META-ANALYSIS OF PROBIOTIC (LACTOBACILLUS) ON PERFORMANCE OUTCOMES AND BLOOD CHOLESTEROL OF LAYING HENS

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Abstract. There are inconsistent results on the effect of dietary lactobacillus supplementation on laying performance. Therefore, this study ascertained the effect of lactobacillus supplementation on laying hen performance. A total of 348 publications were identified from a methodical search done in five databases, of which 12 met the eligibility conditions for the meta-analysis. Eligibility criteria were information on feed intake (FI), feed conversion ratio (FCR), egg production (EP), egg weight (EW), egg mass (EM), Haugh unit (HU), eggshell thickness (EST), egg yolk, blood cholesterol, and moderators (i.e., treatment duration, *Lactobacillus spp.* and supplementation dose, hen age, and strains). Data obtained were analyzed in OpenMEE software, and pooled results showed that FCR, EP, EM, HU, EST, egg yolk, blood and egg yolk cholesterol levels were better in the lactobacillus-supplemented group than the controls, taking significant heterogeneity into consideration. Furthermore, results indicate that studied moderators influenced the results of the meta-analysis and explained the variabilities across studies utilized for the meta-analysis. In conclusion, dietary lactobacillus supplementation improved feed conversion ratio, egg production, aspects of egg quality, and blood cholesterol levels in laying hens and could be added to chicken feed to boost laying performance and egg quality.
Keywords: *beneficial microbes, layers, egg production, egg quality, blood lipid, meta-regression*

Introduction

Eggs are valuable and excellent source of high-quality animal protein and other essential nutrients required for body maintenance and development (Lei, 2020). Increased production and efficient feed conversion ratio are the targets of the modern poultry industry, which can be realized to some extent using specific feed additives or supplements such as probiotics. Probiotics are live microbes included in the feed in the right amounts and proportions to confer health benefits to the host (Mahfuz et al., 2017; Abd El-Hack et al., 2020). Probiotics are presently gaining relevance in health care systems, as well as the poultry and food industries, due to their reported benefits in stimulating the immune system and optimizing nutrient uptake from the gastrointestinal tract (Dowarah et al., 2017; Abd El-Hack et al., 2020). Incorporation of probiotics into livestock feed has gained attention in recent years in the poultry industry because of their capability to modulate the growth of beneficial microbes, increase nutrient digestibility and uptake, stimulate immune responses, and produce B-vitamins, resulting in increased laying rate and quality (Mahfuz et al., 2017; Tang et al., 2017; Alaqil et al., 2020).

Lactobacillus is the most routinely used probiotic bacteria in the lactic acid group. It is abundant in fermented products and has been demonstrated to elicit a beneficial

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influence on chicken performance through modulation of gut microbiota composition (De Cesare et al., 2017; Saleh et al., 2017). Lactobacillus belongs to the phylum ‘Firmicutes’ and is the largest genus in the lactic acid bacteria (LAB) group. During carbohydrate fermentation, lactobacillus generates lactic acid as the principal metabolic end product (Dowarah et al., 2017) and is generally recognized as safe (GRAS) species. Like other probiotics, lactobacillus works through the following mechanisms, as reviewed by Dowarah et al. (2017): (i) reduction of gut pH and biosynthesis of antimicrobial substances such as lactoferrin, organic acids, and bacteriocins; (ii) optimization of nutrient absorption through enhanced digestive ability; (iii) modulation of immune function by improving cytokine production and enhancing macrophage activity; and (iv) modulation of gut microbiota composition by stimulating the growth of healthy microbes via competitive exclusion and alteration of mucin dynamics (Gao et al., 2017; Mahfuz et al., 2017; Qiao et al., 2019).

However, the impact of lactobacillus on health and laying performance is not clear. Alaqil et al. (2020) reported improved FCR, EW and EM in commercial layers (40-week-old Hy-Line brown) fed a yellow corn-soybean meal-based diet with *Lactobacillus acidophilus* (LA) at 0.31×10^7 colony forming units (cfu)/kg diet for 6 weeks. However, Qiao et al. (2019) noticed a 3% and 1.4% reduction in EP and FCR, respectively, in 18-week-old layers fed *L. plantarum* at 1.0×10^9 cfu/mL for 10 weeks compared to layers that received a diet without *L. plantarum* supplementation.

Cholesterol is a structural part of lipid membranes used as a precursor for the biosynthesis of bile acids. Eggs are the principal source of cholesterol in the diet, and studies indicate that one large egg contains about 200 mg of cholesterol and more (Ahn et al., 1999). The adverse health effect of excess dietary cholesterol coupled with its hepatic biosynthesis has been highlighted (Cha and Park, 2019). The hypocholesterolemic properties of lactobacilli have been reported in poultry species (Kalsum et al., 2012; Alaqil et al., 2020; Deng et al., 2020), which could be through deconjugation or excretion of bile salts (Zhai et al., 2019). Despite several studies done in recent years on the impact of probiotic-lactobacillus on laying rate, its effects have not yet been clarified (Alaqil et al., 2020; Deng et al., 2020; Naseem et al., 2021). This ambiguity could be related to a variety of factors, including bacteria count, treatment duration, lactobacillus species or strains, route of administration, hen age or strain, diet composition, and hygiene condition of the farm (Kers et al., 2018; Qiao et al., 2019).

Research has explored the use of a meta-analytic approach to pool studies with inconsistent results to increase statistical power and identify sources of variation among studies addressing the same research questions (Ogbuewu et al., 2021; Hartoyo et al., 2023). However, there is no published study on the meta-analytic effect of lactobacillus on layer productivity. Thus, this study explored the meta-analysis of lactobacillus on the performance outcomes of laying hens.

Materials and methods

Database search and study selection

To achieve the aim of this study, PubMed, Elicit.com, ScienceDirect, Scopus, and Google Scholar were searched for articles on the topic using “Boolean logic operators” and the keywords “laying hens” and “lactobacillus.” The topic was formulated using the PICO (i.e., population, intervention, comparators and outcomes) template, where P = laying hens, I = diets with lactobacillus supplementation, C = diets without

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lactobacillus supplementation, and O = performance parameters. The systematic search was not limited by date of publication or language. Selected articles met the following conditions: (i) randomized controlled experiments with and without lactobacillus supplementation; (ii) studies published in peer-reviewed journals; (iii) trials performed using healthy laying hens offered diets free of other added feed supplements or additives; and (iv) articles stated at least one of the measured outcomes of interest. The measured outcomes of interest were EP, egg quality, FI, FCR, blood, and yolk cholesterol. Reviews, articles that appeared in more than one database, and studies conducted in diseased layers were excluded from the current study. *Figure 1* depicts the article selection flow chart. Three hundred and forty-eight (348) studies were retrieved, and 25 full-text studies were taken based on title and abstract screening. After a thorough screening of the 25 potential full-text papers, 12 publications passed the eligibility conditions for the meta-analysis.

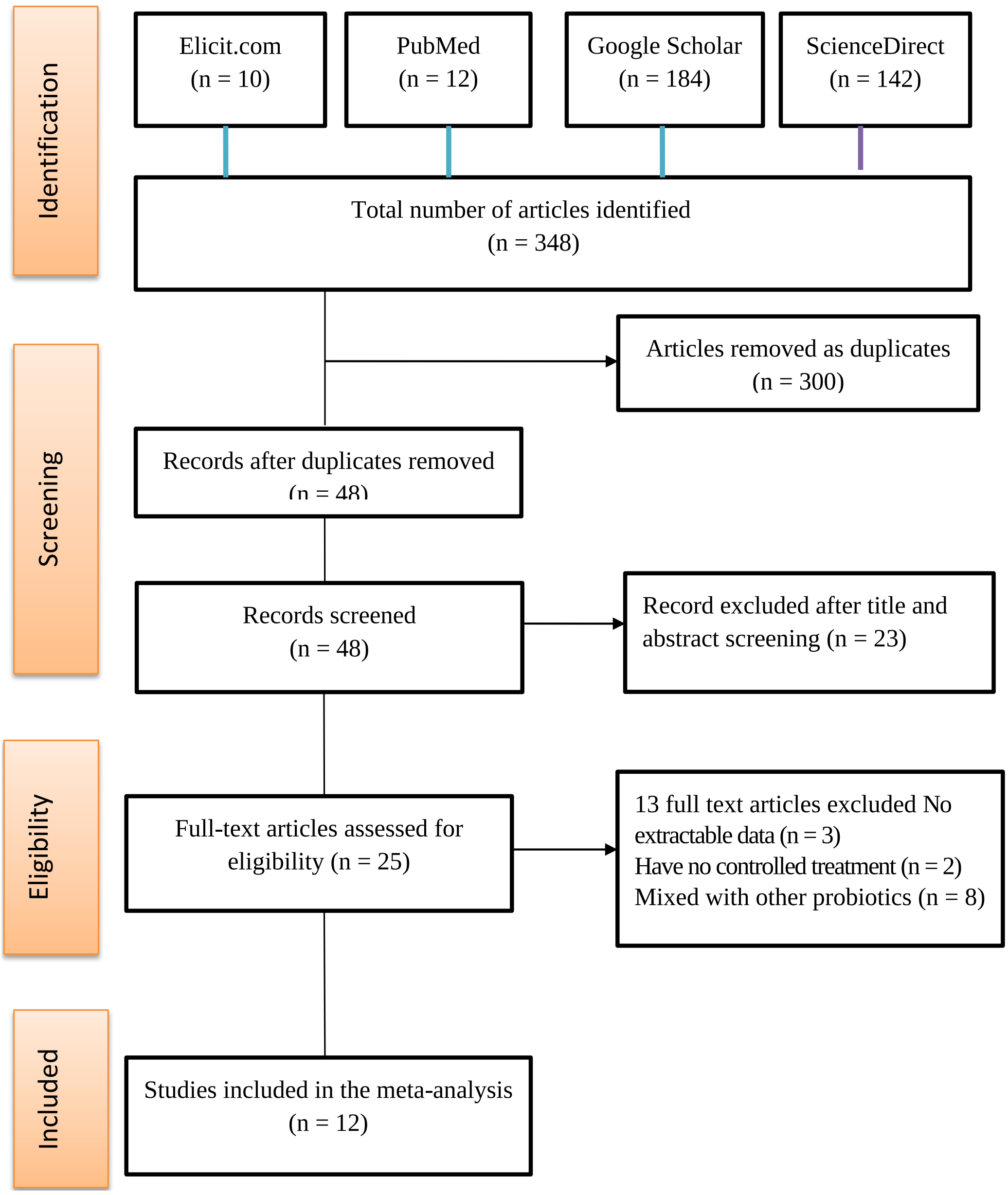

Figure 1. *Article selection flow-chart*

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Database development

Data on study design and moderators: hen age (12-65 weeks), layer strains (Hyline, Lohmann, Leghorn, and Jinghong), *Lactobacillus spp.* (*L. rhamnosus, L. acidophilus, L. plantarum, L. salivarius,* and *L. sporogenes*), supplementation dose, and treatment duration (6-48 weeks) were extracted from each of the 12 studies. Data on the number of layers, the standard deviation (SD) or standard error (SE), and measured outcomes were also extracted. In a situation where a study reported SE and not SD, SD was computed from SE using the method of Higgins and Deeks (2011), as earlier reported by Ogbuewu et al. (2021). The results presented as graphs were digitalized using WebPlotDigitizer (2021). Eight authors whose papers were selected for the meta-analysis were contacted via the corresponding author's email to supply missing information on hen age (n = 1), *Lactobacillus spp.* used (n = 1), and supplementation dose (n = 6). None of the eight authors contacted responded to our email. However, this is not a source of concern as the software used for the analysis has provisions for missing data.

Statistical analysis

OpenMEE, an open-sourced cross-platform statistical software designed and built by Wallace et al. (2016), was employed in all the analyses. Results were expressed as standardized mean differences (SMD) between layers on lactobacillus and control (without lactobacillus supplementation) diets with 95% confidence intervals (CI). This analysis used a random-effects model, and the weights of each article were computed as the inverse of the variance. Stratification analyses were performed using the following moderator variables: (i) hen age (12-65 weeks); (ii) layer strains (Hyline, Lohmann, Leghorn, and Jinghong); (iii) Lactobacillus species (*L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *L. salivarius*, and *L. sporogenes*); and (iv) treatment duration (6-48 weeks). Stratum in subgroup analysis with <3 comparisons were excluded in this analysis. Low sample size was the reason the supplementation dose was not disaggregated in the present study. In addition, *L. rhamnosus, L. salivarius, and L. sporogenes* were not stratified due to low sample size. Heterogeneity across trials was assessed using the Q- and I^2 -statistic as reported by Higgins and Thompson (2002). Sources of heterogeneity were determined using the moderator variables (i) hen age; (ii) layer strains, (iii) *Lactobacillus spp.*; (iv) treatment duration; and (v) supplementation dose. Results were considered significant at the 5% probability level. Sensitivity analysis was done to identify studies having an aberrant influence on the pooled results (Lean et al., 2009). Bias analysis was calculated using the Rosenberg failsafe (Nfs) number. Aspects of the measured outcomes (HU, EM, ST, blood, and yolk cholesterol level) was not subjected to publication and moderator analyses as the number of studies used for their computation were <10 (Borenstein et al., 2020). Pooled results were taken to be robust in the presence of publication bias when Nfs > [5×(n = number of articles) + 10] (Jennions et al., 2013).

Results

Study characteristics

Three hundred and forty-eight articles were identified, and 12 publications met the predefined eligibility criteria with their detailed features presented in *Figure 1* and *Table 1*. The articles utilized for this analysis were published between 1996 and 2023, spanning 27 years.

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Feed intake and feed conversion ratio

Table 2 showed the performance of layers on dietary lactobacillus supplementation. Feed intake was not increased by lactobacillus supplementation. The results of subgroup analysis of FI by moderators are displayed in *Table 3*. Hen strains, hen age, treatment duration, and *Lactobacillus spp.* did not influence FI in layers. However, in comparison to the control, the Hyline strain in the lactobacillus-supplemented group had reduced FI. Similarly, laying hens aged >31 weeks fed *L. acidophilus* recorded a lower FI than the control. There are significant linear relationships between hen strains ($Q_M = 7.2$; $p = 0.027$) and FI in layers, as presented in *Table 4*. There was no publication bias among studies that assessed FI as highlighted in *Table 5*. Results show that lactobacillus improved FCR (SMD = 0.30; 95% CI = 0.16, 0.45; $I^2 = 76\%$; *Table 2*). *Table 6* indicates that layers fed *L. acidophilus* had a lower FCR compared to layers fed *L. plantarum*. *Table 4* demonstrated that FCR was not influenced by hen strains ($Q_M = 5.83$; $p = 0.120$), and treatment duration ($Q_M = 3.7$; $p = 0.717$). In contrast, a linear association existed between aspects of studied moderators: hen age ($Q_M = 46.6$; $p < 0.0001$), supplementation dose ($Q_M = 61.8$; $p < 0.0001$), *Lactobacillus spp.* ($Q_M = 16.1$; $p = 0.003$), and FCR. There was a trace of publication bias across publications that evaluated the impact of lactobacillus supplementation on FCR as shown in *Table 5*. However, Rosenberg's Nfs for the FCR is 246, which is over 4-fold higher than the threshold of 60 needed to consider the mean effect size robust.

Table 1. *Characteristics of included studies*

Study	Country	No of treatment	Covariates					Outcomes extracted
			Inclusion (CFU/kg)	Lactobacillus spp.	Hen strains	TD (weeks)	Hen age (weeks)	
Alaqil et al. (2020)	Saudi Arabia	4	*	*L. acidophilus*	Hyline	6	40	FI, FCR, EP, EW, EM, yolk cholesterol, blood cholesterol
Gallazzi et al. (2008)	Italy	2	1.0×10^9	*L. acidophilus*	Hyline	40	17	FI, FCR, EP, EW, HU, EST,
Haddadin et al. (1996)	Jordan	4	**	*L. acidophilus*	Lohmann	40	25	FI, FCR, EP, EW, EST, yolk cholesterol, blood cholesterol
Loh et al. (2014)	Malaysia	4	nr	*L. plantarum*	Lohmann	12	23	FI, FCR, EP, EW, EM, yolk cholesterol, blood cholesterol
Panda et al. (2008)	India	3	nr	*L. sporogenes*	Leghorn	16	24	FI, FCR, EP, EW, HU, EST, yolk cholesterol
Qiao et al. (2019)	China	2	1.0×10^9	*L. plantarum*	Hyline	10	18	FI, FCR. EP, EW, EM
Xu et a. (2020)	China	2	1.0×10^8	*L. salivarius*	Hyline	4	65	FI, FCR, EP, EW, HU, EST
Forte et al. (2015)	Italy	2	nr	*L. acidophilus*	Hyline	19	16	FCR, EP, EW, HU, yolk cholesterol, blood cholesterol
Abdulrahim et al. (1996)	Jordan	2	4.0×10^6	*L. acidophilus*	Lohmann	16	12	FI, FCR, EP, EW, EST, yolk cholesterol, blood cholesterol
Getachew et al. (2016)	Ethiopia	2	nr	***	Leghorn	12	nr	FI, EP, EW
Liu et al. (2023)	China	2	nr	*L. rhamnosus*	Jinghong	6	63	FCR, EP, EW, HU, EST
Ramasamy et al. (2008)	Malaysia	2	nr	nr	Lohmann	48	16	FI, EP, EW, EM, yolk cholesterol,

TD - treatment duration; nr - not reported; * - $1 \times 10^9/21 \times 10^9/31 \times 10^9$; ** - $0.67\times10^6/2.0 \times 10^6/4.0 \times 10^6$; *** - acidophilus/plantarum; FI – feed intake; FCR – feed conversion ratio; EP – egg production; EW – egg weight; EM – egg mass; HU – Haugh unit; EST – eggshell thickness

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Table 2. *Pooled effect size of lactobacillus supplementation on performance of laying hens*

Outcomes	Dataset	Estimate	95% CI		P value	Heterogeneity	
			Lower	Upper		I^2 (%)	P value
Feed intake	17	−0.06	−0.25	0.03	0.208	0	0.569
Feed conversion ratio	15	−0.25	−0.46	−0.03	0.028	76	<0.001
Egg production	20	0.30	0.16	0.45	<0.001	59	<0.001
Egg weight	20	0.06	−0.03	0.15	0.199	3	0.423
Egg mass	8	0.18	0.04	0.33	0.010	5	0.394
Haugh unit	6	0.22	0.05	0.39	0.010	0	0.508
Eggshell thickness	9	0.34	0.14	0.54	<0.001	52	0.036
Egg yolk cholesterol	14	−1.39	−1.95	−0.84	<0.001	95	<0.001
Blood cholesterol	9	−1.31	−1.59	−1.03	<0.001	69	<0.001

CI confidence interval; I^2 Inconsistency index; *P* probability

Table 3. *Subgroup analyses of moderators on feed intake*

Moderators	Dataset	Estimate	95% CI		SE	P value
			Lower	Upper		
Hen strains						
Hyline	6	−0.21	−0.36	−0.07	0.08	0.005
Leghorn	4	0.11	−0.15	0.37	0.13	0.414
Lohmann	8	0.02	−0.11	0.16	0.07	0.736
Hen age (week)						
<31	12	−0.01	−0.12	0.11	0.06	-0.932
>31	4	−0.25	−0.43	−0.08	0.09	0.005
TD (week)						
6-19	13	−0.11	−0.23	0.01	0.06	0.051
20-48	5	0.05	−0.12	0.21	0.08	0.571
Lactobacillus spp						
L. acidophilus	9	−0.14	−0.27	−0.02	0.07	0.028
L. plantarum	5	0.03	−0.16	0.22	0.10	0.749

CI – confidence interval; TD – treatment duration; SE – standard error

Table 4. *Meta-regression of effect of moderators on response variables*

Outcomes	Moderators	Q_M	DF	p-value	R^2-index (%)
Feed intake	Hen age	12.2	8	0.143	100
	Hen strains	7.2	2	0.027	100
	Treatment duration	11.5	6	0.073	100
	Supplementation dose	3.13	7	0.873	0
	Lactobacillus spp.	2.9	3	0.408	0
Feed conversion ratio	Hen age	46.6	9	4.75e-07	80
	Hen strains	5.83	3	0.120	16
	Treatment duration	3.7	6	0.717	0
	Supplementation dose	61.8	7	6.61e-11	97
	Lactobacillus spp.	16.1	4	0.003	48
Egg production	Hen age	18.7	9	0.028	60
	Hen strains	11.8	3	0.008	53
	Treatment duration	15.4	7	0.031	52
	Supplementation dose	38.9	7	2.06e-06	100
	Lactobacillus spp.	3.31	4	0.507	0
Egg weight	Hen age	16.7	9	0.054	100
	Hen strains	9.56	3	0.023	100
	Treatment duration	10.1	7	0.184	100
	Supplementation dose	3.11	8	0.927	0
	Lactobacillus spp.	13.3	4	0.010	100

DF degree of freedom; Q_M coefficient of moderator; R^2 amount of heterogeneity accounted for by the moderators

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Table 5. *Publication bias assessment*

Outcomes	Estimate	Observed significance	Target significance	Nfs number	n	Nfs > (5*n_{study} + 10)
Feed intake	−0.06	0.208	0.05	0	10	60
FCR	−0.40	<.0001	0.05	246	10	60
Egg production	0.28	<.0001	0.05	163	12	70
Egg weight	0.06	0.192	0.05	0	12	70

n number of study; Nfs fail-safe number; FCR - feed conversion ratio

Table 6. *Subgroup analyses of moderators on feed conversion ratio*

Moderators	Dataset	Estimate	95% CI		SE	P value
			Lower	Upper		
Hen strains						
Hyline	7	−0.37	−0.66	−0.08	0.15	0.012
Lohmann	7	−0.65	−1.22	−0.09	0.29	0.024
Hen age (week)						
<31	12	−0.32	−0.71	0.07	0.20	0.110
>31	5	−0.63	−0.98	−0.27	0.18	<0.001
TD (week)						
6-19	13	−0.33	−0.67	0.02	0.17	0.061
20-48	4	−0.70	−1.30	−0.11	0.30	0.020
Lactobacillus spp						
L. acidophilus	9	−0.80	−1.18	−0.42	0.19	<0.001
L. plantarum	4	0.04	−0.17	0.24	0.11	0.726

C^{+} - number of comparison; CI – confidence interval; TD – treatment duration; SE – standard error

Egg production

Egg production was increased in layers fed lactobacillus compared to controls (*Table 2*). The disaggregation of results by moderators as presented in *Table 7* showed that the Lohmann and Leghorn strains produced more eggs than the Hyline strain. However, Lohmann and Leghorn strains had similar EP. Laying hens aged <31 weeks laid more eggs than layers aged >31 weeks. Treatment duration and *Lactobacillus spp*. influenced EP in layers. Results suggest linear relationships between EP and studied moderators except for *Lactobacillus spp*. as presented in *Table 4*. There was evidence of publication bias among articles that tested the action of lactobacillus on EP (*Table 5*). However, Rosenberg’s Nfs for the FCR is 163, which is over 2-fold higher than the threshold of 70 needed to consider the mean effect size robust.

Egg quality parameters and blood cholesterol

Data on egg quality parameters and blood cholesterol values are displayed in *Table 2*. Layers fed lactobacillus-based diets had similar EW to the control. Effects of moderators on EW as shown in *Table 8,* suggested that laying hens aged >31 weeks produced heavier eggs than those aged <31 weeks. *Table 4* found linear relationships between EW and moderators: hen strains (Q_M = 9.56; p = 0.023) and *Lactobacillus spp*. (Q_M = 13.3; p = 0.010). There was no trace of publication bias across trials that assessed the impact of lactobacillus on EW as presented in *Table 5*. Hens fed *L. acidophilus* produced larger eggs than hens fed *L. plantarum*. Egg mass (SMD = 0.18; 95%

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CI = 0.04, 0.33, I^2 = 4.69%; *Table 2*), HU (SMD = 0.22; 95% CI = 0.05, 0.39, I^2 = 0%; *Table 2*), and EST (SMD = 0.34; 95% CI = 0.14, 0.54, I^2 = 51.51%; *Table 2*) were improved by lactobacillus supplementation. Egg yolk (SMD = −1.39; 95% CI = −1.95, −0.84, I^2 = 95%) and blood cholesterol (SMD = −1.31; 95% CI = −1.59, −1.03, I^2 = 69%) were significantly reduced by lactobacillus supplementation.

Table 7. *Subgroup analyses of moderators on egg production*

Moderators	Dataset	Estimate	95% CI		SE	P value
			Lower	Upper		
Hen strains						
Hyline	7	0.04	−0.10	0.18	0.07	0.578
Lohmann	8	0.43	0.18	0.68	0.13	<0.001
Leghorn	4	0.58	0.32	0.84	0.13	<0.001
Hen age (week)						
<31	13	0.35	0.17	0.54	0.10	<0.001
>31	5	0.06	−0.11	0.23	0.09	0.494
TD (week)						
6-19	15	0.29	0.12	0.46	0.09	<0.001
20-48	5	0.34	0.04	0.65	0.16	0.028
Lactobacillus spp						
L. acidophilus	10	0.36	0.12	0.59	0.12	0.003
L. plantarum	4	0.25	0.01	0.50	0.13	0.045

C^+ number of comparison; CI – confidence interval; TD – treatment duration; SE – standard error

Table 8. *Subgroup analyses of the influence of moderator variables on egg weight*

Moderators	Dataset	Estimate	95% Confidence interval		Standard error	P value
			Lower	Upper		
Hen strains						
Hyline	7	0.10	-0.04	0.24	0.07	0.169
Leghorn	4	0.06	-0.20	0.32	0.13	0.662
Lohmann	8	-0.03	-0.16	0.11	0.07	0.713
Hen age (week)						
<31	13	-0.02	-0.13	0.09	0.06	0.724
>31	5	0.25	0.03	0.48	0.11	0.027
TD (week)						
6-19	15	0.05	-0.08	0.18	0.07	0.424
20-48	5	0.08	-0.08	0.24	0.08	0.315
Lactobacillus spp						
L. acidophilus	10	0.10	-0.03	0.22	0.06	0.137
L. plantarum	5	-0.16	-0.35	0.03	0.10	0.098

Discussion

Probiotic effect

The pooled results revealed that lactobacillus supplementation enhanced FCR and EP in laying hens which is similar to the findings of other researchers (Alaqil et al., 2020; Xu et al., 2020; Liu et al., 2023). This enhancement appears to be related to the action of lactobacillus to optimize the function of the gastrointestinal tracts through

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modulation of gut microbiota composition, production of antimicrobial substances, and competitive exclusion of pathogenic microbes (Mahfuz et al., 2017; Qiao et al., 2019). In addition, this increased EP might be attributed to the ability of lactobacillus to enhance digestion and nutrient uptake in laying hens (Jin et al., 2000). In contrast, Mahdavi et al. (2005) discovered that the inclusion of lactic acid bacteria (LAB) in the laying hen diet had no impact on EP; this disparity could be linked to differences in layer age, genotype, quantity of lactobacillus added to the feed, diet composition, experimental design, etc. Results showed that lactobacillus did not influence FI in laying hens, which contrasted Haddadin et al. (1996), who demonstrated that feeding *L. acidophilus* to Lohmann White laying hens aged 25 weeks increased FI. However, the outcome of this investigation supports the view of Alaqil et al. (2020) and Xu et al. (2020) that lactobacillus had no effect on FI.

The study indicated that layers fed lactobacillus-based diets had improved egg mass with similar EW compared to layers fed standard diets. It is envisaged that supplementation of lactobacillus in a layer diet might optimize nutrient uptake, and this result, in harmony with the findings of Deng et al. (2020), could be interpreted as aligning with this view. Loh et al. (2014) observed that dietary lactobacillus intervention did not influence EW in Lohman brown laying hens. This finding agrees with Kalsum et al. (2012), who discovered that the addition of *L. fermentum* at 5.27×10^8 cells and 2.35×10^9 cells in Japanese quails did not have an effect on EW. This finding confirms the previous report by Panda et al. (2008) that adding *L. sporogenes* to the diet of Leghorn hens increased EP but had no impact on EW. One reason for the lack of a significant effect of in-feed lactobacillus on EW is the inverse association between EP and EW (Panda et al., 2003).

Haugh unit (HU) is considered as a measure of the internal quality of an egg and is calculated as $100 \times \log$ (albumen height $-1.7 \times EW^{0.37} + 7.8$), which was increased by dietary lactobacillus supplementation in the present study. In earlier feeding experiments, feeding probiotic bacteria to layers increased HU (Forte et al., 2015; Xu et al., 2020; Liu et al., 2023). The observed increase in HU in this study suggests the quality of protein in lactobacillus-supplemented diets. This implies that lactobacillus supplementation improves the bioavailability of amino acids required for albumen production.

The eggshell is 96% calcium carbonate, and an egg requires approximately 2-2.5 g of calcium (Al-Batshan et al., 1994). Chickens lay fewer and larger eggs with thinner shells as the hen ages (Jacqueline et al., 2014). This study showed that hens fed lactobacillus produced eggs with thicker shells compared to hens fed a diet without lactobacillus supplementation; implying that lactobacillus may promote better absorption of calcium from the intestine. This finding could be attributed to the proliferation of LAB, which lowers intestinal pH and promotes mineral ionization by upregulating the expression of ITM2C, ABCC9, ITPR2, KNDC1, KCNJ8, and WNK1 genes, which are involved in ion transport (Wilson, 2017; Ogbuewu et al., 2022). This observation implies that lactobacillus could be employed to solve the usual decline in eggshell quality as the hen ages (Darsi and Zhaghari, 2021), as this stability could result in fewer eggs being broken due to thin shells.

Cholesterol is a component of lipid membranes found in all the cells in the body and a precursor of bile acids. Meat and eggs are the primary dietary sources of cholesterol (Ahn et al., 1999). The adverse health effect of excess dietary cholesterol coupled with its hepatic biosynthesis has been highlighted (Cha and Park, 2019). The reduction in

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yolk and blood cholesterol content by lactobacillus in the present study, suggests beneficial effects on hen health and nutrition. This result is consistent with earlier reports showing that dietary lactobacillus decreased yolk cholesterol levels in layers (Loh et al., 2014; Alaqil et al., 2020). This implies that lactobacillus reduces cholesterol in the blood by deconjugating bile salts in the intestinal tract, thus stopping cholesterol from acting as a precursor in the biosynthesis of bile acid. A similar result has been found in animals other than chickens (Kalsum et al., 2012). The mechanism underlying the reduction of cholesterol in the blood and the yolk by dietary lactobacillus supplementation in laying hens is poorly understood. The drop, on the other hand, is probably due to down regulation of the expression of 3-hydroxy-3-methylglutaryl-CoA reductase and related genes in the chicken liver (Deng et al., 2020).

Moderators

Age is a limiting factor on FCR and EP in this meta-analysis, confirming the earlier findings of others (Jacqueline et al., 2014; Dogara et al., 2021; Ogbuewu and Mbajiorgu, 2023), who reported that laying performance is affected by genotype and environmental factors such as nutrition, age, and temperature and their interactions. Egg production in chickens begins between the ages of 18 and 21 weeks (depending on the breed) and increases steadily over the next 6 to 8 weeks, peaking at about 30 weeks of age (Jacqueline et al., 2014). The larger magnitude of effect estimates for FCR and EP in younger pullets compared to older pullets could be explained by a decline in follicular development and recruitment into the rapid growth phase observed as layers advance in age (Joyner et al., 1987). Our results showed a large effect of layer strain as a moderator, and greater than 50% of the treatment effect on FI, EP, and EW was explained by layer strain, corroborating the results of Ogbuewu et al. (2021) that chicken breed and strains affect laying performance. The Lohmann and Leghorn strains fed lactobacillus-supplemented diets recorded higher EP and EW than the Hyline strain, implying that the action of lactobacillus was more pronounced in the Lohmann and Leghorn strains. This could be attributed to the fact that Lohmann and Leghorn strains have similar genetic backgrounds (Silversides et al., 2012).

Lactobacillus spp. and supplementation dose are limiting factors in FCR, EP, and EW in this study and explain greater than 50% of the treatment effect on these parameters. The strong and significant linear relationships between lactobacillus (*L. sporogene* and *L. plantarum*) and FCR in this study indicate that FCR was improved as the level of *L. sporogene* and *L. plantarum* in the ration was increased. Results also indicate that the magnitude of effect size for EP and FCR was larger in layers fed higher doses of lactobacillus than those offered lower doses. Alaqil et al. (2020) found improved FCR and EP in hens fed *L. acidophilus* culture at 3.0×10^9 cfu/kg compared to those fed 1.0×10^9 cfu/kg, implying that inclusion of high doses of lactobacillus in chicken feed would hasten the adherence and colonization process in the gastrointestinal tracts. The probiotic effect of lactobacillus on EP in the current study was evident only in layers administered a high dose of *L. acidophilus* (i.e., 4.0×10^6 cfu/kg) for 16 weeks, confirming the earlier findings that probiotics must be administered at the right amounts to elicit beneficial effect on the host health (Mahfuz et al., 2017; Abd El-Hack et al., 2020). This study also found a positive linear relationship between treatment duration and EP. However, more research is needed to ascertain the effect of supplementing high doses of lactobacillus for more than 16 weeks on EP; as such information is lacking in the literature.

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Publication bias

Publication bias, which is the tendency of journal editors to publish studies with positive results over negative findings, tends to undermine the credibility of the outcomes of meta-analysis. Jennions et al. (2013) stated that a meta-analysis outcome can be deemed robust in spite of publication bias if the fail-safe number is >($5N_{study}$ + 10). Thus, publication bias was not an issue in this study, as it would take a relatively large number of unpublished studies to render the observed significant effect on FCR and EP to non-significant effect.

Conclusion

In summary, it is concluded that feed conversion ratio, egg production, egg mass, Haugh unit, eggshell thickness, blood, and yolk cholesterol content were positively influenced by dietary lactobacillus supplementation in layers. In contrast, this study suggests that feed intake and egg weight were increased by lactobacillus in laying hens. Due to low sample size, the current study did not assess the dose levels of lactobacillus that optimize performance traits in layers. It is recommended that a regression model be used to determine the levels of lactobacillus that optimized all the performance parameters in laying hens. Given the high cost of lactobacillus culture and preparation, future trials should center on determining the economic benefits of using lactobacillus to improve the performance of laying hens. Furthermore, further research should be done to understand the mechanisms in layers fed lactobacillus supplemented diet, as such research data is lacking.

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