Research Article

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Meta-analysis of the potential of dietary *Bacillus* spp. in improving growth performance traits in broiler chickens

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Abstract: *Bacillus* probiotics have been shown to possess several advantages over conventional probiotics, including the capacity to withstand heat during feed manufacturing processes and to be stored for a long time without losing viability, as well as the ability to survive and function in the acidic environment of the chicken gut. However, there are inconsistent results on the effect of *Bacillus* on growth performance of broiler chickens. The objective of this meta-analysis was to assess the effect of dietary *Bacillus* supplementation on feed intake (FI), feed conversion efficiency (FCE), and average daily gain (ADG) in broiler chickens. PubMed, Google Scholar, and Scopus databases were searched for studies that fed diets with and without *Bacillus* to broilers. Pooled estimation revealed that *Bacillus* supplementation improved FCE (standardised mean difference [SMD] = −0.33, 95% confidence interval (CI) −0.39 to −0.28) and increased ADG (SMD = 0.37 g/bird/day, 95% CI 0.28–0.46). In contrast, feed intake (SMD) = 0.03 g/bird/day, 95% CI −0.03 to 0.09) was not significantly different from controls. Subanalysis revealed that broilers fed with *Bacillus* at 0.1–0.5 × 10^6 colony-forming unit (cfu)/g had higher ADG than controls. There is evidence of significant heterogeneity (inconsistency index [I^2] = 80–93%) among the studies included in the meta-analysis. Meta-regression showed that studied moderators (i.e., *Bacillus* spp., duration of supplementation, and broiler strain) explained most of the effect. In conclusion, our results suggest that *Bacillus* supplementation at 0.1–0.5 × 10^6 cfu/g improved FCE and ADG in broiler chickens. However, it is recommended that more research be conducted to determine the *Bacillus* supplementation dose that optimises growth performance indices in broiler chickens.

Keywords: probiotics, *Bacillus*, broiler chickens, growth traits, meta-analysis

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1 Introduction

Chicken production has undergone many changes over the years, moving from free range system to an intensive system. Although intensive chicken production is economical, it is associated with increased stress in birds, leading to impaired immunity and productivity, thus necessitating the use of several growth promoters such as in-feed antibiotics and probiotics to improve chicken and livestock performance [1–4]. Antibiotics are added in animal feed at lower concentrations to enhance growth performance parameters in chickens [5]. However, the ban on the use of antibiotics in chicken rations in many parts of the world because of growing problem of antimicrobial resistance, together with the deposition of antibiotic residues in meat and eggs has led to the search for alternatives. Probiotics is one such alternative, which are live micro-organisms that have the potential to improve host health when given at the right doses [1–3]. Conventional probiotics used in broiler chicken production include *Lactobacillus* spp., *Saccharomyces* spp., *Enterococcus* spp., and *Bifidobacteria* [1,4,5]. However, the use of conventional probiotics in the poultry industry is still problematic due to their inability to withstand heat during pelleting, poor storage life, and their low viability in the harsh environment of chicken gut, all of which led to the search for better probiotics for use in broiler chicken production [6].

The use of *Bacillus* in the broiler chicken industry is on the increase as it has features that address some of the limitations of conventional probiotics. *Bacillus* is a gram-positive bacterium with the potential to form endospores.

Bacillus is moderate in minerals, amino acids, and vitamins [7,8], and has the ability to survive the low pH of the chicken gut [9]. Furthermore, *Bacillus* is stable and viable during feed processing, storage, and gut transit due to its endospore forming capability [8,10,11]. The probable mechanisms by which *Bacillus* spp. limit the proliferation of pathogens include competition for adhesion sites, production of organic acids leading to a reduction in gut pH, and maintenance of normal gut microbiota via competitive exclusion and antagonisms [12–14]. It may also achieve this by production of antimicrobial compounds, improvement in oxidative stability, modulation of immune systems, increase in digestive enzyme activity, and competition for nutrients [12–14].

Bacillus has been demonstrated to increase growth rate and efficiency of digestion in broilers by lowering gut oxygen concentrations [10], inhibiting bacterial metabolism, and increasing nutrient uptake in the small intestine [15]. On the other hand, influence of *Bacillus* on growth rate in broilers are not consistent. Some authors [16–18] found that *Bacillus* enhance growth performance in broilers, while others [19–21] state otherwise. This variation may be due to study design, broiler strain, inclusion level, and duration of *Bacillus* supplementation as reported by Ogbuewu et al. [22]. Currently, there is no study on the impact of *Bacillus* on the performance of broiler chickens using the outcomes of published studies.

One method for combining the results of published studies that assessed the same research questions is meta-analysis [22–24]. It is a statistical method used to resolving disagreements among studies and identifying research gaps and patterns that would not normally be visible in a single study [25,26]. In order to bridge the identified research gaps, the authors quantitatively pool and analyse the results of original investigations that evaluated the effect of *Bacillus* supplementation on growth performance of broiler chickens.

2 Materials and methods

2.1 Data source and search strategy

This meta-analysis followed the guidelines of the Preferred Reporting Items for Systematic Review and meta-analyses. PubMed, Google Scholar, and Scopus databases were searched for published studies that evaluated the impact of *Bacillus* supplementation on growth performance of broiler chickens. The reference list of retrieved studies was also searched for related articles. There was no date and language restriction in our systematic search since there is no published meta-analysis in this area in the literature. The search words were broiler chickens, *Bacillus*, feed intake, FCE, and ADG.

2.2 Eligibility criteria

One hundred and ninety-eight articles were retrieved in a systematic search performed in PubMed, Google Scholar, and Scopus databases and two additional studies were identified from the search performed on the reference list of the retrieved articles. Two hundred published articles were identified and 42 articles satisfied the eligibility conditions for the study as illustrated in Figure 1. To be added in the study, articles must have assessed at least one of the measured outcomes (feed intake (FI), feed conversion efficiency [FCE], or average daily gain [ADG]) in broiler chickens along with a measure of variance such as standard deviation (SD), standard error (SE), or p-value. In addition, *Bacillus* should be the only supplement added to the diet. The details of the 42 articles that met the inclusion criteria for the meta-analysis are shown in Table 1.

2.3 Data extraction and processing

Data on means of FI, FCE, and ADG for the control and treatment groups as well as their measures of variance from each of the 42 studies that met the inclusion criteria were extracted. In addition, information was extracted on the following modifiers: *Bacillus* spp. (*B. subtilis*, *B. coagulans*, *B. amyloliquefaciens*, *B. licheniformis,* and *B. cereus*), duration of supplementation (DOS) of *Bacillus* (1–21, 1–28, 1–35, 1–36, 1–41, 1–42, and 1–49 days), broiler strains (Ross, Cobb, Arbor Acres, Arian, and Hubbard), and supplementation level (SL) of *Bacillus* (0.1–0.5, 0.6–1.0, and >1.0 × 10^6 cfu/g) that we considered a priori to influence trial outcomes of the study for subgroup and meta-regression analyses, where it was provided. The supplementation dose level was categorised based on the level included in the individual studies used for the meta-analysis. When a trial reported SE instead of SD, SD was calculated using the equation ($SD = SE \times \sqrt{n}$) as reported by Higgins and Deeks [27], where "n = number of chickens." In studies with multiple comparisons, the control group was compared with each treatment group separately. A database of 42 articles that met the selection conditions for meta-analysis was created as shown in Table 1.

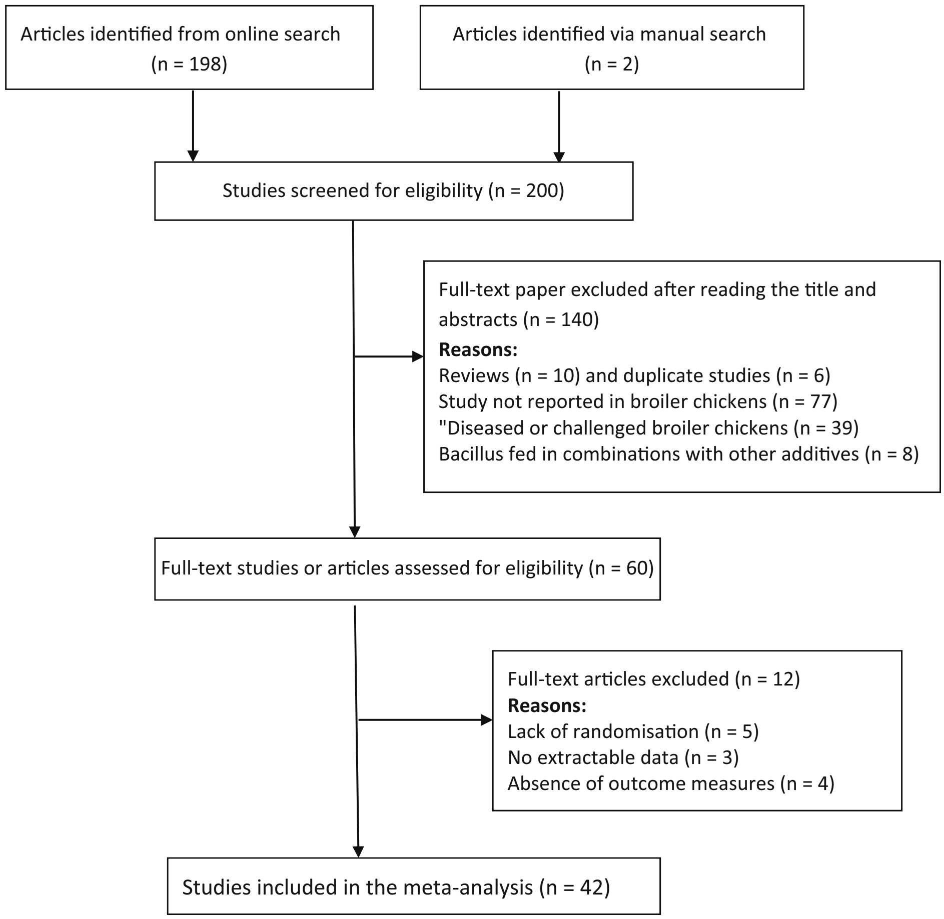

Figure 1: Flow diagram of the paper selection process used for the meta-analysis.

2.4 Statistical analysis

Results were combined using the standardised mean difference (SMD) for random-effects model and presented as 95% CI for each study outcome according to the method of Borenstein et al. [64]. Statistical analysis was performed on outcomes of interest using Open Meta-analyst for Ecology and Evolution (OpenMEE) software [65]. Articles were aggregated using inverse variance method [66]. Bar graphs of publication year were created in Microsoft Excel 2010. SMD was considered significant when the lower and upper CIs did not include zero [25]. SMD values of 0.2, 0.5, and 0.8 were considered as low, moderate, and large, respectively [67]. Subanalysis with fewer than three studies was not reported because of low statistical power. Chi-square (Q) test and the I^2 statistic were used to assess heterogeneity [68]. The I^2 values of 25, 50, and 75% indicate low, moderate, and substantial heterogeneity, respectively [69]. Meta-regression results were considered significant at 5% probability level [70]. Sensitivity analysis was performed using the method of Lean et al. [71] whereas publication bias was examined using funnel plots and Rosenberg's fail-safe number (Nfs). Nfs indicates the number of non-significant, unpublished (or missing) articles that will be required to reduce the overall statistically significant observed result to non-significance. However, according to Rosenberg [72], the results of a meta-analysis is deemed robust regardless of the presence of publication bias when Nfs is greater than "5(n) + 10," where n = number of studies included in the meta-analysis.

3 Results

3.1 Features of studies included in the meta-analysis

Studies which were review papers (n = 10), non-randomised studies (n = 5), and studies on diseased broilers (n = 39) were excluded as shown in Figure 1. Studies not

Table 1: Characteristics of studies included in the meta-analysis of effect of dietary *Bacillus* supplementation in broiler chickens

Ref.	Locations	Datasets	Explanatory variable				Response variables
			***Bacillus* spp.**	**SL ($\times 10^6$ cfu/g)**	**DOS (days)**	**Broiler strain**	
[16]	North Korea	5	*amyloliquefaciens*	0.1–2.0	1–35	Ross	FI, FCE, and ADG
[17]	Germany	2	*subtilis*	0.8	1–42	Cobb	FI and ADG
[19]	Korea	2	*subtilis*	1.0	1–28	Ross	FI, FCE, and ADG
[20]	USA	2	*subtilis*	3.0	1–28	Cobb	FI, FCE, and ADG
[21]	Brazil	3	*subtilis*	3.0–6.0	1–35/1–42	Ross	FI, FCE, and ADG
[28]	Korea	3	*subtilis*	0.3–0.6	1–35	Ross	FI, FCE, and ADG
[29]	Iran	2	*subtilis*	0.2	1–49	Ross	FI and FCE
[30]	China	2	*coagulans*	1.0	1–42	AA	FI, FCE, and ADG
[31]	Tunisia	2	*subtilis*	1.0	1–35	AA	FI
[32]	Korea	2	*subtilis*	1.0	1–35	Ross	FI, FCE, and ADG
[18]	Poland	2	*subtilis*	2.5	1–42	Ross	FI, FCE, and ADG
[33]	Brazil	2	*subtilis*	0.2	1–42	Cobb	FI, FCE, and ADG
[34]	China	2	*amyloliquefaciens*	0.2	1–35	Cobb	FCE and ADG
[35]	Poland	2	*licheniformis*	0.5	1–36	Ross	FI, FCE, and ADG
[36]	Indonesia	2	*subtilis*	0.2	1–56	—	FI, FCE, and ADG
[37]	Australia	2	*amyloliquefaciens*	1.0	1–35	Ross	FI, FCE, and ADG
[38]	Denmark	2	*subtilis*	1.0	1–42	Cobb	FI, FCE, and ADG
[39]	China	5	*subtilis*	0.2–0.5	1–42	AA	FI, FCE, and ADG
[40]	USA	2	*subtilis*	0.5	1–42	Cobb	FI, FCE, and ADG
[41]	USA	2	*subtilis*	0.3	1–42	—	FCE
[42]	USA	2	*subtilis*	3.4	1–41	—	FI and FCE
[43]	Korea	2	*subtilis*	0.1	1–35	AA	FI, FCE, and ADG
[44]	China	5	*subtilis*	0.1–0.25	1–42	AA	FI, FCE, and ADG
[45]	Iran	2	*subtilis*	0.5	1–42	Arian	FI and FCE
[46]	Jordan	2	*subtilis*	1.0	1–35	Hubbard	FI, FCE, and ADG
[47]	Italy	2	coagulans	0.25	1–49	Ross	FI and ADG
[48]	China	3	*licheniformis*	1.0–2.0	1–42	—	ADG
[49]	Australia	4	coagulans	0.1–0.25	1–42	AA	FI, FCE, and ADG
[50]	Korea	4	*subtilis*	0.15–0.45	1–35	Ross	FI, FCE, and ADG
[51]	Indonesia	3	*subtilis*	1.0–2.0	1–42	—	FI and FCE
[52]	Malaysia	2	*subtilis*	1.0	1–28	AA	FCE and ADG
[53]	China	3	*subtilis*	0.4	1–21	AA	FI, FCE, and ADG
[54]	Hungary	2	*subtilis*	0.5	1–42	—	FI and FCE
[55]	China	5	*subtilis*	0.2–0.5	1–21	AA	FI and FCE
[56]	Denmark	2	*subtilis*	0.5	1–42	Ross	FI, FCE, and ADG
[57]	India	2	*subtilis*	0.4	1–35	Cobb	FI
[58]	Taiwan	3	*licheniformis*	1.0 – 3.0	1–35	Ross	FI, FCE, and ADG
[59]	USA	2	*subtilis*	0.5	1–42	Ross	FI, FCE, and ADG
[60]	Singapore	2	*subtilis*	1.0	1–21/1–42	Ross	FI, FCE, and ADG
[61]	China	2	*subtilis*	1.0	1–21/1–42	AA	FI, FCE, and ADG
[62]	China	3	*amyloliquefaciens*	3.0–6.0	1–21/1–42	AA	FI, FCE, and ADG
[63]	China	2	*	1.0	1–21/1–42	Ross	FI, FCE, and ADG

**subtilis*, *licheniformis* and *cereus*; AA – Arbor acres; DOS – duration of study; FI – feed intake; FCE – feed conversion efficiency; ADG – average daily gain.

conducted in broiler chickens ($n = 77$) and trials that had no extractable data ($n = 3$) were discarded. Studies were also removed if they fed *Bacillus* in combination with other growth promoters ($n = 8$) and did not report any outcome of interest ($n = 7$). The characteristics of studies included in the meta-analysis as presented in Table 1 revealed that studies used for the meta-analysis span for 25 years (1995–2020) with 88% of the articles published between 2011 and 2020. In addition, broiler chickens utilised for the meta-analysis were aged between 1 and 49 days. *Bacillus* supplementation doses were ranged from 0.1 to 6.0×10^6 cfu/g feed (Table 1). Thirty-eight studies were used to evaluate the effect of *Bacillus* on FI, whereas, 37 and 33 trials were included to assess the effect of dietary *Bacillus* supplementation on FCE and ADG, respectively. The spatial distribution of studies by country revealed that studies used for this analysis were conducted in 18 countries (Figure 2), with China having the highest number followed by North Korea and USA (Figure 2).

3.2 Probiotic effect

The pooled effect size of 96 datasets, with 29,940 broiler chickens (20,241 for treatment group and 9,699 for control group) revealed that *Bacillus* had no effect on FI (SMD = 0.03 g/bird/day, 95% CI −0.03 to0.09; Figure 3). In contrast, the meta-analysis of 95 datasets, with 17,887 chickens (12,113 for treatment group and 5,774 for control group) suggested that dietary *Bacillus* supplementation significantly improved FCE in comparison with controls (SMD = −0.33, 95% CI −0.39 to −0.28; Figure 4). The analysis of 89 datasets with 18,147 broiler chickens (12,525 and 5,622 for *Bacillus* and control groups, respectively) significantly increased ADG (SMD = 0.37 g/bird/day, 95% CI 0.28–0.46; Figure 5) compared to controls. The magnitude of effect estimate was higher in ADG (0.37) than in FCE (0.33) in the present meta-analysis.

3.3 Stratification analysis

Subanalysis of the effect of studied moderators on FI, FCE, and ADG in broiler chickens on dietary *Bacillus* are presented in Tables 2–4. Results indicate that Cobb strain fed with *Bacillus* supplemented diets at 0.6–1.0 cfu/g (moderate dose) and > 1.0 × 10^6 cfu/g (high dose) for 28 days had significantly reduced FI compared to controls (SMD = −0.09, 95% CI −0.17 to −0.02). Cobb strain fed with *Bacillus* supplemented diets at moderate and high doses for 28 days had significantly reduced feed intake compared to controls. In contrast, Arbor Acres and Ross strains fed with *Bacillus* supplemented diets had similar FI with the controls. Similarly, Arbor Acres and Ross strains fed with *Bacillus* supplemented diets for 1–21, 1–35, 1–42, and 1–49 day had similar FI with the controls. There was no effect of *Bacillus* spp. on FI in broiler chickens. In contrast, Ross, Arbor Acres, and Cobb strains fed with *B. subtilis*, *B. amyloliquefaciens*, *B. coagulans*, and *B. licheniformis* at lower (0.1–0.5 × 10^6 cfu/g), moderate, and higher doses for 1–21 days, 1–28 days, 1–35 days, and 1–42 day had better FCE than controls (Table 3). Broilers fed with *B. subtilis* differed significantly from those offered with *B. coagulans*, but similar to those given *B. amyloliquefaciens*, and *B. licheniformis*. Ross, Arbor Acres, and Cobb strains fed with *B. subtilis*, *B. amyloliquefaciens*, *B. coagulans*, and *B. licheniformis* at 0.1 – 0.5 × 10^6 cfu/g for 1–35 days and 1–42 days had higher ADG than controls. In converse, broilers fed with moderate doses of *Bacillus* spp. for 1–28 days had comparable FCE with controls.

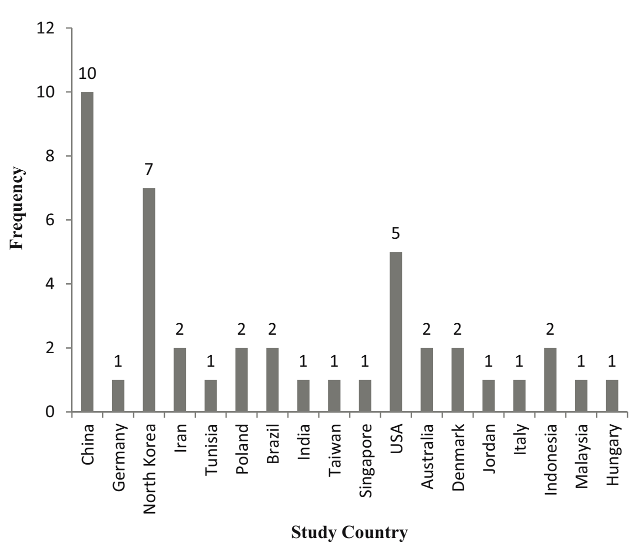

Figure 2: Plots of number of studies from each country.

3.4 Analysis of heterogeneity, meta-regression, and publication bias

Substantial heterogeneity (I^2 = 79.83–92.84%) was observed among the studies used for the analysis (Figures 3–5). In addition, results of subanalysis as shown in Tables 2–4 found that studied moderators did not eliminate the problems of substantial heterogeneity. Table 5 shows that *Bacillus* spp. and supplementation dose were significant predictors of the effect of *Bacillus* on FI in broiler chickens and accounted for approximately 39% of the sources of heterogeneity. Broiler strain and DOS explained about 26% of the factors that led to the inconsistent results among studies on the effect of *Bacillus* on FCE. 12% of the sources of heterogeneity among investigators on the effect of *Bacillus* supplementation on ADG were explained by broiler strain and DOS. Visual examination of the funnel plots as displayed in Figure 6a–c revealed the presence of publication bias among the trials included in the meta-analysis to evaluate the impact of *Bacillus* supplementation on growth performance indices of broiler chickens. The funnel plots were asymmetrical. However,

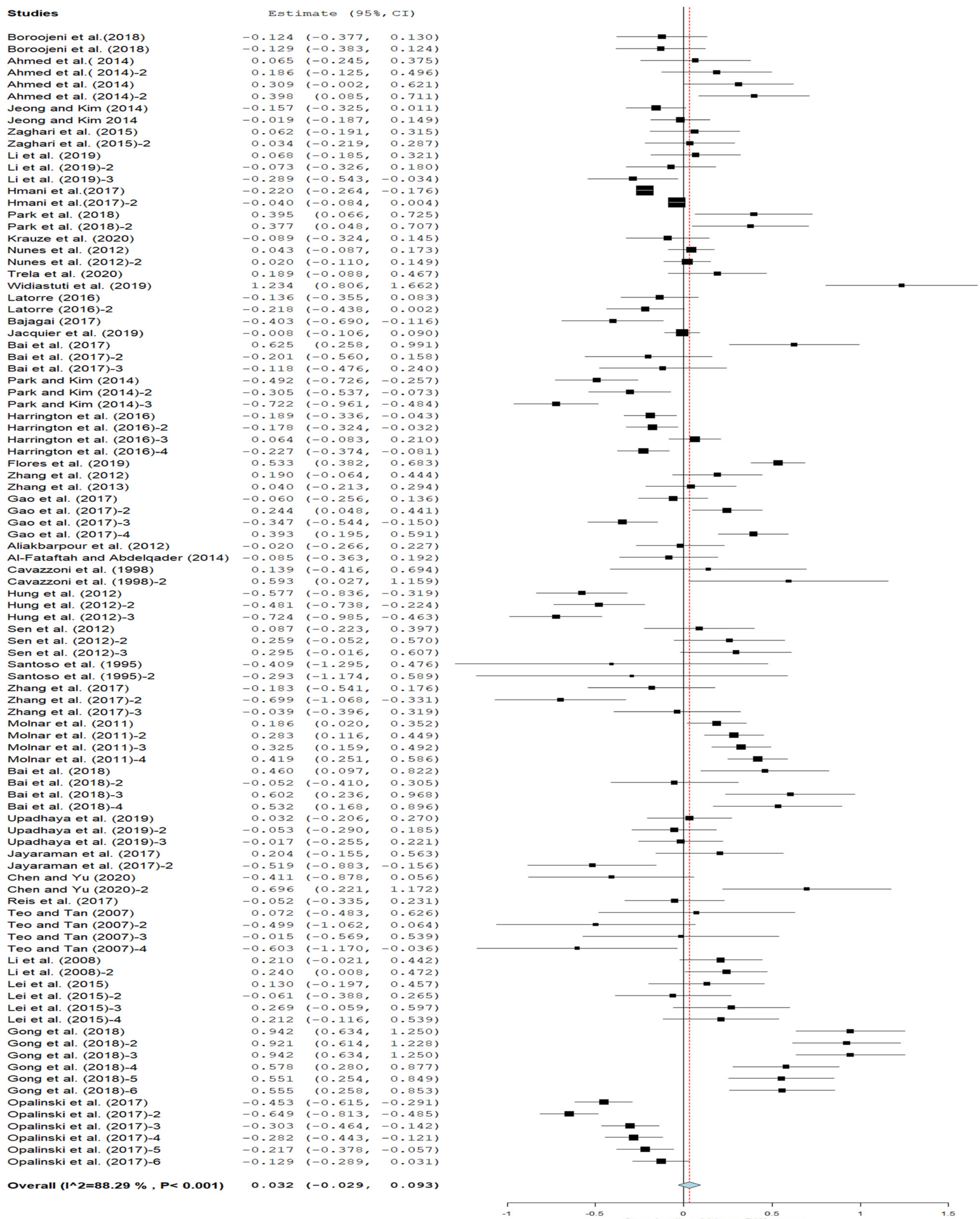

Figure 3: Influence of *Bacillus* supplementation on feed intake in broiler chickens. Pooled estimation (SMD) = 0 (thick line) suggests no effect, SMD > 0 suggests an increase in variables of interest over the controls, and SMD < 0 denotes a decline in variables of interest over the controls. The dotted line with a diamond denotes the cumulative effect size across all studies used for the meta-analysis.

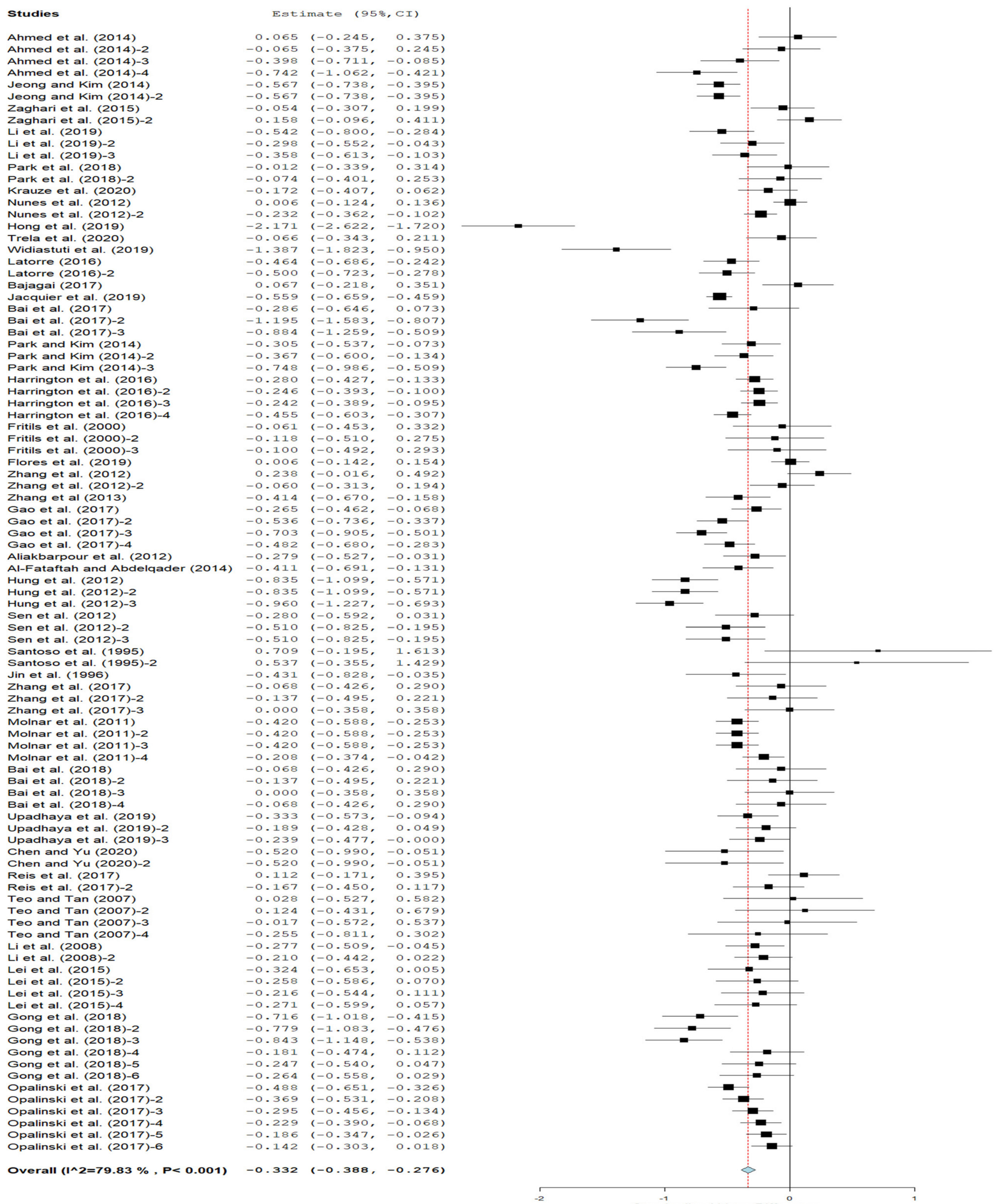

Figure 4: Effect of *Bacillus* supplementation on FCE in broiler chickens. Pooled estimation (SMD) = 0 (thick line) suggests no effect, SMD > 0 suggests an increase in variables of interest over the controls and SMD < 0 denotes a decline in variables of interest over the controls. The dotted line with a diamond denotes the cumulative effect size across all studies used for the meta-analysis.

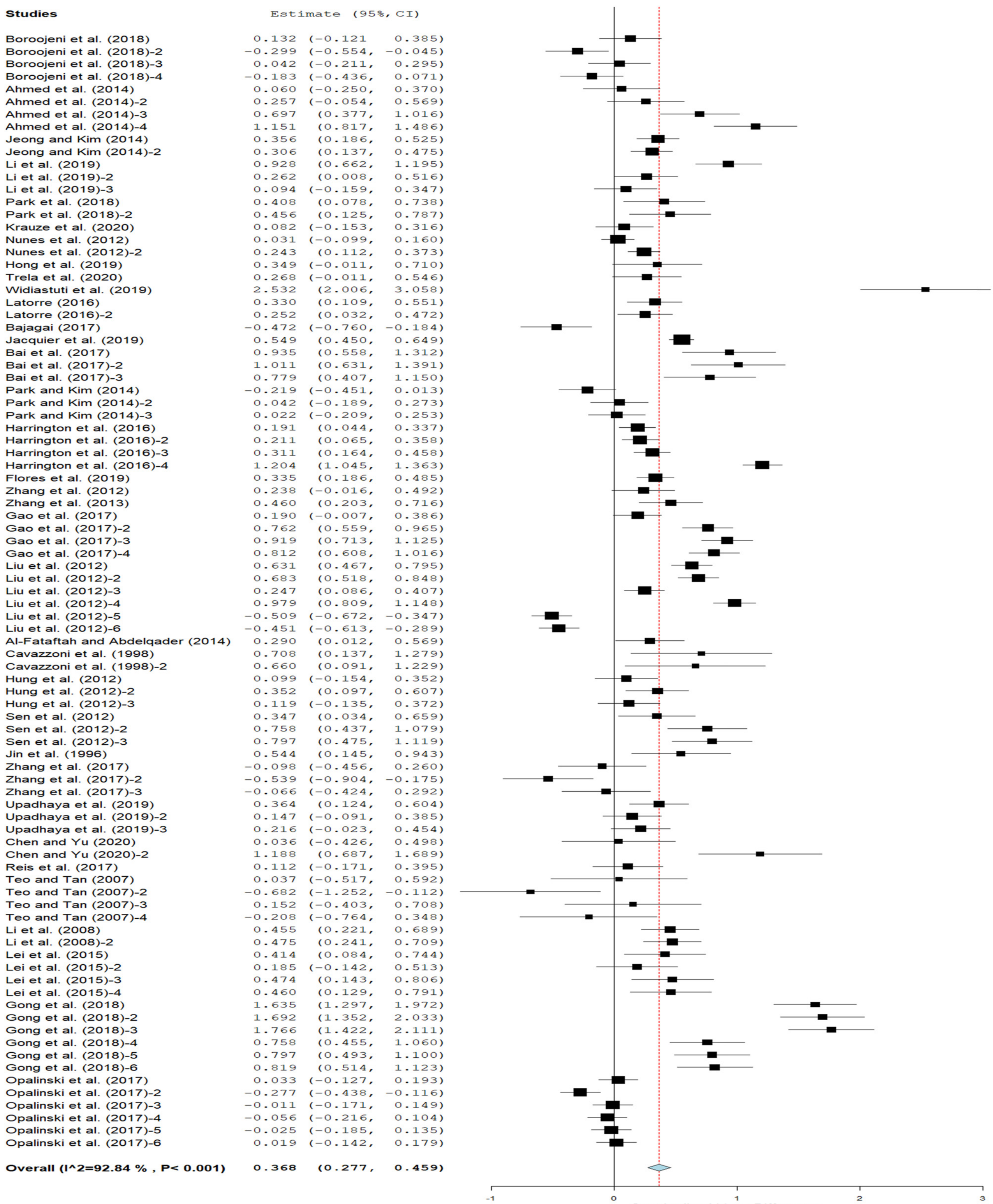

Figure 5: Effect of *Bacillus* supplementation on ADG in broiler chickens. Pooled estimation (SMD) = 0 (thick line) suggests no effect, SMD > 0 suggests an increase in variables of interest over the controls and SMD < 0 denotes a decline in variables of interest over the controls. The dotted line with a diamond denotes the cumulative effect size across all studies used for the meta-analysis.

Table 2: Subgroup analysis of the effect of *Bacillus* probiotics on feed intake of broiler chickens

Subgroups	Model results			Heterogeneity	
	SMD (95% CI)	SE	*P* < 0.05	I^2 test (%)	*P* < 0.05
Broiler strain					
Cobb	−0.09 (−0.17 to −0.02)	0.04	0.017	58.79	0.004
Ross	0.06 (−0.06 to 0.18)	0.06	0.351	89.14	<0.001
Arbor acres	−0.01 (−0.10 to 0.08)	0.05	0.850	86.65	<0.001
Bacillus spp.					
B. subtilis	0.00 (−0.06 to 0.06)	0.03	0.992	87.79	<0.001
B. amyloliquefaciens	0.12 (−0.05 to 0.28)	0.08	0.159	59.43	0.001
B. coagulans	−0.22 (−0.56 to 0.13)	0.18	0.218	86.09	<0.001
B. licheniformis	0.40 (−0.02 to 0.81)	0.21	0.051	85.16	<0.001
SL (10^6 cfu/g)					
0.1–0.5	0.03 (−0.07 to 0.13)	0.05	0.520	85	<0.001
0.6–1.0	0.19 (0.06–0.32)	0.07	0.004	48	0.032
>1.0	0.19 (0.07–0.31)	0.06	0.002	0	0.452
DOS (days)					
1–21	0.23 (−0.02 to 0.49)	0.13	0.070	87.39	<0.001
1–28	−0.37 (−0.58 to −0.17)	0.10	<0.001	74.85	0.003
1–35	0.01 (−0.08 to 0.10)	0.05	0.767	82.92	<0.001
1–42	−0.03 (−0.11 to 0.06)	0.04	0.560	86.90	<0.001
1–49	0.11 (−0.07 to 0.28)	0.09	0.231	8.44	0.351

SMD – standardised mean difference; SE – standard error; I^2 – inconsistency index; cfu – colony forming unit; SL – supplementation level; DOS – duration of supplementation.

Table 3: Subgroup analysis of the effect of *Bacillus* probiotics on FCE of broiler chickens

Subgroups	Model results			Heterogeneity	
	SMD (95% CI)	SE	*P* <0.05	I^2 test (%)	*P* <0.05
Broiler strain					
Cobb	−0.45 (−0.63 to −0.27)	0.09	<0.001	92.56	<0.001
Ross	−0.29 (−0.37 to −0.22)	0.04	<0.001	90.90	<0.001
Arbor Acres	−0.37 (−0.48 to −0.26)	0.06	<0.001	78.72	<0.001
Bacillus spp.					
B. subtilis	−0.29 (−0.35 to −0.24)	0.03	<0.001	75.58	<0.001
B. amyloliquefaciens	−0.41 (−0.74 to −0.09)	0.16	0.012	89.75	<0.001
B. coagulans	−0.79 (−0.97 to −0.62)	0.09	<0.001	43.77	0.149
B. licheniformis	−0.41 (−0.69 to −0.13)	0.14	0.004	69.44	0.011
SL (10^6 cfu/g)					
0.1–0.5	−0.06 (−0.45 to −0.27)	0.05	<0.001	84	<0.001
0.6–1.0	−0.30 (−0.40 to −0.19)	0.05	<0.001	20	0.247
>1.0	−0.28 (−0.49 to −0.08)	0.11	0.008	56	0.025
DOS (days)					
1–21	−0.26 (−0.42 to −0.10)	0.08	0.001	68.64	<0.001
1–28	−0.47 (−0.60 to −0.34)	0.07	<0.001	37.36	0.157
1–35	−0.36 (−0.51 to −0.21)	0.08	<0.001	85.46	<0.001
1–42	−0.34 (−0.41 to −0.28)	0.03	<0.001	76.76	<0.001

SMD – standardised mean difference; SE – standard error; I^2 – inconsistency index; cfu – colony forming unit; SL – supplementation level; DOS – duration of supplementation.

this is not a problem as the Rosenberg's Nfs for the database was 593 (FI), 18,298 (FCE), and 14,592 (ADG) which were 3, 38, and 32 folds above the threshold of 200 (5 × 38 + 10), 195 (5 × 37 + 10), and 175 (5 × 33 + 10) needed to proclaim the mean effect size significant, despite the possibility of publication bias [72].

Table 4: Subgroup analysis of the effect of *Bacillus* probiotics on ADG of broiler chickens

Subgroups	Model results			Heterogeneity	
	SMD (95% CI)	SE	$P < 0.05$	I^2 test (%)	$P < 0.05$
Broiler strain					
Cobb	0.25 (0.06–0.43)	0.09	0.009	93.81	<0.001
Ross	0.36 (0.22–0.51)	0.07	<0.001	91.68	<0.001
Arbor acres	0.41 (0.28–0.55)	0.07	<0.001	84.31	<0.001
Bacillus spp.					
B. subtilis	0.31 (0.21–0.41)	0.05	<0.001	91.44	<0.001
B. amyloliquefaciens	0.35 (0.08–0.63)	0.14	0.011	85.80	<0.001
B. coagulans	0.45 (0.15–0.75)	0.16	0.004	81.70	<0.001
B. licheniformis	0.45 (0.11–0.88)	0.20	0.011	97.25	<0.001
SL (10^6 cfu/g)					
0.1–0.5	0.44 (0.22– 0.66)	0.11	<0.001	89	<0.001
0.6–1.0	0.26 (−0.08 to 0.61)	0.18	0.135	91	<0.001
DOS (days)					
1–21	0.45 (−0.05 to 0.94)	0.25	0.075	95.38	<0.001
1–28	0.14 (−0.05 to 0.33)	0.10	0.153	73.04	<0.001
1–35	0.36 (0.21–0.52)	0.08	<0.001	85.05	0.002
1–42	0.33 (0.21– 0.45)	0.06	<0.001	93.94	<0.001

SMD – standardised mean difference; SE – standard error; I^2 – inconsistency index; cfu – colony forming unit; SL – supplementation level; DOS – duration of supplementation.

Table 5: Meta-regression of the moderator variables

Parameter	Moderators	Q_M	df	$P < 0.05$	R^2 (%)
FI	*Bacillus* spp.	18.00	4	0.001	17.19
	Dosage	2.75	2	0.253	4.00
	Broiler strain	2.13	4	0.711	0.00
	Duration of study	27.90	7	<0.001	21.67
FCE	*Bacillus* spp.	2.77	4	0.597	0.00
	Dosage	0.74	2	0.690	0.00
	Broiler strain	13.20	4	0.010	12.93
	Duration of study	17.60	7	0.014	12.68
ADG	*Bacillus* spp.	8.55	4	0.073	5.44
	Dosage	0.70	2	0.705	0.00
	Broiler strain	1.28	3	0.733	0.00
	Duration of study	19.90	7	0.006	12.29

R^2 – amount of heterogeneity accounted for; df – degree of freedom; Q_M – coefficient of moderators; Q_M – was considered significant at $P < 0.05$.

4 Discussion

4.1 Probiotic effect of *Bacillus* spp.

The results of this meta-analysis demonstrated the beneficial effect of dietary *Bacillus* supplementation on growth performance in broiler chickens. This is consistent with the findings of other authors that found beneficial effect of *Bacillus* supplementation on FCE and ADG in broiler chickens [3,17,18]. These findings also support Frizzo et al. [1] and Hu et al. [73], who reported a positive association between growth performance and probiotics in animals other than broiler chickens. Our meta-analysis results also revealed that broiler chickens fed with *Bacillus* supplemented diets gained weights at comparable FI with the controls. The mechanisms by which *Bacillus* improved growth performance in broiler chickens are not clear. However, the improved FCE and ADG obtained in broilers fed with diets supplemented with *Bacillus* in the present meta-analysis could be credited to the capability of *Bacillus* to limit proliferation of pathogens by competitive exclusion and antagonism and to improve immune systems of the host [9,13,14]. *Bacillus* has been reported to enhance digestive activity which can improve nutrient digestibility in broiler chickens [17,30,32].

4.2 Analysis of moderators

4.2.1 DOS

DOS was a limiting factor in the present meta-analysis. The effect of *Bacillus* on FI was found in studies that fed *Bacillus* for 1–28 days. FCE was enhanced in broilers fed with *Bacillus* for 1–21, 1–28, 1–35, and 1–42 days. Improvement in FCE in broilers fed with *Bacillus* for 1–21 days corroborated the results of Gaggìa et al. [74],

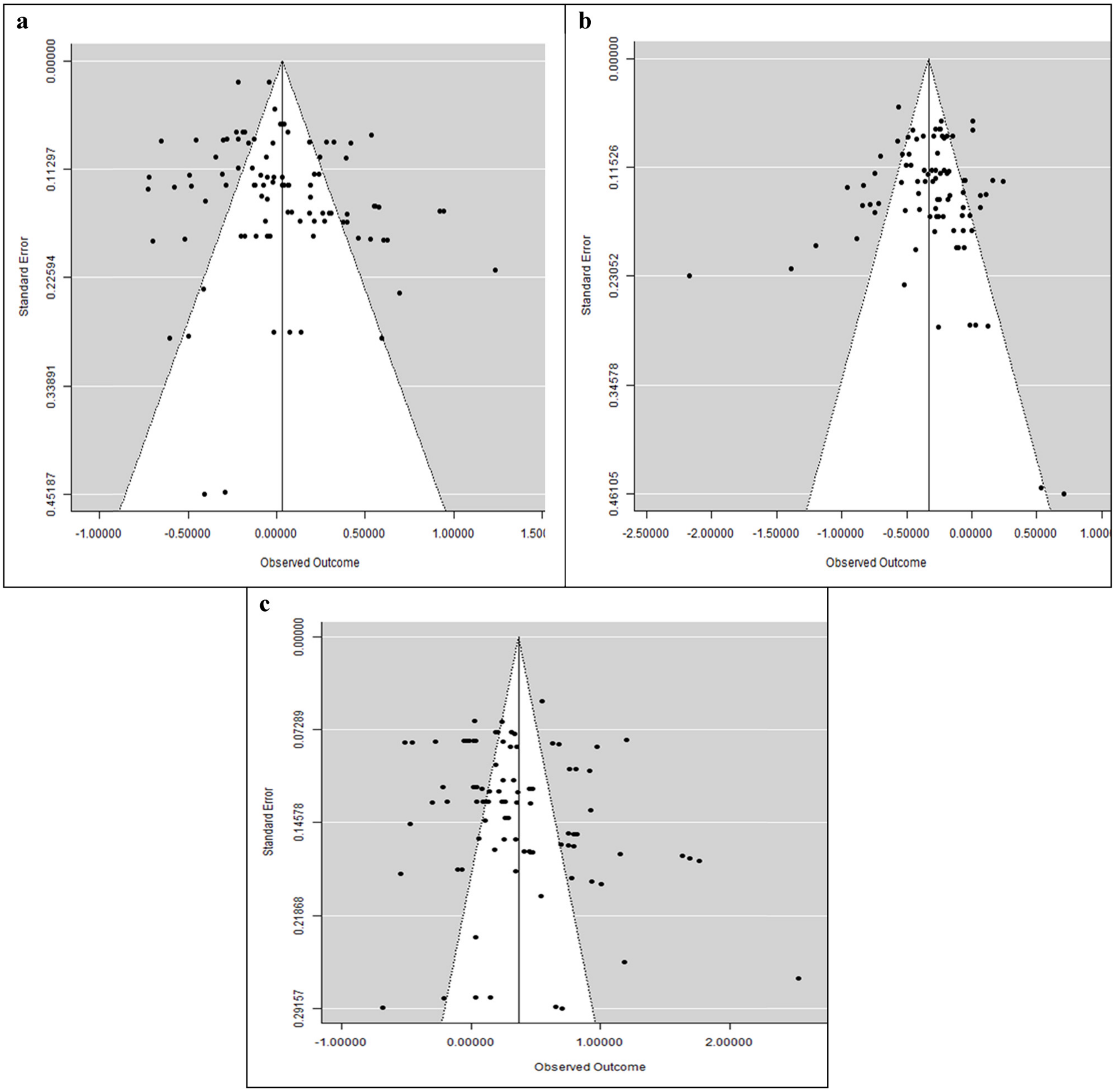

Figure 6: Funnel graphs of the effect of dietary *Bacillus* supplementation on (a) feed intake; (b) FCE; and (c) ADG in broiler chickens.

who observed that probiotic action was evident in chickens during the first few days of life, when the gut microbiota has not been stabilised. Initial colonisation is very relevant to the host because bacteria can modulate the expression of genes in epithelial cells, thus creating a favourable habitat for themselves [75]. Probiotic effect on ADG was identified in trials that fed *Bacillus* for 1–35 and 1–42 days which agrees with Li et al. [30], who noticed significantly higher ADG in broiler chickens fed with *Bacillus* spp. at 1×10^6 cfu/g for 1–21 days. On the other hand, Ahmed et al. [16] revealed that broiler chickens fed with 2.0×10^6 cfu/g *B. amyloliquefaciens* for 1–21 days had no significant effect on ADG. The observed difference could be related to the species of *Bacillus* used as well as the amount added to the diet [5,9].

4.2.2 Broiler chicken strain

Nutrition accounts for about 70% of cost of poultry production under an intensive management system [76], and nutritional strategies that enhance FI and FCE are desirable in the face of rising prices of feed due to high cost of feedstuffs. Probiotic effect on feed intake was evident only in the experiments using Cobb, but not in the experiments using Ross and Arbor acres. This implies that Cobb fed with diets treated with *Bacillus* gained weight at a lower FI compared with controls. FCE is one of the important indices utilised to assess chicken performance. The lower the FCE, the more efficient feed digestion and nutrient utilisation are. Probiotic effect on FCE and ADG was demonstrated in Cobb, Ross, and Arbor Acre strains. The ability of the Cobb strain to gain weight with a reduced FI in this study is a welcome development as it may affect feed cost. However, we could not proceed to ascertain the economics of production of broiler chickens on dietary *Bacillus* in this meta-analysis as such information is lacking in the literature. There is a significant relationship between FCE and broiler strain, which is consistent with the findings of others [77–79]. Although the present study shows evidence of treatment effect on FCE in broiler strains, the influence of other factors such as feed composition, gut health, and indoor temperature known to regulate feed efficiency in chickens could not be ruled out in this meta-analysis [80].

4.2.3 *Bacillus* spp.

Meta-regression analysis demonstrated that the species of *Bacillus* is a limiting factor among the studies included in the analysis and led to the inconsistent results among studies that assessed the effect of *Bacillus* on FI. The non-significant effect of *Bacillus* spp. on FI as shown in our subanalysis results indicates that *Bacillus* spp. has a limited ability to stimulate appetite in broiler chickens. This finding is in harmony with Boroojeni et al. [17] who found that incorporation of *B. subtilis* at 1.6×10^6 cfu/g (starter diet) and 0.8×10^6 cfu/g (grower diet) had no significant effect on FI. In contrast, this result differs with the finding of Ahmed et al. [16], who reported that supplementation of *B. amyloliquefaciens* at 1, 5, 10, and 20 g/kg had no significant effect on FI in Ross 308 broiler chickens and Park et al. [19] who reported significantly increased FI in broilers fed with *B. subtilis* at 1.0×10^6 cfu/g for 1–28 days. The observed differences may be due to the quantity of *Bacillus* included in the diet and type of *Bacillus* species used. On the other hand, subanalysis results indicate that *Bacillus* spp. had beneficial effects on FCE and ADG in broiler chickens. The potential of *Bacillus* spp. to improve FCE and ADG in broilers at a comparable FI with the control supports the findings of Zaghari et al. [29], who found that feed cost per kilogram weight gain was lower in broiler fed with diet supplemented with 0.2 g/kg *B. subtilis* (4×10^9 cfu/g) than in broiler fed with the same diet without *B. subtilis* supplementation. The increased ADG in chickens fed with diets containing supplemental levels of *Bacillus* spp. when compared to control chickens could be credited to the capability of *Bacillus* to boost digestive enzyme activity in the gut, resulting in increased digestion and nutrient uptake, supporting the earlier findings of Mingmongkolchai and Panbangred [12] and Ogbuewu et al. [3] that *Bacillus* organisms enhance the production and secretion of digestive juices and enzymes in chickens.

4.3 Supplementation dose

Meta-regression indicates significant relationship between SL and growth performance variables in broilers. Subanalysis results show that broilers offered with diets containing lower inclusion doses of *Bacillus* ($0.1–0.5 \times 10^6$ cfu/g) had lower FI than birds given moderate and higher doses at 0.6–1.0 and $>1.0 \times 10^6$ cfu/g compared to controls, showing that broilers fed with higher doses of probiotic *Bacillus* consumed more feed than control broilers. However, the higher FI in broilers fed with diets having moderate and high inclusion levels of *Bacillus* did not translate to higher ADG. Interestingly, broilers fed with lower doses of *Bacillus* gained more weight than the controls, implying that these birds were gaining weight at a FI similar to controls. This could be ascribed to the ability of *Bacillus* at certain supplementation dose levels to enhance the secretion of digestive enzymes which assist in feed digestion and nutrient absorption [14]. These findings are consistent with that of other authors [12,14,16], who noticed differences in growth traits between broiler chickens fed with diets supplemented with low and high inclusion levels of *Bacillus*. Taking into account the economic benefits of overall feeding costs in broiler chicken production, subanalysis revealed that SL of $0.1–0.5 \times 10^6$ cfu/g may be the optimal SL for broiler chickens. However, more research is required to determine the optimal supplementation dose of *Bacillus* in the chicken feed that optimised growth performance in broiler chickens using the regression analysis. Broilers offered with *Bacillus* supplemented diets had better FCE than the controls. The better FCE in group offered with *Bacillus* may be credited to the ability of the *Bacillus* to enhance the quality of the diets resulting in higher ADG especially in sub group fed with lower doses of *Bacillus*. The better FCE in broilers given *Bacillus* when compared to the controls supported the findings of other researchers [81–83] who discovered that *Bacillus* improves FCE in broiler chicken via improvement in gut health [83].

4.4 Heterogeneity and publication biases

Heterogeneity is one of the limiting factors in a meta-analysis which typically arises from differences in the study population, type of probiotics used, differences in dose level, and duration of study [3,26]. Our results revealed the existence of significant heterogeneity and this prompted sub group analysis to explain the likely sources of heterogeneity. However, large heterogeneity was still observed within all subgroups of the studied modifiers. Meta-regression showed effect for *Bacillus* spp. and duration of study as moderators for FI, and the duration of study and broiler strain for FCE, implying that not more than 26–39% of the variations across articles used for the current analysis were explained by these moderators, which is similar to the findings of others [3,26]. Meta-regression showed no effect of studied moderators on ADG, implying that none of the studied moderators is a significant predictor of the study effect. These results imply that modifiers other than those studied could be responsible for the unexplained heterogeneity. Thus, more studies are required to ascertain other factors responsible for the unexplained heterogeneity. However, studies included in the analysis were performed in 18 countries of the world showing the validity of our conclusions [25].

Publication bias is one major source of bias in meta-analysis. Even if a meta-analysis produces a mathematically accurate synthesis of the studies included in the analysis, if these studies are a biased sample of all relevant studies, the mean effect computed by the meta-analysis will reflect this bias [84]. The likely explanation for not including all relevant articles in meta-analysis can be the tendency for negative trials or small studies to not be published, either due to editorial bias to only publish results with significant results or authors' aversion to publishing papers with negative results [85]. However, the minimal evidence of publication bias as observed in this meta-analysis is not a problem as the Nfs values were several folds above the thresholds needed to proclaim pooled estimates free from bias [72].

5 Conclusion

Meta-analysis results suggest that *Bacillus* supplementation improved ADG and feed FCE in broilers when compared to controls. Subanalysis showed significant differences among studied moderators (broiler strains, supplementation doses, *Bacillus* species, and DOS). There is a high degree of heterogeneity among studies included in the meta-analysis which could not be removed by the subanalysis. Meta-regression analysis revealed that studied moderators accounted for about 77% of the sources of variation, implying the presence of factors other than those studied in the current meta-analysis. It is therefore recommended that the effect of factors such as indoor rearing temperature, ventilation rate, and relative humidity of the poultry house, among other variables known to influence growth performance in broiler chickens be reported as these factors were not stated in about 90% of studies included in the meta-analysis. This study has standardised the study design for future experiment on the impact of *Bacillus* on broiler chicken productivity.

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