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## Dietary Supplementation of Tannins: Effect on Growth Performance, Serum Antioxidant Capacity, and Immunoglobins of Weaned Piglets – A Systematic Review and Meta-Analysis

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### ADMINISTRATIVE INFORMATION

Support - No external funding.

Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

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**Amendments** - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 22 January 2024 and was last updated on 22 January 2024.

## INTRODUCTION

Review question / Objective This metaanalysis aimed to evaluate the effect of dietary supplementation with tannins on weaned piglets' growth performance, serum antioxidant capacity, and serum immune status. We further explored the heterogeneity of the outcomes by meta-regression analysis to identify the percentage of variation explained by the regression model.

**Condition being studied** Tannins, plant bioactive compounds, have the potential to improve productive performance, reduce oxidative stress, and enhance the immune indices of piglets during weaning. Hence, the objective of this study was to evaluate the effect of dietary supplementation of tannins on weaned piglets' growth performance,

serum antioxidant capacity, and serum immune status using a meta-analysis approach.

## **METHODS**

Search strategy To identify studies that evaluated the effects of tannin supplementation on productive performance, antioxidant status, and immune indices of weaned piglets, a comprehensive literature search in the scientific databases of Web of Science (accessed on 20 October 2023), Scopus (accessed on 20 October 2023), ScienceDirect (accessed on 20 October 2023), PubMed (accessed on 20 October 2023), and Google Scholar (accessed on 20 October 2023) was carried out. The search was limited to the results of papers published between 2010 and 2023. In all the databases, the keywords "tannin," "condensed tannins," "hydrolysable tannin," "weaned pig\*," "growth," "antioxidant status," and "immune indices" were used.

Participant or population Weaned piglets.

**Intervention** Supplementation of different sources of dietary tannins.

**Comparator** Dietary tannins of different sources were supplemented to the basal diets of weaned piglets in the treatment groups and not the control group.

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

Eligibility criteria Inclusion criteria: (1) peerreviewed journal article published in English; (2) studies involving basal diet supplemented with tannins; (3) studies on crossbred weaned pigs; (4) studies with the allotment of weaned pigs randomized; (5) studies with the quantification of tannin doses; (6) studies that reported means of the control and experimental group with variability measures (standard deviation or standard error of mean) and sample size, and (7) studies that reported the parameters of interest. The exclusion criteria included (1) challenged studies, (2) studies with pre- and post-weaning pigs, (3) studies with tannin fed as a replacement ingredient in the diet of weaned pigs, and (4) studies with tannin combined/blended with probiotics/prebiotics/ organic acids or other additives.

**Information sources** To identify studies that evaluated the effects of tannin supplementation on productive performance, antioxidant status, and immune indices of weaned piglets, a comprehensive literature search in the scientific databases of Web of Science (accessed on 20 October 2023), Scopus (accessed on 20 October 2023), ScienceDirect (accessed on 20 October 2023), PubMed (accessed on 20 October 2023), and Google Scholar (accessed on 20 October 2023) was carried out.

Main outcome(s) The response variables or main outcomes were in three categories. The growth performance category included average daily feed intake (ADFI), average daily gain (ADG), final body weight (FBW), and feed conversion ratio (FCR). The antioxidant parameters included: glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), malondialdehydes (MDA), and total antioxidant capacity (T-AOC). The third category, immune indices, included immunoglobulin A (IgA), immunoglobin G (IgG), and immunoglobin M (IgM).

**Data management** Search results from the five databases were pooled in Zotero (Version 6.0.30), and then duplicate publications were removed. The records remaining were independently screened by two reviewers through a two-step process, as previously described by other authors. First, a screening was performed using title and abstract, excluding review papers, stimulated studies (in vitro), and studies not including weaned pigs/ piglets. Pa-pers that passed the title and abstract screening were assessed for eligibility in the second step based on the inclusion and exclusion criteria of the meta-analysis.

Quality assessment / Risk of bias analysis Two researchers independently assessed the study quality using Cochrane Collaboration's Systematic Review Center for Laboratory Animal Experimentation's (SYRCLE) Risk of Bias (RoB) tool for animal studies. 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 bias. Discussions settled the disagreements on assessment with a third researcher.

Strategy of data synthesis All the statistical analysis were performed using the R software (version 4.3.1, The R Foundation for Statistical Computing, 2023-06-16 ucrt) using the "meta" and "metafor" packages. The means of the experimental units (control and treatment) were registered as continu-ous result data. The response variables were analysed through the standardized mean dif-ference (SMD), also called effect size (ES), in which the difference between the means of the experiment and control was standardized using the standard deviation (SD) of the groups with and without tannins. The randomeffects model was used to estimate the effect size, 95% confidence interval (CI), and statistical significance for each trait since it is more conservative than the fixed-effects model.

**Subgroup analysis** Meta-ANOVA (sub-group analysis) tests were conducted to compare the effects of the tannin sources (chestnut, quebracho, carob pods, gallnut microencapsulated tannic acid, gallnut tannic acid, grape seed proanthocyanidins, and chestnut and quebracho blend). Meta-

regression analysis was conducted using effect sizes (SMD) for each outcome ( $n \ge 10$ ) as the dependent variable to examine heterogeneity sources of meta-analysis with tannin dosage (mg/kg), supplementation duration (days), and piglets age at weaning (days) of piglets as a covariate.

Sensitivity analysis Publication bias was analysed 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 numerical data. 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.

Language restriction English.

Country(ies) involved Korea.

**Keywords** tannins; weaned piglets; antibiotics; antioxidants; immunity; performance; early-life nutrition; meta-analysis.

#### **Contributions of each author**

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