



Effect of Prebiotics-Probiotics Synbiotic Supplementation in Feed on Performance, Serum Cholesterol, and Pathogenic Bacteria in Broilers Intestines

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Abstract | The combined effect of probiotics and prebiotics (PPS) in broiler's digestive tract is effective in improving nutrient absorption and broilers health. Therefore, this study aimed to evaluate synbiotic effect of PPS in broilers diets on performance, blood lipid profiles, and pathogenic bacteria in intestines. Synbiotic comprised a combination of prebiotics *Moringa oleifera* leaves and probiotics yeast culture in a ratio of 1:1 (g/g). The results of *in vitro* analysis contained probiotics *Saccharomyces spp.* of 1.7×10^9 cfu/g. Broilers used were 200-day-old chicks (DOC), divided randomly into 4 treatments with 5 replications, each consisting of 10 broilers. The control (treatment A) was given basic feed without the addition of PPS, while treatments B, C, and D were administered feed with PPS 0.2%, 0.4%, and 0.6% respectively. The results showed that supplementation of 0.2% PPS in feed significantly ($P < 0.05$) increased feed efficiency by 3.77% and reduced cholesterol in serum by 24.79% compared to the control. Administration of 0.6% PPS significantly ($P < 0.05$) reduced low-density lipoprotein (LDL) levels, while 0.2% PPS ($P < 0.05$) reduced total cholesterol. *Coliform* and *E.coli* bacteria populations in broilers intestines had no significantly different effect ($P > 0.05$) between treatments. This showed that the addition of 0.2% PPS to commercial rations could increase feed efficiency and reduce total cholesterol levels in broilers. However, the addition of 0.2-0.6% PPS to commercial feed did not have a significant impact on suppressing pathogenic bacteria in broilers intestines. Therefore, the types of prebiotics, probiotics, and their combination, as well as method of administration and concentration were recommended to be explored for further studies.

Keywords | Broilers, Cholesterol, Pathogenic, Probiotics, Prebiotics, Synbiotic

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INTRODUCTION

Antibiotics are the most widely used additives to increase feed conversion, growth rate, and health of broilers. Numerous studies have explored the application

of other products such as probiotics, prebiotics, synbiotic, herbs, and essential oils as viable alternatives due to the ban on antibiotics in broilers feed (Bidura *et al.*, 2017; Callaway *et al.*, 2017; Bidura *et al.*, 2019; Bidura *et al.*, 2020; Candrawati *et al.*, 2023).

According to (Tickle and Codd, 2019; Oke *et al.*, 2024), broilers are susceptible to disease, showing the need for rigorous and intensive maintenance due to high sensitivity to changes in environmental temperature. One disease that easily infects broilers is colibacillosis which is caused by the pathogenic bacteria *Escherichia coli* (da Rosa *et al.*, 2020). In Indonesia, this disease is found in broilers and laying hens across various regions. Therefore, *E. coli* has a significant effect on the economic (Indra *et al.*, 2022), due to a decrease in productivity as well as the quality of the carcasses and eggs produced (Joseph *et al.*, 2023).

Synbiotic is a combination of probiotics and prebiotics (PPS), which are used as supplementation in broilers feed (Gyawali *et al.*, 2019; Swanson *et al.*, 2020). Probiotics are additional feed in the form of live microbes that are beneficial by improving the balance of microorganisms in the digestive tract (Tripathi *et al.*, 2019; Prasanth *et al.*, 2022). Meanwhile, prebiotics are feed ingredients containing oligosaccharides that cannot be digested by the host but provide beneficial effect by stimulating the growth of digestive tract microflora (Prasanth *et al.*, 2022).

Tape yeast is an alternative probiotics that has the potential for significant development (Daniati *et al.*, 2022). Previous study by (Bidura, 2020) showed that supplementation of 0.10-0.30% probiotics *Saccharomyces* spp. N-2 (tape yeast isolate) in the diet of Lohmann Brown layer hens aged 40-50 weeks improved the digestibility of dry and organic matter, with crude protein. The supplementation also reduced the levels of *Coliform* and *E. coli* bacteria, as well as the ammonia gas content in excreta. According to (Candrawati *et al.*, 2023), a potential source of prebiotics administered to broilers is Moringa leaf (*Moringa oleifera*), which is classified as hard-to-dissolve fiber in the digestive tract. The leaf flour contains 3.30% fat, 26.4% protein, 38.50% carbohydrates, 16.80% crude fiber, and 8.5% minerals (Shiriki *et al.*, 2015). Therefore, the presence of synbiotic interactions between PPS was explored to provide more optimal health outcomes (Markowiak and Slizewska, 2017). (Soren *et al.*, 2023) stated that synbiotic possess the ability to improve growth performance, histomorphology, and health in broilers. (Raksasiri *et al.*, 2018) feed conversion ratio (FCR) could be increased through synbiotic while reducing ammonia concentration in the digestive tract. Cholesterol concentration can also be reduced due to the potential of PPS to lower pH, thereby enhancing the health and performance of broilers (Ślizewska *et al.*, 2020).

An increase in feed efficiency and biocontrol pathogenic bacteria can be achieved through the combined effect of plant herbal compounds and probiotics microbes. Generally, bioactive compounds play an important role in broilers feed, serving as a substitute for use of antibiotic growth promoter (AGP). This is attributed to the presence of herbal

leaves in feed to improve digestibility and efficiency, causing a significant reduction in egg yolks (Siti and Bidura, 2022). (Ślizewska *et al.*, 2020) reported that synbiotic can increase the number of beneficial bacteria and suppress the growth of pathogens, thereby increasing broilers performance parameters. PPS can also increase the concentration of lactic acid and short-chain fatty acids (SCFA), along with a decrease in the concentration of branched-chain fatty acids (BCFA) in broilers manure.

Based on the description, this study aimed to explore effect of prebiotics (*Moringa oleifera* leaves) and probiotics (yeast culture) in a ratio of 1:1 (g/g) in feed on performance, serum cholesterol, and pathogenic bacteria in broilers intestines.

MATERIALS AND METHODS

ETHICAL APPROVAL

All test broilers consisting of 200-day-old chicks (DOC) were placed in battery colony cages in a building with a controlled temperature for comfortable conditions. This study was approved by the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

EXPERIMENTAL DESIGN

A total of 200 DOC of healthy male broilers were used in the feeding trial. Rations and drinking water were provided ad libitum, by placing feed containers and drinking water containers in the cage for each treatment. One-day-old male broilers (n=200) were randomly divided into 4 treatments and 5 replications with each consisting of 10 broilers. Synbiotic is a combination of probiotics yeast culture (1.7×10^9 cfu/g) and prebiotics moringa leaves (*Moringa oleifera*) in a ratio of 1:1 (g/g). Broilers in treatment A (control) were given basic feed without PPS, while broilers in treatments B, C, and D had 0.2%, 0.4%, and 0.6% PPS in commercial rations, respectively. The commercial feed used was CP 511 an antibiotics-free product produced by PT. Charoen Phokphan Tbk, Sidoarjo, Indonesia. Nutritional content of broilers feed is shown in Table 1.

PARAMETER MEASURED

Measurements of feed consumption (FCn), final body weight (FBWt), body weight gain (BWGn), and feed conversion ratio (FCR) were carried out every week throughout the observation period (age 0-5 weeks). FCn was calculated based on the difference between feed given and the remaining feed, while FBWt was measured at the end of the study. Before weighing, broilers were fasted for six hours, while drinking water remained available. BWGn is the difference between final body weight and initial body weight. Meanwhile, FCR is a comparison between FCn and BWGn in the same time unit. The lower the FCR value, the higher

Table 1: Nutritional content of broilers feed.

Chemical composition ¹⁾	Treatment				Standard ²⁾
	A	B (0.2%)	C (0.4%)	D (0.6%)	
Gross Energy(kkal/kg)	4130	4130	4130	4130	-
Crude protein (%)	22.74	22.74	22.74	22.74	Min 18
Crude fiber (%)	7.53	7.53	7.53	7.53	Max 6.0
Ash (%)	6.81	6.81	6.81	6.81	-

1: Lab analysis of nutrition and animal feed; 2: Nutrient standards according to SNI (2006).

Table 2: Effect of PPS supplementation in feed on production performance of 5-week-old broilers.

Variable	Treatment				SEM ²⁾
	A	B (0.2%)	C (0.4%)	D (0.6%)	
Initial body weight (g/head)	47.00 ^{a1)}	47.40 ^a	46.70 ^a	46.90 ^a	0.26
FCn (g/head)	3093.6 ^a	2929.9 ^a	2977.95 ^a	2981.9 ^a	67.35
FBWt g/head)	1996.7 ^a	1967.1 ^a	1959.35 ^a	1952.1 ^a	40.10
BWGn (g/head)	1949.7 ^a	1919.7 ^a	1912.65 ^a	1905.2 ^a	40.10
FCR	1.59 ^a	1.53 ^b	1.56 ^{ab}	1.57 ^{ab}	0.02

1: Different alphabets on the same line were significantly different (P<0.05); 2: Standard error of the treatment means.

Table 3: Effect of PPS supplementation in feed on the blood lipid profile of 5-week-old broilers.

Variable Blood lipid profile (mg/dl)	Treatment				SEM ²⁾
	A	B (0.2%)	C (0.4%)	D(0.65)	
LDL	93.73 ^{a1)}	79.44 ^b	82.53 ^b	55.62 ^c	2.74
HDL	59.05 ^{ab}	31.75 ^b	41.80 ^b	87.58 ^a	9.68
Triglycerides	21.03 ^a	34.77 ^b	36.57 ^b	33.04 ^b	1.91
Total cholesterol	156.98 ^a	118.06 ^b	131.74 ^{ab}	149.81 ^a	9.27

1: Different alphabets on the same line were significantly different (P<0.05); 2: Standard error of the treatment means.

feed efficiency. The blood lipid profile was determined by analyzing the blood of 5-week-old broilers using the method (Fan *et al.*, 2021). Determination of pathogenic bacteria *E.coli* and *Coliform* was carried out using Eosine Methylene Blue Agar (EMBA) media. Samples that had been incubated were observed after 24 hours at a storage temperature in an incubator of 37°C. Subsequently, colony counts were carried out using a quebec colony counter (Sudatri, 2021).

STATISTICAL ANALYSIS

Data obtained were analyzed using SPSS (Statistical Product and Service Solutions) software version 25. When the data distribution was homogeneous, further testing was conducted using one-way ANOVA, with a confidence interval of 5% (P<0.05). Meanwhile, when there was a significant effect, further testing was performed using the Duncan test.

RESULTS AND DISCUSSION

Table 2 shows the production performance of 5-week-old broilers given PPS in the ration. The addition of PPS at 0.2% (B), 0.4% (C), and 0.6% (D) caused FCn reduction of 5.29%, 3.74%, and 3.61%, respectively, which was not

significantly different (P>0.05) from the control (A). Broilers that received PPS addition of 0.2% (B), 0.4% (C), and 0.6% (D) showed FBWt reduction by 1.48%, 1.87%, and 2.23%. This result was not significantly different (P>0.05) compared to the control (A). BWGn of broilers that received PPS of 0.2% (B), 0.4% (C), and 0.6% (D) reduced by 1.54%, 1.90%, and 2.28%, although there was no significant difference (P>0.05) compared to the control (A). The administration of PPS at 0.2% (B) significantly (P<0.05) increased feed use efficiency compared to control (A). Meanwhile, PPS of 0.4% (C), and 0.6% (D) did not significantly (P>0.05) increase feed use efficiency compared to control (A).

The results of PPS in rations on the blood lipid profile of 5-week-old broilers are shown in Table 3. Broilers that received PPS treatment of 0.2% (B), 0.4% (C), and 0.6% (D), showed low-density levels. Lipoprotein (LDL) in the blood was reduced by 15.25%, 11.95%, and 40.66%, which was significantly different (P<0.05) from the control (A). The high-density lipoprotein (HDL) content of broilers that received control A was 59.05 mg/dl, while 0.2% (B) and 0.6% (C) PPS were 46.23 and 29.21%, respectively, without significant difference (P>0.05). The triglyceride content

Table 4: Effect of PPS supplementation in feed on *Coliform* and *E.coli* populations in intestines of 5-week-old broilers.

Variable (Colony form unit/g)	Treatment				SEM ²⁾
	A	B (0.2%)	C (0.4%)	D (0.6%)	
<i>E. Coli</i>	2.0 x10 ^{3a1)}	4.85x10 ^{3a}	4.65x10 ^{3a}	8.20x10 ^{3a}	0.24
<i>Coliform</i>	1.70x10 ^{4a}	2.65x10 ^{4a}	5.20x10 ^{4a}	6.80x10 ^{4a}	0.18

1: Different alphabets on the same line were significantly different (P<0.05); 2: Standard error of the treatment means.

in the blood of control (A) was 21.03 mg/dl, while PPS of 0.2% (B), 0.4% (C) and 0.6% (D) had 65.34%, 73.89%, and 57.11%, respectively, which was significantly different (P<0.05). The total cholesterol content in the blood of the control (A) was 156.98 mg/dl, while 0.2% (B) had 24.79%, showing a significant difference (P<0.05). Broilers that received 0.4% (C) and 0.6% (D) total cholesterol content in the blood were 16.08% and 4.57%, showing no significant difference (P>0.05) from the control (A).

The results of PPS in rations against pathogenic bacteria in intestines of 5-week-old broilers are shown in Table 4. Total *E.coli* and *Coliform* bacteria in broilers that received PPS treatment of 0.2% (B), 0.4% (C), and 0.6% (D) were not significantly different (P>0.05) higher compared to the control (A). The analysis results showed that the total *E.coli* bacteria ranged between (2.0 x 10³ cfu/g) – (8.20 x 10³ cfu/g), while the total *Coliform* bacteria varied from (1.7 x 10⁴ cfu/g) to (6.80 x 10⁴ cfu/g).

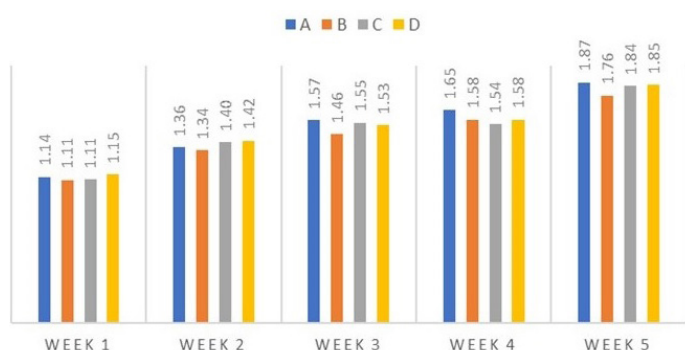


Figure 1: Average FRC value for broilers 1-5 weeks.

The addition of PPS 0.2-0.6% had no different effect on FCn of broilers compared to control (A). Based on the results, there was no difference in feed consumption, due to the ability of PPS in broilers digestive tract to use nutrients efficiently. (Utami and Wahyono 2018) stated that there was no significant difference in feed consumption in broilers given 0.4% (C) and 0.2% (B) PPS. (Sarangi et al., 2016) also stated that the addition of PPS had the same effect on broilers consumption. (Mairizal et al., 2019) stated that the probiotics did not significantly affect broilers' ration consumption. Similarly, there was no substantial difference in FBWt and BWGn due to the provision of PPS at 0.2% (A), 0.4% (B), and 0.6% (D). (Sarangi et al., 2016) reported that the addition of prebiotics, probiotics, and synbiotic did not increase body weight gain and broilers ration con-

sumption. The supplementation of moringa leaf flour to the ration did not affect growth and consumption rate (Utami and Wahyono, 2018). The addition of PPS to commercial broilers feed from 0-5 weeks of age cause a significant improvement feed efficiency in broilers, as shown in Figure 1.

FCR value shows a comparison between the amount of feed consumed and body weight gain. According to (Kolawole et al., 2020), FCR value provides an overview of the level of feed use efficiency. Based on the comparison results, a higher level of feed use efficiency is directly correlated with a lower FCR level (Hasan et al., 2020). In this study, FCR value of broilers that received 0.2% (B) PPS treatment was significantly (P<0.05) lower than the control (A). The results showed a strong correlation to the role of moringa leaf as a source of prebiotics in broilers' digestive tract, which increased the growth of lactic acid bacteria (LAB) and the yeast *Saccharomyces* spp. Therefore, there was an increase in the activity of digestive enzymes, digestibility, and absorption of food substances. (Jeni et al., 2021) stated that the addition of probiotics can increase the growth of beneficial microorganisms, thereby improving the absorption of food substances.

Low-density lipoprotein (LDL) content in the blood of broilers treated with PPS 0.2% (B), 0.4% (C), and 0.6% (D) was significantly lower than the control (A). This was due to the results of leaf fermentation moringa by tape yeast, which produced metabolite products such as SCFA consisting of acetate, butyrate, and propionate. Propionic acid will reduce cholesterol synthesis in the liver by inhibiting the action of HMGCoA reductase enzyme. This was in line with (Kumar et al., 2022), where the administration of non-fat yogurt and inulin from sweet potato flour reduced LDL cholesterol content in hypercholesterolemic mice. In this study, LDL levels ranged from 55.62 – 93.73 mg/dl, as shown in Table 3. (Mardewi et al., 2017) stated that normal LDL levels in broilers are <130 mg/dl. HDL in the blood prevents atherosclerosis by transporting cholesterol from peripheral tissues to the liver to catabolize into acids and bile salts, which enter the small intestines and are excreted with the excreta. Based on the results, the HDL content is still in the normal range of 31.75 – 87.58 mg/dl. This is in accordance with the opinion (Mardewi et al., 2017), where normal HDL levels in broilers are >22 mg/dl. The triglyceride content of broilers treated with PPS 0.2% (B), 0.45% (C), and 0.6% (D) was significantly (P<0.05) higher than

the control (A). This is because the PPS treatment of tape yeast and moringa leaf causes the solubility of nutrients such as fat to increase, thereby improving fat absorption. (Alves-Bezerra and Cohen, 2017) stated that excess fat in the body would be stored in the form of triglycerides and experience a breakdown when there was a lack of energy. (Tada *et al.*, 2020) also stated that the high concentration of triglycerides in the blood comes from high fatty acid synthesis in the liver. In this study, the range of blood triglyceride levels was still within the normal of 21.03-36.57 mg/dl. This is in accordance with the statement (Regar *et al.*, 2019) that normal broilers triglyceride levels range between ≤ 150 mg/dl. The total cholesterol content of broilers that received 0.2% (B) was significantly lower than the control (A). Similarly, (Ebrahimi *et al.*, 2022) stated that some probiotics bacteria could assimilate cholesterol directly from the digestive tract. The administration of synbiotic can also inhibit the action of the HMGCoA reductase enzyme, which plays a role in cholesterol synthesis in the liver. Therefore, the liver will take up cholesterol that is attached to the blood vessels, causing a significant decrease in total blood cholesterol.

Decreased cholesterol and LDL in serum broilers given PPS supplementation may be caused by the presence of saponins in Moringa leaves. A previous study by (Suci *et al.*, 2023) in laying hens reported that the decrease in cholesterol content in egg yolk was caused by an increase in levels of saponin, capable of reducing lipid absorption. As reported by (Elamin *et al.*, 2019) the phytochemical compounds in baobab leaves (*Adansonia digitata*) could function as a natural antioxidant to reduce triglyceride, cholesterol, and LDL levels compared to control. (Adriani *et al.*, 2015) also stated that supplementation of Noni extract in drinking water could reduce total cholesterol, triglycerides, and LDL in broilers serum. Additionally, the reduction in cholesterol was caused by the presence of probiotics. As reported by (Sivamaruthi *et al.*, 2020), probiotics could contribute to the regulation of serum cholesterol concentrations by deconjugating bile acids.

The total population of *E.coli* and *Coliform* bacteria in intestines of broilers that received 0.2-0.6% PPS treatment had the same effect as the control (A), as shown in Table 4. This showed that synbiotic of tape yeast and moringa leaf up to 0.6% have not worked optimally to suppress the growth of pathogenic bacteria in the digestive tract due to the relatively low production of organic acids. Based on the results, pH range in broilers that received synbiotic treatment ranged from 6.48 to 6.60. According to (Puspasari *et al.*, 2014), the acidity level (pH) of broilers small intestines given levels of protein and acetic acid in the diet in the duodenum ranged from 4.17-5.68, jejunum 5-6, and ileum 5.83-6. (Rizal *et al.*, 2019) stated that *E.coli* could grow at pH 4-9. *E.coli*. Moreover, *Coliform* bacteria are a group of

gram-negative bacteria that are normal flora in intestines, but pathogenic when their numbers exceed the threshold. In this study, the presence of *E.coli* and *Coliform* bacteria in the digestive tract was still at normal levels. (Sudatri, 2021) discovered that total *E.coli* was normal in broilers' digestive tract (10^4 - 10^5 cfu/g), while total *coliform* was normal in the digestive tract (4.0×10^6 cfu/g) – (9.4×10^6 cfu/g).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study showed that the addition of 0.2% PPS to commercial rations could increase feed efficiency and reduce total cholesterol levels in broilers. These results provided valuable information for broilers breeders to improve feed efficiency and reduce cholesterol levels. However, the addition of 0.2-0.6% PPS to commercial feed did not have a significant impact on suppressing pathogenic bacteria in broilers intestines. Therefore, the types of prebiotics, probiotics, and their combination, as well as method of administration and concentration were recommended to be explored for further studies.

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NOVELTY STATEMENT

The novelty of this study is that the addition of 0.2% PPS in a ratio of 1:1 (g/g) in commercial feed can increase feed efficiency, while 0.6% PPS is capable of reducing broilers blood cholesterol.

AUTHOR'S CONTRIBUTIONS

To all authors (DPMA, IGM, IGNGB, and NWS) who were included in writing this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this study.

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