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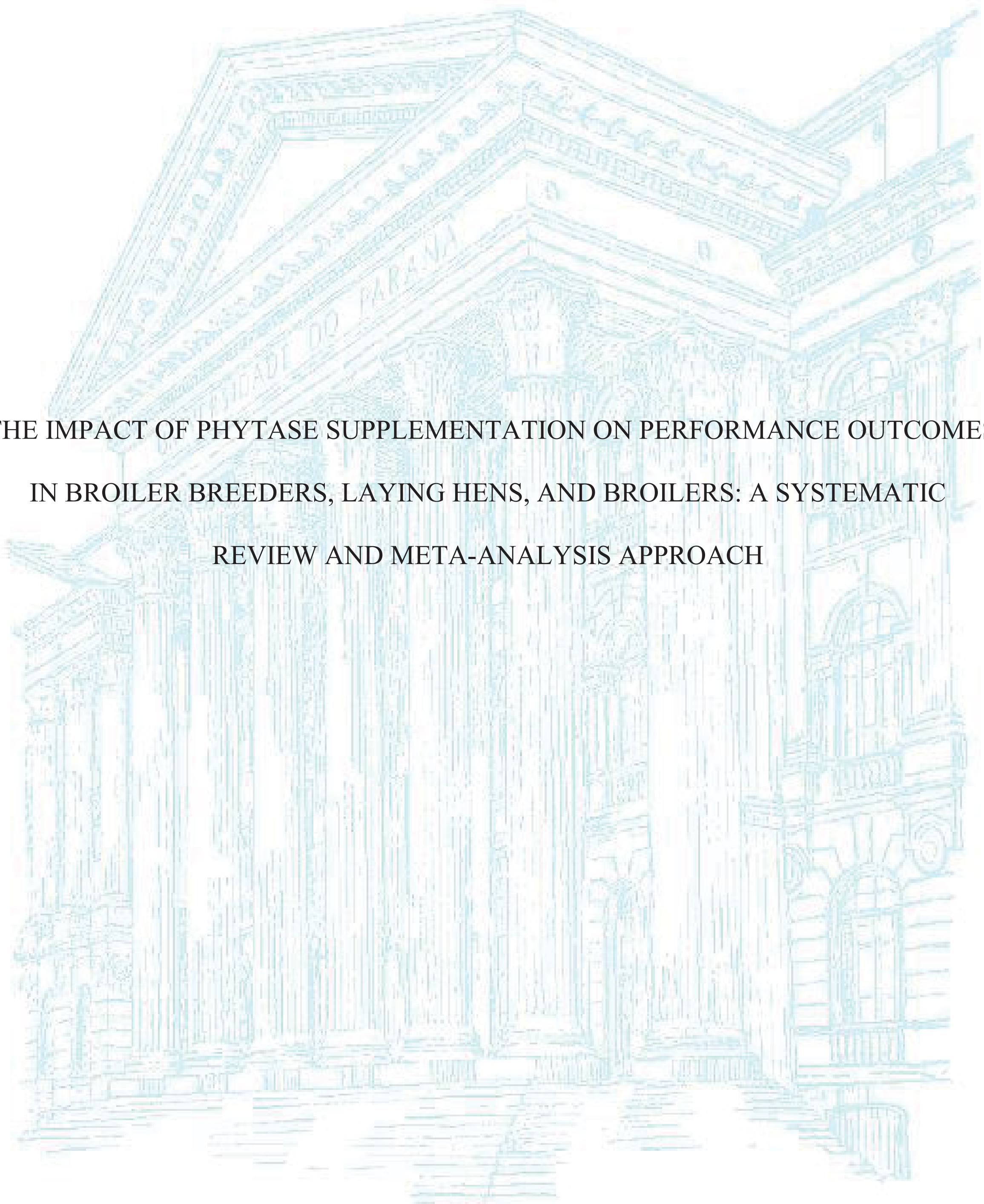

THE IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE OUTCOMES IN BROILER BREEDERS, LAYING HENS, AND BROILERS: A SYSTEMATIC REVIEW AND META-ANALYSIS APPROACH

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Orientador : Prof. Dr. Alex Maiorka
Coorientadora: Profª. Dra. Ines Andretta

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ROBERTO ALEXANDRE YAMAWAKI**, intitulada: **The Impact of Phytase Supplementation on Performance Outcomes in Broiler Breeders, Laying Hens, and Broilers: A Systematic Review and Meta-Analysis Approach**, sob orientação do Prof. Dr. ALEX MAIORKA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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28/03/2025 16:09:03.0
ALEX MAIORKA
Presidente da Banca Examinadora

Assinatura Eletrônica
26/03/2025 17:04:14.0
KEYSUKE MURAMATSU
Avaliador Externo (DSM PRODUTOS NUTRICIONAIS)

Assinatura Eletrônica
27/03/2025 19:49:23.0
CHAYANE DA ROCHA
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
07/04/2025 16:27:44.0
ANTÔNIO MARIO PENZ JUNIOR
Avaliador Externo (CARGILL - BRASIL)

DEDICATION

I dedicate this work to God, my family, my wife Ana Luísa, and our dogs Odinn, Heimdall, and Zeca.

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RESUMO

Manejar os níveis de fósforo (P) na dieta de aves para mitigar impactos ambientais e econômicos é um desafio constante da avicultura. Muitos estudos têm investigado os efeitos da suplementação de fitase (PHY) e sua interação com o fósforo não fítico (NPP), bem como a relação cálcio:NPP (Ca:NPP), no desempenho de matrizes de corte, poedeiras e frangos de corte. No entanto, os resultados observados na literatura apresentam alta variabilidade. Para analisar tais inconsistências, foram realizadas cinco meta-análises para avaliar os efeitos da suplementação de PHY em matrizes de corte (Capítulo 2), poedeiras (Capítulo 3), frangos de corte machos e fêmeas na fase inicial (1–21 dias pós-eclosão) (Capítulo 4) e frangos de corte machos na fase final (22–42 dias pós-eclosão) (Capítulo 5). Essas análises avaliaram o impacto da PHY no desempenho, nos resultados econômicos, no teor de cinzas da tíbia e na digestibilidade de nutrientes. A primeira meta-análise, focada em matrizes de corte, incluiu sete estudos. Foram comparados a produção de ovos (EP), a eclosão, o peso dos ovos (EW) e as proporções relativas de gema, albúmen e casca em relação ao EW entre dietas com e sem suplementação de PHY. Os resultados indicaram que a suplementação de PHY aumentou a EP em 4,85% (P<0,01) e a proporção de gema em 0,24% (P=0,03), enquanto o EW diminuiu 0,53 g (P<0,01) e a proporção de albúmen reduziu 0,34% (P<0,01). Nenhum efeito significativo foi observado na eclosão ou na proporção de casca (P≥0,05). Além disso, um modelo econômico baseado nos resultados dessa meta-análise sugeriu que a suplementação de PHY reduz os custos em US$0,011 ou 3,10% por ovo produzido. A segunda meta-análise examinou os efeitos da suplementação de PHY e sua interação com NPP e a relação cálcio:fósforo fítico (Ca:PP) em poedeiras. Essa análise incluiu 89 estudos, com 38.795 aves entre 24 e 80 semanas de idade. A suplementação de PHY melhorou significativamente (P<0,05) a EP, o consumo diário de ração (ADFI), a massa de ovos (EM), a proporção de albúmen, o teor de cinzas da tíbia, a digestibilidade ileal aparente do fósforo (AIDP) e a utilização de fósforo (P) e nitrogênio (N). Além disso, o uso de PHY melhorou a conversão alimentar (FCR) e a proporção de casca. No entanto, níveis elevados de Ca:PP prejudicaram a EP, a EM, o teor de cinzas da tíbia e a utilização de P, além de aumentar a FCR e a proporção de albúmen e casca em relação ao EW. Os efeitos da suplementação de PHY foram mais pronunciados em dietas com níveis mais baixos de NPP, exceto para a utilização de P. A terceira e quarta meta-análises avaliaram os efeitos da suplementação de PHY e sua interação com NPP e Ca:NPP em frangos de corte machos e fêmeas durante a fase inicial. Uma meta-análise incluiu 98 estudos com 106.476 frangos machos, enquanto a outra analisou sete estudos com 7.052 fêmeas, todos com idade entre 1 e 21 dias pós-eclosão. A suplementação de PHY melhorou significativamente (P<0,05) o desempenho zootécnico em ambos os sexos. Nos machos, PHY também aumentou o teor de cinzas da tíbia, a AIDP, a digestibilidade ileal aparente do nitrogênio (AIDN) e influenciou positivamente a digestibilidade ileal aparente dos aminoácidos, com um efeito maior nos aminoácidos com menor digestibilidade intrínseca, como a cisteína e a treonina. Além disso, o aumento dos níveis de NPP melhorou o desempenho de zootécnico e o teor de cinzas da tíbia. No entanto, altas relações Ca:NPP impactaram negativamente todos os parâmetros avaliados, especialmente em dietas sem suplementação de PHY. A quinta meta-análise quantificou o impacto da suplementação de PHY em frangos machos durante a fase final, utilizando dados de 21 estudos envolvendo 10.706 frangos machos entre 22 e 42 dias de idade. A suplementação de PHY melhorou significativamente (P<0,05) tanto o desempenho zooténico quanto o teor de cinzas da tíbia, com efeitos mais pronunciados em dietas com baixo NPP. Além disso, o aumento dos níveis de NPP na dieta melhorou ambos os parâmetros. No entanto, altas relações Ca:NPP impactaram negativamente todos os parâmetros avaliados, especialmente em dietas sem suplementação de PHY. Os resultados dessas meta-análises destacam o impacto positivo da suplementação de PHY, reforçando seus benefícios na melhoria do desempenho, dos resultados econômicos e da utilização de nutrientes em matrizes de corte, poedeiras e frangos de corte durante as fases inicial e final.

Palavras-chave: Fitase; Meta-análise; Matrizes pesadas; Poedeiras; Frangos de corte.

ABSTRACT

Managing dietary phosphorus (P) levels in poultry diets to mitigate environmental and economic concerns has remained a complex challenge for decades. Numerous studies have investigate the effects of phytase (PHY), and its interaction with non-phytate phosphorus (NPP) and calcium to NPP ratio (Ca:NPP) on broiler breeders, laying hens, and broiler performance, however, results in the literature have shown high variability. To address these inconsistencies, five meta-analyses were conducted to evaluate the effects of PHY supplementation in broiler breeders (Chapter 2), laying hens (Chapter 3), male and female broilers during the starter phase (1–21 days post-hatch) (Chapter 4), and male broilers during the finisher phase (22–42 days post-hatch) (Chapter 5). These meta-analyses assessed PHY's impact on performance and economic outcomes, tibia ash content, and nutrient digestibility. The first meta-analysis, which focused on broiler breeders, included seven studies. It compared egg production (EP), hatchability, egg weight (EW), and the relative proportions of yolk, albumen, and shell weight to EW between diets with and without PHY supplementation. The results showed that PHY supplementation increased EP by 4.85% (P<0.01) and yolk ratio by 0.24% (P=0.03), while EW decreased by 0.53 g (P<0.01) and albumen ratio by 0.34% (P<0.01). No significant effects were observed on hatchability or shell ratio (P≥0.05). Additionally, an economic model based on this meta-analysis results indicated that PHY supplementation reduced costs by $0.011 or 3.10% per egg produced. The second meta-analysis examined the effect of PHY and its interaction with NPP and calcium to phytate phosphorus ratio (Ca:PP) in laying hens. This meta-analysis comprised 89 studies, with 38,795 hens between 24 and 80 weeks of age. PHY supplementation significantly (P<0.05) enhanced EP, average daily feed intake (ADFI), egg mass (EM), albumen proportion, tibia ash content, apparent ileal digestibility of P (AIDP), and the utilization of phosphorus (P) and nitrogen (N). PHY also reduced the feed conversion ratio (FCR) and shell proportion. However, higher Ca:PP levels impaired EP, EM, tibia ash content, and P utilization, while also increasing the FCR and the albumen and shell proportion relative to EW. The effects of PHY supplementation were more pronounced in diets with lower dietary NPP levels, except for P utilization. The third and fourth meta-analyses evaluated the effect of PHY supplementation and its interaction with NPP and Ca:NPP in male and female broilers during the starter phase. One meta-analysis included 98 studies involving 106,476 male broilers, while the other analyzed seven studies with 7,052 female broilers, all aged 1 to 21 days post-hatching. PHY supplementation significantly (P<0.05) enhanced the growth performance in both male and female broilers. In males, PHY also improved tibia ash content, AIDP, apparent ileal digestibility of nitrogen (AIDN), and positively affected the apparent ileal digestibility of amino acids, with a more pronounced effect on those with lower inherent digestibility, such as cysteine and threonine. Additionally, increasing NPP levels improved growth performance and tibia ash content. However, high dietary Ca:NPP ratios negatively affected all measured parameters, particularly in diets without PHY supplementation. The fifth meta-analysis quantified the impact of PHY supplementation on male broiler during the finisher phase, using data from 21 studies involving 10,706 male broilers aged 22 to 42 days post-hatching. PHY supplementation significantly (P<0.05) improved both growth performance and tibia ash content, with more pronounced effects observed in low-NPP diets. Additionally, increasing dietary NPP levels enhanced both growth performance and tibia ash content. However, high dietary Ca:NPP ratios negatively impacted all evaluated parameters, particularly in diets without PHY supplementation. The findings from these meta-analyses highlight the positive impact of PHY supplementation, reinforcing its benefits in improving performance, economic outcomes and nutrient utilization in broiler breeders, laying hens, and broilers during both the starter and finisher phases.
Keywords: Phytase; Meta-analysis; Broiler breeders; Layers; Broilers.

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CHAPTER V - IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, AND TIBIA ASH CONTENT OF BROILERS FROM 22 TO 42 DAYS POST-HATCH: A META-ANALYSIS.

SUMMARY

GENERAL INTRODUCTION

The growing expansion of the global population, along with the rising per capita income, has increased the global demand for grains and animal-based proteins. By 2050, the global population is estimated to be 50% larger than at the beginning of the 21st century, requiring twice the current demand for grains (TILMAN et al., 2002). Therefore, increasing food production without compromising environmental integrity and public health is one of the greatest challenges of our time (TILMAN et al., 2002).

In this context, poultry meat production serves as an important protein source. Currently, chicken meat is the second most consumed animal protein worldwide, with an estimated annual production of over 102 million tons (ABPA, 2024). Poultry production stands out due to its highly developed processes and the efficient feed conversion of birds (BROCH et al., 2018), making it one of the most sustainable and economically viable food production systems (DE VRIES AND DE BOER, 2010). Despite birds exhibiting an excellent feed conversion efficiency into animal protein (BROCH et al., 2018), some plant-based ingredients may contain antinutritional factors for poultry, such as non-starch polysaccharides and, most notably, phytate phosphorus (PP) (CHOCT et al. 2010; SELLE et al., 2023).

Phosphorus (P) is an essential mineral that plays a crucial role in various metabolic processes in poultry, as well as in skeletal mineralization, together with calcium (Ca) (BOUGOUIN et al., 2014). However, the majority of P in plant-based feed is bound to phytic acid and its salts, making it largely unavailable for digestion in poultry and thereby represents a significant challenge in poultry nutrition (COWIESON et al., 2016). Consequently, to meet the daily phosphorus requirements of poultry, animal-derived meals, such as meat and bone meals or costly and non-renewable inorganic P sources must be supplemented in feed (HERVO et al., 2023). Additionally, any excess P that is not absorbed is excreted in manure, potentially contributing to environmental issues such as surface water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023).

Since its introduction more than three decades ago, exogenous phytase (PHY) has become one of the most widely adopted strategy by nutritionist to mitigate the antinutritional effect of PP and improve the digestibility of P, Ca, and other nutrients, including energy and amino acids in monogastric animals such as pigs and poultry (ADEOLA AND COWIESON, 2011; DERSJANT-LI et al., 2014; SELLE et al., 2023). Over the past decades, several studies have reported the beneficial effects of PHY on performance, bone mineralization, and nutrient digestibility in laying hens (AHMADI AND RODEHUTSCORD, 2012; RODEHUTSCORD et al., 2023), broilers (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; FARIDI et al., 2015; COWIESON et al., 2017; KERMANI et al., 2023; NUAMAH et al., 2024), and broiler breeders (BERRY et al., 2003; BHANJA et al., 2005; NUSAIRAT et al., 2018). However, there is considerable variability in the reported effects of PHY supplementation.

To address a better understanding of this heterogeneous body of research and translate findings into practical applications, systematic reviews and meta-analyses have become essential tools. These statistical techniques allow for a quantitative assessment of PHY effects (ST-PIERRE, 2007; LOVATTO et al., 2007). Moreover, systematic reviews and meta-analyses rank at the top of the evidence hierarchy and provide the highest level of evidence among research methodologies (MYUNG, 2023).

This PhD thesis applies a systematic review and meta-analysis approach across five chapters to quantify the impact of PHY supplementation on poultry performance, focusing on broiler breeders, laying hens, and broilers. The first chapter presents a comprehensive literature review aimed to describe the overall metabolism of P and PHY, as well as an overview of the systematic review and meta-analysis process. The remaining four chapters comprise the systematic review and meta-analyses performed, each addressing the effects of PHY supplementation in different areas of poultry production. Chapter two focus on the effect of PHY supplementation on performance, economic outcomes and egg quality traits in broiler breeders. In chapter three, a systematic review and meta-analysis was conducted to assess the effect of PHY on performance, egg quality, tibia ash content, and nutrient digestibility in laying hens. Additionally, in chapter four, a systematic review and meta-analysis was developed to measure the effect of PHY on performance, egg quality, tibia ash content, and nutrient digestibility in broilers during the starter phase (1 to 21 days post-hatching). Finally, in chapter five, systematic review and meta-analysis was performed to investigate the effect of PHY on performance, and tibia ash content in broilers during the finisher phase (22 to 42 days post-hatching). Through this approach, this thesis aims to provide a robust and evidence-based evaluation of PHY supplementation, contributing valuable insights to poultry nutrition research and industry practices.

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CHAPTER I - LITERATURE REVIEW

1. PHOSPHORUS IN POULTRY DIETS

Phosphorus (P) is the second most abundant mineral in the poultry body (PROSZKOWIEC-WEGLARZ AND ANGEL, 2013). Approximately 80% of P is stored in combination with calcium (Ca) as hydroxyapatite crystals in the bones, while the remaining 20% is distributed in body fluids and other tissues as dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{2-}) (WAGNER, 2023). Due to its high reactivity with oxygen, P exists in the body exclusively as phosphate ions (WAGNER, 2023). Phosphorus is not only essential as a structural component of bones and cell membranes but also plays a pivotal role in various physiological processes. These include the formation of muscular tissue, nutrient absorption, enzymatic activation, osmotic regulation, acid-base balance, adenosine triphosphate (ATP) synthesis, and DNA formation. Additionally, P may act as a co-factor for numerous enzymes (PEACOCK, 2021; VALENTE JUNIOR et al., 2024).

However, most P found in cereal grains and meals used in poultry diets is present in the form of phytic acid, or phytate (PP) when in salt form, accounting for 60% to 80% of the total P content, as shown in Table 1 (SELLE AND RAVINDRAN, 2009). Nevertheless, poultry have negligible or nearly absent endogenous enzymes to break down PP (ABUDABOS, 2012), resulting in reduced P availability (RABOY, 2009). This limitation represents a significant challenge in poultry nutrition (COWIESON et al., 2016). To meet the daily nutritional requirements of poultry, additional sources of P may be included in their diets (HERVO et al., 2023). Phosphorus derived from inorganic sources, such as mono-, di-, or triphosphates, is obtained from phosphate rocks and offers nearly 100% bioavailability, as inorganic Ca, with P content varying between sources (HAMDI et al., 2017). Unlike Ca, these inorganic P sources are finite, expensive, and projected to be depleted in the coming decades (KUMAR et al., 2016). Beyond increasing feed costs, excessive P in poultry diets may contribute to excessive amounts of non-absorbed P in manure, potentially leading to environmental issues such as surface water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023). Phosphorus-rich excreta can leach into groundwater, promoting the growth of algae and aquatic plants and exacerbating water eutrophication (KNOWLTON et al., 2004). Animal-derived meals, such as meat and bone meals, also offer a high P content and could serve as alternative sources for poultry diets.

However, these ingredients may present a risk of contamination, with reported *Salmonella* prevalence ranging from 8.7% to 50% (JIANG, 2016).

TABLE 1. Weight mean (and range) of total phosphorus (P) and phytate-P concentrations, and proportion of phytate-P of total P, in important poultry feed ingredients

Feed ingredient	Total P (g kg^{-1})	Phytate-P (g kg^{-1})	Proportion (%)
Cereals			
Barley	3.21 (2.73 - 3.70)	1.96 (1.86 - 2.20)	61.0 (59 - 68)
Maize	2.62 (2.30 - 2.00)	1.88 (1.70 - 2.20)	71.6 (66 - 85)
Sorghum	3.01 (2.60 - 3.09)	2.18 (1.70 - 2.46)	72.6 (65 - 83)
Wheat	3.07 (2.90 - 4.09)	2.19 (1.80 - 2.89)	71.6 (55 - 79)
Oilseed meals			
Canola meal	9.72 (8.79 - 11.50)	6.45 (4.00 - 7.78)	66.4 (36 - 76)
Cottonseed meal	10.02 (6.40 - 11.36)	7.72 (4.90 - 9.11)	77.1 (70 – 80)
Soyabean meal	6.49 (5.70 - 6.94)	3.88 (3.54 - 4.53)	59.9 (53 - 68)
By-products			
Rice bran	17.82 (13.40 - 27.19)	14.17 (7.90 - 24.20)	79.5 (42 - 90)
Wheat bran	10.96 (8.02 – 13.71)	8.36 (7.00 - 9.60)	76.3 (50 - 87)

Adapted from SELLE AND RAVINDRAN, 2009.

2. PHYTATE PHOSPHORUS AND PHYTASE FOR POULTRY

The PP molecule consists of six phosphate groups attached to an inositol ring [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$] (Figure 1) (SOMMERFELD et al., 2018; SELLE et al., 2023). Phytic acid has 12 reactive sites and is a strongly negatively charged polyanionic molecule, particularly within the pH range of the digestive tract. These properties enable it to bind di- and trivalent minerals, forming highly stable and insoluble complexes with cations, such as Ca, potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), and manganese (Mn) (COWIESON et al., 2009; DERSJANT-LI et al., 2014).

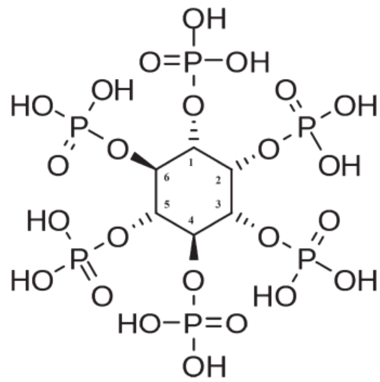

FIGURE 1. Structure of phytic acid [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$] (DERSJANT-LI et al., 2014).

One of the most critical factors influencing PP's interaction with dietary components is the pH of the digestive tract (AMERAH et al., 2014). In the gizzard and proventriculus of birds, where pH ranges from 2.5 to 5.0, most PP chelates become more soluble, making them more susceptible to hydrolysis by PP-degrading enzymes, which exhibit peak activity at acidic pH levels (LIEBERT et al., 1993). However, as the intestinal pH approaches neutrality, the formation of minerals and phytic acid complexes occurs mainly at the higher small intestinal pH (MAENZ et al., 2011). Animal diets typically contain higher levels of Ca compared to other cations. Consequently, phytic acid primarily complexes with Ca in the small intestine, where the elevated pH further strengthens the formation of insoluble and indigestible Ca-PP complexes (LIU et al., 2013; DERSJANT-LI et al., 2014).

Other cations, such as Mg and Zn, also tend to form chelates with phytic acid at higher intestinal pH levels (BASSI, 2024). A synergistic effect may occur when two cations interact with phytic acid, for instance, Ca enhances the absorption of Zn, forming a Ca-Zn phytate complex, while Mg can increase the precipitation of Zn with PP, albeit to a lesser extent than Ca (KONIETZNY AND GREINER, 2003). The extent of precipitation increases with higher dietary Ca levels and a greater Ca:P ratio, resulting in more extensive interactions between Ca, other ions, and phytic acid (BASSI, 2024). Phytate-P can increase endogenous losses of minerals such as sodium (Na) in poultry (COWIESON et al., 2004). As a negatively charged molecule, PP can bind to sodium, reducing its availability in the intestinal lumen. Additionally, PP irritates the gut lining, stimulating the secretion of mucus and bicarbonate, which are rich in sodium. This results in increased intestinal secretions and greater water and electrolyte loss, as Na plays a key role in maintaining osmotic balance in the gastrointestinal tract. Furthermore, PP can inhibit the activity of intestinal sodium transporters and chelate dietary proteins, potentially stimulating hydrochloric acid (HCl) secretion in the proventriculus and leading to further Na excretion (SELLE et al., 2012). Consequently, Na deficiency can have an impact on the activity of Na–K-ATPase in the gastrointestinal tract, which is involved in the absorption of nutrients, reducing the activity of Na–K-ATPase in the gastrointestinal tract in broilers (COWIESON et al., 2008).

Moreover, PP can chemically interact with dietary polysaccharides, proteins, amino acids, and enzymes, inhibiting their activity and negatively impacting protein and carbohydrate digestibility (COWIESON et al., 2009; DERSJANT-LI et al., 2014; VALENTE JUNIOR et al., 2024). Phytate-P non-selectively binds to proteins and has been shown to inhibit enzymes such as trypsin and α-amylase, thereby reducing protein digestibility in animals (DERSJANT-LI et al., 2014). Phytic acid can interact with proteins across a wide pH range. In acidic pH, such as in the stomach, it binds to basic amino acids like arginine, histidine, and lysine, forming protein–PP complexes (DERSJANT-LI et al., 2014). In the small intestine, where the pH rises above the isoelectric point of proteins, phytic acid can bind to proteins indirectly through cations, forming protein–mineral–PP complexes (SELLE et al., 2000; SELLE et al., 2023). These complexes are insoluble, resistant to enzymatic hydrolysis, and significantly reduce the efficiency of protein digestion and utilization complexes (DERSJANT-LI et al., 2014). Additionally, phytic acid can negatively affect nutrient digestibility by interacting with endogenous enzymes, making PP-bound proteins resistant to pepsin digestion (DERSJANT-LI et al., 2014). Furthermore, it can increase endogenous amino acid losses by stimulating the secretion of digestive enzymes and mucins while reducing the reabsorption of endogenously secreted amino acids in the small intestine (WOYENGO AND NYACHOTI, 2013). Additionally, Ca phytate may contribute to the formation of metallic soaps in the gut lumen, which can reduce the digestion of saturated fats (COWIESON et al, 2008). These antinutritional effects will lead to reduced nutrient utilization, increased maintenance protein and energy costs, and reduced energy availability for production.

Microbial phytase (PHY) is the most widely used exogenous enzyme in feed for monogastric animals. PHY reduces the antinutritional effect of PP and improve the digestibility of P, Ca, amino acids and energy, while mitigating the environmental impacts of P excretion (DERSJANT-LI et al., 2014). The first generation of commercially available microbial PHY was introduced in 1991. Since then, advancements have led to new generations of PHYs with varying efficacy (ADEOLA AND COWIESON, 2011; SELLE et al., 2023). While there is no universally standardized international unit for measuring PHY activity, Engelen et al. (1994) introduced the concept of PHY activity in PHY units (FTU), which is typically defined as the amount of enzyme required to release 1 mmol of inorganic phosphate per minute from 0.0051 mol L^{-1} sodium PP at pH 5.5 and 37°C (AOAC, 2000). Alternate abbreviations for PHY units, such as FTY and U, have been adopted for various PHY suppliers, all established under a similar set of parameters (COWIESON et al., 2018; BASSI, 2024). However, as the pH in the stomach of animals is significantly lower than pH 5.5, the actual *in vivo* activity may differ from standard measurements (DERSJANT-LI et al., 2014). Furthermore, various factors may influence PHY activity *in vivo*, including enzyme characteristics, dietary composition, and animal-related factors. Additionally, the effectiveness of microbial PHY in releasing inorganic P depends on dietary PP concentration, the source of PP, species, the age of the animals, dietary mineral concentrations, PHY source, and PHY dosing (ADEOLA AND COWIESON, 2011; DERSJANT-LI et al., 2014; SELLE et al., 2023).

Phytase (PHY), is an enzyme capable of breaking down PP into lower phytate esters and myo-inositol (WALK et al., 2018), as shown in Figure 2. This process allows the previously bound P to be available to the animal while also improving Ca, amino acid and energy digestibility and utilization (COWIESON et al., 2006). PHYs are classified into two categories depending on their hydrolysis activity: 3-phytases (EC 3.1.3.8) liberate P at the C3 position of the PP molecule, while 6-phytases (EC 3.1.3.26) cleave at the C6 position. PP hydrolysis occurs sequentially from myo-inositol hexakisphosphate (IP_6) to lower inositol phosphates (IP_1), releasing up to six inorganic P moieties. Additionally, the P residue at the C2 position is more resistant to hydrolysis compared to other positions (COBAN AND DEMIRCI, 2017). The complete degradation pathway of phytic acid, as characterized by Kempapidis et al. (2020), proceeds as follows: $IP_6 \rightarrow IP_5 \rightarrow IP_4 \rightarrow IP_3 \rightarrow IP_2 \rightarrow IP_1 \rightarrow$ free inositol. This process ultimately generates myo-inositol, which can be absorbed in the gastrointestinal tract (BELLO et al., 2019), along with six inorganic phosphorus moieties.

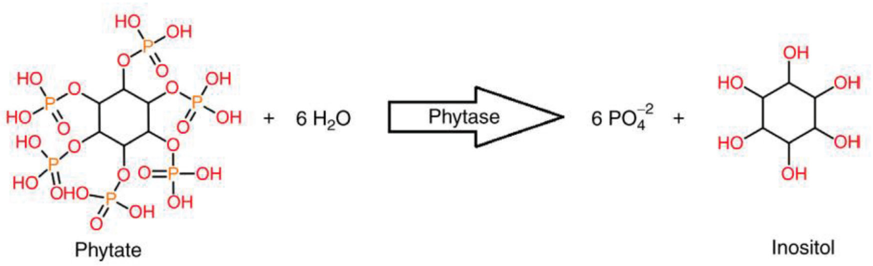

FIGURE 2. Phytate hydrolysis reaction catalyzed by phytase enzyme (CHEMDOODLE, 2016).

The supply of myo-inositol, a product of PP degradation, has garnered increasing attention as PHY supplementation becomes more widespread (MORAN et al., 2019). This extra release of nutrients is referred to as the "extra-phosphoric effect” and contributes to the improved growth performance often observed with PHY supplementation (COWIESON et al., 2011; COWIESON et al., 2016; MOITA et al., 2022). In addition, these nutrients have been found to positively influence the microbiota, immune response, and antioxidant status in nonruminant animals (DERAKHSHAN et al., 2023), which is reflected in improved intestinal morphology and integrity (AMIRI et al., 2021; MOITA et al., 2021). Reducing undigested phytic acid in the intestine can significantly influence the intestinal environment (VALENTE JUNIOR et al., 2024). One key effect is a reduction in digesta pH, which fosters a favorable environment for the growth of beneficial bacteria while inhibiting the proliferation of harmful microbes (MOITA et al., 2021). In addition, PHY may potentially exert a positive impact on the microbiota by inducing intestinal alkaline phosphatase activity (LIU et al., 2010). Additionally, to its role in dephosphorylating myo-inositol monophosphate, intestinal alkaline phosphatase performs several functions, including dephosphorylating bacterial lipopolysaccharide (LPS) and preventing bacterial transepithelial passage, thereby contributing to intestinal health and barrier integrity (LIU et al., 2010). The beneficial effect of PHY supplementation in monogastric animals and the harmful effects of PP molecule is summarized in Figure 3.

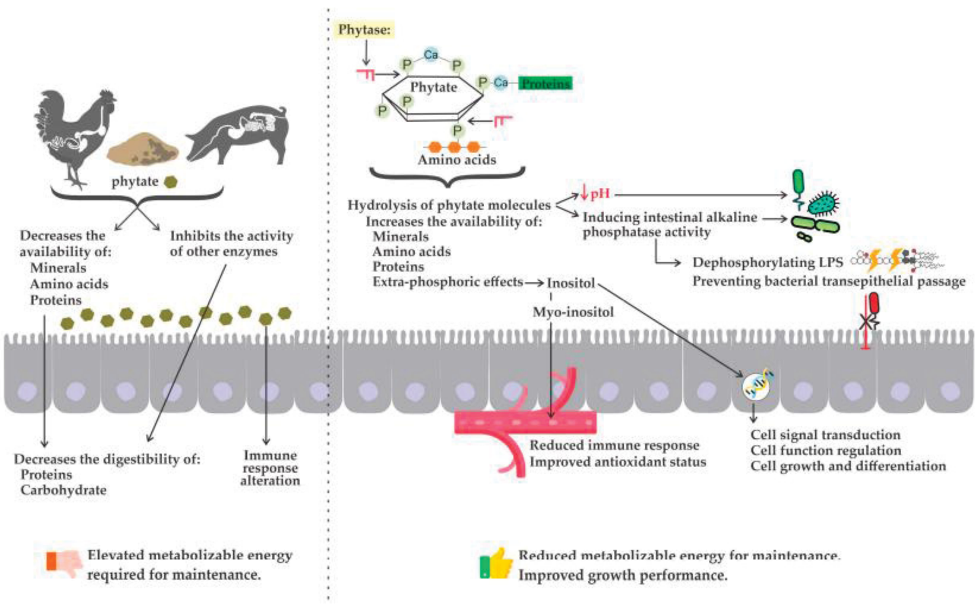

FIGURE 3. Harmful effects of a phytate molecule and the beneficial effects of phytase supplementation in monogastric diets (VALENTE JUNIOR et al., 2024).

3. PHYTASE IN BROILER BREEDER AND LAYING HEN DIETS

Exogenous PHY enzymes have been included in laying hens and broiler breeders' diets over the past years. PHY has shown beneficial effects by enhancing the bioavailability of nutrients, improving digestibility, and helping to eliminate several anti-nutritional factors, leading to better broiler breeder performances (BERRY et al., 2003; BHANJA et al., 2005; NUSAIRAT et al., 2018). Furthermore, improving the digestibility of feed ingredients increases the capacity for the transfer of nutrients from the hen's diet to the egg (GRANGHELLI et al., 2023). The enhanced nutrient availability through the degradation of PP to myo-inositol can also be linked with the extra phosphoric effects of the PHY supplementation (BEESON et al., 2017). Myo-inositol plays a vital role in many physiological processes, including lipid transport and the functioning of coenzyme Q_{10} (GRANGHELLI et al., 2023). Coenzyme Q_{10}, a crucial antioxidant, aids mitochondrial functions, which not only improves energy metabolism but also facilitates nutrient transfer to the embryo through the egg yolk (HUBER et al., 2016). Studies have shown that high doses of dietary PHY increase free myo-inositol concentrations in the gizzard, leading to improved weight gain and feed conversion in broilers (WALK et al., 2014).

Numerous reviews and meta-analyses have reported the positive effects of PHY supplementation on laying hen performance and nutrient digestibility (AHMADI AND RODEHUTSCORD, 2012; BOUGOUIN et al., 2014; RODEHUTSCORD et al., 2023). Ahmadi and Rodehutscord (2012) conducted a meta-analysis that included 14 feeding trials with laying hens aged 36 to 76 weeks, fed corn-soybean meal-based diets. They observed that in diets supplemented with 300 FTU/kg, the optimum level of non-phytate phosphorus (NPP) for optimizing egg production, egg mass, and feed conversion ratio (FCR) was reduced from 2.20 to 1.50 g/kg of diet, 2.20 to 1.40 g/kg of diet, and 2.20 to 1.50 g/kg of diet, respectively, when compared to diets not supplemented with PHY. Additionally, the meta-analysis by Bougouin et al. (2014) showed that layers receiving exogenous PHY at 371 FTU/kg were associated with a 5.02 percentage unit increase in P retention.

4. PHYTASE IN BROILER DIETS

PHY is widely used in the feeding of broilers to mitigate the detrimental effects of PP on nutrient utilization and performance (BASSI, 2024). Improvements in growth performance and bone mineralization of broiler chickens resulting from exogenous PHY supplementation have been described (SELLE AND RAVINDRAN, 2007; DERSJANT-LI et al., 2014; SELLE et al., 2023). Positive effects of PHY supplementation have also been observed in key parameters such as increased bone ash and bone resistance, stemming from greater availability and deposition of calcium Ca and P (LEE et al., 2017); enhanced the bone quality (BRADBURY et al., 2018; KIM et al., 2017); improved performance (BONEY AND MORITZ, 2017; SENS et al., 2021); and increased carcass yield (KRISELDI et al., 2021). Additionally, PHY was shown to be more effective in improving growth performance and tibia ash compared to increasing NPP levels (BASSI, 2024). This is due to the higher digestibility of amino acids and Na enabled by PHY, in addition to the improved P digestibility (DERSJANT-LI AND KWAKERNAAK, 2019).

In recent years, there has been growing interest in the so-called extra-phosphoric effect, which may result from supplementing higher doses of PHY in the broiler diet, beyond the typical industry standards maximum of 2,500 units/kg (COWIESON et al, 2011; BONEY AND MORITZ, 2017). This concept focuses on targeting as many PP molecules in the gastrointestinal tract as possible to enhance the breakdown of phytic acid into lower esters. This process promotes the release of nutrients beyond P, prevents the complexation of PP salts, and accelerates the elimination of PP from the tract (WALK et al., 2013). Ultimately, this process generates myo-inositol, which is absorbed in the gastrointestinal tract and contributes to various processes such as cell metabolism, gluconeogenesis, regulation of glucose transport, and protein synthesis (COWIESON et al., 2014; HUBER et al., 2015; LEE AND BEDFORD, 2016; BELLO et al., 2019).

Although the effects of PHY supplementation in poultry diets have been well recognized and extensively described since its introduction through numerous studies and reviews (RAVINDRAN et al., 1995; ANGEL et al., 2002; COWIESON et al., 2006; SELLE et al., 2007; SELLE et al., 2012; LEI et al., 2013; WOYENGO et al., 2013; DERSJANT-LI et al., 2014; MOSS et al., 2018; SELLE et al., 2023), several studies have reported significant variations in the impact of added PHY on performance and nutrient digestibility in broilers and laying hens (KEBREAB et al., 2012; BOUGOUIN et al., 2014; NUAMAH et al., 2024).

5. META-ANALYSIS

A valuable approach for analyzing the extensive and heterogeneous body of published information on PHY supplementation in poultry diets, particularly the variation in results across studies, involves developing databases and applying statistical techniques to enable a quantitative evaluation of PHY effects. Such evaluations can be effectively conducted through systematic literature reviews and meta-analyses. Positioned at the top of the evidence hierarchy, systematic reviews and meta-analyses typically provide the highest level of evidence among all study designs (MYUNG, 2023) (Figure 4).

FIGURE 4. LEVELS OF EVIDENCE PYRAMID AMONG DIFFERENT STUDY DESIGNS.

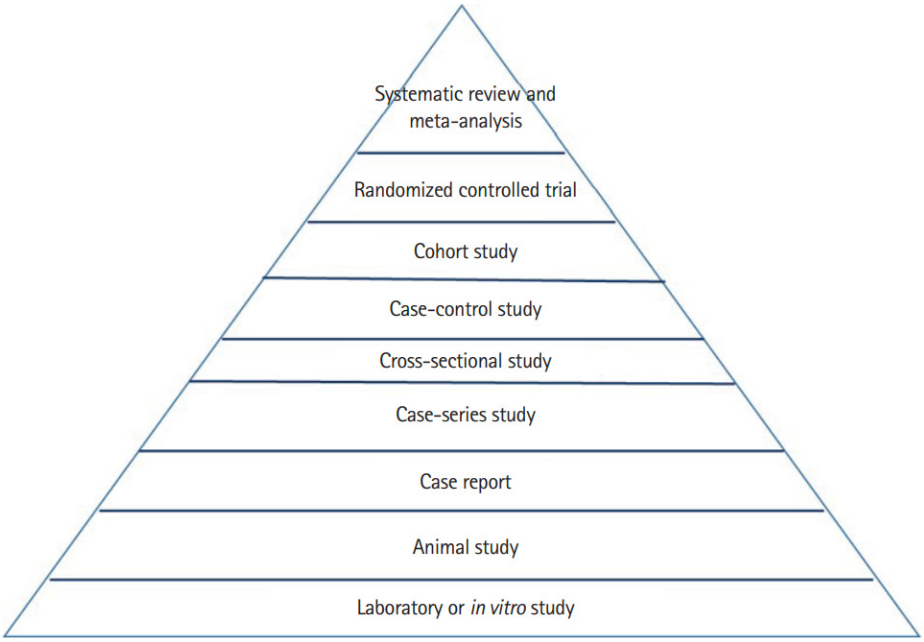

Figure 4. Levels of evidence pyramid among different study designs (Adapted from MURAD et al., 2016; MYUNG, 2023).

A systematic review is a structured evaluation of a clearly formulated research question, employing systematic and explicit methods to identify, select, and critically appraise relevant studies, as well as to collect and analyze data from the included research. Statistical methods, such as meta-analysis, may be used to analyze and synthesize the findings of the selected studies (HIGGINS AND GREEN, 2006; MYUNG, 2023). Furthermore, it addresses a specific, narrowly defined question using explicit search strategies, predefined selection criteria, and structured data extraction and appraisal, with or without the application of quantitative methods like meta-analysis (MYUNG, 2023).

Meta-analysis has gained significance as an effective approach for analyzing complex phenomena, as it employs quantitative methods to aggregate data from multiple independent studies, leading to more robust and dependable conclusions (ST-PIERRE, 2007). This method allows for the transformation of research results into practical applications (POLYCARPO et al., 2017). By addressing the variability between studies, it provides a more extensive and thorough understanding of the subject, overcoming the limitations inherent in individual experiments, which are often constrained by specific experimental conditions (LOVATTO et al., 2007). Meta-analysis also has the power to reduce biases and inaccuracies in publications and to expand the a priori validity domain of the model (SAUVANT et al., 2019). Additionally, they can help to increase knowledge by relating different variables and the effects of PHY on poultry performance and nutrient digestibility, improving prediction models such as the optimum NPP level for laying hens diets (AHMADI AND RODEHUTSCORD, 2012) and broiler diets (LÉTOURNEAU-MONTMINY et al., 2010; KERMANI et al. 2023), as well, quantifying the effect of PHY in the P utilization in poultry (BOUGOUIN et al., 2014). Meta-analysis tool has become a widely used alternative in animal production in recent years, with an annual exponential growth of 15% in the number of publications, mainly due to its statistical power, lower risks and ethical implications, excellent cost-benefit ratio, and practicality (SAUVANT et al., 2019). The main advantage of these tools is their ability to evaluate and measure the different effects of already published data, integrating the results of different studies, extracting information, and creating models that allow a better understanding and prediction of the topic (LOVATTO et al., 2007; BOUGOUIN et al., 2014; SAUVANT et al., 2019).

In a meta-analysis, two basic types of models are used: fixed-effect models and random-effects models (RODRIGUES AND ZIEGELMANN, 2010). The fixed-effect model assumes that the effect of interest is the same in all studies and that the observed differences between them are due only to sampling errors (variability within studies). This model is useful when homogeneity is assumed among the studies included in the meta-analysis (MAZIN AND MARTINEZ, 2009; RODRIGUES AND ZIEGELMANN, 2010). Random-effects models created from meta-analyses allow estimating the heterogeneity of effects and provide an opportunity to explore the factors that explain this heterogeneity (BOUGOUIN et al., 2014), as well as the magnitude and direction of effects (SAUVANT et al., 2008). Additionally, the random-effects model assumes that the effect of interest is not the same across all studies. This model considers that the studies included in the meta-analysis form a random sample from a hypothetical population of studies. Thus, although the effects from the studies are not considered equal, they are connected through a probability distribution, presumably normal. The model incorporates a measure of variability in the effects between the different studies (RODRIGUES AND ZIEGELMANN, 2010). This model is used when the studies are heterogeneous (MAZIN AND MARTINEZ, 2009). When the variability between studies is not purely random, we say that the studies are heterogeneous, and the random-effects model is recommended (RODRIGUES AND ZIEGELMANN, 2010). Although this model may be preferable due to its greater scope, some precautions must be taken when using it. If the number of studies is very small, the estimation of the variance between studies will not have good precision (RODRIGUES AND ZIEGELMANN, 2010). Some test such as are Cochran's Q test or Higgins and Thompson's I^2 statistic can be used to check for the existence of heterogeneity between studies (HIGGINS et al., 2003; RODRIGUES AND ZIEGELMANN, 2010).

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CHAPTER II - IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE AND EGG QUALITY TRAITS IN BROILER BREEDERS: A META-ANALYSIS

ABSTRACT

This meta-analysis aimed to determine the effect of phytase (PHY) supplementation on performance and egg quality traits in broiler breeders. A systematic review across multiple databases identified 2,162 studies, of which seven met all eligibility criteria and were included in the analysis. The meta-analysis compared egg production (EP), hatchability, egg weight (EW), and the yolk, albumen, and shell weight ratios relative to EW between diets supplemented with or without PHY. Heterogeneity and publication bias were also assessed. Diets containing PHY increased EP by 4.85% (P<0.01) and yolk ratio by 0.24% (P=0.03), while EW decreased by 0.53 g (P<0.01) and albumen ratio by 0.34% (P<0.01). No significant effects were observed on hatchability or shell ratio (P≥0.05). Meta-regression and subgroup analyses revealed that diets with phytate phosphorus (PP) levels of 2.5 g/kg or higher and calcium below requirements enhanced the effect of PHY on EP but reduced EW. Additionally, diets with vitamin D levels below 3,000 IU/kg and non-phytate phosphorus levels below requirements negatively impacted EW. PHY treatment lasting longer than 10 weeks and diets with vitamin D levels below 3,000 IU/kg resulted in significant increases in yolk ratio. PHY supplementation affected shell ratio only in diets containing PP levels below 2.5 g/kg. An economic model based on these meta-analysis results indicated that PHY supplementation could reduce costs by \$0.011 or 3.10% per egg produced. PHY supplementation enhances EP and modifies egg quality traits by increasing yolk content while reducing both EW and albumen. The positive economic impact of PHY suggests that its inclusion in broiler breeder diets is beneficial and should be considered in most circumstances.

Keywords: Poultry, phytase, meta-analysis, phytate, meta-regression, egg quality

1. INTRODUCTION

Phosphorus (P) is one of the most important minerals in animal nutrition given its major roles in many body functions, such as the maintenance of growth performance. Along with calcium (Ca), P is extremely important for mineral deposition in the skeleton and subsequent bone mineralization (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014). However, almost two-thirds of the total seed P is complexed with myo-inositol to form phytic acid and its salts, drastically reducing its bioavailability to animals (BOUGOUIN et al., 2014; ABBASI et al., 2019). Consequently, a considerable amount of P in poultry diets is provided in the form of phytate P (PP), with limited availability (SELLE et al., 2023).

In addition to the negligible availability of non-phytate P (NPP) in cereal grains, phytase (PHY), a digestive enzyme catalyzing the release of P from the PP complex, cannot be synthesized at sufficient levels in the poultry intestine (APPLEGATE et al., 2003). Therefore, other inorganic P sources need to be added to poultry diets to meet daily nutritional requirements (HERVO et al. 2023). However, inorganic P sources are both expensive and non-renewable (BOUGOUIN et al., 2014). Beyond the increased feed costs, excess P in poultry diets can be excreted in manure, potentially leading to environmental issues such as surface water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023). Supplementing poultry diets with exogenous PHY enzymes significantly increases PP utilization. Since its introduction in 1991, PHY has become one of the most widely used exogenous enzymes in poultry nutrition (SELLE et al., 2023).

Several studies have reported the effects of PHY supplementation in broilers, broiler breeders, and laying hens; however, a wide discrepancy in the effect size of PHY on performance and P digestibility has been reported (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014). Understanding the effect size of exogenous PHY on performance and P utilization is crucial for determining the appropriate amount of inorganic P needed to meet nutritional requirements, thereby optimizing both sustainability and economic outcomes (BOUGOUIN et al., 2014).

Meta-analyses have become a valuable research approach due to their ability to quantitatively combine data from previous independent studies, thereby providing more robust and reliable inferences (ST-PIERRE, 2007). Another justification for the use of meta-analyses is their capacity to translate study outcomes into practical and applicable knowledge. Unlike single experiments, which are limited by their specific conditions, meta-analytic methods systematically account for heterogeneity among studies, offering a broader and more comprehensive understanding of the topic (LOVATTO et al., 2007).

Numerous systematic reviews and meta-analyses have studied the effects of PHY on performance and P digestibility in layers and broilers (LÉTOURNEAU-MONTMINY et al., 2010; AHMADI AND RODEHUTSCORD, 2012; BOUGOUIN et al., 2014; FARIDI et al., 2015; COWIESON et al., 2017; KERMANI et al., 2023). However, there is limited information on the effects of PHY supplementation on production parameters and egg quality traits in broiler breeders. To the best of our knowledge, no other meta-analysis has specifically evaluated the effects of PHY supplementation on broiler breeders. Additionally, many fundamental questions regarding PP and PHY in this category of animals remain unanswered (SELLE et al., 2023).

Therefore, this study aimed to estimate the effect size of PHY supplementation on production parameters and egg quality traits in broiler breeders through a systematic review and meta-analysis. Furthermore, based on the results of the meta-analysis, a mathematical model was developed to predict the economic impact of PHY supplementation in various scenarios.

2. MATERIAL AND METHODS

This study relied solely on data obtained from the published literature and did not involve the use of animals; thus, approval from the Institutional Animal Care and Use Committee was not required.

2.1. SEARCH AND DATA FILTERING

An extensive online search for studies on PHY performance and P digestibility in broiler breeders was conducted until July 2024. The search focused on scientific articles published in peer-reviewed journals and utilized various online databases, including PubMed, Web of Science, and Scopus. References from the identified publications were also reviewed to identify any additional relevant articles.

The research question was framed using the “PICo” strategy (ANDRETTA et al., 2021), identifying the “Population” as “broiler breeders”, the “Interest” as “phytase”, and the “Context” as “performance” OR “phosphorus retention”. Synonyms and alternative terms for population and context were included to refine the final search strategy.

The resulting database of PHY studies was then exported to reference management software (EndNote X9; Philadelphia, PA, USA), where duplicate references were identified and removed. The relevance and quality of the studies were critically evaluated by initially reviewing titles and abstracts, followed by an in-depth review of the full texts.

The main criteria for selecting publications were: (1) studies describing *in vivo* experiments with commercial broiler breeders; (2) evaluations of flock performance and egg traits using diets supplemented with PHY and non-supplemented control diets; and (3) studies reporting daily egg production (EP), egg weight (EW), or the ratios of yolk, albumen, and shell weight to EW, along with measures of sample variance, such as standard deviation (SD), standard error (SE), and sample size (n). Phosphorus (P) retention data were only available in one study (Bhanja et al., 2005) and were therefore not included as a variable for further analysis. Preselected manuscripts were excluded if they involved a sanitary challenge, combined PHY with another enzyme, lacked a control treatment, or exhibited significant inconsistencies in the statistical design, methodology, or outcome data.

The screening process leading to the final database is shown in Figure 1. Initially, 2,162 references were evaluated, with successive exclusions made based on titles and abstracts. Full-text reviews were conducted on 23 references to assess their eligibility. After detailed analysis, 16 references were excluded based on the previously established criteria. The final list of the seven selected studies is presented in Table 1.

2.2. DATA SYSTEMATIZATION AND CODING

The methodology used for database construction and data encoding was based on established literature (LOVATTO et al., 2007; BOUGOUIN et al., 2014). However, some studies did not present their full dietary nutrient composition. To address this, the nutrient composition of all diets was recalculated using the Practical Program for Formulation of Rations (PPFR; GARCIA-NETO, 2008) based on the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024). This approach ensured a comprehensive and consistent dietary composition across studies. The variability in the diet composition of the dataset is presented in Table 2.

An electronic spreadsheet database was created to record the number of participants (replications per treatment and birds per replication), means, and their corresponding SD for both the control and PHY treatment groups. These data were then used to calculate the weighted mean difference (WMD), assess heterogeneity, and evaluate publication bias for each dependent variable.

2.3. DATABASE DESCRIPTION AND DATA ANALYSIS

Six separate WMD analyses were performed to summarize the effect size of PHY supplementation on each dependent variable (EP, hatchability, EW, and the ratios of yolk, shell, and albumen weight to EW). In cases where more than one concentration of PHY or level of dietary NPP was tested in the same study, each treatment was considered as one observation in the meta-analysis.

2.4. STATISTICAL ANALYSIS

2.4.1. EFFECT SIZE FOR EACH DEPENDENT VARIABLE

The weighted mean difference (WMD) with a 95% confidence interval (CI) was used to estimate the effect size of PHY supplementation on each dependent variable. The pooled effect estimate represents a weighted average of all included study group comparisons, with the weight assigned to each result being inversely proportional to its variance (FENG et al., 2021). This approach gives greater weight to larger trials and lesser weight to smaller trials (COOPER AND HEDGES, 1994). The normal distribution of the studentized residuals of the WMDs was assessed using the Shapiro-Wilk test.

2.4.2. HETEROGENEITY AND PUBLICATION BIAS

Heterogeneity and variation across studies were assessed using the significance levels of the chi-squared statistic associated with the I^2 statistic (BORENSTEIN et al., 2009). As described by VIEIRA et al. (2017), heterogeneity was considered significant at $P<0.10$, because the chi-square test shows a relatively low power to detect heterogeneity among a small number of trials. The I^2 statistic was calculated to describe the percentage of total variation between studies owing to heterogeneity rather than chance (SALAMI et al., 2022). Regardless of the chi-squared and I^2 statistics' results, heterogeneity was accounted for in the meta-analysis by applying random-effects models to estimate the overall effects of PHY supplementation on the selected variables and their statistical significance (VIEIRA et al. 2017).

Although significant heterogeneity across studies was not observed for all variables ($P>0.10$), subgroups of categorical study characteristics were created to evaluate their potential impact on the meta-analysis. These subgroups were categorized based on treatment duration (less than or greater than or equal to 10 weeks), dietary PHY concentrations (less than or greater than or equal to 500 FTU/kg), PP (less than or greater than or equal to 2.5 g/kg), Vitamin D (less than or greater than or equal to 3,000 UI/kg), Ca:NPP ratio (less than or greater than or equal to 10.5), and NPP and Ca requirements based on ROSTAGNO et al. (2024) (less than or greater than or equal to 100%).

Meta-regression was selected as a screening test to evaluate whether there was a statistically significant relationship ($P<0.05$) between the categorical subgroups and the explanatory variables. When statistical significance was found, further subgroup analyses were performed to provide a better understanding of the model and the potential impact of each subgroup on the meta-analysis outcome. Meta-regression was not applied to the hatchability variable due to the limited number of comparisons (fewer than ten), which is generally considered insufficient for reliable meta-regression analysis.

The treatment period was identified as a potential source of variation because mild P feed deficiency may not affect performance traits over several months, as P can be mobilized from the skeleton (RODEHUTSCORD et al., 2023). The median PHY treatment period across all studies included in the meta-analysis dataset was 10 weeks, thus subgroups were defined as less than 10 weeks or greater than or equal to 10 weeks. PHY dose subgroups were defined because feed is typically enriched with 250–500 FTU/kg; however, using more than 500 FTU/kg can enhance the benefits of PHY supplementation (JOUDAKI et al., 2023). The PP content in diets depends on the levels of PP in the feedstuffs used, but practical poultry diets generally contain approximately 2.5 g/kg PP (MOSS et al., 2018; SELLE et al., 2023). The PP dietary subgroups were categorized according to the approach described by VIEIRA et al. (2017). Vitamin D subgroups were based on levels being below or above 3,000 IU/kg, as ATENCIO et al. (2006) and the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) suggest that the vitamin D requirement for broiler breeders is around 3,000 IU/kg of feed. Subgroups for the Ca:NPP ratio, non-phytate phosphorus (NPP), and calcium (Ca) requirements were also defined in accordance with the recommendations provided by ROSTAGNO et al. (2024).

Publication bias in the meta-analysis for all dependent variables was examined both graphically using funnel plots and statistically using Egger's test (VIEIRA et al., 2017). Publication bias assessed with Egger's test was considered significant when *P*<0.05 (SALAMI et al., 2022). WMD, heterogeneity and publication bias analyses were performed using the meta-package (version 6.5-0) in R (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria).

2.5. ECONOMIC IMPACT

The results from significant WMD parameters (*P*<0.05) were used to build an economic model that estimated the effects of PHY supplementation on the total egg (TE) production costs of broiler breeders. The simulation considered the target TE produced by each broiler breeder at 64 weeks of age, following the information on the management guides of the breeds included in the meta-analysis database (COBB-VANTRESS, 2020; AVIAGEN, 2021a; AVIAGEN, 2021b). Additionally, the model incorporated a PHY dose of 500 FTU and the average TE production costs provided by the local poultry agroindustry.

Performance data from animals supplemented with PHY and their control groups were used in the equations, with the differences between results were used to estimate the economic impact. The performance data and costs associated with the TE production model are presented in Table 3.

Equations for economic impact simulation:

(1) TE produced per broiler breeder housed at 64 weeks of age with PHY supplementation (units): $TE_{PHY+} = \alpha + (\frac{\alpha \text{ x } \beta}{100})$, where α: TE produced per broiler breeder housed at 64 weeks of age based on the mean numbers of the breeder management guides, and β: performance gain in TE production from supplemented PHY diets as obtained from the meta-analysis.

(2) Feed conversion ratio per egg (g/unit): $FCR_{PHY-} = \frac{\gamma \text{ x } 1000}{\alpha}$, if treatment without PHY, or $FCR_{PHY+} = \frac{\gamma \text{ x } 1000}{TE\ PHY+}$, if treatments with PHY, where γ: Cumulative feed consumption from day one to 64 weeks of age (kg); α: TE produced per broiler breeder housed at 64 weeks of age (units) based on mean numbers of the breeder management guides and TE_{PHY+}: TE produced per broiler breeder housed at 64 weeks of age with PHY supplementation (units).

(3) Cost of feed ($/kg): $CF_{PHY-} = \frac{\Delta}{1000}$, if treatment without PHY, or $CF_{PHY+} = \frac{\Delta + \varepsilon}{1000}$, if treatments with PHY, where: Δ: Feed cost without PHY ($/ton) and ε is the PHY cost (500 FTU/ton) ($/ton).

(4) Feeding cost per egg ($/egg): $FCE_{PHY-} = CF_{PHY-} \times (FCR_{PHY-} / 1000)$ in the absence of PHY or $FCE_{PHY+} = CF_{PHY+} \times (FCR_{PHY+} / 1000)$ in the presence of PHY.

(5) Other costs per egg (excluding cost of feed) ($/egg): $OC_{PHY-} = \frac{(\zeta \times \alpha)/}{\alpha}$, if treatment without PHY, or $OC_{PHY+} = \frac{(\zeta \times \alpha)/}{TE\ PHY+}$, if treatment with PHY, where ζ: Other costs per Egg (excluded cost of feed) ($/egg).

(6) Total cost per egg ($/egg): $TCE_{PHY-} = FCE_{PHY-} + OC_{PHY-}$, if treatment without PHY, or $TCE_{PHY+} = FCE_{PHY+} + OC_{PHY+}$, if treatment with PHY.

(7) Total eggs produced per 1,000,000 broiler breeders between 1 day old to 64 weeks of age (units): EY_{PHY-}: $\eta \times \alpha$, if treatment without PHY, or $EY_{PHY+} = \eta \times A$, if treatment with PHY, where η: 1,000,000 population size of broiler breeders between 1 day old to 64 weeks of age.

A sensitivity analysis was conducted using the key variables "EP" and "PHY price" to assess how variations in these factors could influence the economic outcomes and profitability of the production system (SALTELLI et al., 2000). To identify scenarios in which PHY supplementation would have a positive economic impact, the range of EP differences between PHY+ and PHY− treatments was adjusted from 0% to 5%, and the price of PHY was modified, ranging from half its current cost to up to five times higher. PHY supplementation was deemed economically viable when the economic impact was greater than zero, denoted in the table by the term “USE”. PHY supplementation should be avoided when the impact value was equal to or less than zero, indicated by the term “AVOID”. The feed cost ($/kg) for the PHY+ group was calculated by adding the cost of PHY (500 FTU/ton) to the base diet cost. Since the primary objective of this analysis was to evaluate the economic impact of PHY on broiler breeder production parameters, the potential economic benefits of replacing inorganic P in the diet were not considered.

3. RESULTS

3.1. DATABASE CHARACTERIZATION

The seven studies included in this meta-analysis comprised a total population of 2,204 broiler breeders, aged between 25 to 72 weeks. The most common breed found was Ross 708 (n=3), followed by Cobb-Vantress 500 (n=2) and Ross 308 (n=1); one study did not mention the poultry breed. All studies utilized a corn-soybean meal-based diet. The phytase types varied among the studies, with one study using 3-phytase (EC 3.1.3.8) and the others using 6-phytase (EC 3.1.3.26). Additionally, five of the PHYs were isolated from bacteria, and two were isolated from fungi. Phytase (PHY) inclusion levels also varied between studies, ranging from 250 to 1200 FTU/kg.

3.2. META-ANALYSIS RESULTS

There were no observed outliers and the studentized residuals of the WMDs fit a normal distribution for all variables. The data used to estimate the overall effects of PHY supplementation on the selected variables were synthesized and presented in forest plots (Figures 2-7), with individual studies referenced by author and year of publication.

PHY supplementation was found to increase egg production by 4.85% (P<0.01, Figure 2, Table 4) while decreasing egg weight by 0.53 g (P<0.01, Figure 4, Table 6). It also enhanced yolk ratio by 0.24% (P=0.03, Figure 5, Table 7) and reduced albumen ratio by 0.34% (P<0.01, Figure 7, Table 8). Hatchability (P=0.85, Figure 3, Table 2) and shell ratio (P=0.72, Figure 6, Table 9) were not affected by PHY supplementation.

Significant heterogeneity among studies was observed only for yolk ratio (P=0.02, I^2=47%) and shell ratio (P<0.01, I^2=56%), indicating that the overall results for these variables may be influenced by one or more qualitative moderators used to categorize the dataset. To address this heterogeneity, meta-regression and subgroup analysis were conducted (Tables 4-9). Meta-regression screening analysis showed that the PHY treatment period (P≤0.01) and vitamin D levels (P≤0.01) were qualitative moderators that significantly influenced the overall results of PHY supplementation on yolk ratio. Consequently, subgroup analysis demonstrated that a minimum treatment period of 10 weeks was required to detect a significant effect of PHY on yolk ratio (WMD=0.38%, P<0.01). Additionally, the effect of PHY on yolk ratio was observed exclusively in diets where vitamin D levels were below 3,000 IU/kg (WMD=0.39%, $P<0.01$). Meta-regression results further showed that the dietary concentration of PP was the only source of variation that significantly influenced the overall results for shell ratio ($P=0.02$). Subgroup analysis indicated that only diets containing PP at concentrations below 2.5 g/kg showed a significant decrease in shell ratio when supplemented with PHY (WMD=-0.29%, $P=0.05$).

Although heterogeneity among studies was not significant for the remaining variables, a more detailed meta-regression and subgroup analysis were conducted to explore potential sources of variation. Even though the overall pooled effect was consistent, diets containing PP ($P=0.03$) and Ca requirements ($P\leq0.01$) at different concentrations were shown to enhance the effectiveness of PHY on egg production. PHY supplementation had a greater effect on egg production diets containing PP concentrations greater than or equal to 2.5 g/kg (WMD=5.59%, $P<0.01$) compared to diets with PP concentrations below than 2.5 g/kg (WMD=3.57%, $P<0.01$). Additionally, the effect of PHY was higher in diets with Ca requirements based on Rostagno et al. (2024) below 100% (WMD=9.07%, $P<0.01$) than in diets containing Ca requirements exceeding 100% (WMD=3.22%, $P<0.01$). Meta-regression analysis also demonstrated that diets containing PP ($P=0.02$), vitamin D ($P=0.04$), NPP requirements ($P=0.05$) and Ca requirements ($P=0.02$) were significant sources of variation affecting the impact of PHY on EW. In line with the overall pooled effect, subgroup analysis showed that PHY supplementation negatively affect EW in diets with PP concentrations greater than or equal to 2.5 g/kg (WMD=-0.74g, $P<0.01$); vitamin D concentrations below 3,000 IU/kg (WMD=-0.78g, $P<0.01$); and NPP and Ca requirements below 100% (WMD=-0.68g, $P<0.01$ and WMD=-1.85g, $P<0.01$, respectively). No significant effects of the qualitative moderators were found on PHY supplementation for hatchability and albumen ratio.

Publication bias was evaluated both graphically, using funnel plots, and statistically, via Egger's test. Despite some natural inconsistencies due to the limited number of comparisons, particularly for hatchability (n=7), no significant publication bias was detected across the variables ($P>0.05$). The funnel plots and Egger's test results are presented in Figure 8.

EP, EW, and yolk and albumen ratios were significantly affected by PHY supplementation ($P<0.05$). However, only EP was incorporated into the PHY economic model, as the other variables, while potentially enhancing offspring performance, did not directly impact TE production costs. PHY supplementation in the diet of broiler breeders resulted in increased EP and a reduction in TE production costs by $0.011 or 3.10% per egg, leading to a total increase of 5,900,000 eggs per 1 million broiler breeders raised from 1 day old to 64 weeks of age (Table 10).

Sensitivity analysis (Table 11) indicated that PHY supplementation would have a positive economic impact in most scenarios, with two exceptions: (1) if the price of PHY increases more than threefold compared to the current price and the difference in egg production between PHY+ and PHY− is no greater than 0.15%, and (2) if there is no variation in egg production due to PHY supplementation. In all other scenarios, PHY supplementation is likely to have a positive economic impact on egg production costs.

4. DISCUSSION

The effects of exogenous PHY supplementation on numerous biochemical pathways related to skeletal integrity and animal performance have been well-documented (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; KERMANI et al., 2023; SELLE et al., 2023). Previous meta-analyses have examined the impact of PHY supplementation on performance and P digestibility in layers and broilers (LÉTOURNEAU-MONTMINY et al., 2010; AHMADI AND RODEHUTSCORD, 2012; BOUGOUIN et al., 2014; FARIDI et al., 2015; COWIESON et al., 2017; VIEIRA et al., 2017; KERMANi et al., 2023). However, to date, no other meta-analysis has specifically reported the effects of PHY supplementation on broiler breeders.

4.1. PERFORMANCE

In contrast to most individual studies, which did not detect significant differences in broiler breeder performance between diets with or without PHY supplementation, the meta-analysis results revealed a positive overall effect of PHY supplementation. On average, PHY increased EP by 4.85% compared to the control group. This effect may be attributed to the larger number of observations included in the pooled analysis which typically narrows the confidence interval of the overall effect and increases the likelihood of detecting statistical significance (VIEIRA et al., 2017).

Although heterogeneity between studies was not significant, further investigation using meta-regression and subgroup analysis revealed that the overall benefit of PHY supplementation on EP was consistent. Notably, these findings suggest that PHY may positively affect EP even in diets that meet or exceed NPP requirements, according to ROSTAGNO et al. (2024). However, the effect of PHY on EP can be enhanced in diets containing PP concentrations greater than or equal to 2.5 g/kg compared to those with PP concentrations below 2.5 g/kg. Diets with higher PP concentrations may improve the effectiveness of PHY, as the release of phytate-P in the digestive tract of poultry is directly correlated with PP concentration in the diet (MOSS et al., 2018). This increased PP concentration potentially provides more substrate for PHY degradation, enhancing its efficiency. Another qualitative moderator observed to enhance the effect of PHY on EP was the Ca concentration in broiler breeder diets. Subgroup analysis showed that diets containing Ca levels below the recommendations of the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) demonstrated an outstanding effect of PHY on EP compared to diets exceeding the Ca requirements. The polyanionic nature of PP molecules allows them to efficiently chelate divalent cations such as Ca, leading to the formation of insoluble mineral-PP complexes. These insoluble Ca-PP complexes are resistant to enzymatic hydrolysis by PHY, reducing its efficacy (ADEKOYA AND ADEOLA, 2023). Additionally, broiler breeder diets have higher Ca concentrations compared to broiler diets, as Ca is crucial not only for bone mineralization but also for eggshell formation in broiler breeders (MANANGI et al., 2009; ROSTAGNO et al., 2024). Therefore, the best strategy observed to maximize the effect of PHY on EP is not to exceed the Ca requirements outlined in the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) for broiler breeder diets.

Exogenous PHY supplementation did not significantly improve hatchability in broiler breeders. Moreover, neither the potential sources of variation examined in the subgroup analysis, nor any individual studies included in the meta-analysis demonstrated a significant effect of PHY supplementation on hatchability.

4.2. EGG QUALITY TRAITS

A negative effect of PHY supplementation was observed in the pooled analysis of studies on EW, leading to a decrease of 0.53 g in EW. Although heterogeneity between studies was not significant for EW, in-depth meta-regression and subgroup analyses revealed that PHY supplementation affected EW only under certain conditions. Specifically, this effect was observed when diets contained PP levels greater than or equal to 2.5 g/kg, vitamin D levels below than 3,000 IU/kg, and NPP and Ca requirements below than those recommended by ROSTAGNO et al. (2024). Remarkably, dietary PP levels below 2.5 g/kg and Ca levels below the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) also enhanced the efficiency of PHY on EP. Given that egg mass is calculated by multiplying EW by EP, this suggests a possible negative correlation between EP and EW, where PHY supplementation improves EP but reduces EW. PHY supplementation showed a significant negative effect on EW only in diets containing NPP levels below those recommended by ROSTAGNO et al. (2024). PHY can catalyze the release of P from the PP complex, significantly increasing the availability of NPP in poultry diets (BOUGOUIN et al., 2014). This ability can be beneficial in diets that fall below NPP recommendations, as it helps meet the NPP requirements, thereby increasing EP, though potentially at the expense of reducing EW. Another important source of variation, vitamin D can enhance PP utilization in poultry diets by increasing Ca absorption from the digesta, which reduces the availability of Ca for forming insoluble Ca-PP complexes and directly enhances phosphate absorption across the lumen (LIEM et al., 2009). Vitamin D levels of 3,000 IU/kg or higher may further improve phosphate absorption across the lumen and its resorption by the kidneys (LIEM et al., 2009), thereby reducing the substrate available for PHY activity. To the best of our knowledge, no study has yet investigated the correlation between PHY supplementation and vitamin D levels in broiler breeders, highlighting the need for further research on this topic.

PHY supplementation increased the yolk ratio by 0.24% and decreased the albumen ratio by 0.34%, indicating a possible inverse relationship between albumen and yolk ratios. However, the shell ratio was not affected by PHY supplementation in broiler breeder diets. Additionally, significant heterogeneity was found in the yolk ratio. Meta-regression and subgroup analysis revealed that the yolk ratio was only influenced by PHY treatment periods lasting 10 weeks or longer. This suggests that P can be mobilized from the skeleton, and that a moderate P deficiency may not adversely affect the yolk ratio over a period of several weeks (RODEHUTSCORD et al., 2023). PHY supplementation in diets with vitamin D levels below 3,000 IU/kg demonstrated an increase in the yolk ratio, suggesting that suboptimal vitamin D levels may enhance PHY efficacy. This is consistent with previous findings for performance and EW, where lower vitamin D levels may not sufficiently improve P absorption and resorption, leaving more available substrate for PHY activity. The chemical form and bioavailability of dietary minerals can also influence changes in the mineral content of egg yolk (RICHARDS, 1997). Phytic acid has the ability to chelate minerals, proteins, and carbohydrates

(DERSJANT-LI et al., 2015), forming complexes that render these nutrients unavailable (KESHAVARZ, 2003). However, PHY catalyzes the release of P from PP, preventing the formation of these complexes in the gastrointestinal tract and allowing the absorption of free nutrients from the diet (MARTINEZ ROJAS et al., 2018). In addition, PHY supplementation can increase NPP levels, which in turn may raise the percentage of yolk, as much of the P from the decomposition of PP is incorporated into the yolk as phosphorylated lipids or proteins (JIANG et al., 2015).

Furthermore, a meta-regression and subgroup meta-analysis were performed to address the significant heterogeneity observed in shell ratio. Contrary to the non-significant overall effect of PHY supplementation, a significant decrease in shell ratio was observed in diets containing PP levels below 2.5 g/kg. This finding contrasts with the results for EP, where PP levels of 2.5 g/kg or higher showed greater PHY efficiency. These contrasting effects highlight the need for further research into the correlation between PHY supplementation and shell ratio at different dietary PP levels. No significant heterogeneity or effects from qualitative moderators were observed in the meta-regression or subgroup analysis for the albumen ratio. Therefore, we recommend that the overall effects from the pooled analyses be considered the primary results for this variable in the meta-analysis.

4.3. PHYTASE ECONOMIC IMPACT

The use of exogenous enzymes in poultry diets has become a well-established practice over the years. These enzymes are added to enhance the digestibility and absorption of nutrients by acting directly on indigestible components, many of which are anti-nutritional factors. Supplementing broiler diets with 500 FTU/kg of PHY can reduce production costs by 0.81% per kilogram of live weight compared to diets without PHY (KRABBE et al., 2024).

The meta-analysis results demonstrated that supplement broiler breeder diet with PHY can reduce the cost per egg produced by $0.011 or 3.10%. Additionally, PHY supplementation can resulted in an increase in total egg production by approximately 5,900,000 eggs per 1 million broiler breeders raised from 1 day old to 64 weeks of age.

5. CONCLUSION

PHY supplementation improves EP in broiler breeders and alters egg quality traits by increasing the yolk ratio while reducing both EW and albumen ratio. Supplementing broiler breeder feed with PHY demonstrates a positive economic impact on EP profitability and should be considered in most circumstances.

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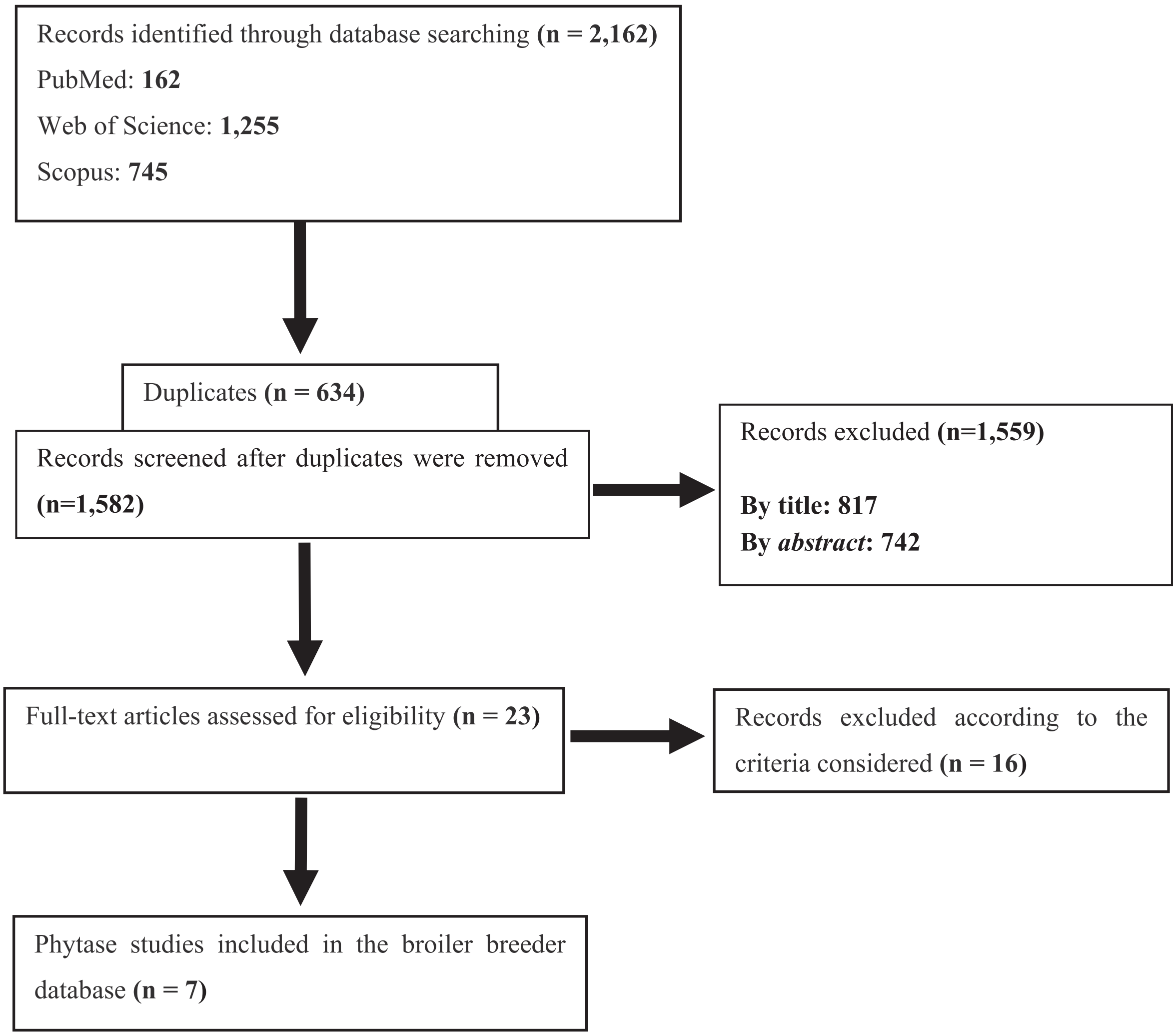

FIGURE 1. Study selection diagram for the meta-analysis focused on phytase supplementation in broiler breeders.

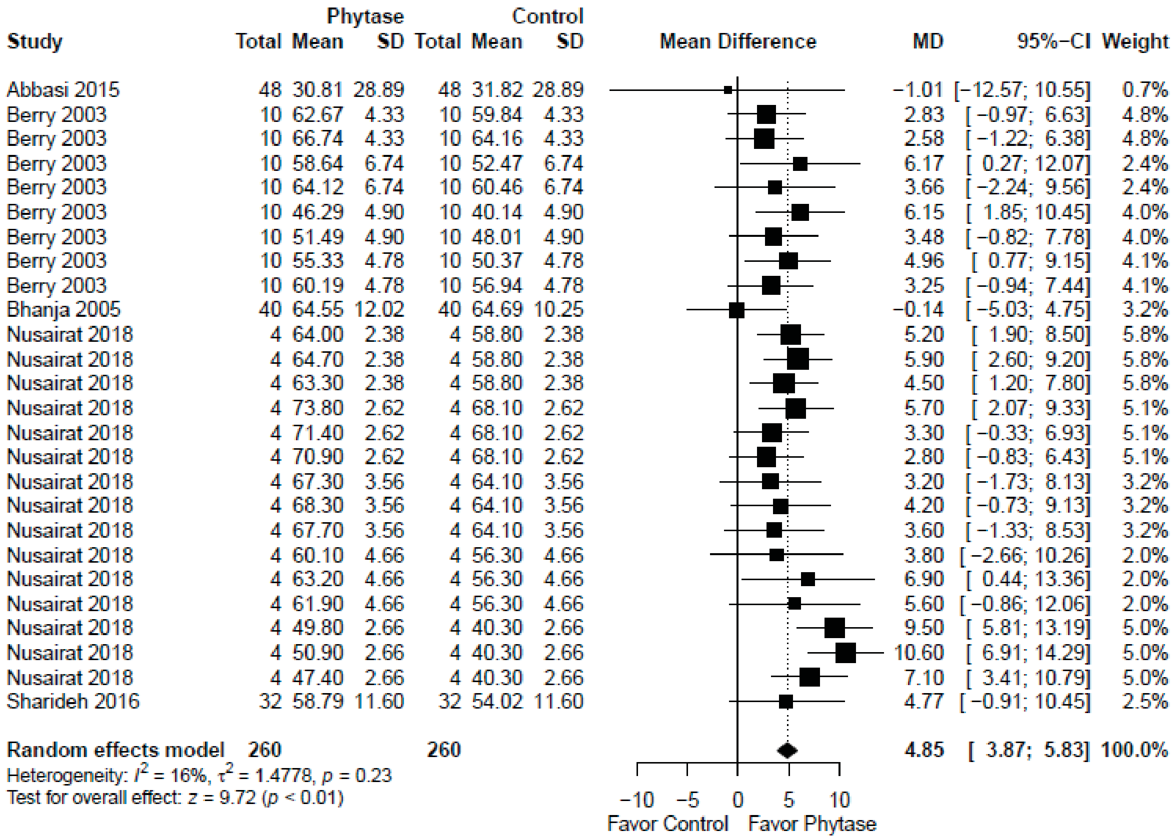

FIGURE 2. Forest plot of the impact of phytase supplementation on egg production in broiler breeders.

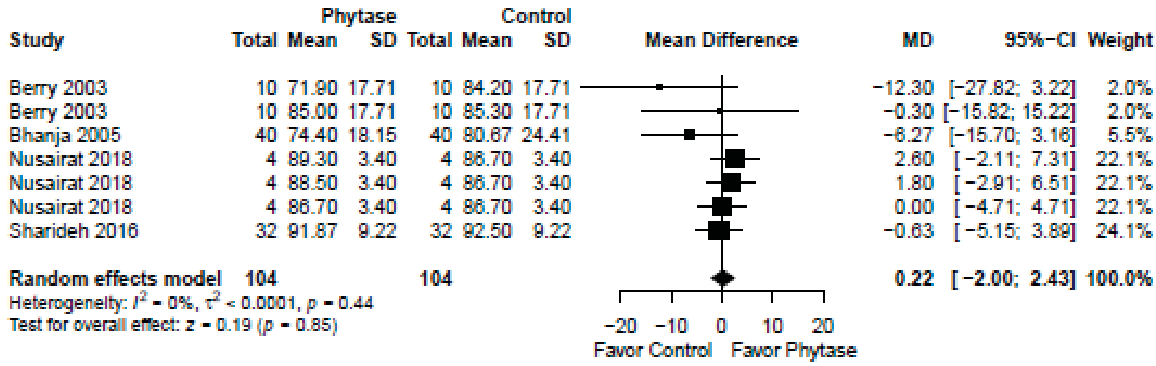

FIGURE 3. Forest plot of the impact of phytase supplementation on hatchability in broiler breeders.

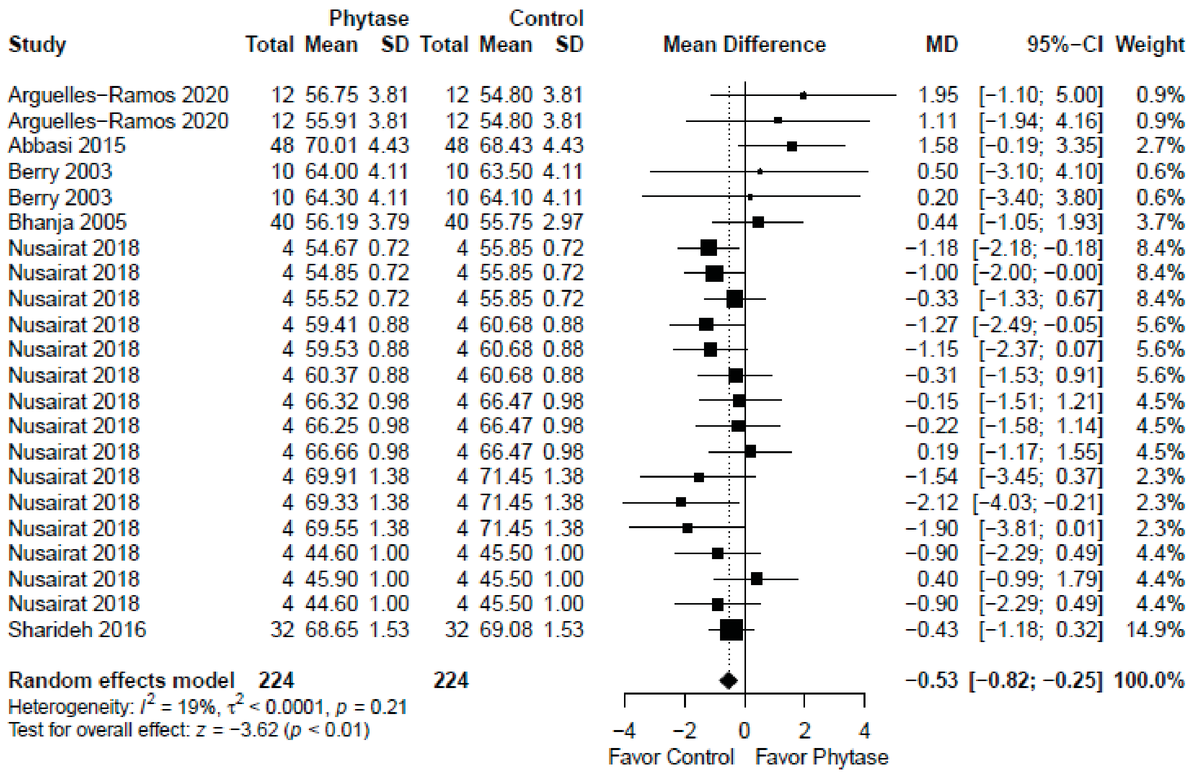

FIGURE 4. Forest plot of the impact of phytase supplementation on egg weight in broiler breeders.

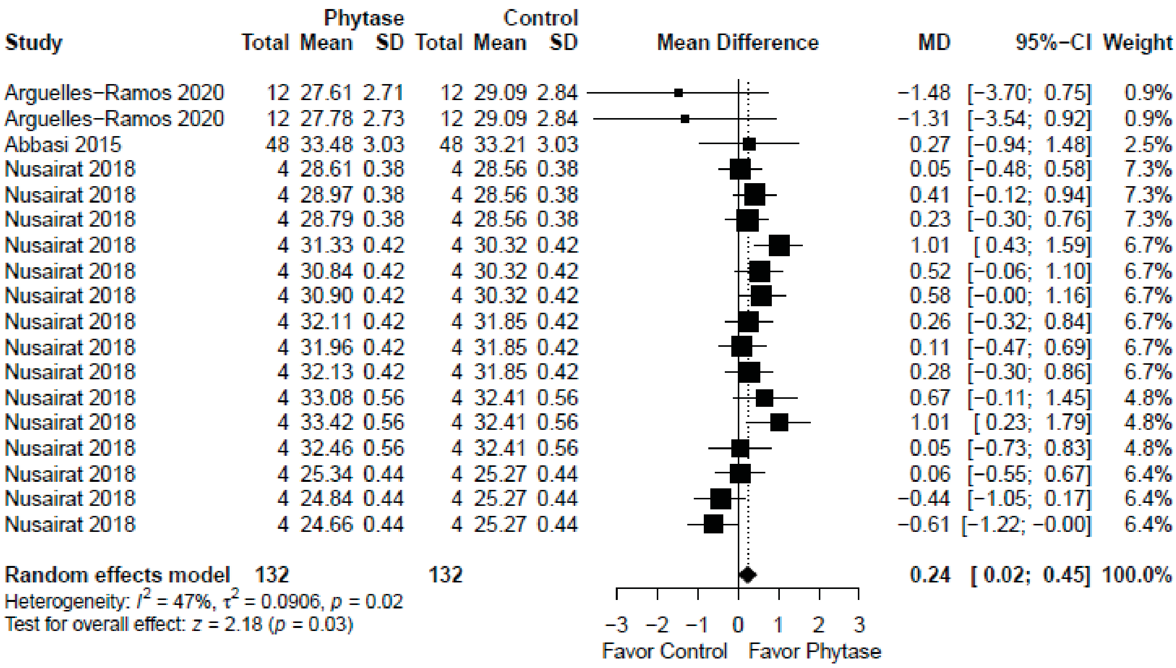

FIGURE 5. Forest plot of the impact of phytase supplementation on yolk ratio (% egg weight) in broiler breeders.

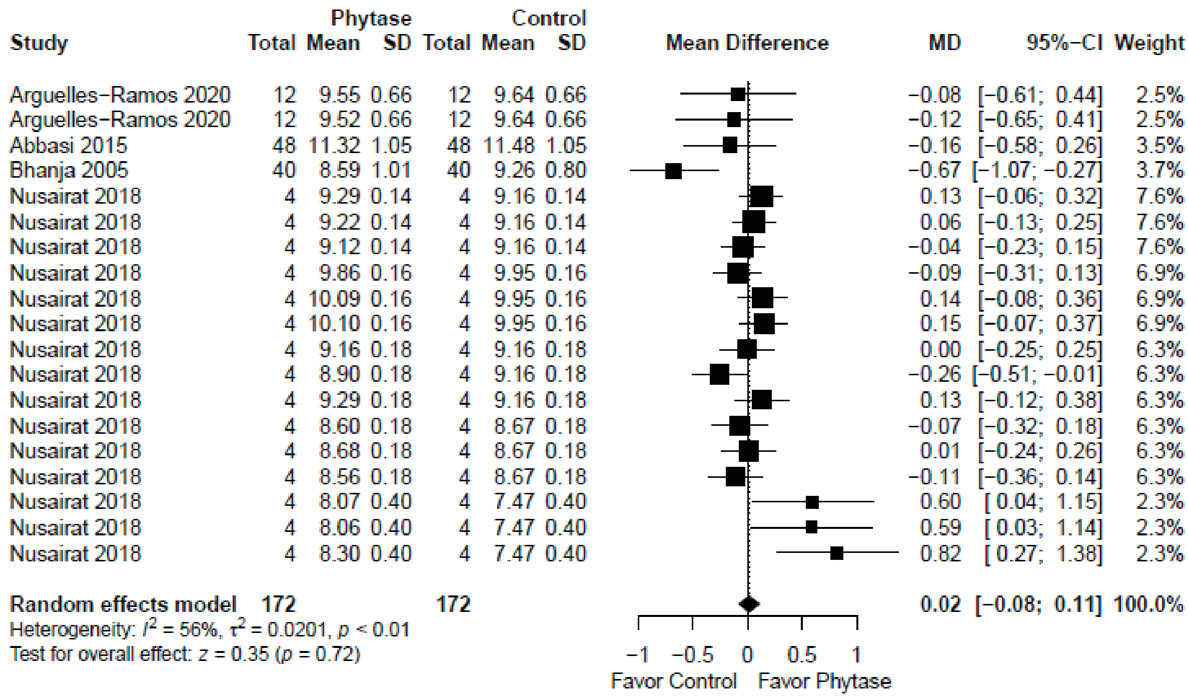

FIGURE 6. Forest plot of the impact of phytase supplementation on shell ratio (% egg weight) in broiler breeders.

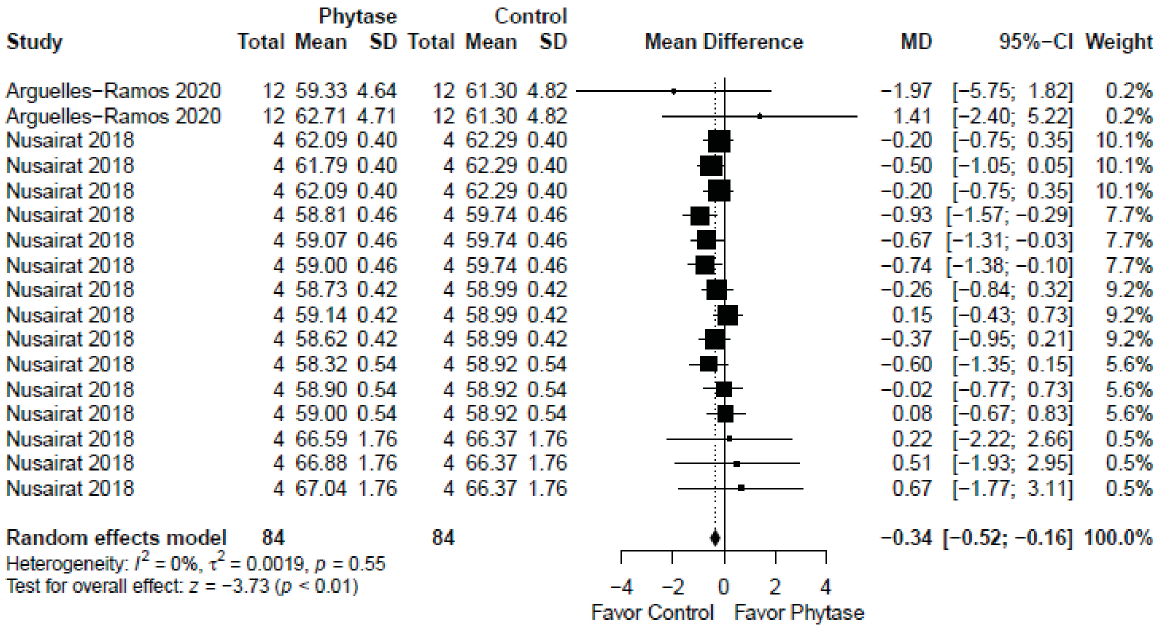

FIGURE 7. Forest plot of the impact of phytase supplementation on albumen ratio (% egg weight) in broiler breeders.

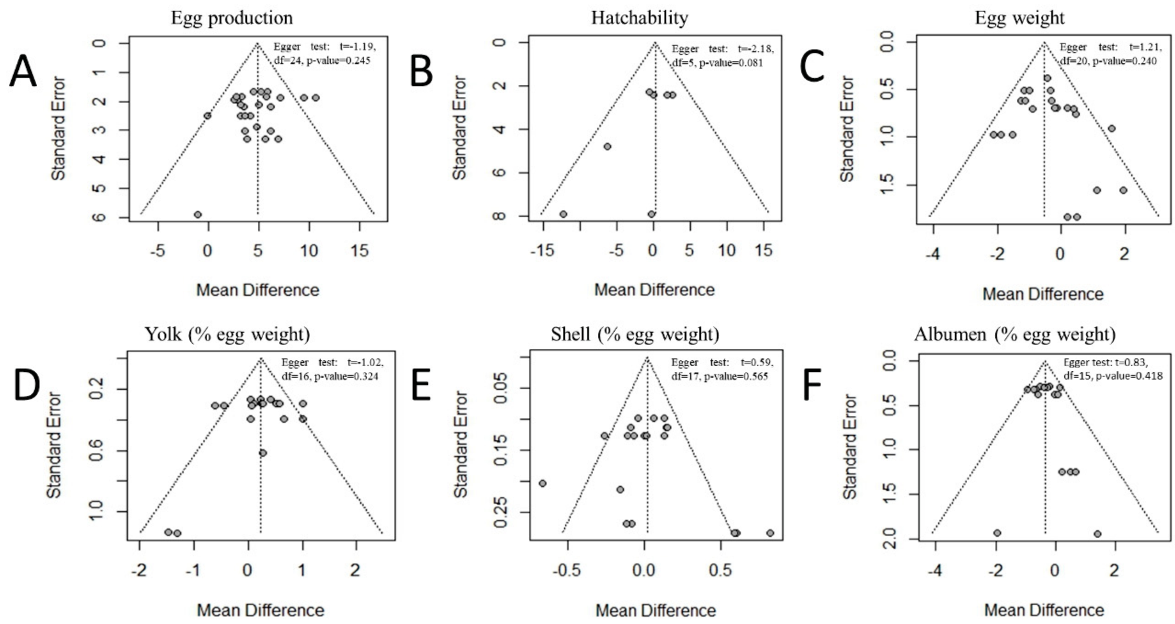

FIGURE 8. Funnel plots of phytase supplementation on (A) egg production, (B) hatchability, (C) egg weight, (D) yolk ratio (%egg weight), (E) shell ratio (% egg weight) and (F) albumen ratio (% egg weight) in broiler breeders.

TABLE 1. Summary of phytase supplementation studies on broiler breeder production in terms of location, journal, and year of publication.

Code	Study	Country	Journal	Year of publication
1	Arguelles-Ramos et al.	USA	Revista Brasileira de Ciência Avícola	2020
2	Abbasi et al.	Iran	Journal of Applied Animal Research	2015
3	Berry et al.	USA	Journal of Applied Poultry Research	2003
4	Bhanja et al.	India	Asian-Australasian Journal of Animal Sciences	2005
5	Nusairat et al.	USA	International Journal of Poultry Science	2018a
6	Nusairat et al.	USA	International Journal of Poultry Science	2018b
7	Sharideh et al.	Iran	Journal of Agricultural Science and Technology	2016

TABLE 2. Diet composition variation included in the dataset for broiler breeder experiments.

Ingredient, g/kg	N[1]	Mean	Min	Max	SD
Corn	7	629.4	0.0	707.2	149.9
Soybean Meal	7	194.2	133.5	306.0	47.8
Wheat Bran	2	45.3	0.0	166.0	66.4
Limestone	4	25.9	0.0	71.8	32.6
Corn Starch	2	25.5	0.0	506.7	113.3
Oyster Shell Grit	2	16.5	0.0	85.4	30.0
Calcium Carbonate	3	16.0	0.0	76.6	26.1
Poultry Fat	4	7.6	0.0	21.1	7.3
DCP	5	7.1	0.0	17.2	6.1
Alfalfa Meal	1	4.4	0.0	87.8	19.6
Corn Gluten Meal	3	4.1	0.0	10.0	5.0
Salt	7	4.1	1.7	5.0	1.2
Inert Filer	3	4.1	0.0	25.9	8.9
Cellulose	1	3.7	0.0	74.5	16.7
Deoiled Rice Bran	1	1.8	0.0	16.0	4.6
Mineral Premix	7	1.7	1.0	3.0	0.8
Vitamin Premix	7	1.6	0.2	3.5	1.1
L-Lysine	3	1.6	0.0	7.0	2.8
Corn Oil	2	1.3	0.0	15.1	4.0
DL-Methionine	7	1.1	0.3	3.3	0.6
Choline Chloride	3	0.95	0.0	2.0	0.95
Phosphoric Acid	1	0.5	0.0	9.5	2.1
Selenium Premix	2	0.4	0.0	1.0	0.5
Sodium Bicarbonate	2	0.4	0.0	2.2	0.7
Coccidiostat	2	0.2	0.0	0.5	0.3
Phytase	7	0.2	0.0	0.7	0.2
L-Threonine	1	0.2	0.0	0.8	0.2

[1]The number of studies that included the ingredient in their poultry diet composition.

TABLE 3. Inputs for estimating the economic impact of phytase (PHY) supplementation in broiler breeder diets.

Inputs	
Average of total egg (TE) produced per broiler breeder housed at 64 weeks of age[1], units	180.7
Weekly egg production variation with phytase[2], %	4.85
Cumulative feed consumption from 1 day old to 64 weeks of age[1], kg	54.93
Feed cost without phytase[3], $/ton	340
Phytase cost (500 FTU/ton)[3], $/ton	0.55
Other costs per egg (excluding cost of feed)[3], $/egg	0.15

[1] Broiler breeder rearing livability (%), cumulative feed consumption (kg), and total hatching egg (HE) produced per hen housed at 64 weeks of age based on average values of breeder management guides (Cobb-Vantress, 2020; Aviagen, 2021a; Aviagen, 2021b).

[2] Variation between diets with or without phytase supplementation obtained by the meta-analysis.

[3] Feed cost ($/ton), phytase cost ($/ton), and other costs per egg (excluding cost of feed) ($/egg) were provided by the poultry agroindustry.

TABLE 4. Summary of weighted mean differences for the egg production variable included in the meta-analysis and their subgroup analyses by different factors.

	Egg production								
	Nº of comparisons (k)	Meta-regression model		Subgroups analysis	Random effects model			Heterogeneity	
		P	R^2		WMD	95%CI	*P*	I^2	*P*
Pooled overall effect	26				4.85	3.87 – 5.97	<0.01	16%	0.23
Phytase dose (FTU/kg)	26	0.84	0%						
	26	0.03	50.2%						
PP (g/kg)	12			< 2.5 g/kg	3.57	2.16 – 4.90	<0.01	0%	0.82
	14			≥ 2.5 g/kg	5.59	4.31 – 6.87	<0.01	25%	0.18
Ca:NPP ratio	11	0.10	25.1%						
Phytase Treatment Period (weeks)	26	0.19	17%						
Vitamin D (UI/kg)	26	0.24	0%						
NPP requirement based on Rostagno et al (2024)	26	0.10	25.1%						
	26	<0.01	100%						
Ca requirement based on Rostagno et al (2024)	23			≥ 100%	4.14	3.22 – 5.05	<0.01	0%	0.98
	3			< 100%	9.07	6.94 – 11.20	<0.01	0%	0.40

TABLE 5. Summary of weighted mean differences for the hatchability variable included in the meta-analysis and their subgroup analyses by different factors.

Hatchability							
	Subgroups	Nº of comparisons (k)	Random effects model			Heterogeneity	
			WMD	95%CI	*P*	I^2	*P*
Pooled overall effect		7	0.22	-2.00 – 2.43	0.85	0%	0.44
Phytase dose (FTU/kg)	≥ 500 FTU	4	0.33	-2.82 – 3.47	0.84	17%	0.31
	< 500 FTU	3	0.10	-3.04 – 3.25	0.95	11%	0.32
PP (g/kg)	< 2.5 g/kg	4	-2.69	-7.21 – 1.83	0.24	0%	0.41
	≥ 2.5 g/kg	3	1.47	-1.25 – 4.19	0.29	0%	0.74
Ca:NPP ratio	≥ 10.5	5	0.51	-2.07 – 3.09	0.70	30%	0.22
	< 10.5	2	-0.60	-4.94 – 3.73	0.78	0	0.97
Phytase Treatment Period (weeks)	≥ 10 weeks	7	0.22	-2.00 – 2.43	0.85	0%	0.44
	< 10 weeks	-	-	-	-	-	-
Vitamin D (UI/kg)	≥ 2000 UI/kg	4	-2.69	-7.21 – 1.83	0.24	0%	0.41
	< 2000 UI/kg	3	1.47	-1.25 – 4.19	0.29	0%	0.74
NPP requirement based on Rostagno et al (2024)	≥ 100%	5	0.51	-2.07 – 3.09	0.70	30%	0.22
	< 100%	2	-0.60	-4.94 – 3.73	0.78	0	0.97
Ca requirement based on Rostagno et al (2024)	≥ 100%	7	0.22	-2.00 – 2.43	0.85	0%	0.44
	< 100%	-	-	-	-	-	-

TABLE 6. Summary of weighted mean differences for the egg weight variable included in the meta-analysis and their subgroup analyses by different factors.

Egg weight									
	Nº of comparisons (k)	Meta-regression model		Subgroups analysis	Random effects model			Heterogeneity	
		P	R^2		WMD	95%CI	*P*	I^2	*P*
Pooled overall effect	22				-0.53	-0.82 - -0.25	<0.01	19%	0.21
Phytase dose (FTU/kg)	22	0.53	0%						
PP (g/kg)	22	0.02	0%						
	7			< 2.5 g/kg	0.45	-0.43 – 1.34	0.31	10%	0.35
	15			≥ 2.5 g/kg	-0.74	-1.07 - -0.41	<0.01	0%	0.51
Ca:NPP ratio	22	0.22	0%						
Phytase Treatment Period (weeks)	22	0.36	0%						
Vitamin D (UI/kg)	22	0.04	100%						
	8			≥ 3000 UI/kg	0.01	-0.77 – 0.79	0.97	28%	0.18
	14			< 3000 UI/kg	-0.78	-1.15 – -0.40	<0.01	0%	0.60
NPP requirement based on Rostagno et al (2024)	22	0.05	0%						
				≥ 100%	0.59	-0.62 – 1.80	0.34	37%	0.17
				< 100%	-0.68	-1.00 - -0.35	<0.01	0%	0.46
Ca requirement based on Rostagno et al (2024)	22	0.02	100%						
	19			≥ 100%	-0.44	-0.74 – 0.14	0.21	10%	0.34
	3			< 100%	-1.85	-2.96 - -0.75	<0.01	0%	0.91

TABLE 7. Summary of weighted mean differences for the yolk ratio (% of egg weight) variable for each dependable variable included in the meta-analysis and their subgroup analyses by different factors.

Yolk ratio (% of egg weight)									
	Nº of comparisons (k)	Meta-regression model		Subgroups analysis	Random effects model			Heterogeneity	
		P	R^2		WMD	95%CI	*P*	I^2	*P*
Pooled overall effect	18				0.24	0.02 – 0.45	0.03	47%	0.02
Phytase dose (FTU/kg)	18	0.78	0%						
PP (g/kg)	18	0.26	0%						
Ca:NPP ratio	18	0.90	0%						
Phytase Treatment Period (weeks)	18	<0.01	86%						
				≥ 10 weeks	0.38	0.21 – 0.55	<0.01	17%	0.27
				< 10 weeks	-0.33	-0.72 – 0.07	0.10	21%	0.28
Vitamin D (UI/kg)	18	<0.01	86%						
	6			≥ 3000 UI/kg	-0.36	-0.80 – 0.07	0.08	10%	0.36
	12			< 3000 UI/kg	0.39	0.18 – 0.60	<0.01	14%	0.31
NPP requirement based on Rostagno et al (2024)	18	0.26	0%						
Ca requirement based on Rostagno et al (2024)	18	0.20	5.2%						

TABLE 8. Summary of weighted mean differences for the albumen ratio (% of egg weight) variable for each dependable variable included in the meta-analysis and their subgroup analyses by different factors.

	Albumen ratio (% of egg weight)								
	Nº of comparisons (k)	Meta-regression model		Subgroups analysis	Random effects model			Heterogeneity	
		P	R^2		WMD	95%CI	*P*	I^2	*P*
Pooled overall effect	17				-0.34	-0.52 - -0.16	<0.01	0%	0.55
Phytase dose (FTU/kg)	17	0.39	0%						
PP (g/kg)	17	0.97	0%						
Ca:NPP ratio	-	-	-						
Phytase Treatment Period (weeks)	17	0.25	0.3%						
Vitamin D (UI/kg)	17	0.29	0%						
NPP requirement based on Rostagno et al (2024)	17	0.97	0%						
Ca requirement based on Rostagno et al (2024)	17	0.42	5.2%						

TABLE 9. Summary of weighted mean differences for the shell ratio (% of egg weight) variable for each dependable variable included in the meta-analysis and their subgroup analyses by different factors.

Shell ratio (% of egg weight)									
	Nº of comparisons (k)	Meta-regression model		Subgroups analysis	Random effects model			Heterogeneity	
		P	R^2		WMD	95%CI	*P*	I^2	*P*
Pooled overall effect	19				0.02	-0.08 – 0.11	0.72	56%	<0.01
Phytase dose (FTU/kg)	19	0.94	0%						
PP (g/kg)	19	<0.01	55%						
	4			< 2.5 g/kg	-0.29	-0.58 – 0.01	0.05	38%	0.19
	15			≥ 2.5 g/kg	0.05	-0.03 – 0.12	0.24	50%	0.01
Ca:NPP ratio	19	0.58	0%						
Phytase Treatment Period (weeks)	19	0.26	0%						
Vitamin D (UI/kg)	19	0.15	0%						
NPP requirement based on Rostagno et al (2024)	19	0.47	0%						
Ca requirement based on Rostagno et al (2024)	19	0.56	0%						

TABLE 10. Simulation of the economic impact of phytase (PHY) supplementation in broiler breeder diets (1 day old – 64 weeks of age).

Variables[1]	Treatments		Variation (%)
	PHY+	PHY-	
Total eggs produced per broiler breeder housed at 64 weeks of age, units	186.6	180.7	+3.27
Feed conversion, g/egg	294	304	-3.17
Cost of feed, $/kg	0.341	0.340	+0.16
Feeding cost per egg, $/egg	0.100	0.103	-3.01
Other costs per egg (excluded cost of feed), $/egg	0.145	0.150	-3.17
Total cost per egg, $/egg	0.245	0.253	-3.10
Total eggs produced per 1,000,000 broiler breeders between 1 day old to 64 weeks of age, units	186,600,000	180,700,000	+3.27

[1]Inputs for building the economic model of PHY supplementation in broiler breeder diets are presented in Table 3.

TABLE 11. Sensitivity analysis of phytase (PHY) supplementation in broiler breeder diets according to variations in PHY prices and variations in egg production rates with or without PHY.

		Variation in PHY prices					
		X/2	X[1]	2X	3X	4X	5X
Variation between egg production (PHY+ and PHY-; %)	0,00	AVOID[2]	AVOID	AVOID	AVOID	AVOID	AVOID
	0,15	USE[3]	USE	USE	AVOID	AVOID	AVOID
	0,30	USE	USE	USE	USE	USE	USE
	0,50	USE	USE	USE	USE	USE	USE
	1,00	USE	USE	USE	USE	USE	USE
	2,00	USE	USE	USE	USE	USE	USE
	3,00	USE	USE	USE	USE	USE	USE
	4,00	USE	USE	USE	USE	USE	USE
	5,00	USE	USE	USE	USE	USE	USE

[1]X: current price of exogenous phytase.
[2]AVOID: the situation where it is possible to raise broiler breeders without PHY with no economic losses.
[3]USE: the situation where it is indicated to supplement with PHY.

CHAPTER III - IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, TIBIA ASH CONTENT, AND NUTRIENT DIGESTIBILITY IN LAYING HENS: A META-ANALYSIS

ABSTRACT

A meta-analysis was performed to quantify the effect of phytase (PHY) supplementation and its relationship with dietary non-phytate phosphorus (NPP) and calcium (Ca) to phytate phosphorus (PP) ratio (i.e., Ca:PP) on performance, egg quality, tibia ash content, and nutrient digestibility in laying hens. The database comprised 89 studies, with 38,795 hens between 24 and 80 weeks of age. Egg production (EP), average daily feed intake (ADFI), egg mass (EM), feed conversion ratio (FCR), tibia ash, shell and albumen proportion relative to egg weight, phosphorus (P) and nitrogen (N) utilization, and apparent ileal digestibility of P (AIDP) were considered as model outputs. All variables were analyzed using a linear mixed model, which included the effects of PHY, dietary NPP, and Ca:PP levels, as well as their interactions. PHY supplementation significantly ($P<0.05$) enhanced EP, ADFI, EM, albumen proportion, tibia ash content, AIDP, and P and N utilization. PHY supplementation also reduced the FCR and shell proportion. However, increased Ca:PP levels negatively affected EP, EM, tibia ash content, and P utilization, while also increasing the FCR and the albumen and shell proportion relative to egg weight. PHY supplementation had a more pronounced effect on all variables in diets with lower dietary NPP levels, except for P utilization. The interaction between Ca:PP and PHY was significant for shell and albumen proportions and P utilization. Dietary NPP levels exhibited a quadratic effect on EP, FCR, EM, albumen proportion, AIDP, and P utilization. A strong relationship between the observed and predicted values was observed across all models. The positive impact of PHY supplementation suggests that its inclusion in laying hen diets is beneficial and should be considered under most circumstances.

Keywords: Layers, phytase, meta-analysis, phytate, digestibility, egg quality.

1. INTRODUCTION

Phosphorus (P) is one of the most important minerals in poultry and the second most abundant element in their bodies (PROSZKOWIEC-WEGLARZ AND ANGEL, 2013). Alongside calcium (Ca), P plays an extremely important role in mineral deposition within the skeleton, contributing to subsequent bone mineralization (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014). Additionally, P is the third most expensive nutrient in laying diets and typically constitutes 0.40% to 0.48% of non-phytate phosphorus (NPP) for growing pullet diets and from 0.35% to 0.45% NPP for laying hen diets (HY-LINE, 2024). Nevertheless, approximately two-thirds of the P in plant-based feedstuffs is bound to phytic acid [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$], with poultry not being able to utilize phytate-P (PP) efficiently (SELLE et al., 2023), drastically reducing its bioavailability (BOUGOUIN et al., 2014). In addition to this limited availability of NPP in plant-based feedstuffs, phytase (PHY), the enzyme responsible for catalyzing the release of P from the PP complex, is not produced in sufficient quantities by poultry in the intestine (APPLEGATE et al., 2003). Therefore, other inorganic P sources may be added to poultry diets to meet daily nutritional requirements (HERVO et al. 2023). However, these sources are both costly and non-renewable (BOUGOUIN et al., 2014). In addition to increased feed costs, excessive dietary P can be excreted in manure, potentially leading to environmental issues such as surface water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023).

Introduced in 1991, PHY has become one of the most widely used exogenous enzymes in poultry nutrition (SELLE et al., 2023). Exogenous PHY may improve P availability in the intestinal lumen by hydrolyzing $InsP_6$ into lower myo-inositol phosphate isomers and inorganic phosphate, thereby significantly increasing PP utilization (AKTER et al., 2016). Numerous studies have reported the effects of PHY supplementation in laying hens; however, a wide discrepancy in the effect of PHY on performance, tibia ash content, and nutrient digestibility has been reported (AHMADI AND RODEHUTSCORD, 2012; RODEHUTSCORD et al., 2023). Measuring the effect of exogenous PHY on performance and nutrient digestibility is critical for determining the optimal level of inorganic P supplementation needed to meet nutritional requirements while simultaneously improving sustainability and cost-effectiveness (BOUGOUIN et al., 2014). Additionally, many fundamental questions regarding the relationship between PP and PHY remain unanswered (SELLE et al., 2023).

Meta-analysis is a relevant approach to analyze complex phenomena and quantify combined data from independent studies, providing more robust and reliable inferences (ST-PIERRE, 2007). Meta-analyses have the ability to translate study outcomes into practical and applicable knowledge. Unlike single experiments, which are limited by their specific conditions, meta-analytic methods systematically address the heterogeneity among studies, providing a broader and more comprehensive understanding of the topic (LOVATTO et al., 2007).

Most meta-analyses have focused on quantifying the effects of PHY levels on the performance of laying hens (AHMADI AND RODEHUTSCORD, 2012). However, to the best of our knowledge, the impact of PHY on tibia ash content and nutrient digestibility in laying hens has not yet been evaluated using a meta-analytic approach. Furthermore, advancement in laying hen genetic selection, the emergence of new generations of PHY, and numerous studies in this field underscore the need for updated analyses. Therefore, this study aimed to estimate the effect of PHY supplementation on production parameters, egg quality traits, tibia ash content, and nutrient digestibility in laying hens through a systematic review and meta-analysis.

2. MATERIAL AND METHODS

This study relied solely on data obtained from the published literature and did not involve the use of animals; thus, approval from the Institutional Animal Care and Use Committee was not required.

2.1. SEARCH STRATEGY AND DATA FILTERING

A structured online search for indexed publications on the effects of PHY supplementation on performance, tibia ash content, and energy, nitrogen and P digestibility in layers was performed until November 2024. The search focused on scientific articles published in peer-reviewed journals within different online database sources, including PubMed, Web of Science, and Scopus. In addition, references from the identified publications were reviewed to identify any additional relevant articles.

The research question was developed using the “PICo” strategy (ANDRETTA et al., 2021), where the “Population” was defined as “layers”, the “Interest” as “phytase”, and the “Context” as “performance” OR “tibia ash content” OR “phosphorus digestibility” OR “energy digestibility" OR "nitrogen digestibility" OR "amino acid digestibility". Synonyms and alternative English terms for the population and context were added to refine the final search strategy.

The database of PHY studies was exported to reference management software (EndNote X9; Philadelphia, PA, United States), where duplicate references were identified and removed. The relevance and quality of the studies were critically evaluated by initially reviewing titles and abstracts, followed by an in-depth analysis of the full texts.

The main criteria for selecting publications were as follows: (1) studies describing *in vivo* experiments with commercial layer breeds; (2) studies evaluating PHY supplemented diets that had non-supplemented control diets; and (3) studies reporting performance data, including daily egg production (EP), average daily feed intake (ADFI), egg weight (EW), egg mass (EM), and feed conversion ratio (FCR) calculated by EM. Egg traits included yolk, shell, and albumen proportions relative to EW. Additionally, studies evaluating the effects of PHY on tibia ash, P, and Ca concentrations were also included, as well as those assessing nutrient digestibility parameters, such as retention and apparent ileal digestibility (AID) of dry matter (DM), P, Ca, nitrogen (N), and amino acids.

Preselected manuscripts were excluded if they involved a sanitary challenge, combined PHY with another enzyme, lacked a control treatment, or showed significant inconsistencies in the statistical design, methodology, or outcome data. Experiments involving layer diets supplemented with super-dosing PHY (i.e., > 2,500 FTU/kg; COWIESON et al., 2011) were removed from the analysis due to data limitations. One phytase unit (FTU) was defined throughout studies as the amount of enzyme that releases one μmol of inorganic P from 5.1 mmol/l sodium phytate per minute at pH 5.5 at 37°C (DERSJANT-LI et al., 2015). Moreover, the ages considered ranged from 24 to 86 weeks of age and only the studies performed in the first production cycle were kept for further analysis. To ensure an accurate evaluation of the effect of PHY in the models, only the PHY supplemented group and the PHY negative control were considered, ensuring non-supplemented diets were matched to the same NPP levels with the supplemented group.

The article selection process generated a database, as shown in Figure 1. Initially, 3,807 references were identified, and successive exclusions were made based on titles and abstracts. Full-text reviews of 329 references were conducted to assess their eligibility. After an in-depth analysis, 240 publications were excluded based on the previously established criteria. The final databased included 89 publications, as presented in Figure 1.

2.2. DATA SYSTEMATIZATION AND CODING

The methodology for building the database, encoding data, and defining dependent and independent variables followed the approaches described in the literature (LOVATTO et al., 2007). To address a comprehensive and consistent dietary composition across studies, experiments that did not provide a complete dietary nutrient composition were recalculated using the Practical Program for Formulation of Rations (PPFR; GARCIA-NETO, 2008), based on the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024).

The retention values for DM, P, Ca, Na, and amino acids were included in the meta-analysis only if the experiment applied the following equation:

$$Retention\ (\%) = \frac{\left[\left(Feed\ intake\ x\ Nutrient_{feed}\right) - \left(Excreta\ output\ x\ Nutrient_{excreta}\right)\right]}{\left(Feed\ intake\ x\ Nutrient_{feed}\right)}$$

The AID values for DM, P, Ca, Na, and amino acids were included on the meta-analysis dataset only if the experiment applied the following equation:

$$AID\ (\%) = \left[1 - \left(\frac{Dietary\ marker_{feed}\ x\ Mineral\ _{digesta}}{Dietary\ marker_{digesta}\ x\ Mineral_{feed}}\right)\right]\ x\ 100$$

2.3. STATISTICAL ANALYSIS

A meta-analytical approach was applied to model the dependent variables. As described by ANDRETTA et al. (2012), the meta-analysis proceeded through sequential analytical steps: 1) graphical (to assess the biological coherence of the data and ensure database quality), 2) correlation (to identify potential relationships between the dependent and explanatory variables), and 3) variance-covariance (to obtain the prediction equations) analyses. All variance-covariance analyses were performed considering the study effect as a random-effect class variable in the model due to the differences among studies in the database (ST-PIERRE, 2001). Two separate models were used to evaluate the effects and interactions of PHY supplementation. The first model assessed the effect of PHY and NPP. The second model analyzed the effects of PHY and the Ca:PP. After testing all other independent variables, only those showing statistical significance ($P<0.05$) or tendencies ($P<0.10$) were maintained. Variables such as EW, yolk proportion relative to EW, tibial Ca and P content, retention of DM, energy, Ca and AA, and AID of DM, energy, N, and AA were not significantly affected by PHY supplementation (P>0.10). These variables were therefore excluded from further model analyses. As described by Létourneau-Montminy et al. (2010) and Ahmadi and Rodehutscord (2012), the response of each dependent variable (EP, ADFI, EM, FCR, shell and albumen proportion relative to EW, tibia ash content, retention of P, energy, and N, and AID of P) to the main statistically significant variables, NPP, Ca:PP, and PHY, was analyzed using a full quadratic model, as follows:

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{NPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{NPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{NPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk}, [1]$$

and,

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{CaPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{CaPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{CaPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk}, [2],$$

where Y_{ijk} represents the value of the dependent variable Y in experiment *i* with level *j* of NPP in equation [1] or Ca:PP in equation [2], and level *k* of PHY. The parameter α is the overall intercept with a fixed effect, while α_i is the random effect of experiment *i*. The coefficients β_1 and β_2 represent the linear effects, β_3 and β_4 capture the quadratic effects, and β_5 demonstrates the interaction effect. The term e_{ijk} represents the residual error.

The distribution of random effects and residual errors was checked to be normal. Internally studentized residuals (ISR) were assessed to detect outliers, with values greater than 3 (|ISR| > 3) identified and deleted from further analysis (FARIDI et al. 2015). Across all models, more than 95% of ISR values fell within the range of -2 to 2, demonstrating a good fit of the models. Model accuracy was evaluated based on the significance levels of the estimated parameters, variance of the error estimate and its approximate standard error (SE), coefficient of determination (R^2), and root mean square error (RMSE). Model adequacy was further assessed by plotting residuals (observed minus predicted values) against the predicted values of Y to test for linear prediction bias (ST-PIERRE, 2001). The optimization process was carried out using the partial derivatives of the models obtained for each dependent variable (LETOURNEAU-MONTMINY et al., 2010).

Data analysis was performed using the general linear model (GLM) procedure in Minitab 22 software (Minitab Inc., State College, PA), with graphs being generated using the ggplot2 package in R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1. DATABASE CHARACTERIZATION

The complete list of studies included in the meta-analysis on laying hen diets supplemented with PHY is detailed in Table 1. The variability in diet composition across the dataset is summarized and presented in Table 2. The 89 studies included in this meta-analysis comprised a total population of 38,795 laying hens aged between 24 and 80 weeks. In our final dataset, studies were performed in the United States (US) (n=14), Brazil (n=13), Iran (n=8), Canada (n=7), Poland (n=6), the Czech Republic (n=6), China (n=5), Mexico (n=5), Turkey (n=5), Germany (n=4), the Republic of Korea (n=4), India (n=3), Spain (n=3), France (n=2), Egypt (n=1), England (n=1), Iraq (n=1), and the Netherlands (n=1). Most treatments utilized a corn-soybean meal-based diet (83.2%), followed by wheat-soybean meal-based diets (13.2%) and sorghum-soybean meal-based diets (3.6%). Additionally, most diets consisted solely of grain-based feedstuffs (78.6%), whereas a smaller proportion included animal-based feedstuffs (21.4%). The feed diets were presented in various forms: mashed (25.1%), pelleted (2.4%), and unspecified forms (72.5%). The investigated diets were supplemented with mineral P sources, including dicalcium phosphate (66.3%), monodicalcium phosphate (23.9%), monocalcium phosphate (4.9%), tricalcium phosphate (3.3%), monosodium phosphate (0.8%), and calcium monophosphate (0.8%). The most common breed found in the experiments was Lohmann Brown (n=131), followed by Hy-line Brown (n=103), Hy-Line W36 (n=96), Isa Brown (n=82), Babcock B300 (n=70), Isa White (n=68), Lohmann LSL Lite (n=66), Hisex Brown (n=55), Hy-Line White (n=25), Hy-line W98 (n=24), Bovans White (n=20), Babcock 600 (n=18), DeKalb Delta White (n=16), Bovans Goldline (n=12), Hy-Line W80 (n=12), Lohmann LSL - Classic (n=12), Lohmann Pink-Shell (n=12), Bovans Brown (n=11), Nick Brown (n=11), Lohmann Tradition (n=8), Nick Chicks White (n=8), Lohmann White (n=4), Nick-Brown (n=4), 87 studies did not mention the poultry breed. The phytase types varied among the studies, with 262 treatments (53.4%) using 3-phytase (EC 3.1.3.8) and the others 229 treatments (46.6%) using 6-phytase (EC 3.1.3.26). Additionally, PHY were derived from *Aspergillus niger* (47.5%), *Escherichia coli* (30.5%), *Hansenula polymorpha* (6.7%), *Peniophora lycii* (3.8%), *Trichoderma ressei* (3.5%), *Buttiauxella sp.* (2.7%), *Citrobacter braakii* (2.3%), *Aspergillus oryzae* (1.9%), and *Serratia odorifera* (1.1%) and PHY inclusion levels ranged from 60 to 2000 FTU/kg.

3.2. THE EFFECT OF PHY SUPPLEMENTATION AND DIETARY NPP AND CA:PP RATIO IN LAYING HEN DIETS

The summary statistics for the dependent and independent variables included in the models are presented in Table 3. The effects of different levels of dietary supplemental PHY and NPP on EP, ADFI, FCR, EM, and shell and albumen proportions are described in Table 4 and illustrated in Figure 2. The impacts of varying dietary supplemental PHY and NPP levels on tibia ash content, P utilization, AID of P, and N utilization are detailed in Table 5 and shown in Figure 3. Additionally, models evaluating the effects of dietary PHY levels and Ca:PP ratio on EP, FCR, EM, shell and albumen proportions to EW, tibia ash and P utilization are described in Table 6 and shown in Figure 4. The plots of residuals (observed minus predicted) against the predicted values from the PHY and NPP mixed model analyses, are illustrated in Figures 5 and 6, respectively. The residual plots for the PHY and Ca:PP ratio mixed model analyses are shown in Figure 7. The optimum levels of NPP required to improve the responses of EP, EM, and FCR in laying hen diets supplemented or not supplemented with PHY are described in Table 7.

3.2.1. PRODUCTION PERFORMANCE AND EGG TRAITS

The response to incremental NPP levels followed a quadratic behavior in most cases, except for ADFI and shell proportion relative to EW (Table 4 and Figure 2). Increasing dietary NPP had a more pronounced effect on EP, ADFI, FCR, EM, and shell and albumen proportions to EW in diets without PHY than diets with PHY supplementation. (PHY x NPP; $P<0.05$) (Table 4; Figure 2). PHY supplementation also positively affected EP and EM while reducing the intercept for FCR and shell proportion in models that included different levels of the Ca:PP ratio and dietary PHY (Table 6 and Figure 4). Increasing the Ca:PP ratio had a negative impact on EP, EM, and albumen proportion, but increased the FCR and shell proportion to EW.

Exogenous PHY supplementation increased EP, with greater effects observed at lower dietary NPP levels (PHY × NPP; $P<0.001$). Compared with diets without PHY, supplementation with 300 FTU/kg increased EP by 1.19%, 0.89%, 0.59%, and 0.21% in laying hen diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively. Additionally, diets added PHY at 300 FTU/kg levels showed quadratic effect on EP for NPP ($P=0.033$), with a plateau at 0.30% dietary NPP (Figure 2). The EP model incorporating PHY and Ca:PP also showed quadratic effect for PHY and Ca:PP ($P<0.05$). PHY supplementation at 300 FTU/kg increased EP by 0.61% across different Ca:PP ratio levels, with a plateau at 25.6 ratio of Ca to PP (Figure 4).

The ADFI model showed a linear effect for dietary NPP *(P*=0.002) and quadratic effect for PHY (*P*=0.011). PHY supplementation at 300 FTU/kg increased ADFI by 1.15g, 0.84g, 0.52g, and 0.21g for dietary NPP levels of 0.1%, 0.2%, 0.3%, and 0.4%, respectively (Figure 2). PHY supplementation exhibited a linear effect (*P*<0.001) and NPP demonstrated quadratic effect for FCR model (*P*=0.023). Compared to diets without PHY supplementation, PHY added at 300 FTU/kg reduced FCR by 0.017, 0.011, 0.006, and 0.001 for diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively, with a plateau at 0.29% dietary NPP (Figure 2). In the FCR model incorporating different levels of PHY and Ca:PP, PHY supplementation showed linear effect for PHY (*P*=0.001) and quadratic effect for Ca:PP for FCR (*P*=0.004). PHY supplementation at 300 FTU/kg enhanced FCR by 0.007 across different Ca:PP levels. However, increasing the Ca:PP ratio above 15 led to a deterioration in FCR. Compared to a Ca:PP ratio of 15, FCR worsened by 0.014, 0.032, 0.053, 0.079, 0.109, and 0.142 in laying hens fed diets with Ca:PP ratios of 20, 25, 30, 35, 40, and 45, respectively (Figure 4).

The EM model containing PHY and NPP showed significant quadratic effect (*P*<0.05). Diets supplemented with 300 FTU/kg compared to diets without PHY increased EM by 0.96, 0.70, 0.43, and 0.16g/hen/day in diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively, with a plateau at 0.32% dietary NPP (Figure 2). The EM model incorporating PHY and Ca:PP variables showed quadratic effect for PHY (*P*=0.018, respectively) and only a tendency for a quadratic effect for Ca:PP (*P*=0.056). PHY supplementation at 300 FTU/kg increased EM by 0.52 g/hen/day across different Ca:PP levels. Nevertheless, a Ca:PP ratio greater than 15 reduced EM by 0.06, 0.15, 0.25, 0.36, 0.50, and 0.66g/hen/day in diets with Ca:PP ratios of 20, 25, 30, 35, 40, and 45, respectively (Figure 4).

The shell and albumen proportions relative to EW were both influenced by PHY, which showed a quadratic effect for shell proportion (*P*=0.006) and a linear effect for albumen proportion (*P*=0.002). It was also observed quadratic effect of NPP levels in albumen proportion relative to EW (*P*=0.018), with a plateau at 0.26% dietary NPP for diets supplemented with PHY at 300 FTU/kg (Figure 2). Additionally, the shell and albumen proportions were affected by PHY and Ca:PP levels. The shell proportion model showed a linear effect for Ca:PP (*P*<0.001) and quadratic effect for PHY (*P*<0.001), while the albumen proportion exhibited a linear effect for PHY (*P*<0.001) (Figure 4).

3.2.2. TIBIA ASH CONTENT AND NUTRIENT DIGESTIBILITY

The tibia ash content model was influenced by PHY and NPP dietary levels. Quadratic effects were observed for PHY (P=0.004), along with a linear effect for NPP (P=0.001). Diets supplemented with 300 FTU/kg increased tibia ash by 1.4%, 0.95%, 0.49%, and 0.03% in diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively (Figure 3). Additionally, tibia ash content was significantly affected by PHY and the interaction between PHY and Ca:PP levels. PHY showed a positive linear effect on tibia ash (P<0.001), whereas the interaction between PHY and Ca:PP negatively affected tibia ash content (P=0.028). Moreover, a Ca:PP ratio greater than 15 reduced tibia ash content. In diets supplemented with 300 FTU/kg of phytase compared to diet without PHY supplementation, tibia ash decreased by 0.18%, 0.36%, 0.53%, 0.71%, 0.89%, and 1.07% in diets with Ca:PP ratios of 20, 25, 30, 35, 40, and 45, respectively (Figure 4).

The P utilization model was affected by PHY and NPP levels. PHY exhibited a linear effect (P<0.001) while NPP showed quadratic effect on P utilization (P=0.006). Diets supplemented with 300 FTU/kg of PHY enhanced P utilization by 1.31% across different dietary NPP, and the highest P utilization value was observed at 0.25% dietary NPP (Figure 4). PHY and Ca:PP levels also affected P utilization, with quadratic behavior observed for Ca:PP levels (P=0.076), and a linear pattern noted for PHY (P<0.001). PHY supplementation at 300 FTU/kg increased P utilization by 1.25% across various Ca:PP levels. However, P utilization decreased in diets with a Ca:PP ratio greater than 15 across all PHY levels. Compared to a Ca:PP ratio of 15, P utilization was reduced by 2.49%, 4.34%, 5.55%, 6.12%, 6.04%, and 5.32% in laying hens fed diets with Ca:PP ratios of 20, 25, 30, 35, 40, and 45, respectively (Figure 4).

PHY and NPP also influenced the AID of P. PHY had a positive impact on the AID of P, showing both linear (P<0.001) and quadratic effects (P=0.024), whereas NPP exhibited a quadratic effect on the AID of P (P=0.073). Compared to diets without PHY, supplementation with 300 FTU/kg of PHY increased the AID of P by 14.9%, 9.8%, 4.7%, and -0.4% in diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively (Figure 3). N utilization was positively affected by PHY, showing both linear and quadratic behaviors (P<0.001 and P=0.062, respectively). Additionally, PHY supplementation had a more pronounced effect on N utilization in diets with lower dietary NPP levels (PHY × NPP; P=0.006). Diets supplemented with 300 FTU/kg of PHY compared to diets without PHY increased N utilization by 1.81%, 1.43%, 1.06%, and 0.69% in diets containing 0.1%, 0.2%, 0.3%, and 0.4% dietary NPP, respectively (Figure 3).

3.3. PERFORMANCE OF STATISTICAL MODELS

The residual plots against the predicted values presented in Figures 5, 6, and 7 indicate the adequacy of the dietary percentage of NPP in laying hens fed different dietary supplemental PHY levels. The residuals displayed a good distribution around zero across the different models. For each dependent variable with values of 5.3%, 6.5%, 7.8%, 7.1%, 4.1%, 1.9%, 9.9%, 17.2%, 16.5%, 16.3%, for EP, ADFI, FCR, EM, shell and albumen proportion relative to EW, tibia ash, P utilization, AID of P, and N utilization, respectively.

In addition, good residuals were observed on models containing different Ca:PP levels in laying hen diets with different dietary PHY levels. For each dependent variable with values of 5.3%, 7.7%, 6.9%, 4.4%, 1.0%, 10.0%, 17.2% for EP, FCR, EM, shell and albumen proportion to EW, tibia ash, and P utilization, respectively.

4. DISCUSSION

Phosphorus is an essential mineral that plays important roles in the genomic and physiological processes of laying hens, including bone development, regulation of key enzymes, and skeletal mineralization (ABBASI et al., 2019). However, almost two-thirds of the P in plant-based feedstuffs exists in the form of PP, which has reduced bioavailability in laying hens (SELLE et al., 2023). To address this limitation, exogenous PHY is commonly supplemented to enhance PP utilization and release NPP into the digestive tract, which plays a critical role in improving NPP in laying hen diets. To the best of our knowledge, this is the first meta-analysis to evaluate the impact of PHY on egg quality traits, tibia ash content, and nutrient digestibility. Furthermore, it updates the understanding of the effect of PHY on the overall performance of laying hens. The models used in this meta-analysis demonstrated good adequacy, as indicated by the RMSE, R^2, and residual plots against the predicted values.

4.1. PERFORMANCE AND EGG QUALITY TRAITS

The response to incremental levels of dietary NPP and PHY supplementation showed quadratic behaviors in most models, including EP, EM, FCR, albumen proportion relative to EW, P utilization, AID of P, and N utilization. However, the positive effect of PHY supplementation diminished when dietary NPP exceeded the plateau level, consistent with the models described by Ahmadi and Rodehutscord (2012) in laying hens and Majeed et al. (2020) in broilers. As observed by Rodehutscord et al. (2002) and Nie et al. (2013), laying hens have an increased capacity to absorb P when dietary NPP levels are low, whereas the extra P released by PHY supplementation is efficiently absorbed. However, when the absorption capacity of laying hens is exceeded due to the saturation of P transporters, no additional benefit is observed from exogenous PHY or higher dietary NPP levels.

The optimal dietary NPP levels required to maximize the EP and EM responses were 0.35% and 0.37%, respectively, for laying hens aged 24 to 80 weeks in diets without PHY supplementation. Moreover, a dietary NPP level of 0.31% improved the FCR. These values align with the primary breeder guide recommendations, which suggest NPP levels between 0.32% and 0.55% during the laying phase (HENDRIX GENETICS, 2020; LOHMANN TIERZUCHT, 2024; HY-LINE, 2024). Increasing dietary PHY levels reduces NPP requirements in laying hen diets. At a PHY supplementation level of 300 FTU/kg, the best EP, EM, and FCR were achieved with dietary NPP of 0.30%, 0.32%, and 0.29%, respectively. This may indicate that 300 FTU/kg of PHY can release up to 0.05% of NPP from the diet. These findings align with Guo et al. (2018), who suggested an NPP level of 0.32% for maximum EP, but differed from Ahmadi and Rodehutscord (2012), who reported that the optimal dietary NPP levels for maximizing EP and EM and enhanced FCR in diets with 300 FTU/kg of PHY were 0.15%, 0.14%, and 0.15%, respectively, for hens aged 36 to 76 weeks. Ahmadi and Rodehutscord (2012) also suggested that 300 FTU/kg of PHY could release up to 0.07% of NPP. Moreover, PHY supplementation showed a greater effect at lower dietary NPP levels. According to the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024), which recommend a dietary NPP level of 0.22% during the laying phase, similar performance in EP, EM, and FCR was observed at NPP levels of 0.12%, 0.13%, and 0.17%, respectively, when 300 FTU/kg of PHY was included. This indicates that PHY at this dosage could release up to 0.10% of NPP in diets with reduced NPP levels.

Differences in NPP requirements may be attributed to several factors, including the wider age range of laying hens evaluated in the present study (24 to 80 weeks) compared to Ahmadi and Rodehutscord (2012), who considered hens from 36 to 76 weeks of age. Other contributing factors include variations in diet composition and the duration of PHY treatment. Younger laying hens may require higher NPP levels to support their growth and egg production. Unlike Ahmadi and Rodehutscord (2012), who included only studies fed with corn-soybean meal-based diets, the current meta-analysis also considered diets based on wheat-soybean meal and sorghum-soybean meal in the dataset. According to Selle and Ravindran (2007), wheat bran and sorghum can contain approximately 4.4 and 1.2 times more PP (g kg^{-1}) than maize. Besides, a proportion of studies also included animal-based feedstuffs in their laying hen diets (21.4%). The variability in feed ingredient composition likely influenced the estimated minimum NPP requirements, as different feed ingredients have varying P fractions and digestibility (RODEHUTSCORD et al., 2023). Furthermore, the duration of PHY supplementation may influence NPP requirements, as a mild P deficiency can be compensated over time by skeletal P mobilization (RODEHUTSCORD et al., 2023). In the present meta-analysis, the average duration of PHY treatment was 7 weeks, whereas in the meta-analysis by Ahmadi and Rodehutscord (2012), the evaluated trials had an average duration of 12 weeks.

In addition, PHY supplementation improved performance over non-supplemented diets, regardless of variations in Ca:PP levels. However, increasing the Ca:PP ratio had a negative impact on performance, such as EP, EM, and FCR. Although broiler chickens have demonstrated a higher potential for PP utilization due to endogenous PHY activity (RODEHUTSCORD et al., 2022), research indicates that the endogenous PHY activity in laying hens is significantly lower (VAN DER KLIS et al., 1997; MAROUNEK et al., 2010). Phytate P molecules have a polyanionic nature that enables them to efficiently chelate divalent cations, such as Ca, resulting in the formation of insoluble mineral-PP complexes. These complexes reduced the PHY efficacy (ADEKOYA AND ADEOLA, 2023), as they can directly bind to the active sites of the enzyme (BOUGOUIN et al., 2014). Moreover, laying hen diets are formulated with higher Ca levels than broiler diets, as Ca is crucial not only for bone mineralization but also for eggshell formation (MANANGI et al., 2009). The Ca:PP plateau level was achieved at 25.6 when PHY was supplemented at 300 FTU/kg. However, Ca:PP ratios exceeding 15 negatively affected the FCR and EM across different PHY supplementation levels. PHY supplementation and increased dietary NPP levels positively influenced ADFI in laying hens. The P deficiency may suppress appetite in both laying hens (ZHAI et al., 2022) and broilers through a complex mechanism involving hypothalamic hormones and the gut (ADERIBIGBE et al., 2022). This reduction in ADFI may lead to decreased energy consumption, impairing EP, FCR, and EM. Additionally, diets added with PHY increased EM but did not significantly influence EW. Since EM is calculated by multiplying EW by EP (ZHAI et al., 2013), and PHY supplementation did not significantly affect EW, it can be suggested that PHY supplementation enhances EM primarily by improving EP.

The shell proportion relative to EW was negatively influenced by the interaction between PHY and NPP. However, the interaction between Ca:PP and PHY increased the shell proportion. Laying hens can exploit their Ca reserves in the bone to lay eggs (ZHAI et al., 2022). When dietary Ca requirements for eggshell formation are not met, the deficit can be filled via bone mineral mobilization (ZHAI et al., 2022). Nevertheless, bone Ca resorption results in an excess of P due to the higher Ca:P ratio in the eggshell compared to hydroxyapatite in bone (CLUNIES et al., 1992). Consequently, elevated plasma P concentrations during shell formation may increase P excretion (MILES et al., 1984). When not strongly influenced by shell formation, plasma P concentration reflects the dietary P intake (BOORMAN AND GUNARATNE, 2001). PHY supplementation may enhance ileal digestible P levels, thereby elevating plasma P concentrations and increasing P influx into the liver (ZHAI et al., 2022). Consequently, elevated dietary or plasma P levels were associated with a decline in shell quality, such as reduced shell percentage and thickness (EL BOUSHY, 1979), and a steady decrease in egg specific gravity with increased daily P intake (MILES et al., 1983).

PHY supplementation and NPP negatively influenced the albumen proportion relative to EW, indicating a possible inverse relationship between albumen and yolk proportions to EW. Although PHY did not have a significant effect on the yolk proportion, Zyla et al. (2012) reported that PHY supplementation was associated with higher yolk weights. This may be attributed to PHY's ability to release NPP in the diet, which increases the yolk proportion. A significant portion of the P released from the decomposition of PP is incorporated into the yolk as phosphorylated lipids or proteins (JIANG et al., 2015). Furthermore, lower-phosphorylated inositol phosphates and free myo-inositol have been shown to exert positive effects on energy and protein metabolism. These benefits are observed alongside the release of chelated nutrients such as starch, amino acids, and trace minerals, which contribute to the so-called 'extra-phosphoric' effects of phytase (GONZALEZ-UARQUIN et al., 2020). Additionally, the interaction between Ca:PP levels and PHY supplementation increased the albumen proportion relative to EW across different PHY levels. Complex insoluble Ca-PP is formed in the crop of the laying hen when pH exceeds 5.0 (NOLAN AND DUFFIN, 1987; CLASSEN et al., 2016). These insoluble complexes limit the PHY ability to release P from PP (SELLE et al., 2009). Consequently, less P is available for incorporation into the yolk, indirectly increasing the albumen proportion relative to EW.

4.2. TIBIA ASH CONTENT AND NUTRIENT DIGESTIBILITY

A positive effect of PHY supplementation and increased NPP dietary levels was observed on tibia ash content. However, a negative correlation was noted between higher Ca:PP levels and tibia ash content. Tibial characteristics are considered critical factors in measuring P absorption in poultry, as P deficiency in the feed can lead to bone disorders (REN et al., 2023). In addition, the relationship between tibial characteristics and growth performance serves as a key marker for identifying P deficiency (SELLE AND RAVINDRAN, 2007). The medullary bone acts as a dynamic reservoir of Ca, which hens may mobilize for both growth and eggshell calcification. This process inevitably leads to structural bone losses during the laying period, although adequate nutrition can mitigate these effects (WHITEHEAD AND FLEMING, 2000; ZHAI et al., 2022). The observed increase in tibia ash content with PHY supplementation or higher dietary NPP levels suggests that hens may require less mineral mobilization from structural bones, probably because of more medullary bone formation and less overall bone resorption. However, the formation of insoluble Ca-PP compounds can reduce the effectiveness of PHY in releasing P from PP. This limitation may necessitate greater mineral mobilization from the bones to compensate for insufficient P availability.

Exogenous PHY can increase energy and nutrient availability in the feed (MOURA et al., 2023) by hydrolyzing phosphate groups, which may have implications for the digestibility of energy and other nutrients (KORNEGAY AND QUIAN, 1996). As the phosphate groups are removed from the myo-inositol phosphates, other compounds, including amino acids (KORNEGAY AND QUIAN, 1996) and certain minerals (HAMDI et al., 2015) such as Ca, zinc, and manganese, may also be released. The AID and utilization of P were positively affected by both PHY supplementation and dietary NPP levels. The highest P utilization was observed at a dietary NPP level of 0.25% across various PHY levels, which collaborates with many studies in the literature (BELLO AND KORVER, 2019; PONGMANEE et al., 2020; JAVADI et al., 2021; ZHAI et al., 2022). However, an increase in the Ca:PP ratio negatively affected P utilization. Beutler (2009) observed that increasing dietary Ca from 2.5 to 5.5%

linearly decreased the digestibility of P, Ca, energy, and protein due to an increase in Ca-PP complex formation in the gastrointestinal tract of laying hens.

PHY supplementation positively influenced N utilization, particularly in low-P diets. The impact of PP on N digestion primarily arises from PP-protein complexes present in feedstuffs or formed de novo in the gastrointestinal tract under acidic conditions. These complexes restrict the PHY access to its substrate and hinder the digestion of resistant protein complexes by pepsin (SELLE et al., 2000). The addition of PHY to poultry diet partially prevents the formation of PP-protein complexes by hydrolyzing PP prior to their formation, thus enhancing protein digestibility (LIU et al., 2007). However, PHY supplementation did not significantly affect the utilization and AID of AA or energy. This lack of significance could be attributed to the limited number of studies meeting the inclusion criteria for the dataset, underscoring the need for further research to better understand these effects.

5. CONCLUSION

PHY supplementation may enhance EP, ADFI, EM, the albumen proportion relative to EW, tibia ash, AIDP, P and N utilization, while reducing the FCR and shell proportion relative to EW in laying hens. However, increased Ca:PP ratios negatively affected EP, EM, tibia ash, and P utilization, while increasing the FCR and the albumen and shell proportions relative to EW.

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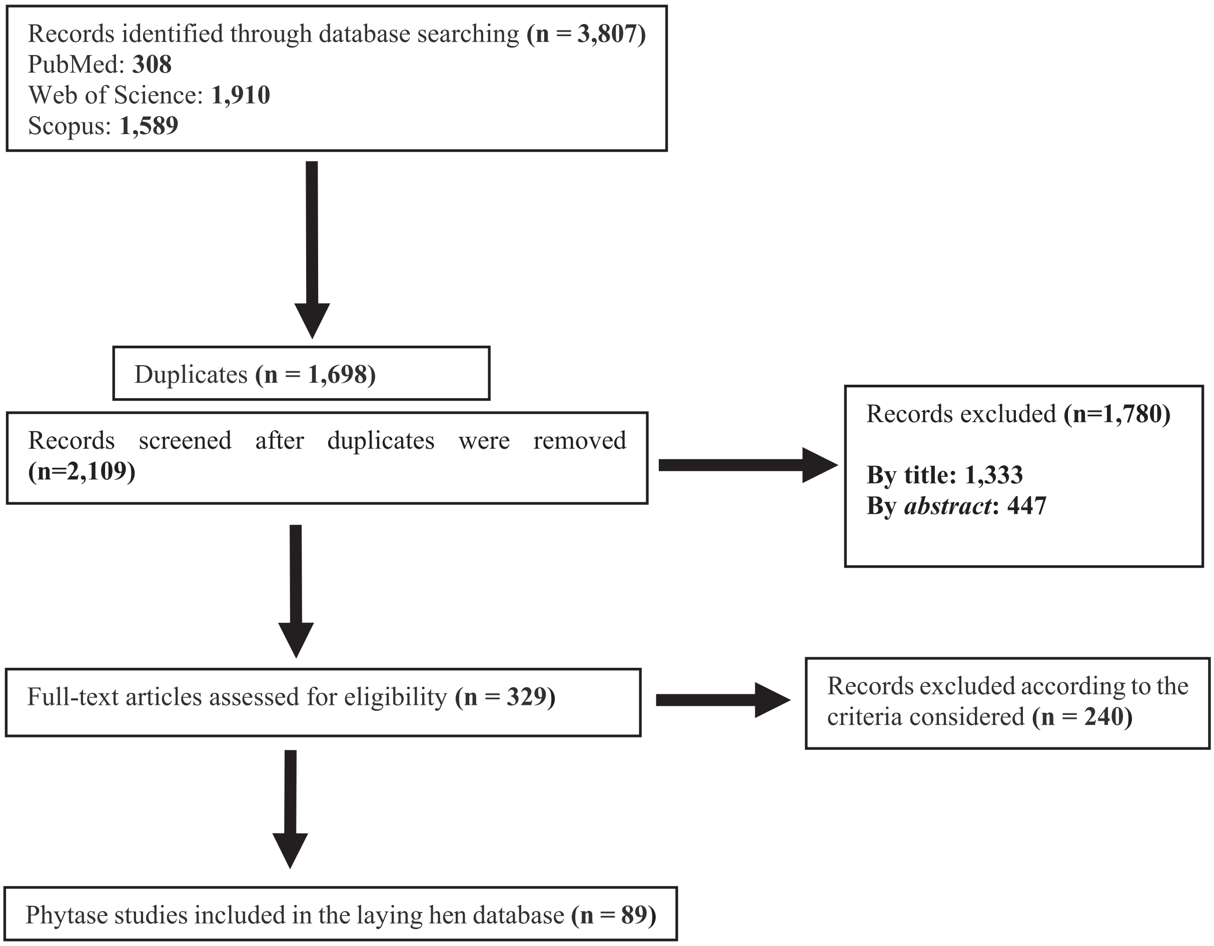

FIGURE 1. Study selection diagram for the meta-analysis focused on phytase supplementation in layers.

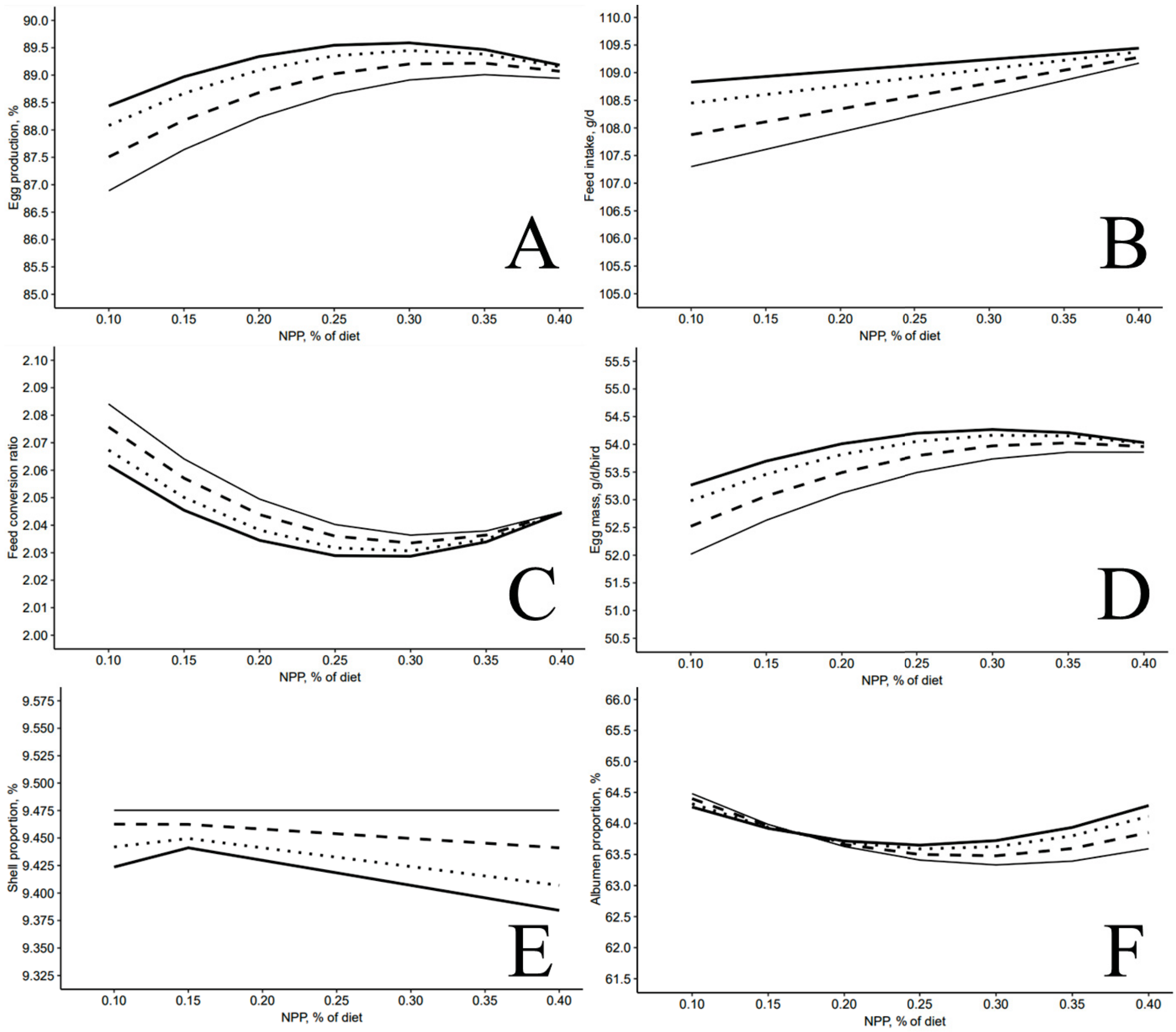

FIGURE 2. Response of egg production, feed intake, feed conversion ratio, egg mass, shell and albumen proportion to egg weight dietary percentage of non-phytate phosphorus (NPP) in laying hens fed with different dietary supplemental phytase.

Description of curve types and equations for egg production, feed intake, feed conversion ratio, egg mass, shell and albumen proportion to egg weight: Curve types: Solid Thin Curve – 0 FTU/kg of diet; Dashed Thick Curve – 150 FTU/kg of diet; Dotted Curve – 300 FTU/kg of diet; Solid Thick Curve – 400 FTU/kg of diet. (A) Egg production (%) = 84.90 + 0.005 × Phytase + 23.18 × NPP - 0.12 × 10^{-7} × Phytase^2 – 32.7 × NPP^2 - 0.011 Phytase × NPP (RMSE = 4.722, R^2 = 0.904). (B) Feed intake (g/d) = 106.68 + 0.005 × Phytase + 6.238 × NPP - 0.1 × 10^{-6} × Phytase^2 - 0.011 Phytase × NPP (RMSE = 8.311, R^2 = 0.96). (C) Feed conversion ratio = 2.14 - 0.75 × 10^{-6} × Phytase – 0.670 × NPP + 1.076 × NPP^2 + 0.185 × 10^{-6} × Phytase × NPP (RMSE = 0.204, R^2 = 0.92). (D) Egg mass (g/d/bird) = 50.42 + 0.004 × Phytase + 18.416 × NPP - 0.98 × 10^{-8} × Phytase^2 – 24.571 × NPP^2 - 0.009 Phytase × NPP (RMSE = 4.73, R^2 = 0.90). (E) Shell proportion to egg weight (%) = 9.48 + 0.18 × 10^{-8} × Phytase^2 - 0.57 × 10^{-5} × Phytase × NPP (RMSE = 0.51, R^2 = 0.87). (F) Albumen proportion to egg weight (%) = 65.89 - 0.001 × Phytase – 16.852 × NPP + 0.008 × Phytase × NPP (RMSE = 1.50, R^2 = 0.94).

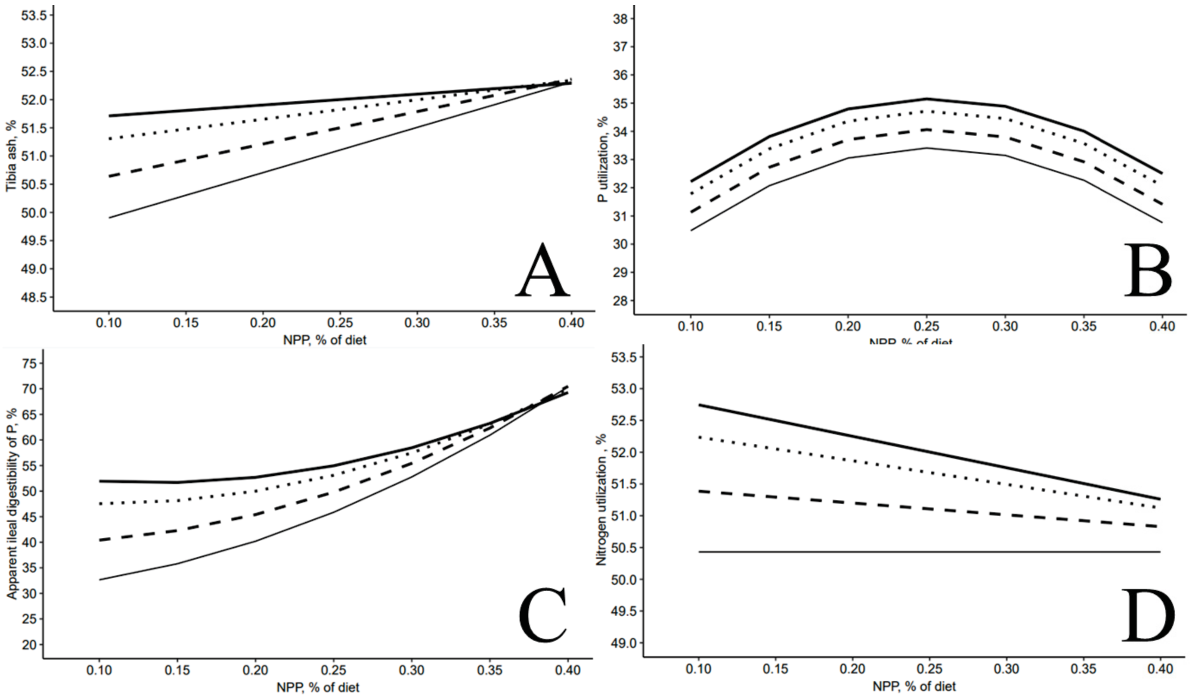

FIGURE 3. Response of tibia ash, utilization and apparent ileal digestibility of phosphorus (P), energy metabolizable and nitrogen (N) utilization to dietary percentage of nonphytate phosphorus (NPP) in laying hens fed with different dietary supplemental phytase.

Description of curve types and equations for tibia ash, nitrogen utilization and phosphorus utilization, and digestibility: Curve types: Solid Thin Curve – 0 FTU/kg of diet; Dashed Thick Curve – 150 FTU/kg of diet; Dotted Curve – 300 FTU/kg of diet; Solid Thick Curve – 400 FTU/kg of diet. (A) Tibia ash (%) = 49.10 + 0.007 × Phytase + 8.025 × NPP - 0.15 × 10^{-7} × $Phytase^2$ - 0.015 × Phytase × NPP (RMSE = 5.95, R^2 = 0.96). (B) P utilization (%) = 25.43 + 0.004 × Phytase + 62.919 × NPP – 123.94 × NPP^2 (RMSE = 7.18, R^2 = 0.75). (C) Apparent ileal digestibility of P (%) = 30.14 + 0.071 × Phytase - 0.14 x 10^{-6} × $Phytase^2$ + 251.469 × NPP^2 – 0.170 × Phytase × NPP (RMSE = 9.25, R^2 = 0.81). (D) N utilization (%) = 50.43 - 0.008 × Phytase – 0.23 × 10^{-7} × $Phytase^2$ - 0.012 × Phytase × NPP (RMSE = 10.10, R^2 = 0.96).

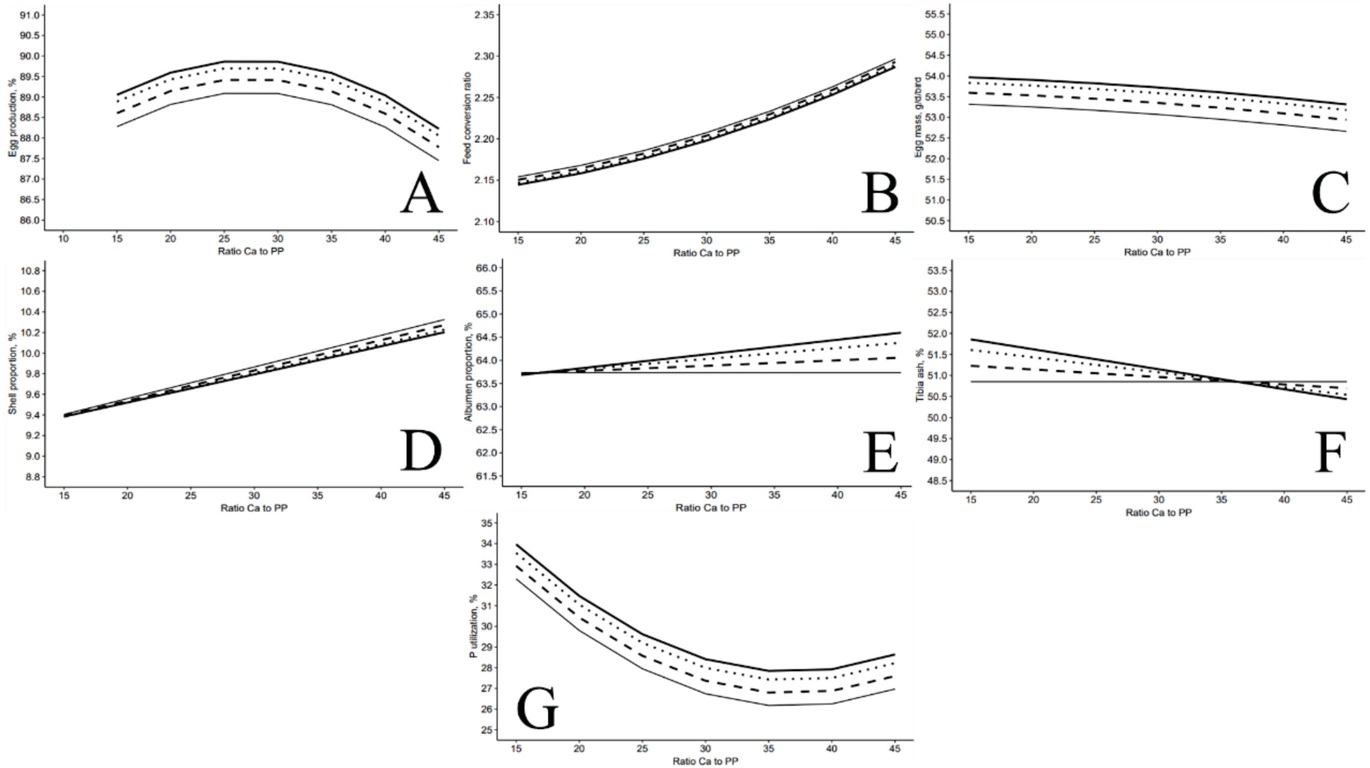

FIGURE 4. Response of egg production, feed conversion ratio, egg mass, shell and albumen proportion to egg weight (EW), tibia ash and phosphorus utilization to the ratio of calcium (Ca) to phytate phosphorus (PP) in laying hen diets fed with different dietary supplemental phytase.

Description of curve types and equations egg production, feed conversion ratio, egg mass, shell and albumen proportion to EW, tibia ash and phosphorus utilization to the ratio of Ca to PP: Curve types: Solid Thin Curve – 0 FTU/kg of diet; Dashed Thick Curve – 150 FTU/kg of diet; Dotted Curve – 300 FTU/kg of diet; Solid Thick Curve – 400 FTU/kg of diet. (A) Egg production (%) = 85.52 + 0.003 × Phytase + 0.254 × Ca:PP - 0.12 × 10^{-7} × Phytase^2 – 0.49 × 10^{-4} × Ca:PP^2 (RMSE = 4.720, R^2 = 0.895). (B) Feed conversion ratio = 2.14 - 0.23 × 10^{-6} × Phytase – 0.006 × Ca:PP + 0.79 × 10^{-6} × Ca:PP^2 (RMSE = 0.158; R^2 = 0.956). (C) Egg mass (g/d/bird) = 53.40 + 0.002 × Phytase – 0.92 × 10^{-8} - 0.36 × 10^{-5} × Ca:PP^2 (RMSE = 3.708, R^2 = 0.907). (D) Shell proportion to EW (%) = 8.95 + 0.031 × Ca:PP + 0.18 × 10^{-8} × Phytase^2 - 0.85 × 10^{-7} × Phytase × Ca:PP (RMSE = 0.411, R^2 = 0.880). (E) Albumen proportion to EW (%) = 63.73 - 0.001 × Phytase + 0.76 × 10^{-6} × Phytase × Ca:PP (RMSE = 1.255, R^2 = 0.917). (F) Tibia ash (%) = 50.85 + 0.004 × Phytase - 0.12 × × 10^{-5} × Phytase × Ca:PP (RMSE = 5.153, R^2 = 0.956). (G) P utilization (%) = 43.63 + 0.004 × Phytase - 0.948 × Ca:PP + 0.12 × 10^{-3} × Ca:PP^2 (RMSE = 5.873, R^2 = 0.752).

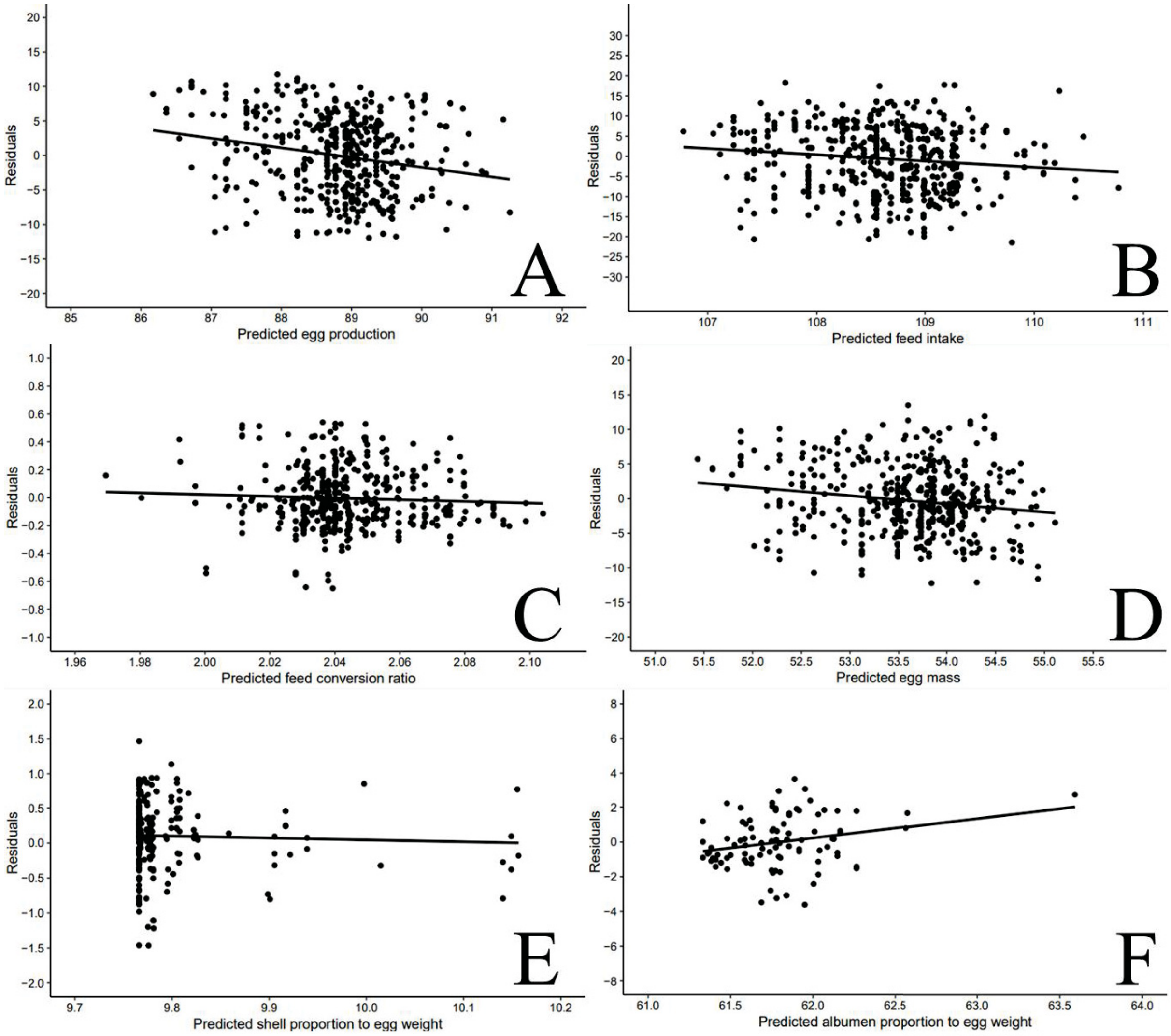

FIGURE 5. Plot of residuals (observed minus predicted) against predicted egg production (EP), feed intake (FI), and feed conversion ratio (FCR), egg mass (EM), and shell and albumen proportion to egg weight (EW) from the phytase and non-phytate phosphorus mixed model analysis.

The line represents the regression of residuals on predicted EP (A) [Y = 123.7 - 1.394 × predicted EP; $R^2 = 0.004$; $P = < 0.001$], on predicted FI (B) [Y = 167.8 - 1.550 × predicted FI; $R^2 = 0.001$; $P = 0.008$], and on predicted FCR (C) [Y = 1.24 - 0.611 × predicted FCR; $R^2 = 0.0001$; $P = 0.244$], on predicted EM (D) [Y = 63.9 - 1.197 × predicted EM; $R^2 = 0.003$; $P = 0.001$], on predicted shell proportion to EW (E) [Y = 2.73 - 0.269 × predicted shell proportion to EW; $R^2 = 0.000$; $P = 0.552$], and on predicted albumen proportion to EW (F) [Y = -70.8 + 1.145 × predicted albumen proportion to EW; $R^2 = 0.005$; $P = 0.016$].

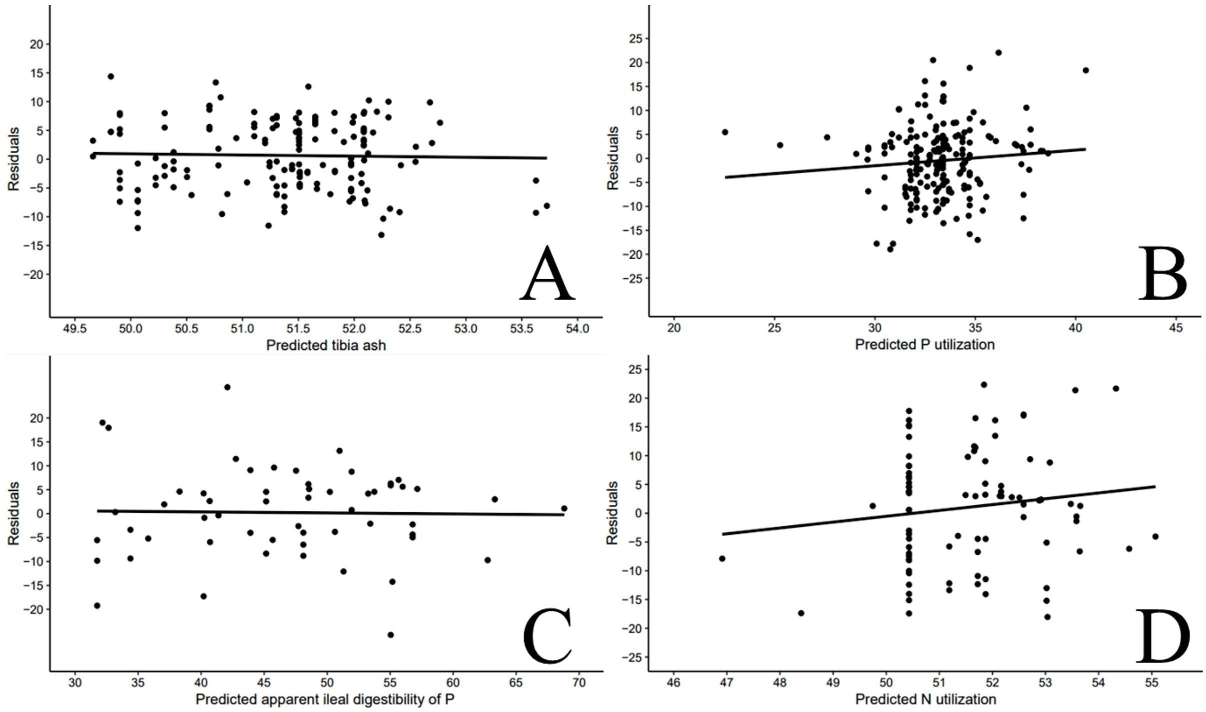

FIGURE 6. Plot of residuals (observed minus predicted) against predicted tibia ash (TA), phosphorus (P) utilization and apparent ileal digestibility (AID) of P, and nitrogen utilization from the phytase and non-phytate phosphorus mixed model analysis.

The line represents the regression of residuals on predicted TA (A) [Y = 11.0 - 0.201 × predicted TA; R^2 = 0.000; P = 0.725], on predicted P utilization (B) [Y = -11.32 + 0.326 × predicted P utilization; R^2 = 0.000; P = 0.191], on predicted apparent ileal digestibility of P (C) [Y = 1.19 - 0.201 × predicted AP; R^2 = 0.000; P = 0.886], and on predicted nitrogen utilization (D) [Y = -51.3 + 1.015 × predicted nitrogen utilization; R^2 = 0.001; P = 0.209].

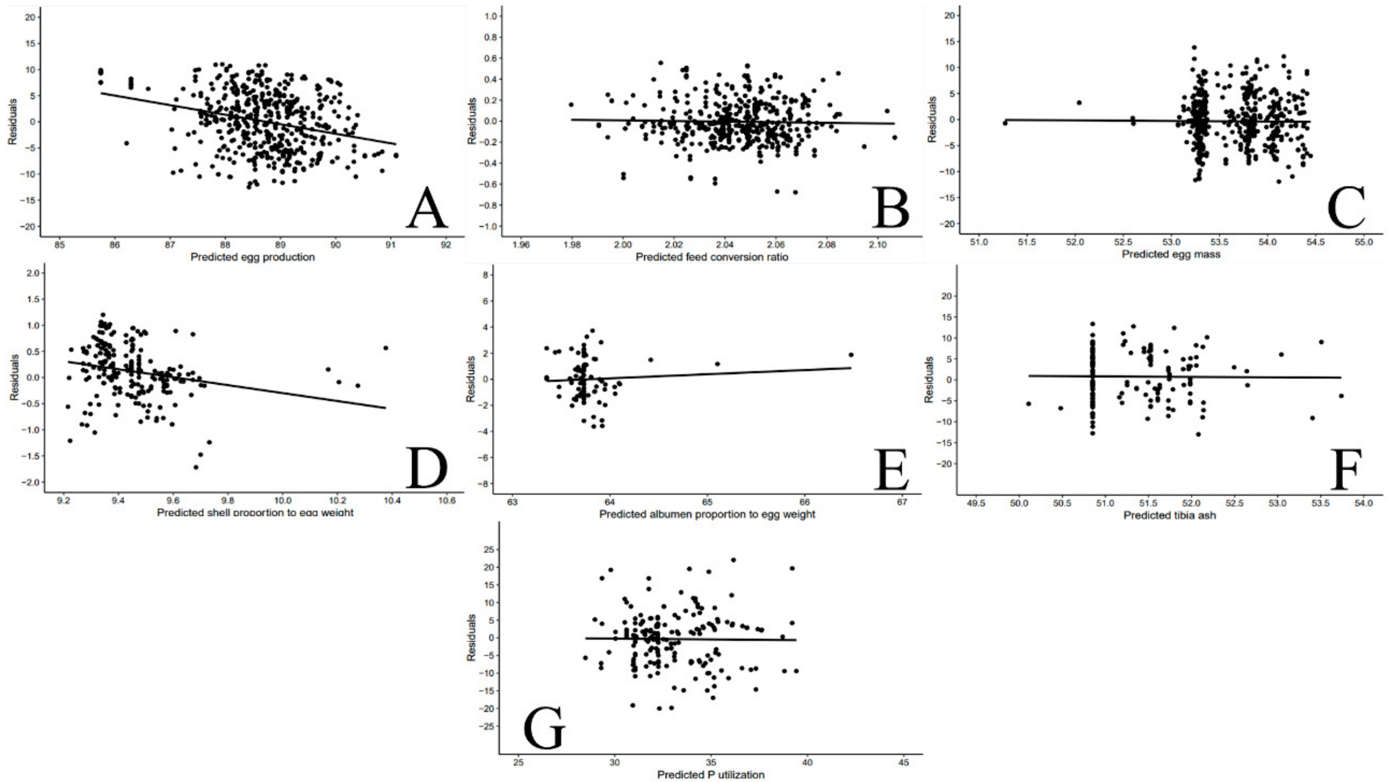

FIGURE 7. Plot of residuals (observed minus predicted) against predicted egg production (EP), feed conversion ratio (FCR), egg mass (EM), shell and albumen proportion to egg weight (EW), tibia ash (TA) and phosphorus (P) utilization from the phytase and calcium to phytate phosphorus ratio mixed model analysis.
The line represents the regression of residuals on predicted EP (A) [Y = 134.0 - 1.510 × predicted EP; $R^2 = 0.004$; $P = < 0.001$], on predicted FCR (B)[Y = 0.58 - 2.87 × predicted FCR; $R^2 = <0.0001$; $P = 0.568$], on predicted EM (C)[Y = 5.5 - 0.109 × predicted EM; $R^2 = <0.0001$; $P = 0.829$], on predicted shell proportion to EW (D) [Y = 7.31 - 0.760 × predicted shell proportion to EW; $R^2 = 0.002$; P = 0.001], on predicted albumen proportion to EW (E)[Y = -20.3 + 0.318 × predicted albumen proportion to EW; $R^2 = <0.0001$; $P = 0.493$], on predicted TA (F)[Y = 16.4 - 0.307 × predicted TA; $R^2 = <0.0001$; $P = 0.667$], and on predicted P utilization (G) [Y = 3.91 - 0.130 × predicted P utilization; $R^2 = <0.0001$; $P = 0.585$].

TABLE 1. Summary of phytase supplementation studies on layer production in terms of location, journal, and year of publication.

Code	Study	Country	Journal	Year of publication
1	Kamińska	Poland	Journal of Animal and Feed Sciences	1996
2	Kamińska et al.	Poland	Journal of Animal and Feed Sciences	1997
3	Van Der Klis et al.	Netherlands	Poultry Science	1997
4	Carlos et al.	United States	Poultry Science	1998
5	Punna et al.	United States	Poultry Science	1999
6	Scott et al.	United States	Poultry Science	1999
7	Um et al.	South Korea	Poultry Science	1999
8	Jacob et al.	Canada	Asian-Australasian Journal of Animal Sciences	2000
9	Keshavarz	United States	Poultry Science	2000a
10	Keshavarz	United States	Poultry Science	2000b
11	Sohail et al.	United States	Journal of Applied Poultry Research	2000
12	Jalal et al.	United States	Poultry Science	2001
13	Scott et al.	Canada	Canadian Journal of Animal Science	2001
14	Ceylan et al.	United States	Poultry Science	2003
15	Jamroz et al.	Poland	Journal of Animal and Feed Sciences	2003
16	Keshavarz	United States	Poultry Science	2003
17	Lim et al.	South Korea	Poultry Science	2003
18	Roland Sr et al.	United States	Journal of Applied Poultry Research	2003
19	Çabuk et al.	Turkey	South African Journal of Animal Science	2004
20	Keshavarz et al.	United States	Poultry Science	2004
21	Casartelli et al.	Brazil	Brazilian Journal of Poultry Science	2005
22	Ciftci et al.	Turkey	International Journal of Poultry Science	2005
23	Francesch et al.	Spain	British Poultry Science	2005
24	Liebert et al.	Germany	Poultry Science	2005
25	Musapuor et al.	Iran	International Journal of Poultry Science	2005
26	Panda et al.	India	British Poultry Science	2005
27	Zyła et al.	Poland	Journal of Animal and Feed Sciences	2005
28	Silversides et al.	Canada	Poultry Science	2006
29	Liu et al. et al.	China	Poultry Science	2007
30	Pereira et al.	Brazil	Acta Scientiarum - Animal Sciences	2007
31	Tossenberger et al.	United States	Poljoprivreda	2007
32	Kannan et al.	India	Research Journal of Agriculture and Biological Sciences	2008
33	Nezhad et al.	Iran	Asian Journal of Animal and Veterinary Advances	2008
34	Silva et al.	Brazil	Brazilian Journal of Animal Science	2008
35	Silva et al.	Brazil	Brazilian Journal of Animal Science	2008
36	Agbede et al.	Germany	Archiv Fur Geflugelkunde	2009a
37	Agbede et al.	Germany	British Poultry Science	2009b
38	Ligeiro et al.	Brazil	Brazilian Journal of Animal Science	2009
39	Silversides et al.	Canada	Journal of Applied Poultry Research	2009
40	Viana et al.	Brazil	Brazilian Journal of Animal Science	2009
41	Yan et al.	South Korea	Asian-Australasian Journal of Animal Sciences	2009
42	Ziaei et al.	Iran	Pakistan Journal of Biological Sciences	2009
43	Junqueira et al.	Brazil	Brazilian Journal of Animal Science	2010
44	Lima et al.	Brazil	Brazilian Journal of Animal Science	2010
45	Hassanien et al.	Egypt	Asian Journal of Poultry Science	2011
46	Kozlowski et al.	Poland	Journal of Animal and Feed Sciences	2011a
47	Kozlowski et al.	Poland	Veterinarija ir Zootechnika	2011b
48	Lei et al.	China	British Poultry Science	2011
49	Meyer et al.	United States	Journal of Applied Poultry Research	2011
50	Ruesga Gutiérrez et al.	Mexico	Australian Journal of Basic and Applied Sciences	2011

51	Vieira et al.	Brazil	Brazilian Journal of Poultry Science	2011
52	Brunelli et al.	Brazil	Semina-Ciencias Agrarias	2012
53	Ebling et al.	Brazil	Ciencia Rural	2012
54	Englmaierová et al.	Czech Republic	Czech Journal of Animal Science	2012
55	Koksal et al.	Turkey	International Journal of Poultry Science	2012
56	Sari et al.	Turkey	International Journal of Poultry Science	2012
57	Tahmasbi et al.	Iran	British Poultry Science	2012
58	Deniz et al.	Turkey	British Poultry Science	2013
59	Gao et al.	China	Animal Feed Science and Technology	2013
60	Wang et al.	China	British Poultry Science	2013
61	Englmaierová et al.	Czech Republic	Czech Journal of Animal Science	2014
62	Rao et al.	India	Animal Feed Science and Technology	2014
63	Englmaierová et al.	Czech Republic	Czech Journal of Animal Science	2015
64	Mirzaee et al.	Iran	South African Journal of Animal Science	2015
65	Mohebbifar et al.	Iran	Animal Feed Science and Technology	2015
66	Vargas-Rodríguez et al.	Mexico	International Journal of Poultry Science	2015
67	Englmaierová et al.	Czech Republic	Italian Journal of Animal Science	2017
68	Musilova et al.	Czech Republic	Czech Journal of Animal Science	2017
69	Martinez Rojas et al.	Mexico	Journal of Applied Animal Research	2018
70	Rojas et al.	Mexico	Veterinaria Mexico	2018
71	Skrivan et al.	Czech Republic	Czech Journal of Animal Science	2018
72	Taylor et al.	England	British Poultry Science	2018
73	Bello et al.	Canada	Poultry Science	2019
74	Fernández et al.	Mexico	Poultry Science	2019
75	Habibollahi et al.	Iran	Journal of Applied Poultry Research	2019
76	Baghban-Kanani et al.	Iran	Asian-Australasian Journal of Animal Sciences	2020
77	Bello et al.	Canada	Poultry Science	2020
78	Pongmanee et al.	Germany	Poultry Science	2020
79	Farias et al.	Brazil	Brazilian Journal of Poultry Science	2021
80	Javadi et al.	Spain	Animals	2021
81	Jing et al.	Canada	Animal	2021
82	Zhai et al.	France	Poultry Science	2022
83	Eltahan et al.	South Korea	Poultry Science	2023
84	Hamed et al.	Iraq	Archives of Razi Institute	2023
85	Hervo et al.	France	Poultry Science	2023
86	Jlali et al.	Spain	Journal of Poultry Science	2023
87	Moura et al.	Brazil	Poultry Science	2023
88	Pirzado et al.	China	Animals	2024
89	Rama Rao et al.	United States	British Poultry Science	2024

TABLE 2. Diet composition variation included in the dataset for laying hen experiments.

	N[1]	Mean[2]	Minimum	Maximum	SD
Year of publication	89	2009	1996	2024	7
Age at starting phytase treatment, weeks	89	45.3	24	80	14.5
Phytase treatment duration, weeks	89	7.52	1	40	7.25
Phosphorus total, g/kg of diet	89	0.44	0.30	1.50	0.11
Phytate phosphorus, g/kg of diet	89	0.22	0.05	1.03	0.06
Calcium, g/kg of diet	89	3.64	0.83	6.30	0.49
Ca:NPP	89	18.0	3.61	76.4	7.78
Metabolic energy, Kcal/kg of diet	89	2,763	2,530	3,108	98.1
Crude protein, g/kg of diet	89	16.3	13.0	18.7	0.97
Vitamin D3, IU/kg of diet	89	2,619	500	6,000	1,090
Copper, mg/kg of diet	89	11	5	125	14
Zn, mg/kg of diet	89	70	10	169	25
Iron, mg/kg of diet	89	57	9	160	24
Mn, mg/kg of diet	89	80	8	800	66
I, mg/kg of diet	89	1.1	0.2	7.7	0.9
Se, mg/kg of diet	89	0.22	0.05	0.87	0.11
Sodium, g/kg of diet	89	0.16	0.03	0.55	0.06
Total lysine, g/kg of diet	89	0.82	0.31	1.15	0.09
Digestible lysine, g/kg of diet	89	0.72	0.27	0.97	0.09
Total methionine, g/kg of diet	89	0.37	0.24	0.50	0.05
Digestible methionine, g/kg of diet	89	0.34	0.22	0.48	0.06
Total methionine + cysteine, g/kg of diet	89	0.65	0.38	0.93	0.07
Digestible methionine + cysteine, g/kg of diet	89	0.58	0.38	0.76	0.07
Total threonine, g/kg of diet	89	0.61	0.23	0.89	0.07
Digestible threonine, g/kg of diet	89	0.53	0.20	0.84	0.07

[1]The number of studies that reported poultry diet composition.
[2] Year of publication: Mode.

TABLE 3. Summary statistics for the data used in the meta-analysis[1].

Item	n	N	Mean	SD	Min.	Max.
Independent variable						
Phytase (FTU/kg[2])	889	89	287	347	0.00	2,000
NPP[2] (%)	889	89	0.22	0.09	0.08	0.55
Ca to PP ratio	889	89	17.42	6.63	3.61	76.4
Dependent variable						
Egg production, %	477	63	88.37	5.33	76.25	99
Feed intake, g/d	418	65	108.03	8.36	86.80	126.91
FCR[4], by egg mass	337	61	2.04	0.21	1.39	2.58
Egg mass, g/d	411	61	53.06	4.62	41.60	67.10
Shell proportion, %	177	33	9.56	0.46	7.94	10.94
Albumen proportion, %	90	20	63.70	1.59	60.21	68.35
Tibia ash, %	141	24	52.12	5.93	38.10	64.20
Phosphorus utilization, %	188	28	32.76	7.89	11.80	58.87
Apparent ileal digestibility of phosphorus, %	53	11	47.72	12.37	12.50	69.80
N utilization, %	88	16	52.56	10.37	31.00	75.99

[1]n, number of observations; N, number of publications; SD, standard deviation; Min., minimal value of the parameter: Max., maximal value of the parameter. [2] One phytase unit (FTU) was defined as the amount of enzyme that releases one µmol of inorganic P from 5.1 mmol/l sodium phytate per minute at pH 5.5 at 37◦C (Dersjant-Li et al., 2015). [3]NPP, non-phytic phytate; [4]FCR, feed conversion ratio.

TABLE 4. The effects of phytase supplementation and dietary non-phytate phosphorus (NPP), along with their parameter estimates for the mixed model applied to laying hen performance and egg quality traits.

Laying hens performance models									
Model	Egg production (%)			Average daily feed intake (g/d)			Feed conversion ratio		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	84.90	1.13	<0.001	106.68	0.94	<0.001	2.14	0.036	<0.001
Phytase	0.005	0.0008	<0.001	0.005	0.001	<0.001	-0.75×10^{-6}	0.20×10^{-6}	<0.001
NPP	23.176	8.204	0.005	6.238	1.952	0.002	-0.670	0.247	0.007
Phytase^2	-0.12×10^{-7}	0.48×10^{-8}	0.020	-0.1×10^{-6}	0.1×10^{-6}	0.011	-	-	NS
NPP^2	-32.663	15.289	0.033	-	-	NS	1.076	0.471	0.023
Phytase x NPP	-0.011	0.002	<0.001	-0.011	0.003	<0.001	0.185×10^{-6}	0.71×10^{-6}	0.010
R^2		0.90			0.96			0.92	
RMSE*		4.72			8.31			0.20	
Egg quality traits models									
	Egg mass (g/d/bird)			Shell proportion (%)			Albumen proportion (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	50.42	1.00	<0.001	9.48	0.08	<0.001	65.89	0.92	<0.001
Phytase	0.004	0.0007	<0.001	-	-	NS	-0.001	0.0004	0.002
NPP	18.416	7.341	0.012	-	-	NS	-16.852	6.743	0.014
Phytase^2	-0.98×10^{-8}	0.40×10^{-8}	0.015	0.18×10^{-8}	0.64×10^{-9}	0.006	-	-	NS
NPP^2	-24.571	13.863	0.077	-	-	NS	27.795	11.519	0.018
Phytase x NPP	-0.009	0.002	<0.001	-0.57×10^{-5}	-0.27×10^{-5}	0.041	0.008	0.002	<0.001
R^2		0.90			0.87			0.94	
RMSE*		4.73			0.51			1.50	

*RMSE, Root mean square error

TABLE 5. The effects of phytase supplementation and dietary non-phytate phosphorus (NPP), along with their parameter estimates for the mixed model applied to tibia concentration and phosphorus (P), and nitrogen (N) digestibility of laying hen.

Tibia ash content and nutrient digestibility models												
Model	Tibia ash (%)			P utilization (%)			Apparent ileal digestibility of P (%)			N utilization (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	49.10	1.17079	<0.001	25.43	2.82	<0.001	30.14	4.31	<0.001	50.43	2.18	<0.001
Phytase	0.007	0.001	<0.001	0.004	0.001	<0.001	0.071	0.011	<0.001	0.008	0.002	<0.001
NPP	8.025	2.398	0.001	62.919	23.084	0.007	-	-	NS	-	-	NS
Phytase^2	-0.15×10^{-7}	0.73×10^{-8}	0.004	-	-	NS	-0.14×10^{-6}	0.60×10^{-7}	0.024	-0.23×10^{-7}	0.12×10^{-7}	0.062
NPP^2	-	-	NS	-123.943	44.544	0.006	251.469	137.098	0.073	-	-	NS
Phytase x NPP	-0.015	0.005	0.004	-	-	NS	-0.170	0.066	0.012	-0.012	0.004	0.006
R^2		0.96			0.75			0.81			0.96	
RMSE		5.95			7.18			9.25			10.10	

*RMSE, Root mean square error

TABLE 6. The effects of phytase supplementation and the calcium to phytate phosphorus (Ca:PP) ratio in laying hen diets, including parameter estimates from the mixed model applied to laying performance, tibia ash content, egg quality, and phosphorus (P) digestibility in laying hen.

Laying hens performance and tibia ash content models

Model	Egg production (%)			Feed conversion ratio			Egg mass (g/d/bird)			Tibia ash (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	85.525	1.51	<0.001	2.136	0.040	<0.001	53.398	0.470	<0.001	50.852	1.053	<0.001
Phytase	0.003	0.001	<0.001	-0.2×10^{-4}	0.7×10^{-5}	0.001	0.002	0.0005	<0.001	0.004	0.001	<0.001
Ca to PP ratio	0.254	0.117	0.030	-0.006	0.002	0.009	-	-	NS	-	-	NS
Phytase^2	-0.1×10^{-6}	0.5×10^{-7}	0.014	-	-	NS	-0.9×10^{-6}	0.4×10^{-6}	0.018	-	-	NS
Ca to PP ratio^2	-0.005	0.002	0.011	0.7×10^{-4}	0.3×10^{-4}	0.004	-0.4×10^{-3}	0.2×10^{-3}	0.056	-	-	NS
Phytase x Ca:PP ratio	-	-	NS	-	-	NS	-	-	NS	0.1×10^{-3}	0.5×10^{-4}	0.028
R^2		0.89			0.96			0.91			0.96	
RMSE*		4.71			0.16			3.71			5.15	

Egg quality traits and phosphorus digestibility models

	Shell proportion (%)			Albumen proportion (%)			P utilization (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	8.946	0.160	<0.001	63.733	0.306	<0.001	43.629	6.362	<0.001
Phytase	-	-	NS	-0.1×10^{-2}	0.3×10^{-3}	<0.001	0.004	0.001	<0.001
Ca to PP ratio	0.031	0.008	<0.001	-	-	NS	-0.948	0.503	0.063
Phytase^2	0.2×10^{-6}	0.6×10^{-7}	<0.001	-	-	NS	-	-	NS
Ca to PP ratio^2	-	-	NS	-	-	NS	0.013	0.007	0.076
Phytase x Ca:PP ratio	0.8×10^{-5}	0.4×10^{-5}	<0.001	0.8×10^{-4}	0.2×10^{-4}	<0.001	-	-	NS
R^2		0.88			0.94			0.75	
RMSE*		0.41			1.25			5.84	

*RMSE, Root mean square error

TABLE 7. Calculated optimum levels of non-phytate phosphorus (NPP) (percentage of diet) required to maximize the response of egg production, egg mass, and minimize the feed conversion ratio (FCR) in diets with or without phytase for laying hens (age 24–80 weeks).

	Phytase (FTU /kg)[1]			
Item	0	150	300	400
Egg production, %	0.35	0.33	0.30	0.29
Egg mass, g/hen/day	0.37	0.35	0.32	0.30
FCR	0.31	0.30	0.29	0.28

[1] One phytase unit (FTU) was defined as the amount of enzyme that releases one µmol of inorganic P from 5.1 mmol/l sodium phytate per minute at pH 5.5 at 37◦C (Dersjant-Li et al., 2015).

CHAPTER IV - IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, TIBIA ASH CONTENT, AND NUTRIENT DIGESTIBILITY IN BROILERS FROM 1 TO 21 DAYS POST-HATCH: A META-ANALYSIS

ABSTRACT

Balancing dietary phosphorus (P) levels in broiler diets to address environmental and economic concerns has been a challenge over the last decades. Numerous studies have investigated the effects of phytase (PHY), non-phytate phosphorus (NPP), and the dietary calcium (Ca) to NPP ratio (Ca:NPP) on broiler performance with highly variability results reported in the literature. To quantify the effect of PHY supplementation on both male and female broilers, two separate meta-analyses were conducted. The first meta-analysis included 98 studies involving 106,476 male broilers, while the second analyzed 7 studies with 7,052 female broilers, all aged 1 to 21 days post-hatching. The collected data were analyzed using a mixed model, incorporating the effects of PHY, dietary NPP, and Ca:NPP levels, as well as their interactions. The models evaluated average daily gain (ADG) and average daily feed intake (ADFI) in female broilers, as well as ADG, ADFI, feed conversion ratio (FCR), tibia ash content, and the apparent ileal digestibility (AID) of P (AIDP), nitrogen (AIDN), and amino acids in male broilers. PHY supplementation significantly ($P<0.05$) enhanced the growth performance of both male and female broilers. In male broilers, PHY also improved tibia ash content, AIDP, AIDN, and positively affected the AID of amino acids, with a more pronounced effect on amino acids with lower inherent digestibility, such as cysteine and threonine. Additionally, increasing NPP levels led to improvements in growth performance and tibia ash content. However, high dietary Ca:NPP ratios negatively affected all measured parameters, particularly in diets without PHY supplementation. The findings highlight the positive impact of PHY supplementation, reinforcing its benefits in improving performance and nutrient utilization in both male and female broilers during the starter phase.

Keywords: Broiler, phytase, meta-analysis, starter phase, digestibility.

1. INTRODUCTION

Phosphorus (P) is an essential nutrient for poultry, supporting growth and bone formation, as well as the development of the nervous system, nucleic acid synthesis, and energy metabolism (VALENTE JUNIOR et al., 2024). In combination with calcium (Ca), P plays a key role in skeletal formation and mineralization, serving as a reserve that can be mobilized to support nearly all metabolic processes, ensuring proper bone development and strength (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; VALENTE JUNIOR et al., 2024). However, the availability of P from plant-based feed ingredients is limited, as approximately two-thirds of P is bound to phytic acid [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$] (SOMMERFELD et al., 2018; SELLE et al., 2023).

Poultry, like other monogastric animals, are unable to efficiently utilize phytate-P (PP) because of their lack of sufficient endogenous enzyme activity (SOBOTIK et al., 2023). Furthermore, PP can chelate with other minerals, amino acids, and enzymes in the gastrointestinal tract, inhibiting their activity and reducing nutrient digestibility (SINGH AND SATYANARAYANA, 2015; VALENTE JUNIOR et al., 2024). A high dietary Ca content may further limit P bioavailability by forming insoluble complexes with PP (SELLE et al., 2023). To meet the daily nutritional requirements of broilers, other inorganic P sources are commonly added to poultry diets (HERVO et al. 2023). However, these sources are expensive and non-renewable (BOUGOUIN et al., 2014). Beyond the increased feed costs, excessive dietary P may be excreted in manure, contributing to environmental issues, such as surface water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023).

Since its introduction, phytase (PHY) has become one of the most widely used exogenous enzymes in poultry nutrition (Selle et al., 2023). Exogenous PHY may hydrolyze $InsP_6$ into lower myo-inositol phosphate isomers and inorganic phosphate, thereby enhancing the digestibility and utilization of P, Ca, minerals, amino acids and energy (COWIESON et al., 2006; AKTER et al., 2016; WALK et al., 2018). Although the effects of supplemental PHY on broiler growth performance, mineralization, and nutrient digestibility have been extensively studied, significant heterogeneity effect exists across individual experiments and meta-analysis (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; FARIDI et al., 2015; KERMANI et al., 2023; NUAMAH et al., 2024). Several aspects may influence in the effect of PHY supplementation in broiler diets, such as age and broiler sex. Assessing the effect of PHY during the starter phase of broilers, typically from 1 to 21 days post-hatching, is particularly important, as nutrient utilization and PHY efficacy can vary with age (BABATUNDE et al., 2019a; 2019b). This phase is marked by rapid growth and serves as the foundation for meat deposition in later stages (BATAL AND PARSONS, 2002; BABATUNDE et al., 2019b). Broilers exhibit higher P utilization during this period, potentially enhancing PHY efficacy, particularly toward the end of the second week of growth (BABATUNDE et al., 2019b). Moreover, advances in the genetic selection have resulted in distinct differences in growth performance, carcass yield, and nutrient requirements between male and female broilers (ENGLAND et al., 2023), potentially impacting P digestibility (RAVINDRAN et al., 2004) and PHY efficacy.

Evaluating the impact of exogenous PHY on performance and nutrient digestibility is crucial for optimizing inorganic P supplementation, enhancing sustainability, and improving cost-effectiveness (BOUGOUIN et al., 2014). Systematic literature reviews and meta-analyses are relevant tools for addressing variability across studies. Meta-analysis is a relevant approach for analyzing complex phenomena because it uses quantitative methods that combine data from previous independent studies, providing more robust and reliable inferences (ST-PIERRE, 2007). This approach can transform research findings into practical knowledge (POLYCARPO et al., 2017). By systematically accounting for heterogeneity across studies, it offers a broader and more comprehensive understanding of the topic, surpassing the limitations of single experiments that reflect specific experimental conditions (LOVATTO et al., 2007).

Previous meta-analyses have focused on quantifying the effects of PHY supplementation on broiler performance, bone mineralization, and nutrient digestibility (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; FARIDI et al., 2015; COWIESON et al., 2017; KERMANI et al., 2023; NUAMAH et al., 2024). However, advancements in broiler genetic selection, the emergence of new generations of PHY, and numerous studies in this field have underscored the continuous need for updated analyses. Additionally, modelling the relationship between PHY and non-phytate phosphorus (NPP) requirements, as well as the dietary Ca to NPP ratio for optimal growth performance and skeleton mineralization in both male and female broilers during the starter phase, remains an area requiring further investigation. Therefore, this study aimed to estimate the effect of PHY supplementation on performance, tibia ash content, and nutrient digestibility in male and female broilers from 1 to 21 days post-hatching through a systematic review and meta-analysis approach.

2. MATERIAL AND METHODS

This study exclusively used data from the published literature and did not involve the use of animals, eliminating the need for approval from the Institutional Animal Care and Use Committee.

2.1. SEARCH STRATEGY AND DATA FILTERING

An online search was performed to identify indexed publications on the effect of PHY supplementation on performance, tibia ash content, and digestibility of P, nitrogen (N), and amino acids in male and female broiler chickens from 1 to 21 days post-hatching. The search, completed by December 2024, focused on peer-reviewed scientific articles published in databases such as PubMed, Web of Science, and Scopus. To ensure thorough coverage, the reference lists of the selected articles were also examined for additional relevant studies. The research question was developed using the "PICo" strategy (ANDRETTA et al., 2021), where the "Population" was defined as "broilers", the "Interest" as "phytase", and the "Context" as "performance" OR "tibia ash concentration" OR "phosphorus digestibility" OR "energy digestibility" OR "nitrogen digestibility" OR "amino acids digestibility". Synonyms and alternative English terms for the population and context were added to refine the final search strategy. PHY studies were exported to a reference management software (EndNote X9; Philadelphia, PA, USA) to identify and remove duplicate entries. The relevance and quality of the studies were assessed through a two-step process: initial screening of titles and abstracts, followed by a detailed review of the full texts.

The primary criteria for selecting publications were: (1) studies describing *in vivo* experiments involving commercial broiler chicken breeds, (2) experiments with non-supplemented control diets and diets supplemented with commercial PHY (3) studies reporting performance data, including average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), as well as the effects of PHY on tibia ash, P and Ca concentrations, and the apparent ileal digestibility (AID) of dry matter

(DM), P (AIDP), Ca (AIDCa), N (AIDN), and amino acids, and (4) Studies published after the year 2000.

Preselected manuscripts were excluded if they involved a sanitary challenge, did not specify the sex of the broilers or were performed using non-sexed broilers, combined PHY with another enzyme, lacked a control treatment, or had notable inconsistencies in the statistical design, methodology, or outcome data. Studies involving broiler diets with super-dosing PHY (i.e.,>2,500 FTU/kg) were excluded due to insufficient data. One phytase unit (FTU) was defined as the enzyme quantity that releases one μmol of inorganic P from 5.1 mmol/l sodium phytate per minute at pH 5.5 at 37°C (DERSJANT-LI et al., 2015). Only studies involving broilers aged 1 to 21 days post-hatching, or those that included experiments conducted partially within this period, were considered. To accurately assess the effects of PHY, only non-supplemented diets that matched the same NPP levels as the PHY-supplemented group were included in the meta-analysis.

Two meta-analyses were conducted to evaluate the effects of PHY supplementation in broilers aged 1 to 21 days. One analysis focused exclusively on male broilers, while the other examined female broilers. The screening process for both meta-analyses is presented in Figure 1. Initially, 5,710 references were identified across both meta-analyses, with successive exclusions made based on titles and abstracts. Full-text reviews were performed for 543 references in male broiler meta-analysis and 43 references in the female broiler meta-analysis to determine eligibility. After a detailed review, 445 publications were excluded from the male broiler meta-analysis, and 36 studies were removed from the female broiler meta-analysis, in accordance with the pre-established criteria. The final male broiler meta-analysis included 98 publications, while the female broiler meta-analysis comprised 7 publications. Notably, the studies by Scheideler et al. (2000) and Wu et al. (2004) included experiments involving both sexes and were included in both meta-analyses. The final lists of selected studies for the male and female meta-analyses are presented in Tables 1 and 2, respectively.

2.2. DATA SYSTEMATIZATION AND CODING

The methodology for constructing the database, encoding data, and defining dependent and independent variables followed the approaches described by Lovatto et al. (2007). To ensure consistency and comprehensiveness in dietary composition across studies, experiments that lacked complete dietary nutrient information were recalculated using the Practical Program for Formulation of Rations (PPFR; GARCIA-NETO, 2008), based on the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024). The variability in diet composition within the male and female broiler datasets is summarized in Table 3.

The AID values for DM, P, Ca, and amino acids were included in the meta-analysis dataset only when they were calculated in the study using the following equation:

$$AID\ (\%) = \left[1 - \left(\frac{Dietary\ marker_{feed}\ x\ Mineral\ _{digesta}}{Dietary\ marker_{digesta}\ x\ Mineral_{feed}}\right)\right]\ x\ 100$$

2.3. STATISTICAL ANALYSIS

A meta-analytical approach was used to model the dependent variables. Following the methodology described by Andretta et al. (2012), the meta-analysis was conducted through sequential analytical steps, including graphical, correlation, and variance-covariance analyses. All variance-covariance analyses were performed considering the study effect as a random-effect class variable in the model due to the differences among the studies in the meta-analysis database (ST-PIERRE, 2001). Mixed models were developed separately for male and female broilers aged from 1 to 21 days post-hatching. PHY and NPP, as well as PHY and Ca:NPP ratio, were assessed in separate models. Independent variables were tested, and only those showing statistical significance ($P < 0.05$) or tendency ($P < 0.10$) were maintained in the final models.

Following the methodology described by Létourneau-Montminy et al. (2010), the response of each dependent variable, including ADG, ADFI, FCR, tibia ash content, and AIDP, AIDN, and amino acids for the male broiler meta-analysis, and ADG and ADFI for the female broiler meta-analysis, were evaluated using a full quadratic model. The model assessed the effects of the main statistically significant independent variables, including PHY, NPP, and Ca:NPP ratio, for both male and female broiler meta-analyses, as shown below:

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{NPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{NPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{NPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk},\ [1]$$

and,

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{CaNPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{CaNPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{CaNPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk},\ [2],$$

where Y_{ijk} represents the value of the dependent variable Y in the experiment *i* with the level *j* of NPP in equation [1] or Ca:NPP in equation [2], and level *k* of PHY. The parameter α is the overall intercept with fixed effect, while α_i is the random effect of experiment *i*. Coefficients β_1 and β_2 represent the linear effects, β_3 and β_4 capture the quadratic effects, and β_5 demonstrates the interaction effect. The term e_{ijk} represents the residual error.

The distributions of random effects and residual errors were checked to follow a normal distribution. Internally studentized residuals (ISR) were analyzed to detect outliers, with values greater than 3 ($|ISR| > 3$) identified and excluded from further analysis (FARIDI et al. 2015; KERMANI et al., 2022). Across all models, more than 95% of ISR values fell within the range of -2 to 2, indicating a good fit of the models. Model accuracy was evaluated based on the significance levels of the estimated parameters, variance of the error estimate and their standard error (SE), coefficient of determination (R^2), and root mean square error (RMSE). Model adequacy was further assessed by plotting residuals (observed minus predicted values) against the predicted values of Y to check for linear prediction bias (ST-PIERRE, 2001). The optimization process was carried out using the partial derivatives of the models obtained for each dependent variable (LETOURNEAU-MONTMINY et al., 2010).

Data analysis was conducted using the general linear model (GLM) procedure in the Minitab 22 software (Minitab Inc., State College, PA, USA). Graphs were generated using the ggplot2 package in R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1. DATABASE CHARACTERIZATION

The meta-analysis for male broiler chickens aged 1 to 21 days post-hatching included 98 studies, encompassing a total population of 106,476 male broilers. Of the 780 treatments included in this meta-analysis, Cobb 500 was the most frequently observed breed (n=276), followed by Ross 308 (n=199), Cobb 400 (n=56), Ross 708 (n=34), Ross 708 x Hubbard (n=29), Arbor Acres (n=18), Cobb 700 (n=3), Ross PM3 (n=2), and VenCobb (n=2). Additionally, 161 treatments did not specify the broiler breed. PHY supplementation varied across the studies. Among the 410 treatments that used PHY, 28.5% utilized 3-phytase (EC 3.1.3.8), while 63.9% used 6-phytase (EC 3.1.3.26). The remaining 7.6% of treatments did not specify the PHY type. PHY enzymes were derived from various organisms, including *Escherichia coli* (47.6%), *Aspergillus niger* (26.6%), *Buttiauxella sp.* (5.6%), *Aspergillus oryzae* (4.9%), *Citrobacter braakii* (3.2%), *Peniophora lycii* (1.7%), *Serratia odorifera* (1.5%), *Trichoderma reesei* (1.4%), *Aspergillus niger* (0.7%), *Bacillus subtilis* (0.7%), *Enterobacter sakazakii* (0.7%), and *Aspergillus ficuum* (0.5%). Additionally, 4.9% of treatments did not specify the donor organism for the exogenous PHY.

Furthermore, the final dataset for the male broiler meta-analysis included studies conducted in the United States of America (USA) (n=26), followed by Brazil (n=22); New Zealand (n=7); Australia, and India (n=6); Spain (n=5); Canada (n=4); Turkey (n=3); China, Croatia, Iran, Pakistan, Thailand, and United Kingdom (n=2); and France, Malaysia, Netherlands, Peru, Republic of Korea, Scotland, and Taiwan (n=1). Most experiments used corn-soybean meal-based diets (81.3%), with smaller proportion using corn-wheat-soybean meal-based diets (11.4%) and wheat-soybean meal-based diets (7.3%). Only grain-based feedstuffs were the included in 69.6% of the diets, while the remaining 30.4% also included animal-based feedstuffs. The feed was presented in various forms: mash (38.3%), crumble (13.4%), pellet (8.5%), cold pellet (4.0%), crumble/pellet (3.8), or in unspecified forms (32.0%). Diets were classified as cold-pelleted diets when the maximum temperature during the pelleting process did not exceed 70°C (RAVINDRAN et al., 2008). Mineral P supplementation was primarily provided through dicalcium phosphate (78.1%), followed by monocalcium phosphate (20.1%), and tricalcium phosphate (1.8%). Moreover, the dataset for male broilers revealed that diets with a Ca:NPP ratio lower than 2:1 accounted for 6.3%, while 61.1% of the diets had a ratio between 2:1 and 3:1, diets with a Ca:NPP ratio between 3:1 and 4:1 constitute 21.3% of the dataset, while those exceeding 4:1 represented 11.3%.

The female broiler meta-analysis included 7 studies, covering a total population of 7,052 female broilers aged 1 to 21 days post-hatching. Of the 31 treatments included in this meta-analysis, Ross 308 was the most frequently reported breed (n=25), followed by Cobb 500 (n=6). Among the 22 treatments that used PHY, 4.5% utilized 3-phytase (EC 3.1.3.8), while 45.5% used 6-phytase (EC 3.1.3.26). The remaining 50% did not specify the PHY type. The exogenous phytase sources for female broilers included: *Escherichia coli* (45.5%), *Trichoderma ressei* (27.3%), *Aspergillus niger* (22.7%), and *Peniophora lycii* (4.5%). PHY inclusion levels ranged from 0 to 2,500 FTU/kg in both male and female broiler meta-analyses. The final dataset for the female broiler meta-analysis included studies conducted in China (n=2), New Zealand, Poland, Scotland, the United Kingdom, and the USA (n=1). Female broilers were predominantly fed corn-soybean meal-based diets (77.4%), with the remaining portion receiving wheat-soybean meal-based diets (22.6%). Grain-based feedstuffs alone were used in 51.6% of diets, while the remaining 48.4% incorporated animal-based feedstuffs. Feed presentation formats included: mash (58.1%), cold pellet (16.1%), or unspecified forms (25.8%). Diets were supplemented with mineral P sources, including dicalcium phosphate (64.0%), and monocalcium phosphate (36.0%). Additionally, 67.7% of the dataset included diets with a Ca:NPP ratio between 2:1 and 3:1, whereas the remaining 32.3% had a ratio between 3:1 and 4:1.

3.2. META-ANALYSIS RESULTS

Summary statistics for the dependent and independent variables included in the meta-analyses for both male and female broilers aged from 1 to 21 days are presented in Table 4. The effects of different levels of dietary supplemental PHY and NPP on ADG, ADFI, FCR, tibia ash content, AIDP, and AIDN in male broilers are detailed in Table 5 and illustrated in Figure 2. The effects of PHY supplementation, dietary NPP, and Ca:NPP ratio on the performance of female broilers are shown in Table 6 and in Figure 3. The effects of dietary PHY levels and Ca:NPP ratio on ADG, ADFI, FCR, tibia ash content, and AIDN in male broilers are presented in Table 7 and Figure 4. The effects of dietary PHY supplementation on the AID of various amino acids in male broilers are summarized in Table 8. The relationship between amino acid digestibility in the control diet/ingredient and the effect of PHY is shown in Figure 5. Optimal NPP levels required to maximize ADG, ADFI, and tibia ash content, and minimize the FCR in male broiler diets supplemented or not supplemented with PHY are provided in Table 9. Residual plots (observed minus predicted) from the PHY and NPP mixed model analyses for male and female broilers are shown in Figures 6 and 7, respectively. Residual plots from the PHY and Ca:NPP ratio models in male broilers are shown in Figure 8, while the residuals for the AID of various amino acids are presented in Figure 9.

3.3. IMPACT OF PHY SUPPLEMENTATION ON THE PERFORMANCE OF MALE AND FEMALE BROILERS

Dietary PHY supplementation increased the intercept for ADG and ADFI in both male and female broilers from 1 to 21 days post-hatching, while decreasing the intercept for FCR in male broilers with varying dietary levels of NPP and PHY. In the female broiler meta-analysis, PHY supplementation did not significantly affect FCR (P>0.10), and FCR was therefore excluded from further model development. In male broilers, the response of ADG to dietary NPP followed a quadratic pattern. However, increasing dietary NPP levels in female broilers did not result a significant effect (P>0.10). Exogenous PHY supplementation significantly increased ADG, with the greatest effects observed at lower dietary NPP levels (PHY × NPP; P<0.001). The optimal NPP levels required to maximize ADG in male broilers from 1 to 21 days of age were 4.81g/kg of diet for diets without PHY supplementation, and 4.51, 4.24, 3.95, and 3.66g/kg of diet for diets supplemented with 500, 1,000, 1,500, and 2,000 FTU/kg of PHY, respectively. In female broilers, PHY supplementation had significant quadratic effect (P=0.013), with the PHY plateau observed at 2,125 FTU/kg. Both male and female broilers were affected by PHY supplementation and the dietary Ca:NPP ratio. In male broilers, exogenous PHY showed a greater effect at higher dietary Ca:NPP ratios, with significant quadratic effect (P<0.001). In female broilers, only a linear effect was observed (P=0.008). A quadratic effect of the dietary Ca:NPP ratio was noted in both male and female broilers (P=0.004 and P<0.001, respectively). Additionally, PHY demonstrated greater efficiency in diets with higher Ca:NPP ratios in both male and female broiler meta-analyses (PHY × Ca:NPP; P<0.001).

The male broiler meta-analysis for ADFI revealed significant quadratic effect (P<0.05) of dietary NPP and PHY levels. PHY supplementation increased ADFI, with a more pronounced effect in low-NPP diets (PHY × NPP; P<0.001). The optimal NPP levels required to maximize ADG were 4.80 g/kg of diet for diets without PHY supplementation, and 4.47, 4.14, 3.80, and 3.47 g/kg of diet for diets supplemented with 500, 1,000, 1,500, and 2,000 FTU/kg of PHY, respectively. In female broilers, PHY displayed quadratic effect (P<0.05), with a plateau at 1,957 FTU/kg. Male and female broilers were impacted by the PHY supplementation and the dietary Ca:NPP ratio. PHY exhibited a significant quadratic effect in both male and female broilers (P<0.05). The dietary Ca:NPP ratio showed a significant linear effect in both groups (P<0.05).

Moreover, PHY showed higher effect in diets with higher Ca:NPP ratios in both male and female broiler meta-analyses (PHY × Ca:NPP; $P<0.001$).

Diets supplemented with PHY improved the FCR in male broilers. PHY and dietary NPP had significant quadratic effect ($P<0.05$) on FCR, with the most substantial impact observed in low-NPP diets (PHY × NPP; $P=0.007$). The optimal NPP levels to enhanced FCR were 4.79 g/kg of diet for diets without PHY supplementation, and 4.44, 4.09, 3.74, and 3.39 g/kg of diet for diets supplemented with 500, 1,000, 1,500, and 2,000 FTU/kg of PHY, respectively. The dietary Ca:NPP ratio and PHY supplementation also showed significant quadratic effect ($P<0.05$) on FCR in male broilers. FCR was more strongly influenced by PHY supplementation in diets with higher Ca:NPP ratios (PHY × Ca:NPP; $P<0.001$).

3.4. TIBIA ASH CONTENT AND NUTRIENT DIGESTIBILITY

In male broilers, tibia ash content was influenced by PHY supplementation and dietary NPP levels, showing significant quadratic effect ($P<0.05$). More pronounced effect of PHY supplementation on tibia ash content was observed at lower dietary NPP levels (PHY × NPP; $P<0.001$). The optimal NPP levels to maximized tibia ash content were 5.26 g/kg of diet for diets without PHY, and 4.99, 4.71, 4.42, and 4.15 g/kg of diet for diets supplemented with 500, 1,000, 1,500, and 2,000 FTU/kg of PHY, respectively. PHY supplementation and the Ca:NPP ratio significantly affected tibia ash content, following a quadratic pattern ($P < 0.05$). Additionally, PHY supplementation had a greater effect in diets with higher Ca:NPP ratios (PHY × Ca:NPP; $P<0.001$). In the female broiler meta-analysis, the impact of PHY supplementation on tibia ash content was not significant ($P>0.10$), and as a result, tibia ash content was excluded from subsequent model development.

In male broilers, PHY supplementation positively impacted AIDP and AIDN ($P<0.05$). However, dietary NPP levels and the interaction between PHY and NPP did not significantly affect AIDP or AIDN ($P>0.10$). A significant interaction between PHY and the Ca:NPP ratio was observed for AIDN, with a more pronounced effect of PHY in diets with lower Ca:NPP ratios. Additionally, PHY supplementation had a positive overall impact on AIDN ($P<0.05$). In the male broiler meta-analysis, the AID of Ca and energy were not significantly affected by PHY supplementation ($P>0.10$). Similarly, in the female broiler meta-analysis, AIDP, AIDN, and the AID of Ca, energy, and amino acids showed no significant effects (*P*>0.10). As result, these variables were excluded from further model development.

3.4.1 APPARENT ILEAL DIGESTIBILITY OF AMINO ACIDS

The mean digestibility coefficient of amino acids in the control diets/ingredients was 0.82, whereas in diets with added PHY (regardless of dose) it increased to 0.85, representing an average enhancement of 2.6 percentage points (*P*<0.001). Although PHY supplementation enhanced the digestibility of all amino acids, the degree of improvement varied, ranging from 1.1 percentage points for tryptophan (TRP) to 6.1 percentage points for cysteine (CYS). Figure 5 illustrates the relationship between amino acid digestibility in the control group and the average response to PHY. A quadratic fit (R^2=0.27; P<0.001) demonstrated that the effect of PHY was less pronounced in diets or ingredients with higher inherent amino acid digestibility. The equation was as follow: % change in amino acid digestibility with phytase = $88.42 - (181.9 \times C) + (94.71 \times C^2)$, where 'C' is the digestibility coefficient of the amino acid in the control diet.

Additionally, PHY significantly influenced the AID of several amino acids in male broilers from 1 to 21 days of age, such as: alanine (ALA), arginine (ARG), aspartic acid (ASP), cysteine (CYS), glutamic acid (GLU), glycine (GLY), histidine (HIS), isoleucine (ILE), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), of proline (PRO), serine (SER), threonine (THR), tryptophan (TRP), tyrosine (TYR), valine (VAL). There were insufficient data to assess the impact of PHY on the AID of asparagine (ASN) and glutamine (GLN). Moreover, dietary NPP and Ca:NPP ratio did not significantly influence the AID of any amino acids (P>0.10).

3.5. MODEL ADEQUACY

The residual plots against the predicted values, shown in Figures 6 and 7, indicate the adequacy of the model assessing dietary NPP levels in broilers fed different PHY levels. Residuals displayed a good distribution around zero across the different models, indicating a good fit. In the male broiler meta-analysis, the residuals differed from the predicted values by 13.7%, 11.9%, 5.4%, 4.5%, 10.1%, and 4.1% for ADG, ADFI, FCR, tibia ash content, AIDP, and AIDN, respectively. In the female broiler meta-analysis, residuals for ADG and ADFI differed by 7.3% and 10.7%, respectively.

Additionally, the models evaluating the effects of different Ca:NPP ratios in broiler diets with varying PHY supplementation are shown in Figures 7 and 8 and displayed well-distributed residuals. For male broilers, residual differences were 12.8%, 11.3%, 5.3%, 8.2%, and 4.0% for ADG, ADFI, FCR, tibia ash content, and AIDN, respectively. For female broilers, residuals were 7.3% and 10.7% for ADG and ADFI, respectively.

Residuals from the meta-analysis assessing the effect of PHY supplementation on the AID of amino acids in male broilers are presented in Figure 9 and demonstrated a good distribution across various amino acids. The observed residual differences were 3.6%, 2.5%, 4.8%, 7.0%, 2.5%, 3.2%, 3.1%, 3.0%, 3.1%, 3.2%, 3.1%, 4.2%, 4.0%, 4.8%, 4.2%, 3.8%, 4.6%, and 4.1% for the AID of ALA, ARG, ASP, CYS, GLU, GLY, HIS, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR, and VAL, respectively.

4. DISCUSSION

The rapid increase in broiler growth rate driven by the continuous genetic advances, the emergence of research combined with the development of new generations of PHY, and the growing concern about environmental pollution highlights the importance of continuous meta-analyses revising broiler nutrient requirements, as well as evaluating P, Ca, and amino acid digestibility in modern broiler strains, assessing dietary P sources, and addressing the broiler requirements at different physiological stages (FALLAH et al., 2020; NUAMAH et al., 2024).

4.1. IMPACT OF EXOGENOUS PHY ON GROWTH PERFORMANCE

Exogenous PHY can influence broiler performance, often correlating with increased nutrient digestibility (DILGER et al., 2004; OLUKOSI et al., 2008; WOYENGO et al., 2010). PHY breaks down PP complexes, releasing P and other essential nutrients, which enhance the performance of broilers fed low-NPP diets (PIRGOZLIEV et al., 2011) or diets low in both Ca and NPP (WALK et al., 2013). The models fitted in the current study demonstrated that PHY supplementation enhanced ADG and ADFI in both male and female broilers, while improved FCR in male broilers from 1 to 21 days post-hatching. These findings are in line with previous meta-analyses that concluded that PHY may enhance growth performance in male broilers during the starter phase (LETOURNEAU-MONTMINY et al., 2010; FARIDI et al., 2015; KERMANI et al., 2023; NUAMAH et al., 2024). To the best of our knowledge, this is the first meta-analysis to correlate PHY supplementation with growth performance in female broilers during the starter phase. Evaluating the impact of PHY supplementation during the starter phase is essential, as broilers in this period exhibit higher protein and energy utilization and digestibility compared to older birds, driven by rapid organ and tissue growth with a more pronounced demand for P (BATAL AND PARSONS, 2002; HUANG et al., 2005; ABUDABOS, 2012). Furthermore, Babatunde et al. (2019a) reported greater PHY efficiency in improving growth performance and P and Ca utilization in low-P diets at day 14 post-hatching compared to broilers fed the same diets at day 22 post-hatching.

The models fitted suggest that the optimal dietary NPP levels required to maximize ADG and ADFI are 4.80 g/kg and 4.81 g/kg of diet, respectively, or approximately 260 mg of NPP per day for male broilers aged 1 to 21 days post-hatching, in diets without PHY supplementation. Moreover, the optimal NPP level to improve the FCR was calculated as 4.79 g/kg. This daily NPP intake aligns with the recommendation from the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024), which suggests an NPP intake of 260 mg/day for male broilers in this age range. Letourneau-Montminy et al. (2010) estimated that dietary NPP levels of 4.4, 4.5, and 4.1 g/kg of diet were required to optimize ADG, ADFI, and gain-to-feed, respectively, in male broiler from 1 to 21 days of age fed corn-soyabean meal diets containing 10 g/kg of Ca. Faridi et al. (2015) reported slightly higher NPP requirements of 4.71, 4.91, and 4.43 g/kg of feed to optimize ADG, ADFI, and feed efficiency, respectively, in broilers fed corn-soybean meal diets with 10 g/kg of Ca. Recently, Kermani et al. (2023) updated these requirements, demonstrating that NPP levels of 4.77, 4.97, and 4.52 g/kg of feed were necessary to optimize ADG, ADFI, and FCR in male broilers from 1 to 21 days post-hatching fed with corn-soybean meal-based diets. Fallah et al. (2020), applying the response surface method, suggested that that optimal dietary levels of Ca and NPP to maximize performance in male broilers aged 1 to 21 days were, respectively, 6.23 and 4.30 g/kg for average weight gain (AWG), 5.41 and 4.11 g/kg for average feed intake (AFI), and 7.18 and 4.11 g/kg for FCR. Differences in the optimal NPP requirements of broilers may be attributed to several factors, including diet composition. Unlike previous meta-analyses, the current meta-analysis included wheat-based diets alongside corn-soybean meal diets. PHY supplementation may hydrolyze PP in soybean meal and maize more effectively than it does in wheat (SELLE AND RAVINDRAN, 2007). This discrepancy may be partially associated with the propensity and storage sites of PP in the grains (RAVINDRAN et al., 1999). In wheat, PP is more likely to form complexes with proteins compared to maize (SELLE AND RAVINDRAN, 2000).

Moreover, the addition of increasing levels of PHY to broiler diets has attracted the attention of many researchers (COWIESON et al., 2011). Increasing PHY supplementation from conventional doses (500 FTU/kg of diet) to higher doses, up to 2000 FTU/kg of diet, may offer economic benefits when the value of the obtained nutrients exceeds the cost of additional PHY (COWIESON et al., 2017). The models fitted in the meta-analysis suggested that increasing PHY levels from 500 to 2000 FTU/kg of diet reduced the NPP requirement for optimal ADG (4.51 vs. 3.66 g NPP/kg of diet), ADG (4.47 vs. 3.47 g NPP/kg of diet), and FCR (4.44 vs. 3.39 g NPP/kg of diet). It is important to highlight that the meta-analysis included studies using different generations of PHY enzymes, which may have resulted in a lower estimated NPP release compared to what could be achieved with the most recent enzyme technologies. Kermani et al. (2023) observed similar effects of increasing PHY doses on the reduction of dietary NPP requirements in male broilers. These improvements are primarily attributed to the release of P in NPP-deficient diets and the reduction in the negative effects of PP on the digestibility of Ca, iron, zinc, starch, lipids, and amino acids. This process enhances nutrient absorption and increases the energy available in the diet (WALK et al., 2014; NUAMAH et al., 2024). Higher PHY doses can also lead to complete dephosphorylation of $InsP_6$, releasing myo-inositol, which plays a crucial role in promoting growth rate and feed efficiency. Myo-inositol contributes to cell growth and metabolism, fat deposition and transport, gluconeogenesis, regulation of glucose transport, and protein synthesis (COWIESON et al., 2014; HUBER et al., 2015; LEE AND BEDFORD, 2016).

The models fitted for PHY supplementation in female broilers from 1 to 21 days demonstrated positive effects on growth performance. A quadratic effect was observed, with plateaus at 2,125 FTU/kg and 1,957 FTU/kg for ADG and ADFI, respectively. In current meta-analysis, higher PHY doses did not further enhance the performance, likely because of the limited PP substrate available for degradation. Due to continuous genetic selection, male and female broilers differ in growth performance, carcass part weights, and nutrient requirements (ENGLAND et al., 2023). Ravindran et al. (2004) reported that female broilers had lower AIDP than males, in both low-P and adequate P diets. However, AIDP improvements were more pronounced with increased dietary P. Despite these insights, only a limited number of studies have evaluated the effects of PHY supplementation in female broilers during the starter phase, underscoring the need for further research to better understand these effects.

Furthermore, the impact of PHY supplementation in broiler growth performance may be expected because of the crucial role of P in energy metabolism, fat transportation, amino acid and protein synthesis (PROSZKOWIEC-WEGLARZ AND ANGEL, 2013). Fallah et al. (2020) suggested that the growth-inhibiting effects of NPP deficient diets could be explained by the role of P in modulating appetite and metabolism. A low-NPP diet may depress feed intake, but this adverse effect can be mitigated by increasing dietary NPP levels either directly or through PHY supplementation (ROUSSEAU et al., 2016). Furthermore, increased P mineral, and nutrient availability can change the viscosity of the diets and improve their feed intake (SELLE et al., 2000; AMERAH et al., 2014).

Our findings demonstrated that an increased dietary Ca:NPP ratio negatively affected growth performance in both male and female broilers. Consistent with these results, previous meta-analyses have reported that higher Ca:NPP ratios, driven by increased Ca content, negatively affected growth performance (LETOURNEAU-MONTMINY et al., 2010; FARIDI et al., 2015; KERMANI et al., 2023; NUAMAH et al., 2024). The utilization of Ca and P in broilers is influenced by the relationship between these minerals in the diet, as they exhibit antagonistic interactions within the poultry intestine (BAVARESCO et al., 2020). High Ca:NPP levels can reduce P availability in the gut lumen by promoting the formation of flocculent calcium phosphate (Ca_2PO_4) precipitates (KERMANI et al., 2023), which is more pronounced in low-NPP diets (BAR et al., 2003). Excess Ca can bind to PP molecules, thereby reducing the solubility of $InsP_6$ and limiting its accessibility to phosphatases in the digestive tract (SELLE AND RAVINDRAN, 2007). Additionally, Ca competes with exogenous PHY for the same active sites, potentially reducing the enzyme's effectiveness (POINTILLART et al., 1985). Létourneau-Montminy et al. (2010) reported that a lower dietary Ca to NPP ratio achieved optimal performance in very low-NPP diets, suggesting that it may need to be reduced below the conventional 2:1 ratio. The models fitted in the current study indicated that a Ca:NPP ratio below the conventional 2:1 ratio yielded the best growth performance in both male and female broilers. However, it is important to note that the database may be biased towards experiments with Ca:NPP ratios below 2:1, as these represent a limited number of observations. Most data points were from diets with a Ca:NPP ratio between

2:1 and 3:1 for both male and female broilers. This highlights the need for further experiments to evaluate the effect of PHY in diets with very low Ca:NPP ratios.

4.2. IMPACT OF PHY SUPPLEMENTATION ON TIBIA ASH CONTENT

Tibia ash content is regarded as an effective way to assess PHY efficacy, mineral utilization and deposition in broilers (LALPANMAWIA et al., 2014). An increase in tibia ash content is a good indicator of improved bone mineralization, reflecting the enhanced availability of minerals released from mineral-PP complexes by exogenous PHY (SOBOTIK et al., 2023). Tibia ash reduces the sensitivity to dietary changes as broilers age. PHY supplementation is more effective at increasing tibia ash during the early growth phase, with bone mineral peaking at approximately four weeks of age. Beyond this age, while tibia growth continues, bone density does not increase significantly due to a larger surface area (TALATY et al., 2009; NUAMAH et al., 2024). Our findings indicate that PHY supplementation increases tibia ash content. The optimization results for dietary NPP levels required to maximize tibia ash in male broilers aged 1 to 21 days post-hatching, whether with or without PHY supplementation, were higher than those for other performance traits, which is aligned with previous meta-analyses (LÉTOURNEAU-MONTMINY et al., 2010; FARIDI et al., 2015; KERMANI et al., 2023; NUAMAH et al., 2024). Liu et al. (2017) reported that, at a given Ca level, higher NPP levels are needed to maximize bone mineralization compared to optimizing growth rate. This can be attributed to the fact that approximately 75 to 85% of P is retained in bones, highlighting that tibia ash content is a more sensitive indicator of NPP requirements than growth performance measures in broiler chickens (FALLAH et al., 2020; NUAMAH et al., 2024). Tibia ash was negatively affected by higher Ca:NPP ratios in male broiler diets. Previous meta-analyses have shown that high dietary Ca levels negatively impact tibia ash content, with higher optimal dietary NPP levels needed to mitigate this effect (LETOURNEAU-MONTMINY et al., 2010; FARIDI et al., 2015; KERMANI et al., 2023). High-Ca diets, particularly when combined with low-NPP diets, increase pH in the proximal segments of the gastrointestinal tract, notably in the gizzard. This elevated pH creates favorable conditions (alkalinity) for the formation of insoluble PP-Ca complexes, further limiting the availability of both Ca and P (SELLE et al., 2009a; BEDFORD AND ROUSSEAU, 2017).

4.3. IMPACT OF PHY SUPPLEMENTATION ON THE APPARENT ILEAL DIGESTIBILITY OF PHOSPHORUS, NITROGEN, AND AMINO ACIDS

Exogenous PHY supplementation increases the availability of P, Ca, energy, and other nutrients in the feed by hydrolyzing phosphate groups, which may affect nutrient digestibility (BOUGOUIN et al., 2014). In our study, AIDP was significantly improved by PHY supplementation. Male broilers fed diets containing 1,000 FTU/kg of PHY showed a 26.1 percentage points increase in AIDP compared with those fed diets without PHY. This finding is consistent with that of Cowieson et al. (2017), who reported a 23.1 percentage points improvement in AIDP in broilers aged 21 to 28 days following PHY supplementation.

Evaluating amino acid responses to PHY in broilers during the starter phase is critical because of the rapid growth of bones and tissues as well as the higher mineral, P, and amino acid requirements (RAVINDRAN AND ABDOLLAHI, 2021). Endogenous amino acid losses are elevated during the first week post-hatching and decrease with advancing age (BARUA et al., 2021). As a result, the risk of amino acid deficiency is greater in young birds, and deficiency in key limiting amino acids, such as lysine, can significantly impair muscle development and body weight (TESSERAUD et al., 1996). Furthermore, the potential for PHY to enhance amino acid digestibility is relatively high in young birds, as the ileal digestibility of protein and amino acids is inherently lower in younger birds compared to older ones (BATAL AND PARSONS, 2002; LI et al., 2015).

The fitted models indicated that PHY supplementation enhanced the AID of N and several amino acids. The *de novo* formation of binary protein–PP complexes at a pH below the isoelectric point (iP) of proteins in the gut is crucial (SELLE et al., 2000). A negatively charged PP molecule can form binary protein–PP complexes with proteins carrying a net positive charge at a pH less than iP. At pH values exceeding their iP, with proteins carrying a net negative charge, a cationic bridge (usually Ca^{2+}) links PP and proteins in ternary complexes, thereby reducing protein digestibility (SELLE et al., 2023). Furthermore, the ability of pepsin to digest proteins bound to PP in insoluble protein aggregates is compromised (SELLE et al., 2023). Another possible mode of action is that PP may induce increases in endogenous amino acid flows and the amino acid composition of pepsin and mucin (COWIESON et al., 2004; 2008). The addition of PHY to poultry diets partially prevents the formation of PP-protein complexes by hydrolyzing PP prior to their formation, thus enhancing protein digestibility (LIU et al., 2007).

Our findings show that PHY supplementation improves ileal amino acid digestibility in broilers by approximately 2.6 percentage points. This aligns with the observations of Selle and Ravindran (2007), who reported an increase of approximately 3 percentage points, but is lower than the 4.1 percentage points improvement described by Cowieson et al. (2017) for broilers aged 21 to 28 days. Nevertheless, our results align with those of Cowieson et al. (2017), who observed that PHY supplementation had a more pronounced effect on diets with lower inherent amino acid digestibility. Among the amino acids, cysteine showed the greatest improvement in AID in the diets supplemented with PHY. Additionally, PHY supplementation improved AIDN in diets with higher Ca:NPP levels. Ca is primarily derived from limestone, which has a high acid-binding capacity (LAWLOR et al., 2005). Consequently, as dietary Ca levels increase, gut pH tends to increase as well (SELLE et al., 2012). Moreover, Ca can interact not only with PP but also with proteins, including soya protein (LAWLOR et al., 2005; SELLE et al., 2012).

5. CONCLUSION

The current meta-analysis quantified the responses of male and female broilers during the starter phase (1–21 days of age) to PHY, dietary NPP, Ca:NPP ratios, and their interactions. The models demonstrated that exogenous PHY supplementation enhanced growth performance in both male and female broilers, as well as tibia ash content, AIDP, and AIDN in male broiler chickens. These positive effects were more pronounced in low-P diets. Also, increasing dietary NPP levels improved growth performance and tibia ash content. PHY supplementation positively affected the mean digestibility coefficient of amino acids, with a more pronounced effect on amino acids with lower inherent digestibility. However, high dietary Ca:NPP ratios adversely affected all variables, particularly in diets without PHY supplementation. Given the complexity of interactions between Ca, NPP, and exogenous PHY, and the limited number of studies involving female broilers during the starting period or diets with Ca:NPP levels below the conventional 2:1 ratio, further research is necessary to better understand their interactive effects on P utilization in broiler chickens.

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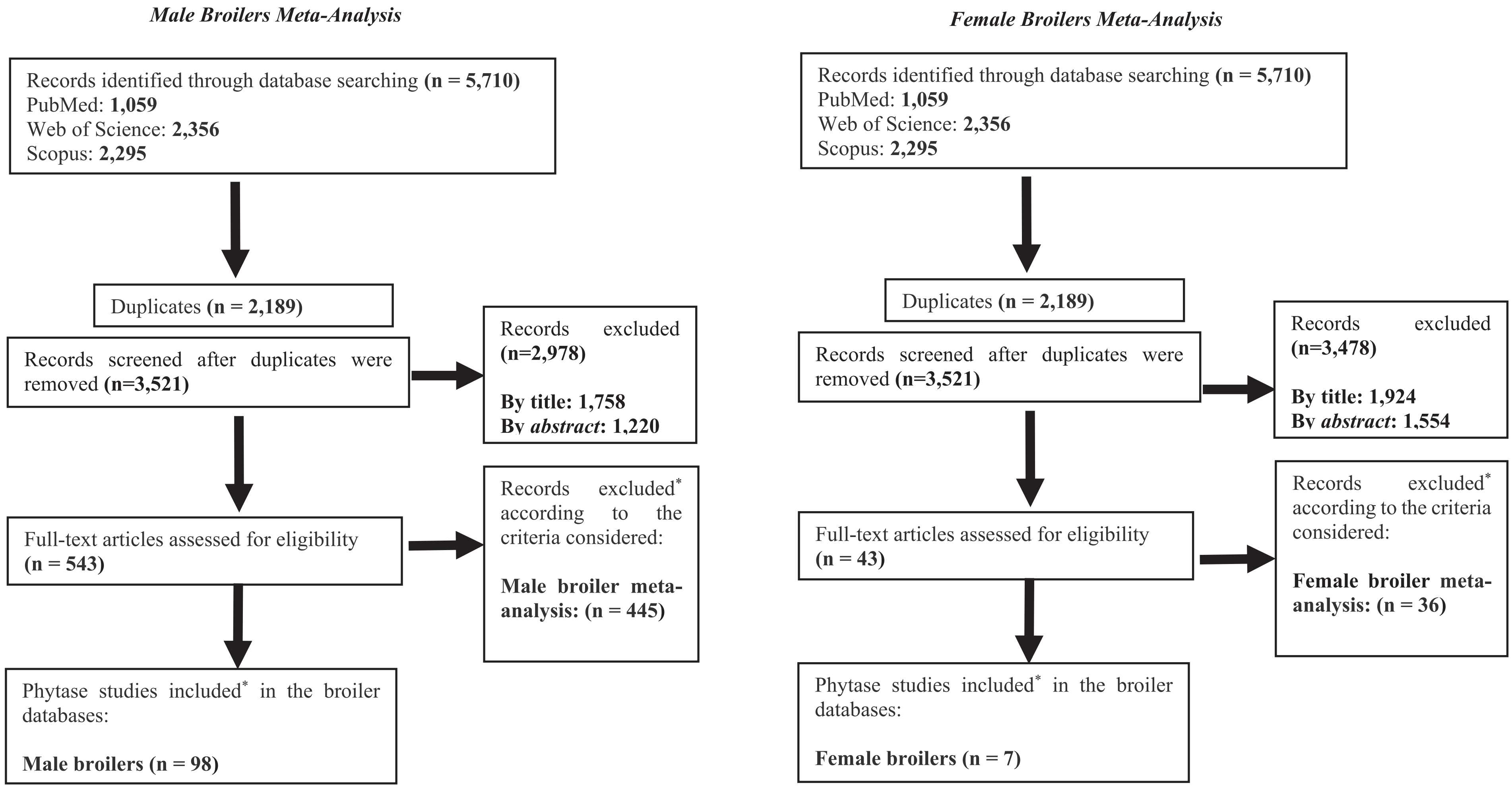

FIGURE 1. Study selection diagram illustrating the selection process for two separate meta-analyses investigating the effects of phytase supplementation in male and female broilers from 1 to 21 days post-hatch.

**Note: Scheideler et al. (2000) and Wu et al. (2004) were included in both the male and female meta-analyses.*

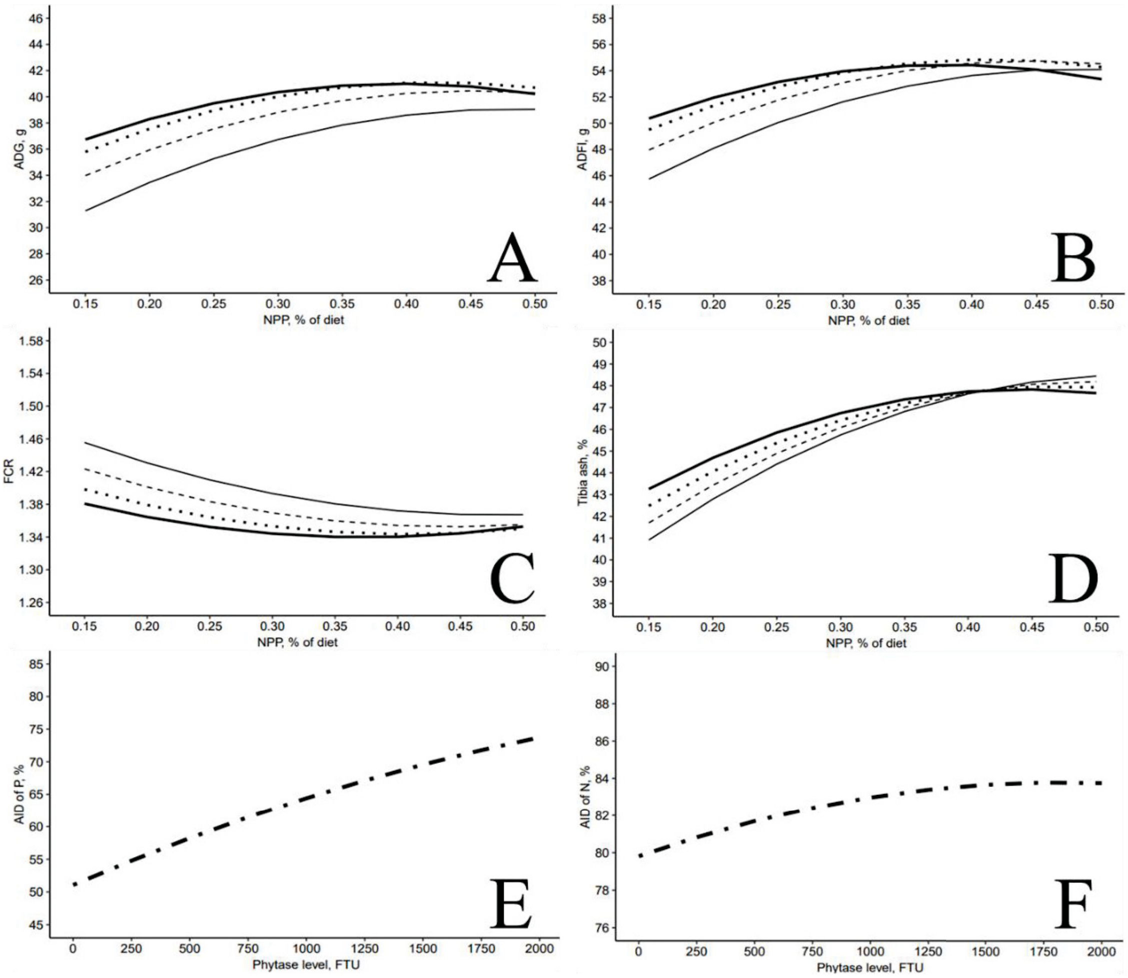

FIGURE 2. Response of average daily gain (ADG, g), average daily feed intake (ADFI, g), feed conversion ratio (FCR), tibia ash (%), apparent ileal digestibility of phosphorus (AIDP, %), and apparent ileal digestibility of nitrogen (AIDN, %) to dietary percentage of non-phytate phosphorus (NPP) in male broilers from 1 to 21 days of age, fed with different dietary supplemental phytase.

Description of curve types and equations for ADG, ADFI, FCR, tibia ash, AIDP, and AIDN. Curve types depicted in panels (A), (B), (C), and (D) correspond to the following dietary phytase levels, were solid thin curve – 0 FTU/kg of diet; dashed thick curve – 500 FTU/kg of diet; dotted curve – 1,000 FTU/kg of diet; solid thick curve – 1,500 FTU/kg of diet. Dot-dash curves in panels (E) and (F) represent the responses of AIDP and AIDN to phytase levels, respectively. (A) ADG (g) = $22.65 + 0.75 \times 10^{-4} \times$ Phytase $+ 68.214 \times$ NPP $- 0.17 \times 10^{-7} \times$ Phytase^2 – $70.87 \times$ NPP^2 $- 0.81 \times 10^{-4} \times$ Phytase $\times$ NPP (RMSE = 5.22, $R^2 = 0.93$). (B) ADFI (g) = $36.383 + 0.66 \times 10^{-4} \times$ Phytase $+ 73.975 \times$ NPP $- 0.13 \times 10^{-7} \times$ Phytase^2 $- 77.094 \times$ NPP^2 $- 0.10 \times 10^{-3} \times$ Phytase $\times$ NPP (RMSE = 6.24, $R^2 = 0.95$). (C) FCR = $1.555 - 0.89 \times 10^{-6} \times$ Phytase $- 0.785 \times$ NPP $+ 0.15 \times 10^{-9} \times$ Phytase^2 $+ 0.82 \times$ NPP^2 $+ 0.11 \times 10^{-5} \times$ Phytase $\times$ NPP (RMSE = 0.08, $R^2 = 0.92$). (D) Tibia ash (%) = $33.682 + 0.25 \times 10^{-4} \times$ Phytase $+ 56.236 \times$ NPP $- 0.14 \times 10^{-9} \times$ Phytase^2 $- 53.417 \times$ NPP^2 $- 0.60 \times 10^{-4} \times$ Phytase $\times$ NPP (RMSE = 4.51, $R^2 = 0.91$). (E) AIDP (%) = $51.059 + 0.50 \times 10^{-3} \times$ Phytase $- 0.22 \times 10^{-7} \times$ Phytase^2 (RMSE = 5.76, $R^2 = 0.77$). (E) AIDN (%) = $79.808 + 0.44 \times 10^{-4} \times$ Phytase $- 0.12 \times 10^{-7} \times$ Phytase^2 (RMSE = 3.36, $R^2 = 0.86$).

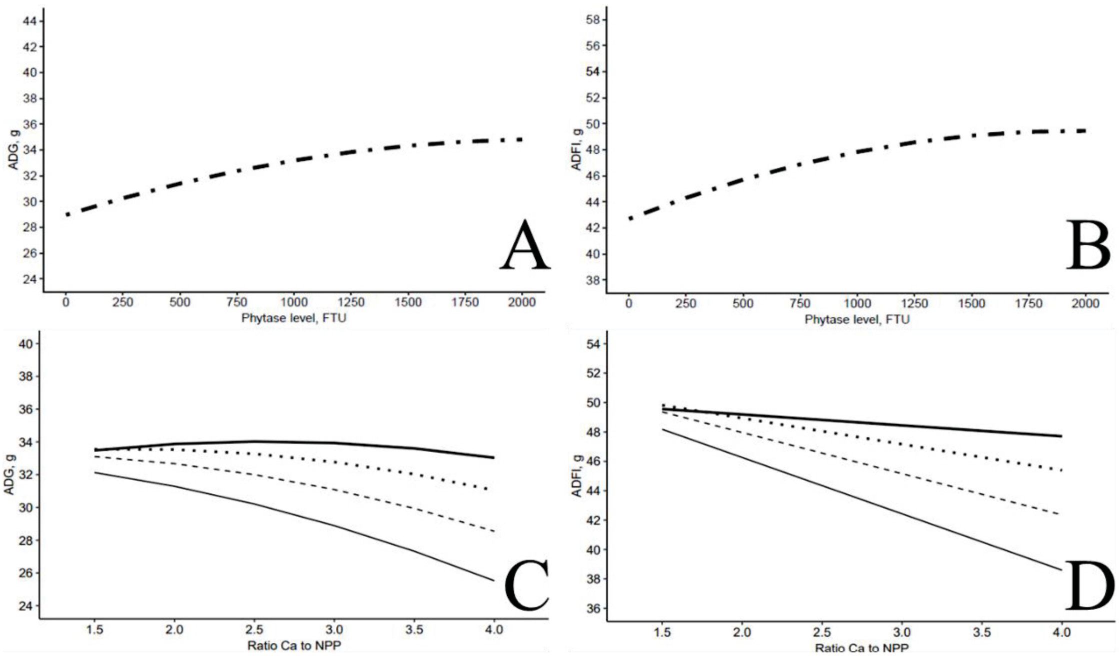

FIGURE 3. Response of average daily gain (ADG, g) and average daily feed intake (ADFI, g) to dietary percentage of non-phytate phosphorus (NPP) and calcium (Ca) to NPP ratio in female broilers from 1 to 21 days of age, fed with different dietary supplemental phytase.

Description of curve types and equations for ADG, and ADFI. In panels (A), and (B), dot-dash curves represent the responses of ADG and ADFI to phytase levels, respectively. In panels (C), and (D), the following curve types illustrate the effects of different dietary phytase levels: solid thin curve – 0 FTU/kg of diet; dashed thick curve – 500 FTU/kg of diet; dotted curve – 1,000 FTU/kg of diet; solid thick curve – 1,500 FTU/kg of diet. (A) ADG response to different dietary NPP = 28.954 + 0.55 × 10^{-4} × Phytase - 0.13 × 10^{-7} × Phytase^2 (RMSE = 2.34, R^2 = 0.89). (B) ADFI response to different dietary NPP = 42.686 + 0.69 × 10^{-4} × Phytase - 0.18 × 10^{-7} × Phytase^2 (RMSE = 4.95, R^2 = 0.94). (C) ADG response to different dietary Ca to NPP ratios = 28.954 + 0.55 × 10^{-4} × Phytase - 0.13 × 10^{-7} × Phytase^2 (RMSE = 2.34, R^2 = 0.89). (D) ADFI response to different dietary Ca to NPP ratios = 42.686 + 0.69 × 10^{-4} × Phytase - 0.18 × 10^{-7} × Phytase^2 (RMSE = 4.95, R^2 = 0.94).

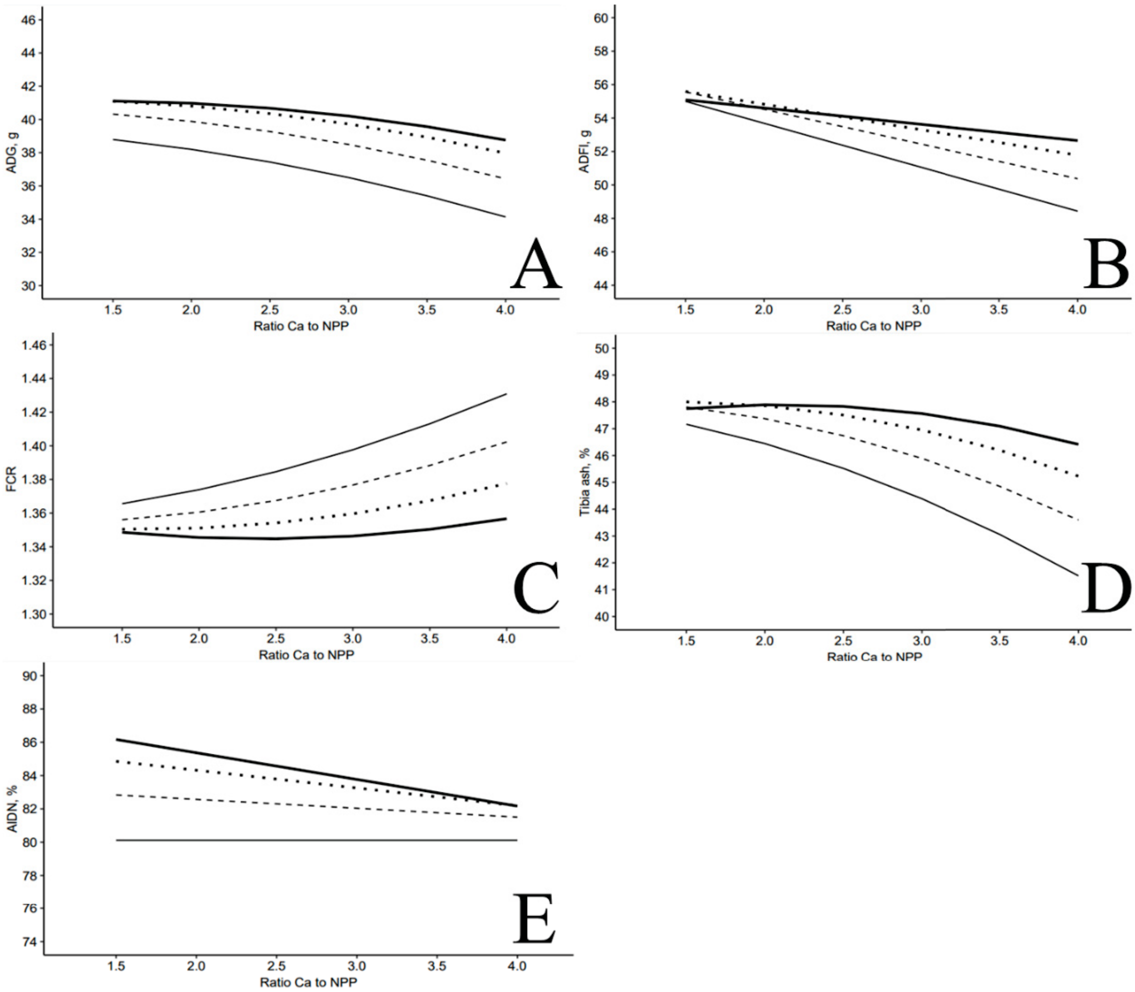

FIGURE 4. Response of average daily gain (ADG, g), average daily feed intake (ADFI, g), feed conversion ratio (FCR), tibia ash (%), and apparent ileal digestibility of nitrogen (AIDN, %) to calcium to non-phytate phosphorus (NPP) ratio in male broiler chicken diets fed with different dietary supplemental phytase.

Description of curve types and equations for ADG, ADFI, FCR, Tibia ash, and AIDN. Curve types depicted in panels (A), (B), (C), (D), and (E) correspond to the following dietary phytase levels, were solid thin curve – 0 FTU/kg of diet; dashed thick curve – 500 FTU/kg of diet; dotted curve – 1,000 FTU/kg of diet; solid thick curve – 1,500 FTU/kg of diet. (A) ADG (g) = $39.56 + 0.29 \times 10^{-4} \times$ Phytase - $0.15 \times 10^{-7} \times$ Phytase^2 – $0.339 \times$ Ca:NPP^2 + $0.62 \times 10^{-5} \times$ Phytase × Ca:NPP (RMSE = 4.92, $R^2 = 0.94$). (B) ADFI (g) = 58.942 - 2.628 × Ca:NPP - $0.11 \times 10^{-7} \times$ Phytase^2 + $0.11 \times 10^{-4} \times$ Phytase × NPP (RMSE = 5.95, $R^2 = 0.96$). (C) FCR = 1.355 + $0.77 \times 10^{-10} \times$ Phytase^2 + $0.48 \times 10^{-4} \times$ Ca:NPP^2 - $0.15 \times 10^{-6} \times$ Phytase × Ca:NPP (RMSE = 0.07, $R^2 = 0.92$). (D) Tibia ash (%) = 44.646 + $0.89 \times 10^{-5} \times$ Phytase + 2.216 × Ca:NPP - $0.19 \times 10^{-9} \times$ Phytase^2 - 0.665 × Ca:NPP^2 (RMSE = 4.22, $R^2 = 0.90$). (E) AIDN (%) = 80.116 + $0.77 \times 10^{-4} \times$ Phytase - $0.14 \times 10^{-7} \times$ Phytase^2 - $0.11 \times 10^{-4} \times$ Phytase × Ca:NPP (RMSE = 3.30, $R^2 = 0.87$).

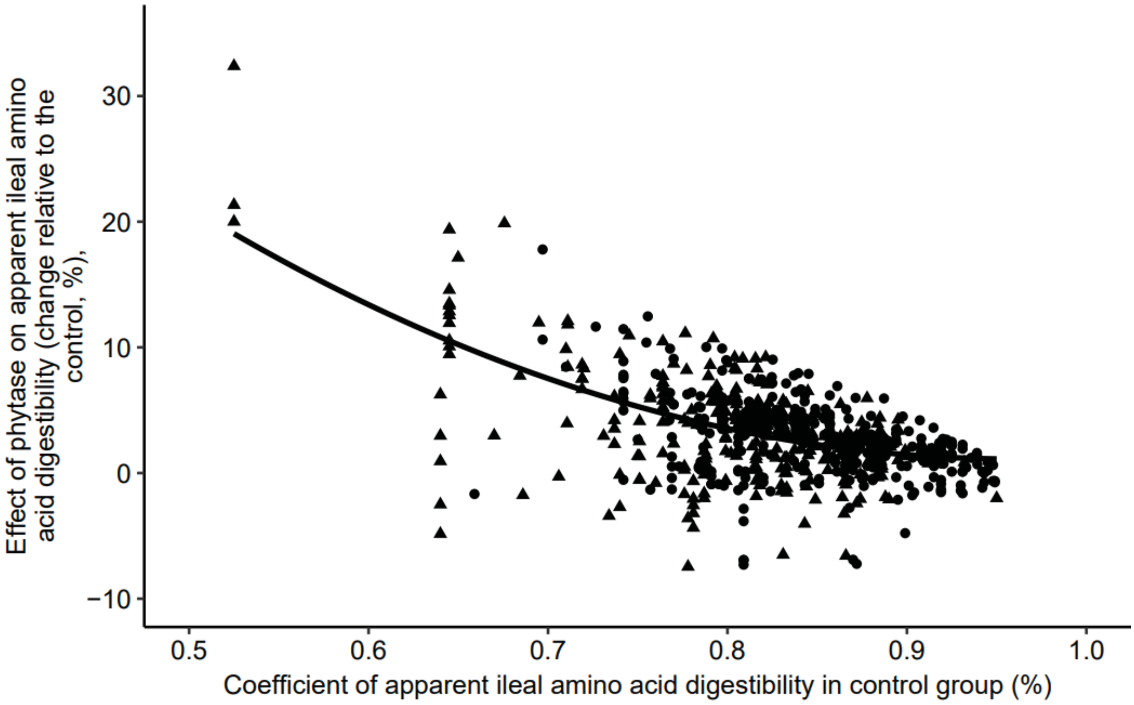

FIGURE 5. Relationship between amino acid digestibility in the control diet/ingredient and the effect of phytase.

The solid line represents the best polynomial fit ($R^2 = 0.31$; **P** < 0.001). Each point represents an individual amino acid: circles indicate essential amino acids, while triangles represent non-essential amino acids. The equation is as follow: % change in amino acid digestibility with phytase = 88.42 – (181.9 × C) + (94.71 × C^2), where ‘C’ is the digestibility coefficient of the amino acid in the control diet.

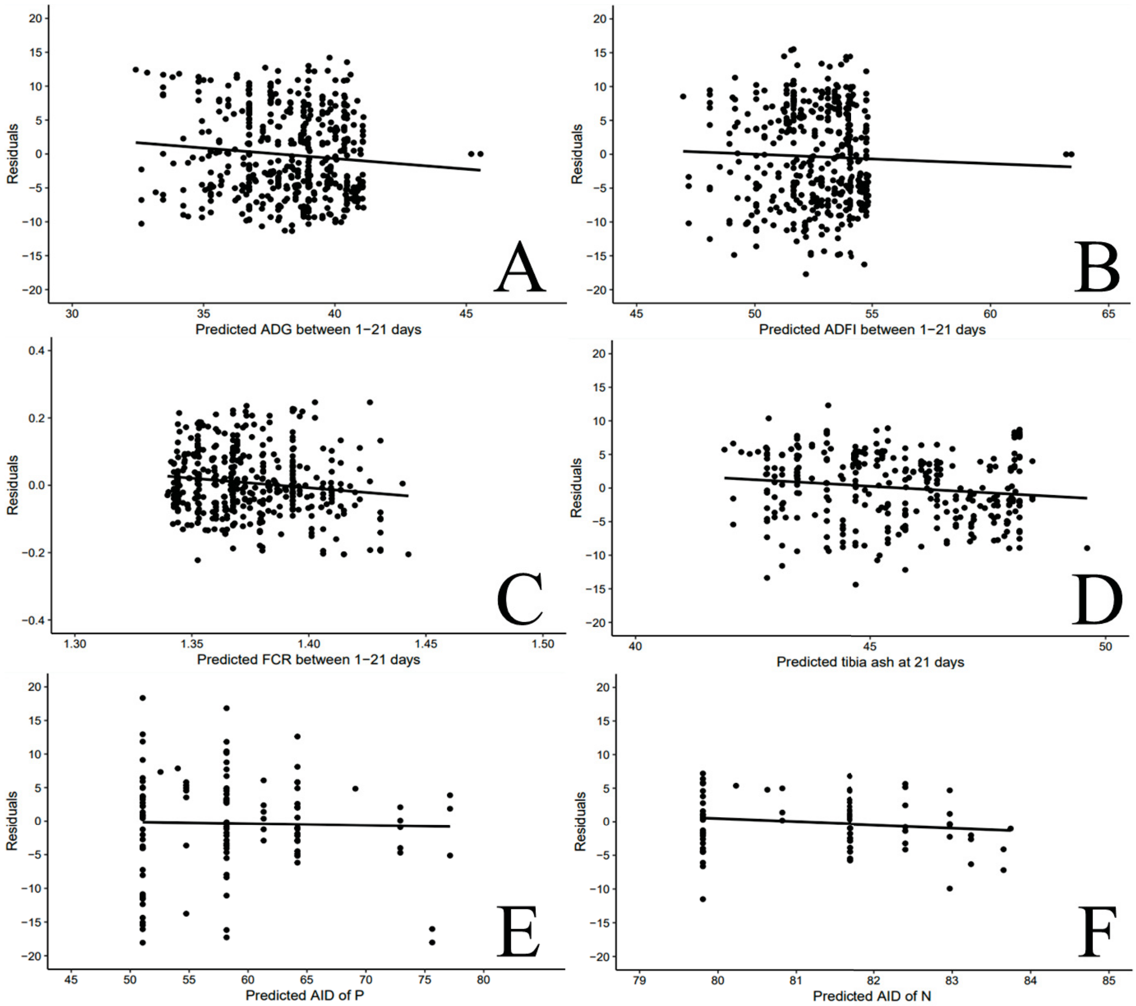

FIGURE 6. Plot of residuals (observed minus predicted) against predicted average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), tibia ash, apparent ileal digestibility of phosphorus (AIDP), and apparent ileal digestibility of nitrogen (AIDN) in male broilers from 1 to 21 days of age, fed with different dietary supplemental phytase and non-phytate phosphorus levels.
The line represents the regression of residuals on predicted ADG (A) [Y = 11.76 - 0.311 × predicted ADG; $R^2 = 0.001$; $P = 0.027$], on predicted ADFI (B) [Y = 6.94 - 0.139 × predicted ADFI; $R^2 <0.001$; $P = <0.001$], on predicted FCR (C) [Y = 0.789 - 0.569 × predicted FCR; $R^2 = 0.001$; $P = <0.001$], on predicted tibia ash (D) [Y = 34.92 - 0.776 × predicted tibia ash; $R^2 = 0.13$; $P = 0.001$], on predicted AIDP (E) [Y = 1.17 - 0.0259 × predicted AIDP; $R^2 <0.001$; $P = 0.066$], and on predicted AIDN (F) [Y = 36.8 - 0.453 × predicted AIDN; $R^2 <0.001$; $P = 0.317$].

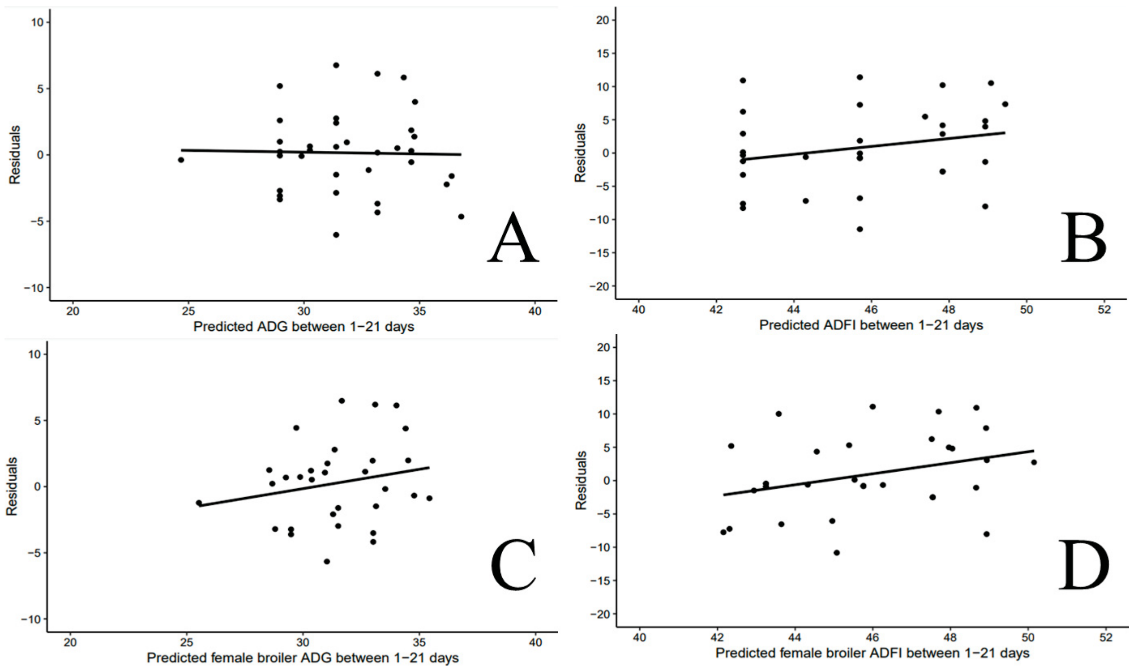

FIGURE 7. Plot of residuals (observed minus predicted) against predicted average daily gain (ADG), and average daily feed intake (ADFI) in female broilers from 1 to 21 days of age, fed with different dietary supplemental phytase (PHY), non-phytate phosphorus (NPP) levels and calcium to non-phytate phosphorus (Ca:NPP) ratios.

The line represents the regression of residuals on predicted ADG incorporating PHY and NPP (A) [Y = 1.00 - 0.026 × predicted ADG; R^2 <0.001; *P* = 0.916], on predicted ADFI incorporating PHY and NPP (B) [Y = -26.3 + 0.594 × predicted ADFI; R^2 <0.001; *P* = 0.654], on predicted ADG incorporating PHY and Ca:NPP (C) [Y = -8.92 + 0.292 × predicted ADG; R^2 <0.001; *P* = 0.262], on predicted ADFI incorporating PHY and Ca:NPP (D) [Y = -27.8 + 0.629 × predicted ADFI; R^2 <0.001; *P* = 0.124].

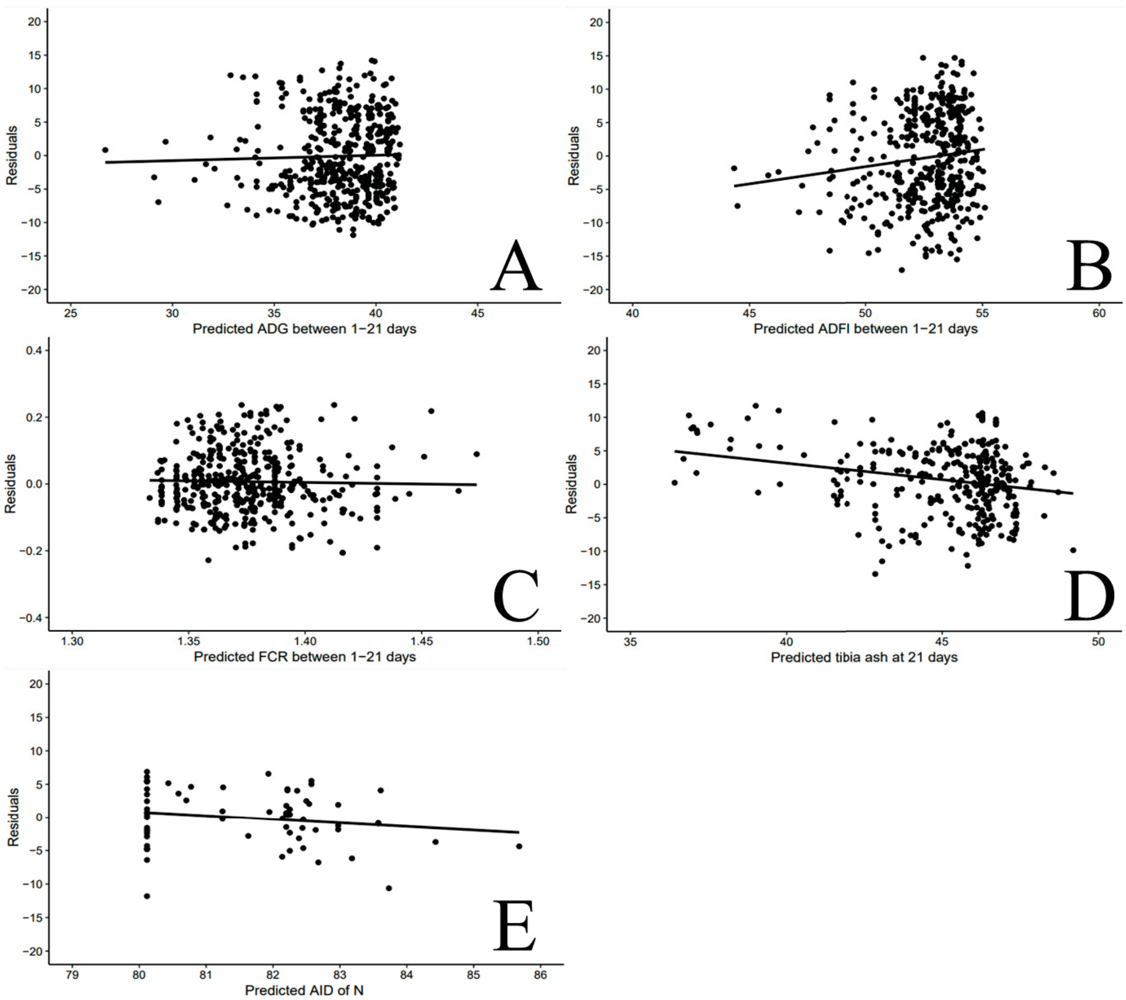

FIGURE 8. Plot of residuals (observed minus predicted) against predicted average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), tibia ash, and apparent ileal digestibility of nitrogen (AIDN) in male broilers from 1 to 21 days of age, fed with different dietary supplemental phytase and calcium to non-phytate phosphorus ratios.

The line represents the regression of residuals on predicted ADG (A) [Y = -3.17 + 0.080 × predicted ADG; $R^2 < 0.001$; $P = 0.544$], on predicted ADFI (B) [Y = -27.3 + 0.513 × predicted ADFI; $R^2 = 0.001$; $P = 0.007$], on predicted FCR (C) [Y = 0.136 - 0.094 × predicted FCR; $R^2 < 0.001$; $P = 0.635$], on predicted tibia ash (D) [Y = 7.12 - 0.151 × predicted tibia ash; $R^2 = 0.001$; $P = 0.210$], and on predicted AIDN (E) [Y = 44.7 - 0.548 × predicted AIDN; $R^2 = 0.002$; $P = 0.145$].

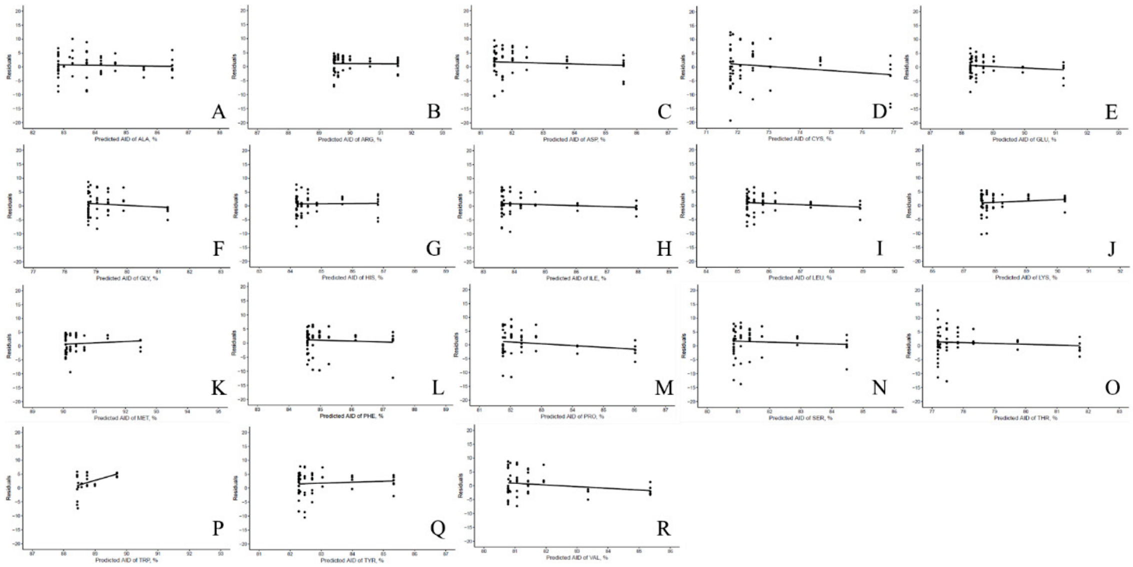

FIGURE 9. Plot of residuals (observed minus predicted) against predicted apparent ileal digestibility (AID) of alanine (Ala), AID of arginine (Arg), AID of aspartic acid (Asp), AID of cysteine (Cys), AID of glutamine (Glu), AID of glycine (Gly), AID of histidine (His), AID of isoleucine (Ile), AID of leucine (Leu), AID of lysine (Lys), AID of methionine (Met), AID of proline (Pro), AID of serine (Ser), AID of threonine (Thr), AID of tryptophan (Trp), AID of tyrosine (Tyr), AID of valine (Val) in male broilers from 1 to 21 days of age, fed with different dietary supplemental phytase and non-phytate phosphorus levels.
The line represents the regression of residuals on predicted AID of ALA (A) [Y = 14.0 - 0.159 × predicted AID of ALA; R^2 <0.001; P = 0.705], on predicted AID of ARG (B) [Y = 8.2 - 0.078 × predicted AID of ARG; R^2 <0.001; P = 0.806], on predicted AID of ASP (C) [Y = 26.6 - 0.304 × predicted AID of ASP; R^2 <0.001; P = 0.702], on predicted AID of CYS (D) [Y = 55.7 - 0.760 × predicted AID of CYS; R^2 = 0.001; P = 0.852], on predicted AID of GLU (E) [Y = 48.8 - 0.545 × predicted AID of GLU; R^2 = 0.001; P = 0.654], on predicted AID of GLY (F) [Y = 37.5 - 0.464 × predicted AID of GLY; R^2<0.001; P = 0.629], on predicted AID of HIS (G) [Y = -5.3 + 0.071 × predicted AID of HIS; R^2 <0.001; P = 0.562], on predicted AID of ILE (H) [Y = 28.4 - 0.328 × predicted AID of ILE; R^2 <0.001; P = 0.591], on predicted AID of LEU (I) [Y = 38.5 - 0.439 × predicted AID of LEU; R^2 = 0.001; P = 0.834], on predicted AID of LYS (J) [Y = -40.2 + 0.470 × predicted AID of LYS; R^2 <0.001; P = 0.876], on predicted AID of MET (K) [Y = -42.8 + 0.484 × predicted AID of MET; R^2 <0.001; P = 0.310], on predicted AID of PHE (L) [Y = 27.7 - 0.313 × predicted AID of PHE; R^2 <0.001; P = 0.941], on predicted AID of PRO (M) [Y = 54.5 - 0.651 × predicted AID of PRO; R^2 = 0.002; P = 0.324], on predicted AID of SER (N) [Y = 28.1 - 0.326 × predicted AID of SER; R^2 <0.001; P = 0.889], on predicted AID of THR (O) [Y = 24.4 - 0.297 × predicted AID of THR; R^2 <0.001; P = 0.821], on predicted AID of TRP (P) [Y = -284 + 3.23 × predicted AID of TRP; R^2 =0.012; P = 0.030], on predicted AID of TYR (Q) [Y = -26.5 + 0.341 × predicted AID of TYR; R^2 <0.001; P = 0.633], and on predicted AID of VAL (R) [Y = 50.2 - 0.609 × predicted AID of VAL; R^2 = 0.002; P = 0.441].

TABLE 1. Summary of phytase supplementation studies included in the meta-analysis of male broilers, categorized by location, journal, and year of publication.

Code	Study	Country	Journal	Year of publication
1	Paik et al.	Republic of Korea	Asian-Australasian Journal of Animal Sciences	2000
2	Scheideler et al.	United States	Journal of Applied Poultry Research	2000
3	Waldroup et al.	United States	Poultry Science	2000
4	Yan et al.	United States	Poultry Science	2000
5	Camden et al.	New Zealand	Animal Science	2001
6	Viveros et al.	Spain	Poultry Science	2002
7	Wu et al.	New Zealand	British Poultry Science	2003
8	Yan et al.	United States	Journal of Applied Poultry Research	2003
9	Cavalcanti et al.	United States	International Journal of Poultry Science	2004
10	Pintar et al.	Croatia	Czech Journal of Animal Science	2004
11	Timmons et al.	United States	Journal of Applied Poultry Research	2004
12	Wu et al.	New Zealand	Journal of Applied Poultry Research	2004a
13	Wu et al.	New Zealand	British Poultry Science	2004b
14	Pintar et al.	Croatia	Czech Journal of Animal Science	2005
15	Silva et al.	Brazil	Brazilian Journal of Animal Science	2006
16	Yan et al.	United States	International Journal of Poultry Science	2006
17	Ao et al.	United States	British Poultry Science	2007
18	Centeno et al.	Spain	British Poultry Science	2007
19	Leytem et al.	Canada	Poultry Science	2008
20	Olukosi et al.	United States	British Journal of Nutrition	2008
21	Santos et al.	Brazil	Journal of Applied Poultry Research	2008
22	Surek et al.	Brazil	Ciencia Rural	2008
23	Woyengo et al.	Canada	Animal Feed Science and Technology	2008
24	Ravindran et al.	New Zealand	Poultry Science	2008
25	Akyurek et al.	Turkey	Journal of Animal and Veterinary Advances	2009
26	Amerah et al.	New Zealand	Animal Production Science	2009
27	Laurentiz et al.	Brazil	Brazilian Journal of Animal Science	2009
28	Han et al.	China	Asian-Australasian Journal of Animal Sciences	2009
29	Lü et al.	China	Journal of Poultry Science	2009
30	Manangi et al.	United States	International Journal of Poultry Science	2009
31	Selle et al.	Australia	Animal Feed Science and Technology	2009b
32	Vaz et al.	Brazil	Brazilian Journal of Veterinary Research and Animal Science	2009
33	Tiwari et al.	India	British Poultry Science	2010
34	Woyengo et al.	Canada	Poultry Science	2010
35	Donato et al.	Brazil	Brazilian Journal of Animal Science	2011
36	Junqueira et al.	Brazil	Acta Scientiarum - Animal Sciences	2011
37	Powell et al.	United States	Poultry Science	2011
38	Shaw et al.	United States	The Journal of Poultry Science	2011a
39	Shaw et al.	United States	Journal of Applied Poultry Research	2011b
40	Rutherfurd et al.	New Zealand	Poultry Science	2012
41	Walk et al.	United States	Poultry Science	2012
42	Donato et al.	Brazil	Brazilian Journal of Poultry Science	2013
43	dos Santos et al.	Thailand	Asian-Australasian Journal of Animal Sciences	2013
44	Günal	Turkey	Animal Production Science	2013
45	Olukosi et al.	United States	Poultry Science	2013
46	Vaz et al.	Brazil	Arquivo Brasileiro de Medicina Veterinaria e Zootecnia	2013

47	Walk et al.	United States	Poultry Science	2013
48	Wilkinson et al.	Australia	Journal of Applied Poultry Research	2013
49	dos Santos et al.	Thailand	Journal of Applied Poultry Research	2014
50	Midilli et al.	Turkey	Kafkas Universitesi Veteriner Fakultesi Dergisi	2014
51	Walk et al.	United States	Poultry Science	2014
52	Kiarie et al.	Canada	Asian-Australasian Journal of Animal Sciences	2015
53	Krishna et al.	India	Asian Journal of Animal Sciences	2015
54	Wu et al.	Australia	Poultry Science	2015
55	Peceros et al.	Peru	Revista de Investigaciones Veterinarias del Peru	2016
56	Ribeiro Jr et al.	Brazil	Animal Feed Science and Technology	2016
57	Islam et al.	Malaysia	Cogent Food & Agriculture	2017
58	Lee et al.	United Kingdom	British Poultry Science	2017a
59	Lee et al.	India	Animal Nutrition	2017b
60	Pieniazek et al.	United States	Poultry Science	2017
61	Schmeisser et al.	France	Journal of Animal Physiology and Animal Nutrition	2017
62	Scholey et al.	United Kingdom	Animal	2017
63	Broch et al.	Brazil	Animal Feed Science and Technology	2018
64	Hamdi et al.	Spain	Animal Feed Science and Technology	2018
65	Walk et al.	Scotland	Poultry Science	2018
66	Walk et al.	India	Poultry Science	2018
67	Widodo et al.	Australia	Poultry Science Journal	2018
68	Freitas et al.	Brazil	Asian-Australasian Journal of Animal Sciences	2019
69	Dessimoni et al.	Brazil	Semina: Ciencias Agrarias	2019
70	dos Santos et al.	Brazil	Canadian Journal of Animal Science	2019
71	Lee et al.	India	Poultry Science	2019
72	Walk et al.	India	Journal of Animal Science	2019
73	Wang et al.	United States	Journal of Applied Poultry Research	2019
74	Babatunde et al.	United States	Poultry Science	2020
75	Bavaresco et al.	Brazil	Journal of Applied Poultry Research	2020
76	Broch et al.	Brazil	Journal of Applied Poultry Research	2020
77	Davin et al.	Netherlands	Journal of Applied Animal Nutrition	2020
78	Dessimoni et al.	Brazil	Anais da Academia Brasileira de Ciencias	2020
79	Mohiti-Asli et al.	Iran	Italian Journal of Animal Science	2020
80	Chuang et al.	Taiwan	Animal Bioscience	2021
81	Jacob et al.	Brazil	Semina: Ciencias Agrarias	2021
82	Olukomaiya et al.	Australia	Animal Feed Science and Technology	2021
83	Ayres et al.	United States	Journal of Applied Poultry Research	2021
84	Lamp et al.	United States	Journal of Applied Poultry Research	2022
85	Anwar et al.	Pakistan	Brazilian Journal of Poultry Science	2022
86	Barrilli et al.	Brazil	Ciencia Rural	2022
87	Dersjant-Li et al.	Australia	British Poultry Science	2022
88	Gulizia et al.	United States	Journal of Applied Poultry Research	2022
89	Hernandez et al.	United States	Animals	2022
90	Javadi et al.	Spain	Animals	2022
91	Bertechini et al.	Brazil	Anais Da Academia Brasileira De Ciencias	2022
92	Anwar et al.	Pakistan	Animals	2023
93	Moradi et al.	Iran	Animals	2023
94	de França et al.	Brazil	Livestock Science	2023
95	Bowen et al.	United States	Journal of Applied Poultry Research	2023
96	Jlali et al.	Spain	Agriculture-Basel	2023

97	Bowen et al.	United States	The Journal of Applied Research	2023
98	Krabbe et al.	Brazil	Brazilian Journal of Poultry Science	2024

TABLE 2. Summary of phytase supplementation studies included in the meta-analysis of female broilers, categorized by location, journal, and year of publication.

Code	Study	Country	Journal	Year of publication
1	Scheideler et al.	United States	Journal of Applied Poultry Research	2000
2	Wu et al.	New Zealand	Journal of Applied Poultry Research	2004
3	Pirgozliev et al.	Scotland	Poultry Science	2007
4	Banaszkiewicz	Poland	Acta Veterinaria (Beograd)	2009
5	Liu et al.	China	Archives of Animal Nutrition	2009
6	Pirgozliev et al.	United Kingdom	British Journal of Nutrition	2013
7	Shi et al.	China	Revista Brasileira de Ciencia Avicola	2022

TABLE 3. Diet composition variation included in the dataset for male and female broiler experiments.

	N[1]		**Mean[2]**		**SD**		**Minimum**		**Maximum**	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Phosphorus total, g/kg of diet	89	7	0.56	0.54	0.09	0.06	0.24	0.49	0.81	0.70
Phytate phosphorus, g/kg of diet	89	7	0.26	0.29	0.05	0.03	0.10	0.25	0.41	0.37
Calcium, g/kg of diet	89	7	0.82	0.82	0.13	0.15	0.50	0.50	1.19	1.00
Metabolic energy, Kcal/kg of diet	89	7	3,040	3,001	92.4	95.2	2,701	2,794	3,217	3,146
Crude protein, g/kg of diet	89	7	21.5	21,4	1.25	1.36	15.0	18.1	26.9	23.1
Vitamin D3, UI/kg of diet	89	7	2,729	2,451	1,196	1,658	800	1,000	6,614	5,000
Copper, mg/kg of diet	89	7	10	7	6.5	2.7	2	2	39	10
Zn, mg/kg of diet	89	7	79	67	44.6	26.2	15	14	210	100
Iron, mg/kg of diet	89	7	55	70	25.7	27.8	15	16	127	100
Mn, mg/kg of diet	89	7	91	92	42.2	31.8	18	16	200	125
I, mg/kg of diet	89	7	1.1	0.8	0.6	0.4	0.2	0.3	3	1.9
Se, mg/kg of diet	89	7	0.22	0.21	0.16	0.07	0.02	0.01	1.00	1.34
Sodium, g/kg of diet	89	7	0.18	0.16	0.03	0.03	0.12	0.11	0.52	0.2
Total lysine, g/kg of diet	89	7	1.27	1.22	0.13	0.12	0.85	1.05	1.63	1.34
Digestible lysine, g/kg of diet	89	7	1.15	1.16	0.10	0.12	0.84	0.94	1.47	1.31
Total methionine, g/kg of diet	89	7	0.58	0.58	0.09	0.07	0.30	0.45	0.78	0.75
Digestible methionine, g/kg of diet	89	7	0.55	0.57	0.08	0.12	0.28	0.44	0.77	0.73
Total methionine + cysteine, g/kg of diet	89	7	0.92	0.90	0.08	0.07	0.67	0.81	1.08	0.98

Digestible methionine + cysteine, g/kg of diet	89	7	0.85	0.88	0.09	0.13	0.68	0.73	1.09	1.03
Total threonine, g/kg of diet	89	7	0.85	0.80	0.08	0.06	0.58	0.68	1.12	0.9
Digestible threonine, g/kg of diet	89	7	0.76	0.70	0.07	0.06	0.57	0.58	1.01	0.76

[1]The number of studies that reported poultry diet composition.

TABLE 4. Summary statistics for the data used in the meta-analyses of male and female broiler aged 1 to 21 days post-hatched[1].

Item[2]	n	N	Mean	SD	Min.	Max.
Male broiler meta-analysis						
Independent variable						
Phytase (FTU/kg)	632	98	560	571	0.00	2,500
Non-phytate phosphorus (%)	632	98	0.30	0.09	0.15	0.52
Calcium to non-phytate phosphorus ratio	632	99	2.9	0.9	1.1	6.2
Dependent variable						
Average daily gain, g	471	80	37.9	6.2	22.4	54.0
Average daily feed intake, g	442	78	52.2	7.3	34.3	68.5
Feed conversion ratio	425	76	1.38	0.09	1.13	1.67
Tibia ash content, %	324	39	46.7	5.1	29.4	56.9
Apparent ileal digestibility of Phosphorus, %	117	22	57.5	9.7	33.0	76.8
Apparent ileal digestibility of Nitrogen, %	74	15	81.2	4.1	68.3	88.5
Female broiler meta-analysis						
Independent variable						
Phytase (FTU/kg)	31	7	770	831	0.00	2,500
Non-phytate phosphorus (%)	31	7	0.29	0.03	0.25	0.37
Ratio calcium to non-phytate phosphorus	31	7	2.9	0.6	2.0	4.0
Dependent variable						
Average daily gain, g	31	7	31.7	4.2	24.3	41.2
Average daily feed intake, g	31	7	46.6	7.2	34.2	59.6

[1]n, number of experiments; N, number of studies; SD, standard deviation; Min., minimal value of the parameter: Max., maximal value of the parameter. [2]NPP, non-phytate phosphorus; FCR, feed conversion ratio; FTU, phytase unit.

TABLE 5. The effects of phytase supplementation and dietary non-phytate phosphorus (NPP), along with their parameter estimates for the mixed model applied to male broiler from 1 to 21 days of age in performance, tibia ash content and apparent ileal digestibility of phosphorus (AIDP) and apparent ileal digestibility of nitrogen (AIDN).

Male broiler growth performance models									
	Average daily gain (g)			Average daily feed intake (g/d)			Feed conversion ratio		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	22.65	2.621	<0.001	36.383	2.767	<0.001	1.555	0.049	<0.001
Phytase	0.75×10^{-4}	0.86×10^{-5}	<0.001	0.66×10^{-4}	0.89×10^{-5}	<0.001	-0.89×10^{-6}	0.16×10^{-6}	<0.001
NPP	68.214	15.154	<0.001	73.975	15.813	<0.001	-0.785	0.287	0.007
Phytase^2	-0.17×10^{-7}	0.24×10^{-8}	<0.001	-0.13×10^{-7}	0.25×10^{-8}	<0.001	0.15×10^{-9}	0.45×10^{-10}	0.001
NPP^2	-70.870	21.349	0.001	-77.094	22.303	0.001	0.820	0.405	0.044
Phytase x NPP	-0.81×10^{-4}	0.22×10^{-4}	<0.001	-0.10×10^{-3}	0.23×10^{-4}	<0.001	0.11×10^{-5}	0.41×10^{-6}	0.007
R^2		0.93			0.95			0.92	
RMSE*		5.22			6.24			0.08	
Tibia ash content and apparent ileal digestibility of nutrients models									
	Tibia ash (%)			AIDP (%)			AIDN (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	33.682	2.298	<0.001	51.059	1.488	<0.001	79.808	1.157	<0.001
Phytase	0.25×10^{-4}	0.38×10^{-5}	<0.001	0.5×10^{-3}	0.16×10^{-4}	<0.001	0.44×10^{-4}	0.97×10^{-5}	<0.001
NPP	56.236	13.838	<0.001	-	-	NS	-	-	NS
Phytase^2	-0.14×10^{-9}	0.24×10^{-10}	<0.001	-0.22×10^{-7}	0.47×10^{-8}	<0.001	-0.12×10^{-7}	0.44×10^{-8}	0.009
NPP^2	-53.417	20.107	0.001	-	-	NS	-	-	NS
Phytase x NPP	-0.60×10^{-4}	0.14×10^{-4}	<0.001	-	-	NS	-	-	NS
R^2		0.91			0.77			0.86	
RMSE*		4.51			5.76			3.36	

*RMSE, Root mean square error

TABLE 6. The effects of phytase supplementation, dietary non-phytate phosphorus (NPP), and the calcium-to-NPP (Ca:NPP) ratio on the performance of female broilers from 1 to 21 days of age, along with parameter estimates for the mixed model applied.

Female broiler growth performance models						
Models incorporating Phytase and NPP						
	Average daily gain (g)			Average daily feed intake (g/d)		
	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	28.954	1.302	<0.001	42.686	2.691	<0.001
Phytase	0.55×10^{-4}	0.12×10^{-5}	<0.001	0.69×10^{-4}	0.16×10^{-4}	<0.001
NPP	-	-	NS	-	-	NS
Phytase^2	-0.13×10^{-7}	0.48×10^{-8}	0.013	-0.18×10^{-7}	0.65×10^{-8}	0.012
NPP^2	-	-	NS	-	-	NS
Phytase x NPP	-	-	NS	-	-	NS
R^2		0.89			0.94	
RMSE*		2.34			4.95	
Models incorporating Phytase and Ca:NPP ratio						
	Average daily gain (g)			Average daily feed intake (g/d)		
	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	33.213	1.397	<0.001	53.930	3.174	<0.001
Phytase	-	-	NS	-	-	NS
Ca:NPP	-	-	NS	-3.834	0.728	<0.001
Phytase^2	-0.10×10^{-7}	0.36×10^{-8}	0.008	-0.15×10^{-7}	0.47×10^{-8}	<0.001
Ca:NPP^2	-0.481	0.092	<0.001	-	-	NS
Phytase x Ca:NPP	0.16×10^{-4}	0.30×10^{-5}	<0.001	0.20×10^{-4}	0.40×10^{-5}	<0.001
R^2		0.92			0.96	
RMSE*		2.50			4.71	

*RMSE, Root mean square error

TABLE 7. The effects of phytase supplementation and the calcium to non-phytate phosphorus (NPP) (Ca:NPP) ratio in diets on performance, tibia ash content, apparent ileal digestibility of phosphorus (AIDP), and apparent ileal digestibility of nitrogen (AIDN) in male broilers from 1 to 21 days of age, along with the parameter estimates for the mixed model applied.

Male broiler growth performance models

	Average daily gain (g)			Average daily feed intake (g/d)			Feed conversion ratio		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	39.556	0.698	<0.001	58.942	0.986	<0.001	1.355	0.012	<0.001
Phytase	0.29×10^{-4}	0.71×10^{-5}	<0.001	-	-	NS	-	-	NS
Ca:NPP ratio	-	-	NS	-2.628	0.227	<0.001	-	-	NS
Phytase^2	-0.15×10^{-7}	0.24×10^{-8}	<0.001	-0.11×10^{-7}	0.21×10^{-8}	<0.001	0.77×10^{-10}	0.38×10^{-10}	0.046
Ca:NPP^2	-0.339	0.035	<0.001	-	-	NS	0.48×10^{-4}	0.65×10^{-5}	<0.001
Phytase x Ca:NPP	0.62×10^{-5}	0.21×10^{-5}	0.004	0.11×10^{-4}	0.13×10^{-5}	<0.001	-0.15×10^{-6}	0.24×10^{-7}	<0.001
R^2		0.94			0.96			0.92	
RMSE*		4.92			5.95			0.07	

Tibia ash content and apparent ileal digestibility of nitrogen models

	Tibia ash (%)			AIDN (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	48.090	0.820	<0.001	80.116	1.218	<0.001
Phytase	-	-	NS	0.77×10^{-4}	0.19×10^{-4}	<0.001
Ca:NPP ratio	-	-	NS	-	-	NS
Phytase^2	-0.89×10^{-8}	0.19×10^{-8}	<0.001	-0.14×10^{-7}	0.46×10^{-8}	0.003
Ca:NPP^2	-0.411	0.304	<0.001	-	-	NS
Phytase x Ca:NPP	0.12×10^{-4}	0.11×10^{-5}	<0.001	-0.11×10^{-4}	0.51×10^{-5}	0.042
R^2		0.93			0.87	
RMSE*		3.73			3.30	

*RMSE, Root mean square error

TABLE 8. The effects of phytase supplementation, along with parameter estimates from the mixed model, on the apparent ileal digestibility of various amino acids in male broilers from 1 to 21 days of age post hatch.

Apparent ileal digestibility of amino acids	n	N	Apparent ileal amino acid digestibility coefficients, %				Phytase Delta, %		Phytase level (FTU/kg of diet) effect model				
			Control group		Phytase group				*P*-Value		R^2	RMSE	Equation
			Mean	SE	Mean	SE	Mean	SE	Phytase	Phytase^2			
Essential Amino Acids (EAA)													
Arginine	61	10	88.9	1.03	90.3	1.02	1.6	0.28	NS	<0.001	0.87	2.57	$89.49 + 0.52 \times 10^{-8} \times \text{Phytase}^2$
Histidine	59	9	82.9	1.20	85.4	1.17	3.0	0.36	NS	0.001	0.78	2.62	$84.20 + 0.66 \times 10^{-8} \times \text{Phytase}^2$
Isoleucine	57	9	82.5	1.52	85.1	1.50	3.2	0.43	NS	<0.001	0.84	2.52	$83.59 + 0.11 \times 10^{-7} \times \text{Phytase}^2$
Leucine	57	9	84.5	1.34	86.5	1.32	2.4	0.35	NS	<0.001	0.88	2.66	$85.30 + 0.90 \times 10^{-8} \times \text{Phytase}^2$
Lysine	57	9	86.8	1.57	88.5	1.56	1.9	0.26	NS	<0.001	0.89	2.89	$87.57 + 0.67 \times 10^{-8} \times \text{Phytase}^2$
Methionine	57	9	89.6	1.24	90.9	1.22	1.5	0.27	NS	0.001	0.84	2.81	$90.07 + 0.60 \times 10^{-8} \times \text{Phytase}^2$
Phenylalanine	57	9	84.0	1.58	85.5	1.55	1.8	0.76	NS	0.003	0.85	3.53	$84.59 + 0.68 \times 10^{-8} \times \text{Phytase}^2$
Threonine	57	9	75.9	1.92	78.9	1.89	3.9	0.66	NS	<0.001	0.79	3.28	$77.19 + 0.11 \times 10^{-7} \times \text{Phytase}^2$
Tryptophan	26	3	88.0	3.34	88.9	3.33	1.1	0.23	NS	0.038	0.95	3.45	$88.43 + 0.56 \times 10^{-8} \times \text{Phytase}^2$
Valine	57	9	79.9	1.61	82.3	1.59	2.9	0.41	NS	<0.001	0.89	3.37	$80.78 + 0.11 \times 10^{-7} \times \text{Phytase}^2$
Sum of EAA			84.5	1.30	86.5	1.28	2.3	0.14	NS	<0.001	0.32	4.30	$85.18 + 0.82 \times 10^{-8} \times \text{Phytase}^2$
Non-Essential Amino Acids (NEAA)													
Alanine	61	10	83.3	1.69	84.5	1.67	1.4	0.20	NS	<0.001	0.90	3.07	$83.19 + 0.92 \times 10^{-8} \times \text{Phytase}^2$
Asparagine	0	0	-	-	-	-	-	-	-	-	-	-	-

Aspartic acid	61	10	80.1	1.84	83.1	1.82	3.7	0.55	NS	<0.001	0.89	3.96	$81.44 + 0.10 \times 10^{-7} \times Phytase^2$
Cysteine	59	9	69.7	2.46	74.0	2.40	6.1	1.08	NS	0.001	0.82	5.12	$71.77 + 0.13 \times 10^{-7} \times Phytase^2$
Glutamine	0	0	-	-	-	-	-	-	-	-	-	-	-
Glutamic acid	59	10	87.4	1.10	89.4	1.08	2.4	0.32	NS	<0.001	0.89	2.29	$88.28 + 0.13 \times 10^{-8} \times Phytase^2$
Glycine	57	9	77.5	1.53	80.4	1.49	3.7	0.53	NS	<0.001	0.83	3.28	$78.76 + 0.11 \times 10^{-7} \times Phytase^2$
Proline	54	8	80.7	1.97	83.4	1.95	3.3	0.48	NS	<0.001	0.88	3.31	$81.76 + 0.11 \times 10^{-7} \times Phytase^2$
Serine	57	9	79.7	2.09	82.2	2.07	3.1	0.56	NS	<0.001	0.89	3.94	$80.85 + 0.91 \times 10^{-8} \times Phytase^2$
Tyrosine	57	9	81.6	1.89	83.3	1.86	2.1	0.47	NS	0.002	0.83	3.87	$82.28 + 0.76 \times 10^{-8} \times Phytase^2$
Sum of NEAA			79.2	1.53	81.8	1.50	3.4	0.23	NS	<0.001	0.36	5.28	$80.15 + 0.96 \times 10^{-8} \times Phytase^2$

[1]n, number of experiments; N, number of studies

TABLE 9. Estimated levels of non-phytate phosphorus (NPP) (g/kg of diet) required to optimize growth performance traits and tibia ash content in male broiler chicken from 1 to 21 days post-hatch, in diets with or without phytase supplementation.

	Phytase (FTU /kg of diet)				
	0	500	1000	1500	2000
Average daily gain, g/bird	4.81	4.51	4.24	3.95	3.66
Average daily feed intake, g/bird	4.80	4.47	4.14	3.80	3.47
Feed conversion ratio	4.79	4.44	4.09	3.74	3.39
Tibia ash, g/100 g dry matter	5.26	4.99	4.71	4.42	4.15

FTU, phytase unit.

CHAPTER V - IMPACT OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, AND TIBIA ASH CONTENT IN MALE BROILERS FROM 22 TO 42 DAYS POST-HATCHING: A META-ANALYSIS

ABSTRACT

Non-phytate phosphorus (NPP) and dietary calcium (Ca) requirements for male broilers decrease with age. However, the finisher phase (22 to 42 days post-hatching) remains an essential period, as it accounts for nearly two-thirds of total feed intake and overall growth. Balancing dietary NPP levels during this phase to optimize both environmental and economic outcomes has been a longstanding challenge for poultry nutritionists. Several studies have investigated the effects of phytase (PHY), NPP, and the dietary Ca to NPP ratio (Ca:NPP) on performance and tibia ash content in broilers from 22 to 42 days, with highly variable results reported in the literature. To quantify the impact of PHY supplementation on male broilers, a meta-analysis was conducted using data from 21 studies involving 10,706 male broilers aged 22 to 42 days post-hatching. The collected data were analyzed using a mixed model that incorporated the effects of PHY, dietary NPP, and Ca:NPP levels, as well as their interactions. The models evaluated average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and tibia ash content. PHY supplementation significantly ($P < 0.05$) improved both growth performance and tibia ash content, with more pronounced effects observed in low-NPP diets. Additionally, increasing dietary NPP levels enhanced both growth performance and tibia ash content. However, high dietary Ca:NPP ratios negatively impacted all evaluated parameters, particularly in diets without PHY supplementation. The findings from this meta-analysis highlight the positive impact of PHY supplementation, reinforcing its benefits in improving performance and tibia ash content in male broilers during the finisher phase.

Keywords: Broiler, phytase, meta-analysis, finisher phase, performance.

1. INTRODUCTION

Calcium (Ca) and phosphorus (P) are essential minerals for numerous physiological functions in broilers (VALENTE JUNIOR et al., 2024). They are also the first and second most abundant minerals stored in the broiler's skeleton, primarily in the form of hydroxyapatite (NUAMAH et al., 2024). Maintaining a balanced ratio of these minerals in the diet is crucial, as they play a pivotal role in bone development, skeletal strength, and metabolic processes (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; VALENTE JUNIOR et al., 2024). Despite the broiler's efficiency in converting feed into muscle, its ability to utilize P from plant-based feed ingredients is limited (NUAMAH et al., 2024). This is because most dietary P is bound to phytic acid [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); InsP6], making it unavailable for absorption (SOMMERFELD et al., 2018; SELLE et al., 2023). Furthermore, poultry lack sufficient endogenous phytase enzymes to efficiently hydrolyze the salt form of phytic acid, known as phytate-P (PP), further restricting P availability (SOBOTIK et al., 2023).

Phytate-P (PP) can bind to minerals, amino acids, and enzymes in the gastrointestinal tract, reducing their bioavailability and impairing nutrient digestion (SINGH AND SATYANARAYANA, 2015; VALENTE JUNIOR et al., 2024). The presence of high dietary Ca further exacerbates this issue by forming insoluble complexes with PP, making P even less accessible for absorption (SELLE et al., 2023). To ensure broilers receive adequate P, inorganic sources are frequently incorporated into poultry diets (HERVO et al., 2023). However, these supplements are both costly and non-renewable resources (BOUGOUIN et al., 2014). Additionally, excess P that is not absorbed by the birds is excreted in manure, leading to environmental concerns such as water eutrophication (BOUGOUIN et al., 2014; SELLE et al., 2023).

A widely used strategy in the poultry industry to improve P utilization is the inclusion of exogenous enzymes, such as phytase (PHY) (SELLE et al., 2023). PHY break down $InsP_6$, releasing myo-inositol phosphate isomers and inorganic phosphate, also known as non-phytate phosphorus (NPP). This process enhances the digestibility and bioavailability of P, Ca, and other nutrients, including minerals, amino acids, and energy (COWIESON et al., 2006; AKTER et al., 2016; WALK et al., 2018). Additionally, several aspects may modulate the effect of PHY supplementation and the NPP requirements for broilers, such as sex and broiler age.

The NRC (1994) recommendations for broiler diets suggest that the NPP and Ca levels for male broilers during the starter phase (1 to 21 days post-hatching) should be 4.5 g/kg and 10.0 g/kg of diet, respectively. In the subsequent finisher phases (22 to 42 days), these levels decrease to 3.5 g/kg and 9.0 g/kg of diet, respectively. In contrast, the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) recommend slightly different values. For male broilers from 1 to 21 days, the suggested NPP and Ca levels are 4.81 g/kg and 10.1 g/kg of diet, respectively. From 22 to 42 days, these values drop to 3.15 g/kg and 6.61 g/kg of diet, respectively. The discrepancies between these recommendations can be attributed to advancements in broiler genetic selection, as modern strains differ significantly from older ones in terms of growth rate, feed intake, feed conversion efficiency, nutrient utilization, and bone structure characteristics (DHANDU AND ANGEL, 2003). However, both the NRC and the Brazilian Tables agree on the progressive reduction of NPP and Ca levels as broilers transition from the starter to the finisher phases. Additionally, the Ca:NPP ratio remains consistent at 2.10 throughout the broiler's life in the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024). On the other hand, the NRC (1994) guidelines recommend an increase in this ratio, from 2.22 in the starter phase to 2.57 in the finisher phases..

The reduction in NPP and Ca requirements after the starter phase can be attributed to the critical role this initial stage plays in supporting rapid growth, which serves as the foundation for meat deposition in later stages (BATAL AND PARSONS, 2002; BABATUNDE et al., 2019). During this period, P utilization is higher, and PHY efficacy may also be enhanced, particularly toward the end of the second week of growth (BABATUNDE et al., 2019). As skeletal development slows after this rapid growth phase, the bird's requirement for NPP and Ca decreases. Although NPP and Ca requirements decrease with age, evaluating NPP needs and PHY effects, along with the Ca:NPP ratio during the finisher phases, remains crucial. This phase account for nearly two-thirds of the total feed intake and overall growth in male broilers (VALABLE et al., 2018; AVIAGEN, 2022, COBB-VANTRESS, 2022; HUBBARD, 2023). Moreover, determining precise NPP requirements and PHY efficacy in later growth stages can maximize both environmental and economic benefits.

Meta-analysis and systematic literature reviews are well-stablished scientific methods for that examine complex phenomena, as they integrate data from multiple independent studies using quantitative techniques, resulting in more robust and reliable conclusions (ST-PIERRE, 2007). This approach can convert research findings into practical knowledge (POLYCARPO et al., 2017). Furthermore, assessing the impact of exogenous PHY on performance, along with determining NPP requirements during the later stages of broiler growth, is crucial for optimizing inorganic P supplementation, promoting sustainability, and improving cost-effectiveness (BOUGOUIN et al., 2014).

To the best of our knowledge, only one previous meta-analysis has evaluated the effects of PHY supplementation on performance and bone mineralization in male broilers aged 22 to 42 days (NUAMAH et al., 2024). However, the relationship between different phytase unit (FTU) levels, NPP requirements, and the dietary Ca:NPP ratio for optimizing growth performance and skeletal mineralization in this phase remains insufficiently explored. Therefore, this study aimed to estimate the effects of PHY supplementation on performance and tibia ash content in male broilers from 22 to 42 days post-hatching using a systematic review and meta-analysis approach.

2. MATERIAL AND METHODS

This study exclusively used data from the published literature and did not involve the use of animals, eliminating the need for approval from the Institutional Animal Care and Use Committee.

2.1. META ANALISYS DATA SEARCH STRATEGY

A systematic online search was performed to detect indexed publications assessing the impact of PHY supplementation on performance and tibia ash content in male broiler chickens aged 22 to 42 days post-hatching. The search, finalized in December 2024, targeted peer-reviewed articles available in scientific databases, including PubMed, Web of Science, and Scopus. To enhance the comprehensiveness of the systematic review, the reference lists of the selected studies were also screened for additional relevant publications. The research question was created using the PICo framework (ANDRETTA et al., 2021), where "Population" was defined as "broilers", "Interest" as "phytase", and "Context" as "performance", or "tibia ash concentration". To improve the search strategy, synonyms and alternative English terms for both the population and context were incorporated. Studies on PHY were then exported to a reference management software (EndNote X9; Philadelphia, PA, USA) for duplicate removal. The relevance and quality of the selected studies were evaluated through a two-stage screening process, beginning with an initial review of titles and abstracts, followed by an in-depth assessment of the full texts.

The criteria for selecting publications were: (1) *in vivo* experiments performed on commercial broiler chicken breeds, (2) experiments that included both non-supplemented control diets and diets supplemented with commercial PHY, (3) studies presenting performance data, including average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), the effects of PHY on tibia ash content, and (4) studies published from 2000 onward. Manuscripts selected for initial screening were excluded if they met any of the following criteria: (1) did not use male broiler chickens or did not specify the sex of the broilers, (2) involved a sanitary challenge, (3) combined PHY with another enzyme, (4) conducted studies lacking a control treatment, or (5) exhibited significant inconsistencies in the statistical design, methodology, or reported outcomes. Additionally, studies utilizing super-dosing PHY (>2,500 FTU/kg) were excluded due to insufficient available data. Only studies performed with broilers aged 22 to 42 days post-hatching, or those that included experiments conducted partially within this period, were considered. To ensure a precise evaluation of PHY effects, only non-supplemented diets (negative control) with matching NPP levels to those in the PHY-supplemented group were included in the meta-analysis.

The screening process for the meta-analysis is presented in Figure 1. Initially, 5,710 studies were identified, with further successive exclusions made based on titles and abstracts. Full-text reviews were performed for 54 references to assess their eligibility. After an in-depth review, 33 publications were excluded from the meta-analysis, in accordance with the pre-established criteria. Thus, this meta-analysis comprised 21 publications. The final list of the selected studies for the meta-analysis is presented in Table 1.

2.2. META-ANALYSIS DATABASE CONSTRUCTION

The methodology for building the database, data encoding, and variable definition was based on the approach described by Lovatto et al. (2007). To maintain consistency and comprehensiveness in dietary composition across studies, experiments lacking full nutrient details were recalculated using the Practical Program for Formulation of Rations (PPFR; GARCIA-NETO, 2008), referencing the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024). The variability in diet composition within the dataset is summarized in Table 2.

2.3. STATISTICAL ANALYSIS

A meta-analytical approach was employed to model the dependent variables, following the methodology described by Andretta et al. (2012). The meta-analysis was conducted through a sequential analytical process, incorporating graphical, correlation, and variance-covariance analyses. To address variability among studies in the meta-analysis database, all variance-covariance analyses treated the study effect as a random-effect in the model (ST-PIERRE, 2001). Mixed models were developed for the male broilers from 22 to 42 days post-hatching. The relationships between PHY and NPP, as well as PHY and the Ca:NPP ratio, were analyzed in separate models to avoid collinearity issues. Independent variables were tested, and only those demonstrating statistical significance ($P < 0.05$) or a tendency ($P < 0.10$) were retained in the final models. Following the approach described by Létourneau-Montminy et al. (2010), the response of each dependent variable, including ADG, ADFI, FCR, and tibia ash content, were evaluated using a full quadratic model. The model incorporated the statistically significant independent variables, including PHY, NPP, and the Ca:NPP ratio, as detailed below:

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{NPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{NPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{NPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk}, [1]$$

and,

$$\mathrm{Y_{ijk}} = \alpha + \alpha_i + \beta_1 \mathrm{CaNPP}_{ij} + \beta_2 \mathrm{PHY}_{ik} + \beta_3 \left[\mathrm{CaNPP}_{ij}\right]^2 + \beta_4 [\mathrm{PHY}_{ik}]^2 + \beta_5 \mathrm{CaNPP_{ij}}\ x\ \mathrm{PHY}_{ik} + \mathrm{e}_{ijk}, [2],$$

where Y_{ijk} denotes the value of the dependent variable Y in the experiment *I,* at the level *j* of NPP (for equation [1]) or Ca:NPP (for equation [2]), and level *k* of PHY. The parameter α represents the overall fixed- effect intercept, while α_i accounts for the random effect of experiment *i*. The coefficients β1 and β2 correspond to the linear effects, β3 and β4 capture the quadratic effects, and β5 represents the interaction effect. The term e_{ijk} represents the residual error.

The random effects and residual errors were examined to ensure they followed a normal distribution. To identify potential outliers, internally studentized residuals (ISR) were investigated, with values exceeding 3 (|ISR| > 3) flagged and excluded from further analysis (FARIDI et al., 2015; KERMANI et al., 2022). Across all models, over 95% of ISR values fell within the range of -2 to 2, confirming a good model fit. Model accuracy was assessed using multiple criteria, including the significance of estimated parameters, error variance and standard error (SE), coefficient of determination (R^2), and root mean square error (RMSE). Model adequacy was further assessed by plotting residuals (observed minus predicted values) against the predicted values of Y to check for linear prediction bias (ST-PIERRE, 2001). For optimization, the partial derivatives of the models for each dependent variable were calculated, following the methodology described by Létourneau-Montminy et al. (2010). Data analysis was performed utilizing the general linear model (GLM) procedure in the Minitab 22 software (Minitab Inc., State College, PA, USA). Graphs were generated using the ggplot2 package in R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1. DATABASE CHARACTERIZATION

The meta-analysis for male broiler chickens aged 22 to 42 days post-hatching comprised 21 studies, encompassing a total population of 10,706 male broilers. A total of 86 treatments were included in this meta-analysis, Ross 308 was the most frequently observed breed (n=49), followed by Cobb 500 (n=22), and Arbor Acres (n=15). PHY supplementation varied across the studies, among the 59 treatments that used PHY, 40.7% used 3-phytase (EC 3.1.3.8), while 37.3% utilized 6-phytase (EC 3.1.3.26). The remaining 22.0% of treatments did not specify the PHY type. PHY enzymes were derived from various organisms, including *Aspergillus niger* (44.1%), *Escherichia coli* (28.7%), *Trichoderma reesei* (8.5%), *Enterobacter sakazakii* (5.1%), *Serratia odorifera* (5.1%), *Buttiauxella sp.* (3.4%), and *Peniophora lycii* (3.4%). Additionally, 1.7% of treatments did not specify the donor organism for the exogenous PHY. Furthermore, the final dataset for the meta-analysis included studies conducted in Iran (n=7), Brazil (n=3); China, Spain, Turkey, and United States of America (USA) (n=2); and Czech Republic, Malaysia, and New Zealand (n=1). Most treatments used corn-soybean meal-based diets (77.9%), with smaller proportion using corn-wheat-soybean meal-based diets (16.3%) and wheat-soybean meal-based diets (5.8%). Only grain-based feedstuffs were the included in 82.6% of the diets, while the remaining 17.4% also included animal-based feedstuffs. The feed was presented in various forms: mash (36.0%), cold pellet (5.8%), pellet (4.7%), or in unspecified forms (53.5%). Diets were classified as cold-pelleted diets when the maximum temperature during the pelleting process did not exceed 70°C (RAVINDRAN et al., 2008). Mineral P supplementation was primarily provided through dicalcium phosphate (89.0%), followed by monocalcium phosphate (6.1%), and tricalcium phosphate (4.9%). Additionally, the dataset revealed that diets with a Ca:NPP ratio lower than 2:1 accounted for 15.2%, while 26.7% of the diets had a ratio between 2:1 and 3:1, diets with a Ca:NPP ratio between 3:1 and 4:1 constitute 17.4% of the dataset, diets with a Ca:NPP ratio between 4:1 and 5:1 constitute 9.3% of the dataset, while those exceeding 5:1 represented 31.4%.

3.2. META-ANALYSIS RESULTS

Summary statistics for the dependent and independent variables included in the meta-analysis of male broilers aged from 22 to 42 days is presented in Table 3. The effects of various levels of dietary supplemental PHY and NPP on ADG, ADFI, FCR and tibia ash content are detailed in Table 4 and illustrated in Figure 2. The effects of dietary PHY levels and Ca:NPP ratio on ADFI, FCR and tibia ash content are presented in Table 5 and Figure 3. Residual plots (observed minus predicted) from the PHY and NPP mixed model analyses are shown in Figure 4, while the residuals from the PHY and Ca:NPP are presented in Figure 5.

3.3. IMPACT OF PHY SUPPLEMENTATION ON THE PERFORMANCE AND TIBIA ASH CONTENT

Dietary PHY supplementation increased the intercept for ADG and ADFI in male broilers from 22 to 42 days post-hatching, while reducing the intercept for FCR depending on dietary NPP and PHY levels. The interaction between exogenous PHY supplementation and dietary NPP levels showed a tendency to increase ADG (PHY × NPP; $P<0.10$) and a significant increase in ADFI (PHY × NPP; $P<0.05$), with the strongest effects observed at lower dietary NPP levels. Additionally, PHY supplementation and increasing dietary NPP exhibited a significant linear positive effect on both ADG and ADFI ($P<0.05$). The interaction between PHY supplementation and the dietary Ca:NPP ratio on ADG was not significant (*P*>0.10), and as a result, ADG was excluded from subsequent model development. However, ADFI was significantly affected by PHY supplementation and the dietary Ca:NPP ratio, with greater PHY effects observed at higher Ca:NPP ratios and significant linear effect (*P*<0.05).

Diets supplemented with PHY improved FCR in male broilers aged 22 to 42 days post-hatching. PHY exerted a significant linear effect (*P*<0.05), while dietary NPP influenced FCR through significant quadratic effect (P < 0.05), with a tendency for a greater impact in low-NPP diets (PHY × NPP; *P*<0.10). The dietary Ca:NPP ratio and PHY supplementation also showed significant quadratic effect (*P*<0.05) on FCR. The impact of PHY supplementation was more pronounced in diets with higher Ca:NPP ratios (PHY × Ca:NPP; *P*<0.001). Additionally, PHY supplementation displayed significant quadratic effects (*P*<0.05) on FCR, whereas the dietary Ca:NPP ratio had a significant positive linear effect (*P*<0.05) in male broilers.

Tibia ash content in male broilers aged 22 to 42 days was influenced by PHY supplementation and dietary NPP levels, showing significant quadratic effect (*P*<0.05) for PHY supplementation, and a linear effect (*P*<0.05) for increasing dietary NPP levels. The impact of PHY supplementation on tibia ash content was more pronounced at lower dietary NPP levels (PHY × NPP; *P*<0.05). Furthermore, the interaction between PHY supplementation and the Ca:NPP ratio had a significant effect on tibia ash content, with a stronger PHY response observed in diets with higher Ca:NPP ratios (PHY × Ca:NPP; *P*<0.05). Additionally, PHY supplementation influenced tibia ash content following a quadratic pattern (*P*<0.05).

3.4. MODEL ADEQUACY

The residual plots against the predicted values, shown in Figures 4 and 5, indicate the adequacy of the model assessing various dietary NPP and Ca:NPP ratio levels in broilers fed different PHY levels. Residuals displayed a good distribution around zero across the different models, indicating a good fit. The residuals differed from the predicted values by 15.9%, 17.8%, 11.2%, and 11.8% for ADG, ADFI, FCR, and tibia ash content, respectively, for the models including various dietary NPP. The residuals differed from the predicted values by 17.1%, 11.1%, and 9.1% for ADFI, FCR, and tibia ash content, respectively, for the models evaluating the effects of different Ca:NPP ratios in broiler diets with varying PHY supplementation.

4. DISCUSSION

Microbial PHY is one of the most used nutritional strategies in the poultry industry to enhance P and nutrient digestibility, consequently improving broiler performance while reducing production costs and environmental pollution. Various studies have examined the interactions and effects of PHY, NPP, and the Ca:NPP ratio on broiler performance, with highly variable results reported in the literature. Previous meta-analyses have assessed the effects of PHY supplementation on broiler performance and tibia ash content in male broilers during the starter phase (LÉTOURNEAU-MONTMINY et al., 2010; BOUGOUIN et al., 2014; FARIDI et al., 2015; COWIESON et al., 2017; KERMANI et al., 2023; NUAMAH et al., 2024). However, to the best of our knowledge, only one previous meta-analysis has evaluated the effects of PHY supplementation on tibia ash content and performance in male broilers during the finisher phase (NUAMAH et al., 2024). Additionally, during the study selection process for the systematic review and meta-analysis, we observed fewer studies addressing the interactions between PHY, dietary NPP, and Ca in broilers during the finisher phase compared to the starter phase. This highlights the need for further research focusing on this specific physiological stage of broiler development.

4.1. IMPACT OF PHYTASE SUPPLEMENTATION ON GROWTH PERFORMANCE AND TIBIA ASH CONTENT

Exogenous PHY supplementation in broiler diets can positively influence performance, and tibia ash content often correlating with increased nutrient digestibility (OLUKOSI et al., 2008; WOYENGO et al., 2010). PHY can hydrolyze PP complexes, releasing P and other essential nutrients. This process improves the performance of broilers fed diets with low NPP levels (PIRGOZLIEV et al., 2011) or those deficient in both Ca and NPP (WALK et al., 2013). The current meta-analysis showed that PHY supplementation improved ADG, ADFI, tibia ash content, and FCR in male broilers from 22 to 42 days post-hatching. These findings are in line with the meta-analysis developed by Nuamah et al (2024), who reported that PHY enhanced growth performance and tibia ash content in male broilers during the finisher phase. Assessing the impact of PHY supplementation during the finisher phase is crucial for reducing environmental pollution, lowering production costs, and enhancing broiler performance. Although NPP and Ca requirements are lower during the finisher phase compared to the starter phase, this phase represent almost two-thirds of the total overall growth and the feed intake in the male broilers production cycle (VALABLE et al., 2018).

The fitted models indicate a positive impact of PHY supplementation on ADFI, with the strongest effects observed at lower dietary NPP levels. While low-NPP diets may reduce feed intake, this adverse effect can be mitigated by either directly increasing dietary NPP levels or through PHY supplementation (ROUSSEAU et al., 2016). Diets supplemented with 1,000 FTU of PHY increased ADFI by 6.5 g/day in male broilers fed an NPP level of 1.50 g/kg from 22 to 42 days of age. However, no significant improvement in ADFI was observed with PHY supplementation in diets containing NPP levels above 2.90 g/kg, suggesting that this concentration meets the dietary NPP requirements for ADFI. These findings align with the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024) and Han et al. (2009), who reported optimal dietary NPP concentrations for ADFI of 3.15 g/kg and 2.90 g/kg, respectively. Similarly, Han et al. (2009) conducted a trial with low-NPP diets (1.3 g/kg) and found that PHY supplementation at levels of 500, 1,000, and 1,500 FTU/kg of diet was equivalent to increasing dietary NPP levels by 0.49, 0.68, and 0.86 g/kg, respectively, for ADFI in male broilers aged 22 to 42 days. Our findings demonstrate that an increased dietary Ca:NPP ratio negatively affects growth performance in male broilers during the finisher phase. Interestingly, PHY supplementation had a more pronounced effect in diets with higher Ca:NPP ratios compared to those with lower ratios. Several mechanisms may explain this inverse relationship, as elevated Ca:NPP levels can reduce P availability in the gut lumen by promoting the formation of calcium phosphate (Ca_2PO_4) precipitates (KERMANI et al., 2023). Additionally, excess Ca binds to PP molecules, reducing the solubility of $InsP_6$ and limiting its accessibility to phosphatases in the digestive tract (SELLE AND RAVINDRAN, 2007). Another potential mode of action is that Ca competes with exogenous PHY for the same active sites, potentially reducing the enzyme’s efficacy (POINTILLART et al., 1985).

Additionally, supplementation with 1,000 FTU of PHY increased ADG by 3.2 g/day in male broilers fed diets containing an NPP level of 3.00 g/kg from 22 to 42 days of age. Nuamah et al. (2024) reported an even greater improvement, with an increase in ADG of 7.8 g/day in broilers fed diets with an average NPP of 3.00 g/kg and supplemented with an average dose of 875 FTU/kg of feed during the same period. In the present study, PHY supplementation exhibited the strongest effects at lower dietary NPP levels. Specifically, diets containing 1.5 g/kg of NPP and supplemented with 1,000 FTU of PHY resulted in an ADG increase of 6.7 g/day. Likewise, Han et al. (2009) observed that PHY supplementation at levels of 500, 1,000, and 1,500 FTU/kg of diet was equivalent to increasing dietary NPP levels by 0.6, 0.78, and 0.96 g/kg, respectively, for ADG in male broilers from 22 to 42 days. Although a higher dietary Ca:NPP ratio, in interaction with PHY supplementation, influenced ADFI, no significant effect on ADG was observed in male broilers from 22 to 42 days of age.

In the present meta-analysis, PHY supplementation significantly improved FCR. This finding agrees with the meta-analysis conducted by Nuamah et al. (2024), who reported an improved in FCR in male broilers fed PHY-supplemented diets from 22 to 42 days of age. Additionally, the fitted models indicate that PHY exhibited its strongest effects at lower dietary NPP levels. According to the dietary NPP recommendations from the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2024), which recommend an NPP level of 0.34% during the finisher phase for male broilers, similar performance in FCR was observed at NPP levels of 0.27%, when 500 FTU/kg of PHY was included. This suggests that PHY at this dosage can release up to 0.07% of NPP in diets with reduced P levels.. It is important to highlight that the meta-analysis included studies using different generations of PHY enzymes, which may have resulted in a lower estimated NPP release compared to what could be achieved with the most recent enzyme technologies. The positive impact of PHY supplementation on broiler FCR may be attributed to its ability to release P in NPP-deficient diets and mitigate the negative effects of PP on nutrient digestibility. By breaking down PP complexes, PHY improves the bioavailability of essential nutrients such as Ca, minerals, amino acids, and enzymes, thereby enhancing overall feed efficiency (WALK et al., 2014). Furthermore, an increased dietary Ca:NPP ratio negatively affected FCR in male broilers during the finisher phase, suggesting that excessive dietary Ca reduces P availability and impairs feed efficiency (KERMANI et al., 2023).

Our findings indicate that PHY supplementation increases tibia ash content, with the greater effects observed at lower dietary NPP levels. Diets supplemented with exogenous PHY enhanced the availability of minerals releasing them from mineral-PP complexes (SOBOTIK et al., 2023). Diets supplemented with 1,000 FTU of PHY increased tibia ash content by 4.9% in male broilers fed an NPP level of 1.50 g/kg, while enhanced the tibia ash content by 1.0% in diets with NPP level of 3.0 g/kg. Han et al. (2009) described that PHY supplementation at levels of 500, 1,000, and 1,500 FTU/kg of diet was equivalent to increasing dietary NPP levels by 0.77, 0.95, and 1.14 g/kg, respectively, for tibia ash content in male broilers from 22 to 42 days. The effects of PHY were more pronounced in diets with higher Ca:NPP ratios compared to those with lower ratios. Furthermore, an increased dietary Ca:NPP ratio negatively impacted tibia ash content in male broilers during the finisher phase. Diets with high Ca levels, especially when paired with low-NPP diets, elevate pH in the upper gastrointestinal tract, particularly in the gizzard. This increase in alkalinity promotes the formation of insoluble PP-Ca complexes, which further restricts the availability of both Ca and P (SELLE et al., 2009; BEDFORD AND ROUSSEAU, 2017).

Several meta-analyses reported a greater effect of PHY on tibia ash content during the starter phase (FARIDI et al., 2015; KERMANI et al., 2023) compared to the findings of the present meta-analysis, which focused on the finisher phase. PHY supplementation appears to be more effective in enhancing tibia ash content during early growth, as birds in the starter phase require higher Ca and P deposition in bones to support the rapid weight gain (TALATY et al., 2009; BABATUNDE et al., 2020). Bone mineralization peaks at approximately four weeks of age. Beyond this point, although tibia growth continues, bone density does not increase significantly due to the expansion of bone surface area (TALATY et al., 2009; NUAMAH et al., 2024). Furthermore, Scholey and Burton (2017) suggested that the femur may be a more appropriate bone for estimating ash content in broilers at six weeks of age, as mineralization is still actively occurring in the femur compared to the tibia.

5. CONCLUSION

Exogenous PHY supplementation improved both growth performance as tibia ash content, with more pronounced effects observed in low-P diets. Also, increasing dietary NPP levels enhanced growth performance and tibia ash content. However, high dietary Ca:NPP ratios impared all evaluated parameters, particularly in diets without PHY supplementation. Given the complex interactions between Ca, NPP, and exogenous PHY, along with the limited number of studies involving broilers during the finisher period, further research is necessary to better understand their interactive effects on P utilization in broiler chickens.

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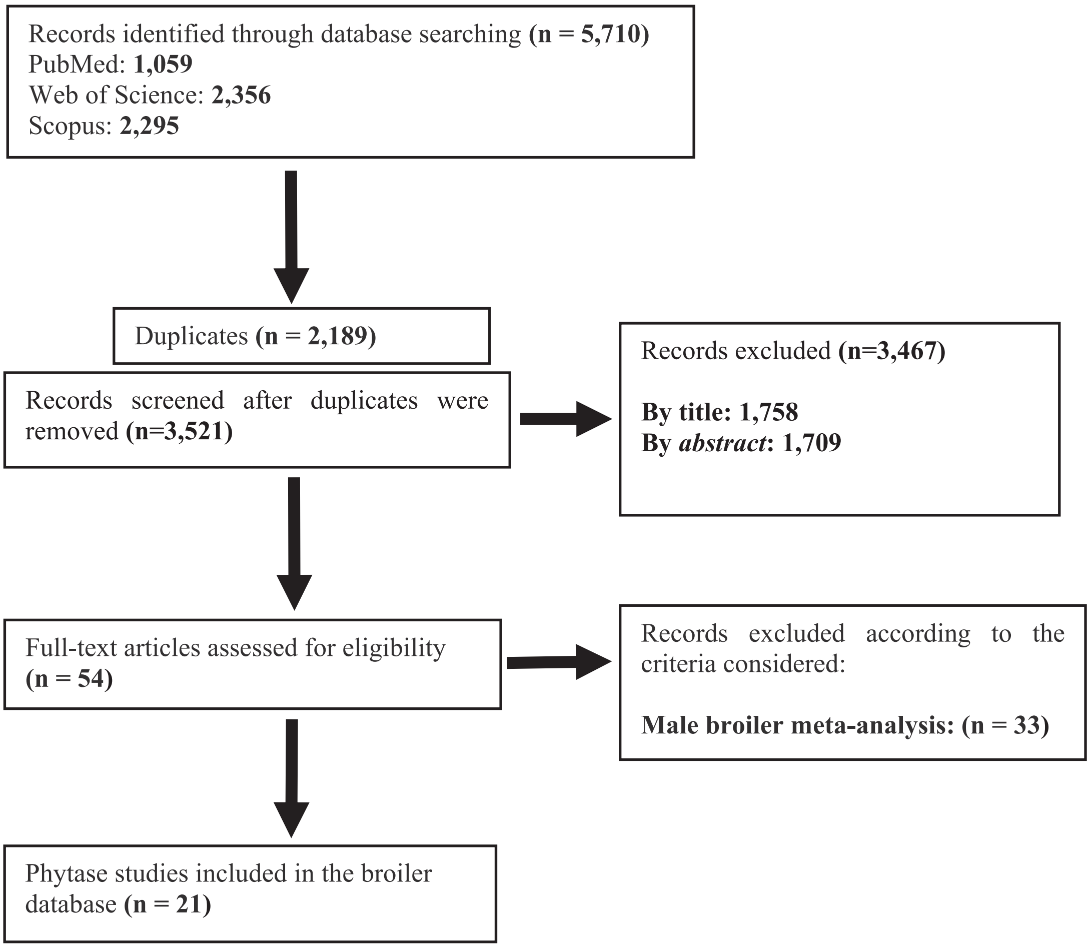

FIGURE 1. Study selection diagram illustrating the selection process for the meta-analyses investigating the effect of phytase supplementation in male broilers from 22 to 42 days post-hatch.

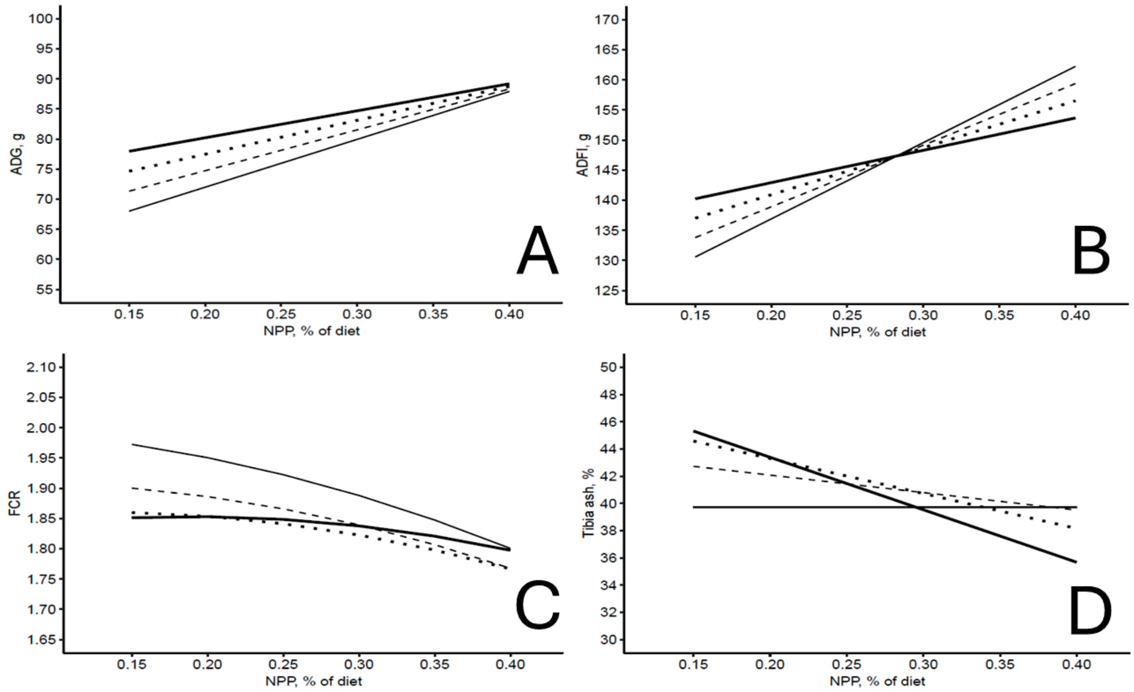

FIGURE 2. Response of average daily gain (ADG, g), average daily feed intake (ADFI, g), feed conversion ratio (FCR), and tibia ash (%) to dietary percentage of non-phytate phosphorus (NPP) in male broilers from 22 to 42 days of age, fed with different dietary supplemental phytase.

Description of curve types and equations for ADG, ADFI, FCR, and tibia ash. Curve types depicted in panels (A), (B), (C), and (D) correspond to the following dietary phytase levels, were solid thin curve – 0 FTU/kg of diet; dashed thick curve – 500 FTU/kg of diet; dotted curve – 1,000 FTU/kg of diet; solid thick curve – 1,500 FTU/kg of diet. (A) ADG (g) = 56.08 + 0.10 × Phytase + 79.589 × NPP - 0.023 × Phytase × NPP (RMSE = 12.09, $R^2 = 0.94$). (B) ADFI (g) = 111.56 + 0.014 × Phytase + 126.71 × NPP - 0.049 × Phytase × NPP (RMSE = 25.74, $R^2 = 0.98$). (C) FCR = 2.001 - 0.22×10^{-5} × Phytase + 0.64×10^{-9} × Phytase^2 + 1.25 × NPP^2 + 0.31×10^{-5} × Phytase × NPP (RMSE = 0.21, $R^2 = 0.98$). (D) Tibia ash (%) = 39.734 + 0.011 × Phytase - 0.23×10^{-7} × Phytase^2 - 0.026 × Phytase × NPP (RMSE = 4.98, $R^2 = 0.94$).

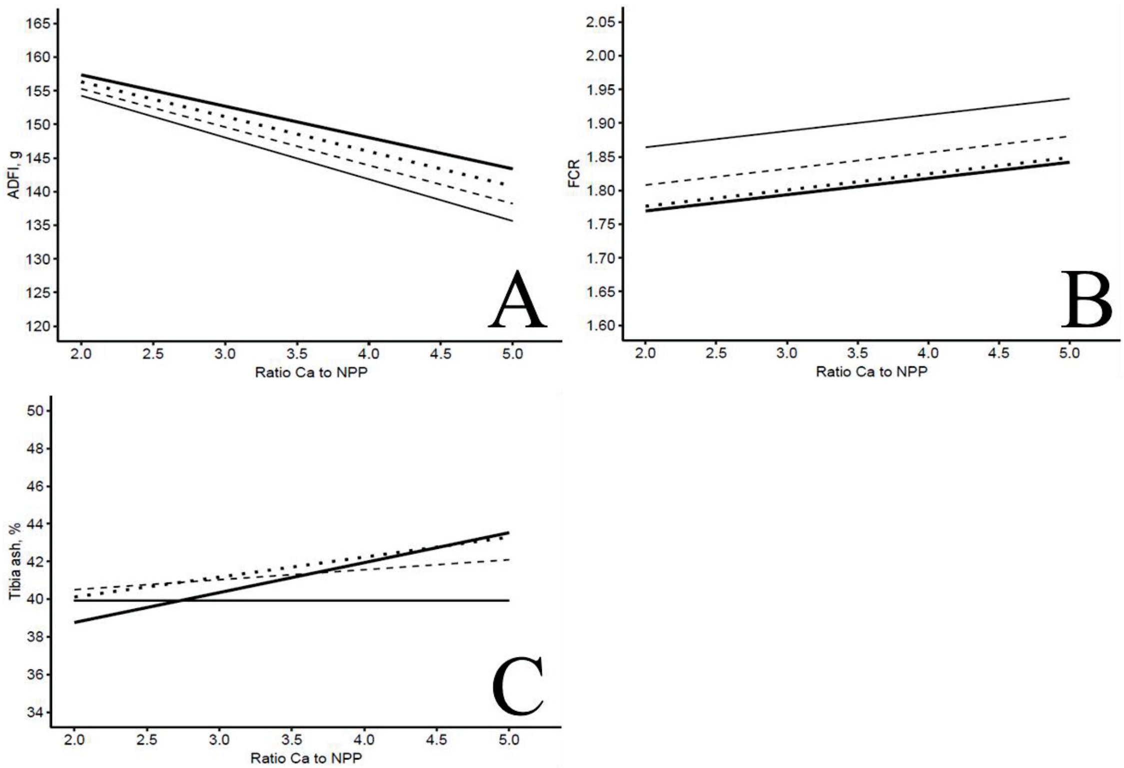

FIGURE 3. Response of average daily feed intake (ADFI, g), feed conversion ratio (FCR), and tibia ash (%) to calcium to non-phytate phosphorus (NPP) ratio in male broiler from 22 to 42 days of age, fed with different dietary supplemental phytase.

Description of curve types and equations for ADFI, FCR, and Tibia ash. Curve types depicted in panels (A), (B), and (C) correspond to the following dietary phytase levels, were solid thin curve – 0 FTU/kg of diet; dashed thick curve – 500 FTU/kg of diet; dotted curve – 1,000 FTU/kg of diet; solid thick curve – 1,500 FTU/kg of diet. (A) ADFI (g) = 166.69 - 6.211 × Ca:NPP + 0.10×10^{-4} × Phytase × Ca:NPP (RMSE = 24.84, $R^2 = 0.97$). (B) FCR = 1.816 - 0.14×10^{-5} × Phytase + 0.024 × Ca:NPP + 0.49×10^{-9} × Phytase^2 (RMSE = 0.21, $R^2 = 0.98$). (C) Tibia ash (%) = 39.927 - 0.19×10^{-7} × Phytase^2 + 0.11×10^{-4} × Phytase × Ca:NPP (RMSE = 4.75, $R^2 = 0.94$).

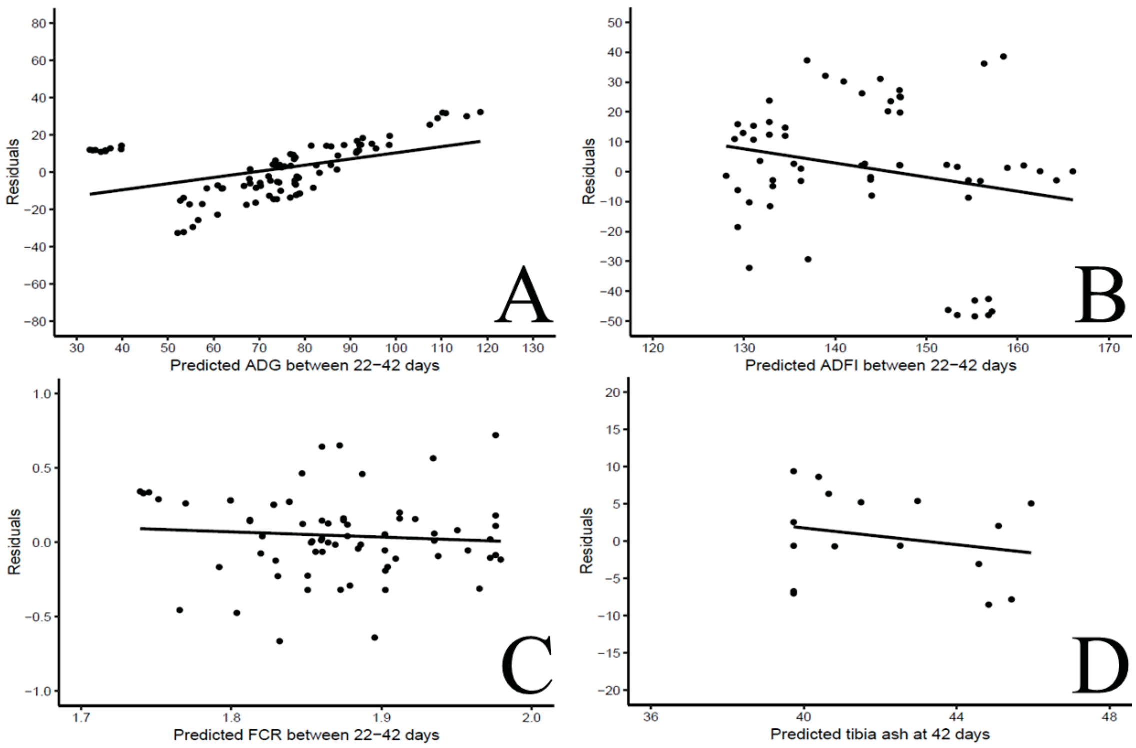

FIGURE 4. Plot of residuals (observed minus predicted) against predicted average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and tibia ash content in male broilers from 22 to 42 days of age, fed with different dietary supplemental phytase and non-phytate phosphorus levels. The line represents the regression of residuals on predicted ADG (A) [Y = -22.74 + 0.331 × predicted ADG; $R^2 = 0.18$; $P = 0.001$], on predicted ADFI (B) [Y = 162.1 - 1.095 × predicted ADFI; $R^2 = 0.09$; $P = 0.074$], on predicted FCR (C) [Y = 0.70 - 0.351 × predicted FCR; $R^2 < 0.001$; $P = 0.514$], and on predicted tibia ash (D) [Y = 24.1 - 0.560 × predicted tibia ash; $R^2 < 0.001$; $P = 0.405$].

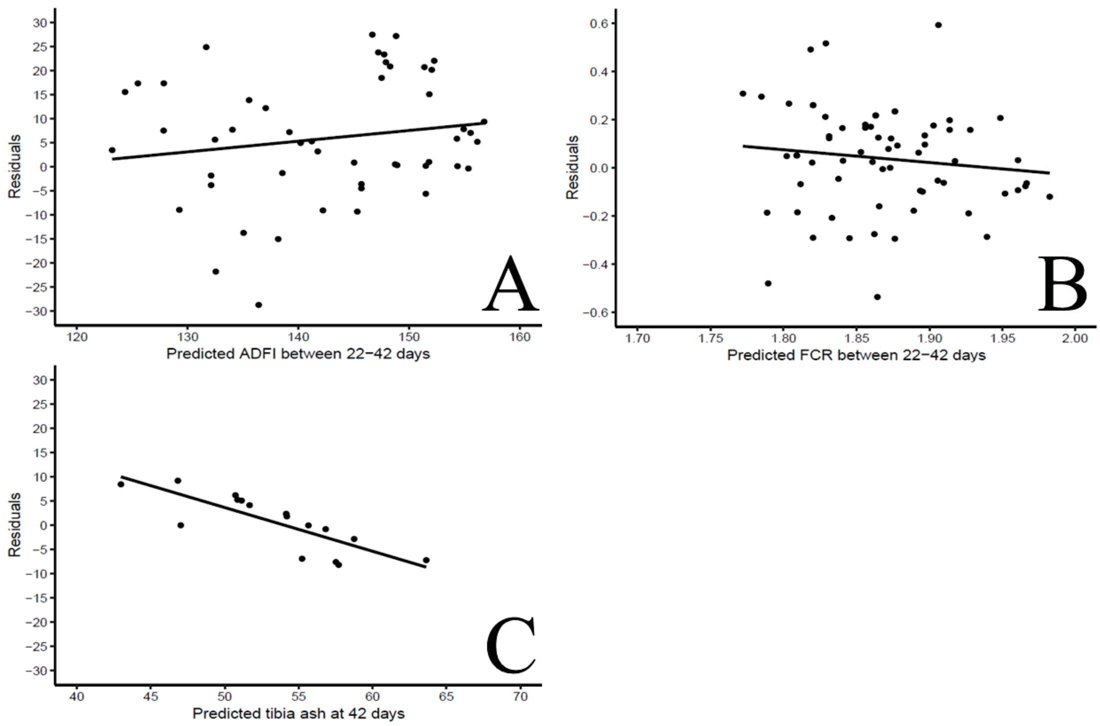

FIGURE 5. Plot of residuals (observed minus predicted) against predicted average daily feed intake (ADFI), feed conversion ratio (FCR), and tibia ash in male broilers from 22 to 42 days of age, fed with different dietary supplemental phytase and calcium to non-phytate phosphorus ratios.

The line represents the regression of residuals on predicted ADFI (A) [$Y = 117.3 - 0.785 \times$ predicted ADG; $R^2 = 0.003$; $P = 0.077$], on predicted FCR (B) [$Y = -0.23 + 0.148 \times$ predicted ADFI; $R^2 < 0.001$; $P = 0.819$], and on predicted tibia ash content (C) [$Y = 48.76 - 0.902 \times$ predicted FCR; $R^2 = 0.63$; $P < 0.001$].

TABLE 1. Summary of phytase supplementation studies included in the meta-analysis of male broilers aged 22 to 42 days, categorized by location, journal, and year of publication.

Code	Study	Country	Journal	Year of publication
1	Viveros et al.	Spain	Poultry Science	2002
2	Dilger et al.	United States	Poultry Science	2004
3	Wu et al.	New Zealand	Journal of Applied Poultry Research	2004
4	Zobač et al.	Czech Republic	Czech Journal of Animal Science	2004
5	Han et al.	China	Journal of Applied Poultry Research	2009
6	Manangi et al.	United States	International Journal of Poultry Science	2009
7	Nourmohammadi et al.	Iran	American Journal of Animal and Veterinary Sciences	2010
8	Junqueira et al.	Brazil	Acta Scientiarum - Animal Sciences	2011
9	Ceylan et al.	Turkey	Journal of Animal and Feed Sciences	2012
10	Nourmohammadi et al.	Iran	Italian Journal of Animal Science	2012
11	Jiang et al.	China	Italian Journal of Animal Science	2013
12	Midilli et al.	Turkey	Kafkas Universitesi Veteriner Fakultesi Dergisi	2014
13	Taheri et al.	Iran	British Poultry Science	2015a
14	Taheri et al.	Iran	Poultry Science Journal	2015b
15	Taheri et al.	Iran	British Poultry Science	2015c
16	Tizziani et al.	Brazil	Brazilian Journal of Animal Science	2016
17	Islam et al.	Malaysia	Cogent Food & Agriculture	2017
18	Nafea et al.	Iran	Biochemical and Cellular Archives	2019
19	Mohiti-Asli et al.	Iran	Italian Journal of Animal Science	2020
20	Javadi et al.	Spain	Animals	2022
21	França et al.	Brazil	Livestock Science	2023

TABLE 2. Diet composition variation included in the dataset for male broiler aged 22 to 42 days post-hatched experiments.

	N[1]	Mean	Minimum	Maximum	SD
Phosphorus total, g/kg of diet	20	0.53	0.35	1.06	0.17
Phytate phosphorus, g/kg of diet	20	0.29	0.22	0.70	0.12
Calcium, g/kg of diet	20	0.81	0.51	1.19	0.14
Metabolic energy, Kcal/kg of diet	20	3,085	2,914	3,227	96.0
Crude protein, g/kg of diet	20	19.3	17.0	22.3	0.95
Vitamin D3, IU/kg of diet	20	2,670	360	8,800	1,928
Copper, mg/kg of diet	20	7	2	13	2.8
Zn, mg/kg of diet	20	70	17	107	22
Iron, mg/kg of diet	20	57	14	80	21
Mn, mg/kg of diet	20	80	24	125	24
I, mg/kg of diet	20	0.9	0.2	2.0	0.5
Se, mg/kg of diet	20	0.19	0.03	0.33	0.07
Sodium, g/kg of diet	20	0.16	0.09	0.21	0.03
Total lysine, g/kg of diet	20	1.07	0.90	1.42	0.12
Digestible lysine, g/kg of diet	20	0.99	0.80	1.30	0.12
Total methionine, g/kg of diet	20	0.45	0.32	0.60	0.08
Digestible methionine, g/kg of diet	20	0.41	0.24	0.58	0.09
Total methionine + cysteine, g/kg of diet	20	0.75	0.34	0.98	0.13
Digestible methionine + cysteine, g/kg of diet	20	0.69	0.48	0.86	0.10
Total threonine, g/kg of diet	20	0.75	0.53	0.84	0.07
Digestible threonine, g/kg of diet	20	0.67	0.53	0.74	0.05

[1]The number of studies that reported poultry diet composition.

TABLE 3. Summary statistics for the data used in the meta-analyses of male broiler aged 22 to 42 days post-hatched[1].

Item[2]	n	N	Mean	SD	Min.	Max.
Male broiler meta-analysis						
Independent variable						
Phytase (FTU/kg)	86	21	531	578	0.00	2,500
Non-phytate phosphorus (%)	86	21	0.24	0.09	0.13	0.43
Calcium to non-phytate phosphorus ratio	86	21	3.8	1.8	1.2	6.9
Dependent variable						
Average daily gain, g	86	21	77.4	14.7	52.1	118.5
Average daily feed intake, g	79	19	146.0	33.6	91.8	231.6
Feed conversion ratio	79	19	1.90	0.27	1.17	2.69
Tibia ash content, %	21	5	44.7	6.33	32.7	52.7

[1]n, number of experiments; N, number of studies; SD, standard deviation; Min., minimal value of the parameter: Max., maximal value of the parameter. [2]NPP, non-phytate phosphorus; FCR, feed conversion ratio; FTU, phytase unit.

TABLE 4. The effects of phytase supplementation and dietary non-phytate phosphorus (NPP), along with their parameter estimates for the mixed model applied to male broiler from 22 to 42 days of age in performance, and tibia ash content.

Male broiler growth performance and tibia ash content models	Average daily gain (g)			Average daily feed intake (g/d)			Feed conversion ratio			Tibia ash (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	56.077	5.282	<0.001	111.56	11.44	<0.001	2.001	0.080	<0.001	39.735	3.101	<0.001
Phytase	0.010	0.003	0.001	0.14×10^{-3}	0.40×10^{-4}	0.001	-0.22×10^{-5}	0.55×10^{-6}	<0.001	0.11×10^{-3}	0.32×10^{-4}	0.009
NPP	79.589	15.051	<0.001	126.71	21.71	<0.001	-	-	NS	56.236	13.838	<0.001
Phytase^2	-	-	NS	-	-	NS	0.64×10^{-9}	0.19×10^{-9}	0.001	-0.23×10^{-7}	0.10×10^{-10}	0.062
NPP^2	-	-	NS	-	-	NS	-1.249	0.361	0.001	-	-	NS
Phytase x NPP	-0.023	0.014	0.092	-0.49×10^{-3}	0.19×10^{-3}	0.012	0.32×10^{-5}	0.17×10^{-5}	0.066	-0.026	0.14	0.096
R^2		0.94			0.98			0.98			0.94	
RMSE*		12.09			25.74			0.21			4.98	

*RMSE, Root means square error

TABLE 5. The effects of phytase supplementation and the calcium to non-phytate phosphorus (NPP) (Ca:NPP) ratio in diets on performance, and tibia ash content in male broilers from 22 to 42 days of age, along with the parameter estimates for the mixed model applied.

Male broiler growth performance and tibia ash content models									
	Average daily feed intake (g/d)			Feed conversion ratio			Tibia ash (%)		
	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	111.562	11.437	<0.001	1.816	0.081	<0.001	39.926	3.002	<0.001
Phytase	0.014	0.004	NS	-0.14×10^{-5}	0.32×10^{-6}	<0.001	-	-	NS
Ca:NPP ratio	126.711	21.709	<0.001	0.024	0.009	0.011	-	-	NS
Phytase^2	-	-	NS	0.49×10^{-9}	0.19×10^{-9}	0.007	-0.19×10^{-7}	0.85×10^{-8}	0.049
Ca:NPP^2	-	-	NS	-	-	NS	-	-	NS
Phytase x Ca:NPP	-0.049	0.019	0.012	-	-	NS	0.11×10^{-4}	0.29×10^{-5}	0.005
R^2		0.98			0.98			0.94	
RMSE*		24.84			0.21			4.75	

*RMSE, Root means square error

FINAL CONSIDERATIONS

Phosphorus (P) plays a crucial role in various metabolic processes within the animal body, making it one of the most nutritionally and economically significant minerals in poultry diet formulations. Microbial phytase (PHY) supplementation is widely recognized as the primary exogenous enzyme used in feed for monogastric animals. PHY reduces the antinutritional effects of phytate phosphorus (PP), enhancing the digestibility of P, calcium (Ca), and other nutrients while also mitigating the environmental impact of inorganic P excretion. Given the variability in published results over the past decades regarding the effects of PHY supplementation in different poultry species, systematic reviews and meta-analyses have emerged as the most reliable and effective tools for synthesizing data from independent studies and translating findings into practical, applicable knowledge. Several previous meta-analyses have evaluated the impact of PHY supplementation on performance, tibia ash content, and nutrient digestibility in laying hens and broilers. However, advancements in broiler genetic selection, the development of new PHY generations, and an increasing number of studies in this field highlight the ongoing need for updated analyses.

To the best of my knowledge, the first meta-analysis conducted in this study was the first to focus on broiler breeders. Additionally, further analyses, including meta-regression and subgroup analyses, were performed to gain a deeper understanding of the factors influencing PHY's effects. The results demonstrated that PHY supplementation enhances egg production (EP) and modifies egg quality traits by increasing the yolk ratio while reducing egg weight (EW) and albumen ratio. Furthermore, dietary vitamin D levels of 3,000 IU/kg or higher negatively influenced PHY's effect on EW and yolk ratio relative to EW. To date, no study has examined the relationship between PHY supplementation and vitamin D levels in broiler breeders, emphasizing the need for further research on this interaction. An economic model based on the meta-analysis results revealed a positive economic impact of PHY supplementation on EP profitability, reinforcing the recommendation that PHY supplementation should be considered in most cases for broiler breeders. Additionally, it was identified a need for more research on the effects of PHY supplementation on male fertility in roosters and its potential impact on the performance of broiler breeder offspring.

A meta-analysis was also conducted on laying hens aged 24–80 weeks of age, marking the first study to demonstrate the positive effects of PHY supplementation on EP, average daily feed intake (ADFI), egg mass (EM), albumen proportion, tibia ash content, apparent ileal digestibility of phosphorus (AIDP), and P and nitrogen (N) utilization. Furthermore, to the best of our knowledge, this was the first meta-analysis to quantify how increased Ca:PP levels negatively affected EP, EM, tibia ash content, and P utilization, while increasing feed conversion ratio (FCR), albumen proportion, and eggshell proportion relative to EW.

A systematic review and meta-analyses on broilers during the starter phase (1–21 days post-hatching) updated the NPP requirements and assessed PHY's effects on performance, bone mineralization, and nutrient digestibility. These analyses accounted for advancements in broiler genetics, the emergence of new PHY generations, and recent research. Notably, this was the first meta-analysis to quantify the positive effects of PHY supplementation in female broilers during the starter phase. The final meta-analysis focused on male broilers during the finisher phase (22–42 days). To the best our knowledge, only one previous meta-analysis evaluated PHY's effects on performance and bone mineralization in male broilers during this period. However, this study was the first to quantify the relationship between different PHY unit (FTU) levels, NPP requirements, and the dietary Ca:NPP ratio for optimizing growth performance and skeletal mineralization. The current thesis highlights the importance of the continuous need for updated by meta-analysis on the NPP and Ca requirements, as well as the effect of new generation of PHY in different poultry species, such as broiler breeders, laying hens and broilers. Moreover, it was observed that high dietary Ca:NPP ratios adversely affected several performance variables across different poultry species, particularly in diets without PHY supplementation. Given the complexity of interactions between Ca, NPP, and exogenous PHY, and the limited number of studies involving female broilers during the starting period or diets with Ca:NPP levels below the conventional ratio usually utilized in poultry industry, therefore further research is necessary to better understand their interactive effects on P utilization across different poultry species.

This thesis underscores the importance of continuous meta-analyses to update NPP and Ca requirements and evaluate the effects of new PHY generations in different poultry species, including broiler breeders, laying hens, and broilers. Moreover, high dietary Ca:NPP ratios were found to negatively impact several performance variables across various poultry species, particularly in diets without PHY supplementation. Given the complexity of interactions among Ca, NPP, and exogenous PHY, further studies are necessary to better understand their effects on P utilization across poultry species.

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