

# INPLASY

## Differential effects of in-ovo feeding metabolic regulators, amino acids, vitamins, and biotics on broiler growth performance: A meta-analysis

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### ADMINISTRATIVE INFORMATION

**Support** - National Taiwan University.

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

**Conflicts of interest** - None declared.

**INPLASY registration number:** INPLASY202660076

**Amendments** - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 16 June 2026 and was last updated on 16 June 2026.

### INTRODUCTION

**Review question / Objective** Objective: To quantitatively evaluate the long-term effectiveness of in-ovo feeding of amino acids, metabolic regulators, vitamins, and biotics on market-age growth performance and breast muscle yield in commercial broilers.

**PICOS Framework:**

**Population (P):** Healthy commercial broiler chickens, including Cobb, Ross, and Arbor Acres (AA) strains. **Intervention (I):** In-ovo feeding or injection with exogenous bioactive substances (amino acids, metabolic regulators, vitamins, and biotics) during embryogenesis.

**Comparison (C):** Sham-injected control group (Control-sham) and/or non-injected control group (Non-injection).

**Outcome (O):** Market-age growth performance, specifically body weight (BW) and feed conversion ratio (FCR) on day 35 and/or day 42 post-hatch, as well as day 42 breast muscle yield.

**Study Design (S):** Randomized controlled trials (RCTs).

**Condition being studied** Embryonic nutritional limitations and the long-term effectiveness of early-life in-ovo interventions on post-hatch growth performance and myogenesis in commercial broiler chickens.

### METHODS

**Search strategy** Electronic literature searches were conducted on March 23rd, 2026, across six databases: PubMed, Web of Science, CABI, Scopus, AGRICOLA, and EBSCOhost.

The search strategy used the following keywords: ("in ovo feeding" OR "in ovo injection" OR "in ovo administration" OR "in ovo supplementation" OR "intra-amniotic") AND ("broiler\*" OR "chicken\*") AND ("muscle\*" OR "myogenesis" OR "meat" OR "breast" OR "intestine\*" OR "gut" OR "villus" OR "growth performance" OR "body weight") NOT

("vaccin\*" OR "virus\*" OR "infect\*" OR "layer\*" OR "breeder\*" OR "quail\*" OR "duck\*").

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**Participant or population** Healthy (performance > 80% compared to commercial objectives) commercial broiler chickens, specifically including Cobb, Ross, and Arbor Acres (AA) strains.

**Intervention** In-ovo feeding or injection with exogenous bioactive substances (categorized into nutrients, bioactives, and biotics) during embryogenesis.

**Comparator** Treatments including a sham-injected control group (Control-sham) and/or a non-injected control group (Non-injection).

**Study designs to be included** Randomized controlled trials (RCTs) involving commercial broilers. Exclusion criteria apply to non-commercial broiler strains, trials applying challenge models, and growth performance outliers (defined as body weight lower than 80% of the commercial performance objectives corresponding to the breed).

**Eligibility criteria** Randomized controlled trials (RCTs) involving commercial broilers. Exclusion criteria apply to non-commercial broiler strains, trials applying challenge models, and growth performance outliers (defined as body weight lower than 80% of the commercial performance objectives corresponding to the breed).

**Information sources** Comprehensive electronic literature searches were conducted across six major electronic databases: PubMed, Web of Science, CABI, Scopus, AGRICOLA, and EBSCOhost. All database searches were performed on March 23rd, 2026. No grey literature or trial registers were searched, and no direct contact with study authors was made.

**Main outcome(s)** Quantitative measurement of market-age growth performance, specifically Body Weight (BW) and Feed Conversion Ratio (FCR) on day 35 and/or day 42 post-hatch.

**Additional outcome(s)** Breast muscle yield, analyzed to evaluate the commercial value of the carcass after in-ovo injection.

**Data management** Data were extracted and managed using standardized Microsoft Excel spreadsheets.

**Quality assessment / Risk of bias analysis** The methodological quality and risk of bias of the included studies will be assessed using the Cochrane risk-of-bias tool for randomized trials (version 2, RoB 2). The assessment covers six domains: randomization process, intervention adherence (using a per-protocol approach), missing outcome data, outcome measurement, selective reporting, and overall risk of bias.

**Strategy of data synthesis** Data synthesis will be conducted using a random-effects model via Comprehensive Meta-Analysis software (version 3). Treatment effects for primary outcomes will be quantified using Hedges's  $g$  and 95% confidence intervals (CIs). The degree of heterogeneity across studies will be evaluated using  $I^2$  and Cochran's  $Q$  statistics. Potential publication bias will be quantified via visual inspection of funnel plots, supplemented by Egger's regression tests for analyses containing 10 or more datasets.

**Subgroup analysis** Pre-planned subgroup analyses will be conducted based on broiler strains (Cobb, AA, Ross) and types of injected substances (Nutrients & Bioactives vs. Biotics). The category of Nutrients & Bioactives will be further subcategorized into "Vitamins & Cofactors" and "Amino acids & Metabolic Regulators". An inclusion threshold of a minimum of three independent studies per subgroup will be applied.

**Sensitivity analysis** The robustness of the study will be quantified via sensitivity analysis using the one-study removal method to determine any statistically significant changes in the summary effect size.

**Language restriction** No language restrictions.

**Country(ies) involved** Taiwan (R.O.C.).

**Keywords** In-ovo injection; Embryogenesis; Myogenesis; Poultry production; Simulated hatching signal; Systematic review.

**Contributions of each author**

Author 1 - Liang-en Yu - Conceptualized the study, developed search strategies, conducted literature screening, data extraction, and quality

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assessment, performed formal statistical analysis, interpreted data, generated illustrations, and drafted the original manuscript.

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Author 2 - Ti-Chun Chang - Participated in literature searching, conducted independent study screening, performed data extraction, and executed methodological quality and risk-of-bias assessment.

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Author 3 - Yuan-Yu Lin - Project supervision and funding acquisition, critically reviewed and edited the manuscript, and approved the final version for submission.

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