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Growth performance, internal organ weight and intestinal microbial population in broiler fed a synbiotic of purslane (*Portulaca oleracea* L.) extract and *Lactobacillus plantarum*

Yusri SAPSUHA[1] (✉), Sundari SUNDARI[2], Nur SJAFANI[1], Amran NUR[3], Zauzah ABDULLATIF[4], Suryati TJOKRODININGRAT[4]

[1] Department of Animal Science, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia
[2] Department Biology Education, Faculty of Teacher Training and Education, Universitas Khairun, Ternate, North Maluku, Indonesia
[3] Department of Pharmacology, Biomedicine, Medical Faculty, Universitas Khairun, Ternate, North Maluku, Indonesia
[4] Department of Agriculture, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia

✉ Corresponding author: yus_ara01@yahoo.co.id

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ABSTRACT

This study aimed to evaluate the effectiveness of adding a synbiotic consisting of purslane extract and *L. plantarum* on the growth performance, internal organ weight, and intestinal microbial population of broiler chickens. The treatment started when the broilers were 8 days old with an average body weight of 132.20 ± 0.95 g. The feed ingredients included yellow corn, soybean meal, meat bone meal, palm oil, DL-methionine, bentonite, limestone, premix, chlorine chlorite, and NaCl, which were formulated accordingly. The study used a completely randomized design with four treatments and four replications, namely T0 (chicks offered diet without additive), T1, T2, and T3 (chicks offered diet with 0.5%, 1%, and 1.5% of the additive purslane extract and *L. plantarum* synbiotic). The results showed that synbiotic administration significantly enhanced ($P < 0.05$) growth performance, internal organ weights such as the heart, pancreas, and bursa of Fabricius, and increased the number of lactic acid bacteria while significantly reducing ($P < 0.05$) coliform bacteria in the ileum and cecum during the rearing period.

Keywords: synbiotic, purslane, growth performance, intestinal microbial, internal organs

INTRODUCTION

As concerns over antibiotic resistance increase and the ban on the use of antibiotics as growth promoters (AGPs) in several countries, including Indonesia, synbiotics have become an appealing alternative. Synbiotics can reduce reliance on antibiotics in broiler chicken production by naturally enhancing the performance and health of chickens (Leite et al., 2020; Prentza et al., 2022). By improving intestinal health and increasing nutrient absorption, synbiotics can enhance broiler chicken production performance, including more efficient feed conversion, faster growth, and higher production levels (Beski and Al-Sardary, 2015; Sapsuha et al., 2023).

Synbiotics are a combination of prebiotics and probiotics that work synergistically to improve the health of the digestive system (Sarangi et al., 2016; Sugiharto, 2016). Prebiotics are dietary fibers that cannot be digested by the human body but can be digested by beneficial bacteria in the intestines (Rehman et al., 2020). Prebiotics provide nutrition for these beneficial bacteria, supporting their growth and survival (Sugiharto, 2016). Probiotics are live microorganisms that provide health benefits when consumed in adequate amounts. Probiotics help maintain the balance of intestinal flora, enhance digestion, and strengthen the immune system

(Bai et al., 2013; Pourakbari et al., 2016). One type of beneficial probiotic is *Lactobacillus plantarum*, which, according to Wang et al. (2017) and Peng et al. (2016), positively affects the growth of broiler chickens, reduces *E. coli* content in the cecum, and increases the number of lactic acid bacteria in the cecum and ileum.

Historically, purslane (*Portulaca oleracea* L.) (Figure 1) has been considered a weed that can inhibit the growth of agricultural plants, but it turns out to contain highly beneficial bioactive compounds such as flavonoids, alkaloids, and polysaccharides. These compounds exhibit various pharmacological effects including antibacterial, antioxidant, hypoglycemic, hypolipidemic, and neuroprotective properties (Zhou et al., 2015; Rahimi et al., 2019).

Figure 1. Purslane (*Portulaca oleracea* L.)

The use of purslane as an alternative to AGPs is expected to support the sustainability of broiler chicken farming without increasing production costs. Research results indicate that the use of purslane extract can improve the performance of broiler chickens (Habibian et al., 2018). In addition to being a phytogenic material, purslane also contains oligosaccharides and polysaccharides, which can function as prebiotics, as nearly all oligo and polysaccharides are known to be prebiotics (Zhou et al., 2015).

Based on the potential of synbiotics, the use of purslane extract combined with *Lactobacillus plantarum* is expected to serve as an effective synbiotic that can enhance the performance of broiler chickens, replacing the role previously played by antibiotic growth promoters (AGPs). However, studies on the use of purslane extract as a synbiotic with *L. plantarum* are not widely found in the literature. Therefore, this study aims to test the effect of administering the synbiotic combination of purslane extract and *L. plantarum* on improving performance, intestinal microbial population, and internal organ weight in broiler chickens.

MATERIALS AND METHODS

Making synbiotics

Purslane was sourced from the vicinity of the Universitas Khairun campus in Ternate, North Maluku Province, Indonesia. Before use, the purslane was air-dried and ground into powder. The purslane extract was prepared by macerating 1 kg of purslane powder in 4 litres of 96% ethanol solution for 3 × 24 hours. During the maceration process, the mixture was stirred twice daily, in the morning and evening. The resulting filtrate was then filtered and evaporated using a rotary evaporator to produce the purslane extract. The production of the synbiotic began by mixing 5 ml of purslane extract per 100 ml of distilled water with 10 ml of *L. plantarum* (bacterial concentration of 1×10^9 cfu/ml). The synbiotic was stored in a refrigerator until used.

In vivo experiment

This research has been approved by the Animal Research Ethics Committee of the Faculty of Agriculture at Universitas Khairun, with the number: 09/KEPH/PH/2023. A total of 200 unsexed broiler chickens were raised in open cages and in healthy conditions. The treatment involved the administration of synbiotic purslane extract and *L. plantarum* as a feed additive. The treatments included T0 (chicks offered diet without additive), T1, T2, and T3 (chicks offered diet with 0.5%, 1%, and 1.5% of the additive purslane extract and *L. plantarum* synbiotic). Each treatment was repeated five times with ten chickens per repetition. Feed treatments began on day 8 after rearing and were mixed with the basic feed (Table 1). Feed and water were provided ad libitum until day 35. All chickens were vaccinated with a commercial Newcastle disease (ND) vaccine on day 4 via eye drops and on day 18 through drinking water. Throughout the study, no chickens became sick or died.

The performance of broiler chickens was measured including body weight, feed consumption, and feed conversion at the end of the rearing period. After the calculation of viability percentage and FCR, the European Broiler Index (EBI) was used to evaluate the growing performance of broilers as suggested by Marcu et al. (2013) as follows:

$$EBI = \frac{Viability\ (\%) \times ADG\ (g/chick/day) \times 100}{FCR\ (kg\ feed/kg\ gain) \times 10}$$

The weights of internal organs such as the heart, liver, proventriculus, gizzard, pancreas, spleen, thymus, bursa of Fabricius, duodenum, jejunum, ileum, and cecum were measured on day 35. Total coliform bacteria were counted using MacConkey agar medium (Merck KGaA) and incubated at 38 °C for 24 hours under aerobic conditions. Coliform bacteria that grew on the agar medium appeared red, and bacterial colonies were then counted. Total lactic acid bacteria were counted on de Man, Rogosa, and Sharpe agar medium (MRS; Merck KGaA) and incubated anaerobically at 38 °C for 48 hours.

Data Analysis

Analysis of variance (ANOVA, SPSS version 16.0) was run to statistically analyse the data based on a completely randomized design. Duncan's multiple range test was used to distinguish statistically distinct means ($P < 0.05$).

Table 1. Feed compositions of broilers as a starter (days 1-21) and finisher (days 22-35)

Items	%, unless otherwise noted	
	Starter (days 1-21)	Finisher (days 22-35)
Yellow corn	56.42	60.46
Soybean meal	32.1	28,61
Meat Bone Meal	4.81	4.26
Palm oil	2.22	2.22
DL-methionine	0.41	0.41
Bentonite	1.09	1.09
Limestone	1.25	1.25
Premix[1]	1.32	1.32
Chlorine chlorite	0.06	0.06
NaCl	0.32	0.32
Calculated composition:		
Metabolizable energy (kcal/kg)[2]	3,072	3,167
Crude protein (%)	22.18	20.64
Crude fibre (%)	3.87	3.88
Crude fat (%)	5.10	5.27
Methionine	0.82	0.80
Lysine	1.40	1.39
Ca	1.00	1.00
P	0.80	0.80
Analyzed composition:		
Metabolizable energy (kcal/kg)[2]	2,902	3,061
Crude protein (%)	21.14	19.36
Crude fibre (%)	3.24	3.16
Crude fat (%)	4.44	4.82

[1] Premix contained (per kg of diet) of vit. A 7,750 IU, vit D3 1,550 IU, vit E 1.88 mg, vit B1 1.25 mg, vit B2 3.13 mg, vit B6 1.88 mg, vit B12 0.01 mg, vit. C 25 mg, folic acid 1.50 mg, Ca-d-pantothenate 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, BHT 25 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, Choline chloride 500 mg, DL-methionine 900 mg, $CaCO_3$ 641.5 mg, Dicalcium phosphate 1500 mg

[2] Metabolizable energy was predicted based on formulation (Bolton, 1967) as follow: 40.81 {0.87 [CP + 2.25 crude fat + nitrogen-free extract] + 2.5}

RESULTS AND DISCUSSION

Broiler performance

Synbiotic supplementation in feed affects the growth performance of broiler chickens. Statistical analysis results show that the administration of synbiotic purslane extract and *L. plantarum* has an impact on the average growth performance of broiler chickens (Table 2). According to Duncan's test, the average final body weight and weight gain were higher ($P < 0.05$) in treatments T2 and T3 compared to treatments T0 and T1. Feed consumption was also higher ($P < 0.05$) in treatments T2 and T3 compared to treatments T0 and T1. Meanwhile, more efficient feed conversion was observed in treatments T0 and T1 compared to T2 and T3.

The administration of 1.5% purslane extract and *L. plantarum* in the feed significantly improved weight gain and feed conversion in broiler chickens in this study, suggesting a possible synergistic effect of the provided synbiotic. The bioactive components in purslane and the probiotic *L. plantarum* may facilitate enhanced nutrient digestion through increased secretion of digestive fluids, influenced by microbial growth control in the intestines. This condition leads to improved feed utilization and efficiency, thereby enhancing broiler growth performance. These results are consistent with the findings of Sapsuha et al. (2023), who observed improved villi growth, protein digestion, weight gain, and feed conversion in broiler chickens fed with a synbiotic blend of nutmeg meat extract and *L. plantarum*. Previous studies also demonstrated positive effects on weight gain, feed intake, and feed conversion in broiler chickens fed diets containing synbiotics compared to those fed diets without synbiotics or antibiotics (Yuanita et al., 2019; Abdel-Wareth et al., 2019). The improved feed conversion observed in broiler chickens given the purslane extract and *L. plantarum* synbiotic indicates better feed utilization than the control group (T0). Other studies have also revealed that the use of synbiotics in broiler chicken feed can enhance intestinal digestibility, ultimately boosting broiler growth (Bogucka et al., 2019; Mohammed et al., 2018).

Internal organ weight

Statistical analysis results indicate that the administration of the synbiotic combination of purslane and *L. plantarum* affects the average weight of internal organs in broiler chickens (Table 3), and according to Duncan's test, the average weight of internal organs showed significant differences ($P < 0.05$). The weight of the heart significantly increased ($P < 0.05$) in T3 compared to T0, T1, and T2. Meanwhile, the weight of the pancreas and bursa of Fabricius in T2 and T3 significantly increased ($P < 0.05$) compared to T0 and T1.

Table 2. Performance of broiler chickens

Items	Treatment groups				SE	*P* - value
	T0	T1	T2	T3		
Initial BW (g)	132.37	131.78	132.64	132.01	0.97	0.55
Final BW (g)	1667.82^a	1669.89^a	1734.56^b	1798.42^c	62.57	<0.01
Weight gain (g)	1535.45^a	1538.11^a	1601.92^b	1666.41^c	62.49	<0.01
Feed intake (g)	2428.84^a	2437.34^a	2497.84^b	2521.03^b	66.03	0.04
FCR	1.58^c	1.58^c	1.56^b	1.51^a	0.05	0.03
EBI	301.38^a	302.17^a	318.48^b	341.03^c	31.29	<0.01

^{a,b,c} In the same row, different superscripts indicated a significant variation ($P < 0.05$), SE: standard error, BW: body weight, FCR: feed conversion ratio, EBI: European Broiler Index.

Table 3. Internal organ weight of broiler chicks fed treatment diets

Items	Treatment groups				SE	*P* - value
	T0	T1	T2	T3		
Liver	50.21	51.32	49.78	50.3	0.95	0.15
Heart	8.23[a]	8.98[a]	9.17[a]	9.98[b]	0.79	<0.01
Proventriculus	9.11	8.79	8.88	8.87	0.33	0.48
Gizzard	32.84	31.98	32.16	32.11	1.20	0.70
Pancreas	5.87[a]	5.12[a]	6.64[b]	6.56[b]	0.69	<0.01
Spleen	1.87	2.12	2.01	1.98	0.30	0.65
Thymus	2.87	2.75	2.98	2.78	0.32	0.48
Bursa Febricus	0.54[a]	0.67[a]	1.12[b]	0.98[b]	0.27	<0.01

[a,b] On the same row, different superscripts indicated a significant variation ($P < 0.05$), SE: standard error.

Our current research results show that chickens fed a diet containing 1.5% synbiotic of purslane and *L. plantarum* have relatively higher heart weights (Table 3). The increase in heart weight in this study is likely due to improved availability and digestibility of nutrients for cell metabolism as a result of the administration of the purslane and *L. plantarum* synbiotic. These nutrients need to be distributed to cells, and in this case, the heart plays a crucial role in the circulation of nutrients, causing the heart to work harder and thereby leading to enhanced development of the cardiac muscle (Horhoruw and Rajab, 2019). Synbiotics can also affect physiological activities in poultry, including metabolism and the cardio-muscular system (Sarangi et al., 2016). This increase in activity may contribute to the increased heart weight.

The research results indicate that the weight of the bursa of Fabricius in chickens given the synbiotic of purslane and *L. plantarum* increased compared to the control group. The bursa of Fabricius is a specialized lymphoid organ found in poultry, playing a crucial role in the development of the immune system, particularly in the production of B cells essential for the body's immune response to infections (Peng et al., 2017). The increase in the weight of the bursa of Fabricius from the administration of the purslane and *L. plantarum* synbiotic is likely due to its beneficial effects on intestinal microbiota without producing toxins. Peng et al. (2017) reported that a decrease in the weight of the bursa of fabricius could be caused by feed contaminated with aflatoxins, a group of cytotoxic and carcinogenic mycotoxins produced by Aspergillus flavus. Furthermore, Cazaban et al. (2015) stated that toxins could adversely affect health, including the immune development of organs like the bursa of Fabricius.

Microbial population

Statistical analysis results indicate that the administration of the synbiotic of purslane and *L. plantarum* affects the intestinal microbiota population in both the ileum and caecum (Table 4), and according to Duncan's test, the average intestinal microbiota population shows significant differences ($P < 0.05$). The population of lactic acid bacteria (LAB) significantly increased ($P < 0.05$) in T3 compared to T0, T1, and T2, in both the ileum and caecum. Conversely, the population of coliform bacteria significantly decreased ($P < 0.05$) in T3 compared to T0, T1, and T2, in both the ileum and caecum.

Table 4. Bacteria population in the intestine of broiler chickens

Items	Treatment groups				SE	*P* - value
	T0	T1	T2	T3		
Ileum (log cfu/g)						
LAB	9.83[a]	10.25[ab]	10.69[b]	11.24[c]	0.71	<0.01
Coliform	7.53[b]	7.12[b]	7.02[b]	6.18[a]	0.58	0.02
Caecum (log cfu/g)						
LAB	10.44[a]	10.93[b]	11.23[b]	11.85[c]	0.56	<0.01
Coliform	9.44[b]	9.16[b]	8.94[b]	8.11[a]	0.65	<0.01

[a,b,c] In the same row, different superscripts indicated a significant variation ($P < 0.05$), SE: standard error

The administration of the synbiotic consisting of purslane and *L. plantarum* can increase the population of LAB in the intestines, both in the ileum and caecum. Synbiotics, a combination of prebiotics and probiotics, can promote the growth and activity of beneficial bacteria, including LAB, within the digestive tract. The prebiotics in the synbiotic provide food for probiotic bacteria like *Lactobacillus*, thereby helping to increase their number and activity in the intestines (Sapsuha et al., 2023). Prebiotics fermented by beneficial bacteria can produce metabolites in the form of short-chain fatty acids (SCFA). SCFAs such as acetate, butyrate, and propionate can enhance the growth of LAB in the intestines (Sugiharto, 2016). These results align with research by Śliżewska et al. (2020), which reported that synbiotic administration can increase the population of lactic acid bacteria and decrease coliform in the intestines. *L. plantarum*, a lactic acid bacterium, produces lactic acid which can maintain the pH of the digestive tract of broiler chickens at acidic levels (Sapsuha et al., 2023). Acidic conditions in the digestive tract, particularly in the ileum and caecum, support the balance of microflora by increasing the population of lactic acid bacteria and decreasing pathogenic bacteria. Moreover, acidic conditions can enhance the effectiveness of nutrient absorption processes by the intestinal walls, thereby meeting nutritional needs, maintaining health status, and increasing productivity (Mabelebe et al., 2014).

CONCLUSION

Treatment with 1.5% of the additive synbiotic of purslane and *L. plantarum* to broiler chickens improved performance, and intestinal microbial population and did not have a detrimental effect on the internal organ weight of broiler chickens.

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