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Research Article
Evaluation of Bacteria from *Gallus domesticus* as a Potential Probiotic in Broiler Chicks: Effects on Growth Performance and Feed Conversion Ratio

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Abstract

Objective: The aim of this study was to isolate, characterize and evaluate the potential of probiotic bacteria from the Indonesian domestic chicken. **Methodology:** Probiotic bacteria were isolated using the medium Mann Rogosa Sharpe Agar (MRSA). Seven isolates were obtained, five of which had potential as probiotics and were referred to as Probiotic Bacteria (PB): PBA, PBB, PBD, PBE and PBG. A variety of tests were performed to characterize the bacteria, including evaluating their resistance to acidity and bile salts. **Results:** The results showed that the two isolates of probiotics (PBD and PBG) resistance to acidity (pH 3) and bile salts (5%) could inhibit the growth of the pathogenic bacteria *Escherichia coli* and *Salmonella typhi.* Two isolates were selected, PBD and PBG, to give as a probiotic solvent to broiler chickens. A total of 40 broiler chickens were divided into four treatments with 8 repetitions. The treatments were R0 (without probiotic), R2 (probiotic PBD), R3 (probiotic PBG) and R4 (a mixture of probiotics PBD and PBG). The results showed that probiotics containing different bacteria in chicken feed influenced the body weight gain of broilers, but did not significantly affect Feed Conversion Ratio (FCR). The obtained results confirm the effect of probiotic that was isolated from domestic chicken provided increased body weight gain and meat quality. **Conclusion:** The visual differences found in this study were due to the increased health of the broiler chickens that were given probiotics in comparison to those that were not given probiotics.

Key words: Probiotic, FCR, broiler, weight gain, *Gallus domesticus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The domestic chicken (*Gallus domesticus*) can easily adapt to its environment and is more resistant to disease and weather than the broiler chicken[1,2]. The endurance capabilities of the domestic chicken in terms of its diet can influence the microflora in the intestine, this microflora adapts to its environment so as to form a community of bacteria that are resistant to the habitat in which the chicken lives[1,3].

The presence of bacteria in the digestive tract of chickens is partially due to the contamination of the surrounding environment with bacteria, which then enter the body through the consumption of chick feed[4]. The age of chickens also affects the different types of bacteria present[1,5]. In chicks, digestion occurs fastest in the anterior part of the small intestine[6]. Most of the chicken's digestive organs are acidic, with a pH of 3-4 and contain bile salts, so that the microbes used as probiotics should be resistant to both of these conditions[7,8].

In Indonesia, commercial broiler chicks are reared in large-scale farms and fed with antibiotics. Antibiotics can disrupt the balance of bacteria in the digestive tract of broilers and are a potential hazard to human health because of their residue in broiler meat[9-12]. Probiotics containing bacteria that can potentially boost the balance of bacteria in the digestive tract are given to broiler chicks at a young age[13-15]. Broiler chickens with balanced bacteria conditions will have more endurance and resistance, especially against intestinal pathogens[16,17]. Probiotics have some beneficial effects when used in feed because they prevent the reaction of pathogenic bacteria, boost the immunity of animals, supply enzymes to help with digestion and produce antimicrobial substances that improve the health of the host[18-27].

Microbes can be selected as candidate probiotics and isolated from the gut of chickens, so that when tested on broilers, they can potentially grow and thrive in the intestine. Therefore, this experiment aimed to isolate and characterize potentially probiotic bacteria from the gut of domestic poultry (*G. domesticus*) from South Sulawesi, Indonesia. The study also aimed to examine the effect of probiotic bacteria that was isolated from *G. domesticus* on the growth and Feed Conversion Ratio (FCR) of commercial broiler chicks.

MATERIALS AND METHODS

Isolation of probiotic bacteria: A sampling of domestic poultry (*G. domesticus*) was conducted at Luwu Timur Regency, South Sulawesi, Indonesia. The inner walls of the chicken intestine were scraped and then inserted into physiological, sterile NaCl solution and diluted with graded dilution. De Mann Rogosa Sharpe Agar (MRSA) medium was inoculated with 1 mL of dilution and $CaCO_3$ 1% was added, then the medium was incubated for 24-48 h at a temperature of 37°C.

Purification, morphology and making stock isolates of bacteria probiotics: The initial stage of purification involved the selection of a single colony that was surrounded by a clear zone in the MRSA medium and incubating it at 37°C for 48 h. The purification step could be done 2-3 times, to be sure that the colony obtained was absolutely pure. The morphology of each colony formed after purification was then observed. The observations included the form of the colony (shape), the shape of the edge (margin), the color, the colony surface (elevation) and the smell (odor). Each of the different colonies formed after purification was then inoculated on a slant MRSA medium for further testing.

Resistance to gastric acidity (pH): Resistance to acidity was tested using MRSB medium supplemented with 0.1 N HCl to obtain pH 2.5-3.0 (according to the pH of the stomach). Positive results were indicated by the bacterial growth in the medium HCl-MRSB and negative results were indicated by no growth of bacteria.

Biochemical characteristics and resistance to bile salts: The MRSB medium was supplemented with synthetic bile salts (ox bile) at concentrations of 1 and 5%. A total of 1 oose from each bacterial isolate was taken from the stock culture and used to inoculate the MRSB-bile salts. They were then incubated for 2-3 h at a temperature of 37°C. The number of bacterial colonies growing before and after incubation was measured. Biochemical characteristics were studied using the Methyl Red-Voges Proskauer (MR-VP) test, catalase test and Triple Iron Sugar Agar (TISA) motility test.

Inhibitory power against bacterial pathogens: The inhibitory power test was performed to confirm that isolates had excellent potential as probiotic bacteria to inhibit the growth of pathogenic bacteria. The pathogenic bacteria used were *Salmonella typhi* and *Escherichia coli*. The activities of antibiotics were tested using Mueller Hinton Agar (MHA) medium and the agar diffusion method using a paper disk. Each paper disk was immersed in the supernatant of bacteria for 15 min. About 1 mL suspension of test bacteria was transferred to a sterile petri dish and MHA medium was added

(at 45°C), then allowed to solidify. Paper disks that had been soaked in MHA media solidified with a distance of 20 mm and were incubated at 37°C. Observations were conducted for 48 h and the diameter of the inhibition zone that had formed was measured.

Broiler chicken feed and the addition of probiotics: Starter probiotics were made by dilution methods. About 9 mL of 0.9% NaCl physiological saline was transferred into a test tube and homogenized. Stock bacteria were taken by using syringe as much as 0.5 mL then inserted into the tube dilution. The number of probiotic bacteria was calculated using SPC (Standard plate) to obtain a bacterial density of 55% T on a spectrophotometer. Probiotics diluted (55% T) are then mixed in broiler chicken feed at a dose of 10 mL kg^{-1} of feed.

Each probiotic isolate was sprayed onto the artificial feed, which was then given to broiler chickens. The chicken feed used is artificial feed (rice bran 30 g, refined corn 40 g and fish meal 30 g). Feed added a solution of probiotics based on its kind that is: R0 (without probiotic), R2 (probiotic PBD), R3 (probiotic PBG) and R4 (a mixture of probiotics PBD and PBG). The feed was given *ad libitum* every day for 6 weeks. The chickens were kept according to the standard method of maintenance of broilers. The changes that occurred during the 40 days period were recorded and at the end of each week, chickens were weighed, the feed intake was recorded and the feed conversion ratio was calculated.

Research design and statistical analysis: Completely Randomized Design (CRD) with four treatments was used in this study. Each treatment used 8 broiler chicks (replications). The data were statistically analyzed using analysis of variance (ANOVA) and then continued using Duncan's test to observe whether differences between treatments were significant at a probability level of 0.05 (95% confidence interval). Analyses were performed in SPSS software v.16.

RESULTS AND DISCUSSION

Seven isolates were obtained from the domestic chicken intestine as candidate probiotic bacteria. Three isolates had the form of short bacilli (PBA, PBF and PBG), three isolates had the form of cocci (UN, PBC and PBD) and one isolate was a long bacillus (PBE). Three of the isolates were Gram-positive bacteria and the other four were Gram-negative. Lactic acid bacteria vary in their characteristics but they are always Gram-positive. They can be rod-shaped, such as *Lactobacillus*, or coccus-shaped, such as *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Enterococcus* and *Pediococcus*[28].

Candidate probiotics from lactic acid bacteria are required to work in the digestive tract of the host, so they should be selected based on several criteria, including resistance to acidity and bile salts, which can damage the cell walls of bacteria, including resistance to acidity, bile salts (which can damage the cell walls of bacteria) and enzymes or metabolites that are used in defense[29]. Four isolates (PBA, PBD, PBE and PBG) could grow well under acidic condition (pH 3) with bile salts (5%), characterized by the presence of turbidity and the amount of sediment at the bottom of the tube. The isolate UN produced high levels of turbidity but low quantities of sediment. The isolates PBC and PBF showed no turbidity or sediment. This is consistent with the statements of Li *et al.*[28] and Madigan *et al.*[30] that potentially probiotic bacteria are fermentative microorganisms that can live in low pH conditions.

The bacteria used as a probiotic agent must not only be resistant to low pH, but also to bile salts, in order to survive the delicate broiler chick intestines. This is related to the function of bile salts as an emulsifier in the digestion of fat[31,32]. Two of the probiotic bacterial isolates were found to be resistant to both pH and bile salts isolates PBD and PBG. They exhibited stable growth during the acidity test and with 1 and 5% bile acid, indicating the maximum results. These two isolates were selected and identified using biochemical tests.

The results showed that the isolated bacteria had antibacterial properties against *Escherichia coli* and *Salmonella typhi*, whereby incubation periods of 24 and 48 h produced a clear zone (Fig. 1). The diameter of the inhibition zone against *Escherichia coli* with a 24 h incubation period was 23.04 mm for PBD and 25.03 mm for PBG. After an incubation period of 48 h, there was not an increase in the diameter of the clear inhibition zone. For *Salmonella typhi* the diameter of the inhibition zone after 24 h was 11.05 mm for PBD and in PBG the diameter was 16.07 mm. After 48 h, the diameters were 11.06 and 16.08 mm for PBD and PBG, respectively. The results showed that the inhibitory zone of the two isolates for *Escherichia coli* was above 20 cm. According to Li *et al.*[28], when the zone of inhibition is above 20 cm, there is a strong inhibitory effect. This suggests that both isolates had a strong inhibitory effect against *Escherichia coli*.

The results showed an increase in the inhibitory zone from 24-48 h. This suggests that both isolates are bactericidal,

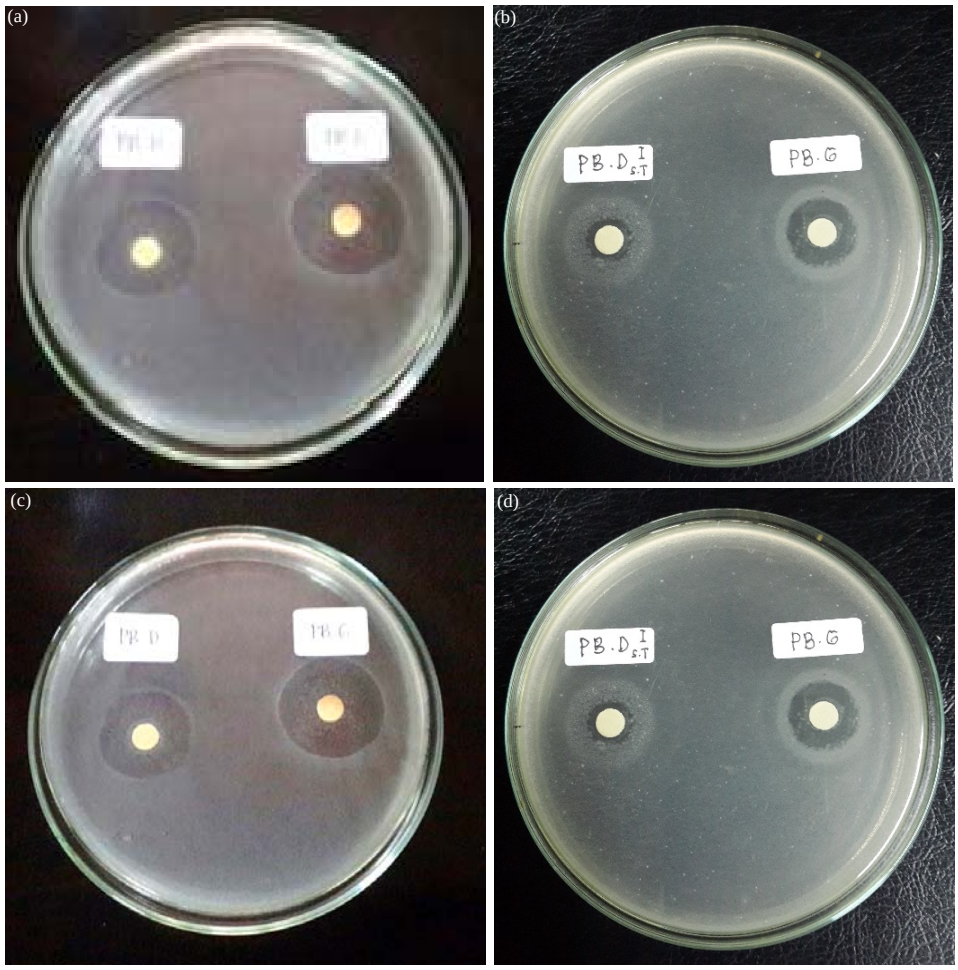

Fig. 1(a-d): Test of power Inhibitory Zone (IZ) of probiotic bacteria isolates (a) *Escherichia coli* 24 h (IZ of PBD 23.04 cm and PBG 23.04 cm), (b) *Salmonella typhi* 24 h (IZ of PBD 11.05 cm and PBG 16.07 cm), (c) *Escherichia coli* 48 h (IZ of PBD 25.03 cm and PBG 25.03 cm) and (d) *Salmonella typhi* 48 h (IZ of PBD 16.07 cm and PBG 16.08 cm)

meaning that they are able to kill and stop the physiological activity of other bacteria. According to Gillespie *et al.*[33], when the inhibition zone formed after an incubation period of 48 h remains the same or increases, the bacteria can be described bactericidal. This indicates that the bacteria that were isolated from domestic poultry could be described as bactericidal.

The ability of the PBD and PBG isolates to inhibit the growth of pathogenic bacteria (*Escherichia coli* and *Salmonella typhi*) will certainly benefit the hosts. This is because probiotic bacteria should be able to produce anti-microbial substances (bacteriocins), providing beneficial effects to their host[22,34]. The ability of the isolates PBD and PBG to inhibit the growth of pathogenic bacteria is in accordance with the results of Oh and Jung[20] and Angmo *et al.*[27], which stated that probiotic bacteria have this ability. In contrast to most bacteriocins, PBD and PBG significantly inhibited two major foodborne Gram-negative pathogens, *Escherichia coli* and *Salmonella typhi*, which are listed as serious and moderate hazards by World Health Organization (WHO)[28,35].

Appearance of broiler chickens: Broiler chicken can grow fast and there are some indicators for the healthy one that are actively move, clean body and no physical disability. This study evaluated visual appearance, liveliness and organoleptic quality (taste, texture and aroma of the meat). The result showed that there is a significant difference in visual appearance between R0 and R4. It can be indicated by dirt, health, feather, skin and meat appearance. Moreover, the feces of R0 was very pungent compared with other treatments. Probiotics could improve the health of broiler chicken. Probiotics have the main role in skin and meat appearance. Skin of broiler chicken tends to more yellow and

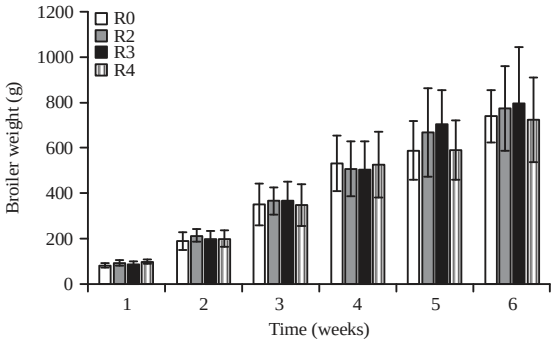

Fig. 2: Graph of broiler weight gain from week 1-6. R0: Without probiotic, R2: Probiotic PBD, R3: Probiotic PBG and R4: Probiotic mixture PBD and PBG

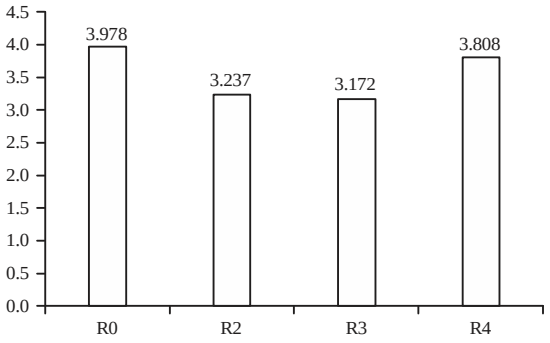

Fig. 3: Graph broiler feed conversion for 6 weeks. R0: Without probiotic, R2: Probiotic PBD, R3: Probiotic PBG and R4: Probiotic mixture PBD and PBG

the meat has a smoother texture. This result was supported by Mojgani *et al.*[18] and Alves *et al.*[19], who found that probiotics could improve the health of broiler chickens. Giving probiotics to chickens can affect the taste of the meat, due to changes in the levels of protein, albumin and cholesterol[1,36].

Weight gain of broiler chickens: The result of broiler chicken weight comparison showed that there are a significant different among groups (Fig. 2). The treatment has an effect on weight gain of broiler chicken. The R3 group has body weight average of 796 g compared with R2 and R4 groups have a body weight of 774 and 769.75 g, respectively. This result is similar to the previous study conducted by Souza *et al.*[1] and Toghyani *et al.*[36]. They stated that probiotic has a positive effect on body weight. The probiotics promote body repair by improving digestibility and nutrient absorption in the digestive tract. They do this by producing enzymes to help digest some foods[25], butyric acid[37], propionic acid[38], lactic acid[39] and bacteriocins[40], which serve to improve the mucosal and intestinal villi and suppress harmful bacteria.

Feed conversion ratio: The results showed that there was a difference in ratio conversion between treatments that were fed the probiotic and the control (Fig. 3). An ANOVA at the 95% confidence level indicated that the conversion ratio of broiler chickens from week 1-6 showed no significant results ($p = 0.650$, $F = 0.624$). This indicates that there was non significant effect of treatment on the feed conversion of broiler chickens, therefore rejecting H1. The best feed conversion was obtained in R3 treatment (probiotic PBG), followed by R4 (a mixture of probiotics), R0 (without probiotic) and lastly, R2 (probiotic PBD) (Fig. 3). The results showed that there was no effect of probiotics on FCR. This is supported by Toghyani *et al.*[36], Olnood *et al.*[15] and Wang and Gu[41], who found that the addition of probiotics to broiler chicken diets did not significantly affect FCR.

The obtained results confirm the effect of probiotic that was isolated from domestic chicken provided increased body weight gain and meat quality. The visual differences found in this study were due to the increased health of the broiler chickens that were given probiotics in comparison to those that were not given probiotics. Moreover, the way of probiotic administration in the feed employed in this study provides both strain viability and efficacy, which are of crucial importance in extensive farming. However, obtained results showed that probiotic that was isolated from domestic chicken did not decrease FCR. Therefore, further study needs to be done to maximize the use of probiotic and feed that will lower the value of FCR.

CONCLUSION

Seven probiotic isolates were obtained from bacteria in the digestive tract of domestic poultry originating from Luwu Timur, South Sulawesi, Indonesia. Tests on the resistance of the bacteria to acidity and bile salts revealed that only two isolates had potential as probiotics because they had a steady growth. One isolate was a positive coccus (PBD) and the other was a positive bacillus (PBG). Both isolates could inhibit the growth of the pathogenic bacteria *Escherichia coli* and *Salmonella typhi.* Adding the probiotics to chicken feed influenced the weight gain of broilers, but did not significantly affect the FCR. The most effective treatment was R3 (Probiotic PBG), which produced an average body weight of 796 g and a feed conversion ratio of 3.1721 g.

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