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Research Article

Meta-Analysis of Bacillus in Laying Hen Nutrition: Feed Intake, Feed Conversion Ratio and Egg Productive Indices

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Abstract: This data synthesis study aimed to explore the impact of bacillus additive on performance of laying hens. Three bibliographic databases were scanned, with no restrictions on language and date. The inclusion criteria were randomised and controlled trials using non-diseased laying hens and published in peer-reviewed journals. The eligibility criteria were also information on measured performance parameters and studied moderators. Eighteen (18) studies passed the eligibility conditions for the study and were analysed in OpenMEE software. Results were displayed as Standardised Mean Difference (SMD) at 95% confidence intervals (CI). Results showed that bacillus supplementation improved feed conversion ratio (SMD = -0.21; 95% confidence intervals (CI) = -0.29, -0.12) and increased hen-day egg production (SMD = 0.21; 95% CI = 0.16, 0.27)]. Feed intake was not affected by dietary bacillus supplementation. In contrast, bacillus supplementation increased egg weight (SMD = 0.08; 95% CI = 0.03, 0.12) and egg mass (SMD = 0.27; 95% CI = 0.16, 0.38) compared with the control. Restricted subgroup and mixed effect meta-regression analyses showed that pooled results were changed by the studied moderators. In conclusion, results suggested that bacillus supplementation could be added to laying hen diets to improve performance indices.

Keywords: Probiotic-Bacillus, Laying Hens, Egg Production, Meta-Regression, Meta-Analysis

Introduction

The target of the commercial poultry industry is to increase nutrient uptake, which can be met to a certain degree by administering certain feed additives. Probiotics, one such additives are non-pathogenic microorganisms given at right amounts to improve the host's performance and health (Rajput et al., 2013). Lactobacillus and Bifidobacterium are some of the probiotics used in animal production to date to enhance productivity (Arsène et al., 2021). Studies indicate that probiotics modulate intestinal microbiota composition and boost the immune system, which in turn leads to improvements in production parameters in poultry (Xiang et al., 2019; Ogbuewu and Mbajiorgu, 2020; Ogbuewu and Mbajiorgu, 2022a-b). The manner in which probiotics improve chicken performance is not well understand. However, it could achieve this via the following mechanisms of action: direct nutritional effect, modulation of gut microbiota through competitive exclusion, and stimulation of immune responses (Arsène et al., 2021; Pang et al., 2022; Elghandour et al., 2024).

Bacillus, one such probiotic, is a gram-positive bacterium that is currently utilised as a feed supplement in the livestock industry. *B. subtilis* (BS), *B. velezensis* (BV), *B. licheniformis* (BL), *B. coagulans* (BC), and *B. amyloliquefaciens* (BA) are some of the species of bacillus used as feed additives. One advantage of bacillus over conventional probiotics used in livestock production is the ability to form spores that resist heat during feed pelleting process (Ogbuewu et al., 2022). Furthermore, bacillus is ideally suited as feed additives due to its ability to produce a variety of digestive enzymes and can be kept for extended periods while retaining its viability. Bacillus also performs well in the high pH of the chicken intestine (Arsène et al., 2021; Ogbuewu et al., 2022). The intracellular digestive enzymes released by bacillus facilitate the breakdown of feed in the chicken gut, leading to an improvement in nutrient utilisation (Arsène et al., 2021). Studies suggest that bacillus produces antimicrobial active substances such as organic acids, hydrogen peroxide, and bacteriocins that interfere with the ability of pathogens to colonise the walls of the intestine in chicken (Shi et al., 2020; Ogbuewu et al., 2022).

Results of bacillus supplementation in laying hen performance are inconclusive. Some investigators found that bacillus improves performance parameters of chickens (Ogbuewu and Mbajiorgu, 2022a). In addition, dietary bacillus increased feed intake, laying rate, and egg quality, as well as enhanced feed efficiency, immune functions, and oxidative status in laying hens (Liu et al., 2018; Upadhaya et al., 2019; Ye et al., 2020). However, other authors reported that bacillus had no effect on performance indices of commercial layers (Ribeiro et al., 2014; Forte et al., 2015; Fathi et al., 2018). These disparities can be linked to variables such as diet composition, hen genetics, bacillus strains, dosage, and the environment in which these layers were reared (Ogbuewu and Mbajiorgu, 2020).

Given that the aim of commercial poultry industry is to enhance feed utilisation and optimise egg production, while consuming less feed. Thus, it is important to use meta-analytical method to ascertain the factors that contribute to variable results in laying hens offered bacillus-enriched diets. Meta-analysis is a statistical tool that combines findings of similar studies to resolve inconsistent findings, increase statistical strength, identify research gaps, and suggest future research paths. Presently, there is little or no study on productivity of layers fed bacillus-based rations. Hence, this data synthesis aimed to explore the influence of dietary bacillus on performance outcomes of laying hens.

Table 1: Search query

Bibliographic engine	Keywords	Retrieved studies
PubMed	(“probiotics” OR “bacillus species”) AND (“feed intake” OR “feed-egg ratio” OR “FCR”) AND (“commercial laying hens” OR “commercial layers”) AND (laying performance” OR “egg production” OR “laying rate” OR egg quality)	315
Scopus	(“probiotics” OR “bacillus species”) AND (“feed intake” OR “feed-egg ratio” OR “FCR”) AND (“commercial laying hens” OR “commercial layers”) AND (laying performance” OR “egg production” OR “laying rate” OR egg quality)	348
Google Scholar	(“probiotics” OR “bacillus species”) AND (“feed intake” OR “feed-egg ratio” OR “FCR”) AND (“commercial laying hens” OR “commercial layers”) AND (laying performance” OR “egg production” OR “laying rate” OR egg quality)	205

Materials and Methods

Database Sources and Literature Searching

Three bibliographic databases (PubMed, Google Scholar, and Scopus) were scanned for publications on the topic. Bibliographic databases were scanned following PRISMA guidelines (Mohe et al., 2009). A reference list of relevant articles identified during the review process was also searched for other important studies. The keywords (bacillus, feed intake, layers, feed to egg ratio, laying performance, egg quality, and probiotic-bacillus) were blended with Boolean logic operators as provided in Table (1). The title was structured following PICO template. This stands for population (layers); Intervention (probiotic bacillus); Comparators (diets without probiotic bacillus); and the Outcomes (analysed variables of interest).

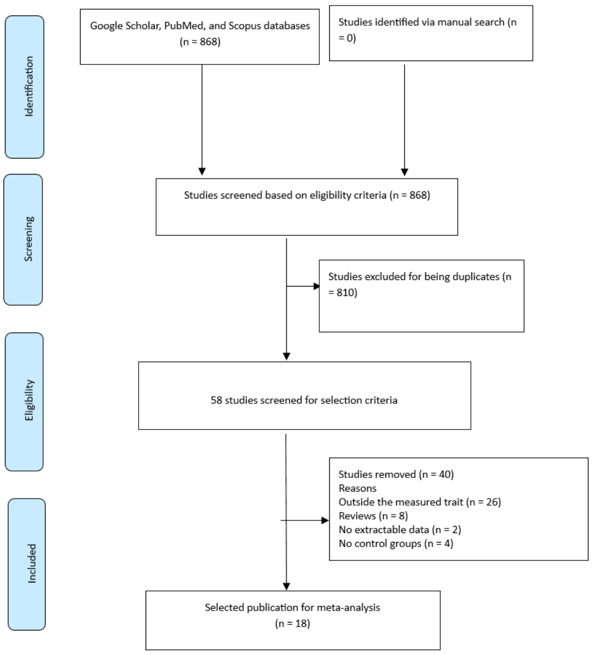

Fig. 1: Publication selection steps

Screening for Eligibility

Identified publications were reviewed for eligibility by the first and the second authors, and disagreements were settled via discussion and consensus. The current meta-analysis employed a 2-step screening method. In the first step, articles that failed to fulfill the criteria for inclusion based on the title and abstract were not used for the analysis. Thereafter, full-text screening was conducted on potentially relevant studies that passed the first stage of screening. The included articles should meet the following criteria (i) Be an original article published in any language; (ii) Randomised and controlled studies that evaluated the effects of bacillus in laying hen performance; (iii) Measured at least the performance outcomes of interest in laying hens, and (iv) All the diets used must be free of other probiotics. All review articles on the impacts of dietary bacillus on laying hen performance were excluded. Also excluded were duplicate studies and publications outside the measured outcomes. Based on the eligibility criteria, 18 peer-reviewed publications were used for the study. The PRISMA selection flow chart and features of the 18 articles used for the study were presented in Figure (1) and Table (2), respectively. The included studies were evaluated for risk of bias through RoB 2.0 (Cochrane risk-of-bias in randomised trial) as shown in Table (3).

Table 2: Characteristics of included publications

Study location	Moderators						References
	LS	*B. spp.*	BC[1]	Dosage(%)	[2]DI	[3]LA	
China (Asia)	HL	BC	1.26 - 9.35E+05	0−0.20	56	602	1
China (Asia)	LM	BS	6.00E+09	0−0.10	210	175	2
China (Asia)	IB	BA			42	196	3
China (Asia)	HL	BA		0−0.06	70	196	4
China (Asia)	HL	BL	2.00E+10	0−0.09	56	196	5
Korea (Asia)	HL	BS/BL		0−0.05	189	126	6
Egypt (Africa)	HS	BS	1.50E+08	0−0.10	84	154	7
China (Asia)	HL	BS	2.0 - 3.06E+08		42	567	8
Italy (Europe)	HL	BS		0−0.05	112	126	9
China (Asia)	HL	BS	1.00E+09	0−0.50	70	203	10
Taiwan (Asia)	LH	BS/BA	1.00E+09	0−0.30	56	455	11
Poland (Europe)	LM	BS			168	126	12
China (Asia)	HL	BV		0−0.20	42	343	13
China (Asia)	XBB	BS	1.00E+09		56	175	14
Korea (Asia)	HL	BS	1.00E+09	0−0.05	35	280	15
Canada (North America)	SW	BS	1.1 - 2.2E+08	0−0.50	203	133	16
China (Asia)	HL	BS	1.00E+10		168	196	17
Brazil (South America)	HL	BS			140	175	18

XBB Xuefeng black bone; HL hyline; LM Lohmann; BL Bacillus licheniformis; BS Bacillus subtilis; BC Bacillus coagulans; LH Leghorn; IB Isa Brown; BA Bacillus amyloliquefacien; HS hisex; SW Shaver white; BV Bacillus velezensis,
[1]BC Bacillus count
[2]DI duration of intervention,
[3]LA layer age in days; 1 - Wang et al. (2023); 2 - Xu et al. (2006); 3 - Tang et al. (2018); 4 - Zhou et al. (2020); 5 - Lei et al. (2013); 6 - Upadhaya et al. (2019); 7 - Abd El-Hack et al. (2016); 8 - Liao et al. (2023); 9 Forte et al. (2015); 10 - Chen et al. (2019); 11 - Tsai et al. (2023); 12 - Sobczak and Kozłowski (2015); 13 - Ye et al. (2020); 14 - Liu et al. (2018); 15 - Shi et al. (2020); 16 - Neijat et al. (2019); 17 - Guo et al. (2017); 18 - Ribeiro et al. (2014)

Data Extraction

Data on first author's surname, year the article was published, location (Egypt, China, Italy, Canada, Brazil, Korea, and Poland), number of layers in the control and experimental groups were retrieved from each study that met the inclusion criteria. Production and egg quality data were FI, FCR, HDEP, EW, and EM. Data were also extracted on selected moderators layer age (126-602 days), layer strains (HS, LM, HL, XBB, SW, and IB), *Bacillus spp. (*BA, BV, BS and BL), bacillus dosage (0-0.5%), and duration of intervention (35−210 days).

Table 3: Study quality assessment

Publications	Bias domains					Overall bias
	R	DB	OB	MO	SR	
Chen et al. (2019)	LR	LR	LR	LR	LR	LR
Xu *et al.* (2006)	LR	LR	LR	LR	LR	LR
Tsai *et al.* (2023)	LR	LR	LR	LR	LR	LR
Ribeiro et al. (2014)	LR	LR	SC	LR	LR	SC
Ye et al. (2020)	LR	LR	LR	LR	LR	LR
Forte et al. (2015)	LR	LR	LR	LR	LR	LR
Upadhaya et al. (2019)	LR	LR	LR	LR	LR	LR
Abd El-Hack *et al.* (2016)	LR	LR	LR	LR	LR	LR
Guo et al. (2017)	LR	LR	SC	LR	LR	SC
Liu et al. (2018)	LR	LR	LR	LR	LR	LR
Tang et al. (2018)	LR	LR	SC	LR	LR	SC
Neijat et al. (2019)	LR	LR	SC	LR	LR	SC
Shi *et al.* (2020)	LR	LR	LR	LR	LR	LR
Zhou et al. (2020)	LR	LR	SC	LR	LR	SC
Lei et al. (2013)	LR	LR	LR	LR	LR	LR
Liao *et al.* (2023)	LR	LR	LR	LR	LR	LR
Sobczak & Kozłowski (2015)	LR	LR	LR	LR	LR	LR
Wang *et al.* (2023)	LR	LR	LR	LR	LR	LR

LR low risk; SC some concern; RB randomisation bias; DB deviation bias; OB outcome bias; MB measurement bias; SB selection bias

Statistical Analysis

Data on measured outcomes were computed in OpenMEE software as described by Wallace *et al.* (2017). Data generated were analysed through random-effects model method using a standard method (DerSimonian and Laird 2015). The effect estimate was computed via Hedges'd (Hedges and Olkin, 1985) also called SMD. Publication bias was determined using the procedures of Rosenberg (2005), whereas heterogeneity was calculated via the Q−and I^2−statistic (Higgins and Thompson, 2002). To determine the sources of variations, meta-regression was conducted based on Layer Strain (LS), Layer Age (LA), dosage, Duration of Intervention (DI), bacillus species and count, whereas subgroup analyses were planned based on LS, LA, bacillus species, dosage, and DI. Meta-regression test was conducted in all analysed outcomes due to the fact that a non-significant test for between-study variance does not ensure homogeneity among publications used for the data synthesis (Baker et al., 2009). A stratum having < 3 comparisons was removed because of poor statistical power (Koricheva et al., 2013). According to Jennions et al. (2013), pooled estimates were considered valid in presence of publication bias when Rosenberg fail-safe number (Nfs) is greater than 5× [(T) +10], where T is the number publications. In the present study, findings presented as graphs were extracted using WebPlotDigitizer (Rohatgi, 2021). Forest plots and restricted subgroup analysis results were said to be significant when zero is not part of 95% CIs (Koricheva et al., 2013). Furthermore, meta-regression and publication bias analysis were deemed significant when the probability value was <5%.

Results

Literature Overview

Eight hundred and sixty-four articles were collected from the search executed on the three bibliographic databases of which a total of 18 met the inclusion criteria. Figure (1) shows the article selection flow chart, while Table (1) presents the characteristics of eligible publications. The eligible publication were published between 2006 and 2023. The risk of bias among candidate publications was 72% for "low risk" and 28% for "some concern" indicating that there was no publication that satisfied the eligibility conditions which was not included in this meta-analysis.

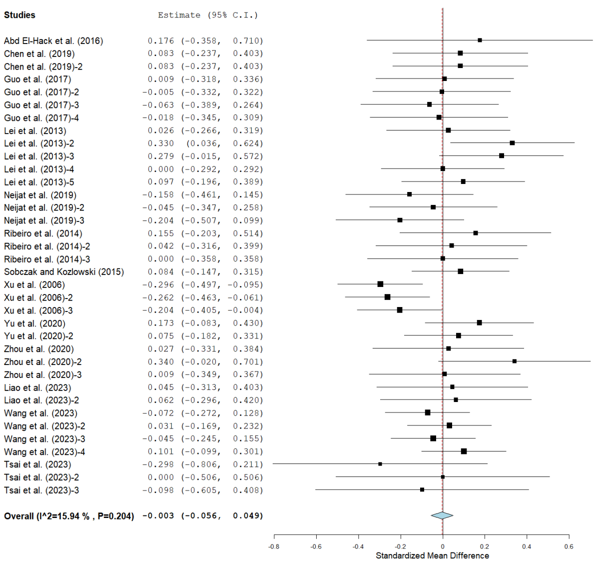

Fig. 2: Feed intake of layers on bacillus diets. The vertical line is the line of no effect, while the horizontal lines emerging from the square boxes represent the 95% confidence interval. The square boxes indicate the weight of the individual study. Dotted vertical line and sky-blue diamond represent the pooled estimates. The width of the sky-blue diamond represents the confidence limits. The points to the left and right of the thick vertical line represent an increase and decrease in feed intake, respectively. I^2 = heterogeneity

Feed Intake (FI) and Feed Conversion Ratio (FCR)

Feed intake and FCR of laying hens fed bacillus-based diets are presented in Figs. (2-3). In comparison with the control, laying hens on probiotic bacillus additive had similar FI without statistical heterogeneity [Inconsistency index (I^2)-statistic = 16%; P = 0.206; Fig. 2]. The influence of moderators on FI in layers fed bacillus is presented in Table (4). Feed intake was significantly affected in HL layers fed BS and BL for 7−70 and 147−210 days. However, FI was not affected by layer age and dosage. The pooled results showed that layers offered bacillus had better FCR than the control with the presence of substantial heterogeneity (I^2-statistic = 72%; p<0.001) as shown in Fig. (3). Table (5) displayed the effect of moderators on FCR. Layer strains (LM, HL, and SW) aged from 126-217 days fed BA and BS at at a level less than 0.01% and 0.1-0.5% for 7-70 and 147-210 days had enhanced FCR.

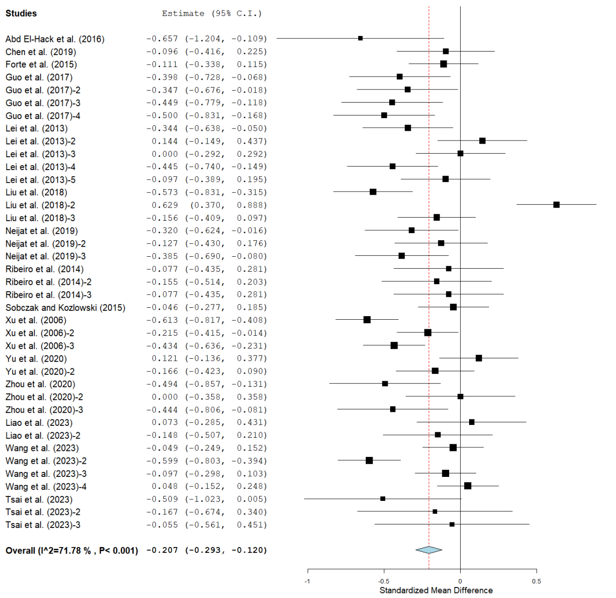

Fig. 3: FCR in laying hens fed bacillus-enriched diets. The points to the left and right of the thick vertical line represent an increase and decrease in FCR, respectively

Data as presented in Figure (4) indicate that laying hens on bacillus supplementation had higher HDEP (SMD = 0.21; 95% CI 0.16, 0.27) than the control without evidence of between-study variance (I^2−statistic = 25%; P = 0.073; Fig. 4). Hyline layer aged 126−217, 224−504, and >504 days (Table 6) offered BS, BL, BC, and BA for 7–70, 147–210, and 147–210 days had significantly increased HDEP. However, bacillus had no effect on HDEP in Shaver white and leghorn layers. The impacts of probiotic bacillus intervention on EW and EM are presented in Figs. (5-6), respectively. Layers that received bacillus recorded better EW and EM than the controls. Restricted subgroup analyses based on the studied moderators on EW and EM are shown in Tables (7-8), respectively. Lohmann and XBB aged from 126 to

217 days given diets supplemented with *B. substilis* at 0.10–0.50% for 77−140 and 147−210 days had significantly increased EW (Table 7). Furthermore, Hyline strain aged 126−217 and > 504 days fed BS, BL, BC, and BA at < 0.10% for 7–70 and 77−140 days had significantly heavier EM (Table 8).

Table 4: Feed intake of layers on dietary bacillus

Moderators	Strata	Dataset	SMD	(95% CI)		Het	PV
				Lower	Upper		
LS	Lohmann	6	−0.11	−0.26	0.03	56	*
	Hyline	23	0.06	0.01	0.12	0	ns
	SW	3	−0.14	−0.31	0.04	0	ns
	Leghorn	3	−0.13	−0.42	0.16	0	ns
LA (d)	126−217	25	−0.01	−0.08	0.07	33	ns
	224−504	5	0.05	−0.10	0.21	0	ns
	>504	6	0.01	−0.08	0.10	0	ns
Dosage (%)	< 0.10	13	0.04	−0.06	0.14	45	*
	0.10−0.50	10	−0.04	−0.16	0.08	32	ns
DI (d)	7−70	21	0.06	0.01	0.13	0	ns
	77−140	4	0.08	−0.11	0.27	0	ns
	147−210	11	−0.14	−0.22	−0.06	5	ns
Bacillus spp.	BS	21	−0.08	−0.15	−0.02	0	ns
	BC	4	0.04	−0.10	0.10	0	ns
	BL	5	0.15	0.02	0.28	0	ns
	BA	4	0.09	−0.10	0.28	0	ns

*SW Shaver white; d days; LS layer strain; LA layer age; DI duration of intervention. BS B. substilis; BL B. licheniformis; BA B. amyloliquefaciens; BC B. coagulans; PV probability value; ns not significant; * significant*

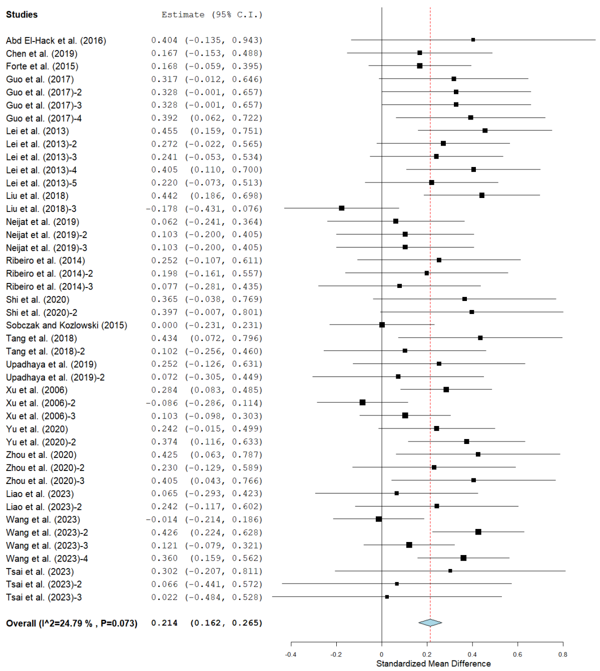

Fig. 4: HDEP of layers on dietary bacillus. The left and right side of the no effect line represent an increment and reduction in HDEP, respectively

Table 5: FCR of layers on bacillus supplementation; Hen-Day Egg Production (HDEP), Egg Weight (EW), and Egg Mass (EM)

Moderators	Strata	Dataset	SMD	(95% CI)		Het	PV
				Lower	Upper		
LA (d)	126−217	28	−0.24	−0.34	−0.13	73	*
	224−504	5	−0.10	−0.30	0.09	28	ns
	>504	6	−0.14	−0.35	0.08	80	*
LS	Lohmann	5	−0.29	−0.50	−0.08	77	*
	Hyline	24	−0.19	−0.28	−0.09	60	*
	XBB	3	−0.03	−0.72	0.65	95	*
	Shaver white	3	−0.28	−0.45	−0.10	0	ns
	Leghorn	3	−0.24	−0.54	0.05	0	ns
Dosage (%)	<0.10	14	−0.22	−0.36	−0.08	77	*
	0.10−0.50	9	−0.21	−0.36	−0.06	50	*
DI (d)	7−70	23	−0.14	−0.27	−0.02	76	*
	77−140	5	−0.15	−0.29	0.00	0	ns
	147−210	11	−0.35	−0.46	−0.24	47	*
Bacillus spp.	BS	24	−0.24	−0.36	−0.12	73	*
	BL	5	−0.15	−0.36	0.07	62	*
	BA	4	−0.27	−0.52	−0.01	43	ns
	BC	4	−0.17	−0.45	0.11	87	*

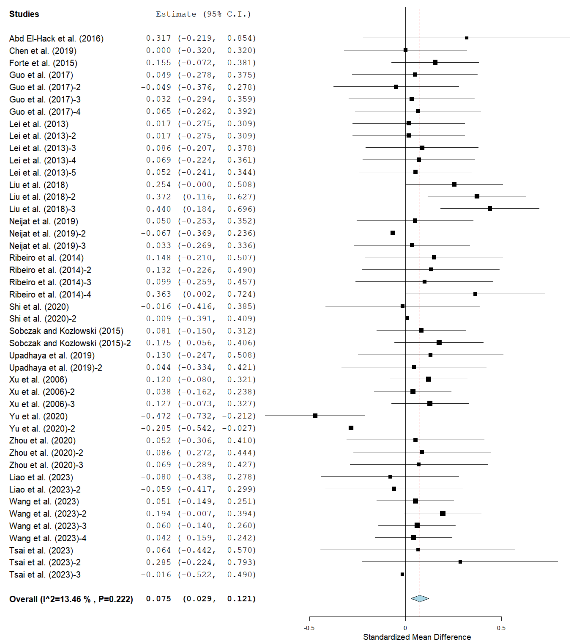

Fig. 5: Plots of the impact of bacillus on egg weight. The points to the left and right of the thick vertical line represent an increase and decrease in egg weight, respectively

Analysis of Moderators and Publication Bias

Table (9) reveals that feed intake in laying hens was influenced by the moderators with the exception of dosage and layer age. Furthermore, result showed that layer strains, dosage, and *Bacillus spp*. had an impact on EW in laying hens. In contrast, the studied moderators did not influence FCR, HDEP, and EM. Table (10) shows the presence of publication bias for FCR, HDEP, EM, and EW. There was no publication in feed intake as the observed significance was less than the target significance of 0.05.

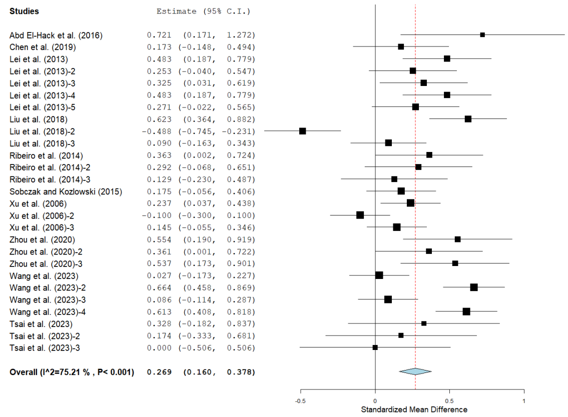

Fig. 6: Plots of the impact of bacillus on egg mass. The left and right side of the no effect line represent an increment and reduction in egg mass, respectively

Table 6: Hen day egg production of layers on dietary bacillus

Moderators	Strata	Dataset	SMD	(95% CI) Lower	Upper	Het	PV
DI (d)	7−70	27	0.25	0.19	0.32	28	ns
	77−140	4	0.172	0.02	0.33	0	ns
	147−210	13	0.15	0.06	0.24	21	ns
LS	Lohmann	5	0.09	−0.05	0.23	46	ns
	Hyline	27	0.26	0.21	0.32	0	ns
	Shaver white	3	0.09	−0.09	0.26	0	ns
	Leghorn	3	0.13	−0.16	0.42	0	ns
Dosage (%)	< 0.10	18	0.27	0.20	0.33	11	ns
	0.10−0.50	9	0.15	0.03	0.27	23	ns
Bacillus spp.	BC	4	0.22	0.02	0.42	75	*
	BL	6	0.29	0.17	0.42	0	ns
	BS	26	0.17	0.10	0.24	20	ns
	BA	6	0.29	0.14	0.44	0	ns
LA (d)	126−217	31	0.20	0.14	0.27	25	ns
	224−504	7	0.29	0.15	0.42	0	ns
	>504	6	0.21	0.05	0.36	61	*

Table 7: Egg weight of layers on bacillus

Moderators	Strata	Dataset	SMD	(95% CI) Lower	Upper	Het	PV
LA (d)	126−217	22	0.13	0.06	0.19	0	ns
	224−504	17	0.02	−0.07	0.11	36	ns
	>504	6	0.07	−0.03	0.16	0	ns
LS	Lohmann	6	0.10	0.01	0.19	0	ns
	Hyline	29	0.03	−0.02	0.09	6	ns
	XBB	3	0.36	0.21	0.50	0	ns
	Shaver white	3	0.01	−0.17,	0.18	0	ns
	Leghorn	3	0.11	−0.18	0.40	0	ns
Dosage (%)	< 0.10	18	0.08	0.02	0.14	0	ns
	0.10−0.50	9	−0.03	−0.21	0.14	62	*
DI (d)	7−70	25	0.05	−0.03	0.13	45	*
	77−140	6	0.18	0.05	0.32	0	ns
	147−210	14	0.07	0.00	0.14	0	ns
Bacillus spp.	BS	29	0.12	0.07	0.18	0	ns
	BL	5	0.05	−0.08	0.17	0	ns
	BA	4	0.06	−0.14	0.25	0	ns
	BC	4	0.09	−0.01	0.19	0	ns

Table 8: Egg mass values of layers fed bacillus-enriched diets

Moderators	Strata	Dataset	SMD	(95% CI) Lower	Upper	Het	PV
LA	126−217	20	0.26	0.14	0.38	72	*
	224−504	3	0.17	-0.13	0.46	0	ns
	>504	4	0.35	0.02	0.68	91	*
LS	Lohmann	5	0.12	−0.01	0.25	37	ns
	Hyline	15	0.36	0.24	0.48	63	*
	XBB	3	0.08	−0.55	0.70	94	*
	Leghorn	3	0.17	−0.13	0.46	0	ns
Dosage (%)	< 0.10	13	0.37	0.24	0.50	68	*
	0.10−0.50	7	0.14	−0.03	0.31	41	ns
DI (d)	7−70	19	0.30	0.15	0.44	79	*
	77−140	4	0.32	0.12	0.52	7	ns
	147−210	4	0.11	−0.04	0.26	52	ns
Bacillus spp.	BS	14	0.18	0.03	0.33	74	*
	BL	5	0.36	0.23	0.49	0	ns
	BA	4	0.41	0.19	0.62	18	ns
	BC	4	0.35	0.02	0.68	92	*

XBB Xuefeng black bone

Table 9: Relationship between moderators and measured outcomes in layers

Outcomes	Moderators	Intercept	Q_M	dof	P-value	R^2 (%)
FI	Layer age	−0.01	0.29	2	0.864	0
	Layer strains	0.18	15.6	4	0.004	98
	Dosage	−0.04	0.99	1	0.321	0
	Duration of intervention	0.08	16.4	2	< 0.001	100
	Bacillus spp.	−0.08	11.3	4	0.023	70
	Bacillus count	0.18	26.4	13	0.015	100
FCR	Layer age	−0.24	1.12	2	0.572	0
	Layer strains	−0.66	3.8	5	0.578	0
	Dosage	−0.22	0.00	1	0.987	0
	Duration of intervention	−0.18	4.98	2	0.083	12
	Bacillus spp.	−0.24	1.65	4	0.799	0
	Bacillus count	−0.66	10.4	13	0.659	0
HDEP	Layer age	0.21	0.70	2	0.706	0
	Layer strains	0.40	9.10	6	0.168	23
	Dosage	0.15	2.73	1	0.098	20
	Duration of intervention	0.26	3.13	2	0.209	11
	Bacillus spp.	0.17	3.55	4	0.470	5
	Bacillus count	0.40	12.3	13	0.502	8
EW	Layer age	0.12	3.57	2	0.167	13
	Layer strains	0.32	17.8	5	0.003	100
	Dosage	−0.05	4.06	1	0.044	61
	Duration of intervention	0.19	2.31	2	0.315	0
	Bacillus spp.	0.12	26.4	4	2.58e-05	100
	Bacillus count	0.32	11.1	13	0.599	0
EM	Layer age	0.26	0.63	2	0.731	0
	Layer strains	0.72	7.39	4	0.117	16
	Dosage	0.15	3.71	1	0.054	22
	Duration of intervention	0.34	1.88	2	0.390	0
	Bacillus spp.	0.18	3.19	3	0.363	5
	Bacillus count	0.72	10.9	7	0.145	21

FI feed intake; EW egg weight; FCR feed conversion ratio; HDEP hen day egg production; dof degree of freedom; Q M coefficient of moderators; P probability; R 2 coefficient of determination

Table 10: Publication bias calculation

Outcomes	Intercept	OS	TS	Nfs	N	5*n+ 10
Feed intake	-0.01	0.720	0.05	0	13	75
FCR	-0.20	<. 001	0.05	768	15	85
Hen-day egg production	0.05	<.001	0.05	767	18	100
Egg weight	0.08	0.004	0.05	100	17	95
Egg mass	0.25	0.001	0.05	582	10	60

SMD standardised mean difference; n number of study; N number of publications; Nfs fail-safe number; OS observed significance; TS target significance

Discussion

Probiotic Effect

This study indicates that dietary bacillus supplementation enhanced FCR in laying hens at comparable feed intake with the control. Research indicates that bacillus encourage the growth of normal gut microbes, enhance the release of digestive enzymes, improve intestinal barrier functions as well as the histoarchitecture of the small intestine in poultry, thus, improving feed digestion and absorption capacity of the villi (Chen et al., 2019; Ogbuewu et al., 2022). The improved FCR in the present study conforms with the results of others, who reported that bacillus improve digestion and nutrient utilisation, especially energy and protein (Ogbuewu et al., 2022).

Eggs are rich sources of beneficial nutrients for humans, and their composition is influenced by dietary manipulation (Ogbuewu et al., 2021). Chickens perform at their best when there is a balance between healthy and destructive gut microbes. The ability of bacillus to modulate intestinal microbiota composition and enhance nutrient uptake in chickens has been highlighted (Chen et al., 2019; Ogbuewu et al., 2022). This may explain the enhanced HDEP in laying hens fed bacillus-enriched diets. Another possible reason for the enhanced HDEP is the ability of bacillus-based diets to support the production of egg precursors (vitellogenins and apolipoprotein) in the liver in response to sex hormones (follicle stimulating hormones and luteinizing hormones) that increased egg production via follicular development and increased ovulation (Zhou et al., 2020). This meta-analysis suggests that bacillus can be included layer ration to increase EW and EM, confirming the earlier statement that bacillus intervention improves external egg quality traits (Xu et al., 2022). The improved egg quality in layers given bacillus-enriched diets could be linked to the capability of bacillus to release protease needed for protein digestion and uptake from the intestines (Ogbuewu and Mbajiorgu, 2022a). This finding supports the view of Wang et al. (2023), who reported no difference in EW between layers fed diet with and without bacillus. The variations in egg weight and mass could be connected to differences in the amount of bacillus incorporated into the diets, layer age, and chicken genetics. *et al.* (2023), who reported that the inclusion of *B. coagulans* in layer diet at 0.01 and 0.02% for 56 days increased HDEP, EW, and EM in 86-week-old Hyline layers. However, several variables such as environment, layer age, and hen genetics could influence EW and EM (Trachoo and Boudreaux, 2006). The addition of *B. coagulans* at 1.0×10^6 cfu/g to the rations of Hailan laying hens (42-week-old) for 35 days increased HDEP by 4% (Xu et al., 2022), supporting the findings of this study. Other researchers (Xu et al., 2022; Wang et al., 2023) found that bacillus increased EW and EM in commercial layers, which support the findings of this meta-analysis. In contrast, this finding is at variance with Tsai

Analysis of Moderators

Layer Age

Layer age was not a significant predictor of analysed performance outcomes in laying hens. However, the probiotic effect on FCR was evident in only studies that used layers aged 126–217 days, which support the view of Lv et al. (2022) that age affects the efficiency of digestion and nutrient uptake. In addition, HDEP was increased in trials that used layers aged 7−126, 224−504, and > 504 days. The effect of bacillus on EW was more pronounced only in experiments that included hens aged 126 to 217 days. The significantly higher EW in experiments that used layers aged 126–217 days relative to the control is ascribed to the enhance quality of nutrients in the test diets, resulting in heavier EW. However, the comparable EW in studies that used layers aged 224−504 days and >504 days compared with control indicates that bacillus-enriched diets are unable to support the production of heavier yolk, which increases EW in laying hens as they progress in age (Cunnigham and Sanford, 1974). The action of bacillus on EM was observed only in studies that used layers aged 126−217 days and > 504 days. The superiority of layers from feeding trials that used hens aged >504 days in terms of EM compared to those experiments that used hens aged 126−217 and 224−504 days corroborates the result of Joyner et al. (1987), who noticed that older hens lay fewer but larger eggs than the younger hens.

Dosage

The effect of bacillus dosage on aspects of performance parameters of laying hens fed certain feed additives has been highlighted (Ogbuewu and Mbajiorgu, 2020; Xu et al., 2022; Wang et al., 2023). The present result found a strong linear relationship between EW and bacillus supplementation in laying hens, implying that EW varies with a unit change in the level of bacillus in the feed. Bacillus effect on FCR and HDEP was observed in experiments that fed bacillus at a level less than 0.10% or 0.10–0.50%, suggesting that this may be the optimum dose levels of bacillus for layers. This finding is also in harmony with Abd El-Hack et al.

(2016) and Xu et al. (2022), who observed a dose-dependent effect of bacillus on FCR and HDEP in laying hens. However, the probiotic effect on EW and EM was found only in studies that included bacillus in layer diet at < 0.10%. Dietary bacillus improved feed intake and EM in layers from studies that fed bacillus at < 0.10% and 0.10−0.50%.

Layer Strains

Chicken genetics have been found to influence egg production and quality (Sharma et al., 2022). Presently, there is a scanty publication on the influence of layer strains on production parameters of poultry in response to dietary bacillus supplementation. The present results indicate that layer strain is a predictor of feed intake and EW. Feed intake and HDEP were improved in studies that used Hyline strain. Similarly, Lohmann, Xuefeng Black Bone (XBB), Hyline, and Shaver white strains had enhanced FCR. Our results indicate that EW and EM were increased only in trials that used Lohmann and XBB strains. The significantly higher EW in experiments that used XBB strain compared with experiments that used Shaver white, confirms the earlier report that genetics affects chicken performance (Sebola et al., 2015). The better EW observed in the XBB strain fed bacillus suggests that the gut microbiota is diverse and more abundant in indigenous chicken strains than in commercial layer strains (Ogbuewu et al., 2022).

Duration of Intervention

The current findings suggest that one of the factors contributing to inconsistent feed intake in layers fed bacillus-based diets is treatment duration. The higher FI in experiments that fed bacillus-enriched diets for 7–70 days than those fed the same diets for 147−210 days suggests that bacillus have a greater impact on feed intake at the early stage of lay. This finding supports the view of Wang *et al.* (2023), who reported that Lohmann strain fed bacillus for 35 days consumed more feed. FCR and HDEP were enhanced in studies that offered bacillus for 7−70, 77−140, and 147−210 days. Similarly, the probiotic impact on EM was found in trials that offered bacillus to laying hens for 7−70 and 147−210 days. However, the probiotic-effect was more pronounced in studies that offered bacillus to laying hens for 77−140 and 147−210 days.

Bacillus Count and Bacillus spp.

The results showed that *Bacillus spp.* contributed to the variable EW and FI in layers fed bacillus-based diets. The higher EW recorded in layers fed *B. substilis* compared to controls suggests that the bacillus promotes production of large eggs. Probiotic effect on FI was found in studies that administered BS and BL, whereas the impact on FCR was found in trials that incorporated BS and BA. The probiotic impact on EW was noticed only in studies that fed *B. substilis*. In contrast, probiotic effect on HDEP and EW was found in experiment that fed BC, BS, BA, and BL.

Source of Heterogeneity and Bias Analysis

This study utilised 18 peer-reviewed articles, enabling the conclusion that the addition of bacillus to laying hen diets improves performance outcomes considering the presence of significant heterogeneity. The selected moderators accounted most of the heterogeneity variance in this study. One of the commonest types of bias that can affect the validity and generalization of conclusions in meta-analyses is publication bias. Meta-analyses are prone to publication bias because studies without positive outcomes are less likely to be accepted for publication owing to the lack of interest by both the editors and the authors to publish research with negative results and, therefore, results from meta-analyses may overstate a treatment effect (Hopewell et al., 2009). Rosenberg's Nfs which is the additional results without positive outcomes required to alter significant outcomes to non-significance, could help in detecting the presence of publication bias (Rosenberg, 2005). The fact that the observed significance for all the performance indices except for feed intake was less than the target significance of 5% in this study ruled out the likelihood of publication bias. However, the result of feed intake according to Jennions *et al.* (2013) can be regarded valid despite the existence of publication bias as a substantial amount of unreported negative or insignificant research would be required to make the P value to be over 0.05.

Limitations and Strengths of the Analysis

The findings of this study are limited to laying hens and cannot be generalised to other avian species. There are variations in the quantity of bacillus incorporated into the rations, layer age, and strains, and the location where the study was conducted, and this may affect the validity and generalization of conclusions. Regardless of these limitations, this is the first study to characterise the influence of bacillus supplementation on the performance of laying hens to increase statistical power, resolve conflict, identify research gaps, and suggest the direction for future research.

Conclusion

This study included 18 peer-reviewed studies that evaluated the performance of layers and revealed that bacillus is effective in improving performance outcomes of laying hens at a comparable feed intake with the controls. The probiotic effect was related to layer strains, layer age, duration of intervention, dosage, and *Bacillus spp*. Subgroup analysis revealed that bacillus can be incorporated to the diet of up to 0.5% to enhance performance traits in laying hens. Results suggest that moderators were predictors of bacillus effect on performance outcomes in laying hens and accounted for most of the between and within study variance.

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Author's Contributions

All authors equally contributed to this study.

Ethics

The authors state that they have no competing interest to declare.

Data Availability

Data will be made available on reasonable request.

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