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Original Research Article

Supplementation of Synbiotic Content of *Moringa oleifera* Extract and *Lactobacillus* to Improve Growth Performance in Starter Phase Diet of Broiler Chicken

Widya P. Lokapirnasari[1]*, Mirni Lamid[1], Rochmah Kurnijasanti[2], Nova Teriyanto[3], Ajeng T. Kartika[3], Evania H. Chandra[4], Kornelia K. Riong[4], Andreas B. Yulianto[5]

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ABSTRACT

Prebiotics are substrates that can be used to increase the growth of probiotics. Oligosaccharides, polysaccharides and galacto-oligosaccharides have important functions as prebiotics to improve intestinal health through increased growth of beneficial bacteria. Synbiotics are combinations of probiotic bacteria and prebiotic food ingredients. This study examined the synbiotic effects of *Moringa oleifera* extract as prebiotic and probiotic incorporating *Lactobacillus fermentum* and *Lactobacillus plantarum* on the growth performance starter phase of broiler chickens. The dietary treatments comprised of the starter basal diet control and supplemented with 1% prebiotic + 1% probiotic, 2% prebiotic + 1% probiotic and 1% prebiotic + 2% probiotic, 2% prebiotic + 2% probiotic, respectively. The results showed that there is a significant difference ($p<0.05$) between control and treatment. Furthermore, the supplementation of 1% prebiotic + 2% probiotic produces the highest body weight values. Meanwhile, the lowest feed conversion ratio (FCR) and the highest feed efficiency were supplemented with 1% prebiotic + 2% probiotic, 2% prebiotic + 2% probiotic, 1% prebiotic + 1% probiotic, which was similar to the supplementation with 2% prebiotic + 1% probiotic, while the lowest feed consumption was found in 2% prebiotic + 2% probiotic. Therefore, based on the results of this study, it can be concluded that supplementation of 1% to 2% prebiotic and 1% to 2% probiotic has the ability to enhance the production performance of starter phase broilers compared to the control.

Keywords: Broiler, *L. fermentum*, *L. plantarum*, *Moringa oleifera* extract.

Introduction

Antibiotic growth promoters (AGPs) are used to describe any medicine that destroys or inhibits bacteria and is administered at a low sub-therapeutic dose. They are widely used for growth promoters, suppression of diseases and stress, including the maintenance and intensification of livestock farming. However, its use has been banned in industrialized countries (EC Regulation No. 1831/2003), while in Indonesia it is regulated under the Animal Husbandry and Animal Health Law No. 18, 2009 in conjunction with Law No 41 / 2014. In relation to the prohibition of its usage, it is necessary to provide an alternative, such as Lactic acid bacteria (LAB), which are a group of probiotic bacteria used to boost growth performance, feed efficiency and productivity by increasing the balance of microbes in the gut thereby improving the health of the host.[1-3] Several studies have shown that probiotics enhance the growth performance of broilers, as well as the probiotic *Bifidobacterium spp*, *L.acidophilus* and *Lactobacillus casei* WB 315.[4,5]

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The characteristics of *Lactobacillus fermentum* (*L. fermentum*) probiotic includes the ability to survive at under pH 2.0; 2.5 and 3.0, inhibitory effect against pathogenic strains of *E. coli* O157: H7 and *Salmonella enteritidis*.[6] *L. fermentum* probiotic has been shown to adhere to the intestinal epithelium of poultry, as well as tolerance to bile salts and gastric juice. *L. fermentum* showed antagonistic effects on pathogenic bacteria such as *Salmonella* sp. SS11 and *Shigella* sp. SS10.[7]

Furthermore, to boost the growth of probiotics, prebiotics is added. They are defined as feed ingredients that are unhydrolyzed in the upper digestive tract, and they selectively stimulate the growth activities of several bacteria in the intestine in order to improve intestinal health. Prebiotic oligosaccharides include mannan-oligosaccharides (MOS) and fructo-oligosaccharides (FOS) which tend to be fermented by *Lactobacillus plantarum*.[8] The addition of xylo-oligosaccharides shows a significant difference in the growth performance of broilers.[9] The use of supplements, namely probiotics and prebiotics (synbiotics) improves the health and productivity of poultry.[10] *Moringa oleifera* is a potential medicinal plant that is used to enhance the immunity and intestinal health of broilers, as well as reduce the number of *E.coli* in the intestines.[11, 12] They are known to contain important nutrients such as crude protein (25.6%), amino acids, crude fiber (4.38%), extract ether (4.39%), carbohydrates (43.88%), energy (1296.00 kJ/g), minerals (calcium (27.9%), phosphorus (0.26%), vitamins, β-carotene and polysaccharides.[13-15] However, the effects of these synbiotic on the starter phase of broiler chicken were not clarified. Therefore, the purpose of this study is to analyze the effect of synbiotics containing prebiotic *Moringa oleifera* extract and a combination of *L.fermentum* and *L. plantarum* probiotics on the growth performance (body weight, average daily weight gain,

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feed consumption, efficiency and conversion ratio) of broilers during the starter phase.

Materials and Methods

Isolate preparation

Two *Lactobacillus fermentum* and *Lactobacillus plantarum* were separately cultured in de Man-Rogosa-Sharpe (MRS broth) (Oxoid, Thermo Fisher Scientific Inc) at 37°C for 24 h[16] for the preparation and cultivation of probiotic A dose of 1% and 2% probiotic culture was prepared by dissolving 10 ml and 20 ml respectively in 1000 ml drinking water. In addition, the concentration of the probiotic was adjusted as 10^8 Colony Forming Unit (cfu/mL).

Extraction of Moringa oleifera

2000 g leaf powder of *M.oleifera* was macerated for 3x24 hours at room temperature in 2000 ml of 70% ethanol and then filtered using Whatman paper. The extraction process was repeated severally by the renewal of the solvent after which the solvent was removed by rotary evaporation in order to obtain *Moringa oleifera* extract (60°C, 50 rpm).[17,18]

In this study, 50-day old chicks (DOC) were used, and their treatment was divided into 5 ($n = 10$). This experiment was carried out for 3 weeks (Day 1-21 of hatching).

Bodyweight gain

The body weight gains were detected weekly by subtracting the weight obtained the previous week from the currently realized one. However, this was carried out every week until the age of 21 days.

Feed consumption

This was calculated weekly by subtracting the quantity of feed consumed from the remaining amount.

Feed efficiency

It was calculated by multiplying the average feed consumed by the chicks and the weight gained by 100.

Feed conversion ratio:

The feed conversion ratio (FCR) was calculated by determining the average weight gained and feed consumed by the chicks in each treatment.

FCR = AFC/ABW

Where FCR = Feed Conversion Ratio, AFC = Average Feed Consumed (g) and ABW = Average Body Weight gain (g)

Statistical analysis

All the data obtained were evaluated by adopting the analysis of variance (ANOVA) method using the Statistical Product and Service Solution (SPSS, IBM Corporation, USA) for Windows 22.0 program. However, supposing a notable difference was detected, another test was carried out using the Duncan's Multiple Range Test method at a significant level of 5% to determine the most effective treatment.

Results and Discussion

The average values of feed consumption are shown in Table 1 and Figure 1. This shows significant differences ($p < 0.05$) between treatment and control. The highest feed consumption was discovered in the supplementation of 1% prebiotic + 1% probiotic, while the least was found in 2% prebiotic + 2% probiotic. The use of probiotics enhances feed consumption because it contains an appetite-stimulating effect. The results from this study are consistent with the research carried out by Rowghani et al., (2007), which started that feeding broilers with probiotics significantly improve their feed intake and efficiency.[19] The results show that high probiotic and prebiotic supplementation tend to reduce feed consumption. This is also consistent with the research carried out by Biggs *et al.* (2007), which stated that the use of high doses of oligosaccharides reduces MEn and AA digestibility.[20] The use of probiotics containing a combination of *L. fermentum* and *L.plantarum* were consistent with the results from the research carried out by Loh *et al.* (2010), which stated that the use of *L. fermentum* boosts lactic acid bacteria count, affects the height of intestinal villi as well as increases the concentration of faecal volatile fatty acid.[21] The average body weight gain of prebiotic and probiotic supplementation in the starter phase of broilers is shown in Table 1 and Figure 2. Furthermore, it showed a significant difference ($p<0.05$) between the treatment and control. The highest average body weight gain was detected in the supplementation of 1% prebiotic + 2% probiotic, and it is similar to the groups supplemented with 2% prebiotic + 1% probiotic and 1% prebiotic + 1% probiotic. The least was discovered in the control group. Probiotic supplementation increases average body weight gain because it tends to improve nutrient and dry matter digestibility, crude protein and most amino acids as well as energy, in 21-day-old chickens at the ileal region, in addition it also boosts growth performance in the starter phase.[22] The combination of *Moringa oleifera* extract and probiotics *L. fermentum* and *L.plantarum* was discovered to boost average body weight gain, and this is in accordance with the research carried out by Wang (2016) which stated that probiotic supplementation triggers the development as well as increases the total length of the small intestine, and this describe for more digestive activities and absorption thereby increasing the use of nutrients to improve average daily gain.[23]

The average values of feed conversion ratio (FCR) are shown in Table 1 and Figure 3. According to the FCR results, there was a significant difference ($p < 0.05$) between the treated and untreated (control). The least FCR was detected in the supplementation of 1% prebiotic + 2% probiotic, 2% prebiotic + 2% probiotic, 1% prebiotic + 1% probiotic respectively and was similar to the 2% prebiotic + 1% probiotic. The highest was discovered in the untreated (control) group. Subsequently, the low FCR value is influenced by the low amount of feed consumed, although it leads to an increase in body weight gain. The results from this study indicate that the supplementation of 1% prebiotic + 2% probiotic showed a lower FCR value of 15.94% compared to the control. This is because the quantity of feed consumption which is similar to the control and produced a higher average daily weight gain than in the untreated group. An increase was detected in the average daily weight gain, which was 18% higher than the control. The supplementation of 2% prebiotic + 2% probiotic also shows a lower FCR value of 14.28% compared to the control, this is because the amount of feed consumption was lower in the untreated group. However, it showed a higher average daily weight gain value of 8.03% compared to the control. The supplementation of 1% prebiotic + 1% probiotic showed a lower FCR value of 11.89% compared to the controls. This is related to the higher amount of feed consumption and average daily gain of 16.46% compared to the control. The results from this study are consistent with the research carried out by Loh et al., (2010), which reported that the use of *L. fermentum* and prebiotic enhance growth performance and improved feed conversion ratio in broilers.[21,24] The feed efficiency values for prebiotic and probiotic supplementations in the starter phase broilers are shown in Table 1 and Figure 4. The results show that there was a significant difference ($p < 0.05$) between the treated and control groups. The least feed efficiency was determined in the control, while the highest was 1% prebiotic + 2% probiotic, 2% prebiotic + 2% probiotic and it is similar to the supplemented 2% prebiotic + 1% probiotic and 1% prebiotic + 1% probiotic. The results from this study are consistent with the research carried out by Bai *et al.*, (2013), which stated that the use of *L. fermentum* with Saccharomyces aids to improve feed efficiency compared to the provision of basal feed without probiotic supplements.[25] The use of *Moringa oleifera* extract enhances the growth of *L.plantarum* and *L. fermentum* probiotics. These results are consistent with the study carried out by Yang *et al.* (2006), which stated that the use of *Moringa oleifera* increases the number of Lactobacillus in the ileum of broilers.[11]

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Table 1: The value of feed consumption, average weight gain, feed conversion ratio, feed efficiency and body weight of starter phase broilers by supplementation of prebiotic and probiotic

Treatment	Feed consumption (g/d)	Average weight gain (g/d)	Feed conversion ratio	Feed efficiency (%)	Body weight (g)
Control	$63.00^{b} \pm 1.61$	$39.48^{a} \pm 2.03$	$1.60^{b} \pm 0.08$	$62.67^{a} \pm 2.81$	$826.67^{a} \pm 40.57$
1% prebiotic +1% probiotic	$66.65^{c} \pm 1.04$	$45.98^{bc} \pm 1.55$	$1.43^{a} \pm 0.05$	$68.98^{ab} \pm 1.83$	$956.67^{bc} \pm 31.10$
2% prebiotic +1% probiotic	$64.38^{b} \pm 1.49$	$44.15^{bc} \pm 4.64$	$1.47^{ab} \pm 0.10$	$68.49^{ab} \pm 5.93$	$920.00^{bc} \pm 92.76$
1% prebiotic +2% probiotic	$64.38^{b} \pm 2.19$	$46.93^{c} \pm 3.25$	$1.38^{a} \pm 0.98$	$72.69^{b} \pm 5.38$	$975.67^{c} \pm 64.99$
2% prebiotic +2% probiotic	$59.82^{a} \pm 1.72$	$42.65^{ab} \pm 3.45$	$1.40^{a} \pm 0.18$	$71.49^{b} \pm 7.84$	$890.00^{ab} \pm 68.94$

[a,b,c] means within the same column having significantly different ($p < 0.05$).

The inclusion of prebiotics has a positive effect on the digestive tract as well as modulates an increase in beneficial microbes and a decrease in pathogen bacteria.[10] The results from studies mentioned above stated that the supplementation of *L. plantarum* B1 improves growth performance reduces the number of *E. coli* in cecal, increase lactic acid and short-chain fatty acid (SCFA) in the ileum and caecum of broilers.[26] The addition of prebiotic significantly increased the height and width of the villus, including depth of the crypt in duodenum and ileum.[24] This is related to the optimization of nutrient absorption in order to increase feed efficiency.

The values of body weight in starter phase broilers are shown in Table 1 and Figure 5. It was significantly different ($p < 0.05$) in broilers fed with prebiotic and probiotic compared with those in control, and it is similar to those supplemented with 2% prebiotic + 2% probiotic from 1 to 21 days. The highest body weight was discovered in the supplementation of 1% prebiotic + 2% probiotic and is similar to the supplementation of 1% prebiotic + 1% probiotic and 2% prebiotic + 1% probiotic while the least was detected in the control. This study shows that *Moringa oleifera* extract containing oligosaccharides tends to be fermented by probiotics with *L. plantarum* and *L. fermentum*. These results are consistent with studies which stated that oligosaccharides such as mannan-oligosaccharides (MOS) and fructo-oligosaccharides (FOS) are fermented by Bifidobacteria and Lactobacilli.[8] The results from this study also disclosed that *Moringa oleifera* leaf supplementation shows an increase in the total body weight, average body weight gain, and daily gain, daily feed intake as well as improve feed conversion ratio as previously reported.[14, 27]

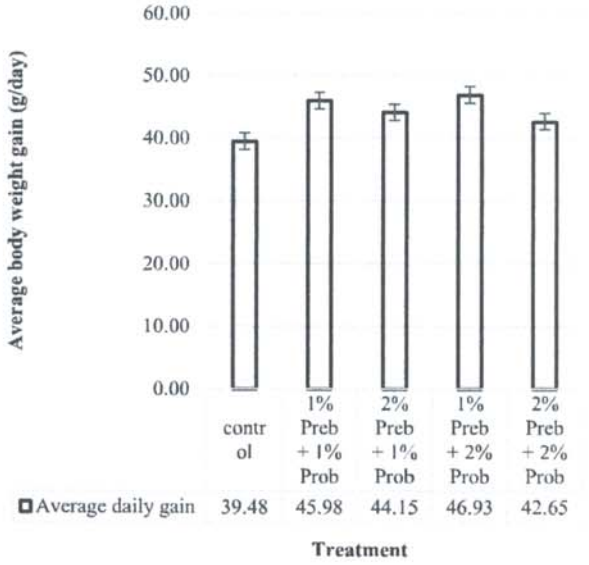

Figure 2: Average body weight gain of Broilers supplemented with prebiotic and probiotic in the starter phase.

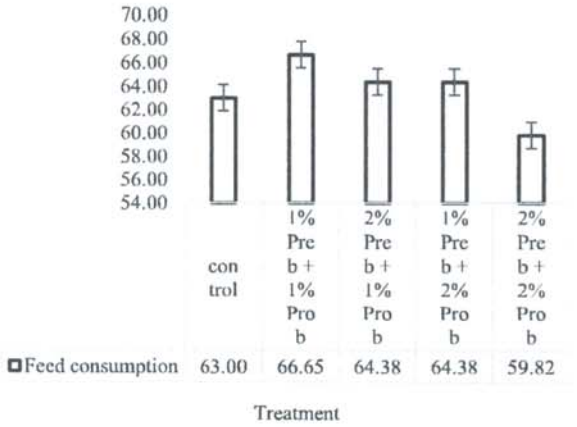

Figure 1: Feed consumption of supplementation prebiotic and probiotic in starter phase broilers

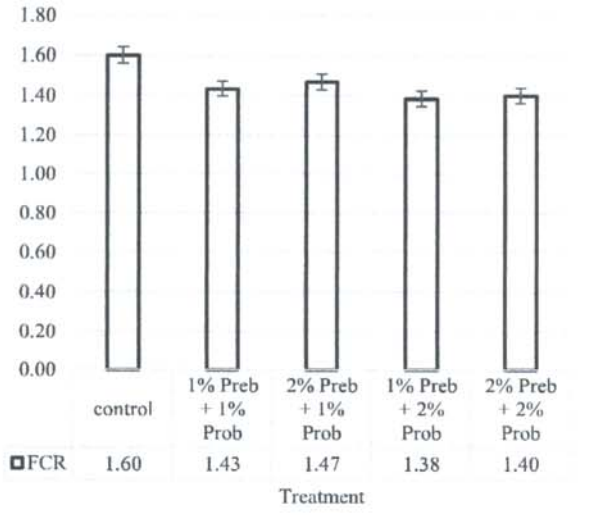

Figure 3: Effect of Supplementation of prebiotic and probiotic in broiler starter phase on feed conversion ratio

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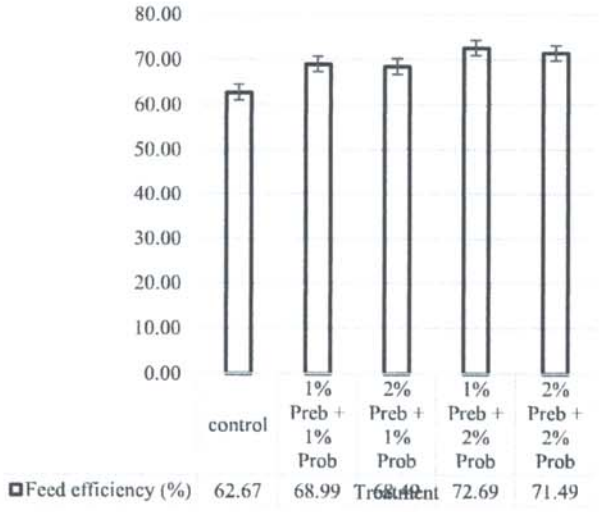

Figure 4: Average feed efficiency of supplementation of prebiotic and probiotic in broiler starter phase diets

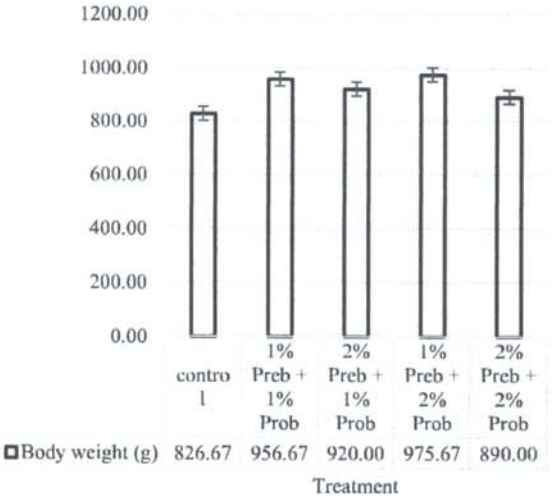

Figure 5: Average body weight of supplementation of prebiotic and probiotic in broiler starter phase diet

Conclusion

The dosage of 1% prebiotic + 2% probiotic supplementation is recommended due to its low concentration yielding, which has a similar effect as 2% prebiotic + 2% probiotic supplementation. Subsequently, the supplementation of 1% prebiotic + 2% probiotic needs to be optimally included in the diets of broiler during the starter phase. Alternatively, a combination of antibiotic growth promoters showed promising symbiotic properties for improved growth performance and productivity of broiler chicken in the starter phase.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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