Research Article

Lactobacillus as Growth Promoter: A Meta-Analysis of Performance, Histology and Microbiota on Broiler Tract Digestive

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Abstract | Meta-analysis of lactobacillus was built to evaluate the performance, histology and microbiota of lactobacillus on digestive tract of broilers. A database was built from previously published article from internet reporting lactobacillus as feed additives in broilers. Articles were strictly selected according to evaluation of title, abstract and parameter which used in the study. Database collected was statistically analyzed using the mixed model method with different study as random effect and level of lactobacillus as fixed effect using SAS program. Lactobacillus as potential feed additive had significant influence ($p<0.05$) to improve performance such as average daily gain (50.28 g), average feed intake (93.57 g) and feed conversion ratio (1.91) of broilers in ameliorate condition of digestive tract by decreasing the amount of *Escherichia coli*. Due to pathogen bacteria decrease, the histologic structures of digestive tract encounter improvement through minimizing damage of villus. In conclusion, lactobacillus supplementation in broilers increase performance due to improvement in the digestive tract and decrease in pathogenic bacteria with 5 x 10^{-7} cfu $\log^{-1}$ *Lactobacillus* population recommendation.

Keywords | Intestinal, Microorganism, Feed efficiency, Mix model, Systematic review

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INTRODUCTION

The European nation aim to develop production that efficiently uses such as feed and renewable energy. Determination of novel solution meet the animal feed requirement balancing of production animals is key to development of animal industry in future trends (Adli, 2021). Indonesia facing several problems such as availability of the raw materials, and producing healthy meat (Adli et al., 2022). One of regulation has been established by government were prohibition of the use of antibiotic growth promoters on broiler chicken production. Prohibition on the use of Antibiotic Growth Promoter (AGP) as feed additives is stipulated in Minister of Agriculture regulation in 2017 concerning the classification of veterinary drugs. The regulation clarifies the mixing of veterinary drugs in feed for therapy based on instructions and under the supervision of a veterinarian.

Prohibition on the use of AGP on broilers can reduce the productivity. However, there is actually a way to keep the broilers performing well by using ingredients derived from nature. Natural ingredients do not cause any side effects on the host and researchers are looking for them to replace antibiotics. Resistance problem has become huge clinical and public health problem nowadays and will face multiresistant disease (Levy, 2002). Phytochemicals (Lillehoj et al., 2018; Prihambodo et al., 2022), probiotics and their derivate (Silva et al., 2020) and other metabolites are potential as antibiotics.

Probiotics are good bacteria with many types. *Lactobacillus* is one of the types of with abundant amount in fermentation products. Every fermentation product mostly produce *Lactobacillus* and it works optimally with the presence of a material providing an optimal environment. This condition results in an increase in the number/population of probiotics in the gastrointestinal tract. An increase in the *Lactobacillus* population results in an increase in the digestibility and absorption of nutrients resulting in an increase in performance. Actually, *Lactobacillus* has various mechanisms to improve performance, but the principal mechanism of *Lactobacillus* is by working anaerobically so pH of the digestive tract drops, and inhibits the development and growth of pathogenic bacteria.

Even mechanism of *Lactobacillus* as alternative antibiotics seems promising, but another report shows different results. Systematic review such as meta-analysis helps researchers find out inconsistency from several studies to conclude. Meta-analysis refers to a quantitative and methodical strategy creating a continuous analysis of previous studies (Hidayat et al., 2021). Meta-analysis can also be used to quantitatively verify the type of findings in a study. Therefore, this study aimed to evaluate, using a meta-analysis of previously published articles, the effects of *Lactobacillus* on the performance and intestine condition of broiler.

MATERIALS AND METHODS

Database development

Database established for this meta-analysis were collected from published articles in multiple search engines for scientific paper such as Google Scholar, Scopus and Science Direct using keywords "lactobacillus" and "broiler". 48 articles have been collected discussing *Lactobacillus* as feed additives for broilers but only 38 articles were chosen as potential articles based on its title and abstract. Diagram flow of article selection in the meta-analysis using Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) method was reported in Figure 1. The parameters chosen were (1) productivity of broilers: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR), (2) histologic structure of intestine: villus height, crypt depth and ratio of villus height and crypt depth and (3) gastrointestinal microbiota specifically in caecum and ileum.

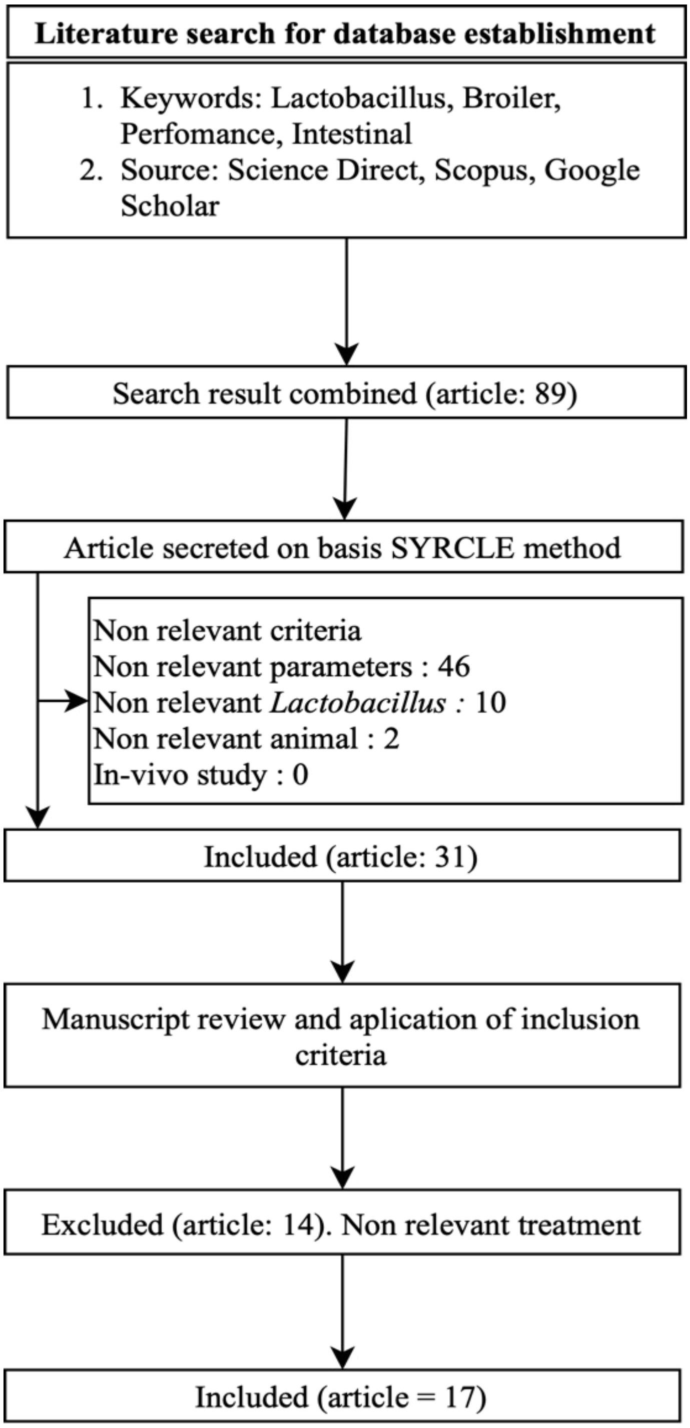

Figure 1: Diagram flow of article selection in the meta-analysis using systematic review centre for laboratory animal experimentation (SYRCLE) method.

After strict evaluation of 38 articles, 17 articles were selected and reported in Table 1 based on their numerical results, confirmed specific species and dosage of the *Lactobacillus*. All parameters have been concluded based on descriptive method and reported in Table 2. All parameters have equal units as a requirement of meta-analysis such as average daily gain and feed intake were expressed in g/day, FCR was g/g, villus height and crypt depth were µm, microbiota population were log cfu/g, and dosage of *Lactobacillus* was in cfu/g.

Table 1: Articles included in the meta-analysis.

No	Article	*Lactobacillus*		Broiler	
		Species	Dosage (cfu/g)	Breed	Age (d)
1	Wu *et al.*	*L. acidophilus*	0 – 1 x 10^9	Cobb	1 – 21
2	Wang *et al.*	*L. plantarum*	0 – 2 x 10^6	Cobb	1 – 42
3	Peng *et al.*	*L. plantarum*	0 – 2 x 10^9	Arbor Acres	1 – 42
4	Li *et al.*	*L. acidophilus*	0 – 4 x 10^6	Arbor Acres	1 – 21
5	Jahromi *et al.*	*Mixed Lactobacillus*	0 – 1 x 10^9	Cobb	1 – 35
6	Shokyazdan *et al*	*L. salivarius*	0 – 1 x 10^9	Cobb	1 – 42
7	Qing *et al.*	*L. johnsonii*	0 – 1 x 10^6	Cobb	1 – 28
8	Chen *et al.*	*Mixed Lactobacillus*	0 – 1 x 10^6	Arbor Acres	1 – 35
9	Wang *et al.*	*L. plantarum*	0 – 1 x 10^8	Arbor Acres	1 – 42
10	Vantsawa *et al.*	*L. acidophillus*	0 – 1 x 10^9	Arbor Acre+	1 – 42
11	Wu *et al.*	*L. acidophillus*	0 – 1 x 10^{10}	Arbor Acres	1 – 42
12	Yang *et al.*	*L. plantarum*	0 – 1 x 10^9	Cobb	1 – 42
13	Cholis *et al.*	*Mixed Lactobacillus*	0 – 1 x 10^8	Lohmann	1 – 42
14	Wang *et al.*	*L. johnsonii*	0 – 1 x 10^6	Cobb	1 – 42
15	Wang *et al.*	*L. plantarum*	0 – 1 x 10^8	Arbor Acres	1 – 42
16	Vineetha *et al.*	*L. plantarum*	0 – 1 x 10^8	Caribo Dhanraja	1 – 35
17	Liu *et al.*	*L. plantarum*	0 – 1 x 10^8	Cobb	1 – 21

Table 2: Descriptive statistics of the chosen article of meta-analysis.

Response parameter	Unit	Mean	SD	Min	Max
Performance					
Starter ADG	g/day	32.97	10.52	20.81	79.19
Starter ADFI	g/day	47.96	11.99	33.65	80.48
Starter FCR	g/g	1.500	0.162	1.280	1.860
Finisher ADG	g/day	64.15	20.67	27.40	89.23
Finisher ADFI	g/day	129.3	35.71	58.09	171.9
Finisher FCR	g/g	1.843	0.203	1.460	2.170
Total ADG	g/day	50.22	10.55	29.97	63.32
Total ADFI	g/day	92.53	16.00	63.54	111.5
Total FCR	g/g	1.880	0.402	1.340	3.280
Intestine histology					
Duodenum villus height	µm	1017	169.1	790.5	1316
Duodenum crypt depth	µm	182.8	40.23	144.6	236.0
Duodenum V: H	µm/µm	5.826	1.756	4.450	9.100
Jejunum villus height	µm	890.9	228.2	527.9	1247
Jejunum crypt depth	µm	154.6	50.97	95.56	237.9
Jejunum V: H	µm/µm	6.035	0.953	4.650	8.130
Ileum villus height	µm	687.1	200.8	349.4	968.0
Ileum crypt depth	µm	155.5	53.67	52.78	223.8
Ileum V: H	µm/µm	4.822	1.243	3.470	4.822
Intestine microbiota					
Caecum *Lactobacillus*	Log cfu/g	8.577	0.308	7.900	9.010
Caecum *E. coli*	Log cfu/g	6.587	0.450	5.920	7.280
Ileum caecum	Log cfu/g	8.251	0.514	7.350	8.980
Ileum *E. coli*	Log cfu/g	5.301	1.258	4.180	7.180

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Statistical analysis

Database were processed for statistical analysis using mixed model procedure in linear and quadratic model for meta-analysis (Sauvant et al., 2008; Prihambodo et al., 2021). Statistical analysis was conducted using SAS on demand for academic with PROC MIXED procedure. *Lactobacillus* addition dosage was used as fixed effect, while the studies were as random effect. The significance value was set as $p<0.05$.

Lactobacillus dosage was used as continuous predictor in which the response variables were regressed using the mathematical model:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij}$$

Where, Yij = dependent variable, B0= overall intercept across all studies (fixed effect), B1= linear regression coefficient of Y on X (fixed effect), B2= quadratic regression coefficient of Y on X (fixed effect), Xij = value of the continuous predictor variable (*Lactobacillus* addition level), si= random effect of study i, bi= random effect of study i on the regression coefficient of Y on X in study i, eij= the unexplained residual error while a low akaike information criteria (AIC) value indicates that the model is better at describing the observed data. The smaller the difference in AIC values between the two models, the smaller the difference in model quality between the two. Meanwhile, intercept is used to assist in understanding how the independent variable affects the dependent variable. The intercept can also provide information about the baseline or initial value of the dependent variable before the independent variable influences it.

Table 3: *Lactobacillus* addition effect on performance of broiler chicken.

Response parameter	Model	n	Parameter estimates				Model estimates	
			Intercept	SE intercept	Slope	SE slope	P-value	AIC
Starter								
ADG	Q	49	32.94	2.460	-0.001	0.001	<0.001	338.7
					0.010	0.018	<0.001	327.9
ADFI	Q	52	48.08	2.620	-0.001	0.001	<0.001	316.5
					0.002	0.008	<0.001	304.3
FCR	Q	52	1.512	0.034	0.001	0.001	<0.001	-58.90
					-0.001	0.001	<0.001	-76.70
Finisher								
ADG	Q	49	66.11	4.281	-0.002	0.002	<0.001	367.8
					0.018	0.026	<0.001	358.1
ADFI	Q	46	128.3	7.914	-0.010	0.002	<0.001	390.5
					0.012	0.034	<0.001	380.4
FCR	Q	46	1.866	0.045	0.001	0.001	<0.001	0.200
					-0.001	0.001	<0.001	-17.00
Total								
ADG	Q	37	49.78	2.403	-0.001	0.001	<0.001	247.1
					0.019	0.017	<0.001	236.7
ADFI	Q	40	91.28	4.117	0.001	0.001	<0.001	271.5
					0.003	0.013	<0.001	260.4
FCR	Q	40	1.881	0.087	0,001	0,001	<0.001	46.70
					-0.001	0.001	<0.001	31.60

SE, standard error; AIC, akaike information criteria; Q, quadratic; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

RESULTS AND DISCUSSION

In fact, all parameters had significant influence ($p<0.05$) due to *Lactobacillus* addition in positive way. Statistical analysis of this study was reported in Table 3. In this study, broilers gained good FCR score in starter, finisher and total phase. Average FCR of its respective phase were 1.5 ± 0.162, 1.843 ± 0.203 and 1.88 ± 0.402 g/g. Those FCR supported ADG in highest weight, namely 32.97 ± 10.52, 64.15 ± 20.67 and 50.22 ± 10.55 g, respectively. It can't be separated due to escalation of intestine histology reported in Table 4.

Good condition in productivity of broilers cannot be separated from well-maintained intestinal ecosystem. Based on Table 5, *Lactobacillus* addition boosted *Lactobacillus* population and reduced *Escherichia coli* population. The population of *Lactobacillus* in intestine especially in caecum and ileum was significantly boosted ($p<0.05$) due to *Lactobacillus* addition in feed to 9.010 and 8.980 log cfu/g, respectively. Opposite result showed by *Escherichia coli*, the addition of *Lactobacillus* suppressed its population to support the performance of broilers.

The histologic structures of organs in digestive tract were significantly affected ($p<0.05$) due to *Lactobacillus* addition both in quadratic and linear model. Duodenum and ileum support the performance improvement in broilers even though jejunum did not. Duodenum and ileum construct good condition in intestine with average unit of 1017 ± 169.1 and 687.1 ± 200.8 µm, respectively and 5.826 ± 1.756 and 4.822 ± 1.243 µm/m for their villus height. Jejunum had a minimum trend due to *Lactobacillus* specifically in its villus height and ratio of villus and crypt depth due to linear decrease in crypt depth.

The results above have demonstrated the effect of *Lactobacillus* addition to broilers specifically in their performance based on their histologic structures and gastrointestinal microbiota. These results are in line with (Jahromi et al., 2017; Wang et al., 2017b; Fesseha et al., 2021), both in mixed or single *Lactobacillus* species. The capability to boost performance is inseparable from the power of *Lactobacillus* to modify or modulate such as regulate the microbial population in the digestive tract thereby influencing the immune response to efficiently absorb nutrients. *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus johnsonii, Lactobacillus salivarius* and mixed *Lactobacillus* were used in this study with each bacterium has its own mechanism to encourage the performance of broilers. *Lactobacillus acidophilus* has a mechanism by directly fermenting nutrients in the stomach (Jin et al., 2000),

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Table 4: *Lactobacillus* addition effect on intestine histology of broiler chicken.

Response parameter	Model	n	Parameter estimates				Model estimates	
			Intercept	SE intercept	Slope	SE slope	P value	AIC
Duodenum								
Villus height	L	49	1001	75.18	17.85	6.571	<0.001	112.5
Crypt depth	Q	52	180.1	18.14	-0.520	0.165	<0.001	78.20
					-0.270	0.473	<0.001	82.50
V:C	L	52	5.750	0.553	0.042	0.042	0.002	37.30
Jejunum								
Villus height	Q	49	865.4	74.47	2.621	1.297	<0.001	318.8
					3.780	4.581	<0.001	324.1
Crypt depth	L	46	147.9	15.60	-0.047	1.129	<0.001	253.7
V:C	Q	46	6.090	0.284	0.018	0.018	<0.001	83.40
					0.011	0.037	<0.001	77.80
Ileum								
Villus height	Q	37	670.8	69.16	-0.590	0.608	<0.001	285.9
					-0.165	3.104	<0.001	287.6
Crypt depth	Q	40	148.4	17.95	-0.010	0.125	<0.001	219.8
					-0.041	0.494	<0.001	215.4
V:C	Q	40	4.958	0.421	-0.002	0.005	<0.001	74.10
					-0.001	0.019	<0.001	63.80

SE, standard error; AIC, akaike information criteria; Q, quadratic; L, linear; V:C, ratio of villus height and crypt depth.

Table 5: *Lactobacillus* addition effect on microbiota population intestine of broiler chicken.

Response parameter	Model	n	Parameter estimates				Model estimates	
			Intercept	SE intercept	Slope	SE slope	P-value	AIC
Ileum								
Lactobacillus	Q	11	8.156	0.308	-0.001	0.001	<0.001	33.4
					0.004	0.001	<0.001	20.2
E. coli	Q	11	5.679	0.574	0.001	0.001	<0.001	32.8
					-0.003	0.001	0.001	22.9
Caecum								
Lactobacillus	Q	11	8.440	0.161	-0.001	0.001	<0.001	27.2
					0.003	0.002	<0.001	13.8
E. coli	Q	11	6.787	0.158	0.001	0.001	<0.001	24.5
					-0.007	0.002	<0.001	11.2

SE, standard error; AIC, akaike information criteria, Q, quadratic.

Lactobacillus plantarum stimulates protective immune responses (Wang et al., 2015), *Lactobacillus johnsonii* assesses changes in lipid metabolism, gut microbiota, gut development, and digestive abilities (Wang et al., 2017). As mentioned above, it can be theoretically meaningful that *Lactobacillus* can replace antibiotics.

Supplementing diets with probiotics is one of the promising methods for preventing and treating bacterial illnesses. It is necessary for lactobacilli to get past physical and chemical barriers, such as stomach acid and bile in the gut, in order to exert health-promoting probiotic effects. Stimulating and modifying digestive tract increase the performance of broilers. One of the indicators representing the optimization of feed to performance is feed conversion ratio (Homma et al., 2021). This study reported that of all phase in broilers with minimum trend in quadratic model, 5 x 10^7 $cfug^{-1}$ was the best dosage of *Lactobacillus*. The *Lactobacillus* addition to FCR parameter was analogous with Huang et al. (2004) and Mountzouris et al. (2010). This meta-analysis also validated overall performance parameters such as ADG and ADFI in all phase. In quadratic model, negative slope indicates maximum trend of *Lactobacillus* in representing the feed intake increase of broilers which in line with previous studies (Abdel-Hafeez et al., 2017; Rehman et al., 2020). The increase in feed intake and palatability (Jia et al., n.d.) is due to natural fermentation products such as acetic acid and biogenic amine (Lee et al., 2020).

Higher ADFI and lower FCR with an increase in ADG at maximum point cannot be separated with the histologic structure of broilers and the capability of *Lactobacillus* to produce digestive enzymes. The growth performance is improved by the secreted amylolytic, cellulolytic, proteolytic, and lipolytic enzymes because they increase the digestibility of starch, protein, and fat components and release the most energy. Furthermore, overall histologic structure of gut showed better condition than control. The high villi of duodenum, jejunum and ileum and supported by low crypt depth are notable parts of digestive tract related to immune health (Wu et al., 2021), stress control (Wu et al., 2021) and nutrient absorption (Cholis et al., 2018). Villus height of duodenum and ileum showed improvement than control with the better results. The longer the villus, the less damage can be caused by external factors. Each *Lactobacillus* species has each capability to increase villus height such as *Lactobacillus acidophillus* by producing enzyme to stimulate small intestine peristalsis (Wu et al., 2021), *Lactobacillus plantarum* by affecting mucosal immunity and the gut barrier (Wang et al., 2015), *Lactobacillus johnsonii* by balancing gut microflora in small intestine thereby healing the damaged mucosa through the renewal of epithelial cells (Dvorak, 2010), *Lactobacillus salivarius* by supporting the gut to reduce the enterocytes damage and renew it (Perić et al., 2010).

All mechanisms of *Lactobacillus* in this study are associated with reducing damage of intestine by producing digestive enzyme (Dudley et al., 2018; Zijlstra et al., 1997; Kyoung et al., 1998; Fathima et al., 2022) due to renewal cell of intestine such as villus height. Table 2 shows the correlation of good intestinal villi and an increase in broiler performance. The primary elements involved in nutrient absorption in the small intestine are villi. Epithelium surface of intestine area is increased by high villi for better nutrient absorption (Loh et al., 2010). Normally, pathogen microflora in intestine invades villi surface (Ritchie et al., 2012; Fathima et al., 2022) by altering their permeability resulting in chronic inflammation of intestine epithelium which leads to a decrease in villi size (Loh et al., 2010). In addition, a defense mechanism against other undesired bacterial colonization from the cecum, or control ileal flora is the bacterial adhesion to the ileal epithelial wall (Khonyoung and Yamauchi, 2012).

In other way, metabolites of *Lactobacillus* producing bacteriocin and organic acids help the immune system of broilers to inhibit the growth of pathogen bacteria. Both in ileum and caecum, *Lactobacillus* reduce the amount of *Escherichia coli* and making them a natural probiotic. The potential use of lactic acid bacteria (LAB)-produced bacteriocins as a non-toxic and secure bio-preservative to increase food safety has garnered a lot of attention (Lv et al., 2018). Bacteriocin is stable in acidic condition (Iranmanesh et al., 2014) and inhibits the growth of *Escherichia coli* (O'Shea et al., 2012) by transporting small ions like K^+ and Na^+ as essential electrolytes through the bacterial cell membrane, promoting cell membrane activities, and maintaining correct enzyme activity. Increased electrolyte release will signify the disrupted permeability barrier (Diao et al., 2014; Iranmanesh et al., 2014). Along with Na^+ and K^+, adenosine triphosphate (ATP), and nucleic acids are ingredients of membrane constituents (Bajpai et al., 2013) to identify certain intracellular components. Leakage markers serve as a measure of the membrane's resistance to a particular antimicrobial agent in comparison to untreated cells.

Due to the lipophilic character of their undissociated state, organic acids have the ability to permeate cell membranes and alter the amounts of related anion and proton in the cytoplasm. Genetic, age, and sperm factors also related to the cell membranes production in the cytoplasm (Kusumawati et al., 2019; Susilawati et al., 2017, 2020). As a result, purine bases and crucial enzymes are affected, and bacterial viability is reduced (Warnecke and Gill, 2005; Gómez-García et al., 2019). *Escherichia coli* is one kind of pathogen bacteria categorized as gram negative bacteria with its membrane cell Gómez-García et al. (2019) aims to form organic acids especially formic acid. In addition to the metabolites, the capability of *Lactobacillus* is one of main factor how *Lactobacillus* work as antibiotic. Alp and Kuleaşan (2019) reported some factors for *Lactobacillus* to bind with intestine such as (a) mucus binding protein; (b) lipoteichoic acid (c) extracellular polysaccharides and (d) flagella and pili. Intestinal mucus has main role as the protection of epithelial surfaces against pathogens by maintaining a favorable environment for digestion thereby allowing the movement of nutrients from the lumen to the underlying epithelium. Douillard et al. (2013) reported pili by *Lactobacillus* increased mucus-binding activity. However, findings about the adhesion mechanism of *Lactobacillus* have not clearly explained. The binding of epitopes on carbohydrate chains and type of several reason become an obstacle and need to be investigated in the future (Nishiyama et al., 2016).

CONCLUSIONS AND RECOMMENDATIONS

The present meta-analysis concludes that overall *Lactobacillus* addition in broilers can increase performance due to improvement in the digestive tract and decrease in pathogenic bacteria with 5 x 10^{-7} cfu log^{-1} *Lactobacillus* population recommendation. Future research in this area is required, specifically in separated *Lactobacillus* strain since different bacterial strains could produce different outcomes.

NOVELTY STATEMENT

The current study, shows the best evaluation and dosage of the addition of various types of lactobacillus in broilers which are seen in performance, histology and digestive tract profile through a meta-analysis approach.

AUTHOR'S CONTRIBUTION

BH, TRP and WW conducted the experiments, analyzed the data, and drafted the article. TRP reviewed the data analysis and revised the draft article. BH and SR supervised the experiment. MB, EAR and FMS designed the experiment, reviewed the data analysis, and revised the article draft.

Ethical approval

Ethical approval is not required for meta-analysis study.

Conflict of interest

The authors have declared no conflict of interest.

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