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Potential of *Lactobacillus* sp. as an alternative substitute for antibiotic growth promoter (AGP) on production performance in broilers infected by *Escherichia coli*

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Abstract

This study investigated the efficacy of probiotics in increasing production performance (feed intake, feed conversion ratio, and body weight gain) in broiler chickens infected by *Escherichia coli*. This experimental study utilized a completely randomized design and involved forty male broilers. The broilers were inoculated with *Escherichia coli* at 21 days of age, and the experiment extended over a 35-days period. The study consisted of 4 treatment groups, with 10 birds in each group. The treatment groups were, P0 (negative control without feed additive plus no *Escherichia coli* infection), P1 (positive control without feed additive but with *Escherichia coli* infection), P2 containing *Zinc Bacitracin* as an antibiotic growth promoter added at 1 g/kg mixed through commercial feed and without probiotic *Lactobacillus* sp. with *Escherichia coli* infection), as well as, P3 (commercial feed without AGP and probiotic *Lactobacillus* sp. added at 5 mL/L through drinking water with *Escherichia coli* infection). A repeated measures general linear model was employed to analyze the data, with subsequent comparisons using Duncan's Multiple Distance tests. The results indicated statistically significant variations ($p<0.05$) in production parameters such as feed intake, feed conversion ratio, and body weight increase across the treatment groups. In conclusion, the administration of probiotic *Lactobacillus* sp. added at 5 mL/L through drinking water improved production performance in broilers infected with *Escherichia coli*.

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Introduction

The chicken farm contributes greatly to the country's income by exporting carcasses, eggs and feathers, which helps control inflation. Chicken is also an important commodity in fulfilling animal protein needs in Indonesia (1). The Republic of Indonesia's 2022 Statistics of Animal Husbandry and Animal Health reported a 60.22% increase in poultry meat consumption by Indonesians in 2021 (2). Chicken farm management is critical to ensure good chicken production, as chicken meat consumption directly impacts chicken production. Some important aspects of chicken farm management include *day-old-chicks* (DOC), housing system, rearing, chicken health management, and feed (3,4). Chicken diseases are numerous and frequently exhibit almost identical symptoms (5). Various factors, such as infection with viruses, bacteria, fungi, protozoa, worms, and lice can cause illness. The most frequent illness in broilers is colibacillosis (6,7). Colibacillosis is an infectious poultry disease caused by the bacterium *Escherichia coli* (*E. coli*). This infection occurs in broilers and layers as well as other poultry including ducks, turkeys, and other avian spp. (8-10). According to Fuhrmann (11), the impact of economic losses from *Escherichia coli* infection on chickens is caused by decreased hatchability, decreased production, high mortality, and additional medical costs. The treatment that still often found in poultry farms in Indonesia is by using antibiotic growth promoter (AGP) as feed additives (4,12). Nevertheless, the widespread use of antibiotics in Indonesian poultry production has raised concerns due to the escalating issue of antibiotic resistance (13). The indiscriminate and excessive use of antibiotics has led to the development of antibiotic resistance, consequently causing reduced productivity and increased mortality rates in poultry. In response to this crisis, the Indonesian government enacted Law No. 18 of 2009, subsequently amended by Law No. 41 of 2014 (Article 22, Paragraph 4C), which explicitly prohibits the inclusion of hormones or antibiotics in animal feed to mitigate the emergence of long-term antibiotic resistance (12). Prohobiting hormone and antibiotic use in feed additives presents an opportunity to develop alternatives to AGP, such as probiotics. These microbial supplements have the potential to enhance livestock production while bolstering chickens' natural defenses against *Escherichia coli* infections (14-17). Based on the previous studies from Yulianto (18) and Lokapirnasari (16), this current study used the administration of probiotic *Lactobacillus* sp. through drinking water.

This study aimed to evaluate the potential of probiotic *Lactobacillus* sp. administered via drinking water to enhance production performance measured by feed intake (FI), feed conversion ratio (FCR), and body weight gain (BWG) in *Escherichia coli*-infected broilers. These findings contribute to the growing body of evidence supporting *Lactobacillus* sp. as a viable alternative to AGP.

Materials and methods

Ethical approval

Prior to commencing this study, formal ethical clearance was secured from the Animal Ethics Commission, Faculty of Veterinary Medicine, University Airlangga (Approval number: 1.KEH.017.01.2024).

Study duration and site

The study was conducted over a 35-day period spanning April to May 2024. The experimental site was located at Mr. Zain's poultry cages within the Al Baihaqi Farm (Panjalu Cattle Feedlot) on Gobang Street, Blabak, Pesantren Sub-district, Kediri City, East Java, Indonesia.

Materials and instruments

The study utilized *Escherichia coli* (1.5 x 108 CFU/mL) obtained from the private collection of Dr. Fidi Nur Aini E. P. D, DVM, MSi, and *Lactobacillus* sp. (1.2 x 109 CFU/mL) from the private collection of Prof. Dr. Widya Paramita Lokapirnasari, DVM, MP. Commercial broiler feed (CP-511) was purchased from PT. Charoen Pokphand Indonesia. *Coliform* and *Escherichia coli*-free well water was used as drinking water. Husk for cage bedding during the brooding period was sourced from a local rice mill on Gobang Street, Blabak, Pesantren Sub-district, Kediri City, East Java. A total of 40 male broiler chicks were procured from PT. Japfa Comfeed Indonesia for the experiment.

The study employed the following equipment: 15 ml and 1 ml syringes, a modified 1.5 L mineral water bottle, masks, gloves, label paper, markers, a hand sprayer, feed and drink bins, cage cleaning tools, trash bags, gasolec (Gasolec™ S8 model; the netherlands), a 250 cm x 200 cm x 200 cm postal cage for brooding 40 chicks for two weeks, individual 35 cm x 20 cm x 45 cm battery cages, and a digital scale for feed and body weight measurement.

Methods

This study utilized a true experimental approach with a completely randomized design (CRD) to evaluate the effects of probiotic *Lactobacillus* sp. administered via drinking water and antibiotic growth promoter (AGP) *zinc bacitracin* added to feed. Four treatment groups were established for this purpose. The groups were divided into K- as a negative control group (P0) with commercial feed without the addition of probiotics and/or AGP and without *Escherichia coli* infection, K+ group as a positive control group (P1) with *Escherichia coli* infection and commercial feed without the addition of probiotics and/or AGP, (P2) *Zinc Bacitracin* as an antibiotic growth promoter added at 1 g/kg mixed through feed and without probiotic *Lactobacillus* sp. and infected with *Escherichia coli*. The (P3) commercial feed without AGP and probiotic *Lactobacillus* sp. was added at 5 mL/L through drinking water and infected with *Escherichia coli*.

Animal trials

This study used broilers purchased from an agent of PT Japfa Comfeed Indonesia. The care process of 1-2 weeks old *day-old-chick* (DOC) (the weight around 201g in 1^{st} week and 425g in 2^{nd} week) was carried out in prepared postal cages. The agent began the vaccination program, so the broilers arrived already vaccinated. At 2 weeks of age, Forty male broilers were selected from more than 100 broilers, then the broilers were moved to battery cages for the study process (2 broilers per cage).

Feeding and drinking

Feed is given twice a day, in the morning at 08.00 am and 15.00 pm. The commercial feed used is CP-511 produced by

PT Charoen Pokphand Indonesia, which is given for 5 weeks from DOC to harvest (35 days). Drinking water is given ad libitum which has been mixed with probiotics *Lactobacillus* sp.

Procedures for giving *Escherichia coli*, AGP, and probiotic *Lactobacillus* sp.

The way to administer *Escherichia coli* bacterium is through oral administration of 0.5 ml per animal which is given at the age of 21 days (19,20); probiotics are given through drinking water. Every time the supply of drinking water is replenished. Probiotics are given as much as 5 ml/liter mixed into drinking water at the age of 8-35 days. The capacity of the modified drinking water bottle is 1.5 liters, so 5 ml of probiotics needed to be mixed in a bottle containing 1 L of water and then mixed until homogeneous. This dose is given based on the best dose carried out in a study on laying hens conducted by Huda (21). Then, AGP (*zinc bacitracin*) is given at the age of 8-35 days at the time of feeding at a dosage according to the recommended commercial packaging (1 kg/ton of feed or 1 g/kg of feed), then the AGP is mixed into the feed given until homogenous.

Data collection

Production performance data were collected weekly, including feed intake, feed conversion ratio, and body weight gain. Feed intake was calculated as the difference between feed offered and feed leftovers. The feed conversion ratio was determined by dividing feed intake by the change in body weight. Body weight gain was calculated as the difference between the current and initial body weights.

Data analysis

Data were analyzed using repeated measures analysis of variance (ANOVA) within a general linear model framework. Post-hoc Duncan's multiple range tests was conducted for pairwise comparisons where significant differences were observed (P<0.05). Statistical analyses were performed using IBM SPSS Statistics for Windows.

Results

Feed intake (FI)

The inclusion of AGP in the feed significantly influenced feed intake (p<0.05). Notably, treatment P2 (*zinc bacitracin* at 1 g/kg feed) exhibited the most pronounced difference compared to the control groups (P0, P1). While no significant differences were observed between P0, P1, and P3, these groups differed significantly from P2. A detailed overview of average feed intake during the final three weeks of the study is presented in Table 1.

Feed conversion ratio (FCR)

The addition of probiotic *Lactobacillus* sp. to drinking water significantly improved the feed conversion ratio (P<0.05). Treatment P3, supplemented with 5 mL/L of *Lactobacillus* sp, exhibited the lowest FCR value compared to all other treatment groups (P0, P1, and P2). While no significant differences were observed between P0, P1, and P2, these groups differed significantly from P3. A detailed overview of the average FCR during the final three weeks of the study is presented in Table 1.

Body weight gain (BWG)

Probiotic *Lactobacillus* sp. supplementation via drinking water significantly enhanced body weight gain (p<0.05). Treatment P3, incorporating 5 mL/L of *Lactobacillus* sp, demonstrated the highest BWG compared to the control groups (P0, P1, and P2). While no significant differences were observed between P0, P1, and P2, these groups differed significantly from P3. A detailed overview of the average BWG during the final three weeks of the study is presented in Table 1.

Table 1: Mean and standard deviation of production performance (feed intake, feed conversion ratio, and body weight gain) during the last 3 weeks of the study (15-35 days of age)

Groups	Mean ± SD for the last 3 weeks		
	FI	FCR	BWG
P0	$732.42^{a} \pm 42.48$	$1.59^{b} \pm 0.06$	$1209.33^{a} \pm 59.69$
P1	$713.90^{a} \pm 28.17$	$1.63^{b} \pm 0.07$	$1166.73^{a} \pm 28.88$
P2	$788.97^{b} \pm 33.02$	$1.65^{b} \pm 0.07$	$1224.00^{a} \pm 51.02$
P3	$733.59^{a} \pm 22.82$	$1.46^{a} \pm 0.04$	$1332.93^{b} \pm 30.46$

Note: (a,b) Different superscript letters within a column indicate statistically significant differences between treatment groups (P<0.05).

Discussion

Treatment P2, supplemented with *zinc bacitracin* (ZnB) at 1 g/kg feed, exhibited significantly higher feed intake compared to the control groups (P0, P1, and P3). This enhancement suggests that ZnB, when employed as an AGP, can stimulate feed consumption in *Escherichia coli*-infected broilers. The increased feed intake is likely attributed to ZnB's ability to suppress the growth of pathogenic *Escherichia coli* within the digestive tract, thereby reducing associated stress (*Bacitracin* is an AGP has an anti-inflammatory effects, which produce cytokine proteins that will reduce the potential for inflammation in the gut and can prevent pain due to inflammation (22)). Consequently, improved nutrient absorption across the intestinal wall is facilitated, as suggested by Pertiwi and Dadi (23) suggested.

Even though *Bacitracin*, as an AGP, has low resistance, consuming it daily will still harm the consumer's health, potentially leading to antibiotic resistance. As a result, an alternative feed additive for broiler feed is required. Probiotics are another option that could potentially assist solve this problem (24).

According to the current study results, the P3 treatment group had the second highest mean of feed intake (addition of 5 mL/L of probiotic *Lactobacillus* sp. added to drinking water). These findings align with those of Hardiawan (24), who proposed probiotics, particularly *Lactobacillus* sp, as a promising alternative for managing *Escherichia coli*-infected broilers.

The results of this study collectively indicate that probiotic *Lactobacillus* sp. administered via drinking water can effectively substitute antibiotic growth promoter (AGP) in enhancing feed intake among *Escherichia coli*-infected broilers. This means that in this study, administering *Lactobacillus* sp. as a probiotic via drinking water can positively impact the financial margins of broiler farms, as evidenced by the low requirement for feed consumption to achieve high body weight.

The P3 treatment group differed significantly from the P0, P1, and P2 treatment groups. This demonstrates that adding *Lactobacillus* sp. (5 mL/L of drinking water) as a probiotic to the drinking water can reduce the feed conversion ratio value of broilers infected with *Escherichia coli*. This decrease in feed conversion ratio value demonstrates that *Lactobacillus* sp. as a probiotic supplementation can effectively reduce FCR in *Escherichia coli*-infected broilers. By inhibiting the growth of pathogenic bacteria within the digestive tract, *Lactobacillus* sp. is believed to enhance nutrient absorption and utilization, as proposed by Palupi (25).

In line with the current study results, the P3 treatment group had the lowest mean feed conversion ratio (adding 5 mL/L of *Lactobacillus* sp. as a probiotic to drinking water). This result is consistent with Abun (26) statement, which states that probiotic treatment results in lower FCR values because feed intake is lower or nearly identical to the negative control. According to Abun (26), the lower the FCR value, the better the feed efficiency of broilers. Apart from that, Govind (27) stated that the FCR value indicates how well the broiler chicken can convert feed intake into body weight. Small changes in the FCR value can impact a farm's financial margins.

The multiple explanations above demonstrate that administering *Lactobacillus* sp. as a probiotic through drinking water can be an alternative to using AGP in feed to reduce the value of the FCR in broilers infected with *Escherichia coli*. This means that in this study, administering *Lactobacillus* sp. as a probiotics through drinking water can have a positive impact on the financial margins of broiler farms, as evidenced by the low FCR value, which indicates that less feed is required to achieve high body weight.

The P3 differed significantly from P0, P1, and P2 treatment groups. This demonstrates that administering *Lactobacillus* sp. (5 mL/L of drinking water) as a probiotic to broilers infected with *Escherichia coli* can increase body weight gain. The observed increase in body weight gain indicates that probiotic *Lactobacillus* sp. effectively inhibits pathogenic bacterial growth within the broiler's digestive tract. Data analysis revealed that the P3 treatment group exhibited the highest average body weight gain during the final three weeks of the study, followed by P2, P0, and P1, respectively.

The positive effects of probiotics on production performance observed in this study align with previous study. As cited in Fesseha (28), proposed that probiotics enhance digestive processes by increasing beneficial microbial populations, bacterial enzyme activity, and intestinal microbial balance, ultimately improving nutrient digestion, absorption, and intake. Similarly, referenced in Hossain (29), reported that probiotic supplementation significantly increased broiler body weight gain and feed efficiency. The improved FCR observed in the probiotic-treated group can be attributed to the rapid proliferation of beneficial gut microbiota. As cited in Hossain (29), a diverse intestinal microbiota enhances nutrient digestibility and consumption, leading to increased growth and improved FCR.

The various explanations given above demonstrate that administering *Lactobacillus* sp. as a probiotic via drinking water can be an alternative to using AGP in feed to increase the value of the BWG in broilers infected with *Escherichia coli*. This means that in this study, administering *Lactobacillus* sp. as probiotics via drinking water can positively impact the financial margins of broiler farms, as evidenced by the high BWG values.

Conclusion

The findings of the current study demonstrate that the administration of probiotic *Lactobacillus* sp. via drinking water significantly enhanced feed conversion ratio (FCR) and body weight gain (BWG) in broiler chickens infected with *Escherichia coli*. While the AGP (*zinc bacitracin*) group exhibited higher feed intake and FI value, the probiotic group demonstrated significantly improved feed conversion ratio and body weight gain. These findings suggest that *Lactobacillus* sp. supplementation can be a promising alternative to AGP for enhancing production performance in broilers facing *Escherichia coli* challenges.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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إمكانية استخدام *Lactobacillus sp* كبديل لمحفز نمو المضادات الحيوية على الأداء الإنتاجي في الدجاج اللاحم المصاب ببكتيريا الإشريكية القولونية

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الخلاصة

هدفت هذه الدراسة إلى التحقق من فعالية البروبيوتيك في زيادة الأداء الإنتاجي (تناول العلف، نسبة التحويل الغذائي، وزيادة وزن الجسم) في دجاج اللحم المصاب ببكتيريا الإشريكية القولونية. استخدمت هذه الدراسة التجريبية تصميمًا عشوائيًا بالكامل وشملت أربعين ديك. تم تطعيم الدجاج اللاحم ببكتيريا الإشريكية القولونية في عمر ٢١ يومًا، وامتدت التجربة على مدى فترة ٣٥ يومًا. تكونت الدراسة من ٤ مجموعات علاجية، تضم كل مجموعة ١٠ طيور. كانت مجموعات العلاج هي، P0 (مجموعة تحكم سلبية بدون إضافات علفية بالإضافة إلى عدم وجود عدوى الإشريكية القولونية)، P1 (مجموعة تحكم إيجابية بدون إضافات علفية ولكن مع عدوى الإشريكية القولونية)، P2 تحتوي على الزنك باسيتراسين كمحفز نمو مضاد حيوي مضاف بمعدل ١ جم/كجم مخلوط من خلال الأعلاف التجارية وبدون بروبيوتيك *Lactobacillus sp* مع عدوى الإشريكية القولونية)، وكذلك، P3 (علف تجاري بدون AGP وبروبيوتيك *Lactobacillus sp* يضاف بمعدل ٥ مل/لتر من خلال مياه الشرب المصابة بعدوى الإشريكية القولونية). تم استخدام نموذج خطي عام للقياسات المتكررة لتحليل البيانات، مع مقارنات لاحقة باستخدام اختبارات دنكان للمسافات المتعددة. وأشارت النتائج إلى وجود فروق ذات دلالة إحصائية ($P<0.05$) في معايير الإنتاج مثل تناول العلف، ونسبة تحويل العلف، وزيادة وزن الجسم عبر مجموعات العلاج. وفي الختام، أدى إعطاء البروبيوتيك *Lactobacillus sp* المضاف بمعدل ٥ مل/لتر من خلال مياه الشرب إلى تحسين الأداء الإنتاجي في الدجاج اللاحم المصاب ببكتيريا *Escherichia coli*.