

Meta-analysis of the effects of organic and inorganic zinc supplementation on performance traits and diarrhea incidence in suckling calves

Abstract

This meta-analysis aimed to investigate and compare the effects of organic and inorganic zinc supplementation on performance traits and diarrhea incidence in suckling calves. A comprehensive literature search identified 15 eligible studies (published between 2019 to 2024), providing data for 44 comparisons on body weight (BW), 70 on average daily gain (ADG), 66 on total dry matter intake (TDMI), 34 on starter intake, 53 on feed conversion ratio (FCR), and 34 on diarrhea incidence. A multilevel random-effects model was employed to estimate the effect size of zinc supplementation, with the effect size expressed as the standardized mean difference (SMD). Publication bias was assessed using funnel plots and Egger's test. Data from the 15 selected studies were analyzed using Stata software (version 18), and heterogeneity among studies was evaluated using the Q- test and the I-squared (I^2) statistic. The results demonstrated that both organic (SMD = 0.64, $p < 0.05$) and inorganic (SMD = 0.72, $p < 0.05$) zinc significantly improved ADG in suckling calves. The highest BW was observed in calves supplemented with organic zinc (SMD = 0.58, $p < 0.05$). Organic zinc also significantly increased starter intake compared to inorganic zinc (SMD = 0.40, $p < 0.05$). Both forms of zinc increased TDMI in suckling calves ($p < 0.05$). A significant reduction in diarrhea incidence (measured as percentage of diarrheic calves in each group) was observed with both zinc sources ($p < 0.05$). Multivariate meta-regression analysis showed that experimental duration and zinc supplementation method were significant sources of heterogeneity for starter intake, ADG, and FCR ($p < 0.05$).

In conclusion, this meta-analysis suggests that organic zinc supplementation has a more profound effect on improving performance traits in pre-weaning suckling calves compared to inorganic zinc sources. Additionally, zinc supplementation (regardless of form) effectively reduces diarrhea incidence in suckling calves. These findings underscore the importance of ensuring adequate zinc levels in the diets of calves to support gastrointestinal health and overall performance.

Keywords Dairy calves, Diarrhea incidence, Meta-analysis, Performance traits

INTRODUCTION

Successful dairy cattle farming requires meticulous management of suckling calves, with nutrition playing a pivotal role during this critical period [1]. The preweaning period is a vulnerable stage during which calves face challenges due to a nascent immune system and an immature gastrointestinal tract [2], making them susceptible to gut microbial imbalances and gastrointestinal infections. In the context of suckling calf nutrition, the utilization of minerals, particularly trace minerals, is highly important. Although trace minerals constitute less than 0.01% of an animal's body weight, they are crucial for cellular function [3]. Furthermore, deficiencies in these elements are more prevalent in young ruminants [3]. Deficiencies in trace minerals such as zinc, copper, manganese, and selenium are commonly observed in young ruminants. Given that animals cannot store substantial amounts of these minerals, daily intake through the diet is essential [4]. Zinc, a crucial trace element, is involved in various metalloenzymes and activates more than 300 different enzymes in animals [5]. It is a critical mineral for the health and productivity of young calves, as it is essential for metabolism, growth, immune function, and antioxidant status [6]. Zinc deficiency can impair the production and secretion of growth hormone and insulin-like growth factor-1 (IGF-1) [7]. Zinc plays a role in numerous biological processes and is recognized as an effective anti-inflammatory and antidiarrheal agent [8]. Diarrhea is a leading cause of calf mortality, particularly in the first two weeks of life, resulting in antibiotic use and economic losses for dairy farms [9]. In recent years, zinc has been used as an anti-diarrheal agent in infants and children [10]. Moreover, zinc functions as a structural component in enzymes, a proton donor at the active site, and an atomic bridge between the substrate and enzyme [11]. It also participates in over 200 enzyme functions related to DNA synthesis, mitosis, cell division, protein synthesis, and carbohydrate metabolism [12]. According to standard nutritional tables, growing calves require approximately 33 mg of zinc per kg of dietary dry matter [1]. The National Research Council [13] recommends 70 mg Zn kg⁻¹ DM for growing calves aged 30 days. However, given that cow milk contains only 3-5 mg Zn kg⁻¹, daily milk consumption may not meet the zinc requirements of suckling calves, potentially leading to reduced appetite, nutrient intake, and growth [14]. Nevertheless, the most critical determinant of zinc requirements in living organisms is the concentration of zinc in the soil and plants of a given region [14]. Zinc levels in surface soils in Iran have been reported to be less than 0.8 mg/kg [15]. Consequently, plants grown in these soils are zinc-deficient and, when consumed as animal feed, can lead to a wide range of adverse effects in livestock, including growth abnormalities [16]. To ensure that growing animals achieve their genetic potential for performance and health, zinc is often supplemented in the diets of animals in zinc-deficient regions. Currently, both inorganic and organic zinc sources are used in human and animal nutrition. Compared with inorganic sources, organic zinc sources,

particularly zinc-methionine, have been shown to have greater bioavailability and utilization in ruminants [17]. Numerous studies have investigated the positive effects of zinc supplementation in growing calves. Inorganic zinc sources, such as zinc oxide [18] and zinc sulfate [19], have been shown to increase growth, strengthen the immune system, and reduce diarrhea symptoms in suckling calves. Seifdavati et al [20] reported that nano zinc oxide supplementation at 30 and 60 mg/kg of dietary dry matter improved weight gain in suckling calves. In a study by Ma et al [21], organic zinc (zinc proteinate) supplementation resulted in significantly greater body weights and an improved feed conversion ratio in preweaning calves than did inorganic zinc (zinc oxide). The percentage of diarrhea incidence was also lower in calves supplemented with zinc proteinate. Wright and Spears [22] reported that zinc deficiency in calves led to reduced appetite, feed intake, growth rate, and feed efficiency. Nagalakshmi et al [23] reported that zinc proteinate supplementation decreased lipid peroxidation and enhanced humoral immunity and superoxide dismutase activity in lamb red blood cells. Research indicates that supplementing 80 mg of zinc per calf daily in the first two weeks of life stimulates growth, enhances immune function, and reduces diarrhea symptoms in suckling calves [19]. The anti-diarrheal effect of zinc has been attributed to improved immune function, reduced pathogenic bacteria populations, and increased beneficial bacteria in the gastrointestinal tract [24]. Despite these studies, the results comparing organic and inorganic zinc sources are inconsistent, with some studies showing no difference in bioavailability [24]. Meta-analysis is a systematic review of quantitative studies based on statistical and mathematical principles. Combining the results of various studies on a common topic provides a more accurate and reliable estimate than individual studies do [22]. This study aimed to compare the effects of organic and inorganic zinc supplementation on performance traits and diarrhea incidence in pre-weaning suckling calves.

MATERIAL AND METHODS

Meta-analysis sources

The data sources for this meta-analysis consisted of published articles related to the utilization of organic and inorganic zinc sources in the diets of suckling calves, specifically those published from 2019 to 2024. The study population included articles published in both Iranian domestic databases (IranDoc, Scientific Information Database [SID], Magiran) and international databases (Scopus, Google Scholar, Web of Science, and PubMed). A systematic literature search was conducted using Persian keywords and their English equivalents, combined in various permutations. The key search terms included: “zinc,” “inorganic zinc supplement,” “organic zinc supplement,” “minerals,” “suckling

calves,” “performance,” “growth,” “dairy calves” “dry matter intake,” “average daily gain,” “body weight,” and “diarrhea incidence”. Google Scholar was searched for up to the first 10 pages of results.

Inclusion and exclusion criteria

Search results from both Persian and English language databases were collected in EndNote software (version 9), and duplicate articles were subsequently removed. Articles found from the search were then included or excluded from the study based on the following criteria: Inclusion Criteria: (1) Articles published in peer-reviewed journals in Persian or English ; (2) Studies that involved the use of organic and inorganic zinc supplementation in suckling calves; (3) Studies with both a treatment group (zinc supplemented) and a control group (no zinc supplementation); (4) Studies providing sufficient data (mean, standard deviation [SD], or standard error [SE] for at least one relevant trait (e.g., grow performance, diarrhea incidence. The exclusion criteria included: (1) Studies lacking quantitative data on zinc supplementation or relevant outcome; (2) Studies with data pertaining to non-suckling calves; (3) Studies in which zinc supplementation was combined with other elements; (4) Review articles, conference abstract, or studies without full-text availability.

Data extraction

The following variables were extracted from each study: first author, publication year, zinc dosage, calf sex, experimental duration, zinc source, and methods of zinc supplementation in milk and starter (Table 1). Additionally, the mean and SD of the studied traits for both the treatment and control groups were extracted (Table 2). The extracted parameters included total dry matter intake (TDMI), average daily gain (ADG), final body weight (BW), starter intake, the feed conversion ratio (FCR), and diarrhea incidence (percentage of diarrheic calves in each group) in suckling calves. After screening, 15 studies met the eligibility criteria and were included in the meta-analysis. The PRISMA flow diagram, illustrating the initial search, screening, and final selection of articles for inclusion in the meta-analysis, is depicted in Fig 1. The number of comparisons made for BW, ADG, starter intake, TDMI, FCR, and diarrhea incidence in this meta-analysis were 44, 70, 34, 66, 53, and 34, respectively.

Effect size calculation

In the present study, given the continuous nature of the data, Hedges's g index (Equation 1) and SMD (Equation 2) and were employed to calculate the effect size.

$$g = \frac{\bar{X}_s - \bar{X}_n}{s_p} \times J \quad \text{Eq (1)}$$

In this equation, g represents the effect size, $\bar{X}_s$ is the mean of the experimental group, $\bar{X}_n$ is the mean of the control group, s_p is the pooled standard deviation, and J is the bias correction factor for the two groups.

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$$\text{SMD} = (\bar{X}_e - \bar{X}_c) / S \quad \text{Eq (2)}$$

In this equation, SMD = Effect size, $\bar{X}_e$ = Mean of the experimental group, $\bar{X}_c$ = Mean of the control group, S = Standard deviation.

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For studies reporting separate SD for the control and experimental groups, Equation 3 was used. For studies reporting the Standard Error of the Mean (SEM), Equation 4 was used to calculate the pooled standard deviation.

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$$s_p = \sqrt{\frac{(n_s - 1)SD_s^2 + (n_N - 1)SD_N^2}{n_s + n_N - 2}} \quad \text{Eq (3)}$$

In Equation 3, s_p is the pooled standard deviation, n_s represents the number of experimental units in the experimental group, n_N represents the number of experimental units in the control group, SD_s represents the standard deviation of the experimental group, and SD_N represents the SD of the control group [26].

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$$s_p = \text{SEM} \times \sqrt{n_p} \quad \text{Eq (4)}$$

In Equation 4, s_p is the pooled standard deviation, SEM represents the SD of the mean for all groups, and n_p represents the total number of experimental units in the control and experimental groups [26].

131 **Weighting of Study Data**

Studies were weighted to determine the contribution of each study to the final results basis on study quality, including higher replication numbers and smaller variances, via Equation 5 [27].

$$W_i = \frac{1}{\text{vari}} \quad \text{Eq (5)}$$

In Equation 5, vari represents the variance of the study, and W_i represents the weight assigned to that study.

136 **Quality assessment**

Two independent investigators assessed methodological quality using the Cochrane Risk of Bias tool version 2 (RoB2) [28]. The following potential bias were evaluated: (D1) Bias arising from the randomization process, (D2) Bias due to deviations from intended interventions, (D3) Bias due to missing outcome data, (D4) Bias in measurement of the outcome, (D5) Bias in selection of the reported result. Each article was classified as being at low risk of bias indicated by a plus (+), unclear risk of bias by a minus (-) or high risk of bias by cross (×) according to the assessment details for risk of bias presented above.

Heterogeneity testing

Heterogeneity (Cochran's Q test) of the effect size variability across studies, attributed to interstudy differences, was assessed via the Q statistic (Equation 6) at a significance level of $\alpha = 0.1$. In meta-analyses with limited study replication, where the Cochran's Q test may exhibit low sensitivity, heterogeneity was further evaluated using the I^2 statistic. I^2 values of 0 indicate no heterogeneity, values between 25 and 50 indicate moderate heterogeneity, and values greater than 50 indicate high heterogeneity among studies. To identify further sources of heterogeneity, it was necessary to perform meta-regression or subgroup analysis [29].

$$I^2 = \frac{Q - (k-1)}{Q} \times 100\% \quad \text{Eq (6)}$$

In Equation 6, Q represents the chi-square heterogeneity statistic (Cochran's Q), and K represents the number of studies.

Subgroup analysis

To investigate the overall effect of zinc sources on the performance of suckling calves, as well as the effect size of organic and inorganic zinc sources on the traits under study, subgroup analysis was employed. To enhance the depth and precision of the current meta-analysis, comprehensive subgroup analyses were conducted. These analyses were performed based on several key factors to more accurately differentiate the effects of various variables on the overall meta-analysis results. Specifically, studies were categorized and examined according to trial duration, classifying them into two groups: ≤ 28 days and > 28 days. Furthermore, the impact of zinc on performance traits was analyzed separately based on the sex of the calves, considering three distinct groups: male, female, and mixed (male + female). The various dosages of zinc supplementation were also meticulously analyzed, with doses grouped into three levels: ≤ 40 , >40 and ≤ 80 , and >80 mg Zn kg^{-1} DM per day. In addition, a detailed comparison was made regarding the

efficacy of organic versus inorganic zinc forms. Finally, the method of zinc supplementation, whether via milk or starter feed, was evaluated for its influence on the performance traits and the incidence of diarrhea in calves. This subgroup approach allows for a deeper understanding of how zinc impacts the performance of suckling calves and significantly contributes to identifying the factors that influence its effectiveness, depending on the specific conditions of each study.

Multivariate meta-regression

To identify heterogeneity sources, multivariate meta-regression ("meta reg" command) was performed for confounding factors (sex, dose, Zn source, supplementation method, experimental duration). Forest plots were generated ("meta forest plot" command).

Dose-Response

A dose-response meta-regression was conducted to investigate the relationship between zinc dose and performance traits and diarrhea incidence in dairy calves. This analysis specifically examined how the response changed across the 20 to 120 mg range of zinc supplementation.

Publication bias

To assess publication bias, a funnel plot was generated. A funnel plot is a method for detecting publication bias, and is based on the principle that the statistical weight of a study increases with its sample size. Therefore, studies with small sample sizes are widely dispersed at the bottom of the plot, whereas studies with large sample sizes are located at the top of the plot, closer to the mean effect. In the presence of bias, the funnel plot becomes asymmetrical. To test for asymmetry in the funnel plot, Egger's test was used with a significance level of 0.05.

Sensitivity Analyses

To assess the stability and robustness of the primary meta-analysis results, a supplementary sensitivity analysis was conducted. In this analysis, only the treatment arm containing the highest level of zinc supplementation (mg/kg DM) from each study was included, and its results were compared with the corresponding control group. This approach was adopted to evaluate whether the effects observed in the initial meta-analysis were predominantly driven by the

strongest zinc interventions across studies, and whether the inclusion of lower zinc levels had diluted the overall findings.

Statistical analysis

The results obtained from this meta-analysis are presented in forest plots, containing key information, such as the overall effect size, SE, and 95% confidence interval (CI), as well as the effect size, SE, and 95% CI for each individual study. The weight of each study was represented by the area of a square centered on the mean effect size of that study, with the size of the square indicating the weight assigned to that study in the final analysis. For data analysis, STATA/MP 18.0 software was used, employing a random-effects model to estimate the effect size, 95% CI, and statistical significance for each trait. This model was chosen because it is more conservative than the fixed-effects model [30-31]. The effect size of zinc supplementation was expressed as the SMD. The SMD values were interpreted as follows: $SMD < 0.2$ indicated a small effect, $0.2 \leq SMD \leq 0.7$ indicated a moderate effect, and $SMD > 0.7$ represented a large effect [32]. Additionally, an SMD with a p -value < 0.05 was considered statistically significant. We applied a multilevel random-effects model to the collected data to account for multiple effect sizes derived from individual studies (i.e., when a single control group was compared with several treatment groups within the same publication). This multilevel random effect modeling approach is the most suitable for such datasets, as it accommodates dependencies arising from multiple effect sizes within a single study [35]. The multilevel framework accounts for the hierarchical structure of the data, incorporating variance at different levels, including within-study and between-study variability. Effect sizes from the same study were treated as nested within a higher-level cluster, rendering this method particularly effective for addressing substantial between-study heterogeneity. By explicitly modeling these variance components, the multilevel meta-analysis approach enhances the precision of treatment effect estimates and facilitates the exploration of potential sources of heterogeneity [34]. Additionally, this model enables the quantification of variation across studies and levels, the estimation of the pooled effect size, and the examination of potential moderators or confounding factors that may explain heterogeneity [34]. The analysis employed the SMD as the effect size metric, which represents a widely used statistical technique in meta-analysis for synthesizing results from studies with differing measurement scales [33].

RESULTS

Study selection workflow and risk of bias assessment

The article searches and selection process is detailed in Fig 1. A total of 459 articles were initially identified from the target databases for screening. After removing 121 duplicate studies and 226 studies that did not meet the inclusion criteria (including studies on non-dairy calves, review articles, articles with only abstracts available, those involving zinc supplementation mixed with other additives, and articles lacking relevant production data), we retained 112 articles for full-text review. Following a thorough full-text evaluation based on predefined protocols, we excluded an additional 97 articles. Thus, 15 eligible articles were included in the final meta-analysis. The extracted data from these studies, comprising study name, publication year, breed, number of calves per treatment, trial duration, zinc dosage, calf sex, and supplementation method (milk or starter feed), are summarized in Table 1. Additionally, Table 2 presents the mean $\pm$ SD values for all investigated traits in both control and zinc-supplemented groups. The risk of bias assessment for each included study is shown in Fig 2.

The assessment results indicated that the risk of bias associated with the randomization process and deviations from intended interventions was low in more than 90% of the studies. This demonstrates the high quality of the design and execution of these studies in these two domains. Regarding bias due to missing outcome data, 65-70% of studies had a low risk of bias, while 20-25% raised "some concerns," and about 5-7% showed a "high risk of bias" for this domain. These results highlight the need for cautious interpretation when working with missing data. Furthermore, over 90% of studies had a low risk of bias in outcome measurement, and 65% of studies demonstrated a low risk of bias in reporting result selection.

Effect of Zinc Supplementation on Performance Traits

Forest plots demonstrating the effects of zinc supplementation (organic vs. inorganic forms) on starter intake, TDMI, ADG, BW, and FCR are presented in Figs 3, 4, 5, 6, and 7, respectively.

Starter Intake

A multilevel random effects model was employed to estimate the SMD for starter intake in dairy calves. The analysis revealed a moderate SMD of 0.29 (95% CI: 0.11-0.47) (Fig 3, Table 4), indicating a statistically significant difference in starter intake between control calves and those receiving zinc supplementation ($p < 0.05$). As shown in Fig 3, the

inorganic zinc form demonstrated no significant effect on starter intake in dairy calves (SMD = 0.17, p = 0.20, I^2 =19.81%). However, the use of the organic form of zinc had a positive and moderate effect on starter intake, leading to an increase compared to the control group (Fig 3, SMD = 0.40, p < 0.05, I^2 =14.39%).

Total Dry Matter Intake

The mean SMD for TDMI, estimated using the multilevel random effects model, was 0.27 (95% CI: 0.14-0.41; p < 0.05; Table 4). Both organic (Fig 4, SMD = 0.24, p < 0.05, I^2 =33.30%) and inorganic zinc (Fig 4, SMD = 0.32, p < 0.05, I^2 =32.33%) supplementation significantly enhanced TDMI in dairy calves.

Average Daily Gain

Seventy comparisons were used to evaluate the effect of zinc supplementation on ADG in dairy calves. The multilevel random effects model results indicated a positive effect of zinc supplementation on ADG (SMD = 0.67, 95% CI: 0.53-0.82, p < 0.05) (Fig 5). Both inorganic (Fig 5, SMD = 0.72, p < 0.05, I^2 =11.18%) and organic zinc (Fig 5, SMD = 0.64, p < 0.05, I^2 =50.69%) significantly improved ADG in dairy calves.

Body Weight

The forest plot illustrating the effect of organic and inorganic zinc sources on BW in dairy calves is shown in Fig 6. The multilevel random effects model results indicated a positive and moderate effect of zinc supplementation on BW (SMD = 0.42, 95% CI: 0.25-0.59) (Fig 6, Table 4), with a statistically significant difference between control calves and zinc supplemented calves (p < 0.05). As shown in Fig 6, both organic and inorganic zinc supplementation significantly enhanced BW, with effect sizes of +0.58 (95 % CI: 0.32-0.84) and +0.27 (0.07-0.46), respectively (p < 0.05).

Feed Conversion Ratio

Figure 7 presents the results of 53 statistical comparisons between treated and control groups regarding the effect of zinc supplementation on FCR. Based on the multilevel random effects model, the SMD for FCR was negative and moderate (SMD = -0.63, 95% CI: -0.80, -0.47) between control calves and those receiving zinc supplementation, indicating that zinc supplementation reduced FCR in dairy calves (Table 4, p < 0.05). Compared to the inorganic zinc form (Fig 7, SMD = -0.50, p < 0.05, I^2 =28.66%), the use of organic zinc supplementation (Fig 7, SMD = -0.79, p < 0.05, I^2 =31.63%) had a significantly greater effect on reducing the feed conversion ratio.

Heterogeneity and Publication Bias

I^2 values below 25% for starter intake (Fig 3) and between 25-50% for TDMI (Fig 4) indicate low and moderate heterogeneity, respectively. The symmetric distribution of all studies around the effect size in the funnel plot suggests an absence of publication bias for the studies included in the meta-analysis for starter intake (Fig 9.A) and TDMI (Fig 9.B). Additionally, Egger's test confirmed the symmetry in the funnel plot for both starter intake and TDMI (Table 6). For ADG, I^2 values ranged between 25-50%, indicating moderate heterogeneity. The asymmetrical distribution of all studies around the effect size in the funnel plot (Fig 9.C) suggests potential publication bias for the ADG data extracted from the included studies. This finding was further supported by a statistically significant ($p < 0.05$) Egger's test for publication bias (Table 6). The I^2 test revealed moderate heterogeneity in the results for BW ($I^2=35.51\%$, $Q=42.58$, $p < 0.05$). The symmetric distribution of all studies around the effect size for BW indicated no publication bias, and Egger's test results also supported this conclusion (Table 6, $p = 0.37$). For FCR, the I^2 value was 32.67%, indicating moderate heterogeneity (Fig 7). The asymmetrical distribution of all studies around the effect size for FCR in the funnel plot (Fig 9.E) suggests possible publication bias in the included studies. This was further confirmed by a significant ($p < 0.05$) Egger's test for asymmetry (Table 6).

Multivariate Meta-Regression

The selected moderators, such as zinc dosage, sex, and zinc source, did not significantly affect the starter intake of calves receiving zinc supplementation (Table 3). However, both trial duration and the method of zinc supplementation (added to milk or starter) significantly influenced starter intake in dairy calves ($p < 0.05$) (Table 3). Meta-regression analysis revealed that the method of zinc supplementation marginally improved TDMI in dairy calves (Table 3, $p = 0.07$). In contrast, other investigated moderators, including zinc dosage ($p = 0.45$, 95% CI: -0.003, 0.007), calf sex ($p = 0.46$, 95% CI: -0.44-0.20), and zinc source ($p = 0.37$, 95% CI: -0.13-0.43), did not significantly influence the TDMI of dairy calves (Table 3).

Meta-regression results showed that zinc dosage ($p = 0.63$) and zinc source ($p = 0.35$) as moderators did not significantly affect the ADG of dairy calves (Table 3). Conversely, the method of zinc supplementation was identified as a significant moderator ($p < 0.05$), and while calf sex showed a trend toward significance ($p = 0.08$). Meta-regression analysis revealed that moderators such as zinc source, dosage, sex, and trial duration were not significant sources of heterogeneity for the BW of dairy calves (Table 3). However, the method of zinc supplementation was identified as a

source of heterogeneity for calf body weight (Table 3). Meta-regression results indicated that, among the moderators, the method of zinc supplementation significantly affected the FCR ($p < 0.05$), while other moderators had no significant effect on FCR (Table 3).

Subgroup Analysis

Subgroup analyses were performed by classifying studies based on dosage, sex, trial duration, and zinc supplementation method (added to milk or starter) (Table 4). The SMDs for starter intake, TDMI, ADG, BW, and FCR across different subgroups are presented in Table 4. The subgroup analysis revealed several notable findings. Specifically, zinc supplementation added to milk at a dose exceeding 80 mg Zn kg⁻¹ DM significantly improved starter intake ($p < 0.05$). Moreover, this effect was more pronounced in female calves when zinc was administered in milk. Furthermore, adding zinc supplementation at a dose exceeding 80 mg Zn kg⁻¹ DM had a positive and moderate effect size on the TDMI of calves ($p < 0.05$). This effect was higher in male calves that received zinc supplementation in milk compared to the control group ($p < 0.05$). The results of the subgroup analysis for ADG and BW indicated that including zinc supplementation at a dose of 40-80 mg Zn kg⁻¹ DM in milk had the greatest impact on the ADG of female calves ($p < 0.05$). Consistent with ADG, zinc supplementation at a dose > 40 and ≤ 80 mg Zn kg⁻¹ DM in milk also had the greatest effect on the body weight of female calves during trial periods > 28 days ($p < 0.05$). The subgroup analysis for FCR showed that zinc supplementation in milk at a dose greater than 80 mg Zn kg⁻¹ DM significantly reduced FCR in female calves.

Interaction Effect of Zinc Source $\times$ Dosage and Sensitivity Analysis

The results regarding the interaction effect of dosage $\times$ zinc source on the performance traits of dairy calves are presented in Table 5. According to Table 5, the interaction effect of dosage and zinc source did not significantly affect performance traits. To evaluate the impact of each study individually and the stability of the study results, a sensitivity analysis was performed by sequentially removing each study and considering the highest level of zinc supplementation, then estimating the overall effect of the remaining studies (Table 7). The findings from the sensitivity analysis provided strong evidence for the stability and reliability of the meta-analytical results regarding the efficacy of organic and inorganic zinc supplements in improving performance traits in dairy calves. As a result, all pooled estimates fell within the range of the overall effect (Table 7), indicating both low sensitivity and high stability in the results of this meta-analysis.

Relationship Between Performance Traits and Zinc Supplementation

In examining the relationship between performance traits and zinc dose, zinc supplementation was considered the independent variable, and calf performance indicators (including starter intake, TDMI, ADG, BW, and FCR) were investigated as dependent variables. The results showed a quadratic relationship between the adjusted performance indicators (starter intake, ADG, BW, and FCR) and zinc supplementation level (Fig 10). Maximum starter intake (Fig 10.A) and TDMI (Fig 10.B) were achieved at zinc level exceeding 80 mg Zn kg⁻¹ DM. In contrast, maximum ADG and BW were observed at zinc level between > 40 and ≤ 80 mg Zn kg⁻¹ DM (Figs 10.C and 10.D, respectively). Notably, with increasing zinc dose (> 80 mg Zn kg⁻¹ DM), ADG exhibited a decreasing trend. With increasing zinc dose (> 80 mg Zn kg⁻¹ DM), the FCR of dairy calves showed an increasing trend (Fig 10.E).

Effect of Zinc Supplementation on Diarrhea Incidence

In this meta-analysis, the incidence of diarrhea was defined as the percentage of calves affected by diarrhea in both control and zinc-supplemented groups. Based on the multilevel random effects model, the SMD for the incidence of diarrhea in dairy calves was estimated to be -0.95 (95% CI: -1.17, -0.74), indicating a substantial effect of zinc supplementation in reducing the occurrence of diarrhea in dairy calves. An I² value of 32.67% suggests moderate heterogeneity ($p = 0.01$). The asymmetry of studies around the effect size for the incidence of diarrhea in the funnel plot (Fig 9.F) indicates the presence of publication bias among the studies included in the meta-analysis. Egger's test also confirmed significant publication bias for diarrhea incidence ($p < 0.05$) (Table 6). Meta-regression analysis showed that the method of zinc supplementation significantly influenced on the incidence of diarrhea in dairy calves ($p < 0.05$). However, no significant relationship was observed between the moderators (including trial duration, dosage, zinc source, and sex) and zinc supplementation (Table 5).

The results from subgroup analyses (summarized in Table 4) revealed several significant differences in diarrhea incidence across various categories. Regarding dose subgroup categorization zinc levels between > 40 and ≤ 80 mg Zn kg⁻¹ DM showed the greatest effect on reducing diarrhea incidence in dairy calves ($p < 0.05$). In trials duration exceeding, >28 days, zinc supplementation had a strong negative effect on diarrhea incidence ($p < 0.05$). Additionally, our subgroup analysis indicated that adding zinc supplementation to milk (compared to starter feed) significantly reduced the incidence of diarrhea in studies that included a combination of male and female calves ($p < 0.05$). The zinc source × dosage interaction did not significantly affect diarrhea incidence in dairy calves. When individual studies

were removed sequentially, and the highest level of zinc consumption was considered, all remaining studies fell within the range of the overall effect. This indicates that the results of the meta-analysis have low sensitivity and high stability (Table 7). The relationship between the dose of zinc supplementation in dairy calf diets and the SMD for diarrhea incidence in dairy calves is shown in Fig 10.F. The meta-regression analysis revealed a quadratic relationship between the dose and the effect size of diarrhea incidence. This relationship suggests that at lower doses, increasing dietary zinc leads to a reduction in diarrhea incidence, but after an optimal point (a dose of 80 mg Zn kg⁻¹ DM), further increases in zinc may have a less significant reducing effect or could even lead to an increase in diarrhea incidence.

DISCUSSION

Performance Traits

The present meta-analysis aimed to systematically evaluate how zinc sources affect calves during the critical pre-weaning stage, which is essential for their subsequent growth and productivity. For example, an increase in starter intake can reshape the rumen fermentation profile, leading to changes in the proportions of volatile fatty acid (VFA) proportion and increased butyrate concentration [36]. Notably, butyrate is more efficiently produced from concentrate fermentation than from compared to roughage, and its key role in stimulating the development of the rumen mucosa [37]. Once the starter is consumed and fermented in the rumen, butyrate is absorbed and transformed into beta-hydroxybutyrate (BHBA) before entering the bloodstream [36]. In addition to meeting the physiological needs of the animal, zinc supplementation provides benefits for feed efficiency and gastrointestinal health at specific life stages. Consistent with the results of the present study, Wright and Spears [22] reported that male calves receiving 20 mg Zn kg⁻¹ DM had significantly higher ADG than those in the control group. Other studies have shown that zinc supplementation in preweaning dairy calves improves growth performance [18]. In contrast to our findings, Wo et al [38] found no significant difference in starter intake between calves fed different levels of zinc proteinate. One of the earliest signs of zinc deficiency in the is appetite reducing, followed by decreased feed intake [39]. In the present study, zinc supplementation enhanced appetite and increased starter and TDMI compared with the control diet. The primary mechanism through which zinc affects appetite may involve its direct influence on the expression of appetite-regulating genes. This is supported by evidence that zinc deficiency alters the production and secretion of appetite-controlling hormones and enzymes [39].

One such enzyme is pyruvate kinase, whose gene expression is regulated by insulin. Zinc deficiency reduces the insulin sensitivity of this enzyme, leading to decreased carbohydrate catabolism and loss of appetite [40]. Furthermore,

zinc deficiency reduces also the production and secretion of growth hormone and insulin-like growth factor-1, both of which play crucial roles in growth and weight gain [41]. The present meta-analysis demonstrated that zinc supplementation in suckling calves significantly increased ADG and BW. This beneficial effect was particularly noted with organic zinc sources in our meta-analysis. The observed benefits of zinc supplementation on performance in specific species may be related to its dual effects on host intestinal tissue and the gut microbiome [42]. Within the host, zinc appears to enhance intestinal villi and intestinal cell health [43], reduce intestinal permeability [44], and induce antioxidant effects in the intestinal mucosa [45]. Building upon this, zinc also positively influences the gut microbiome, which in turn plays a crucial role in animal health and performance by influencing nutrient absorption, metabolism, and immune function [46]. Specifically, bacteria in the microbiome produce beneficial metabolites such as short-chain fatty acids [47]. These metabolites reduce inflammatory markers and lower the pH of the intestinal lumen, thereby limiting the proliferation of potentially pathogenic bacteria [48]. Zinc is an essential element for the bacteria that constitute the microbiome, with zinc-binding proteins accounting for 5% of the bacterial proteome [49], and the microbiota utilizes up to 20% of the dietary zinc consumed by the host [50]. Rajaei-Sharifabadi et al [51] in a study on milk supplemented with various zinc sources (zinc sulfate, zinc-methionine, and Benza[®] Zn) in suckling calves, reported that Benza[®] Zn supplementation improved ADG. In an investigation of different levels of zinc-proteinate supplementation (0, 40, 80, and 120 mg Zn kg⁻¹ DM), Wo et al [38] reported that high levels of zinc supplementation (120 mg Zn kg⁻¹ DM) significantly increased ADG and BW in suckling calves, which is consistent with the results of the present study. In contrast to the results of the present study, previous studies reported that zinc supplementation in whole milk [52] or milk replacer above NRC recommendations [53] did not significantly affect growth performance. Chang et al [18], in a study on the effects of zinc oxide (80 mg/day) and zinc-methionine (80 mg/day), reported that organic and inorganic zinc supplementation did not significantly affect starter and TDMI, but zinc-methionine supplementation significantly increased ADG in newborn calves. I² values (25-50%) for performance traits indicated moderate heterogeneity. This heterogeneity may be attributed to differences in zinc supplement form, dosage, initial weight, animal age, experimental duration, and the form of zinc supplementation (in milk or starter). Regression analysis indicated that at least two covariates (trial duration and method of zinc supplementation) out of the five significantly influenced the five performance outcome variables. These covariates explained 18.44%, 32.13%, 38.39%, 35.51%, and 32.67% of the heterogeneity in starter intake, TDMI, ADG, BW, and FCR, respectively (I² values in Figs 3-7). Other modulating factors did not significantly affect performance traits. This suggests that other

unknown dietary and management-associated factors may influence the effect of zinc sources on pre-weaning dairy calves. Zinc sources in the studies included in this meta-analysis were supplied at recommended dosages (20-120 mg Zn kg⁻¹DM), which were hypothesized to have a beneficial effect on calves. Although zinc sources significantly impacted the five performance outcomes, the heterogeneity for these outcomes was observed to be moderate. Our subgroup analysis further confirmed that lower doses of zinc (≤ 40 mg Zn kg⁻¹ DM) did not affect the performance traits of pre-weaning calves, whereas higher doses (>40 and ≤ 80 mg Zn kg⁻¹ DM) significantly improved ADG and body weight in pre-weaning calves. Researchers have reported that dietary zinc supplementation positively affects calf performance, antioxidant status, and the immune system [54]. However, using high levels of dietary zinc may negatively impact the digestion, absorption, and utilization of other nutrients, potentially leading to environmental contamination due to excess zinc excretion in feces [55]. Subgroup analysis revealed that zinc supplementation in the starter feed had no beneficial effect on performance traits, whereas supplementation in liquid form (milk) significantly and positively influenced performance traits. The notable impact of various supplementation methods on the five measured outcomes likely stems from the route of delivery (solid vs. liquid) [56]. In young calves, solid feed enters the rumen, whereas liquid feed bypasses the rumen and goes directly to the abomasum [57]. Beyond the delivery method, the divergent protein and fiber content of the starter feed may also contribute to these differential effects [58]. Furthermore, the gradual dietary transition calves undergo during weaning- shifting from predominantly liquid diets (milk or milk replacer) to a solid starter diet rich in fermentable carbohydrates [59], is a significant factor. This transition can alter the composition of the ruminal microbiome, potentially leading to an increase in *Proteobacteria* and *Firmicutes* and a decrease in *Bacteroidetes* phylum [60].

Effect of Zinc Supplementation on Diarrhea Incidence

The results of the present study demonstrated that both organic (SMD = -0.93, $p < 0.05$) and inorganic (SMD = -0.98, $p < 0.05$) zinc sources significantly reduced diarrhea incidence in suckling calves compared with the control group, with an overall effect size of SMD = -0.95 ($p < 0.05$). Diarrhea is the most common disease in calves, and often occurs in the first month of life [61]. Several factors contribute to diarrhea in calves, including incomplete intestinal development, inadequate nutrient absorption, impaired immune function, and stress from cold and heat [62]. For decades, zinc has been used as an antidiarrheal agent for the prevention and treatment of diarrhea in infants, children, and animals. Consistent with the results of the present study, Ma et al [63] reported that, compared with the control

diet, zinc-methionine supplementation reduced the incidence of diarrhea from days 8-14 and throughout the study. In that study, zinc oxide had a similar effect to that of zinc-methionine, but the effect was not statistically significant. According to Chang et al [18], the first two weeks of life represent the peak period for diarrhea prevalence in suckling calves. Therefore, zinc-methionine supplementation in the diet of dairy calves early in life is recommended, as it can reduce diarrhea incidence. Feldmann et al [19] also reported that calves fed Zn-Met-enriched feed had 14.7% less diarrhea than unsupplemented calves did, which aligns with the results of the present study. In a study by Liu et al [64], the percentages of diarrhea occurrence in calves receiving zinc-proteinate and zinc oxide were reported to be 10.7% and 16.1%, respectively, which were significantly lower than those in the control group. Zinc-proteinate and zinc oxide reduced diarrhea incidence from 1 to 28 days of age, but zinc oxide had no effect on reducing diarrhea incidence in calves from 1 to 14 days after birth, and zinc supplementation had an antidiarrheal effect after 28 days of age [64]. Recent studies, which have focused primarily on the preventive effect of short-term zinc supplementation in milk on diarrhea in dairy calves, have shown that adding 80 mg Zn kg⁻¹ to milk can reduce diarrhea incidence and improve ADG in dairy calves [51]. The multivariate meta-regression results revealed that diarrhea incidence difference between the experimental and control groups was a source of heterogeneity in the SMD for both the form of zinc supplementation (in milk and starter) and calf sex. Previous reports indicate that whole milk contains 3 to 5 mg of zinc per liter [65]. Given that starter intake is low during the first two weeks of life, it usually results in negligible zinc intake from starter feed. Therefore, the minimal contribution of zinc from starter feed during this period highlights the importance of milk as the primary zinc source for suckling calves and demonstrates the potential efficacy of zinc supplementation strategies in milk. Feldmann et al [19] examined the effects of milk containing 80 mg zinc/L (as zinc-methionine or zinc sulfate) in suckling calves up to 14 days of age. They reported a significant interaction effect between zinc supplementation and growth rate, with male calves in the zinc-methionine group showing increased growth rates. This positive impact of zinc, particularly its ability to support overall health and resilience (acting as a preventive measure against potential issues like diarrhea or impaired immunity), is attributed primarily to enhanced immune responses and increased blood immunoglobulins [66]. Furthermore, Ma et al [63] reported that zinc-methionine supplementation specifically helps maintain the integrity of the intestinal epithelial barrier in dairy calves, further elucidating its anti-diarrheal mechanism. Publication bias is a common challenge in meta-analyses, as it can alter the overall estimated effect of an intervention (such as zinc supplementation) on the outcome being studied (diarrhea incidence). In this meta-analysis, publication bias was assessed through visual inspection of funnel plots and

Egger's test. The obtained funnel plot (Fig 9.F) in this meta-analysis was slightly asymmetrical. This asymmetry indicates possible publication bias. Publication bias can stem from the tendency for studies with negative results not to be published, either due to editorial bias in journals or authors' disinclination to publish papers with unfavorable findings [67]. Evidence of moderate heterogeneity was observed in this meta-analysis, as indicated by the I^2 values [68]. This heterogeneity issue was resolved through subgroup analysis. Subgroup analysis revealed that zinc supplementation within the 40-80 mg Zn kg⁻¹ DM range significantly reduced the incidence of diarrhea in trials lasting over 28 days. Although significant publication bias was detected and sensitivity analysis also showed significant results, it's important to note that the interaction effect between zinc dosage and zinc source was not statistically significant. Collectively, these findings emphasize the critical role of both the supplementation method and optimal dosage in mitigating calf diarrhea. Furthermore, these results highlight the importance of considering potential reporting biases and their influence on the overall effect size in meta-analytic studies.

CONCLUSION

This meta-analysis revealed that zinc supplementation (particularly organic sources) can enhance growth performance and feed efficiency in calves. This improvement was evidenced by an increase in BW and ADG, and a reduction in the FCR, primarily due to increased starter intake and TDMI. Furthermore, the method of supplementation (specifically adding to milk) significantly influenced performance traits and reduced the incidence of diarrhea in suckling calves. Significant heterogeneity was observed for traits such as ADG, FCR, and diarrhea incidence, indicating that the effect of zinc supplementation on these traits was not consistent across different studies. In contrast, no significant heterogeneity was found for TDMI, BW, and starter intake, suggesting that the effect of zinc supplementation on these traits was relatively stable across the studies. The results of Egger's test for FCR, ADG, and diarrhea incidence revealed significant publication bias. This means that studies with positive and significant results for these traits were more likely to be published, while studies with negative or non-significant findings were less likely to be published. Consequently, due to the presence of heterogeneity and publication bias in traits such as FCR, ADG, and diarrhea incidence, the results should be interpreted with caution. Furthermore, subgroup analysis and multivariate meta-regression showed that the effectiveness of zinc intervention significantly increases with longer supplementation duration. Subgroup analysis, along with the correlation between zinc dosage and performance traits, indicated that doses below 40 mg Zn kg⁻¹ DM had no impact on calf performance. Conversely, using doses exceeding

80 mg Zn kg⁻¹ DM could negatively affect the digestion, absorption, and utilization of other nutrients in the diet. This higher dosage also increases the risk of environmental pollution through excessive zinc excretion in feces. Therefore, it's recommended to supply zinc in the diet of suckling calves at level up to 80 mg Zn kg⁻¹ DM. These findings suggest that both zinc sources can serve as effective interventions for managing and reducing gastrointestinal issues during the suckling period. Thus, the use of zinc in both organic and inorganic forms can be recommended as a beneficial strategy for improving calf health and reducing treatment costs in the livestock industry.

CONFLICT OF INTEREST STATEMENT

The authors declare no known conflict of interest.

Author's contributions

The entire study, including conceptualization, methodology, software, validation, formal analysis, investigation, and data curation, was conducted solely by the corresponding author. Additionally, the original draft preparation, review, and editing were performed entirely by the corresponding author.

Data availability

The data that support the findings of this study are not openly available and are available from the corresponding author upon reasonable request.

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Table 1. Characteristic of all selected studies in meta-analysis

Author (year)	Breed	No. of dairy calve/group	Duration length (day)	Zinc source	Dosage (mg Zn kg ⁻¹ DM)	Sex	Supplementation methods
Cheraghi Mashoof et al (2024) ⁶⁶	Holstein	10	77	Zn sulfate, Zn hydroxy chloride, Chelated Zn	20	Male & Female	Starter
Rajae-Sharifabadi et al (2024) ⁵¹	Holstein	10	70	Zn sulfate, Chelated Zn, Zn methionine	80	Female	Milk
Liu et al (2023a) ⁶⁴	Holstein	8	28	Zn proteinate, Zn oxide	80	Male & Female	Milk
Liu et al (2023b) ⁷⁰	Holstein	12	70	Zn proteinate, Zn oxide	40, 80, 120	Female	Milk
Wo et al (2022) Exp 1 ³⁸	Holstein	10	14	Zn proteinate	40, 80, 120	Female	Milk
Wo et al (2022) Exp 2 ³⁸	Holstein	12	28	Zn proteinate, Zn methionine	80	Female	Milk
Dabaghian et al (2023) ⁶⁹	Holstein	10	74	Zn oxide	80	Male & Female	Milk
Karamnejad et al (2022) ⁷¹	Holstein	7	49	Zn methionine	120	Female	Starter
Zaboli et al (2021) ⁷²	Holstein	6	70	Zn sulfate	30	Female	Milk
Ma et al (2020a) ⁶³	Holstein	6	14	Zn oxide, Zn methionine	80	Male	Milk
Ma et al (2020b) ²¹	Holstein	8	14	Zn methionine, Zn oxide	80	Male	Milk
Chang et al (2020) ¹⁸	Holstein	10	14	Zn oxide, Zn methionine	80	Female	Milk
Abdollahi et al (2020) ⁶	Holstein	10	70	Zn oxide	50	Female	Starter
Wei et al (2019) ⁵⁴	Holstein	8	14	Zn oxide	20, 40, 80, 120	Male & Female	Starter
Adab et al (2019) ⁷³	Holstein	24	80	Zn glycine	100	Female	Milk

Table 2. Data description (means and SD between studies)

Parameters	Unit	NC	Mean		SD	
			Control	Treatment	Control	Treatment
Starter intake	g/d	34	189.55	206.86	34.23	38.72
TDMI	g/d	66	1079.81	1118.15	343.42	382.30
ADG	g/d	70	554.75	630.38	181.26	208.22
BW	Kg	44	70.21	74.15	42.98	55.05
FCR	gFI/gA DG	53	2.11	1.94	0.38	0.40
Diarrhea incidence	%	34	26.86	15.60	7.67	7.83

BW= body weight, ADG=average daily gain, TDMI, total dry matter intake= FCR, feed conversion ratio, NC=number of comparisons, SD= standard division

Table 3. A summary of the statistical model and moderators for meta-analysis of performance traits and diarrhea incidence

Item	Estimate	SE	Z-value	p-value	95% CI	
					Lower	Upper
Starter intake						
Multilevel random effect model	0.29	0.15	-0.41	0.001	0.11	0.47
Moderators						
Duration	0.02	0.003	5.09	0.001	0.01	0.02
Dose	0.003	0.002	1.08	0.28	-0.002	0.008
Supplementation methods	-0.55	0.33	-1.14	0.03	-1.02	-0.04
Sex	-0.04	0.14	-0.28	0.77	-0.31	0.23
Zn source	-0.16	0.17	-0.98	0.32	-0.50	0.16
TDMI						
Multilevel random effect model	0.27	0.19	-0.43	0.01	0.14	0.43
Moderators						
Duration	0.01	0.002	3.71	0.001	0.005	0.02
Dose	0.002	0.003	0.74	0.45	-0.003	0.007
Supplementation methods	-0.27	0.21	-1.75	0.07	-0.78	0.03
Sex	-0.12	0.16	-0.73	0.46	-0.44	0.20
Zn source	0.15	0.14	0.99	0.37	-0.13	0.43
ADG						
Multilevel random effect model	0.68	0.18	1.15	0.01	0.53	0.82

Moderators						
Duration	0.006	0.002	2.29	0.02	0.009	0.01
Dose	-0.001	0.002	-0.48	0.63	-0.007	0.004
Supplementation methods	-0.41	0.23	-1.60	0.02	-1.17	-0.17
Sex	-0.38	0.22	-1.74	0.08	-0.82	0.05
Zn source	0.15	0.14	0.93	0.35	-0.15	0.42
BW						
Multilevel random effect model	0.42	0.23	1.01	0.001	0.25	0.59
Moderators						
Duration	0.005	0.003	1.48	0.14	-0.001	0.01
Dose	0.007	0.003	0.24	0.80	-0.005	0.006
Supplementation methods	-0.27	0.14	0.11	0.04	-0.67	-0.09
Sex	-0.14	0.31	-0.45	0.65	-0.75	-0.47
Zn source	-0.21	0.22	-0.93	0.35	-0.66	0.23
FCR						
Multilevel random effect model	-0.63	0.2	-1.17	0.001	-0.08	-0.47
Moderators						
Duration	-0.006	0.004	-1.64	0.01	-0.08	-0.47
Dose	-0.001	0.003	-0.57	0.56	-0.007	0.004
Supplementation methods	0.55	0.13	2.40	0.006	0.19	1.13
Sex	0.26	0.22	1.21	0.22	-0.16	0.69
Zn source	0.07	0.15	0.43	0.66	-0.23	0.36
Diarrhea incidence						
Multilevel random effect model	-0.95	0.62	-0.73	0.001	-1.17	-0.74
Moderators						
Duration	-0.002	0.008	-0.33	0.74	-0.02	0.01
Dose	0.005	0.003	0.17	0.86	-0.006	0.007
Supplementation methods	0.96	0.28	3.45	0.002	0.39	1.53
Sex	-0.19	0.24	-0.82	0.41	-0.65	0.27
Zn source	-0.04	0.22	-0.19	0.84	-0.46	0.38

BW=body weight, ADG=average daily gain, TDMI=total dry matter intake, FCR=feed conversion ratio, SE=standard error

Table 4. Subgroup analysis of the effect of zinc supplementation on performance traits diarrhea incidence of suckling calves

Variable	Covariates	Subgroup	SMD	95% CI		p-value
				Lower	Upper	
Starter intake	Dosage (mg Zn kg ⁻¹ DM)	≤40	0.12	-0.61	0.85	0.75
		>40≤80	0.24	0.05	0.44	0.01

TDMI (kg)	Duration (day)	>80	0.68	0.09	1.27	0.001
		≤28	0.05	-0.15	0.26	0.60
		>28	0.78	0.41	1.15	0.001
	Sex	Male	0.06	-0.41	0.52	0.82
		Female	0.49	0.20	0.78	0.001
		Male & Female	0.02	-0.29	0.33	0.89
	Supplementation methods	Milk	0.35	0.14	0.55	0.001
		Starter	0.12	-0.30	0.54	0.58
	Dosage (mg Zn kg ⁻¹ DM)	≤40	0.18	-0.14	0.50	0.27
		>40≤80	0.31	0.13	0.48	0.001
		>80	0.36	0.09	0.64	0.01
	Duration (day)	≤28	0.09	-0.09	0.27	0.30
		>28	0.41	0.21	0.61	0.001
	Sex	Male	0.47	0.25	0.67	0.001
		Female	0.04	-0.27	0.34	0.81
		Male & Female	0.13	-0.10	0.37	0.27
ADG	Supplementation methods	Milk	0.29	-0.13	0.44	0.001
		Starter	0.26	0.001	0.52	0.05
	Dosage (mg Zn kg ⁻¹ DM)	≤40	0.48	0.25	0.70	0.001
		>40≤80	0.98	0.82	1.17	0.001
		>80	0.35	0.03	0.67	0.01
	Duration (day)	≤28	0.81	0.61	1.01	0.001
		>28	0.57	0.38	0.76	0.001
	Sex	Male	0.73	0.50	0.95	0.001
		Female	1.04	0.70	1.37	0.001
		Male & Female	0.50	0.30	0.69	0.001
	Supplementation methods	Milk	0.85	0.65	1.04	0.001
		Starter	0.46	0.28	0.65	0.001
BW	Dosage (mg Zn kg ⁻¹ DM)	≤40	0.22	0.02	0.43	0.03
		>40≤80	0.72	0.45	0.99	0.001
		>80	0.43	0.04	0.81	0.03
	Duration (day)	≤28	0.41	0.06	0.77	0.02
		>28	0.42	0.23	0.61	0.001
	Sex	Male	0.71	0.45	0.96	0.02
		Female	0.01	-1.95	1.95	0.97
		Male & Female	0.13	-0.07	0.33	0.20
	Supplementation methods	Milk	0.73	0.44	1.03	0.001
		Starter	0.18	0.001	0.37	0.05
FCR	Dosage (mg Zn kg ⁻¹ DM)	≤40	-0.36	-0.57	-0.14	0.001

		>40≤80	-0.86	-1.09	-0.63	0.001
		>80	-0.93	-1.52	-0.34	0.001
	Duration (day)	≤28	-0.73	-0.95	-0.5	0.001
		>28	-0.52	-0.75	-0.28	0.001
	Sex	Male	-0.9	-1.37	-0.42	0.001
		Female	-0.97	-1.23	-0.72	0.001
		Male & Female	-0.39	-0.58	-0.2	0.001
	Supplementation methods	Milk	-0.96	-1.16	-0.75	0.001
		Starter	-0.27	-0.46	-0.08	0.01
Diarrhea incidence	Dosage (mg Zn kg ⁻¹ DM)	≤40	-0.97	-1.44	-0.49	0.001
		>40≤80	-0.98	-1.28	-0.69	0.001
		>80	-0.95	-1.32	-0.48	0.001
	Duration (day)	≤28	-0.93	-1.18	-0.68	0.001
		>28	-1.15	-1.58	-0.72	0.001
	Sex	Male	-0.83	-1.24	0.43	0.001
		Female	-0.79	-1.11	-0.47	0.001
		Male & Female	-1.28	-1.63	-0.93	0.001
	Supplementation methods	Milk	-1.02	-1.27	-0.77	0.01
		Starter	-0.61	-1.07	-0.15	0.01

BW=body weight, ADG=average daily gain, TDMI=total dry matter intake, FCR=feed conversion ratio, SMD=standardized mean difference

Table 5. Regression coefficients for the interaction between zinc source and dosage on performance traits and diarrhea incidence in suckling calves

Item	Coefficient	SE	p-value	95% CI	
				Lower	Upper
BW	0.001	0.004	0.77	-0.007	0.009
ADG	0.004	0.002	0.30	-0.004	0.01
TDMI	-0.005	0.004	0.19	-0.01	0.002
Starter intake	-0.001	0.007	0.88	-0.01	0.01
FCR	0.005	0.004	0.29	-0.004	0.01
Diarrhea incidence	-0.006	0.008	0.47	-0.02	0.01

BW=body weight, ADG=average daily gain, TDMI=total dry matter intake, FCR=feed conversion ratio, SE=standard error

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Table 6. Results of Egger's test for studied traits

Measured outcome	<i>p</i> -value
BW	0.37
ADG	0.001
TDMI	0.34
Starter intake	0.78
FCR	0.01
Diarrhea incidence	0.01

BW=body weight, ADG=average daily gain, TDMI=total dry matter intake, FCR=feed conversion ratio

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Table 7. Sensitivity analysis for performance traits diarrhea incidence in suckling calves

Item	Estimate	95% CI		<i>p</i> -value
		Lower	Upper	
Starter intake	0.29	0.11	0.48	0.001
TDMI	0.30	0.15	0.46	0.001
ADG	0.67	0.51	0.83	0.001
BW	0.43	0.24	0.61	0.001
FCR	-0.61	-0.80	-0.42	0.001
Diarrhea incidence	-1.05	-1.26	-0.84	0.001

BW=body weight, ADG=average daily gain, TDMI=total dry matter intake, FCR=feed conversion ratio

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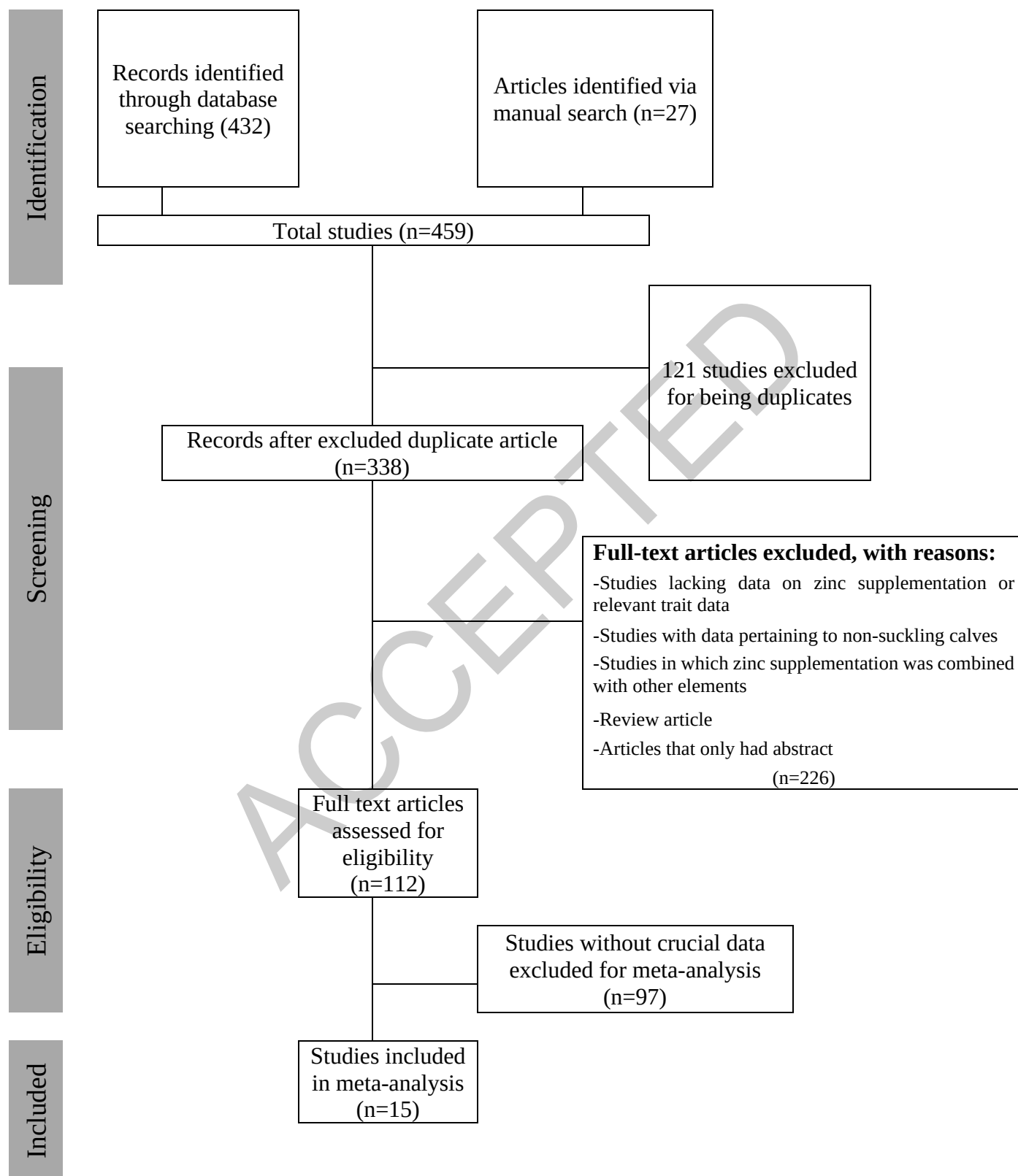


Fig 1. Flowchart of the systematic review (PRISMA) from the initial search to the selection of articles included in this meta-analysis

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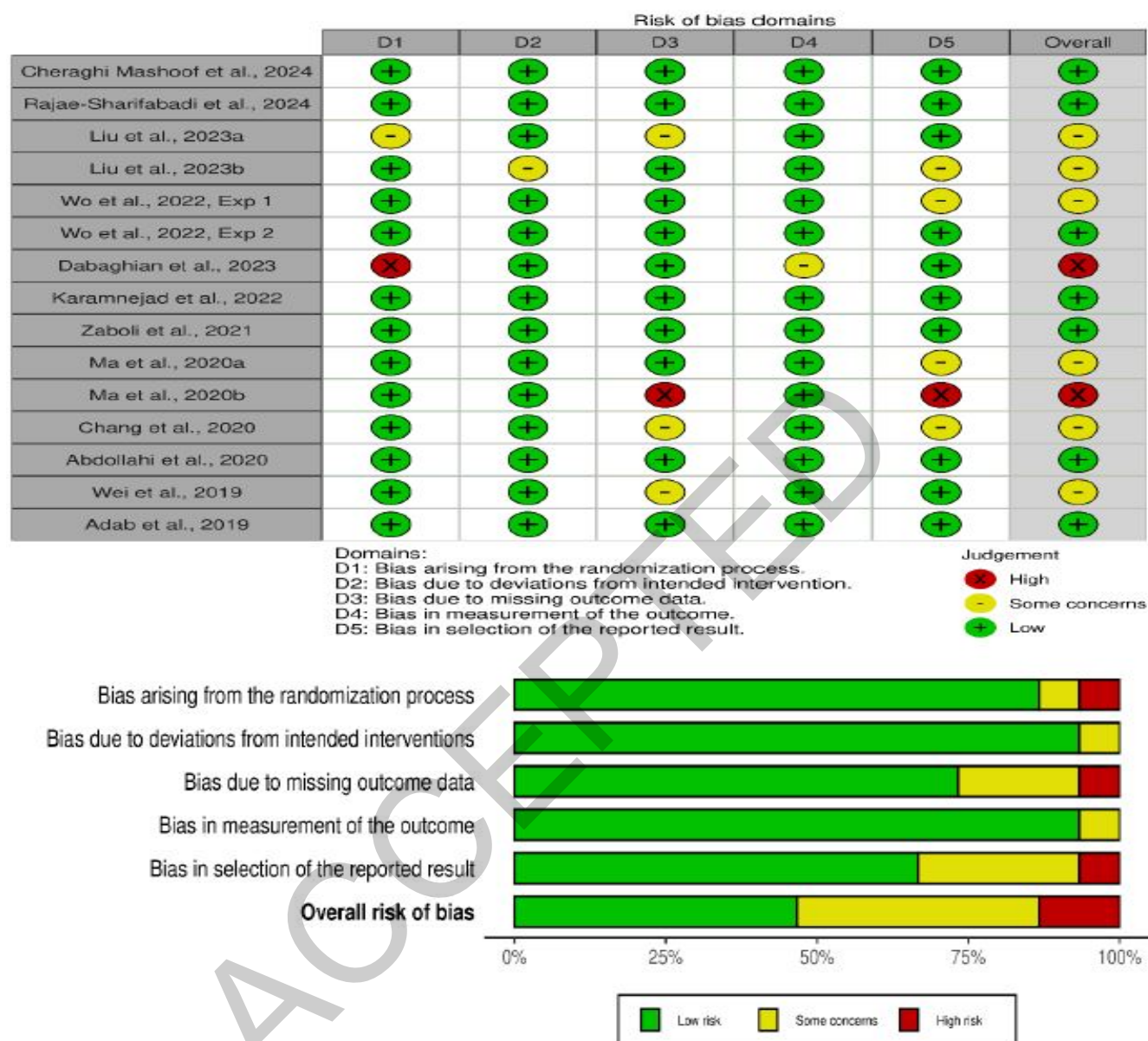
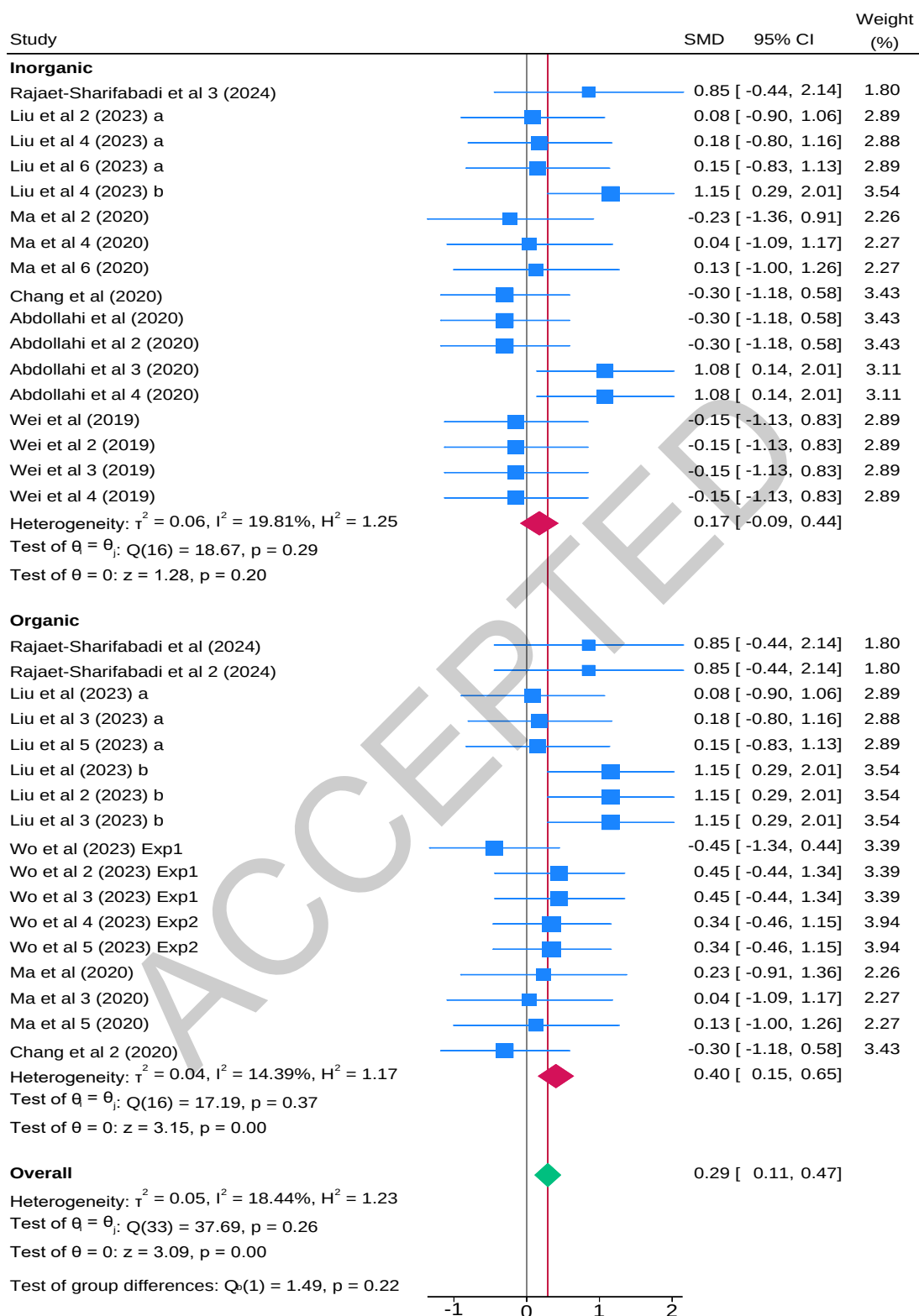
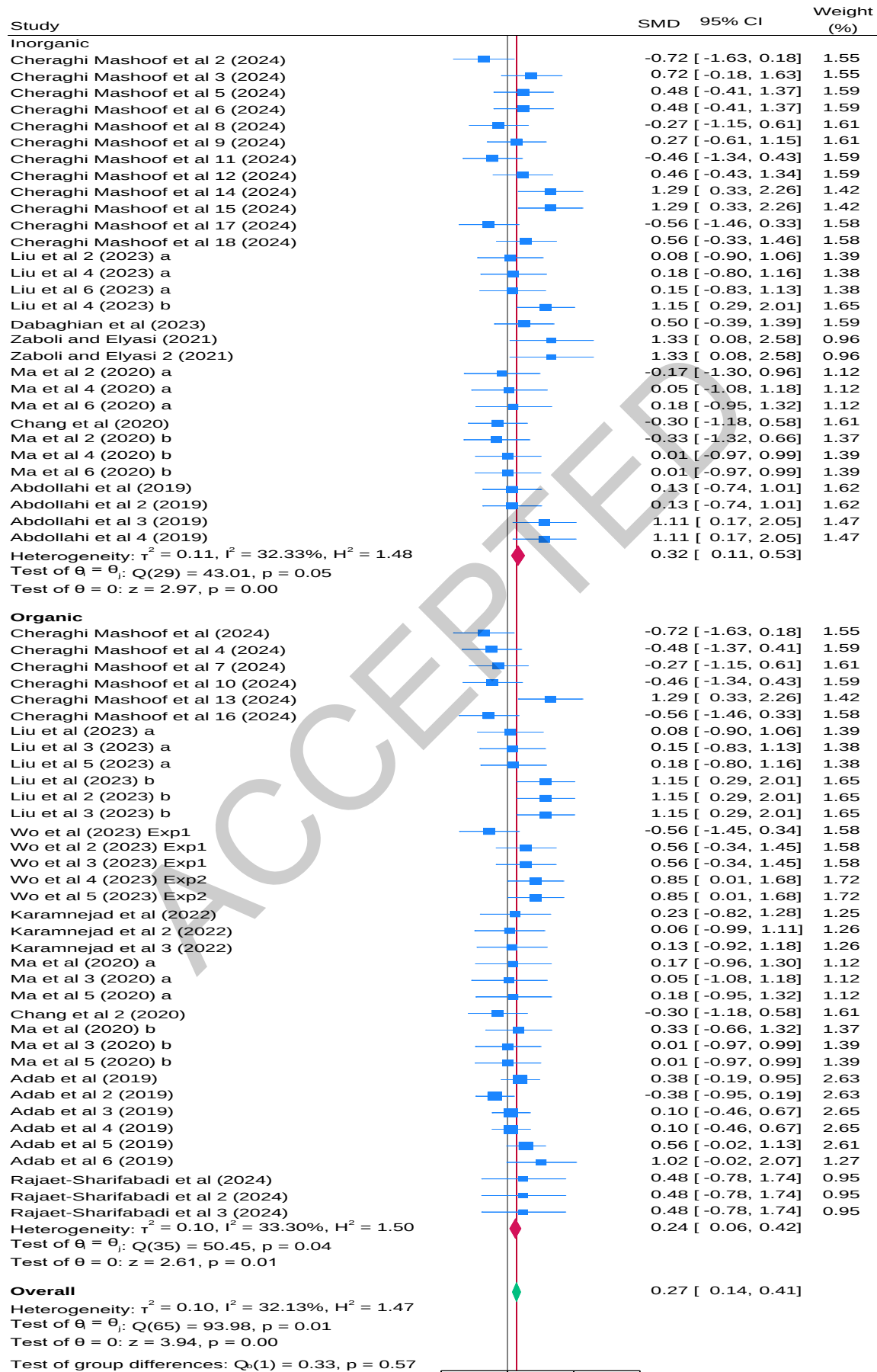


Fig. 2. Risk of bias graph and summary of study risk bias analysis



Multilevel RE meta-analysis

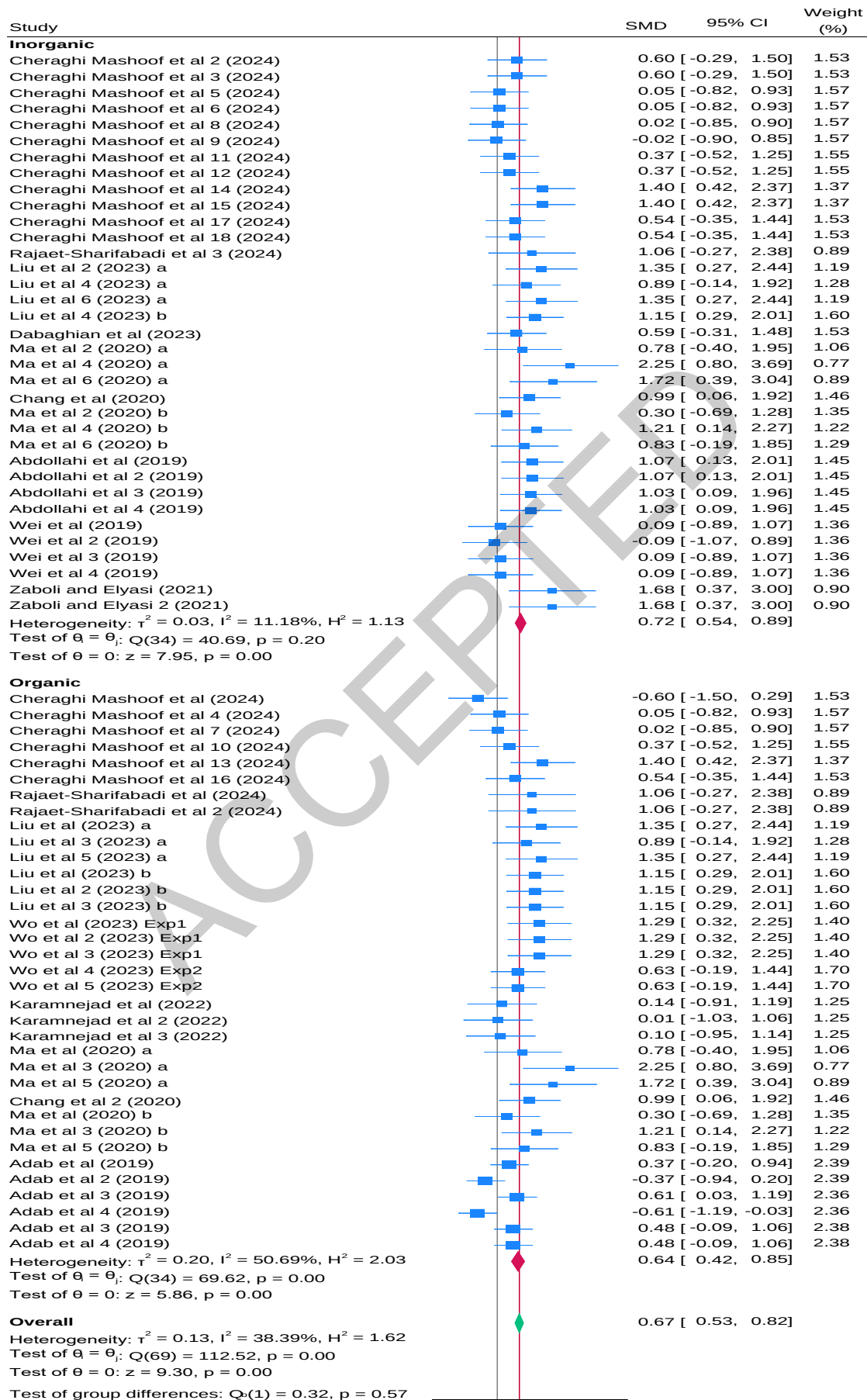
Fig 3. Means and forest plot of SMD with 95% CI for the random effect of zinc supplementation on starter intake of suckling calves



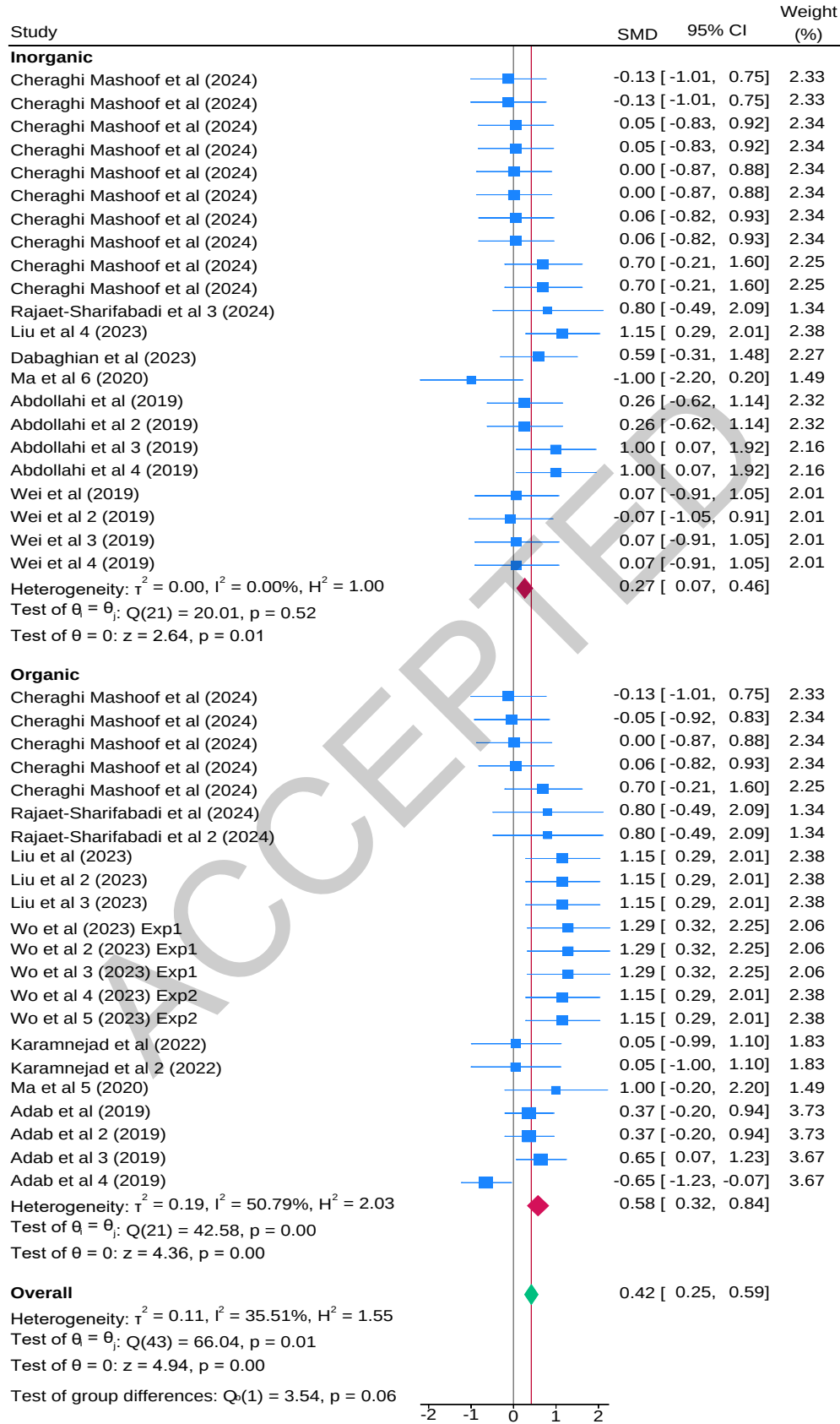
791 **Fig 4.** Means and forest plot of SMD with 95% CI for the random effect of zinc supplementation on the TDMI of suckling calves

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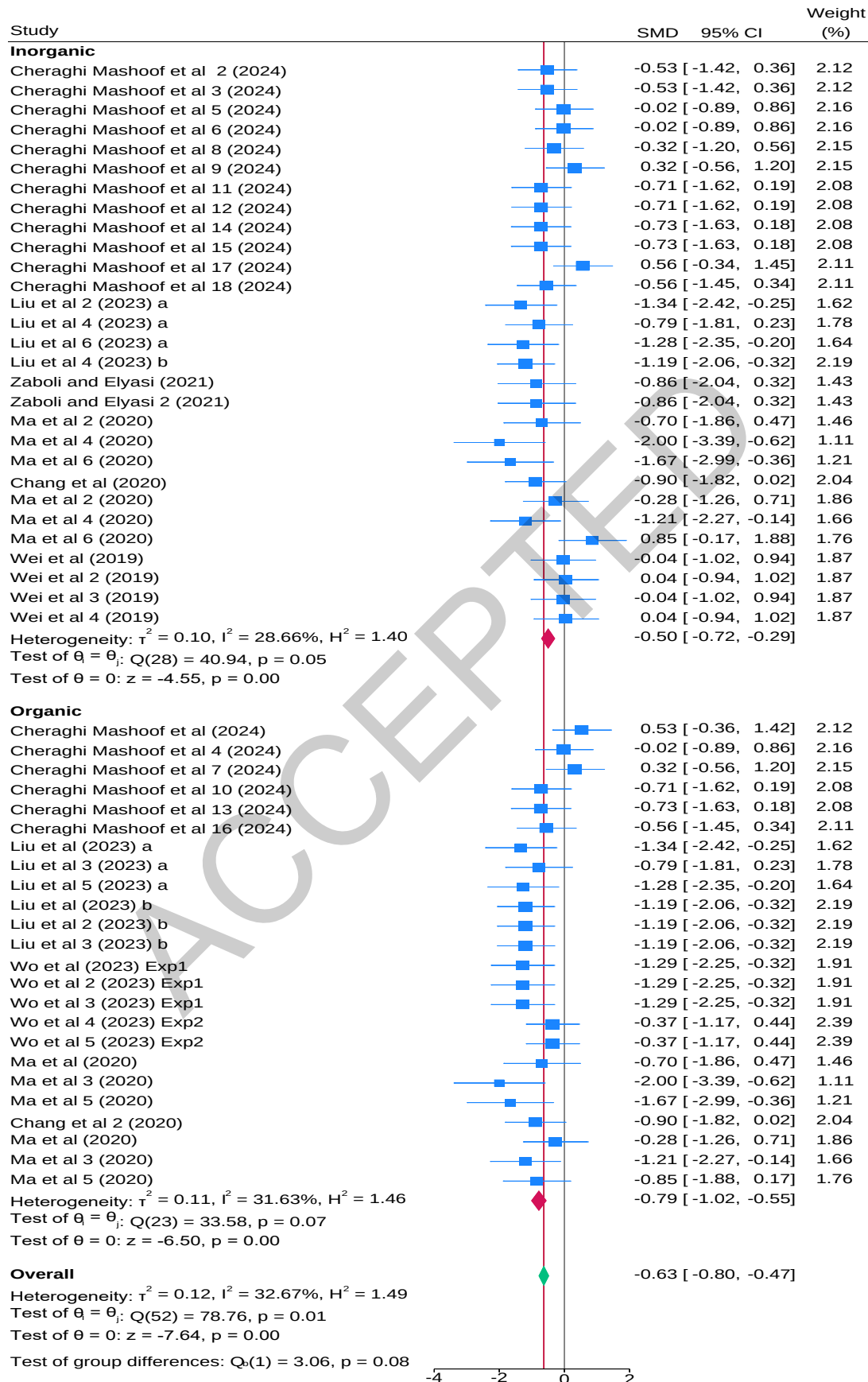
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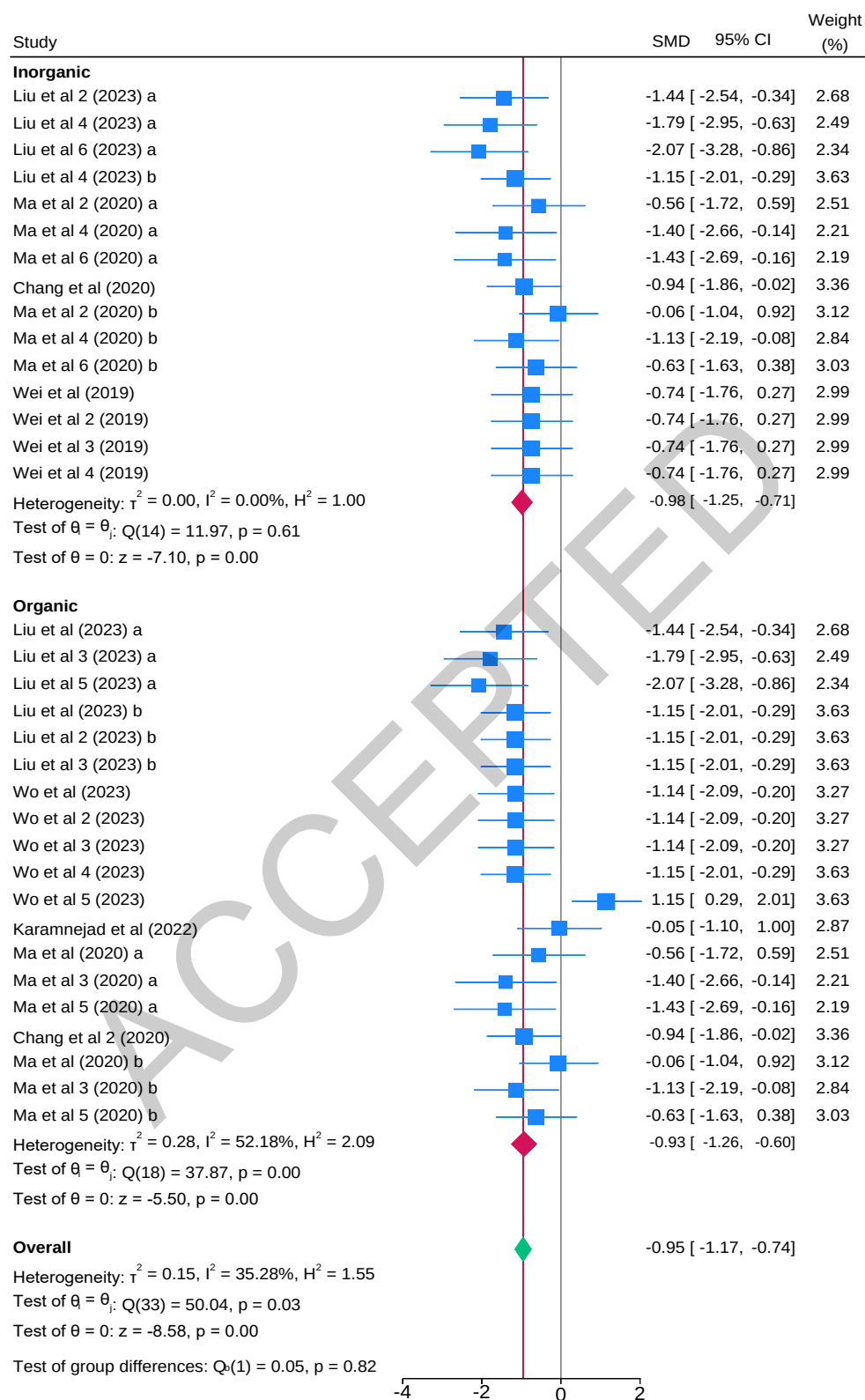


798 **Fig 7.** Means and forest plot of SMD with 95% CI for the random effect of zinc supplementation on the FCR of suckling calves

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Multilevel RE meta-analysis

Fig 8. Means and forest plot of SMD with 95% CI for the random effects of zinc supplementation on diarrhea incidence in suckling calves

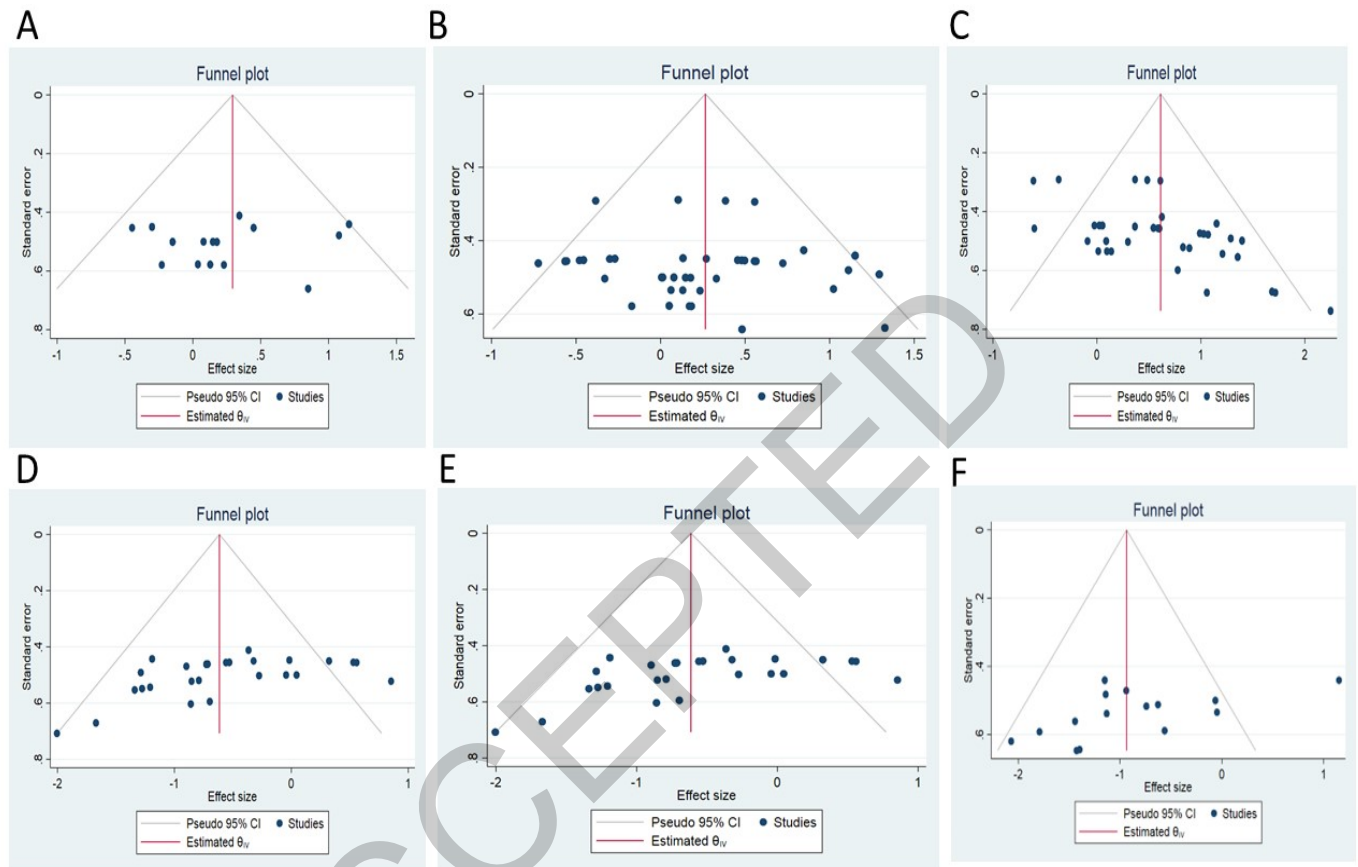


Fig 9. Funnel plots of the effects of zinc supplementation on performance traits and diarrhea incidence in suckling calves: (A) starter intake; (B) TDMI; (C) ADG; (D) BW; (E) FCR and (F) diarrhea incidence

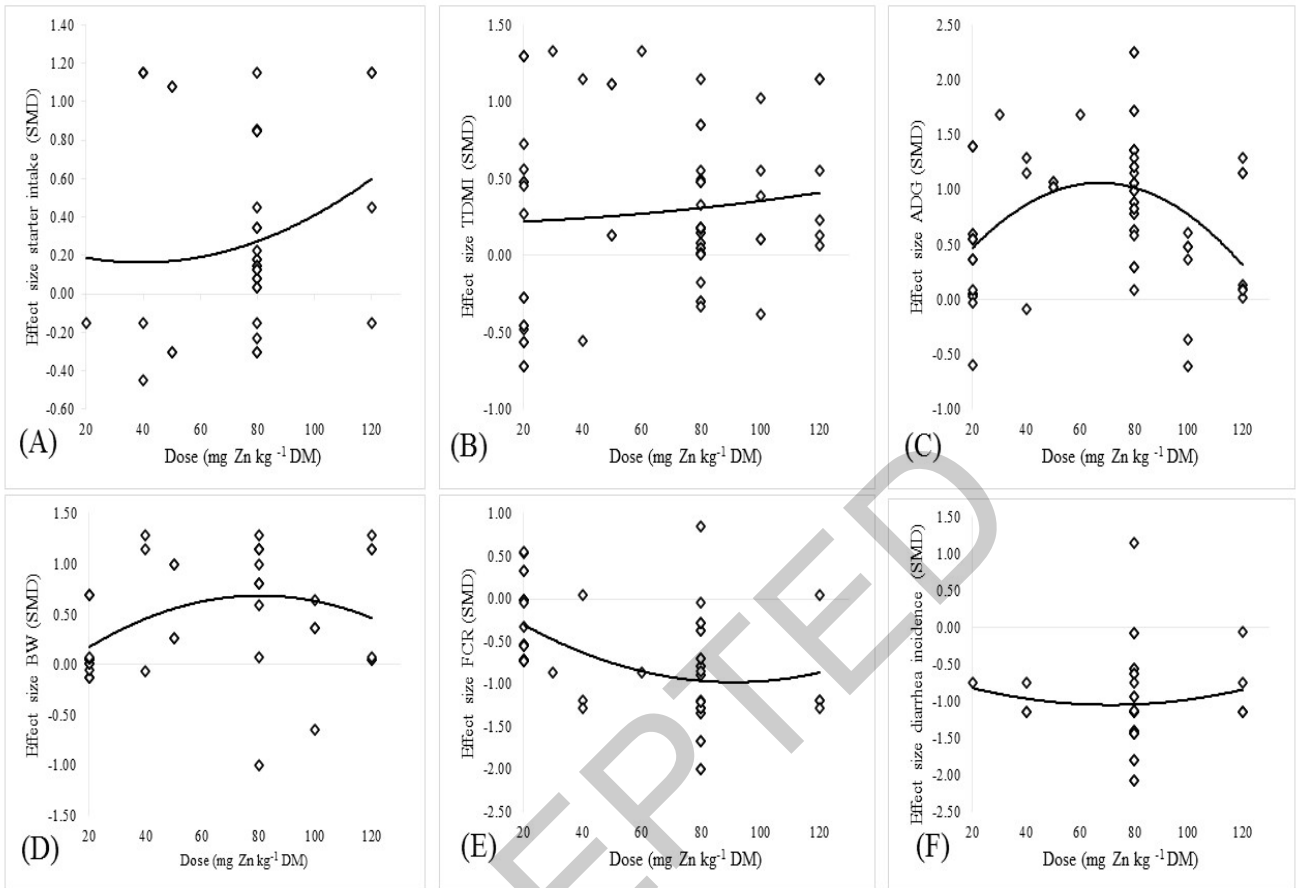


Fig 10. Relationship between zinc supplementation and starter intake (A), TDMI (B), ADG (C), BW (D), FCR (E), and diarrhea incidence (F)