International Platform of Registered Systematic Review and Meta-analysis Protocols

INPLASY

INPLASY202440096

doi: 10.37766/inplasy2024.4.0096

Received: 23 April 2024

Published: 23 April 2024

# Unlocking Phytate with Phytase: A Meta-Analytic View of Meat-type Chicken Muscle Growth and Bone Mineralization Potential

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**ADMINISTRATIVE INFORMATION**

**Support -** No external funding.

**Review Stage at time of this submission -** Data analysis.

**Conflicts of interest -** None declared.

**INPLASY registration number:** INPLASY202440096

**Amendments -** This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 23 April 2024 and was last updated on 23 April 2024.

## INTRODUCTION

**Review question / Objective** Is "exogenous phytase effective in unlocking phytate P in Ca-and-P deficient diet for muscle growth and bone mineralization potential in broiler at different growth phases?

**Rationale** Phosphorus (P), which is second most abundant element in growing meat-type chicken body is considered a vital mineral (Proszkowiec-Weglarz and Angel, 2013). Almost two-thirds of total P in plant source feedstuffs is bound to phytic acid or phytate, and broiler chickens are not able to utilize phytate-P efficiently (Selle and Ravindran, 2007). Due to its bioavailability in monogastric digestion, mineral P must be included in broiler chicken diets to meet P needs. This nutritional strategy to supply mineral P to meet the requirement of growing broilers contribute in their over excretion in the manure (Baradaran et al., 2017). For the challenge of environmental pollution and enormous economic losses, animal nutritionists are exploring the potential of exogenous phytase in unlocking the phytate P in the primary feed ingredient's of monogastric. The inconsistencies of phytase effects on digestibility of nutrients and variants of it been manufactured by animal feed companies globally calls for a need to review studies published within this domain.

**Condition being studied** Growth performance and bone strength and mineralization of broiler chickens fed a Ca-and-P deficient diets in the growth stages of the starter, grower, and finisher phases starter, grower, and finisher phases of growth.

## METHODS

**Search strategy** A comprehensive literature search for English articles published between 2000 and February 2024 was conducted using Web of Science (accessed on 21 February 2024), Scopus (accessed on 22 February 2024), ScienceDirect (accessed on 21 February 2024), PubMed

(accessed on 22 February 2024), Poultry Science (accessed on 21 February 2024), and Google Scholar (accessed on 20 February 2024) online databases. In all the databases, the keywords “phytase supplementation”, “phosphorus”, “broiler chicken”, “growth”, “bone mineralization”, and “blood characteristics” were used.

**Participant or population** Broiler chicken.

**Intervention** Varying doses of exogenous phytase.

**Comparator** Stand-alone Ca-and-P deficient basal diet.

**Study designs to be included** Meta-analysis according to PRISMA.

**Eligibility criteria** Inclusion criteria: (1) peer-reviewed journal article published in English, (2) studies in-volving stand-alone Ca-and-P deficient basal diet supplemented with phytase, (3) studies on broiler strains at either starter or grower-finisher phase, (4) studies with a randomized allotment of broilers, (5) studies with a quantified dose of phytase, (6) studies that reported the means of the control and experimental group with variability measures (standard de-viation or standard error of mean) and sample size, and (7) studies that reported the pa-rameters of interest. The exclusion criteria included (1) challenged studies, (2) studies with phytase fed as a replacement in the diet of starter and grower-finisher broilers, and (4) studies with phytase combined with other exogenous enzymes or additives.

**Information sources** To address the research question, “exogenous phytases’ phytate unlocking efficacy for potential muscle growth and bone mineralization in broiler at different growth phases”, a comprehensive literature search for articles published between 2000 and February 2024 was conducted using Web of Science (accessed on 21 February 2024), Scopus (ac-cessed on 22 February 2024), ScienceDirect (accessed on 21 February 2024), PubMed (ac-cessed on 22 February 2024), Poultry Science (accessed on 21 February 2024), and Google Scholar (accessed on 20 February 2024) online databases. In all the databases, the key-words “phytase supplementation”, “phosphorus”, “broiler chicken”, “growth”, “bone mineralization”, and “blood characteristics” were used.

**Main outcome(s)** 1. Growth performance outcomes: ADFI (g/d); ADG (g/d); BWG (g); FCR 2. Bone mineralization and strength outcomes: Bone breaking strength (Kgf/mm); tibia ash; tibia Ca (g/Kg DM); tibia P (g/Kg DM).

**Data management** After screening, two main categories of data will be extracted independently by two team members from studies identified as relevant data, compiled and constructed into a database using structured spreadsheets created in Google Sheets (Google LLC, USA).

**Quality assessment / Risk of bias analysis** The assessment of the eligible studies’ quality, validity, and potential risk of bias was conducted independently by two team members using Cochrane Collaboration’s Systematic Review Center for Laboratory Animal Experimentation’s (SYRCLE) Risk of Bias (RoB) checklists of items for animal studies [3]. The assessment items included random sequence generation (selection bias), baseline characteristics (selection bias), allocation concealment (selection bias), random housing (performance bias), blinding of participants and personnel (performance bias), random outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. Discussions with a third researcher settled the disagreements in the assessment.

**Strategy of data synthesis** Sixteen (16) separate meta-analyses were carried out to combine estimates of phytase supplementation on growth performance and bone mineralization in broilers across studies using the “meta” and “metafor” packages of R (version 4.3.3, "Angel Food Cake", R Foundation for Statistical Computing Platform). The means of the experimental units (control and treatment) were registered as continuous data, and their effect was calculated with Hedges’ g as the standardized mean difference (SMD), also called the effect size (ES), using methods for the random-effects model. The difference between the means of the treated and the control groups was standardized using the standard error of mean (SEM) of the group's mean with and without exogenous phytase. The random-effects model was used to estimate the effect size since it is more conservative than the fixed-effects model. [4,5]. At a confidence interval (CI) of 95%, calculated SMD with a p-value $\leq 0.05$ was declared statistically significant.

**Subgroup analysis** Meta-ANOVA (sub-group analysis) tests were conducted to compare the effects of the broiler strains and basal diets’ primary ingredients. Meta-regression analysis, on the other hand, was performed using effect sizes estimate (SMD) from each control and phytase

supplementation treatment group comparison for each outcome (PSMD 50%, n ≥ 10) as the dependent variable, and phytase dosage (FTU/kg) and supplementation duration (days) as the independent (or explanatory) variable to examine the source of the meta-analysis detected heterogeneity.

**Sensitivity analysis** Publication bias was analyzed to confirm the study results' validity and assess the risk of bias in individual studies. The funnel plots were drawn to visualize the bias, and Egger's linear test was performed to evaluate the publication bias accurately with numer-ical data [49]. Tests to assess publication bias can be achieved when the variable to be considered is at least ten studies and when significant heterogeneity (Q) is detected with $p \leq 0.05$ because it may lead to false-positive claims [50]. Consequently, funnel plots and Egger's test were only performed for variables that met the criteria above. In cases where statistical evidence of publication bias was found, Duval and Tweedie's "trim-and-fill" method was used to estimate the number of possible missing observations [51].

**Language restriction** English.

**Country(ies) involved** Korea.

**Other relevant information** Extracted data from the eligible studies' calculated chemical composition of stand-alone Ca-and-P deficient basal diet, phytase dosage, and supplementation duration were analyzed using the descriptive statistics procedure of Minitab (Version 21.2, 2022).

**Keywords** Growth performance; Broilers; Exogenous enzyme; Phosphorus; Calcium; Welfare; Meta-analysis.

**Dissemination plans** Publish the findings in the MDPI Animals Journal.

**Contributions of each author**

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