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Effect of Probiotic Administration of *Bacillus subtilis* and *Bacillus coagulans* Isolate on Growth Performance in Broiler Chicken

Berliana Elok Faiqoh[1], Mirni Lamid[1]*, Ratna Damayanti[2], Sri Chusniati[3], Mohammad Anam Al Arif[1], Sunaryo Hadi Warsito[1], Tita Damayanti Lestari[4], Hartanto Mulyo Raharjo[3], Saifur Rehman[3,5], Muhammad Asif Hussain[6]

[1]Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Division of Basic Medical Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [3]Division of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [4]Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, [5]Department of Epidemiology and Public Health, University of Veterinary and Animal Science, Lahore, Pakistan, [6]Department of Animal Nutrition, College of Animal Husbandry and Veterinary Science, and Animal Science, Abdul Wali Khan University, Mardan, Pakistan.
*Corresponding author: mirnylamid@fkh.unair.ac.id

Abstract

This study aimed to determine the effect of probiotic administration of *Bacillus subtilis* and *Bacillus coagulans* isolate in improving feed intake, and body weight, and feed conversion rate (FCR) in broiler chicken. A total of 24 broiler chickens divided into four treatments i.e. (P0) was a group without probiotics, (P1), (P2), and (P3) were experimental treatment groups consist of probiotic 2 mL/day, 4 mL/day, and 6 mL/day per orally. In results, feed intake, body weight, and FCR values in all treatment groups (P1, P2, P3) were significantly different ($p < 0.05$) from (P0) group. Meanwhile, there was no significant difference ($p > 0.05$) between P1 and P3 group. It can be concluded that the effect of *B. subtilis* and *B. coagulans* as probiotics with a dose of 4 mL/day had a noticeable impact on feed intake, body weight, and decreased FCR.

Keywords: *Bacillus coagulans*, *Bacillus subtilis*, broiler chicken, probiotic, zero hunger

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INTRODUCTION

Based on data from the Directorate General of Livestock and Animal Health, the Ministry of Agriculture of the Republic of Indonesia (2020) stated that the amount of broiler meat production in the 2016–2020 period dominates with an average growth of 17.56% per year. The feed cost in the broiler farming business reaches 60–70% of the total production cost (Irawan *et al*., 2020). This condition is an obstacle for broiler chicken farms because of the dependence of chickens on imported feed raw materials such as corn, soybean meal, and fish meal (Resnawati, 2012).

Many breeders use antibiotic growth promoters (AGP) as feed additives to reduce the cost of feed, which is getting higher (Kompiang, 2009). However, these conditions were banned by the Indonesian government because of the risks. Such as increased creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), which can lead to indications of nephrotoxicity and hepatotoxicity (Haque *et al*., 2018). In addition, it can cause antibiotic residues in meat that have adverse effects on consumer health in the long term (Amer and Khan, 2012) and can lead to resistance of pathogenic bacteria to certain antibiotics, thus requiring a continuous increase in dosage to get the effect (Wolfenden *et al*., 2010; Andriani *et al*., 2020).

Probiotics that are widely used are lactic acid bacteria because they can increase the ability of non-specific immunity (Widiyaningsih, 2011). In addition, lactic acid bacteria can also increase the efficiency of digestion and absorption of nutrients (Murhadi *et al*., 2012). One of the microbes that have the potential to be used as probiotics is *Bacillus spp*. Some of the factors that make *Bacillus coagulans* a good probiotic to use,

among others, the bacteria are easy to cultivate in large numbers, the bacteria produce organic acids, and the bacteria can sporulate (Hyronimus *et al*., 2000).

Based on the description above, study on the effect of *B. subtilis* and *B. coagulans* isolates as probiotics was conducted to analyze feed intake, body weight, and feed conversion ratio (FCR) in broiler chickens.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Universitas Airlangga, Animal Care and Use Committee (ACUC) Ethical Clearance No: 801-KE.

Study Period and Location

This study was performed at October–December 2022. The experimental animals were treated in animal laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

Experimental Design

A total of 24 Cobb strain broiler chickens, aged of 20 days were recruited as the study subjects, with no distinction made based on their gender—hence, they were considered unsexed. Before subjecting the broiler chickens to the treatment protocols, a preparatory phase of seven days was implemented to facilitate their adaptation to the experimental environment and conditions.

To facilitate a comprehensive evaluation of the potential impacts of bacterial isolates, these 24 broiler chickens were thoughtfully divided into four treatment groups, each meticulously designed to probe the consequences of different dosages of bacterial isolates. The treatment groups were as follows, (P0) was received no bacterial isolates and was intended to serve as the baseline reference, (P1) was administered 2 mL/day of bacterial isolate orally, (P2) was administered a dosage of 4 mL/day of bacterial isolate, and (P3) was administered a dosage of 6 mL/day of bacterial isolate.

The bacterial isolates used in this study hailed from two prominent strains i.e. *B. subtilis* and *B. coagulans*. The concentration of these isolates was maintained at a concentration of $1x10^7$ CFU/mL, with the source being PT. Centra Biotech Indonesia—a reputable supplier known for its quality biotechnological products.

To ensure the welfare of the broiler chickens during this study, they were housed in specially designed plastic battery cages, each boasting dimensions of 155 cm in length, 55 cm in width, and 55 cm in height. These cages were thoughtfully equipped with dedicated sections for food and water, ensuring the animals had convenient access to sustenance. The broiler chickens were sustained on a commercial broiler chicken feed diet, identified explicitly as HI PROVITE 512-Br.

Data Analysis

The data obtained in this study were analyzed by using SPSS for the Windows 20.0 program with ANOVA followed by Duncan's multiple distance test with a significant level of 5%.

RESULTS AND DISCUSSION

Feed Intake

Based on the results of the data analysis, there was a significant difference ($p < 0.05$) between P0 and P1, P2, and P3. However, P1 was not significantly different ($p > 0.05$) from P3 (Table 1 and Figure 1).

The results of data analysis showed that the administration of *B. subtilis* and *B. coagulans* bacteria isolates as probiotics in P2 treatment at a dose of 4 mL/day was able to increase feed intake in broiler chickens compared to other treatments. This is because probiotics have antimicrobial properties that can balance the microflora in the intestines so that they can inhibit the growth of pathogenic bacteria and make nutrient absorption better (Widiawati *et al*., 2018). The presence of pathogenic bacteria itself can cause damage to the intestinal villi, so it interferes with the absorption of nutrients. This is in line with Lokapirnasari's study (2016), which stated that giving liquid probiotics to feed intake can increase digestibility so that feed intake can increase. When the

digestibility of broiler chickens increases, the absorption of feed and the emptying of the digestive tract is faster, so the broilers become hungry faster (Pratama *et al*., 2021).

The P3 treatment with the highest dose showed lower feed intake than the P2 treatment. These results are thought to be due to competition for nutrients between bacteria, causing the bacterial population and activity of the endoglucanase enzyme to be lower and resulting in slower digestion and absorption of nutrients in the P3 treatment (Sinta *et al*., 2020). In addition, probiotics will not work well if the dose of probiotics given is not appropriate or the way of giving probiotics is not appropriate (Astuti *et al*., 2015). Giving probiotics with high doses causes large bacterial colonization so that they are more competitive in getting nutrients from the larger substrate so that bacteria that lack nutrients will be hampered by their activities and lead to death and cannot have the maximum effect as probiotics. The higher the dose, the more energy is used in metabolism because during this metabolism, the bacteria will produce enzymes and organic acids which result in decreased feed intake (Mas'ad *et al*., 2020; Wardiana *et al*., 2021).

Sen *et al*. (2012) also stated that *Bacillus spp*. administration caused histomorphological changes in broiler intestines, increasing villi height to crypt depth ratio to increase nutrient digestibility and absorption capacity of the small intestine. Broiler chickens fed *Bacillus spp*. supplementation showed a decrease in digest viscosity, which could affect the availability and absorption of nutrients (Latorre *et al*., 2015). Factors that affect feed intake in broilers are body weight, strain, production level, stress level, livestock activity, energy content in feed, and environmental temperature (Sjofjan *et al*., 2016).

Body Weight

Based on the results of the data analysis, there was a significant difference ($p < 0.05$) between P0 and P1, P2, and P3. However, P1 was not significantly different ($p > 0.05$) from P3 (Table 1 and Figure 1).

The results of data analysis showed that the administration of bacterial isolates of *B. subtilis* and *B. coagulans* as probiotics in treatment P2 had the highest weight gain value among all treatments, namely 89.466 g/head. This weight gain was in line with the high P2 treatment feed intake. This shows that the feed consumed by broiler chickens is quite efficient and is widely used for growth. This study supported the opinion of Astini *et al*. (2014) that weight gain is influenced by the amount of feed consumed. The higher the level of feed intake, the higher the resulting weight gain, and vice versa. Increased feed intake will result in high nutrient intake, one of which is protein, which plays a role in increasing the growth of broiler chickens. The use of *Bacillus spp*. for the weight gain of chickens following study by Yang *et al*. (2019), which stated that the use of *Bacillus spp*. after 28 days of treatment showed that the chickens had a higher body weight than the control group.

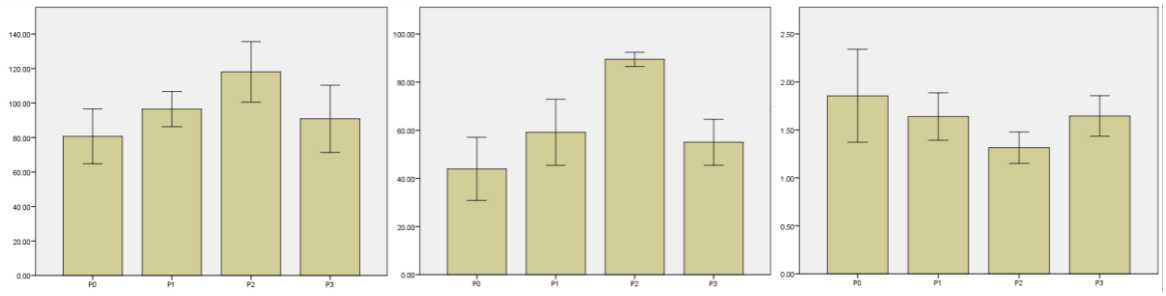
Increasing the dose of probiotics at a dose of 6 mL/day (P3) did not affect body weight gain. This is presumably due to reduced appetite in chickens caused by the suppression of pathogenic bacteria in the small intestine due to competition between pathogenic bacteria and probiotics and the presence of bacteriocin produced from probiotic bacteria (Warwick *et al*., 1995). This shows optimal limits in chickens in their tolerance to microbial populations in the digestive tract (Wiryawan *et al*., 2005). Then, according to Mahdavi *et al*. (2005), probiotic microbes can produce an optimum response in the digestive tract in certain doses.

Azizah *et al*. (2020) stated that probiotics could increase the activity of digestive enzymes so that the decomposition and absorption of feed became well absorbed and could be utilized by chickens to increase body weight. Probiotics play a role in increasing metabolic products that benefit livestock's body. These metabolic products are utilized optimally to form or increase the size of new tissues. Based on Wizna *et al*. (2013) stated that *B. coagulans* bacteria could produce several enzymes to help digest food substances needed for growth in chickens.

Table 1. Feed intake, weight gain and FCR value in all treatment groups

Treatments	Feed intake	Weight gain	FCR
P0	$80.675^{a} \pm 7.929$	$43.966^{a} \pm 6.562$	$1.855^{c} \pm 0.241$
P1	$96.485^{b} \pm 5.135$	$59.133^{b} \pm 6.864$	$1.638^{b} \pm 0.123$
P2	$118.051^{c} \pm 8.780$	$89.466^{c} \pm 1.473$	$1.315^{a} \pm 0.081$
P3	$90.853^{b} \pm 9.764$	$55.016^{b} \pm 4.770$	$1.656^{b} \pm 0.130$

Different superscript in the same column indicate significant different ($p < 0.05$).



a b c

Figure 1. Trend graph of (a) feed intake, (b) weight gain, (c) and FCR.

The factors that affect weight gain are regular feeding and the content of the feed provided also includes everything needed by the livestock (Chandra *et al*., 2022). Then, in terms of the development of the livestock, its balanced between weight gain and harvest time (Salim, 2017).

FCR Evaluation

Based on the results of the data analysis, there was a significant difference ($p < 0.05$) between P0 and P1, P2, and P3. However, P1 was not significantly different ($p > 0.05$) from P3 (Table 1 and Figure 1).

The results of data analysis showed that the administration of *B. subtilis* and *B. coagulans* bacteria isolates as probiotics in P2 treatment was able to reduce the FCR of broiler chickens with the lowest value of 1.315. This is because the feed consumed to the maximum in the growth process can affect the weight gain of chickens. The lower the FCR, the more efficient the feed, which means that the use of the feed is economical (Azizah *et al*., 2020). The FCR is one of the business success factors for farmers (Utomo *et al*., 2022). A comparison of feed intake and weight gain will produce an economic calculation value. This is in line with the effect of efficient probiotics on feed intake and weight gain (Abdurrahman *et al*., 2022).

Based on the P0 group showed the highest FCR compared to the other three treatments. The higher FCR was due to a large amount of feed consumed and not matched by weight gain (Agustono *et al*., 2019). In addition, the condition of the small intestine is less efficient in the process of absorption of food and irritates due to pathogenic bacteria, so food substances that should be needed for livestock growth may be used to repair the irritated surface of the small intestine (Hidayat, 2013). These results are consistent with previous studies using *B. subtilis* probiotics to improve FCR in poultry (Mingmongkolchai and Panbangred, 2018). Wizna *et al*. (2013) stated that the increase in weight gain was in line with the increase in the number of colonies of *B. coagulans* in the small intestine because this *B. coagulans* can benefit the growth of broiler chickens to increase feed efficiency (Hamid *et al*., 2022). In addition, *B. coagulans* can help the digestive process of broiler chickens in digesting food substances. Rodas *et al*. (1996) stated that the efficiency of feed use had a significant effect when probiotics were added to feed that had a balanced composition and nutrition to give the result that the feed given probiotics at a dose of 4 mL/day (P2) was the most efficient dose to achieve optimal growth. Maximum and more profitable when compared to the control treatment (P0).

According to Salim (2017), the FCR is influenced by livestock conditions, livestock digestibility, nation, feed quality and quantity, and environmental factors. The provision of probiotics in the feed will reduce FCR because it increases the digestibility value and feed efficiency in utilizing nutrients in metabolic processes in animal tissues (Lokapirnasari *et al*., 2022).

CONCLUSION

We can concluded that the provision of *B. subtilis* and *B. coagulans* isolates as probiotics at a dose of 4 mL/day can increase the amount of feed intake, weight gain and reduce FCR in broiler chickens.

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AUTHORS' CONTRIBUTIONS

ML: Conceptualization and drafted the manuscript. BEF, RD, SC, MAA, and SHW: Treated the animal laboratory. TDL and HMR: Validation, supervision, and formal analysis. SR and MAH: Performed the statistical analysis and the preparation of table and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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