oil source and reflects its quality. High acidity values indicate greater concentrations of free fatty acids, which are less efficiently digested than triglycerides, particularly in young birds, due to limited bile secretion and reduced pancreatic lipase activity (RODRIGUEZ-SANCHEZ *et al*., 2019). In the present study, the acid index of soybean acid oil source 3 was associated with a high AMEn value, which was comparable to that reported by Calderano *et al*. (2024; 7,830 kcal/kg).

Degummed rice bran oil is a by-product of rice processing derived from the rice germ and inner husk (AL-ABDULLATIF *et al*., 2023). This ingredient has been gaining increasing attention among animal nutritionists not only as an energy source but also for its functional potential, as its unsaponifiable lipid fraction has high nutritional value. In particular, the oil is enriched with γ-oryzanol, tocotrienols, and tocopherols—compounds recognized for their antioxidant properties (BUMRUNGPERT *et al*., 2019). In the present study, the AME and AMEn values obtained for degummed rice bran oil were higher than those reported by Song *et al*. (2022), who observed values of 6,908 and 6,879 kcal/kg, which may reflect differences in rice type and processing conditions between studies.

Regarding alternative meal feedstuffs, poultry viscera meal is produced through acid cooking followed by controlled drying of the entire gastrointestinal tract (REDOY *et al*., 2021). Approximately 10 million tons of viscera are produced annually worldwide (ECD/FAO, 2018), which presents a potential for inclusion in poultry diets. In this study, poultry viscera meal exhibited AMEn values within the range reported in the literature, as described by Silva *et al*. (2010) and de Oliveira *et al*. (2025), who reported values ranging from 2,524 to 4,346 kcal/kg and 3,189 to 3,342, respectively. This result is consistent with its high ether extract content, which favor nutrient digestibility and energy utilization. Although poultry viscera meal is often recognized primarily for its protein, amino acid, and mineral contributions, the present results demonstrate that its energetic value is also substantial and should be properly accounted for in diet formulation, particularly in energy-dense diets.

Although its use in broiler diets is generally limited by its high cost, spray-dried porcine plasma has gained increasing attention in the poultry industry, particularly in production systems that restrict the use of synthetic growth promoters. This growing interest is largely attributed to its content of biologically active components, including immunoglobulins, lipids, peptides, enzymes, amino acids, and other functional factors (BORG *et al*., 2002), which play crucial roles in various biochemical, immunological, and metabolic pathways (PÉREZ-BOSQUE *et al*., 2016).

In the present study, the AMEn value observed for spray-dried porcine plasma was lower than that reported by Calderano *et al*. (2024; 3,234 kcal/kg), which is nutritionally consistent with the compositional differences between the ingredients. Despite the high crude protein content observed in the present study (80.30%), the absence of ether extract likely limited the energetic contribution of the ingredient when compared with the values reported by Calderano *et al*. (2024), who described lower crude protein (71.7%) and detectable lipid content (1.03%). Nonetheless, the high digestibility of spray-dried porcine plasma, as reported by Khadour *et al*. (2022), supports its strategic use as a functional protein source that can also contribute to dietary energy, although to a lesser extent than lipid-containing ingredients.

Whole annatto seed meal presented energy values compatible with its chemical composition, considering its ether extract content (6.60%) and crude fiber level (13.67%), which is associated with differences in energy utilization by birds (MURUGESAN *et al*., 2017). Despite its reduced metabolizable energy, whole annatto seed meal is commonly included in alternative and niche production systems, particularly for pigmentation purposes (GARCIA *et al*., 2010; RODRÍGUEZ-ORTEGA *et al*., 2024).

In addition to its potential in niche markets, this feed ingredient has been widely studied for its antioxidant properties, which may improve animal performance by inhibiting the growth of pathogenic microorganisms (AL-MASOUDI *et al*., 2023). This feature makes it an even more viable alternative for the conventional broiler industry, as it supports intestinal health and feed efficiency, offering benefits in both productivity and risk reduction (AL-SAEEDI *et al*., 2025). However, its energy contribution is often overlooked in feed formulation, the present findings demonstrate that, although limited, whole annatto seed meal provides measurable metabolizable energy and should not be considered an inert ingredient from an energy standpoint.

Future studies should investigate the interactions between chemical quality parameters of alternative feed ingredients, such as acidity, fatty acid profile, and degree of processing, and their effects on metabolizable energy utilization in broilers at different physiological stages. In this context, energy values should be considered alongside the physiological and functional roles of these ingredients, rather than being evaluated in isolation. Moreover, studies addressing the physiological benefits of functional ingredients should also integrate sanitary challenge conditions with performance responses and energy utilization, allowing a more comprehensive assessment of their nutritional and productive value. Additionally, evaluating the consistency of industrial by-products across production batches may help refine energy prediction models and improve formulation accuracy.

In conclusion, the AME and AMEn values determined in the present study, respectively, were 7,023 and 6,841 kcal/kg for soybean acid oil source one, 7,743 and 7,466 kcal/kg for soybean acid oil source two, 8,318 and 7,913 kcal/kg for soybean acid oil source three, 8,554 and 8,457 kcal/kg for distillers corn oil, 8,273 and 7,910 kcal/kg for degummed rice bran oil, 999 and 975 kcal/kg for whole annatto seed meal, 3,388 and 2,753 kcal/kg for spray-dried porcine plasma, and 3,691 and 3,216 kcal/kg for poultry viscera meal.

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.

ACKNOWLEDGMENTS

To the Instituto Nacional de Ciência e Tecnologia em Ciência Animal (INCT–CA), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant No. 425168/2025-5), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Grant No. APQ-08688-25), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES; Finance Code 001) for the financial support.

REFERENCES

ABDOLLAHI, M. R.; WILTAFSKY-MARTIN, M.; RAVINDRAN, V. Application of apparent metabolizable energy versus nitrogen-corrected apparent metabolizable energy in poultry feed formulations: A continuing conundrum. **Animals**, v. 11, n. 8, p. 2174, 2021. DOI: https://doi.org/10.3390/ani11082174.

AL-ABDULLATIF, A.; HUSSEIN, E.; SULIMAN, G.; AKASHA, M.; AL-BADWI, M.; ALI, H.; AZZAM, M. Evaluating rice bran oil as a dietary energy source on production performance, nutritional properties and fatty acid deposition of breast meat in broiler chickens. **Foods**, v. 12, n. 2, p. 366, 2023. DOI: https://doi.org/10.3390/foods12020366.

AL-MASOUDI, Z. A. A. A.; AL-KAFAJI, F. R. Effect of adding annatto seed powder (Bixa orellana) and astaxanthin to the diet in the productive performance of broilers (Ross-308). **IOP Conference Series: Earth and Environmental Science**, v. 1158, n. 5, p. 052021, 2023. DOI: https://doi.org/10.1088/1755-1315/1158/5/052021.

AL-SAEEDI, M. K. I.; NOOR, A. S.; AL-JEBORY, H. H. Effect of in ovo injection annatto seed extract on some blood biochemical traits of broiler chicks (Ross 308). **IJBB**, v. 7, n. 1, p. 01–04, 2025. DOI: https://dx.doi.org/10.33545/26646536.2025.v7.i1a.89.

ALBINO, L. F. T.; TAVERNARI, F. C.; VIEIRA, R. A. **Criação de frango e galinha caipira**. 4. ed. Viçosa, MG: Aprenda Fácil, 2016.

BARZEGAR, S.; WU, S. B.; CHOCT, M.; SWICK, R. A. Factors affecting energy metabolism and evaluating net energy of poultry feed. **Poultry Science**, v. 99, n. 1, p. 487–498, 2020. DOI: https://doi.org/10.3382/ps/pez554.

BORG, B. S.; CAMPBELL, J. M.; POLO, J.; RUSSELL, L. E.; RODRÍGUEZ, C.; RÓDENAS, J. Evaluation of the chemical and biological characteristics of spray-dried plasma protein collected from various locations around the world. **Proceedings of the American Association of Swine Veterinarians**, v. 33, p. 97–100, 2002.

BUMRUNGPERT, A.; CHONGSUWAT, R.; PHOSAT, C.; BUTACNUM, A. Rice-bran oil containing gamma-oryzanol improves lipid profiles and antioxidant status in hyperlipidemic subjects: A randomized double-blind controlled trial. **Journal of Alternative and Complementary Medicine**, v. 25, p. 353–358, 2019. DOI: https://doi.org/10.1089/acm.2018.0212.

CALDERANO, A. A.; GENOVA, J. L.; TAVERNARI, F. C.; ALBINO, L. F. T.; ROSTAGNO, H. S. Composition and nutritional feedstuff value. In: ROSTAGNO, H. S.; ALBINO, L. F. T. (eds.). **Brazilian Tables for Poultry and Swine: Feedstuff composition and nutritional value**. 5. ed. Visconde do Rio Branco, MG: Suprema, 2024. p. 1–230.

DE OLIVEIRA, C. R. C.; RABELLO, C. B. V.; RODRIGUES, P. B.; DA COSTA LOPES, C.; SILVA, B. A.; LOPES, E. C.; LUDKE, M. C. M. M.; DE SOUZA, L. F. A.; NASCIMENTO, J. C. S.; DA SILVA, W. A.; RIBEIRO, A. G.; CARVALHO, L. R. A.; BATISTA DOS SANTOS, M. J. Nutritional composition and prediction of energy values of poultry offal meal and meat and bone meal for broilers. **Tropical Animal Health and Production**, v. 57, n. 7, p. 335, 2025. DOI: https://doi.org/10.1007/s11250-025-04580-8.

DI LENA, G.; ONDREJÍČKOVÁ, P.; PULGAR, J. S. D.; CYPRICHOVÁ, V.; JEŽOVIČ, T.; LUCARINI, M.; CAPRONI, R. Towards a valorization of corn bioethanol side streams: Chemical characterization of post-fermentation corn oil and thin stillage. **Molecules**, v. 25, n. 15, p. 3549, 2020. DOI: https://doi.org/10.3390/molecules25153549.

DURAIN, A. M.; SULABO, R. C.; ADIOVA, C. B.; MERCA, F. E. Concentration of nitrogen-corrected apparent metabolizable energy, nutrient and dietary fiber digestibilities of banana meal in broilers. **International Journal of Agricultural Technology**, v. 21, n. 3, p. 855–864, 2025. DOI: https://doi.org/10.63369/ijat.2025.21.3.855-864.

FREITAS, E. R.; SAKOMURA, N. K.; NEME, R.; SANTOS, A. L. D. Valor energético do óleo ácido de soja para aves. **Pesquisa Agropecuária Brasileira**, v. 40, p. 241–246, 2005. DOI: https://doi.org/10.1590/S0100-204X2005000300007.

GARCIA, E. A.; MOLINO, A. B.; GONÇALVES, H. C.; JUNQUEIRA, O. M.; PELÍCIA, K.; OSERA, R. H.; DUARTE, K. F. Ground annatto seeds (Bixa orellana L.) in sorghum-based commercial layer diets and their effects on performance, egg quality, and yolk pigmentation. **Brazilian Journal of Poultry Science**, v. 12, n. 4, p. 259–264, 2010. DOI: https://doi.org/10.1590/S1516-635X2010000400007.

HILL, F. W.; ANDERSON, D. L. Comparison of metabolizable energy and productive energy determinations with growing chicks. **Journal of Nutrition**, v. 64, n. 4, p. 587–603, 1958. DOI: https://doi.org/10.1093/jn/64.4.587.

KAKHKI, R. A. M.; NAVARRO-VILLA, A.; DE LOS MOZOS, J.; DE VRIES, S.; GARCÍA-RUIZ, A. I. Evaluation of fibrous feed ingredient alternatives to oat hulls as a source of feed structure in broiler diets. **Poultry Science**, v. 103, n. 12, p. 104297, 2024. DOI: https://doi.org/10.1016/j.psj.2024.104297.

KHADOUR, H. V. N.; PARSONS, B. W.; UTTERBACK, P. L.; CAMPBELL, J. M.; PARSONS, C. M.; EMMERT, J. L. Metabolizable energy and amino acid digestibility in spray-dried animal plasma using broiler chick and precision-fed rooster assays. **Poultry Science**, v. 101, n. 5, p. 101807, 2022. DOI: https://doi.org/10.1016/j.psj.2022.101807.

KIM, H.; LEE, S. H.; KIM, B. G. Effects of dietary spray-dried plasma protein on nutrient digestibility and growth performance in nursery pigs. **Journal of Animal Science**, v. 100, n. 1, p. 351, 2022. DOI: https://doi.org/10.1093/jas/skab351.

LOPEZ, G.; LEESON, S. Relevance of nitrogen correction for assessment of metabolizable energy with broilers to forty-nine days of age. **Poultry Science**, v. 86, n. 8, p. 1696–1704, 2007. DOI: https://doi.org/10.1093/ps/86.8.1696.

MURUGESAN, G. R.; KERR, B. J.; PERSIA, M. E. Energy content of select dietary supplemental lipids for broilers, turkeys, and laying hens. **Journal of Applied Poultry Research**, v. 26, n. 4, p. 536–547, 2017. DOI: https://doi.org/10.3382/japr/pfx027.

NATIONAL RESEARCH COUNCIL. **Nutrient requirements for poultry**. 9. rev. ed. Washington, DC: National Academy Press, 1994.

OECD/FAO. **OECD–FAO agricultural outlook 2018**. Paris: OECD Agriculture Statistics Database, 2018.

OKETCH, E. O.; WICKRAMASURIYA, S. S.; OH, S.; CHOI, J. S.; HEO, J. M. Physiology of lipid digestion and absorption in poultry: An updated review on the supplementation of exogenous emulsifiers in broiler diets. **Journal of Animal Physiology and Animal Nutrition**, v. 107, n. 6, p. 1429–1443, 2023. DOI: https://doi.org/10.1111/jpn.13859.

PÉREZ-BOSQUE, A.; POLO, J.; TORRALLARDONA, D. Spray-dried plasma as an alternative to antibiotics in piglet feeds: Mode of action and biosafety. **Porcine Health Management**, v. 2, p. 16, 2016. DOI: https://doi.org/10.1186/s40813-016-0034-1.

REDOY, M. R. A.; RAHMAN, M. M.; AL-MAMUN, M.; ISLAM, K. M. S. Short time preservation of poultry viscera meal and its potentiality as a source of protein in broiler diet. **International Journal of Poultry Science**, v. 20, n. 2, p. 67–75, 2021. DOI: https://doi.org/10.3923/ijps.2021.67.75.

RODRÍGUEZ-ORTEGA, L. T.; SEGOVIA-AZPEITIA, S.; HERNÁNDEZ-GUZMÁN, F. J.; RODRÍGUEZ-ORTEGA, A.; PRO-MARTÍNEZ, A.; ESTRADA-HERNÁNDEZ, M. D. L. L. Effect of annatto and alfalfa on egg yolk pigmentation in Creole hens. **Agro Productividad**, v. 17, n. 10, p. 45–52, 2024. DOI: https://doi.org/10.22004/ag.econ.352095.

RODRIGUEZ-SANCHEZ, R.; TRES, A.; SALA, R.; GUARDIOLA, F.; BARROETA, A. C. Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages. **Poultry Science**, v. 98, n. 3, p. 1341–1353, 2019. DOI: https://doi.org/10.3382/ps/pey458.

SERPA, F. C.; GARCIA, R. G.; BURBARELLI, M. F. C.; KOMIYAMA, C. M.; VALENTIM, J. K.; CASTILHO, V. A. R.; CALDARA, F. R. Emulsifier in diets with different alternative lipid sources: Effects on the health of Japanese quails. **Brazilian Journal of Poultry Science**, v. 25, n. 3, p. eRBCA-2023, 2023. DOI: https://doi.org/10.1590/1806-9061-2023-1800.

SILVA, E. P. D.; RABELLO, C. B. V.; ALBINO, L. F. T.; LUDKE, J. V.; LIMA, M. B. D.; DUTRA JUNIOR, W. M. Prediction of metabolizable energy values in poultry offal meal for broiler chickens. **Revista Brasileira de Zootecnia**, v. 39, n. 10, p. 2237–2245, 2010. DOI: https://doi.org/10.1590/S1516-35982010001000020.

SILVA, E. P. D.; RABELLO, C. B. V.; LIMA, M. B. D.; LUDKE, J. V.; ARRUDA, E. M. F. D.; ALBINO, L. F. T. Poultry offal meal in broiler chicken feed. **Scientia Agricola**, v. 71, n. 3, p. 188–194, 2014. DOI: https://doi.org/10.1590/S0103-90162014000300003.

SONG, M.; TIAN, K.; REN, C.; LIU, Y.; YE, X.; WANG, Y.; ZHAO, F. Impact of chemical composition on metabolizable energy and its prediction models in brewer's spent grains for broilers at different ages. **Poultry Science**, v. 104, n. 8, p. 105323, 2025. DOI: https://doi.org/10.1016/j.psj.2025.105323.

SONG, M.; ZHAO, F.; YE, X.; XIE, J.; SA, R.; ZHANG, G.; WANG, Y. A comparative evaluation on the energetic values and digestibility of fatty acids in rice bran oil and palm oil for broilers. **Poultry Science**, v. 101, n. 8, p. 101954, 2022. DOI: https://doi.org/10.1016/j.psj.2022.101954.

VIEIRA, S. L.; RIBEIRO, A. M. L.; KESSLER, A. M.; FERNANDES, L. M.; EBERT, A. R.; EICHNER, G. Utilização da energia de dietas para frangos de corte formuladas com óleo ácido de soja. **Revista Brasileira de Ciência Avícola**, v. 4, n. 2, p. 127–139, 2002. DOI: https://doi.org/10.1590/S1516-635X2002000200005.

Chapter 2: Effects of phytase supplementation on broiler chickens fed nutrient-deficient diets

Samuel Oliveira Borges[a]*, Tiago Goulart Petrolli[b], Melissa Izabel Hannas[a], Romário Duarte Bernardes[a], Carlos Henrique de Oliveira[a], Kelly Morais Maia Dias[a], Bruno Figueiredo de Almeida[a], Lucimauro da Fonseca[a], Alex Filipe Ramos de Sousa[c], Arele Arlindo Calderano[a]

[a]Department of Animal Science, Federal University of Viçosa, Viçosa, 36570-900, Brazil.

[b]Department of Animal Science, University of the West of Santa Catarina, Xanxerê, 89820-000, Brazil.

[c]Department of Veterinary Medicine, Universidade Federal de Viçosa, Viçosa, 36570-900, Brazil.

Published at Livestock Science 301: e105823, 2025.

Paper history:

Received: August, 13, 2025; Accepted: September, 12, 2025; Available online: September, 13, 2025.

Doi: https://doi.org/10.1016/j.livsci.2025.105823

ABSTRACT

This study aimed to evaluate the effects of different inclusion levels of phytase on the performance, body composition, blood parameters, and bone characteristics of broilers fed reduced-nutrient diets. The experiment was conducted using 1,400 male broilers, allocated to seven treatments with ten replicates and twenty birds per experimental unit, in a completely randomized design. The experimental treatments were: positive control, formulated to fully meet the birds' nutritional requirements; negative control 1 (NC1), negative control 2 (NC2) and negative control 3 (NC3), formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; and NC1, NC2 and NC3 supplemented with 500, 750 and 1000 FTU of phytase. Phytase supplementation improved ($P<0.05$) body weight gain, feed conversion ratio, and final body weight compared to the negative controls, restoring these parameters to levels comparable with the positive control, even in diets simultaneously reduced in available phosphorus (P), total calcium (Ca), total sodium, metabolizable energy, crude protein, and digestible amino acids. Improvements were also observed in serum Ca and P levels, as well as in bone indicators, including the Seedor index, bone breaking strength, and ash and magnesium concentrations, matching those of the positive control ($P<0.05$). Phytase supplementation further enhanced body composition, with greater deposition of lean tissue, protein, and bone in supplemented birds ($P<0.05$). The main contribution of this study is the confirmation of phytase's effectiveness as a nutritional tool in challenging diets, demonstrating its capacity to recover performance and bone quality, with benefits that extend beyond phosphorus utilization.

Keywords: enzyme; phosphorus; nutrition; poultry

1. INTRODUCTION

Plant-based ingredients commonly used in poultry feed formulations contain antinutritional factors such as phytic acid, which dissociates by losing hydrogen ions to form its salt, phytate (JOUDAKI *et al*., 2023). This naturally indigestible compound negatively affects animal growth and development by forming complexes with minerals, amino acids, and carbohydrates, thereby reducing nutrient digestion and absorption (Jain & Sapna, 2016). Furthermore, phytate reduces nutrient availability by inhibiting the activity of digestive enzymes and promoting endogenous nutrient losses through its detrimental effects on the intestinal mucosa (MOITA *et al*., 2021). These effects compromise both the structural and functional integrity of the intestine, trigger inflammatory responses, and ultimately impair nutrient digestion and absorption.

Phytate is structurally composed of an inositol ring esterified with six phosphate groups (OATWAY *et al*., 2001). Phytase specifically targets these phosphate esters, catalyzing the stepwise hydrolysis of phosphate groups from the inositol ring (HIRVONEN *et al*., 2019). With each reaction, one inorganic phosphate is released, and the phytate molecule undergoes progressive dephosphorylation until complete degradation is achieved (PIRES *et al*., 2023). Consequently, its cation-chelating capacity is diminished, leading to the release of previously unavailable nutrients and enhancing their absorption and utilization from the diet.

Broiler diets are predominantly based on plant-derived ingredients, particularly corn and soybean meal, in which the majority of phosphorus (P)—approximately 60%—is present in an unavailable form (RAVINDRAN, 2013; MIRABILE *et al*., 2020; SELIM *et al*., 2022). Therefore, the use of phytase is a common strategy to mitigate the antinutritional effects of phytate. This enzyme enhances digestive efficiency by improving nutrient and energy utilization from feed ingredients (FERNANDES *et al*., 2019). Phytase hydrolyzes phytate, thereby releasing P and other bound minerals, improving amino acid digestion and absorption (MARTÍNEZ-VALLESPÍN *et al*., 2025), and increasing the metabolizability of dietary energy (BERNARDES *et al*., 2022).

In addition to promoting the release of P and other minerals bound to phytate, phytase has been shown to downregulate the expression of pro-inflammatory genes such as interleukin-6, thereby enhancing intestinal integrity (ADEDOKUN; ADEOLA, 2017). It may also upregulate the expression of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, through the increased availability of essential minerals and cofactors (DERAKHSHAN *et al*., 2023). These effects contribute to improved metabolic efficiency, gut health, and nutrient utilization, ultimately supporting enhanced animal performance (WALK; RAO, 2020).

Modern poultry production faces the ongoing challenge of balancing productivity with economic and environmental sustainability (CARDOSO *et al*., 2018). In this context, nutritional strategies have been extensively researched to reduce feed costs without compromising performance. One of the most promising approaches involves the use of exogenous enzymes like phytase in diets formulated with reduced levels of available P, total calcium (Ca), sodium (Na), crude protein, digestible amino acids, and metabolizable energy (ME). Despite its widespread use, the scientific literature still presents contradictory results regarding dosage, inclusion strategy (matrix value versus on-top), and effects on variables beyond performance, such as body composition percentage and physiological parameters. This underscores the necessity for studies that account for ingredient profiles, the presence of enzymatic substrates, and the enzyme's impact on various physiological responses.

Therefore, the hypothesis of this study was that phytase supplementation would restore performance, body composition percentage, and bone quality in broiler chickens fed nutrient-deficient diets. Accordingly, the objective was to evaluate the performance, body composition percentage, blood parameters, and tibia mineral content of broilers fed diets with reduced nutritional levels and varying levels of phytase inclusion.

2. MATERIAL AND METHODS

2.1 Ethical matters

The Animal Care and Use Committee of the Federal University of Viçosa, Brazil, approved all animal procedures (protocol no. 126/2023). The experiment was conducted according to the experimental protocols for using live broilers from the Brazilian College of Animal Experimentation.

2.2 Birds and experimental design

One-day-old male broiler chickens (Cobb 500) were obtained from a commercial hatchery. The birds were reared on the floor according to the management guidelines for the strain until the start of the experiment. They had free access to water and were fed a standard corn/soybean meal-based diet in mashed form. At ninth days of age a total of 1,400 male broilers (291.2 ± 24.75 g) were distributed in a completely randomized design, consisting of seven treatments, ten replicates and twenty birds per experimental unit. The birds were housed in 70 experimental units within a masonry shed, divided into 1.0 × 2.0 m pens lined with wood shavings. Temperature was maintained according to strain management guidelines, and the broilers were exposed to 18 hours of continuous light per day throughout the experimental period.

The experimental treatments were: treatment 1 corresponded to the positive control (PC), formulated to fully meet the birds' nutritional requirements; treatment 2 was the negative control 1 (NC1), formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; treatment 3 consisted of NC1 supplemented with 500 FTU of phytase (50 g/ton); treatment 4 was the negative control 2 (NC2), also formulated with nutrient reductions based on the phytase matrix; treatment 5 corresponded to NC2 supplemented with 750 FTU of phytase (75 g/ton); treatment 6 was the negative control 3 (NC3), likewise formulated according to the nutrient release matrix; and treatment 7 consisted of NC3 supplemented with 1000 FTU of phytase (100 g/ton). The percentage of reductions in nutrients and ME from one treatment to the next (NC1 × PC, NC2 × NC1, and NC3 × NC2) are presented in Table 1.

The phytase used was derived from Pichia pastoris – ACCC 21387 (6-PHYTASE, Spalla®, BTA, Xanxerê, Brazil) with a guaranteed activity level of 10,000 FTU/g.

Table 1. Percentage reduction of nutrients and metabolizable energy (ME) relative to the positive control (PC) and among the negative controls (NC1, NC2, and NC3).

Nutrients and ME	NC1 x PC (%)	NC2 x NC1 (%)	NC3 x NC2 (%)
		9 to 17 day phase	
Crude protein	-1.60	-0.31	-0.36
ME	-1.92	-0.13	-0.13
Calcium	-22.35	-3.62	-2.43
Available phosphorus	-52.96	-15.56	-8.77
Sodium	-15.71	-4.35	-2.60
Digestible Lysine	-2.27	-0.28	-0.56
Digestible Met + Cys	-2.79	-0.36	-0.72
Digestible Arginine	-2.21	-0.33	-0.42
Digestible Threonine	-2.47	-0.85	-0.71
Digestible Tryptophan	-2.26	-0.46	-0.47
Digestible Valine	-2.36	-0.72	-0.36
		18 to 35 day phase	
Crude protein	-1.49	-0.28	-0.33
ME	-1.97	-0.13	-0.13
Calcium	-19.23	-3.00	-2.00
Available phophosforus	-45.51	-11.54	-6.21
Sodium	-14.85	-4.07	-2.42
Digestible Lysine	-2.13	-0.26	-0.52
Digestible Met + Cys	-2.66	-0.34	-0.69
Digestible Arginine	-1.94	-0.38	-0.38
Digestible Threonine	-2.33	-0.79	-0.67
Digestible Tryptophan	-2.06	-0.42	-0.42
Digestible Valine	-1.44	-0.22	-0.34
		36 to 42 day phase	
Crude protein	-1.36	-0.26	-0.30
ME	-2.02	-0.14	-0.14
Calcium	-13.08	-1.90	-1.25
Available phophosrus	-30.89	-6.18	-3.13
Sodium	-14.02	-3.80	-2.26
Digestible Lysine	-1.96	-0.24	-0.48
Digestible Met + Cys	-2.58	-0.33	-0.66
Digestible Arginine	-1.81	-0.34	-0.34
Digestible Threonine	-2.14	-0.73	-0.61
Digestible Tryptophan	-1.84	-0.37	-0.38
Digestible Valine	-1.31	-0.20	-0.31

Values expressed as percentage reduction relative to the reference treatment in each comparison. Calculation: % reduction = [(level in the compared treatment − level in the reference treatment) ÷ level in the reference treatment] × 100 (negative values indicate reduction).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; and NC3: negative control 3, likewise formulated according to the nutrient release matrix.

2.3 Diets

The experimental diets were based on corn and soybean meal and formulated to meet the nutritional recommendations of Rostagno *et al*. (2024) for the phases of 8-17, 18-35, and 36-42 days of age (Table 2, 3 and 4), except for the levels of available P, Ca, Na, ME, crude protein, and digestible amino acids, which were adjusted according to the NC diets. In addition, corn and soybean meal samples were collected for phytic P analysis, whith was performed using the wet extraction method at AB Vista Lab Services (USA: 8151 Peter's Road, Suite 2001, Plantation, FL 33324). The determined phytate P contents were 0.214% for corn and 0.458% for soybean meal.

Table 2. Ingredients and nutritional composition of the experimental diets for the 9 to 17 day phase.

Ingredients (g/kg)	PC	NC1	NC2	NC3
Corn	495.99	532.85	537.53	541.68
Soybean meal	426.04	413.07	411.01	408.91
Soy oil	36.32	18.86	17.17	15.71
Dicalcium phosphate	19.91	11.86	10.75	10.23
Limestone	8.21	10.86	11.27	11.39
Salt	5.15	4.39	4.22	4.11
DL-Methionine – 99%	3.16	2.95	2.93	2.88
Mineral premix	1.35	1.35	1.35	1.35
Vitamin premix	1.30	1.30	130	1.30
Choline chloride – 60%	0.90	0.90	0.90	0.90
L-Lysine HCl – 78.8%	0.78	0.80	0.81	0.80
Salinomycin – 12%	0.55	0.55	0.55	0.55
L-Threonine – 98.5%	0.29	0.21	0.17	0.14
Calculated nutritional values				
Crude protein (g/kg)	235.8	232.6	232.0	231.3
ME, Kcal/kg	2970.00	2910.00	2906.00	2902.00
Ca, (g/kg)	10.32	8.97	8.80	8.69
Available P, (g/kg)	4.92	3.40	3.19	3.09
Na, (g/kg)	2.14	1.84	1.77	1.73
Digestible Lysine, (g/kg)	12.74	12.49	12.46	12.40
Digestible Met + Cys, (g/kg)	9.30	09.06	9.03	8.97
Digestible Arginine, (g/kg)	14.95	14.68	14.63	14.58
Digestible Threonine, (g/kg)	8.41	8.23	8.17	8.12
Digestible Tryptophan, (g/kg)	2.72	2.67	2.66	2.65
Digestible Valine, (g/kg)	9.95	9.82	9.80	9.77

[1]Vitamin premix provided per kg of diet: vitamin A, 9638000 UI; vitamin D3, 2410000 UI; vitamin E, 36100 UI; vitamin K3, 1936 mg; vitamin B1, 2600 mg; vitamin B2, 6450 mg, vitamin B12, 159 mg; vitamin B6, 3610 mg; vitamin B5, 12.95 g; vitamin B3, 39.2 g; vitamin B9, 903.0 mg; biotin, 89.8 mg.

[2]Trace mineral premix provided per kg diet: Mn, 58.36 g; Zn, 54.21 g; Fe, 41.68 g; Cu, 8.31 g; I, 843.00 mg; Se, 250.00 mg.

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; and NC3: negative control 3, likewise formulated according to the nutrient release matrix.

Table 3. Ingredients and nutritional composition of the experimental diets for the 18 to 35 day phase.

Ingredients (g/kg)	PC	NC1	NC2	NC3
Corn	572.44	609.19	613.98	618.13
Soybean meal	366.27	353.40	351.24	349.14
Soy oil	30.04	12.60	10.89	9.43
Dicalcium phosphate	11.98	3.93	2.82	2.30
Limestone	6.04	8.70	9.11	9.23
Salt	4.85	4.09	3.91	3.81
DL-Methionine – 99%	3.32	3.12	3.10	3.05
Mineral premix	1.05	1.05	1.05	1.05
Vitamin premix	1.00	1.00	1.00	1.00
Choline chloride – 60%	0.80	0.80	0.80	0.80
L-Lysine HCl – 78.8%	1.26	1.27	1.29	1.27
Salinomycin – 12%	0.55	0.55	0.55	0.55
L-Threonine – 98.5%	0.35	0.27	0.23	0.20
Calculated nutritional values				
Crude protein (g/kg)	215.1	211.9	211.3	210.6
ME, Kcal/kg	3040.00	2980.00	2976.00	2972.00
Ca, (g/kg)	7.02	5.67	5.50	5.39
Available P, (g/kg)	3.34	1.82	1.61	1.51
Na, (g/kg)	2.02	1.72	1.65	1.61
Digestible Lysine, (g/kg)	11.72	11.47	11.44	11.38
Digestible Met + Cys, (g/kg)	9.02	8.78	8.75	8.69
Digestible Arginine, (g/kg)	13.37	13.11	13.06	13.01
Digestible Threonine, (g/kg)	7.74	7.56	7.50	7.45
Digestible Tryptophan, (g/kg)	2.43	2.38	2.37	2.36
Digestible Valine, (g/kg)	9.05	8.92	8.90	8.87

[1]Vitamin premix provided per kg of diet: vitamin A, 9638000 UI; vitamin D3, 2410000 UI; vitamin E, 36100 UI; vitamin K3, 1936 mg; vitamin B1, 2600 mg; vitamin B2, 6450 mg, vitamin B12, 159 mg; vitamin B6, 3610 mg; vitamin B5, 12.95 g; vitamin B3, 39.2 g; vitamin B9, 903.0 mg; biotin, 89.8 mg.

[2]Trace mineral premix provided per kg diet: Mn, 58.36 g; Zn, 54.21 g; Fe, 41.68 g; Cu, 8.31 g; I, 843.00 mg; Se, 250.00 mg.

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; and NC3: negative control 3, likewise formulated according to the nutrient release matrix.

Table 4. Ingredients and nutritional composition of the experimental diets for the 36 to 42 day phase.

Ingredients (g/kg)	PC	NC1	NC2	NC3
Corn	614.23	650.95	655.57	659.73
Soybean meal	324.31	311.50	309.51	307.41
Soy oil	33.68	16.29	14.62	13.16
Dicalcium phosphate	9.86	1.80	0.70	0.18
Limestone	5.40	8.06	8.47	8.59
Salt	4.57	3.81	3.63	3.53
DL-Methionine – 99%	3.25	3.04	3.02	2.97
Mineral premix	0.90	0.90	0.90	0.90
Vitamin premix	0.85	0.85	0.85	0.85
Choline chloride – 60%	0.70	0.70	0.70	0.70
L-Lysine HCl – 78.8%	1.66	1.66	1.68	1.66
L-Threonine – 98.5%	0.44	0.36	0.31	0.29
L-Valine – 96.5%	0.11	0.03	0.00	0.00
Calculated nutritional values				
Crude protein (g/kg)	199.7	196.5	195.9	195.2
ME, Kcal/kg	3120.00	3060.00	3056.00	3052.00
Ca, (g/kg)	6.04	4.69	4.52	4.41
Available P, (g/kg)	2.87	1.35	1.14	1.04
Na, (g/kg)	1.91	1.61	1.54	1.50
Digestible Lysine, (g/kg)	11.03	10.78	10.75	10.69
Digestible Met + Cys, (g/kg)	8.60	8.36	8.33	8.27
Digestible Arginine, (g/kg)	12.23	11.96	11.92	11.87
Digestible Threonine, (g/kg)	7.28	7.10	7.04	6.99
Digestible Tryptophan, (g/kg)	2.21	2.16	2.15	2.14
Digestible Valine, (g/kg)	8.49	8.29	8.23	8.20

[1]Vitamin premix provided per kg of diet: vitamin A, 9638000 UI; vitamin D3, 2410000 UI; vitamin E, 36100 UI; vitamin K3, 1936 mg; vitamin B1, 2600 mg; vitamin B2, 6450 mg, vitamin B12, 159 mg; vitamin B6, 3610 mg; vitamin B5, 12.95 g; vitamin B3, 39.2 g; vitamin B9, 903.0 mg; biotin, 89.8 mg.

[2]Trace mineral premix provided per kg diet: Mn, 58.36 g; Zn, 54.21 g; Fe, 41.68 g; Cu, 8.31 g; I, 843.00 mg; Se, 250.00 mg.

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; and NC3: negative control 3, likewise formulated according to the nutrient release matrix.

2.4 Performance and sample collection

The broilers' performance was evaluated at 17 and 42 days of age by determining final weight (FW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Mortality was recorded and used to adjust performance data. At 42 days of age, two birds whose body weights were closest to the average of their respective experimental unit were selected for sample collection. One of these birds was used for blood collection to assess serum levels of alkaline phosphatase (ALP), Ca, and P (Cobas c 311; Roche Diagnostics GmbH, Basel, Switzerland), following the manufacturer's instructions. Two birds were slaughtered to evaluate carcass yield (CY) and cut yield (CutY). CY was calculated as the ratio of carcass weight (excluding viscera, head, feet, and neck) and live body weight prior to slaughter, expressed as a percentage. CutY was calculated as the ratio of the weight of the individual cuts and the eviscerated carcass weight, expressed as a percentage. The same birds had their liver, spleen, and intestine collected and weighed to determine relative organ weight (%Relative weight = Organ weight × 100 / Live weight). The right tibia from one of these birds per experimental unit was collected, weighed, and measured for length to calculate the Seedor index. This index was calculated by dividing the bone weight by bone length, as described by Seedor (1995; tibia weight, mg / tibia length, mm). The tibia was manually broken using a press, defatted in Soxhlet extractors (Soxhlet, 1879) and ground in a ball mill for analysis of ash content, Ca, P, potassium (K), and magnesium (Mg). For ash determination, 2 g of air-dried sample were incinerated in a muffle furnace at 550 °C for 4 hours. After complete combustion, the crucibles were placed in a desiccator for 30 minutes, weighed, and the results recorded (AOAC International, 2016). To determine the percentages of Ca, P, K, and Mg in the bone samples, a nitric-perchloric digestion was performed (Sarruge & Haag, 1974), in which 0.5 g of the ground sample was treated with 10 mL of a nitric acid and perchloric acid solution in a 4:1 ratio, under a fume hood. The digestion was carried out on a heating plate, starting at 80 °C and gradually increasing the temperature up to 200 °C, until a clear and crystalline extract was obtained. After cooling, the volume was adjusted to 25 mL with deionized water. The concentrations of Ca, P, K, and Mg were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES – Nolte, 2003; Perkin Elmer Optima 8300 DV instrument), previously calibrated with a multielement solution in the same matrix as the samples. The left tibia from the same bird was subjected to bone-breaking resistance analysis using a compression press (Standarts A., 2003). An apparatus was employed, consisting of a custom metallic support specifically designed to correctly position the bone during the mechanical test. The support featured a fixed base with two parallel rests, establishing a 58 mm free span at the diaphysis, thereby standardizing the support points and minimizing any influence of bone length on the measurements.

2.5 DEXA estimated body composition percentage

Body composition percentage of the birds was estimated using Dual-Energy X-ray Absorptiometry (DEXA). At 42 days of age, another bird with body weight closest to the average of each experimental unit was selected and slaughtered. The estimated amounts of lean, protein, and mineral tissues were adjusted using the equations proposed by Aguiar *et al*. (2024):

Lean tissue (g) = –31.807 + 1.157 × lean mass (DEXA, g; R2 = 0.997);

Protein tissue (g) = –8.8705 + 0.2167 × lean mass (DEXA, g; R2 = 0.989);

Mineral tissue (g) = 7.139 + 1.578 × mineral mass (DEXA, g; R2 = 0.956).

The percentage of each tissue was calculated based on the total body weight of the bird.

2.6 Statistical analysis

The adopted statistical model was:

$$Y_{ik} = \mu + \tau_i + \varepsilon_{ik},$$

where Yik is the value recorded for the response variable observed in the k-th repetition of the i-th level of the tested factor, μ is the overall mean, τi is the effect of the i-th level of the tested factor, and εik is the experimental error associated with the observed Yik value. All collected data were subjected to ANOVA at a 5% significance level using the GLM procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The Shapiro-Wilk test was performed to assess the normality of residuals prior to ANOVA. Dunnett’s test was applied at a 5% significance level to compare the means of the treatment groups with that of the PC group.

3. RESULTS

3.1 Performance

No treatment effect was observed on FI of the birds during the periods from 9 to 17 days and from 9 to 42 days of age ($P > 0.05$; Table 5). However, birds in the NC groups showing lower BWG and FW, as well as worse FCR ($P < 0.05$).

Table 5. Performance results observed during the periods from 9 to 17 days and from 9 to 42 days of age.

Variables	PC	NC1	NC1+500 FTU	NC2	NC2+750 FTU	NC3	NC3+1000 FTU	SEM	*P*-value
					9 - 17 days				
Inicial weight (kg/bird)	0.291	0.290	0.291	0.291	0.291	0.291	0.292		
FI (kg/bird)	0.691	0.692	0.700	0.689	0.702	0.695	0.701	0.001	0.348
BWG (kg/bird)	0.601	0.571*	0.600	0.567*	0.598	0.564*	0.592	0.002	<0.001
FCR	1.15	1.21*	1.17	1.22*	1.17	1.23*	1.18*	0.004	<0.001
FW (kg)	0.892	0.863*	0.891	0.858*	0.888	0.856*	0.884	0.002	<0.001
					9 - 42 days				
FI (kg/bird)	5.135	5.221	5.244	5.128	5.214	5.128	5.181	0.015	0.174
BWG (kg/bird)	3.499	3.368*	3.495	3.299*	3.483	3.235*	3.439	0.014	<0.001
FCR	1.47	1.55*	1.50	1.55*	1.50	1.59*	1.51*	0.005	<0.001
FW (kg)	3.797	3.673*	3.813	3.590*	3.785	3.531*	3.748	0.015	<0.001

FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio; FW: final weight; SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett's test ($P < 0.05$).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

3.2 Serum metabolites

There was no treatment effect on serum ALP levels in the birds at 42 days of age (P > 0.05; Table 6). However, birds in the NC groups showed lower serum Ca and P levels compared to the PC (P < 0.05).

Table 6. Serum levels of broilers at 42 days of age.

Variables	PC	NC1	NC1+ 500 FTU	NC2	NC2+ 750 FTU	NC3	NC3+ 1000 FTU	SEM	*P*-value
				Serum levels					
ALP (U/L)	3731.00	2616.40	2666.20	2191.30	2784.00	3068.70	2959.10	192.19	0.5237
Ca (mg/dL)	10.38	9.30*	10.25	9.23*	10.09	9.18*	10.22	0.08	<0.001
P (mg/dL)	8.34	6.46*	7.86	6.67*	8.03	6.07*	7.66	0.16	<0.001

ALP: Alkaline phosphatase; Ca: Calcium; P: Phosphorus.

SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett’s test (P < 0.05).

PC: positive control, formulated to fully meet the birds’ nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

3.3 Carcass yield, cuts and relative organ weights

No treatment effects were observed on CY and CutY or on the relative weights of the liver, spleen, and intestine in broiler chickens at 42 days of age (P > 0.05; Table 7).

Table 7. CY and CutY and relative weights of liver, spleen, and intestine in broiler chickens at 42 days of age.

Variables	PC	NC1	NC1+500 FTU	NC2	NC2+750 FTU	NC3	NC3+1000 FTU	SEM	*P*-value
					CY, cuts				
Carcass (%)	79.97	78.78	79.95	80.07	80.10	79.63	79.69	0.001	0.113
Breast (%)	40.86	41.95	41.36	41.34	40.09	41.25	41.03	0.001	0.290
Thigh + Drumstick (%)	26.12	25.66	25.52	25.47	26.00	25.18	25.83	0.001	0.355
					Relative organ weights				
Liver (%)	1.592	1.561	1.534	1.508	1.522	1.655	1.495	0.018	0.230
Spleen (%)	0.096	0.090	0.088	0.084	0.094	0.091	0.085	0.001	0.558
Intestine (%)	3.326	3.299	3.106	3.371	3.115	3.242	3.054	0.038	0.163

SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett's test (P < 0.05).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

3.4 Tibia minerals

Birds in the NC3 group showed lower concentrations of ash and Mg in the tibia, whereas birds in the NC1 group exhibited a reduced Mg concentration (P < 0.05; Table 8). No treatment effect were observed on the levels of Ca, P, and K (P > 0.05).

Table 8. Minerals content (%) of the tibia in broiler chickens at 42 days of age.

Variables	PC	NC1	NC1+ 500 FTU	NC2	NC2+750 FTU	NC3	NC3+1000 FTU	SEM	*P*-value
Bone composition									
Ash (%)	48.172	47.175	48.711	47.571	49.380	46.199*	48.325	0.19	<0.001
Ca (%)	22.587	21.788	22.150	22.882	22.489	22.289	21.990	0.22	0.877
P (%)	10.924	10.345	10.729	10.875	10.945	10.582	10.603	0.09	0.610
K (%)	0.722	0.680	0.662	0.693	0.669	0.735	0.674	0.01	0.073
Mg (%)	0.437	0.363*	0.439	0.394	0.456	0.370*	0.434	0.01	<0.001

Ca: Calcium; P: Phosphorus; K: Potassium; Mg: Magnesium.

SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett's test (P < 0.05).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

3.5 Breaking strength and seedor index of tibiae

Only birds in the NC groups exhibiting lower breaking strength compared to the PC group (P < 0.05; Table 9). Only the birds in the NC3 group exhibited a lower Seedor index (P < 0.05; Table 9).

Table 9. Breaking strength and Seedor index of tibiae from broiler chickens at 42 days of age.

				Breaking strength					
Variables	PC	NC1	NC1+500 FTU	NC2	NC2+750 FTU	NC3	NC3+1000 FTU	SEM	*P*-value
Strength (kgf)	43.192	34.846*	43.298	36.536*	42.814	32.085*	40.743	0.78	<0.001
				Seedor index					
Seedor index mg/mm	PC	NC1	NC1+500 FTU	NC2	NC2+750 FTU	CN3	NC3+1000 FTU	SEM	P-value
	184.211	164.5	187.5	165.2	177.0	159.7*	177.8	2.297	0.002

SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett's test (P < 0.05).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

3.6 Percentage of lean, protein, and bone mass of broiler chickens at 42 days of age.

No treatment effects were observed on the percentages of lean and protein mass at 42 days of age (P > 0.05; Table 10). However, birds in the NC groups showed lower bone mass percentage compared to the PC group (P < 0.05).

Table 10. Performance results observed during the periods from 9 to 17 days and from 9 to 42 days of age.

Variables	PC	NC1	NC1+500 FTU	NC2	NC2+750 FTU	NC3	NC3+1000 FTU	SEM	*P*-value
					%				
Lean (%)	93.076	93.751	93.093	91.227	93.873	92.964	92.518	0.260	0.1093
Protein (%)	17.355	17.480	17.359	17.005	17.504	17.330	17.250	0.048	0.1035
Bone (%)	1.900	1.605*	1.920	1.623*	1.834	1.585*	1.832	0.021	<0.001

SEM: standard error of the mean. Means in the same row followed by * differ from the positive control group according to Dunnett's test (P < 0.05).

PC: positive control, formulated to fully meet the birds' nutritional requirements; NC1: negative control 1, formulated with nutrient reductions according to the phytase nutrient release matrix but without enzyme supplementation; NC1+500 FTU: NC1 supplemented with 500 FTU of phytase (50 g/ton); NC2: negative control 2, also formulated with nutrient reductions based on the phytase matrix; NC2+750 FTU: NC2 supplemented with 750 FTU of phytase (75 g/ton); NC3: negative control 3, likewise formulated according to the nutrient release matrix; and NC3+1000 FTU: NC3 supplemented with 1000 FTU of phytase (100 g/ton).

4. DISCUSSION

Phytase is widely recognized for its ability to enhance the digestion and absorption of P bound to phytate (MA *et al*., 2025). More recent studies have shown that its effects also extend to amino acid absorption (MARTÍNEZ-VALLESPÍN *et al*., 2022), energy utilization (PIENIAZEK *et al*., 2017; BERNARDES *et al*., 2022), intestinal integrity (NARI *et al*., 2020), and modulation of oxidative stress (LEE *et al*., 2019). At the skeletal level, the gradual release of P, Ca, zinc, and Mg supports improved mineral deposition and bone strength (NUAMAH *et al*., 2024). Based on these reported effects, we hypothesized that phytase could restore productive performance and bone quality in broilers fed nutritionally restricted diets. The results supported the hypothesis. Birds supplemented phytase showed BWG, FW, FCR comparable to those of the PC group, despite receiving diets with reduced levels of available P, Ca, Na, crude protein, digestible amino acids, and ME. These findings suggest that, overall, the enzyme effectively released sufficient nutrients to support growth under nutritional restriction. Interestingly, although phytase supplementation at 500 and 750 FTU improved FCR in nutrient-deficient diets, the highest dose tested (1000 FTU in the NC3 diet) did not restore this parameter to the level of the positive control. This result may indicate a possible interaction between phytase dose and dietary context, particularly the severity of nutrient reductions in NC3. In more restrictive diets, substrate availability or nutrient release dynamics may not align linearly with increasing enzyme doses, leading to less efficient utilization of released nutrients. This result shows that the effectiveness of phytase depends not only on the enzyme dose, but also on the composition and nutritional adequacy of the basal diet (CHOI *et al*., 2024). These results suggest that phytase nutrient release matrices, especially at higher inclusion levels, should be periodically re-evaluated in highly restrictive dietary scenarios to ensure accurate prediction of enzyme contributions.

Bone deposition was also influenced by the treatments. Broilers in the NC groups showed a reduced bone mass percentage compared to those in the PC group. However, the inclusion of phytase in the diets—regardless of the extent of nutritional reduction—restored bone mass to levels similar to the PC group. In addition, phytase supplementation significantly restored breaking strength, Seedor index, and ash and magnesium concentrations to levels comparable to the PC group, even in the most restrictive diets. These findings align with the well-established role of phytase in releasing essential minerals involved in bone mineralization (ZANU *et al*., 2020a). Additionally, bone mineralization is regulated by hormonal and molecular pathways that are highly sensitive to the animal's nutritional status (CAO *et al*., 2021). For instance, inadequate P availability can impair the activation of osteogenic genes such as RUNX2, BMP-2, and OPG, thereby disrupting osteoblast differentiation and function (LI *et al*., 2022b). It is important to recognize that bone tissue requires both time and a consistent supply of minerals to develop properly. Under extremely restrictive diets, damage may occur early and might not be fully reversible within the short broiler production cycle. Valable *et al*. (2018) demonstrated this by restricting Ca and P intake from 11 to 21 days of age, and then switching to a control diet during the finishing phase. Despite this nutritional correction, bone parameters did not return to control levels, highlighting the long-lasting effects of early mineral deficiencies. In contrast, the present study showed that even with highly deficient diets from the beginning of the experiment—when the birds were still in the early stages of development—bone traits were fully preserved in the phytase-supplemented groups. This difference is likely due to the fact that, unlike Valable *et al*. (2018), our animals received phytase from the start, ensuring continuous mineral release and availability throughout the most critical phases of skeletal development. These findings reinforce the importance of early phytase inclusion in the diet, especially under restrictive nutritional conditions. Although phytase acts specifically on dietary phytate, and its effectiveness depends on substrate availability, the present results suggest that the phytate content in even restrictive basal diets was sufficient to allow a full enzymatic response. Therefore, no physiological ceiling to phytase activity was observed within the conditions tested, in contrast to what might be expected under more extreme limitations (ZHANG *et al*., 2024).

The NC diets led to a reduction in serum Ca and P levels, indicating an impairment in mineral availability and homeostasis. However, the recovery of these levels in birds supplemented with phytase confirms its role in mobilizing minerals complexed with phytate. The study by ZANU *et al*. (2020b), for example, showed that low-Ca and low-P diets supplemented with phytase were effective in maintaining mineral homeostasis. This supports the efficacy of phytase in restoring mineral balance, primarily through the hydrolysis of phytic acid and the subsequent release of previously unavailable nutrients.

Additionally, serum alkaline phosphatase (ALP) levels did not differ statistically between treatments. ALP is produced in various tissues, primarily in the liver and bones; however, in growing birds, serum ALP reflects osteoblastic activity due to its strong correlation with osteoblast number (FARLEY; BAYLINK, 1986; MAGNUSON *et al*., 2025). One of the enzyme's primary functions is to hydrolyze phosphate groups, releasing phosphate ions from dietary inorganic pyrophosphate, which facilitates the crystallization of hydroxyapatite—the principal mineral component of bone (ABALYMOV *et al*., 2020). Despite observed mineral replenishment and support for bone mineralization, serum ALP levels exhibit high variability and decreased sensitivity to dietary changes during later growth stages. Zálešáková *et al*. (2025) report that serum ALP can have a coefficient of variation as high as 78.8%, and unlike other biomarkers, its activity naturally declines with age as osteoblastic activity is most pronounced during early growth phases (LI *et al*., 2022a). Therefore, at the time of sample collection, bone turnover was likely stabilizing, which may explain the lack of a significant ALP response despite improvements in other bone health indicators.

Although CY and CutY, as well as relative organ weights and most body composition variables, showed no significant differences among treatments, reporting these data provides valuable context for interpreting the results. The absence of statistical changes indicates that the feeding strategies applied were safe, since phytase inclusion did not impair carcass yield or organ development (SELLE *et al*., 2023). Even without treatment effects on these parameters, the findings confirm that phytase has a neutral impact on tissue growth and nutrient distribution, supporting that the gains in performance and bone quality occurred without adverse physiological consequences. Taken together, this perspective emphasizes phytase not only as a nutritional aid to enhance growth but also as an additive that helps preserve physiological stability in broilers raised under nutrient-restricted diets.

Future studies could explore the combined use of phytase with other feed additives currently under evaluation in the market that share similar functionalities, or even assess natural alternatives, as a way to broaden the scope of nutritional strategies, especially considering that financial sustainability is increasingly aligned with environmental sustainability. Regardless of the additive tested, it is essential that researchers carefully evaluate the substrate profile of feed ingredients to ensure outcomes that are more representative of practical conditions.

The inclusion of phytase at 500, 750, and 1000 FTU in diets with reduced nutritional levels restored performance, body composition, blood parameters, and tibial mineral content in broiler chickens. The results demonstrate that this enzyme is an effective nutritional tool to enhancing nutrient utilization.

ACKNOWLEDGMENTS

To the Instituto Nacional de Ciência e Tecnologia em Ciência Animal (INCT–CA), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant No. 425168/2025-5), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Grant No. APQ-08688-25), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES; Finance Code 001) for the financial support.

REFERENCES

ABALYMOV, A.; VAN POELVOORDE, L.; ATKIN, V.; SKIRTACH, A. G.; KONRAD, M.; PARAKHONSKIY, B. Alkaline phosphatase delivery system based on calcium carbonate carriers for acceleration of ossification. **ACS Applied Bio Materials**, v. 3, n. 5, p. 2986–2996, 2020. DOI: https://doi.org/10.1021/acsabm.0c00053.

ADEDOKUN, S. A.; ADEOLA, O. The response in jejunal and ileal nutrient and energy digestibility and the expression of markers of intestinal inflammation in broiler chickens to coccidial vaccine challenge and phytase supplementation. **Canadian Journal of Animal Science**, v. 97, p. 258–267, 2017. DOI: https://doi.org/10.1139/CJAS-2016-0093.

AOAC INTERNATIONAL. **Official methods of analysis**. 20. ed. Gaithersburg, MD, USA: Association of Official Analytical Chemist International, 2016.

BERNARDES, R. D.; OLIVEIRA, C. H. D.; CALDERANO, A. A.; FERREIRA, R. D. S.; DIAS, K. M. M.; ALMEIDA, B. F. D.; ALEIXO, P. E.; ALBINO, L. F. T. Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets. **Revista Brasileira de Zootecnia**, v. 51, p. 20210211, 2022. DOI: https://doi.org/10.37496/rbz5120210211.

CAO, S.; LI, T.; SHAO, Y.; ZHANG, L.; LU, L.; ZHANG, R.; HOU, S.; LUO, X.; LIAO, X. Regulation of bone phosphorus retention and bone development possibly by related hormones and local bone-derived regulators in broiler chicks. **Journal of Animal Science and Biotechnology**, v. 12, p. 1–16, 2021. DOI: https://doi.org/10.1186/s40104-021-00610-1.

CARDOSO, E. F.; DONZELE, J. L.; DE OLIVEIRA DONZELE, R. F. M.; SUFIATE, B. L.; SILVA, A. D.; TIZZIANI, T. Non-phytate phosphorus requirement for broilers from 8 to 21 days of age under heat stress conditions. **Tropical Animal Health and Production**, v. 50, p. 317–325, 2018. DOI: https://doi.org/10.1007/s11250-017-1434-1.

CHOI, P. S.; TAJUDEEN, H.; MUN, J. Y.; HA, S. H.; HOSSEINDOUST, A.; PARK, S.; PARK, H. I.; SILVESTRE, P. N.; LOKHANDE, A.; SANTOSH, I.; KIM, J. S. Phytase and nutrient-energy matrix: a strategic approach to enhancing the performance of broiler chickens fed a corn-soybean meal-based diet. **Animal Bioscience**, v. 38, n. 3, p. 522, 2024. DOI: https://doi.org/10.5713/ab.24.0565.

DE AGUIAR, G. A.; DA FONSECA, L.; DE FARIAS, M. R.; BRAGA, G. R.; BARCELLOS, J.; SCHULTZ, É. B.; HANNAS, M. I. Dual-energy X-ray absorptiometry: an effective approach for predicting broiler chicken body composition. **Poultry Science**, v. 103, n. 2, p. 103363, 2024. DOI: http://doi.org/10.1016/j.psj.2023.103363.

DERAKHSHAN, M.; GHASEMIAN, S. O.; GHOLAMI-AHANGARAN, M. The effects of probiotic and phytase on growth performance, biochemical parameters and antioxidant capacity in broiler chickens. **Veterinary Medicine and Science**, v. 9, n. 2, p. 860–866, 2023. DOI: https://doi.org/10.1002/vms3.1075.

FARLEY, J. R.; BAYLINK, D. J. Skeletal alkaline phosphatase activity as a bone formation index in vitro. **Metabolism**, v. 35, n. 6, p. 563–571, 1986. DOI: https://doi.org/10.1016/0026-0495(86)90016-8.

FERNANDES, J. I. M.; HORN, D.; RONCONI, E. J.; BUZIM, R.; LIMA, F. K.; PAZDIORA, D. A. Effects of phytase superdosing on digestibility and bone integrity of broilers. **Journal of Applied Poultry Research**, v. 28, n. 2, p. 390–398, 2019. DOI: https://doi.org/10.3382/japr/pfz001.

HIRVONEN, J.; LILJAVIRTA, J.; SAARINEN, M. T.; LEHTINEN, M. J.; AHONEN, I.; NURMINEN, P. Effect of phytase on in vitro hydrolysis of phytate and the formation of myo-inositol phosphate esters in various feed materials. **Journal of Agricultural and Food Chemistry**, v. 67, n. 41, p. 11396–11402, 2019. DOI: https://doi.org/10.1021/acs.jafc.9b03919.

JAIN, J.; SAPNA, B. S. Characteristics and biotechnological applications of bacterial phytases. **Process Biochemistry**, v. 51, p. 159–169, 2016. DOI: https://doi.org/10.1016/j.procbio.2015.12.004.

JOUDAKI, H.; ARIA, N.; MORAVEJ, R. Microbial phytases: Properties and applications in the food industry. **Current Microbiology**, v. 80, p. 374, 2023. DOI: https://doi.org/10.1007/s00284-023-03471-1.

LEE, S. A.; NAGALAKSHMI, D.; RAJU, M. V.; RAO, S. V. R.; BEDFORD, M. R.; WALK, C. L. Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. **Poultry Science**, v. 98, n. 5, p. 2122–2132, 2019. DOI: https://doi.org/10.3382/ps/pey566.

LI, L.; ZHANG, X.; ZHANG, J.; LIU, M.; ZHAO, L.; JI, C.; ZHANG, J.; HUANG, S.; MA, Q. Growth performance, bone development and phosphorus metabolism in chicks fed diets supplemented with phytase are associated with alterations in gut microbiota. **Animals**, v. 12, n. 7, p. 940, 2022a. DOI: https://doi.org/10.3390/ani12070940.

LI, T.; CAO, S.; LIAO, X.; SHAO, Y.; ZHANG, L.; LU, L.; LIU, Z.; LUO, X. The effects of inorganic phosphorus levels on phosphorus utilization, local bone-derived regulators, and BMP/MAPK pathway in primary cultured osteoblasts of broiler chicks.

Frontiers in Veterinary Science, v. 9, p. 855405, 2022b. DOI: https://doi.org/10.3389/fvets.2022.855405.

MA, B.; ZHANG, Y.; LIU, M.; SHI, C.; ZHOU, Y.; CHEN, M.; YUCHUN, C.; QU, H.; ZHANG, L.; ZHANG, C.; CHEN, G.; HAN, J. Dietary non-phytate phosphorus and phytase interaction affects growth performance, mineral transportation and metabolism in broiler chickens. **Poultry Science**, v. 104, n. 6, p. 105108, 2025. DOI: https://doi.org/10.1016/j.psj.2025.105108.

MAGNUSON, A. D.; BOONSINCHAI, N.; CALDAS, J.; ENGLAND, J.; COON, C. Utilization of Bone Alkaline Phosphatase (BAP) and Tartrate Resistant Acid Phosphatase (TRAP) as Biomarkers of Eggshell Quality and Bone Metabolism in Broiler Breeders and Progeny. **Journal of Animal Physiology and Animal Nutrition**, v. 109, n. 2, p. 601–609, 2025. DOI: https://doi.org/10.1111/jpn.14075.

MARTÍNEZ-VALLESPÍN, B.; ADER, P.; ZENTEK, J. Effect of increasing levels of phytase on performance, prececal nutrient digestibility, intestinal mucosa physiology and immune response in broiler chickens from 1 to 21 days of age. **Poultry Science**, p. 105473, 2025. DOI: https://doi.org/10.1016/j.psj.2025.105473.

MARTÍNEZ-VALLESPÍN, B.; MÄNNER, K.; ADER, P.; ZENTEK, J. Evaluation of high doses of phytase in a low-phosphorus diet in comparison to a phytate-free diet on performance, apparent ileal digestibility of nutrients, bone mineralization, intestinal morphology, and immune traits in 21-day-old broiler chickens. **Animals**, v. 12, p. 1955, 2022. DOI: https://doi.org/10.3390/ani12151955.

MIRABILE, C. G.; ROCHA, G. C.; CAMPOS, P. H. R. F.; FONSECA E SILCA, F.; HANNAS, M. I.; ALBINO, L. F. F. T.; BORGES, S. O.; CALDERANO, A. A. True ileal phosphorus digestibility of soybean meal for male and female broiler chickens. **Animal Feed Science and Technology**, v. 270, p. 114649, 2020. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114649.

MOITA, V. H. C.; DUARTE, M. E.; KIM, S. W. Supplemental effects of phytase on modulation of mucosa-associated microbiota in the jejunum and the impacts on nutrient digestibility, intestinal morphology, and bone parameters in broiler chickens. **Animals**, v. 11, p. 3351, 2021. DOI: https://doi.org/10.3390/ani11123351.

NARI, N.; GHASEMI, H. A.; HAJKHODADADI, I.; FARAHANI, A. K. Intestinal microbial ecology, immune response, stress indicators, and gut morphology of male broiler chickens fed low-phosphorus diets supplemented with phytase, butyric acid, or Saccharomyces boulardii. **Livestock Science**, v. 234, p. 103975, 2020. DOI: https://doi.org/10.1016/j.livsci.2020.103975.

NOLTE, J. **ICP: Emission spectrometry. A practical guide**. Weinheim: Willey-VCH, 2003. 267 p.

NUAMAH, E.; OKON, U. M.; JEONG, E.; MUN, Y.; CHEON, I.; CHAE, B.; ODOI, F. N. A.; KIM, D. W.; CHOI, N. J. Unlocking phytate with phytase: A meta-analytic view of meat-type chicken muscle growth and bone mineralization potential. **Animals**, v. 14, n. 14, p. 2090, 2024. DOI: https://doi.org/10.3390/ani14142090.

OATWAY, L.; VASANTHAN, T.; HELM, J. H. Phytic acid. **Food Reviews International**, v. 17, n. 4, p. 419–431, 2001. DOI: http://doi.org/10.1081/FRI-100108531.

PIENIAZEK, J.; SMITH, K. A.; WILLIAMS, M. P.; MANANGI, M. K.; VAZQUEZ-ANON, M.; SOLBAK, A.; MILLER, M.; LEE, J. T. Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. **Poultry Science**, v. 96, n. 2, p. 370–382, 2017. DOI: https://doi.org/10.3382/ps/pew225.

PIRES, S. M.; REIS, R. S.; CARDOSO, S. M.; PEZZANI, R.; PAREDES-OSSES, E.; SEILKHAN, A.; YDYRYS, A.; MARTORELL, M.; GÜRER, E. S.; SETZER, W. N.; RAZIS, A. F. A.; MODU, B.; CALINA, D.; SHARIFI-RAD, J. Phytates as a natural source for health promotion: A critical evaluation of clinical trials. **Frontiers in Chemistry**, v. 11, p. 1174109, 2023. DOI: https://doi.org/10.3389/fchem.2023.1174109.

RAVINDRAN, V. Feed enzymes: The science, practice, and metabolic realities. **Journal of Applied Poultry Research**, v. 22, n. 3, p. 628–636, 2013. DOI: http://doi.org/10.3382/japr.2013-00739.

ROSTAGNO, H. S.; ALBINO, L. F. T.; CALDERANO, A. A.; HANNAS, M. I.; SAKOMURA, N. K.; PERAZZO, F. G.; ROCHA, G. C.; SARAIVA, A.; DE ABREU, M. L. T.; GENOVA, J. L.; TAVERNARI, F. C. **Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**. 5. ed. Visconde do Rio Branco, MG: Suprema, 2024. 531 p.

SARRUGE, J. R.; HAAG, H. P. **Chemical analysis of plants**. Piracicaba: Escola Superior de Agricultura Luiz de Queiroz, 1974. 56 p.

STANDARTS, A. **Shear and three-point bending test of animal bone**. ANSI/ASAE S459 DEC01, USA, 2003.

SEEDOR, J. G. The biophosphanate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. **Journal of Bone and Mineral Research**, v. 4, p. 265–270, 1995. DOI: http://doi.org/10.1002/jbmr.5650060405.

SELIM, S.; ABDEL-MEGEID, N. S.; KHALIFA, H. K.; FAKIHA, K. G.; MAJRASHI, K. A.; HUSSEIN, E. Efficacy of various feed additives on performance, nutrient digestibility, bone quality, blood constituents, and phosphorus absorption and utilization of broiler chickens fed low phosphorus diet. **Animals**, v. 12, n. 14, p. 1742, 2022. DOI: https://doi.org/10.3390/ani12141742.

SELLE, P. H.; MACELLINE, S. P.; CHRYSTAL, P. V.; LIU, S. Y. The contribution of phytate-degrading enzymes to chicken-meat production. **Animals**, v. 13, n. 4, p. 603, 2023. DOI: https://doi.org/10.3390/ani13040603.

SOXHLET, F. V. Die gewichtsanalytische bestimmung des milchfettes. **Polytechnisches Journal**, v. 232, p. 461–465, 1879.

VALABLE, A. S.; NARCY, A.; DUCLOS, M. J.; POMAR, C.; PAGE, G.; NASIR, Z.; MAGNIN, M.; LÉTOURNEAU-MONTMINY, M. P. Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. **Animal**, v. 12, n. 8, p. 1555–1563, 2018. DOI: https://doi.org/10.1017/S1751731117003093.

WALK, C. L.; RAO, S. R. Increasing dietary phytate has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations in broilers. **Poultry Science**, v. 99, n. 1, p. 290–300, 2020. DOI: http://doi.org/10.3382/ps/pez489.

ZÁLEŠÁKOVÁ, D.; NOVOTNÝ, J.; ŘIHÁČEK, M.; HORÁKOVÁ, L.; MRKVICOVÁ, E.; ŠŤASTNÍK, O.; PAVLATA, L. The blood biochemical parameters intervals and dynamics in modern broiler chickens. **Veterinary and Animal Science**, p. 100465, 2025. DOI: https://doi.org/10.1016/j.vas.2025.100465.

ZANU, H. K.; KHERAVII, S. K.; MORGAN, N. K.; BEDFORD, M. R.; SWICK, R. A. Interactive effect of dietary calcium and phytase on broilers challenged with subclinical necrotic enteritis: 3. Serum calcium and phosphorus, and bone mineralization. **Poultry Science**, v. 99, n. 10, p. 4914–4928, 2020a. DOI: http://doi.org/10.1016/j.psj.2020.04.012.

ZANU, H.; KEERQIN, C.; KHERAVII, S.; MORGAN, N.; WU, S.; BEDFORD, M.; SWICK, R. Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 1. growth performance, intestinal pH, apparent ileal digestibility, cecal microbiota, and tibial mineralization. **Poultry Science**, v. 99, p. 1540–1550, 2020b. DOI: https://doi.org/10.1016/j.psj.2019.11.021.

ZHANG, Q.; WALK, C. L.; COWIESON, A. J.; STAMATOPOULOS, K.; WU, J. L.; SORBARA, J. O. B. Efficacy of a novel phytase in response to low and high phytate diets using a short-term digestibility model in broiler chickens at two ages. **Animal Feed Science and Technology**, v. 307, p. 115832, 2024. DOI: https://doi.org/10.1016/j.anifeedsci.2023.115832.

GENERAL CONCLUSION

This thesis demonstrates that the accurate nutritional characterization of alternative feed ingredients and phytase supplementation represent complementary strategies for improving broiler nutrition. The determination of metabolizable energy values provides the basis for more precise diet formulation, whereas phytase supplementation enhances nutrient utilization in nutritionally reduced diets. Together, these approaches increase formulation flexibility, improve feed efficiency, and support the development of more economically and environmentally sustainable feeding programs.

The first study generated updated AME and AMEn values for eight alternative feed ingredients sources, demonstrating substantial variation among ingredients of similar origin, particularly among lipid sources. These findings reinforce that the use of generalized tabulated values may compromise formulation accuracy and highlight the importance of periodically updating feed composition databases. The energy values generated in this study provide new information that may support future revisions of the Brazilian Tables for Poultry and Swine and improve the nutritional evaluation of alternative ingredients used by researchers, nutritionists, and the feed industry.

The second study demonstrated that phytase supplementation effectively restored productive performance, bone mineralization, serum calcium and phosphorus concentrations, and body composition in broilers fed diets with planned reductions in available phosphorus, calcium, sodium, metabolizable energy, crude protein, and digestible amino acids. These results confirm that phytase can be strategically incorporated into matrix-based formulation programs, allowing reductions in dietary nutrient specifications while maintaining productive responses.

Together, the results indicate that accurate nutritional characterization of alternative feed ingredients expands formulation flexibility, whereas phytase supplementation enables more efficient utilization of these nutrients in practical feeding programs. Consequently, these strategies may reduce dependence on conventional feedstuffs, improve feed cost efficiency, and contribute to more sustainable poultry production through better utilization of agro-industrial coproducts and reduced nutrient excretion.

Despite these contributions, the present research was conducted under controlled experimental conditions using specific ingredient batches and broilers at particular physiological stages. Since the nutritional composition of alternative feed ingredients may vary according to raw material source, industrial processing, and storage conditions, continuous evaluation remains necessary to ensure formulation accuracy.

Future research should expand the nutritional characterization of alternative feed ingredients from different production systems and industrial processes, validate the metabolizable energy values under commercial production conditions, and investigate interactions between ingredient quality, enzyme supplementation, gut health, and productive performance. In addition, incorporating new feed ingredients and updated energy values into national feed composition databases will further strengthen precision nutrition programs for modern broiler production.

Overall, this thesis provides applied information that supports both scientific advancement and practical decision-making in poultry nutrition. Its main contribution lies in demonstrating that accurate feed evaluation and strategic enzyme supplementation are complementary tools that enable more precise, economically competitive, and environmentally sustainable nutritional programs for broiler chickens.