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Lactobacillus as growth promoter: a meta-analysis of performance, histology and microbiota on broiler tract digestive. Commented [BR1]: This article has been proof read. The certificate is attached in attachment section BAMBANG HARTOYO1,*, TRI RACHMANTO PRIHAMBODO1,2, WAHYUNINGSIH3, SRI RAHAYU1, FRANSISCA MARIA SUHARTATI1, MUHAMAD BATA1, EFKA ARIS RIMBAWANTO1 1Animal Science Faculty,

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18**Corresponding author e-mail**: bambang.hartoyo@unsoed.**ac.id ABSTRACT Meta-analysis** of lactobacillus **was** built **to** evaluate **the**

performance, histology and microbiota of lactobacillus on digestive tract

2**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

> 1**was statistically analyzed using** the **mixed model** method with **different** study **as random** effect **and** level **of**

0.05) to improve performance such as

> 29**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, 1 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 INTRODUCTION

> 12**The European nation aim to develop production that efficiently uses such as feed and renewable energy**

.

> 8**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

> 7**prohibition of the use of Antibiotic Growth Promoters**

on Broiler chicken production. Prohibition

> 28**on the use of Antibiotic Growth** Promoter (**AGP**) as **feed** additives **is**

stipulated in Minister of Agriculture regulation in

> 36**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

> 6**et al., 2018**; Prihambodo **et al., 2022**), probiotics **and** their derivate (Silva **et al**

., 2020) and other metabolites are potential as antibiotics. 2 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

> 21**in an increase in the number**/population **of** probiotics in **the** gastrointestinal tract. **An increase in**

the Lactobacillus population results in

> 32**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

> 7**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

> 2**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,

> 19**this study aimed to** evaluate, using **a meta-analysis of** previously **published** articles, **the effects of**

Lactobacillus

> 35**on the performance and** intestine condition **of broiler. MATERIAL AND**

METHOD Ethical approval

> 13**Ethical approval is not** required **for** meta-analysis **study. Database development** Database established for **this meta-analysis**

were collected from published articles in

> 4**multiple search engines for scientific** paper **such as Google Scholar, Scopus** and **Science**

Direct 3 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.

> 2**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

> 3**were (1**) productivity **of** broilers: **average daily gain (ADG), average daily feed intake (ADFI**) and **feed conversion ratio (FCR**

), (2) histologic structure of intestine:

> 14**villus height, crypt depth and ratio of villus height** and **crypt depth and**

(3) gastrointestinal microbiota specifically in caecum and ileum. After strict evaluation of 38 articles, 17 articles were selected and reported in Error! Reference source not found. 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 Error! Reference source not found.. All parameters have equal units as a requirement of meta-analysis such as

> 5**average daily gain and feed intake were** expressed **in**

g/day, FCR was g/g,

> 38**villus height and crypt depth were**

?m, microbiota population were log cfu/g, and dosage of Lactobacillus was in cfu/g. Statistical analysis Database

> 4**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.

> 17**The significance** value **was set** as **p<0.05**. Lactobacillus dosage **was**

used

> 4**as continuous predictor** in which **the response variables were regressed using the mathematical model**

: □□□ = □0 + □1□□□ + □2□□2□ + □□ + □□□□□ + □□□ 4

> 1**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**

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 ?m respectively and 5.826 ? 1.756 and 4.822 ? 1.243 ?m/?m for their villus height. Jejunum had a minimum trend due to Lactobacillus specifically in its

> 5**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.

> 23**These results are in line with** (Jahromi **et al**., 2017; Wang **et al**

., 2017 b; 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,

> 34**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), Lactobacillus plantarum stimulates protective immune responses (Wang et al., 2015), Lactobacillus johnsonii assesses

> 15**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 6 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 107 cfu g-1 was the best dosage of Lactobacillus. The Lactobacillus addition to FCR parameter was analogous with

25**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

22**in line with previous studies** (Abdel-Hafeez **et al**., 2017; Rehman **et al**., 2020). **The**

30**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

10**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 (Wang 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., 7 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., n.d.; Zijlstra

27**et al., 1997**; Kyoung **Park et al., 1998**; Fathima **et al**

., 2022) due to renewal cell of intestine such as villus height. Error! Reference source not found. 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

26**which leads to a decrease in** villi size (Loh **et al., 2010**

). In addition,

20**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 et al., 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

31**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 8 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

33**et al., 2019**; Susilawati **et al**., 2017; Susilawati **et al., 2020**

). As a result,

24**purine bases and** crucial **enzymes are affected, and bacterial viability**

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) CONCLUSION 9 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. AUTHORS CONTRIBUTION BH, TRP and WW conducted the experiments, analyzed

6**the data, and drafted the article**. TRP **reviewed the**

9**data analysis and revised the** draft **article**. BH **and** SR **supervised the experiment**

. MB, EAR and FMS

9**designed the experiment**, reviewed the **data analysis, and revised the article draft**

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11**CONFLICT OF INTEREST The authors declares no conflict of interest regarding the publication of this article**

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