



Effects of Snail-Based Calcium Supplements on Production Performance and Economic Viability in Laying Poultry: A Systematic Review and Meta-Analysis

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Received: January 06, 2026, Revised: February 09, 2026, Accepted: March 03, 2026, Published: March 31, 2026



ABSTRACT

Snail-based calcium may substitute for conventional calcium sources in laying-poultry diets, especially in regions with abundant invasive snails. The effects of snail-based calcium on egg productivity and profitability differ depending on the snail species, processing method, and inclusion level. The present study aimed to estimate the effects of snail-based calcium supplementation on hen-day production and egg weight in laying poultry. Following PRISMA guidelines, nine databases through 2025 were searched for controlled trials comparing snail-derived calcium with conventional sources in laying hens. Out of 825 records, 18 studies met the inclusion criteria after systematic screening for study design, outcome reporting, and variance data. Egg weight and hen-day production were the primary outcomes; economic outcomes were analyzed qualitatively due to limited variance in the data. Using a random-effects model, pooled results indicated that snail-shell calcium significantly reduced egg weight compared to the conventional calcium sources, with no significant effect on hen-day production. Subgroup analysis indicated that quail maintained comparable egg production when fed snail-shell calcium, whereas layer hens experienced a significant decline. Among 26 economic comparisons, 69.2% reported favorable outcomes for snail-based calcium, primarily because lower ingredient costs offset the slight reduction in egg output. Heterogeneity was high for egg weight ($I^2 = 81.6\%$) and hen-day production ($I^2 = 93.5\%$). Snail-based calcium can be viable when processed appropriately and used at species-appropriate inclusion levels. Overall, the meta-analytic results suggested that snail-shell calcium can be a practical, cost-effective alternative when properly processed and used at levels suited to the target poultry species.

Keywords: Calcium supplementation, Golden apple snail, Laying hen, Snail shell, Snail-based calcium

INTRODUCTION

Calcium is essential for eggshell quality, bone density, and laying efficiency in poultry (Clunies et al., 1992). Calcium is supplied through intestinal absorption and bone mobilization, with approximately 60-80% derived from the diet and 20-40% from skeletal reserves (Clunies et al., 1992; Sinclair-Black et al., 2023), corresponding to an approximate ratio of 1:0.4-1:0.25. Accordingly, commercial laying hens are fed diets containing 3.5-4.5% calcium (Rodrigues et al., 2012; Wang et al., 2020). Medullary bone serves as the primary short-term calcium reserve (Sinclair-Black et al., 2023). Insufficient calcium leads to thinner shells, increased breakage, and cage-layer

fatigue (Olgun and Aygun, 2016; Jansen et al., 2020), whereas levels up to 4.5% can improve shell quality and production (An et al., 2016; Wang et al., 2020).

Limestone, oyster shell, and dicalcium phosphate have traditionally been the main calcium sources in poultry diets due to their efficacy and low cost (Castillo et al., 2004). However, mining and processing these traditional calcium sources are energy-intensive and contribute to carbon emissions (Valentini et al., 2020; Djanabou et al., 2025), prompting interest in renewable, locally available alternatives within a circular-economy framework. Snail shells, particularly *Pomacea canaliculata* (golden apple snail), and marine snail by-

products have emerged as potential alternatives (Anizoba et al., 2021; Badejo et al., 2021; Jarabe et al., 2025). In Asia, golden apple snail infestations can reduce rice yields (Lee et al., 2010; Ramli et al., 2017). This snail burden has motivated efforts to convert it into feed-grade calcium (Constantine et al., 2023). Snail shells consist largely of calcium carbonate (CaCO₃), with calcium content comparable to that of limestone (Kaewboonruang et al., 2016; Tepsila and Suksri, 2018), and their use may offer co-benefits for pest management, waste reduction, and circular farming (Subia, 2019; Boakye et al., 2023).

Evidence on snail-based calcium remains inconsistent. Some studies reported comparable egg production and egg weight between snail-shell and conventional calcium sources (Sumiati et al., 2020; Tac-an et al., 2025), whereas others found reduced laying efficiency (Anizoba et al., 2021) or lower profitability (Houndonougbo et al., 2012). These discrepancies might be attributable to differences in snail species, processing methods, inclusion rates, and rearing conditions, complicating the development of standardized recommendations for snail-shell calcium in laying poultry. Moreover, the relationship between production performance and economic returns under snail-shell calcium supplementation remains unclear.

Despite a growing body of individual snail-shell studies, no systematic review or meta-analysis has synthesized evidence on snail-based calcium in laying poultry. The present study aimed to estimate the pooled effects of snail-based calcium supplementation on hen-day egg production and egg weight in laying poultry, identify sources of heterogeneity across studies, and evaluate the economic feasibility of snail-shell calcium as an alternative feed ingredient.

MATERIALS AND METHODS

Search strategy and study selection

This systematic review was conducted in accordance with the PRISMA guidelines (Page et al., 2020). The certainty of evidence for each outcome was assessed using the GRADE approach (Guyatt et al., 2011). No review protocol was pre-registered, and the absence of a pre-registered protocol was acknowledged as a limitation. A literature search was conducted across PubMed, Scopus, ScienceDirect, Web of Science, EMBASE, AGRICOLA, FSTA, CAB Abstracts, and Google Scholar from inception to December 2025; the final search was conducted in January 2026. The PubMed search string was snail shell, snail calcium, golden apple snail, Pomacea, snail meal,

shell powder, laying hen, quail, laying duck, egg production, egg quality, shell thickness, and calcium supplement. The search string was adapted for other databases. Reference lists of retrieved articles were screened, and forward citation searches were conducted in Google Scholar. Relevant conference proceedings were also searched manually.

Eligible studies were controlled trials that compared snail-based calcium with conventional calcium sources, including limestone, oyster shell, or dicalcium phosphate, in laying poultry. Additionally, Eligible studies reported hen-day egg production, egg weight, or economic outcomes. Quantitative synthesis of biological outcomes required variability measures, specifically SD or SEM. Published articles, theses, dissertations, and conference papers in English were eligible. The English-language restriction remains a limitation of the study. Excluded studies used non-laying species, used snail-derived products primarily as protein supplements, failed to identify snail species, lacked variance data for egg weight or hen-day egg production, or used non-experimental designs. Two independent reviewers screened titles and abstracts and evaluated the full texts. Cohen's kappa quantified inter-rater agreement (Cohen, 1960).

The searches identified 800 records, plus 25 additional records from reference screening and citation searching. After duplicate removal (727 records), 98 records remained and were screened by title and abstract, resulting in 74 additional exclusions. The remaining 24 full-text articles were assessed for eligibility, and 6 failed to meet the inclusion criteria, yielding 18 eligible studies published between 2012 and 2025 (Figure 1). The identified studies originated from Indonesia, the Philippines, Samoa, and Nigeria. These studies evaluated laying chickens, quail, and ducks fed shells derived from *Pomacea canaliculata* or *Tympanotonus fuscata* at inclusion rates ranging from 1% to full replacement of conventional calcium sources.

Certainty of evidence

The certainty of evidence for each primary outcome was assessed using the GRADE approach (Guyatt et al., 2011). Evidence from the included trials was initially rated as high certainty and was downgraded across five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Risk-of-bias ratings within GRADE were derived from the SYRCLE assessment. Inconsistency was evaluated using the I² statistic, with values above 75% considered grounds for

downgrading. Indirectness was assessed by comparing study populations, interventions, and outcomes against the target context of commercial laying-poultry production. Imprecision was evaluated by examining the width of the 95% confidence interval relative to a meaningful effect threshold. Publication bias was assessed using Egger's regression test and a funnel plot. Each outcome was rated as high, moderate, low, or very low certainty. Two reviewers independently assessed each domain and resolved disagreements by discussion.

Quality assessment

Risk of bias was assessed using the SYRCLE tool (Hooijmans et al., 2014), adapted for layer nutrition trials. Randomization was assessed at the pen level. Personnel blinding was rated high risk by default because dietary interventions cannot be readily concealed from staff. Detection bias was evaluated through random outcome assessment and assessor blinding. Each of the ten domains of sequence generation, baseline characteristics, random housing allocation, blinding of personnel, random outcome assessment, blinding of assessors, incomplete outcome data, selective outcome reporting, sample size adequacy, and conflicts of interest was rated as low (+), unclear (?), or high (-) risk (Figure 2).

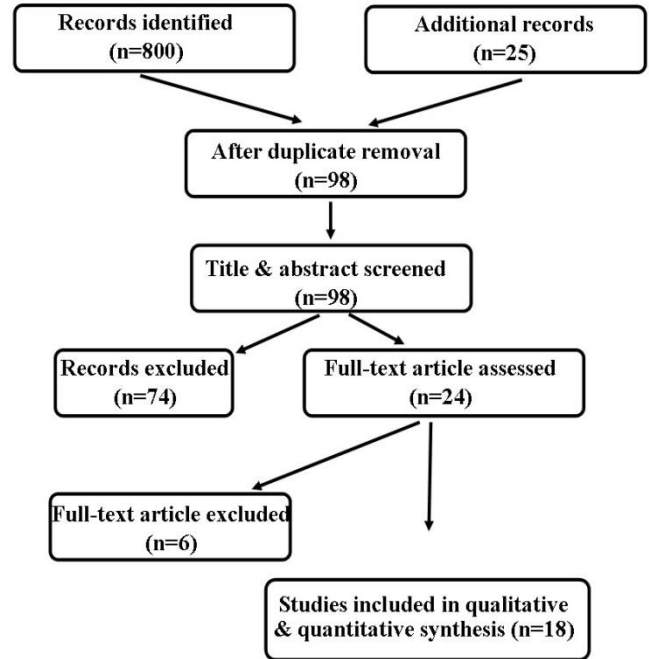


Figure 1. PRISMA flow diagram illustrating the study selection process for the systematic review and meta-analysis of snail-based calcium in laying-poultry diets. A total of 800 records were identified through database searching and 25 through additional sources; after duplicate removal of 727, 98 records were screened, 24 full-text articles were assessed, and 18 studies met all inclusion criteria.

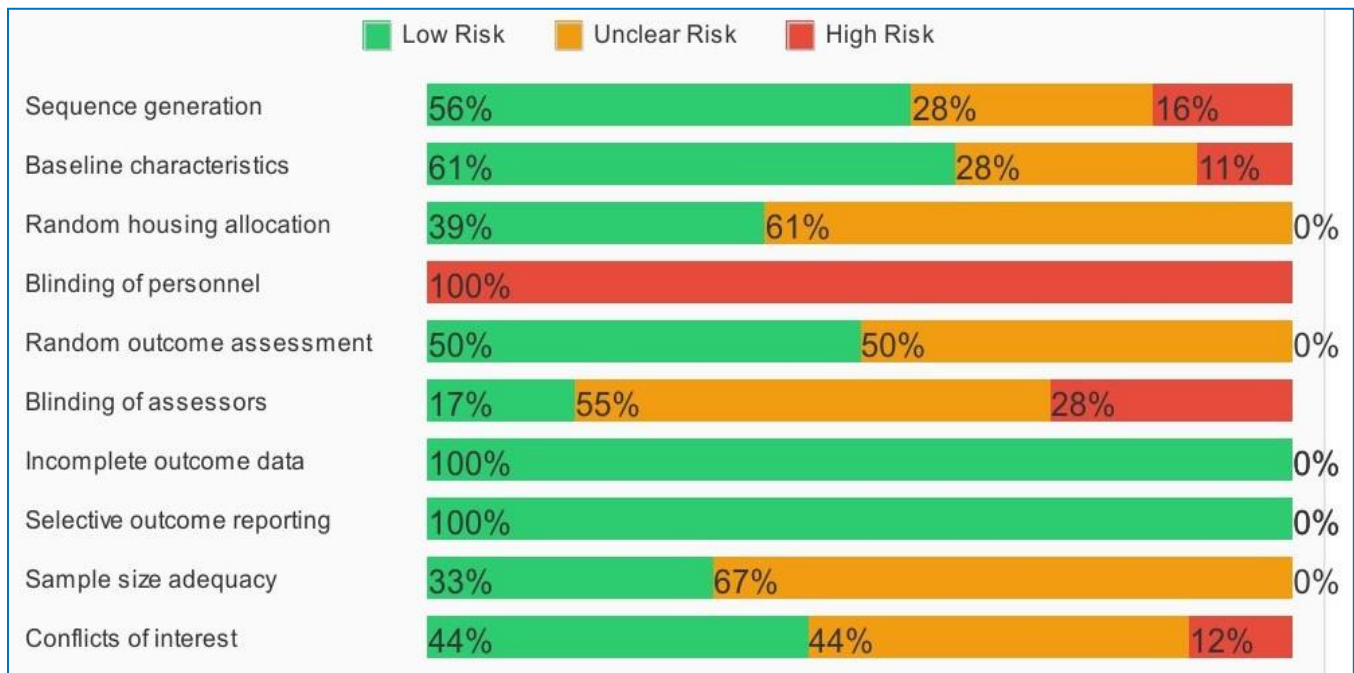


Figure 2. Risk of bias classification across 10 adapted SYRCLE domains for the 18 included studies. Each domain was rated as low risk (green), unclear risk (yellow), or high risk (red). Blinding of personnel was rated high risk in all studies because dietary treatments in poultry feeding trials cannot be concealed from caregivers.

Overall, the study risk of bias was classified with a threshold approach applied to nine domains. The nine domains excluded blinding of personnel. Two or more high-risk ratings across the nine domains resulted in a high-risk classification. One high-risk rating resulted in a moderate-risk classification. Three or more unclear ratings across the nine domains also resulted in a moderate-risk classification. Zero high-risk ratings and fewer than three unclear ratings resulted in a low-risk classification. Blinding of personnel was excluded from the overall classification because dietary intervention studies in poultry inherently carry a high risk in the blinding-of-personnel domain. Across the 18 studies, sequence generation was low risk in 55%, unclear in 28%, and high risk in 17%. Baseline comparability of the treatment group indicated 61% low risk, 28% unclear risk, and 11% high risk. Random housing was low risk in 39% and unclear in 61%. Personnel blinding was high risk in all studies (100%). Random outcome assessment was low risk in 50% and unclear in 50%. Assessor blinding was low risk in 17%, unclear in 55%, and high risk in 28%. Incomplete outcome data and selective reporting were both low risk in all studies. Sample size adequacy was low risk in 33% and unclear in 67%, conflicts of interest were low risk in 45%, unclear in 44%, and high risk in 11%. Overall classification identified 7 of 18 studies (39%) as low risk, 8 studies (44%) as moderate risk, and 3 studies (17%) as high risk of bias. Inter-rater agreement was $\kappa = 0.82-0.88$ for study selection. Inter-rater agreement across bias domains ranged from $\kappa = 0.65$ to 1.00. Inter-rater agreement for overall risk classification was substantial ($\kappa = 0.86$). Sensitivity analysis, which excluded three high-risk studies (Anizoba et al., 2024; Jarabe et al., 2025; Tac-an et al., 2025), produced pooled effect sizes consistent with the primary analysis. For egg weight, the sensitivity analysis yielded a Hedges' g of -0.54 (95% CI: -0.81 to -0.27) compared with -0.566 in the full dataset; for hen-day production, the sensitivity analysis yielded a Hedges' g of -0.11 (95% CI: -0.42 to 0.20) compared with -0.127 in the full dataset. Neither outcome showed a change in direction or statistical significance. A sensitivity analysis produced no change in pooled effect sizes and no change in statistical significance for either outcome.

Data extraction and statistical analysis

Data were independently extracted by two reviewers using a pilot-tested, standardized form capturing study identifiers, animal characteristics, dietary treatments, trial duration, and outcome values with variability measures. Variability was recorded as standard deviation (SD) or standard error of the mean (SEM), depending on which

measure the original study reported. Discrepancies were resolved by consensus or by a third reviewer. The unit of analysis was the pen mean, or individual bird mean, depending on the reporting level. In multi-treatment studies, each snail-calcium treatment was compared against the shared control, with the control sample size divided equally to avoid double-counting.

When original studies reported variability as SEM rather than SD, values were converted using the formula $SD = SEM \times \sqrt{n}$. Data from the 20-40-week production period were prioritized. When unavailable, end-of-trial values were used for studies lasting at least 8 weeks, and the latest reported time point was used for shorter trials. A sensitivity analysis restricted to the 20-40-week production periods confirmed that using end-of-trial or latest time-point values when the target production period was unavailable did not bias the results. The primary outcomes were hen-day egg production (%) and egg weight (g). Effect sizes were calculated as Hedges' g and pooled using DerSimonian-Laird random-effects models in Statsmodels 0.13.5 (Python 3.9; Jackson et al., 2010).

A Hartung-Knapp-Sidik-Jonkman (HKSJ) adjustment served as a sensitivity check. At the same time, the HKSJ-adjusted confidence intervals were wider but did not alter the direction or statistical significance of pooled effects for egg weight or hen-day production. Heterogeneity was assessed using I^2 and Cochran's Q , with I^2 values of 25%, 50%, and 75% interpreted as low, moderate, and high heterogeneity (Higgins et al., 2003). Subgroup analyses were performed by snail species, shell processing method, calcium inclusion level, and trial duration. For hen-day production, subgroup analysis by species reflected baseline production intensity. Quail studies reported baseline hen-day production below 80%, whereas layer hen studies reported baseline production at or above 80%. This species-linked threshold was used to distinguish low-intensity from high-intensity production subgroups in Table 1. Comparisons with $|g| > 5.0$ were excluded as presumed methodological anomalies, removing 3 of 29 (10.3%) egg-weight and 5 of 67 (7.5%) hen-day production comparisons (Table 1). Subgroups with fewer than three comparisons were not analyzed. Duck comparisons were excluded from subgroup analyses but retained in overall estimates. Meta-regression evaluated continuous moderators as potential sources of heterogeneity. Economic outcomes were synthesized qualitatively via vote-counting because most studies lacked variance data. Publication bias was assessed using funnel plots and Egger's regression test. Egger's test was applied only when the number of comparisons reached 10 or more, as recommended for

adequate statistical power, and a significance threshold of $p < 0.10$ was used because Egger's test is known to have limited sensitivity at conventional alpha levels (Table 1). Pearson correlations ($\alpha = 0.05$) between biological and

economic outcomes were computed across the eight studies reporting both outcomes, and no multiple-testing correction was applied because the analysis was exploratory.

Table 1. Meta-analysis results for the standardized mean difference (Hedges' g) in egg weight and hen-day egg production between snail-based and conventional calcium sources in laying poultry (quail, laying hens, and ducks; studies published 2012-2025)

Outcome / Subgroup	n	Hedges' g	95% CI	p	I ² (%)	τ^2	Egger's p
Egg weight (g)	26	-0.566	-0.832, -0.301	<0.001***	81.6	0.373	<0.05*
By Species							
Quail	11	-0.426	-0.705, -0.147	0.003**	68.0	---	---
Laying hens	14	-0.553	-0.948, -0.158	0.006**	82.9	---	---
By sample size (Number of chickens)							
Small (< 20)	9	-1.360	-2.139, -0.581	0.001***	88.3	---	---
Large (\geq 20)	17	-0.267	-0.468, -0.065	0.010*	62.1	---	---
Hen-day production (%)	62	-0.127	-0.426, 0.173	0.406	93.5	1.325	<0.05*
By Species							
Quail (baseline HDP < 80%)	57	-0.019	-0.332, 0.293	0.903	93.1	---	---
Laying hens (baseline HDP \geq 80%) Hen (\geq 80%)	5	-1.300	-2.308, -0.291	0.012*	95.8	---	---
By sample size							
Small (< 20)	13	0.408	-0.480, 1.296	0.368	92.8	---	---
Large (\geq 20)	49	-0.240	-0.561, 0.081	0.143	93.7	---	---

Random-effects model (DerSimonian–Laird). n: Number of independent treatment-versus-control comparisons, Hedges' g : Bias-corrected standardized mean difference, I² = Between-study heterogeneity (%), τ^2 = Between-study variance, Egger's p: Egger's regression test for funnel-plot asymmetry, HDP: Hen-day production. Significance levels are denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

RESULTS

Effects on egg weight

Across 26 comparisons, snail-based calcium was associated with lower egg weight than conventional calcium sources (Hedges' $g = -0.566$; $p < 0.05$; Table 1; Figure 3). A Hedges' g of -0.566 corresponds to an approximate mean difference of 0.5–1.0 g per egg, depending on baseline egg weight and variability. Egg weight reductions were observed in both quail ($g = -0.426$; $p < 0.05$) and laying hens ($g = -0.553$; $p < 0.05$). The egg weight reduction was more pronounced in studies with smaller sample sizes. Trials with fewer than 20 layer chickens per treatment demonstrated a larger negative effect on egg weight ($g = -1.360$; $p < 0.05$), whereas larger studies reported a smaller reduction in egg weight ($g = -0.267$; $p < 0.05$). The difference between small and large studies was statistically significant ($p < 0.05$), suggesting that small-sample studies may overestimate the negative effect because of sampling variability. Statistical heterogeneity was high (I² = 81.6%; Cochran's Q $p < 0.05$), and Egger's test ($p < 0.05$) indicated potential small-study effects. Of the original 29 comparisons, three extreme effect

sizes ($|g| > 5.0$) were excluded as outliers attributable to very small samples ($n < 10$). Excluding these outliers (Houndonougbo et al., 2012), 2 comparisons, and Mankpondji et al. (2012), 1 comparison) did not alter the direction or statistical significance of the pooled estimate.

Effects on hen-day production

Meta-analysis of 62 comparisons, no statistically significant overall reduction in hen-day production was found ($g = -0.127$; $p = > 0.05$; Table 1; Figure 4). However, differences by species were evident. Quail studies (57 comparisons) indicated near-identical hen-day production between snail-calcium and conventional-calcium groups ($g = -0.019$; $p > 0.05$), whereas layer chicken studies (5 comparisons) showed a significant decline in hen-day production ($g = -1.300$; $p < 0.05$). The larger number of quail comparisons largely drove the non-significant overall result. Heterogeneity was very high (I² = 93.5%; $p < 0.05$), warranting cautious interpretation of the overall pooled estimate. Species-level subgroup estimates are more precise because they are less influenced by high between-study variability. Comparisons with effect sizes exceeding $|g| > 5.0$

were treated as outliers and excluded from the primary analysis. A standardized mean difference of 5.0 exceeded three pooled standard deviations, which was widely regarded as indicative of methodological anomalies such as data extraction errors, unit-of-analysis problems, or extremely small sample sizes rather than genuine treatment effects

(Viechtbauer and Cheung, 2010). Sensitivity analysis confirmed the robustness of the pooled estimates after excluding five outliers, including three comparisons from Anizoba et al. (2021), two comparisons from Malvar and Agapito (2020), and one comparison from Anizoba et al. (2022).

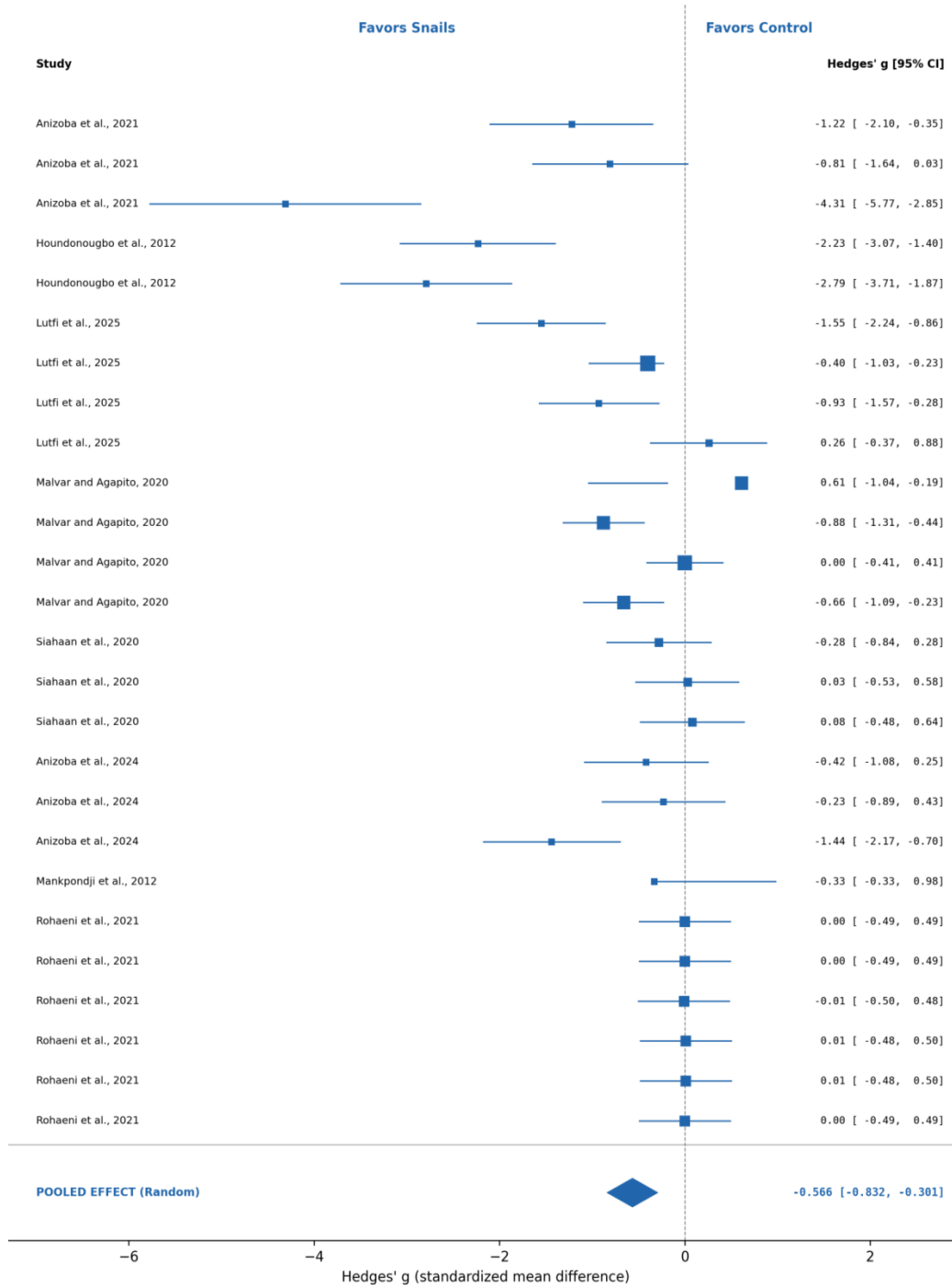


Figure 3. Forest plot showing the standardized mean difference (Hedges' g) in egg weight between snail-based and conventional calcium sources (26 comparisons from 18 studies involving laying hens, quail, and ducks). Negative values indicated that snail-based calcium produced lighter eggs than the conventional source. The diamond represents the overall pooled estimate from the random-effects model.

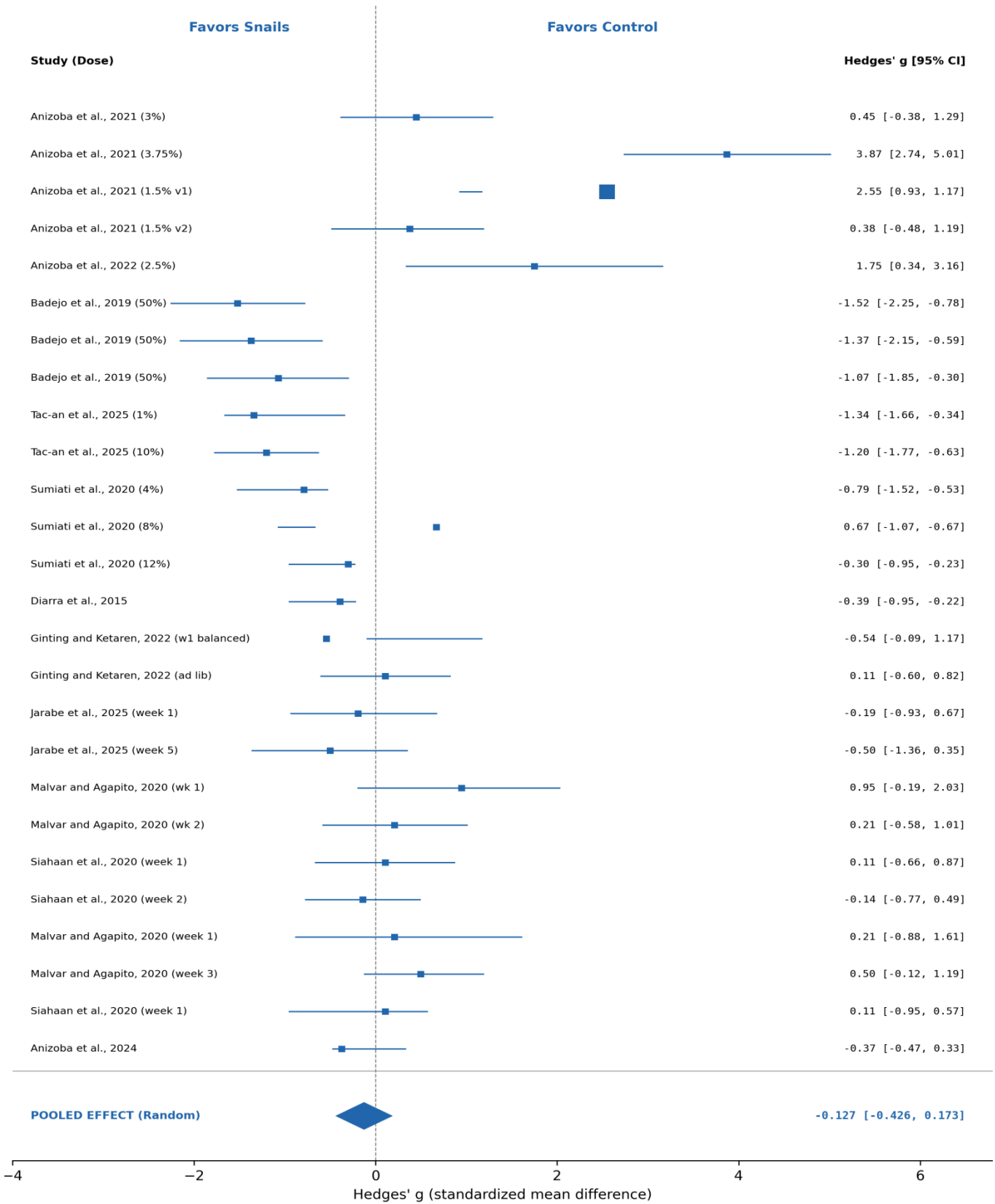


Figure 4. Forest plot showing the standardized mean difference (Hedges' g) in hen-day egg production between snail-based and conventional calcium sources (62 comparisons from 18 studies involving laying hens, quail, and ducks). Negative values indicate that snail-based calcium produced lower hen-day production than the conventional source. The diamond represents the overall pooled estimate from the random-effects model.

Economic viability and biological performance

Across 26 economic comparisons from eight studies, 69.2% (18/26) reported favorable outcomes for snail-based calcium. However, the proportion varied by profitability metric. In all economic feed-efficiency comparisons, snail calcium was the preferred option (4/4, 100%), whereas return on investment (ROI) comparisons were favorable in 10 of 12 cases (83%), income-over-feed-cost comparisons in 3 of 6 (50%), and net income comparisons in only 1 of 4 (25%). This economic feed-efficiency result suggested that economic benefit depended on how profitability was measured. Additionally, economic outcomes differed by region. In Benin, all 5 comparisons favored snail-based calcium (100%). In the Philippines, 10 of 12 comparisons were favorable for snail-based calcium (83%). In Indonesia, 3 of 6 (50%) were favorable, and in Samoa, none of the 3 (0%) were favorable for snail-based calcium. A Fisher's exact test comparing countries with abundant snail resources and high conventional-calcium costs (Benin and Philippines combined, 15/17 favorable) against countries with lower conventional-calcium costs (Indonesia and Samoa combined, 3/9 favorable) indicated a statistically significant difference ($p < 0.01$). However, the small number of comparisons per country limits the reliability of formal statistical inference. The regional differences should be interpreted as descriptive patterns reflecting variation in local shell availability, labor costs for collection and processing, and egg pricing systems. Several studies reported non-linear dose-response relationships in which economic returns improved up to an intermediate inclusion level and then declined, suggesting an optimal replacement threshold beyond which cost savings no longer offset biological trade-offs. Study-level correlations indicated that neither egg weight ($r = 0.177$; $p > 0.05$; Figure 5) nor hen-day production ($r = -0.010$; $p > 0.05$; Figure 6) predicted whether economic outcomes were favorable or unfavorable. The egg weight-production correlation was moderate but non-significant ($r = 0.480$; $p > 0.05$; Figure 7). These correlation results suggested that favorable economics were driven primarily by input cost savings rather than superior biological performance.

Certainty of evidence

The certainty of evidence for each outcome was assessed using the GRADE approach across five domains, namely risk of bias, inconsistency, indirectness, imprecision, and publication bias (Guyatt et al., 2011). A summary of the GRADE assessment is presented in

Table 2. For egg weight, certainty was rated Low. Although the pooled effect was significant ($g = -0.566$, $p < 0.001$, corresponding to an approximate mean difference of 0.5–1.0 g per egg), with consistent direction across subgroups, evidence was downgraded one level for serious risk of bias (39% of studies classified as low risk, 17% as high risk, with universal high risk for personnel blinding and majority unclear risk for assessor blinding and sample size adequacy) and one level for serious inconsistency ($I^2 = 81.6\%$). Egger's regression test was significant for egg weight ($p < 0.05$), indicating funnel plot asymmetry. However, evidence was not downgraded for publication bias because the asymmetry pattern was consistent with greater sampling variability and inflated effect sizes in small studies ($n < 20$ per treatment) rather than selective non-reporting of null findings. This interpretation was supported by the subgroup analysis, which indicated significantly larger effect sizes in small studies ($g = -1.360$) than in large studies ($g = -0.267$; $p < 0.05$). This pattern was characteristic of small-study effects rather than publication bias. Evidence was not downgraded for indirectness or imprecision because the study populations, interventions, and outcomes were directly relevant to the review question and the 95% confidence interval excluded zero. For hen-day production, certainty was rated Very Low. The non-significant pooled effect ($g = -0.127$, $p = 0.406$) was downgraded one level for serious risk of bias (same SYRCLE profile as egg weight, with high risk for personnel blinding in all studies, unclear risk for assessor blinding in 55%, and unclear risk for sample size adequacy in 67%) and two levels for very serious inconsistency ($I^2 = 93.5\%$). Evidence was further downgraded one level for serious imprecision because the wide confidence interval (-0.426 to 0.173) could not exclude either a meaningful reduction or a small improvement in hen-day production. The significant species-specific subgroup difference (quail $g = -0.019$ vs. layer hens $g = -1.300$) reinforced the conclusion that the overall estimate masks important variation by poultry species. Evidence was not downgraded for indirectness. Although Egger's regression test was significant ($p < 0.05$), evidence was not downgraded for publication bias because the asymmetry was attributable to inflated effect sizes in small studies rather than selective non-reporting, consistent with the pattern observed for egg weight.

For economic viability, certainty was rated Very Low. Evidence was downgraded one level for serious risk of bias, one level for serious inconsistency (0-100% favorable outcomes across countries), and one level for serious indirectness. The downgrade in indirectness was applied because economic outcomes depend on market-specific factors, including local calcium source prices, egg pricing systems (count-based vs. weight-based grading), labor costs for shell collection and processing,

and snail species availability, which limit the transferability of economic findings from the study settings to other production contexts. Imprecision and publication bias were not assessed because economic outcomes were synthesized qualitatively. These ratings indicate that while the direction of the egg weight reduction is reliable, the magnitude may change with additional studies. Hen-day production and economic findings should be interpreted as preliminary.

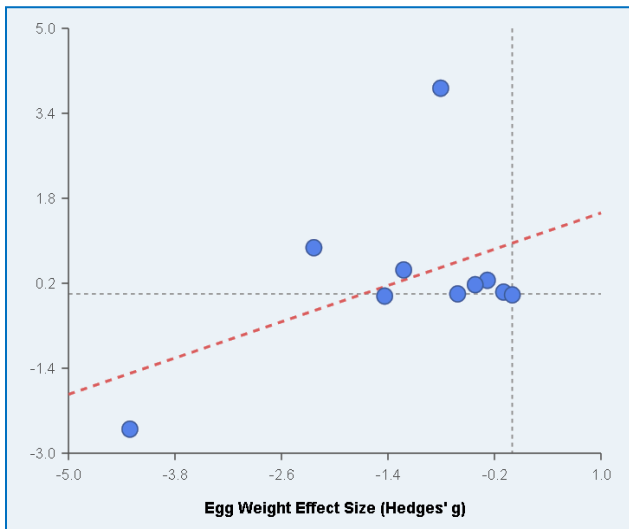


Figure 5. Scatter plot of the study-level relationship between egg weight standardized mean difference (Hedges' g; x-axis) and economic outcome (y-axis) across eight studies that reported both biological and economic data ($r = 0.177$, $p = 0.822$). The dashed line represents the linear regression trend. Each point represents one study-level mean.

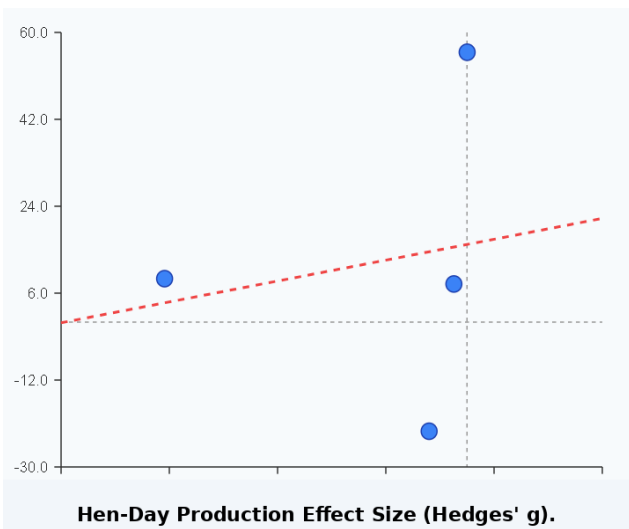


Figure 6. Scatter plot of the study-level relationship between hen-day egg production standardized mean difference (Hedges' g; x-axis) and economic outcome (y-axis) across eight studies that reported both biological and economic data ($r = -0.010$, $p = 0.987$). The dashed line represents the linear regression trend. Each point represents one study-level mean.

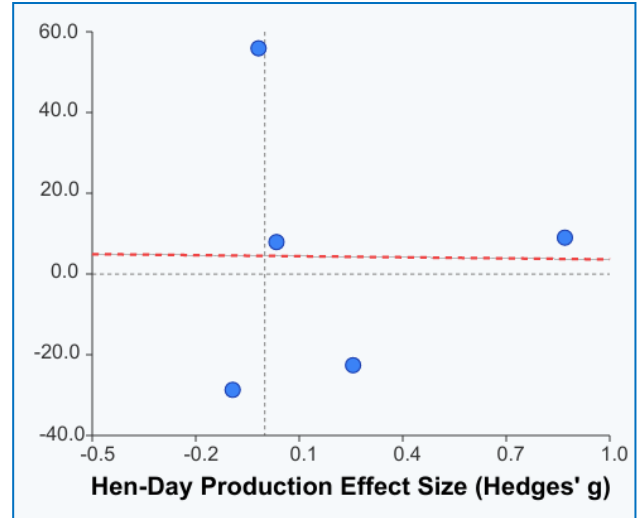


Figure 7. Scatter plot of the study-level relationship between egg weight standardized mean difference (Hedges' g; x-axis) and hen-day egg production standardized mean difference (Hedges' g; y-axis) across eight studies ($r = 0.480$, $p = 0.160$). The dashed line represents the linear regression trend. Each point represents one study-level mean.

DISCUSSION

The observed reduction in egg weight with snail-based calcium may be partly related to differences in how calcium is released from different shell sources during digestion. Snail shells consist primarily of aragonite-form CaCO_3 , whereas many commercial limestones consist of calcite (Hasse et al., 2000; Bonou et al., 2019). Aragonite dissolves more slowly than calcite under acidic conditions (Hasse et al., 2000), which could potentially reduce the availability of ionized calcium during the period of active eggshell formation. Bonou et al. (2019) reported that aragonite can reform as brushite or hydroxyapatite, which may further delay calcium release. Although direct evidence of differential dissolution kinetics in the avian gastrointestinal tract is lacking, such differences could offer a mechanistic explanation for the consistent reduction in egg weight observed across studies, whereas hen-day production remained unaffected. Residual organic matrices and trace minerals present in snail shells might also adhere to CaCO_3 surfaces or form chemical bonds with calcium (Marxen and Becker, 2000), especially when shells are processed without adequate cleaning or heat treatment.

Variation in shell processing might have contributed to the wide range of effects concerning egg weight and hen-day production across numerous studies (Sumiati et al., 2020; Anizoba et al., 2021; Tac-an et al., 2025). When

shells are ground without thorough cleaning or heat treatment, residual organic matter may inhibit the dissolution of CaCO_3 (Marxen and Becker, 2000). Heat treatment has multiple functions. Boiling for 15-20 minutes can inactivate thiaminase and reduce organic residues, thereby improving calcium availability (Kuku et al., 2012; Siahaan et al., 2020). Calcination above $1,000^\circ\text{C}$ converts CaCO_3 to CaO , which dissolves rapidly (Topić Popović et al., 2023).

Particle size is another factor that can influence dissolution kinetics. Coarse particles (≥ 1.5 mm) are retained longer in the gizzard, prolonging exposure to gastric acid, which may be advantageous because shell calcification occurs primarily during the dark period (Fleming et al., 2006; Hervo et al., 2022). In contrast, finer particles pass through the upper gastrointestinal tract more quickly and may deliver calcium when shell-gland demand is lower. The lack of standardized particle-size reporting across trials may account for some of the heterogeneity observed in the pooled estimates of egg weight. Castillo et al. (2004) reported that calcium requirements of high-producing hens can exceed NRC (1994) standards by more than 30%. Therefore, higher calcium demand is consistent with the reduced capacity of high-producing hens to buffer, leading to slower calcium release from snail shells. Age-related decreases in intestinal calcium absorption and medullary bone resorption may further limit calcium availability in older avian species (Saunders-Blades et al., 2009; Gu et al., 2021), and the layer hens included in the meta-analysis were mostly older, high-producing chickens. Quail generally have lower calcium requirements and shorter laying cycles (Bar, 2009), which may reduce vulnerability to slower calcium release and may explain the absence of production losses in that subgroup.

Interpretation considered uncertainty in the pooled estimate during the present study. In the present study, heterogeneity was high ($I^2 = 93.5\%$), and Egger's test indicated small-study effects, consistent with variability in effect sizes across designs and potential inflation of estimates in smaller studies. Subgroup analyses confirmed that the pooled effects were context-dependent, varying by poultry species, production intensity, and shell processing quality. The relationship between biological performance and profitability was also not evident. Study-level correlations indicated that neither egg weight ($r = 0.177$; $p > 0.05$; Figure 5) nor hen-day production ($r = -0.010$; $p > 0.05$; Figure 6) predicted whether economic outcomes favored snail-based calcium. The weak association between biological performance and economic outcomes

suggested that profitability was driven by input cost savings rather than by improvements in egg output. Consistent with the present findings, Houndonoubo et al. (2012) reported a 9.0% economic benefit when periwinkle shell replaced a higher-cost calcium source despite a 2.23 g reduction in egg weight. This cost-driven profitability aligns with the overall finding that 69.2% of economic comparisons favored snail-based calcium, despite a notable reduction in egg weight. The impact of reduced egg weight on revenue depends on whether eggs were sold by count or by weight grade in a specific market.

Under count-based systems, modest reductions in egg mass may have limited direct price consequences, whereas weight-based or graded systems can result in lower prices for lighter eggs. Abanikannda and Leigh (2012) reported that sorting and grading eggs by size resulted in a 15% increase in revenue, with unsorted eggs leading to loss of income, decreased marketability, and increased wastage. These pricing differences may partly explain the regional contrasts observed in the present study. Favorable economic outcomes were more common in count-based markets, such as Benin (100%) and the Philippines (83%), than in weight-based markets, such as Indonesia (50%). In regions where golden apple snails are invasive, shell collection may also provide pest-control benefits that conventional cost calculations may undervalue (Davalos, 2022). Economic variability across studies appeared to relate to processing thoroughness and inclusion level. Trials reporting favorable outcomes often employed more thorough processing methods such as boiling, drying, and fine grinding (Houndonoubo et al., 2012; Kuku et al., 2012; Siahaan et al., 2020). In addition, snail-shell calcium was economically favorable only at inclusion levels that yielded ingredient-cost reductions in a balanced diet (Houndonoubo et al., 2012; Siahaan et al., 2020; Rohaeni et al., 2021). Regional patterns further illustrated the role of local input costs and market structure. In the Philippines, the majority of comparisons (10/12) reported favorable outcomes, which are associated with low acquisition costs for snail materials, high transportation costs for limestone, and count-based pricing (Davalos, 2022; Constantine et al., 2023; Jarabe et al., 2025). The two unfavorable comparisons in the Philippines both involved high replacement rates (75% and 100%). In contrast, all five comparisons in Benin indicated positive outcomes, which were associated with higher prices of imported oyster shells than of locally sourced snail shells (Houndonoubo et al., 2012). Indonesian trials reported mixed outcomes, consistent with the availability of low-

cost limestone and weight-based egg grading. In Samoa, snail-based diets reduced feed costs relative to conventional-calcium controls, but the reduction was insufficient to demonstrate a clear economic advantage when evaluated alongside production performance (Diarra *et al.*, 2015).

Sources of heterogeneity

High heterogeneity for egg weight and hen-day production may reflect differences in experimental conditions rather than a uniform treatment response. Four factors contributed to the observed heterogeneity, including species and production intensity, shell processing methods, non-linear dose-response patterns, and geographic and economic context. Species-specific results indicated that quail maintained hen-day production with snail-shell calcium, whereas layer chickens showed a significant decline in hen-day production. When hens had access to free-choice feeding systems that allowed self-regulation of calcium intake, reductions in egg weight and production were smaller (Mankpondji *et al.*, 2012; Diarra *et al.*, 2016). The existing evidence does not justify a single recommendation across poultry species.

Processing methods varied widely across the examined studies, and differences in heat treatment protocols and particle size distributions contributed to between-study heterogeneity. Coarser particles may increase nocturnal calcium release, whereas fine particles may deliver calcium during daytime hours when shell-gland demand is lower (Fleming *et al.*, 2006; Hervo *et al.*, 2022). Calcination increases solubility but raises processing costs and associated carbon emissions (Valentini *et al.*, 2020; Chen *et al.*, 2024). Non-linear dose-response patterns were reported in the examined studies. Anizoba *et al.* (2021) found that 3.75% periwinkle shell increased production, whereas higher inclusion levels reduced production, possibly through calcium-phosphorus antagonism. Houndonougbo *et al.* (2012) reported similar production responses, indicating that dose-response curves depend on baseline diet composition, snail species, and processing quality. The geographic and economic context also contributed to heterogeneity. Studies from Benin (Houndonougbo *et al.*, 2012) reported 100% favorable economic comparisons, and Philippine studies (Malvar and Agapito, 2020; Davalos, 2022) reported 83% favorable outcomes, whereas Indonesian (Rohaeni *et al.*, 2021; Lutfi *et al.*, 2025; 50%) and Samoan (Diarra *et al.*, 2015) (0%) studies reported lower proportions of favorable results. These differences align with local cost

structures, egg pricing systems, and the availability of different snail species. Where conventional calcium was limited or costly, snail-shell calcium lowered input costs, even with a slight decrease in biological performance. Because I^2 values ranged from 81.6-93.5, the pooled estimates should be viewed as weighted averages across diverse experimental conditions rather than as evidence that snail-shell calcium performs equivalently across different poultry species, inclusion levels, or processing methods. Outcomes depended on species/production intensity, processing parameters, and particle-size distribution, inclusion level relative to diet Ca:P and baseline calcium, and local price ratios and market structure.

A notable gap in the present study was the absence of pooled eggshell quality data. Shell thickness, breaking strength, and shell percentage are important because reduced shell integrity increases breakage losses. Several studies measured these parameters individually (Houndonougbo *et al.*, 2012; Sumiati *et al.*, 2020); however, inconsistent measurement and reporting precluded the possibility of conducting a meta-analysis. Given the remarkable reduction in egg weight, the possibility that snail-shell calcium compromises shell quality cannot be excluded.

Practical implications

Snail-shell calcium can partially replace conventional sources, but the extent of replacement depends on the species. For layer chickens, 25-50% replacement appears to be the maximum replacement, whereas quail can tolerate 50-75% level (Houndonougbo *et al.*, 2012; Mankpondji *et al.*, 2012; Davalos, 2022). Standardized processing, including boiling, drying, controlled grinding, and calcium verification, is essential for ensuring consistent calcium bioavailability from snail-shell sources (Kuku *et al.*, 2012; Siahaan *et al.*, 2020). On-farm monitoring should track egg weight, shell thickness, and skeletal integrity because hen-day production may remain unchanged while egg weight declines. Before commercial adoption, on-farm trials should quantify ingredient, processing, and revenue costs under local market conditions. Reported examples included return on investment improvements across formulations (Jarabe *et al.*, 2025), favorable economic feed efficiency at 5-10% inclusion of Snail-shell (Houndonougbo *et al.*, 2013), and positive returns at 25-50% protein replacement with poorer outcomes at $\geq 75\%$ snail-shell inclusion (Davalos, 2022). Promotion of snail-shell calcium as a feed

ingredient may be the most appropriate in regions where conventional calcium sources are costly or limited, snail shells are locally abundant, processing infrastructure is available, and modest reductions in egg weight are commercially acceptable.

Limitations

This meta-analysis had several limitations. High statistical heterogeneity for both egg weight and hen-day production reduced the precision of the pooled estimates. Therefore, the overall effect sizes should be viewed as averages across diverse experimental conditions, not as universally applicable values. Egger's regression test was significant for both outcomes, indicating small-study effects that may have overstated the pooled estimates. Processing methods were often reported without quantitative parameters, limiting mechanistic inference. Several studies used general terms such as ground (14 studies), calcined (1 study), fermented (1 study), and not described (2 studies) without quantitative specifications; only a subset reported heat-treatment duration/temperature or described calcination procedures.

The geographic focus of the studies was primarily Southeast Asia (11 studies), West Africa (6 studies), and Oceania (1 study), with no studies from temperate climates or commercial systems in Europe, the Americas, or East Asia, limiting the generalizability of the current findings. Economic outcomes were synthesized qualitatively because outcome metrics differed across studies, and variance reporting was limited (SD/SEM reported in 31% of comparisons). Several trials lasted < 12 weeks, which might not have captured longer-term changes in bone reserves and cumulative production effects. Longer trials (≥ 12 weeks) provided more information on sustained outcomes.

An important limitation of this meta-analysis was the lack of pooled data on eggshell quality traits, such as shell thickness, breaking strength, weight, and percentage. Although several studies reported eggshell quality measurements (Houndonougbo et al., 2012; Mankpondji et al., 2012; Sumiati et al., 2020), the data were too limited and inconsistently formatted for quantitative pooling. Since calcium source and bioavailability directly affect shell mineralization, the absence of pooled eggshell quality data prevents this review from determining whether snail-based calcium compromises shell integrity in addition to reducing egg weight. The lack of pooled eggshell quality data is particularly relevant for commercial producers, who experience significant

economic losses due to shell breakage during handling and transport.

CONCLUSION

This systematic and meta-analysis study found that snail-shell calcium reduced egg weight compared to conventional calcium sources but did not significantly affect overall hen-day egg production. Quail demonstrated no significant change in hen-day production; however, layer chickens exhibited a significant reduction in hen-day production ($g = -1.300$; $p = 0.012$). Although this finding in layer chickens was based on only five comparisons, warranting cautious interpretation. Because quail comparisons outnumbered layer chicken comparisons, the non-significant quail results dominated the overall pooled estimate and masked the significant layer-chicken decline. Economic outcomes favored snail-based calcium in 69.2% of comparisons and were primarily driven by local calcium input costs, processing and labor expenses, egg pricing systems, and the inclusion level of snail-shell calcium. Based on the present findings, replacement levels of 25-50% for layer chickens and 50-75% for quail are suggested as practical starting points. Farmers and feed formulators should implement standardized processing protocols, verify calcium content of the processed shell material, and routinely monitor egg weight and eggshell quality to detect any decline over successive production cycles. On-farm cost-benefit analyses under local conditions are essential before commercial scale adoption. Future studies should prioritize longer trials (≥ 24 weeks) to assess cumulative effects on bone health and production, standardized reporting of processing parameters, comparative calcium dissolution kinetics across snail species, and studies conducted in temperate climates and intensive commercial systems.

DECLARATIONS

Acknowledgments

The authors gratefully acknowledge the University of Science and Technology of Southern Philippines, Claveria, Misamis Oriental, Philippines, particularly the Library and the Research and Development Office, for providing the independent reviewers for this systematic review.

Authors' contributions

Siony Miranda Cordova and Imelda Ulep Hebron conceptualized and crafted the methodology. Renante

Decenella Taylaran and Charly Guillermo Alcantara were responsible for the data validation and visualization. Nelda Ruba Gonzaga and Eric Randy Reyes Politud analysed and wrote the original draft. Rudy Mirabueno Camay was responsible for data curation and editing. All authors read and approved the final edition of the manuscript.

Ethical considerations

Ethical issues on plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all the authors. Grammarly, with the AI writing assistant tool (Coda Generative AI), was used for language editing and sentence restructuring during manuscript revision. It was not used for data collection, analysis, or interpretation. All AI-assisted outputs were reviewed and approved by the authors, who take full responsibility for the content and integrity of this study.

Availability of data and materials

Data are available from the corresponding author upon reasonable request.

Funding

This study received no external funding.

Competing interests

The authors have declared that no competing interests exist.

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